**Vectorial potential of *Mansonia* species in the transmission of *Wuchereria bancrofti* and evaluation of mosquito collection methods in Tana-Delta, Coastal Kenya**

**Nancy Mutanu Kinyatta**

**A thesis submitted in partial fulfilment for the degree of Master of Science in Medical Parasitology and Entomology in the Jomo Kenyatta University of Agriculture and Technology**

**2010**

# DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature ……………………………….                Date………………………

**Nancy Mutanu Kinyatta**

This thesis has been presented for examination with our approval as University supervisors.

Signature…………………………………..                Date……………………

**Prof. Zipporah Ng'ang'a**

**JKUAT, Kenya**

Signature...............................................                Date................................

**Dr. Luna Kamau**

**KEMRI, Kenya**

## DEDICATION

This thesis is dedicated to my beloved family; my husband Patrick, son Eric and

Daughter Irene, who have all along been of support to see my progress to victory.

# ACKNOWLEDGEMENT

**TABLE OF CONTENTS**

**CHAPTER ONE**

**CHAPTER TWO**

**2.0 LITERATURE REVIEW** .............................................................**8**

**CHAPTER THREE**

**CHAPTER FOUR**

**CHAPTER FIVE**

## LIST OF TABLES

# LIST OF FIGURES

# LIST OF PLATES

# LIST OF APPENDICES

# LIST OF ABBREVIATIONS AND ACRONYMS

**BP**                Base pairs

**$^{O}$C**                Degrees Celsius

**CBRD**            Centre for Biotechnology Research and Development

**CDC**             Centres for Disease Control and Prevention

**CFA**             Circulating filarial antigen

**DALYs**          Disability Adjusted Life Years

**DEC**             Diethylcarbamazine

**DNA**             Deoxyribonucleic Acid

**ELISA**           Enzyme-linked immunosorbent assay

**G**                 Grams

**GAPELF**       Global alliance program for elimination of lymphatic filariasis

**GPELF**         Global program for elimination of lymphatic filariasis

**HLC**             Human landing catch

**ICT**             Immunochromatograpy test

**IVM**             Integrated vector management

**JKUAT**        Jomo Kenyatta University of Agriculture and Technology

**KEMRI**        Kenya Medical Research Institute

**KG**              Kilograms

**LF**               Lymphatic filariasis

**MDA**            Mass drug administration

**Ml**               Milli litres

**μM**              Micro litres

**MM**             Milli meters

| | |
|---|---|
| **MX** | Molecular xenodiagnosis |
| **NPELF** | National program for elimination of lymphatic filariasis |
| **PCR** | Polymerase chain reaction |
| **PH** | log H+ Concentration |
| **PM** | Pico moles |
| **PSC** | Pyrethrum spray catch |
| **SPSS** | Statistical package for social sciences |
| **SSP 1** | Species-specific primer 1 |
| **TAE** | Tris Acetic Ethylenediaminetetra acetic acid (EDTA) |
| **WHO** | World Health Organization |

# ABSTRACT

Different mosquito species have been incriminated as vectors of lymphatic filariasis (LF). On the Kenyan coast, *Anopheles, Culex* and *Aedes* species have been identified as vectors of LF. This study aimed at determining whether *Mansonia* species are also vectors of LF in Tana-Delta district of Kenya. Secondarily, the study also evaluated mosquito sampling methods. A cross-sectional study was carried out in six villages in the district, where mosquitoes were collected by three methods: Pyrethroid sprays, CDC light Traps and CDC Gravid Traps. Mosquitoes from each collection method were counted to determine the method with the highest catch. A total of 1632 mosquitoes were collected, with 1265 being collected by light traps (77.55%), 311 (19.1%) by pyrethrum sprays, and 56 (3.4%) by gravid traps. The collected mosquitoes were identified to the level of genera. Five mosquito genera were collected: *Culex* species, 1048 (64.2%), *Aedes* species, 188 (11.5%), *Mansonia* species, 236 (14.5%), *Anopheles* species 148 (9.1%), and *Ficalbia* species 12 (0.7%). The prevalence of *Wuchereria bancrofti* in *Mansonia* species was also determined. Fifty *Mansonia* mosquito species were dissected to determine presence of *W. bancrofti* stage III larvae ($L_3$). To identify filarial worms in mosquito specimen, Deoxyribonucleic acid (DNA) was extracted from filarial larvae, amplified by the PCR assays using *W. bancrofti* species-specific primers. Only two out of 50 *Mansonia* species dissected had stage II filarial larvae. Deoxyribonucleic acid (DNA) was also extracted from individual *Mansonia* species, and analyzed by PCR to determine *W. bancrofti* infectivity rates. The PCR analysis was negative for *W. bancrofti*. Light traps were found to be the most efficient method for mosquito

sampling. There was no evidence that *Mansonia* species have significant medical importance in the transmission of *W. bancrofti* since both dissection and PCR assays did not indicate any transmission potential in the mosquitoes. It is therefore recommended that light traps should be used in collecting large numbers of mosquitoes for parasite screening purpose.

**CHAPTER ONE**

## 1.0 INTRODUCTION

### 1.1 Lymphatic filariasis

Lymphatic filariasis (LF) is a chronic parasitic disease of public health and socioeconomic significance in tropical and subtropical countries. More than 128 million people are estimated to be infected in 83 countries, with nearly 1.2 billion people at risk (Michael and Bundy, 1997). The global burden of LF is estimated at 5.78 million disability adjusted life years (DALYs) lost annually (Dreyer *et al.*, 1997). Lymphatic filariasis is caused by three of the threadlike nematodes, namely, *Wuchereria bancrofti, Brugia malayi* and *B. timori* species (Ottesen and Ramachandran, 1995). World Health Assembly in May, 1997 resolved to eliminate lymphatic filariasis by the year 2020 through the Global alliance program for elimination of lymphatic filariasis (GAPELF) in endemic areas (Ottesen, 2000; Molyneux, 2003), using mass drug administration (MDA) with the combination therapy of albendazole and diethylcarbamazine (DEC) or ivermectin (Chodakewitz, 1995; WHO, 2000).

Monitoring of an intervention strategy is an essential component of the elimination programme which involves surveillance of either microfilaraemia or antigenaemia levels in the community or infection rates in the vector populations (Weil *at el.*, 1997; Lammie *et al.*, 2004). When the infections are at low levels, large numbers of mosquitoes need to be dissected to determine the infection rates, thus dissection

becomes less sensitive and highly labour intensive (Fischer *et al.,* 2003). Blood test is the most reliable way to determine if someone is infected with microfilariae due to different symptomatic representations of the disease (Fischer *et al*., 2003). Lymphatic filariasis parasites such as *W. bancrofti* have a nocturnal periodicity, thus blood used in diagnosis is drawn at night between 10pm - 4am (Miranda *et al.*, 2005). Antigen-detection, immunochromatographic test (ICT) detects infection within minutes and can be carried out at any time of the day. Polymerase chain reaction (PCR) a molecular technique is used for parasite detection to overcome the shortcomings of traditional methods such as microscopic examinations of microfilariae in blood samples and mosquito dissections. The use of species-specific oligonucleotide probes and primers in *W. bancrofti* diagnosis (Ramzy, 2002) is an example. The PCR-based assays are less labour intensive and less tedious than dissection. Pool screening of mosquitoes (Ramzy *et al.,* 1997) is cost effective if large numbers of mosquitoes are required for examination. It is possible to detect one infective *Wuchereria* larva in a pool of about 14 – 50 *Anopheles* mosquitoes (Bockarie *et al.*, 2000; Williams *et al*., 2002) and 30 – 50 *Aedes* mosquitoes (Chanteau *et al.*, 2002).

Various mosquito sampling methods are used in entomological studies to ensure large mosquito catches. Three methods have been evaluated in this study to determine the most effective for vector collection. Studies have shown that both biting and feeding behaviour of *Mansonia* species are compatible with a potential for lymphatic filariasis transmission. *Mansonia* species dissection to determine presence

of infective stages (L$_3$) of *W. bancrofti* larvae and *W. bancrofti* DNA detection in the mosquito by PCR was used to determine their transmission potential.

**1.2 Socio - Economic impact of lymphatic filariasis worldwide**

Lymphatic filariasis is a leading cause of permanent and long-term disability worldwide (WHO, 1995). In Coastal-Kenya, LF prevalence of 2% - 27% in human populations has been reported (Mwabobia *et al.*, 2000; Kagai *et al.*, 2008). Although not fatal, people with the disease suffer from pain, disfigurement and sexual disability (Ahorlu *et al.*, 1999). Men and women disfigured by the disease are often rejected by their spouses, families and the community. Affected people are unable to work because of frequent ADL attacks and their disability due to chronic obstructions and this affects their families and their communities. Lymphatic filariasis exerts a heavy social burden especially because of the specific attributes of the disease; chronic complications are often hidden and are considered shameful hence affected persons isolate themselves (Dreyer *et al.*, 1997). For men, genital damage is a severe handicap leading to physical limitations and social stigmatization. For women, shame and taboos are also associated with the disease when affected by lymphoedema, they are considered undesirable and when their lower limbs and genital parts are enlarged they are severely stigmatized and marriage in many situations becomes weak or impossible for the youth (Gyapong *et al.,* 1996b).

Lymphatic filariasis causes economic burden to individuals, households, government-funded and private healthcare systems (Ramaiah *et al.*, 1996; 2000). Economic losses result from the disability associated with acute attacks and chronic

manifestations of the disease (Gyapong *et al.*, 1996b). An acute attack results in several working days lost and sufferers often experience multiple attacks each year (Ramaiah *et al.*, 1996). Lymphatic filariasis also imposes a burden on the health care infrastructure in endemic areas. In Africa, lymphatic filariasis cause almost US $1 billion in losses each year (Haddix *et al.*, 1999), 83% of this loss is due to disability in men with hydrocele. Hydrocele appears to have the greater economic impact because of its prevalence (Lu *et al.,* 1988) coupled with the fact that young adults contribute more to economic development. The surgical management of hydrocele in hospitals causes strain to already overburdened health-delivery systems (WHO, 1997). Prevalence of hydrocele in males above 35years old in Coastal-Kenya ranges from 2-34%, depending on locality (Wijers, 1977a; Estambale *et al.*, 1994a; Wamae *et al.*, 1998; Mwabobia *et al.*, 2000).

## 1.3 Problem statement

*Wuchereria bancrofti* parasites are transmitted by various species of *Culex, Anopheles* and *Aedes* mosquitoes (Bogh *et al.*, 1998). The distribution of *W. bancrofti* is largely determined by climatic conditions which favour survival and abundance of the mosquitoes important in transmission of *W. bancrofti*. *Culex quinquefasciatus, An. gambiae and An. funestus* are the main vectors of bancroftian filariasis in Kenya (Mwandawiro *et al.*, 1997; Kasili *et al.*, 2009). Although *Mansonia* species are also found in the Kenyan coast, studies to document their vectorial capacity in the transmission of *W. bancrofti* in Kenya and their infectivity rate have not been done. This study investigated the role of *Mansonia* species as

vectors of *W. bancrofti*. The detection of filarial infections in the mosquito vectors is an indication that there may be positive individuals in the area.

## 1.4 Study justification

*Wuchereria bancrofti* causal agent of LF is endemic in Coastal–Kenya, with prevalence of 2-27% (Mwabobia *et al.*, 2000; Wamae *et al*., 2001; Kagai *et al*., 2008). The vectors of *W. bancrofti* include, *An. gambiae s.l*., *Cx. quinquefasciatus* and *An. funestus,* the infectivity rates with *W. bancrofti* of the vectors varies from one mosquito species to the other and from one endemic area to another (Mwandawiro *et al*., 1997). At the Kenyan coast, a study by Kasili *et al*. (2009), showed an infection rate for *W. bancrofti* of 16.6%, 4.37% and 1.87% in *An. gambiae s.l*., *Cx. quinquefasciatus* and *An. funestus* respectively. Other mosquitoes such as *Mansonia* may be presented in Coastal Kenya, but their role as vectors of LF has not been investigated in details. In a survey conducted in Shirikisho and Maziwa villages of Tana Delta in 2007, 60% of the mosquito species composition was *Mansonia* species (J. Kagai, preliminary data). The high number of *Mansonia* species obtained from some villages in this district stimulated the need to investigate the role of *Mansonia* species in transmission of *W. bancrofti*. In Kenya, the National lymphatic filariasis elimination programs (NPELF) was launched in 2003 and since then, mass drug administration has been taking place annually in some endemic districts. The incidence of *W. bancrofti* in mosquito vectors is important in the baseline data and during and post eradication period. Xenodiagnosis using mosquito dissection and/or PCR methods is important for surveillance purposes in the effort to eradicate LF in endemic areas. Xenodiagnosis is more acceptable to communities as

it does not directly involve human populations like in the invasive procedures that involve obtaining specimens from humans to detect presence of an infection in an area. Various collection methods are commonly used in entomological studies as a lot of mosquitoes are needed for disease screening.

**1.5 Study hypothesis**

**1.5.1 Null hypothesis**

1. The number of mosquitoes collected by CDC light traps, CDC gravid traps and Pyrethrum spray methods are not the same.

2. *Mansonia* species of mosquitoes are not important vectors of *Wuchereria bancrofti* in Tana-Delta district, Kenya.

**1.5.2 Alternative hypothesis**

1. The number of mosquitoes collected by CDC light traps, gravid traps and Pyrethrum spray methods are the same.

2. *Mansonia* species are important vectors of *Wuchereria bancrofti* in Tana-Delta district, Kenya.

**1. 6 Objectives**

**1.6.1 General objective**

To compare three different mosquito collection methods and to determine the vectorial potential of *Mansonia* species in the transmission of *Wuchereria bancrofti* in Tana Delta district, Kenya.

### 1.6.2 Specific objectives

1. To compare the effectiveness of 3 different mosquito collection methods, namely, CDC light traps, CDC gravid traps and Pyrethrum spray in obtaining mosquito species.

2. To determine the prevalence of *W. bancrofti* in *Mansonia* species in Tana Delta district, Kenya by PCR assays.

3. To determine *W. bancrofti* prevalence ($L_3$) of *Mansonia* species of mosquitoes obtained in Tana delta district, Kenya by dissection.

**CHAPTER TWO**

**2.0 LITERATURE REVIEW**

**2.1 Filariasis**

Filariasis is a disease affecting humans and animals that is caused by nematode parasites of the order Filariidae which are thread like worms (Ottesen and Ramachandran, 1995). Filarial parasites are classified according to the habitat of the adult worms in vertebrate hosts. Cutaneous filariasis is caused by *Loa loa, Onchocerca volvulus* and *Mansonella streptocerca*, the lymphatic filariasis by *Wuchereria bancrofti*, *Brugia malayi,* and *B. timori* (WHO, 1992); and the body cavity filariasis is caused by *M. perstans* and *M. ozzardi* (WHO, 1992).

**Figure 2.1:** Shows a *W. bancrofti* microfilarial worm in a blood film. Mg. X10



**Source;** Lymphatic filariasis: the disease and its control, WHO Technical Report series No. 821, 1992.

**2.2 Epidemiology of lymphatic filariasis**

Lymphatic filariasis is considered as a disease of the poor, and is among the neglected tropical parasitic disease, and is associated with poor sanitation, industrialization and rapid urban population growth in tropics. These conditions create suitable sites for mosquito breeding (Evans *et al*., 1993). In *Wuchereria bancrofti* causing Bancroftian filariasis, humans are the exclusive hosts even though certain strains of *B. malayi* can infect some felines and monkeys (McMohan and Simonsen, 1979). *Wuchereria bancrofti* globally accounts for approximately 90% of all LF infections (WHO, 2001). It is the most common cause of lymphatic filariasis (LF) in Africa (Michael and Bundy, 1997). *Wuchereria bancrofti* is endemic in Africa, India, Southeast Asia, the Pacific Islands, the Caribbean, and South America (Babu *et al.,* 2005). Human brugian filariasis, which is caused by *Brugia malayi* and *B. timori*, affects 13 million people in the oriental region and accounts for 10% of all infections (WHO, 2001; Ottesen *et al.*, 1997). Brugian filariasis is confined to East and South Asia primarily India, Malaysia, Indonesia, the Philippines and China (Jones *et al.,* 1972; Subra and Hebrard, 1975). Globally, one third of the infected people live in India, a third in Africa, and the rest in Americas, the Pacific Islands, Papua New Guinea and South-East Asia (Michael and Bundy, 1997). In Africa, the infections predominate in rural areas where access to health care is mostly inadequate. In Kenya, the only known type of LF inflicting people is caused by *W. bancrofti* which is endemic along the coastal districts of Kwale, Malindi, Lamu, Kilifi and Tana River (Wamae *et al.,* 2001).

## 2.3 Geographical distribution of lymphatic filariasis

Among the agents of lymphatic filariasis, *W. bancrofti* is found in the tropics. Figure 2.2 shows the global distribution of lymphatic filariasis, *Brugia malayi* is limited to Asia and *B. timori* is restricted to some islands of Indonesia. The agent of river blindness, *Onchocerca volvulus*, occurs mainly in Africa, with additional foci in Latin America and the Middle East. *Loa Loa* and *Mansonella streptocerca* are found in Africa, *M. perstans* occurs in both Africa and South America, and *M. ozzardi* occurs only in the American continent.

**Figure 2.2:** Map of lymphatic filariasis endemic countries as of 2004
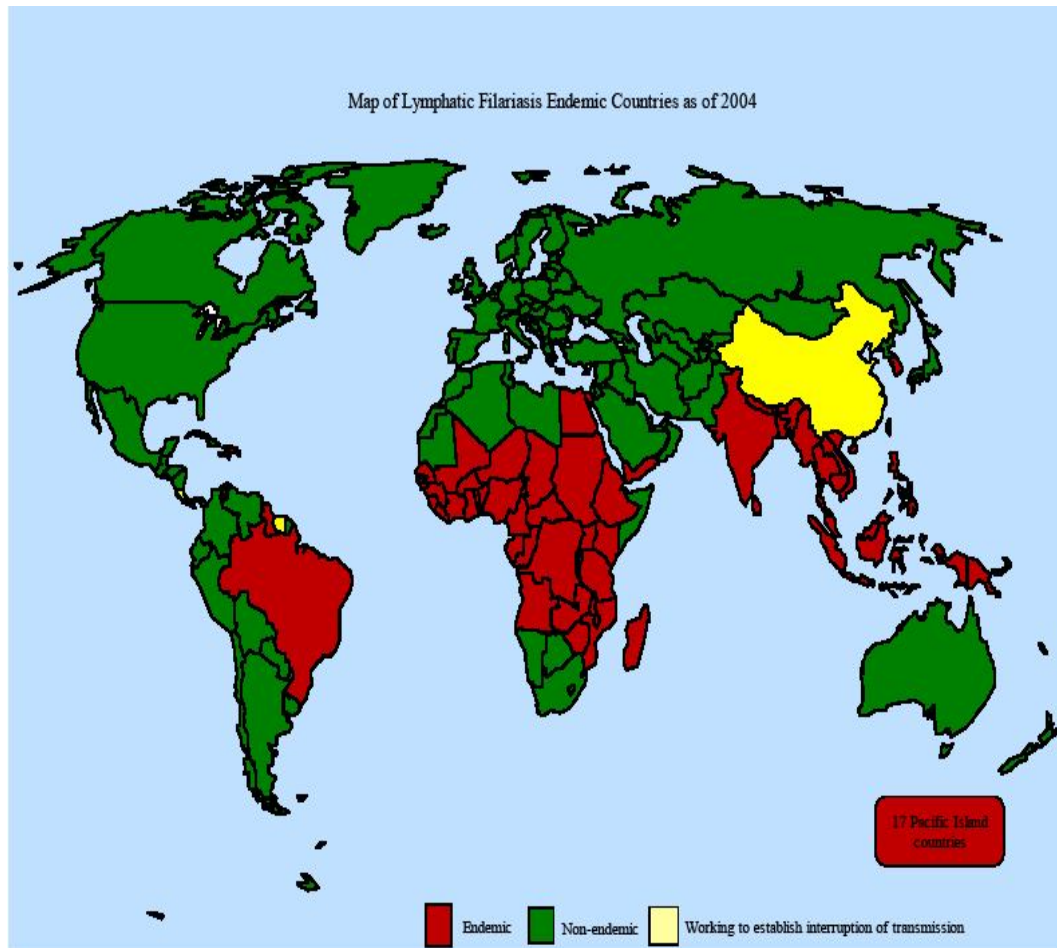


Source: World Health Organisation: Annual report on Lymphatic filariasis ; 2004.

## 2.4 Life-cycle of *Wuchereria bancrofti*

### 2.4.1 Microfilaria pathophysiology and development

Figure 2.3 illustrates the life cycle of *W. bancrofti*. The filarial life cycle, like that of all nematodes, consists of 5 developmental or larval stages in a vertebrate host, an arthropod or intermediate host (vector). Adult female worms produce thousands of microfilariae in the vertebrate host, are released into the peripheral blood and are

ingested by feeding mosquito vectors of *Culex, Anopheles and Aedes* mosquito species. Some microfilariae have a unique circadian periodicity in the peripheral circulation over a 24-hour period; this periodicity is influenced by physical signals like oxygen tension in the blood and body temperature in the host (Scott, 2000). The arthropod vectors such as mosquitoes and flies, also have a circadian rhythm in which they obtain blood meals. The highest concentration of microfilariae usually occurs when the local vector is feeding most actively (Miranda *et al*., 2005). A mosquito ingests the microfilariae during a blood meal (Fig 2.3). After ingestion, the microfilariae lose their sheaths to become first-stage larvae ($L_1$) and some of them work their way through the wall of the proventriculus and cardiac portion of the mosquito's midgut and reach the thoracic muscles, the microfilariae develop into second-stage larvae and subsequently into third-stage (infective larvae) in 7-21 days (WHO, 2002) depending on the ambient temperature and relative humidity. The third-stage infective larvae migrate through the hemocoel to the mosquito's proboscis and can infect another human when the mosquito takes a blood meal. Several hundreds to thousands of infective mosquito bites are necessary to establish infection. Infected mosquitoes are defined as those carrying microfilariae or larvae ($L_1$-$L_3$). Mosquitoes having $L_3$ at any part of the body (proboscis, head, thorax and abdomen) are capable of transmitting the infection to human during blood feeding (WHO, 2000).

During a blood meal, third-stage larvae ($L_3$) are inoculated back into the vertebrate via the skin through the bite of a mosquito, and then migrate to the lymphatics (Fig 2.3). Two moulting stages take place, first to $L_4$ and then to young adults which later, develop into mature adult worms in about 1 year (WHO, 1987) and commonly reside

12

in the lymphatics. The life span of microfilaremia is 1 year (WHO, 1987). The female worms measure 80 to 100 mm in length and 0.24 to 0.30 mm in diameter, while the males measure about 40 mm by 0.1 mm. When mature adults mate, the fertilised females produce mf, which move from the lymphatics into the bloodstream. Adult females produce sheathed microfilariae measuring 260 by 8 µm (McMahon and Simonsen, 1996), with nocturnal periodicity, except the South Pacific microfilariae which have the absence of marked periodicity. The microfilariae migrate into lymph and blood channels moving actively through lymph and blood. The adult parasites may live and produce microfilaria for more than 20 years but on average the life span is shorter (McMahon and Simonsen, 1996).

**Figure 2.3:** Life cycle of *Wuchereria bancrofti* in the host and the vector
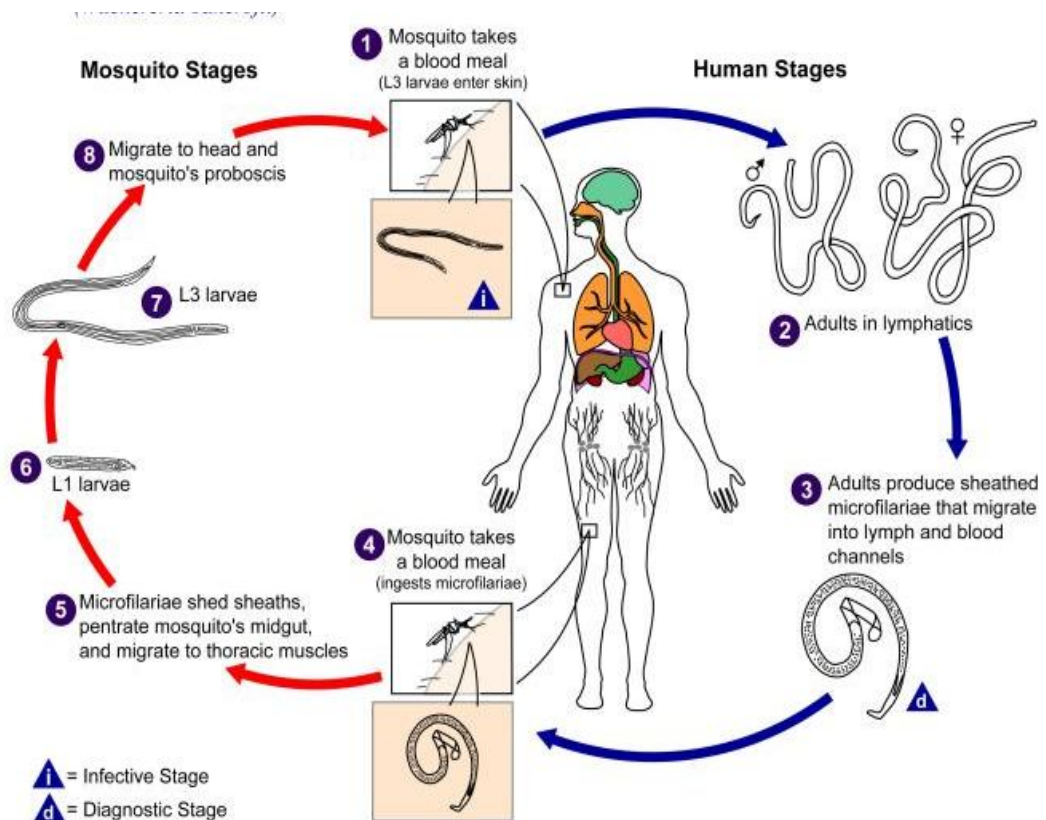


**Mosquito Stages**

① Mosquito takes a blood meal (L3 larvae enter skin)

⑧ Migrate to head and mosquito's proboscis

⑦ L3 larvae

⑥ L1 larvae

⑤ Microfilariae shed sheaths, pentrate mosquito's midgut, and migrate to thoracic muscles

④ Mosquito takes a blood meal (ingests microfilariae)

**Human Stages**

② Adults in lymphatics

③ Adults produce sheathed microfilariae that migrate into lymph and blood channels

▲ i = Infective Stage
▲ d = Diagnostic Stage

## 2.5 Pathology and clinical manifestation of lymphatic filariasis

Clinical features and pathology depend on the sites occupied by developing and mature worms, the number of worms present, length of infections and the immune responses of the host especially to damaged and dead worms. The pathology of lymphatic filariasis is also as a result of the pathogenic potential of the parasites, the tissue response of the host and external bacterial and fungal infections of which most

of the pathology is limited to the lymphatics (Ottesen, 1980). The damage to the lymphatic vessels is mediated both by an immune response to the adult worms and by a direct action of the parasite or the products released by them (Vickery *et al.,* 1991). In the absence of inflammation, marked lymphatic dilation with lymphoedema is seen in experimental animals with immune deficiency and when immuno competent cells are induced, it results in inflammatory granuloma reactions around the parasites and subsequent obstructions of lymphatic vessel occurs leading to lymphoedema (Vickery *et al.*, 1991 ).

Bancroftian filariasis has a wide range of clinical presentations and the symptoms of the infection differ from one endemic area to another and from one individual to another. Clinical presentations are grouped into: asymptomatic (sub clinical), acute, chronic presentations and tropical pulmonary eosinophilia (TPE) of lymphatic filarial disease (Ottesen, 1980). A number of other syndromes may be associated with filarial infections but these may or may not be caused directly by the parasites. The acute form includes inflammatory episodes of the limbs or scrotum. The most common chronic form is the hydrocele and lymphoedema (Estambale *et al.*, 1994a; Wamae *et al.*, 1998).

In endemic areas, a proportion of the population does not show microfilaraemic or clinical manifestation even though they have some degree of exposure to infective larvae (Ottesen, 1992) similar to those who become infected. This is often referred to as the asymptomatic amicrofilaraemic stage of LF. Laboratory diagnostic techniques are not able to determine whether they are infected or not. A considerable proportion

of *W. bancrofti* infected people remain asymptomatic for months or years, even though they have large numbers of circulating microfilaria (Ottesen, 1992), such people are important reservoirs of the infection. Asymptomatic microfilaraemic individuals sometimes do have clinically silent renal abnormalities presenting as microscopic haematuria or proteinuria. Ultrasonography and lymphoscintiographic imaging have also shown that such individuals have dilated and compromised lymphatic functions in the scrotal or lymphatics where the adult worms reside (Freedman *et al*., 1994; Noroes *et al*., 1996).

Acute manifestations of LF occur in the form of acute lymphagities filarial/Acute Adenolymphangitis (ADL), and these consist of intermediate episodes of lymphagities, adenolymphangities, funiculities or epididymo-orchities with fever. These fever episodes occur once or twice a year, although they may occur more frequently (Ramaiah *et al.,* 1996). During the initial stages of the infection there are recurrent episodes of acute inflammation in the lymph vessel or node of the limb and scrotum that are related to bacterial and fungal super infections of the tissues with already compromised lymphatic function. These are termed as filarial fevers or ADLA' (Acute dermatolymphangioadenitis) as that they start peripherally (Dreyer and Piessens, 2000), individuals have features of cellulitis and drain centrally towards lymph nodes. These attacks are associated with transmission intensity (Gyapong *et al.,* 1996a) and host immune response to incoming $L_3$ larvae (Maizels and Denham, 1992). Acute filarial lymphangitis (AFL) another type of 'filarial fever' in which the inflammation starts in the lymph node with extension down the lymphatic tract. The inflammation appears to be immune-mediated in response of the

death of adult filarial worm spontaneously or as a result of treatment with macrofilaricidal drug. AFL is accompanied by fever, headache malaise and cold oedema. Acute filarial lymphangitis (AFL) is common in the scrotal area due to high prevalence of living adult worms of *W. bancrofti* in the lymphatics of the spermatic cold (Noroes *et al.,* 1996; Dreyer *et al*., 1995b).

The chronic form of LF includes elephantiasis, hydrocele and chyluria (Ottesen, 1992). Elephantiasis affects the lower extremities and causes disabling and disfiguring (chronic lymphodema) of the limbs, breasts and the genital area (fig.2.4). Hydrocele condition which affects the male genitalia is fluid filled balloon–like enlargement of the sacs around the testicles which if left untreated can destroy the testicles. Hydrocele is found only with *W. bancrofti* infections and not *Brugia* infections and is the most common clinical manifestation of lymphatic filariasis (Estambale *et al*., 1994a; Wamae *et al*., 1998). It is uncommon in childhood but is seen more frequently post-puberty and with a progressive increase in prevalence with age (Estambale *et al.*, 1994a). In the Kenyan coast, hydrocele in men ranges from 2.0-27.6% (Mwobobia *et al.,* 2000). Chyluria is another form of the chronic filarial syndromes, is caused by the intermittent discharge of intestinal lymph (chyle) into the renal pelvis and subsequently into the urine (McMahon & Simonsen, 1996). The prevalence of chyluria in most endemic areas is very low. Those who develop the chronic form of elephantiasis are usually amicrofilaraemic but have adult worms.

**Figure 2.4**: Elephantiasis of the limbs and the scrotum.



**Source:** Lymphatic filariasis (1992): the disease and its control WHO Technical Report Series No. 821.

Occult filariasis is as a result of hyper responsiveness to filarial antigens derived from microfilaria stages of *W. bancrofti* (Ottesen, 1990). Microfilarial (Mf) are absent in classical clinical manifestation but dead or dying mf are demonstrated in lungs, liver and lymph node biopsies (Webb *et al*., 1960). Patients present with paroxysmal cough and wheezing, low grade fever, scandy sputum with occasional haemoptysis, adenopathy and increased eosinophilia. It affects males twice as often as female and is rarely seen in children (WHO, 1992). X-ray shows diffused nodular mottling and interstial thickening (Ottesen, 1992).

**2.6 Age, sex and lymphatic filariasis patterns**

*Wuchereria bancrofti* infections can start as early as 4-5 years of age with the prevalence of microfilaria increasing gradually with age. The infections peak in early

adulthood and remain stable or decline in later years (Grenfell and Michael, 1992; Kazura *et al.,* 1997). Microfilariae prevalence and mean intensities are higher in males than in females (Brabin, 1990; Chanteau *et al.*, 1995). This has been attributed to difference in exposure due to different activities men and women are involved in, hormonal/pregnancy related effects and differences in sampling time between men and women (Estambale *et al.*, 1994a; Simonsen *et al.*, 1997b). Because of the slow development of the disease, chronic complications are rare in children with the youngest hydrocele cases in 15-20 years old, and its prevalence increases with age (Simonsen *et al.,* 1995a). Leg elephantiasis starts to occur late in life and is more common in men than in women (Simonsen *et al.*, 1995a) while other studies indicate the opposite (Kazura *et al.*, 1984).

**2.7 Mosquitoes as vectors for lymphatic filariasis**

Mosquito are capable of transmitting disease-causing viruses, protozoans, and filarial nematodes. They are the main vectors of lymphatic filariasis. Several species of *Culex, Anopheles*, *Aedes*, and *Mansonia* mosquitoes are involved in the transmission of LF (McMahon *et al.*, 1981). *Culex quinquefasciatus* is the major vector in Africa, Asia, and South America and transmits *W. bancrofti* nocturnally periodically. *Cx. quinquefasciatus* also found to be the main vectors of *W. bancrofti* in the coastal towns and the villages (Wijers and Kinyanjui, 1977; Wijers and Kiilu, 1977). Culicine mosquitoes are important in most urban and semiurban areas. *A*mong *Anophelines; An. gambiae* and *An. funestus* play a significant role in more rural areas of Africa and elsewhere (Kasili *et al.*, 2009). Several *Aedes* species, particularly *Ae. polynesiensis*, are the major vectors in the South Pacific islands where diurnally sub

periodic *W. bancrofti* is common. *Brugia malayi* is primarily transmitted by *Mansonia* and *Anopheles* species and some *Aedes* species. *Brugia timori* is transmitted by *An. barbirostris*. In Kenya, the main vectors of *W. bancrofti* include *Aedes aegypti, culex quinquefasciatus, Anopheles gambiae* and *An. funestus* (Nelson *et al.*, 1962). The importance of these vectors varies from place to place depending on the local ecological conditions (Southgate, 1984). *Mansonia* species; *Ma. uniformis* and *Ma. Africanus* though present, have not yet been incriminated as vectors for *W. bancrofti*.

**2. 8 Incrimination of mosquitoes as vectors for lymphatic filariasis**

This is the association of an organism to disease transmission. It is important to isolate disease vectors and non vectors. Vectors also differ in factors that are related to transmission, for example, they differ in host prevalence, infection rates, seasonal prevalence and also resting behaviour for the purpose of control methods. These transmission factors are used in vector incrimination. The presence of mosquitoes in any area is an indication that they may be able to transmit diseases. The abundance of mosquitoes may indicate a high transmission season especially during the wet season when the breeding of the mosquitoes is high. In rural areas of the Kenyan coast, where *Anopheles* transmit filariasis, transmission levels increase during and immediately after the long rain seasons and decrease during the dry seasons ( Kasili *et al.*, 2009).

The proportion of the vectors infected with *W. bancrofti* parasites is expressed as a percentage or proportion of the infected vectors (mosquitoes). This proportion can be determined by mosquito dissection or PCR assays. Dissection of mosquitoes is used

to look for the larvae stages ($L_1$, $L_2$ and $L_3$) of the parasite in the abdomen, thorax, head and the proboscis of the mosquito. Presence of $L_3$ which is the infective stages of *W. bancrofti* indicates that the mosquito is able to pick the microfilaria during blood intake and is able to support the development of microfilaria to infective stages ($L_3$) through $L_1$ and $L_2$ and subsequent inoculation to human host when it feeds for transmission. In areas of low infection rate, a lot of mosquitoes need to be dissected and this is labour intensive and time consuming (Ramzy, 2002). ELISA and PCR are used but they include expensive procedures.

Different mosquitoes have different host preference. The source of blood taken by the vector can be determined by ELISA or PCR assays. Human Blood Index (HBI) can be determined to indicate that the blood meal was from humans. Human Blood Index (HBI) suggests that the mosquito species may be a vector (Walsh *et al*., 1978; WHO, 1964).

**2.9 Role of *Mansonia* species in transmission of lymphatic filariasis**

*Mansonia* species have long been recognized as the main vectors of human *Brugia malayi,* they are also involved in the transmission of animal filarioids which includes species of the genera *Brugia, Dirofilaria* and *Setaria* (Jones, 1972). The *Mansonia* species belong to the subgenus *Mansonioides*. This subgenus is made up of six species and are grouped into two major categories; the urban-rural type, consisting of *Ma. uniformis, Ma. annulifera, Ma. Indiana/Ma. africana* and *Ma. annulata* which breed in vegetation, choked drains and canals, abandoned ponds or neglected water bodies. The other group comprises of *Ma. bonneae and Ma. dives* breeds mainly in

low lying swamp forests. In the Kenyan coast, two species; *Ma. uniformis* and *Ma. africanus* are common though the prevalence is low (Kasili *et al.*, 2009), but higher in swampy areas (Kagai preliminary data) of Tana River district. *Brugia malayi*, a nocturnal periodic form is principally a rural disease. It occurs in Eastern and Southern Asia including India, West Malaysia, Vietnam, Korea, Thailand, Indonesia, China and some parts of Japan (Jones, 1972; Subra and Hebrard, 1975). It is transmitted mainly by night-biting *Mansonia* mosquitoes, such as *Ma. annulata, Ma. annulifera, Ma. uniformis* and also by various *Anopheles* species (Jones, 1972). These *Mansonia* species breed in more or less permanent waters with floating or rooted aquatic vegetation such as swamps, and ponds. Adults bite mainly outdoors and rest out of doors after feeding, but some species will bite and rest indoors in some areas. The nocturnal subperiodic form of *B. malayi* occurs in West Malaysia, Indonesia, Thailand, and the Philippines and is transmitted by *Mansonia* mosquitoes, mainly by *Ma. annulata, Ma. dives, Ma. bonneae* and *Ma. uniformis*. Larvae occur in habitats with much vegetation, such as swampy forests. Adults bite mainly at night but also during the daytime. The sub periodic form of *B. malayi* is essentially a parasite of swamp monkeys, especially the " leaf monkey (Jones, 1972) and humans become infected when they live near the swamps. Other reservoirs include Macaca monkeys, domestic and wild cats, such as civets, and pangolins. In the Kenyan coast, *Brugia patei, Dirofilaria repens, D. reconditum* and *D. immitis* in dogs and cats are common and *Mansonia africanus* and *Ma. uniformis* being the main vectors ( Nelson *et al.*, 1962). Natural infection rates of mosquitoes with infective larvae of *B. malayi* range from 0.1 – 2 or 3%, which is slightly lower than for *W. bancrofti*, but infection rates vary according to mosquitoes and local conditions (Nelson *et al.*, 1962).

**2.10 Methods of mosquito collection**

Mosquito collection methods differ in their effectiveness. It has often been argued that, if infection rate is the only factor required for monitoring index of transmission in LF control programmes, then large numbers of vector mosquitoes are needed irrespective of the collection method (Goodman *et al.*, 2003; Plichart *et al.*, 2006). Some studies have shown clearly that in terms of numbers and accurate estimation of transmission indices Human Landing Catch (HLC) alone can suffice in situations where vector population densities are high (Goodman *et al.*, 2003). Human Landing Catch and Pyrethrum spray catch (PSC) could be combined where only infection rates are required in the vectors but data from such a combination cannot be used to follow up changes in transmission. Centres for the disease control gravid traps were designed by Dr. Paul Reiter of the centres for Disease Control, Division of Vector Borne Disease for selective capture of gravid *Culex* mosquitoes (Reiter, 1983). Gravid traps baited with oviposition attractants either pheromones or grass hay infusions (Mboera *et al.,* 1999) are effective for sampling gravid mosquitoes. Combination of pheromones and grass infusions yield more mosquitoes as compared to use of one attractant media (Mboera *et al.,* 1999). These traps are able to obtain more mosquitoes when placed far from the natural emergence site (Mboera *et al.*, 1999). Centres for the disease control (CDC) light traps are capable of obtaining a large number of mosquitoes as compared to Pyrethrum sprays and Gravid traps (Mboera *et al.*, 1999)

## 2.11 Diagnosis of lymphatic filariasis

Accurate diagnosis of lymphatic filariasis still remains a problem. In general, diagnosis may be based on clinical, parasitological, histopathological or immunological approaches. For an ideal diagnostic procedure, one requires a method which is sensitive enough to detect low density microfilaremia, accurate in estimating parasite densities, economical, easy to use and above all acceptable to the community. Diagnosis of LF is done both in human populations and mosquito vectors (WHO, 1992; Ramzy *et al.*, 1997). In humans, direct demonstration of the parasites is done microscopically by blood or skin scrapping specimens thick or thin smears. Circulating filarial antigen (CFA) detection is the gold standard method for diagnosing *Wuchereria bancrofti* infections with a great sensitivity (Ottesen, 1984; Weil *et al.*, 1996). Ultrasonography, lymphoscintigraphy, X-ray, and haematology are also used in human population diagnosis (Dreyer *et al.*, 1996c; Ottesen, 1994).

Molecular based tests, Polymerase chain reaction requires collection of human blood, sputum, urine and representative samples of mosquitoes, efficient isolation of DNA from mosquito pools and from human specimens, amplification of parasite DNA sequences, and detection of the amplified product. Species-specific primers (Ssp 1) have been used in PCR to amplify a 188-bp non-coding DNA repeat sequence for diagnosis of *W. bancrofti* (Williams *et al.*, 2002). The amplified product can then be detected by agarose gel electrophoresis, enzyme-linked immunosorbent assay (ELISA), or by DNA test strips (Abbasi *et al*, 1999; Williams *et al.*, 2002; Goodman *et al.,* 2003; Helmy *et al.*, 2004). Pool screening of mosquitoes by PCR allows a far greater number of mosquitoes to be processed at once. Monitoring changes in

mosquito infection rates can provide a timely index of transmission dynamics, as changes in infection patterns in humans could take years to detect (Bockarie *et al.*, 2000). Dissection and examination of female vector mosquitoes for filarial larvae has been used for along time as a diagnostic tool for assessing presence of filarial infection and for monitoring the progress of control programmes in endemic areas (Ramzy *et al.*, 1997; Williams *et al.*, 2002). Mosquito dissection allows calculation of entomological measures of transmission intensity in the endemic areas.

Clinical and epidemiological studies use the presence of microfilaria and clinical disease in the patient or at community level as the diagnostic criteria. Before microfilariae are shed by the adult worms, diagnoses have to be made on the basis of clinical findings as acute manifestation like fever with adenitis, lymphangitis, funiculities or epididymi-orchitis, or as chronic disease manifested as hydrocele or elephantiasis of the legs (Gyapong *et al.,* 1996a). Due to the nocturnal periodicity of *W. bancrofti*, blood sample collection is done during the night (10pm-4am) (McMahon and Simonsen, 1996; Miranda *et al.*, 2005). A diagnostic approach based on ultrasonographic detection of adult worms in the lymphatics has in recent times contributed to the understanding of the clinical picture of the disease (Gyapong *et al.,* 1996a). Correlation studies between infection and disease in endemic communities have shown the disease burden of hydrocele and elephantiasis to be a reliable measure of the prevalence of infection at community level (Gyapong *et al.*, 1996a). Assessment of disease burden could be a useful approach for mapping infection status in parts of Africa where the distribution of lymphatic filariasis is unknown.

Demonstration of microfilarae in the peripheral blood is the most common diagnostic method used in parasitological diagnosis of LF and it is done on blood samples which are collected at night for nocturnal periodicity or during the day for diurnal periodicity. Several approaches have been used to demonstrate presence of microfilariae in blood samples: thick or thin blood smears, Membrane (Nuclepore) filtration method, the counting chamber technique, Knott's method for concentrating microfilariae in low- level micro-filaraemia, DEC provocative test (2mg/Kg) and Quantitative Blood Count (QBC) (Wamae *et al*., 1994; WHO, 1992; McMahon and Simonsen, 1996). These methods differ in their sensitivity, cost and their appropriateness for use in the field settings. The effectiveness of detection of microfilaria in blood is hindered by the large number of infected individuals who are amicrofilaraemic (Chanteau *et al.,* 1994b). The parasitological methods are inconvenienced by the nocturnal periodicity of *W. bancrofti* parasites which requires night blood collection (10.00pm- 4.00pm) (McMahon and Simonsen, 1996; Miranda *et al*., 2005) to coincide with microfilaria in the peripheral blood.

*Wuchereria bancrofti* circulating antigen can be detected in blood or plasma by Immuno Chromatographic Test (ICT), (Weil *et al*., 1997) at any time of the day and is not influenced by nocturnal periodicity of *W. bancrofti*. Circulating filarial antigens (CFA) released by adult worms can also be detected by ICT (Weil *et al*., 1996). Circulating filarial antigens (CFA) detection is regarded as Gold Standard for diagnosing *Wuchereria bancrofti* infection (Weil *et al*., 1996). Immuno Chromatographic Test (ICT) will detect antigen in amicrofilaraemic as well as in clinical manifestations such as lymphoedema, elephantiasis and hydrocoele

conditions (Weil *et al.*, 1997). Immunologically, intradermal tests and antibody detection assays lack specificity and or sensitivity to discriminate between past and present infections. Antigen detection assays provide a more accurate indication of active filarial infection and is useful in mapping the infection status (Wamae *et al.*, 1994). Advantages of ICT are that, it is easy to use in the field setting and can use finger prick day blood samples, which is acceptable by the community as compared to night venous blood required by the other test methods (Njenga and Wamae, 2001). It needs minimum experts and no complicated equipments required (Njenga and Wamae, 2001). A further advantage of antigen detection assays is that they are equally effective on day and night samples irrespective of the periodicity of the parasite. The current draw-backs of this method are relatively high cost and operational issues related to feasibility of use under field conditions (Gyapong *et al.,* 1997). This method however has a limitation in monitoring the effectiveness of control programmes in the short and medium terms due to the fact that antigens can also be detected in the blood even after clearance of the worms.

Serological techniques measure the presence of antibodies or filarial antigens in blood. Antigen detection is non specific due to cross- reaction with other helminth infections, and is limited to its inability to distinguish between exposure, current infection and past infections (Ottesen and Campbell, 1994). Test kit utilising Monoclonal antibody AD12 (Weil *et al.*, 1996) and Monoclonal antibody Og4C3 (Chanteau *et al.*, 1994b; Lammie *et al.,* 1994; Weil *et al.*, 1996) are commercially available both detecting circulating adult worm antigens. Monoclonal antibody AD12 based antigen detection detects whether an individual is positive or negative

without indicating the intensity of infection. It is ideal for field studies for rapid diagnosis. The Og4C3 antigen detection is an ELISA test useful for both serum and filter paper blood specimen (Chanteau *et al.*, 1994b; Lammie *et al.,* 1994). It can quantify indirectly the intensity of infection measured as antigen units.

Ultrasonography using a 7.5 MHz or 10 MHz probe can locate and visualize the movements of living adult worms of *W. bancrofti.* Ultrasonography is used in the scrotal lymphatics of asymptomatic males with microfilaraemia or adult worms in the female breasts (Dreyer *et al.,* 1996c). The constant thrashing movements described as filarial dance sign can be visualized.

Another method sometimes used for diagnosis of lf is lymphoscintigraphy. The structure and function of the peripheral lymphatic systems of the involved limbs can be assessed by lymphoscintigraphy after injecting radio-labelled albumin or dextran which is safe (Ottesen and Campbell, 1994) in the web space of the toes. The structural changes can be imaged using a Gamma camera. Lymphoscintigraphy can also be used to directly demonstrate lymphatic dilation and obstruction can even in early clinically asymptomatic stage of the disease (Witte *et al*., 1993; Freedman *et al.*, 1994).


X-ray is helpful in the diagnosis of tropical pulmonary eosinophilia. Pictures will show interstial thickening, diffused nodular mottling (Ottesen, 1990). Chest X-ray shows increased bronchovascular markings and patchy infiltrates (Ottesen, 1992). Haematology shows an increase in eosinophil count due to the erythrocyte sedimentation rate increase, this indicates early infection of lymphatic filariasis (Ottesen, 1992).

Polymerase chain reaction (PCR) technique is a molecular technique which is rapid and specific (Zhong *et al.*, 1996) developed to detect infectious parasites DNA like filarial parasite DNA in blood and mosquito samples and other specimens. Polymerase chain reaction (PCR) technique used in disease diagnosis can differentiate filariae species in humans, the reservoir host and the mosquito vector in endemic areas (Thanomsub *et al.,* 2000).

## 2. 12 Management and control of lymphatic filariasis

Though LF is not fatal, the control of infections is very vital since chronic elephantiasis cases are not curable. Management of cases is important to avoid many working hours lost when one has episodic fevers and bacterial infections which worsen the situation. Lymphatic filariasis has been identified by the International Task Force for Disease Eradication as one of the six eradicable or potentially eradicable infectious diseases (CDC, 1993).

The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 (Molyneux and Zagaria, 2002). National Programme to Eliminate Lymphatic Filariasis (NPELF) were launched in different endemic areas, in Kenya the program started in 2003 (Molyneux, 2003) and annual MDA is going on in most parts of the Kenyan coast like Kilifi, Malindi, Kwale districts and not yet started in other areas like Tana River district. The main goal of these programmes is to eliminate LF in endemic areas by year 2020 (Ottesen, 2000; Molyneux, 2003). The GPELF/ NPELF use a twin pillar strategy to combat the disease. One component being to stop the

spread of infection by interrupting the transmission, and the second one being to alleviate the suffering of affected individuals by morbidity control. Interruption of transmission is through chemotherapy and vector control. Since the official launch of the GPELF, almost 2 billion doses of once-yearly anti-filarial drug treatment have been administered to over 570 million people through National Programs in 48 countries of the world's 83 endemic countries (Ottesen *et al.*, 2008). The efforts of GPELF has already prevented 6.6 new filarial cases from developing in children and stopped progression of the disease in 9.5 million people (BBC World Service, 2008).

**2.12.1 Chemotherapy of lymphatic filariasis**

Chemotherapy is carried out by identifying the districts in which LF is endemic and then community-wide (mass treatment) programmes implemented to treat the entire at-risk population for about 4-6 years (Ottesen and Ramachandran, 1995). In most countries, the programme is based on yearly administration of single doses of two drugs combination; albendazole plus either diethylcarbamazine (DEC) or albendazole and ivermectin. Albendazole and Ivermectin are given in areas where LF is co-endemic with either onchocerciasis or loiasis (Dreyer *et al.*, 1995a). The combined drugs are significantly more effective than treatment with a single drug alone, yielding up to 80-99% clearance in microfilaremia within days and remain low up to six months after treatment (WHO, 1992). The use of common table/ cooking salt fortified with DEC in the endemic region for a period of 1-2 year is an alternative community-wide regimen with equal effectiveness. These drugs kill infectious LF larvae that circulate in the blood and deprive mosquitoes of their ability to transmit the disease. The interruption of transmission is possible since there are

generous donors who donate the drugs free of charge to the endemic areas. In 1998 the global healthcare company SmithKline Beecham donated albendazole drugs free of charge, for as long as necessary to ensure success of the elimination programme in collaboration with the World Health Organization in its elimination efforts. Merck and Co., Inc. has also expanded its ongoing Mectizan® (ivermectin) Donation Programme to include treatment of lymphatic filariasis where appropriate, and the creation of additional partnerships with other private, public and international organizations, including the World Bank with the aim of strengthening the prospects for success of these elimination efforts.

Diethylcarbamazine (DEC) is the drug of choice in treating lymphatic filariasis (WHO, 1992). Diethylcarbamazine is effective against mf (Microfilariacidal) and lowers mf level even in single dose. It is only effective against adult worms in 50% of patients in sensitive cases. The dosage is 6mg/Kg body weight single dose (WHO, 1992; 1994). Adverse reactions are mostly due to the rapid destruction of mf which is characterised by fever, nausea, myalgia, sore throat, cough and headache (Dreyer *et al.*, 1998). Diethylcarbamazine is the drug of choice in the treatment of TPE and has no effect on the treatment of ADL. This drug is not used in areas where LF is co-endemic with Loaloasis or onchocerciasis because of its severe reaction effect due to rapid death of microfilaria (WHO, 1992; 1994).

Ivermectin is the drug of choice in co-endemic areas of LF and Onchocerciasis or Loaloasis (Chodakewitz, 1995; Dreyer *et al.*, 1995a). Ivermectin directly acts on mf and has no action on adult worms. Ivermectin is effective against mf

(Microfilariacidal) and lowers mf levels even in a single dose of 200μg – 400μg/Kg body weight. It has no action on TPE. With ivermectin, microfilariae reappear faster than with DEC so repeated treatment is required. Adverse reactions are lesser but similar to those of DEC and are characterised by fever, nausea, myalgia, sore throat, cough and headache (Noroes *et al.*, 1997; Dreyer *et al.*, 1998).

Albendazole (ABZ) kills adult worms and has no action on microfilariae. The dosage is 400mg/kg/twice day /2 weeks .The action of the drug is enhanced by a combination of DEC or Ivermectin (WHO, 1992). Albendazole induces severe adverse reactions in hydrocele cases due to sudden death of adult worms.

Diethylcarbamazine (DEC) (0.2-0.4 % w/w) - fortified salt is used for a period of 9-12 months for clearing microfilaria. Diethylcarbamazine (DEC) - fortified is simple, cheap and effective in reducing or eliminating lymphatic filariasis. Diethylcarbamazine incorporated into iodized salt is well tolerable and safe in pregnancies (WHO, 1994; Meyrowitsch and Simonsen, 1998).

### 2.12.2 Surgical treatment of hydroceles

Surgery is the definitive management of hydrocele. Aspiration of hydrocele fluid in areas where resources are scarce is an alternative method to chemotherapy (Musa *et al.*, 1995; Dreyer *et al.,* 1998), the recurrence rate of the disease is high with aspiration of fluid. At the Kenyan coast the prevalence of hydrocelectomy ranges from 2.0%- 27.6% (Mwobobia *et al.*, 2000.)

**2.12. 3 Vector Control**

With integration of the control of lymphatic filariasis with other parasitic disease control programmes, such as malaria, vector control has become possible. Integrated Vector Management (IVM) uses a strategic approach of controlling mosquitoes in addition to the other LF control and management methods. Sustained vector control is achieved by use of several techniques which involves anti larval measures, anti adult measures and personal prophylaxis (WHO, 2004). Anti larval measures include chemical control by use of mosquito larvicidal oil, Pyrosene oil and Organo phosphorous compounds such as Temephos, Fenthion. The selection of control methods is based on the knowledge of the vector biology and disease transmission. Other control programmes include collaboration with the health sector and with other public and private sectors that impact on vector breeding such as agricultural sector/ irrigation. Involvement of local community and other stakeholders' interest in removal of vegetation near houses and drainage of stagnant waters around homesteads help in removal of mosquito breeding places. Community participation has indicated success in removing aquatic plants in controling *Mansonia* species (Panicker *et al*., 1992). Upgrading the quality of pit latrines in urban areas is another control measure especially for *culex* mosquito species (Mwandawiro *et al*., 1997). Biological predators such as larvae eating fish are also important in control. Use of insecticide; Anti adult measures such as indoor residual sprays using DDT, HCH and Dieldrin. Space spraying with pyrethroids is also recommended in vector control. Good management practices, personal awareness such as personal prophylaxis including reduction of man-mosquito contact by using mosquito nets, screening of

houses, and wearing of protective clothes at night (WHO, 2004) are also control measures.

Biocides are used as biological control of mosquitoes whereby toxins producing bacteria *Bacillus sphaericus* or *Bacillus thuringiensis* are used for controling larvae of *Culex quinquefasciatus,* the main vector of LF in most endemic areas of the world (Hougard *et al*., 1993). The microbial agent has significant residue effects against *Cx. quinquefasciatus* and *Cx. pipiens* in highly polluted breeding areas. Biocides are also effective for controling *Mansonia* species vectors of Brugian filariasis in certain regions (Cheong and Yap, 1985). Biological control is environmentally safe and suitable for integrated control programmes with community participation. The bacterium can persist and recycle under field conditions for about 3 months (Hougard *et al*., 1993).

Polystyrene beads are used to control mosquito vector breeding in closed water systems such as pit latrines (Mwandawiro *et al*., 1997). This is effective in urban areas in endemic areas (Maxwell *et al.,* 1990) for controling mosquitoes which breed in foulest water surfaces such as *Culex* species, the beads prevent the mosquitoes from laying eggs on the water surface and help in killing mosquito larvae.

Use of Insecticide –Impregnated bed nets (ITN) is well implemented in the control of *Anopheles* in malaria control programmes in most of endemic countries (WHO, 2004). This is one of the integrated disease control programs because LF will be controlled in general mosquito control. Synthetic pyrethroids with long-lasting residual effects can be successful in controling adult mosquitoes (Rozendaal, 1989).

Long-lasting insecticide treated nets (LLINs) recommended by WHO, are pre-treated bed nets and the residual effects can last 4-5 years withstanding 10 or more washes. Indoor Residual spraying (IRS) is the application of long lasting chemicals on the walls and roofs of houses and domestic animal shelters. These chemicals reduce the lifespan and the density of the vectors and hence reduce transmission intensity (WHO, 2004).

Larval mosquito control is done by eliminating or reducing the source of breeding areas. This is through filling of mosquito breeding sites, draining standing waters, covering water- storage containers and reducing vegetation around the houses. Larviciding is treating water bodies to kill or prevent larval development. Environmental management includes physical, chemical and biological approaches to control both larvae and adult mosquitoes. Proper housing through house screening of doors and windows without large ventilations and holes allowing mosquitoes in the house also helps in the control of adult mosquitoes (WHO, 2004).

Reduction of man-vector contact is through personal protection by wearing long sleeves and trousers in the evenings or at night especially when outdoors. Use of mosquito repellents helps in reducing human-vector contact. New repellent formulations (soap with DEET and permethrin as active ingredients) have good efficacy against *Mansonia* adults and residual protection when applied on human skin (Abu Hassn and Narayanan, 1992). Mosquito coils containing knockdown synthetic pyrethroids also give good protection against *Culex* and *Mansonia* species. Other measures of reducing man-vector contact include observing outdoor working hours because most mosquitoes bite early evening or at dusk and early closing of

house doors and windows to avoid mosquitoes from entering. Zoo prophylaxis is another strategy of man-vector contact by putting animals near houses to divert vectors from man (WHO, 2004).

## 2.12.4 Community participation in LF control

For a successful control program based on integrated vector and filarial disease control, community participation plays a major role (Panicker *et al.*, 1992). The type and the level of community involvements depend on the characteristics of the target community. Community directed treatments cover a wide area and reach more people (88%) as compared to health based treatment (46.5%) (Wamae *et al.*, 2006). Involving the community in mosquito control is found to be more effective. However there are problems encountered in involving the community; most people have the perception that filariasis is not serious because it is not fatal. The slow progression of the disease to chronic form hinders people from participating in control. The absence of clinical cure among people with elephantiasis and lack of knowledge of the cause of the disease hinders community participation in control efforts.

## 2.12.5 Morbidity Control and Management of lymphatic filariasis

Controling Morbidity or relief of suffering to the infected individuals is the second strategy used by WHO/GPELF in its elimination efforts (Addiss *et al.*, 1994; Pani *et al.*, 1995) after transmission reduction by chemotherapy and vector control. To alleviate the suffering caused by the disease, it is necessary to implement community education programmes to raise awareness in affected patients. This promotes the benefits of intensive local hygiene and the possible improvement, both in the damage

that has already occurred and in preventing the debilitating and painful, acute episodes of inflammation. This is through Community-level care of those with disease by managing lymphoedema, acute inflammatory attacks and hydrocele repair programmes. Early treatment with drugs may destroy the adult worms and logically prevent the later development of lymphoedema. Once lymphoedema is established there is no cure and the "foot care programme" may offer relief and prevent acute attacks thus preventing further progression of the swelling (Ottesen *et al.*, 1997).

## 2.12.6 Treatment and Prevention of Filarial fever (Adenolymphangitis- ADL)

Filarial fever (the acute attacks of ADL) is the most distressing aspect of LF, which results in considerable economic loss and deterioration of quality of life. Prompt treatment and prevention of ADL are important. Adenolymphangitis is seen both in early and late stages of the disease. It is triggered by the infection and inflammation of the skin and affected area by entry of bacteria or fungus through the entry lesions (Addiss *et al.*, 1994; Pani *et al.,* 1995). The skin becomes warm, tender, painful red and swollen. Patients develop fever, headache, chills and sometimes nausea and vomiting. Peeling and darkening of skin is also common. Repeated attacks increase the size of the legs. Management includes symptomatic treatment like relieving pain and care of entry lesions. In patients with late stages of oedema, long term antibiotic therapy using oral Penicillin or long acting parentral Benzathil Penicillin are used to prevent bacterial infections (Addiss *et al.*, 1994; Pani *et al.,* 1995). Lymphoedema management helps in elimination of the bad odour, prevents and heals entry lesions, reduces of the size of the lymphoedema, prevents disability and economic losses. This is through community based patient self help groups by practicing hygiene,

cleaning one another on the affected parts, exercise to avoid accumulation of the fluid, elevation of foot and use of proper foot wares ( Addiss *et al*., 1994).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study site

The study was carried out at Tana Delta district of Coast Province, Kenya. This district was curved off from Tana River district in October 2007. Tana Delta district has three divisions; Garsen, Kipini and Tarasaa. It has an area of $16,013.4km^{2.}$ The population in both Tana River and Tana Delta is estimated to be 250,000 with about 134,000 being in Tana River district (United Nations, 2007). The rainfall ranges between 220-900mm per year. The altitude ranges between 0-200m. The average temperature is $30^{o}C$. The major ethnic groups are the Pokomo, many of who are farmers, and the Orma and Wardey, who are predominantly nomadic. Rainfall in this district is erratic, with rainy seasons in March-May and October-December. Flooding is regular, caused by heavy rainfall in upstream areas of the Tana River. Lymphatic filariasis is common in the district as the environmental conditions and human economic activities favour mosquito development. The houses in Tana Delta are grass thatched houses both walls and roofs, grass roofed mud walled houses and galvanized iron sheet roofed houses with brick walls.

**Figure 3.1:** A map of divisions and villages in Tana River and Tana Delta Districts
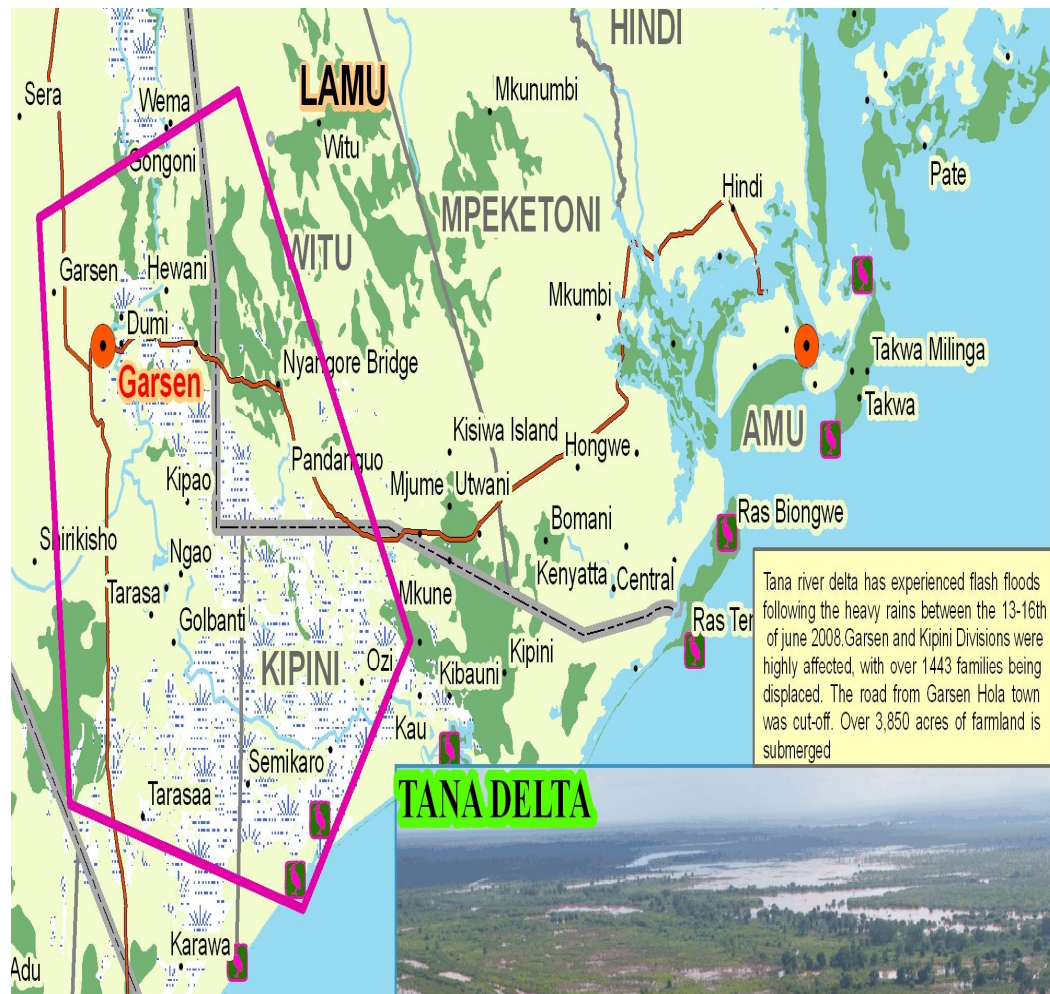


**Source:** United Nations information Centre. Nairobi, 2007

**3.2 Study design**

This was a cross sectional study whereby six villages were selected randomly. Six homesteads were randomly selected from each village. Three mosquito collection methods; Light traps, Gravid traps and Pyrethrum sprays were used in mosquito collection.

### 3.3 Mosquito sampling

Mosquitoes were collected inside the houses of willing household heads who gave consent for their houses to be used. Collection of mosquitoes was done for 10 days during the month of May in six villages of Tana Delta district which were randomly selected. The villages included Idsowe, Chakamba, Kisiwani farm, Kilelengwani, Onindo and Hewani. Six homesteads per village were selected randomly. Three mosquito collection methods; CDC Gravid traps, CDC Light traps and Pyrethrum sprays were used in collection of mosquito vectors for analysis. Pyrethrum spray catch (PSC) was applied once a week in each of the selected houses whereas light traps and CDC gravid traps were set at the same time in the same houses once a week for two weeks. The villages were divided in such a way that the homesteads sprayed in the first week had traps set during the second week and vice versa. For good comparison all the methods of collection were used in the same houses but different times for the traps and sprays.

### 3.3.1 Pyrethrum spray catch

Pyrethrum sprays were applied in six randomly selected houses in each village between 7.00pm and 10.00pm. White sheets were spread on the beds, chairs, under beds, and on the floor in randomly selected six rooms (1 room/house) per village and the rooms sprayed with pyrethroid insecticide formulation (Raid® Insecticide: Tetramethrin, Allethrin, Deltamethrin). The knocked down mosquitoes were collected after 5-10 minutes (WHO, 1975). The knockdown mosquitoes were picked and put in labelled petri dishes in cool box, they were transported to the laboratory for counting, sorting to species and dissection and then preserved under silica gel for

PCR work. Plate 3.1 shows further spraying of the knocked down mosquitoes to prevent them from escaping.

**Plate 3.1:** Pyrethrum spraying of the knocked down mosquitoes



### 3.3.2 CDC Light trap

Six rooms in each village were selected randomly for the light trap collection. In each room, a miniature CDC light trap with a standard 6V 100 mA incandescent bulb and powered by 4 dry cell batteries ( Plate 3.2) was hung from 6.00pm to 6.00am

(WHO, 1975). The trapping nets were removed from the traps carefully not to let the mosquitoes escape and the mosquitoes killed by exposing them to chloroform. The mosquitoes were sorted to species, dissected and then preserved under silica gel for analysis by PCR.

**Plate 3.2:** light trap set for mosquito collection
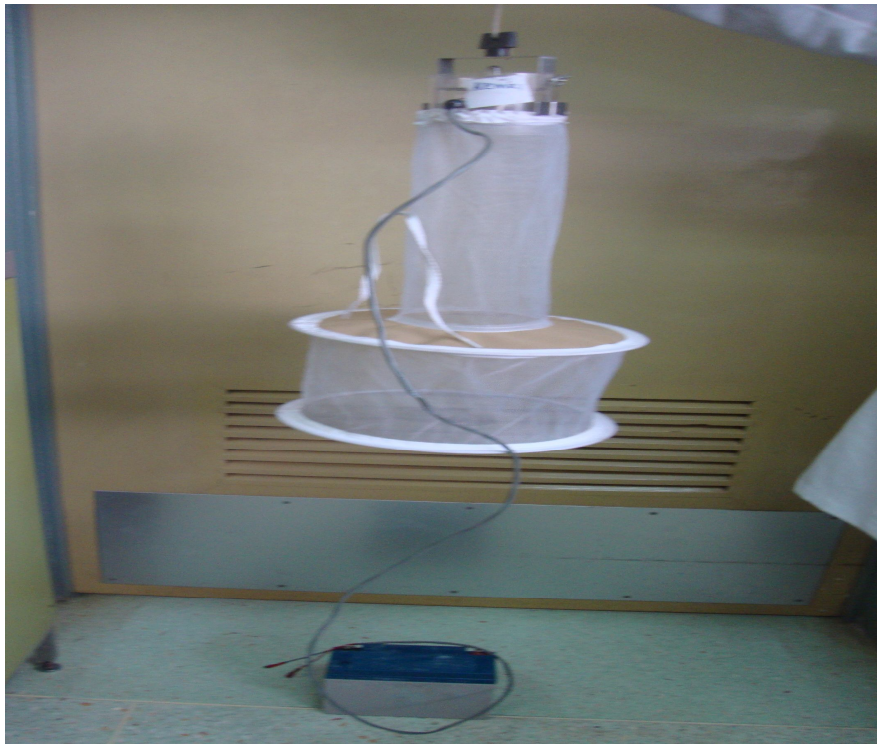


### 3.3.3 Gravid Traps

Gravid traps containing hay infusions to attract gravid mosquitoes for oviposition were used (Plate 3.3) (Reiter, 1983). Grass infusion hay was used as mosquito oviposition attractant (Mboera *et al.,* 1999) which was effective when prepared and used a fresh per night. The hay infusion was prepared by composing 0.5g hay to 114 litres of water and incubated for 5 days (Reiter, 1983). New hay medium (Reiter,

1983) was used each night to avoid bad odour reaching people in the rooms and which sometimes act as a repellent to the mosquitoes when used for several nights. Traps were placed in randomly selected houses where the light traps were set, a trap per house. Mosquitoes were removed in the morning by first exposing them to chloroform and putting them in labelled petri dishes with moist filter papers in cool box. The mosquitoes were sorted out in to species, a few dissected and the rest preserved in silica gel for analysis by PCR. Obtained larvae were preserved for filarial species determination by PCR.

**Plate 3.3:** Gravid Trap set inside a house

### 3.4 Mosquito identification and dissection

All mosquitoes were counted and classified according to the method and village of collection. Female mosquitoes and other arthropods were identified to genera level based on the morphological characteristics under a standard dissecting microscope and with the help of taxonomic identification keys (Wharton, 1962; Peyton and Scanlon, 1966; Gillet; Rattanarithikul, 1982; Gillett and Coetzee, 1987.). Dissection for filarial parasites was performed on the head, thorax and abdomen of *Mansonia* mosquitoes as described by Leemingsawat *et al.* (1987). This was done between 6-12 hours of the mosquito collection. Fifty *Mansonia* species were dissected on saline solution using a dissecting microscope X10 to determine the presence and the larvae stages ($L_1$, $L_2$ and $L_3$) of *Wuchereria bancrofti* in the mosquito. Parasite larvae stages identification was done on observation (Chandler and Read, 1969), $L_1$ is sausage shaped, $L_2$ is motile and short and $L_3$ is very motile, long and infective. The larvae were stained using Giemsa to improve observation. Infection rates and infectivity rates were determined. The larvae obtained from dissection were preserved for filarial species determination by PCR.

### 3.5.1 Deoxyribonucleic acid (DNA) Amplification by Polymerase Chain Reaction Assays

Deoxyribonucleic acid (DNA) extraction was done as by (Abbasi *et al.*, 1999). The extracted DNA was amplified in a PCR thermocycler, detection and analysis of the PCR products was done through gel electrophoresis. Polymerase chain reaction (PCR) was used to amplify Ssp1 repeat of the DNA, the primer sequences were 5' CAACCAGAATACCATTCATCC 3' and 5'CGTGATGGCATAAAGTAGCG 3'

identified by Zhong *et al.(* 1996) to amplify a 188-bp product in gDNA of *W. bancrofti*. Nested PCR was carried out on a few randomly selected PCR products for results confirmation. Table 3.1 shows the reagents used in preparation of master mix for PCR (Zhong *et al.,* 1996).

**Table 3.1: PCR Premix**

| Reagent | Final conc. in PCR Per reaction-1X |
|---|---|
| 10X PCR Buffer (LABSCO, Germany) | 5ul |
| 5pmol direct primer (I) (LABSCO, Germany) | 0.5ul |
| 5pmol reverse primer (II) (LABSCO, Germany) | 0.5ul |
| 100mM dNTPs mix (Promega, USA) | 0.5ul |
| DNase-free water (Sigma, USA) | 43.5 ul |
| Taq DNA polymerase (KEMTAQ, Kenya) or AmpliTaq Gold™ (Roche). | 0.5ul |

The samples were run on Gene Amp® PCR system 9700 under the following predetermined programme.

| | | |
|---|---|---|
| Primary denaturation | 95$^o$C | 5 minutes |
| Denaturation | 95 $^o$C | 1 min |
| Annealing | 54$^o$C | 1 min | 35 cycles |
| Extension | 72$^o$C | 1 min |
| Final Extension | 72$^o$C | 10 min |
| Hold | 4 $^o$C | |

### 3.5.2 PCR product analysis

Target gene amplification was confirmed by analysis using agarose gel electrophoresis (Plate 4.1). Two percent (2%) (W/V) of agarose gel was prepared by dissolving 3.0g of molecular grade agarose (Sigma) in 150ml of 1 X TAE buffer. 2µl of loading buffer (Promega) were added to 10µl of the amplified product of each sample and loaded in the wells. The gel was run on a horizontal electrophoresis tank (Bio Rad®). A marker (DNA ladder), positive and negative controls were included in the gel. DNA amplified products were visualized as bands under ultraviolet (UV) light on a transilluminator and the results were recorded and photographed using Polaroid camera and films were taken for reference.

### 3.5.3 Scoring of the bands

The positive bands in the gel electrophoresis were recorded as those appearing at the position equivalent to 188bp (non-coding DNA sequence in *W. bancrofti* Ssp1 repeat DNA sequence) of the DNA positive control marker (Zhong *et al*., 1996). The results were recorded along with the other specimen collection parameters such as mosquito species, method of collection, place of collection (indoor) and the time of collection.

**3.6 Ethical clearance**

Ethical clearance for this study was obtained from the Scientific Steering and Ethical review committees of Kenya Medical Research Institute (See appendices). Mosquito sampling involved entry into people's houses. Before the study implementation, villagers were given information about the aim of the study. The willing participants gave consent for setting of traps and the spraying of the houses with pyrethrum spray.

**3.7 Data management and analysis**

Data entry and validation were done in Microsoft excel 2003 version. The data was analysed using the statistical software, SPSS Version 10.0. Generalized linear model univariate analysis was used to test any statistical differences among the mosquito collection methods. Generalized linear model univariate analysis was also used in analysing the variables which were influencing mosquito collection, such as village of collection, sampling method and mosquito species. T-test was used for independent samples to test the role of *Mansonia* species in the transmission of *W. bancrofti*.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Mosquito collection methods analysis

A total of 1632 female mosquitoes were collected. Among these, 1265 were collected by Light traps (77.55%), 311 (19.1%) by Pyrethrum sprays and 56 (3.4%) by Gravid traps (Table 4.1). The three methods were significantly different (df =2, f=13.808, p= <0.0001) at 95% confidence interval (Table 4.1) in terms of efficiencies in collecting mosquitoes. Other arthropod species were also collected such as houseflies, moths, black ants, spiders and cockroaches (Table 4.2).

**Table 4. 1: Number of mosquitoes collected using each of the three collection method and the statistical difference of the methods**

| Collection methods | No. of mosquitoes | Percent |
|---|---|---|
| Light traps | 1265 | 77.5 |
| Gravid traps | 56 | 3.4 |
| Pyrethrum spray | 311 | 19.1 |
| Total mosquitoes | 1632 | 100.0 |
| Statistical difference of the three methods | F-value = 13.808 | Significance= .0001 |

**Table 4.2: Other arthropods trapped during mosquito collection**

| Collection method | Moths | Black ants | House flies | Crickets | Beetles | cockroaches | Spiders | Butter flies |
|---|---|---|---|---|---|---|---|---|
| Light | 180 | 4 | 6 | 1 | 28 | 1 | 3 | 5 |
| Gravid | 15 | 1 | 5 | 0 | 4 | 1 | 5 | 2 |
| Spray | 30 | 6 | 24 | 1 | 5 | 10 | 10 | 20 |

## 4.2 Mosquito species caught

Five mosquito genera were collected, namely, *Culex, Aedes, Anopheles* and *Mansonia* species. *Culex* mosquito species were 1048 (64.2%), *Aedes* species 188 (11.5%), *Mansonia* species 236 (14.5%), *Anopheles* species 148 (9.1%) and *Ficalbia* species 12 (0.7%) (Table 4.3).

**Table 4.3: Mosquito species composition collected in the study area**

| Mosquito species | No. of mosquitoes | Percent |
|---|---|---|
| *Culex* | I048 | 64.2 |
| *Aedes* | 188 | 11.5 |
| *Mansonia* | 236 | 14.5 |
| *Anopheles* | 148 | 9.1 |
| *Ficalbia* | 12 | .7 |
| Total | 1632 | 100.0 |

**4.2.1 Mosquito species obtained by each of the collection method**

Different mosquito species had different preference for different collection methods. Out of 1048 *Culex* mosquito species obtained, light trap caught the highest number (970) compared to pyrethrum spray which had 45 and gravid traps 33 *Culex* mosquitoes (Fig 4.1). Most of the *Aedes* species (176) were caught by spray method as compared to light traps which had 12 *Aedes* mosquitoes and none of the *Aedes* species were obtained by gravid traps (Fig 4.1). One hundred and fifty three *Mansonia* species were obtained by light traps, pyrethrum spray had 71 and gravid traps had 12 mosquitoes of *Mansonia* species. One hundred and thirty *Anopheles* species mosquitoes were obtained by light traps, 11 by gravid traps and 7 by pyrethrum spray. All the *Ficalbia* species were obtained by pyrethrum spray. The mosquito species obtained by each method were significantly different (df= 4, f=8.617, p= <0.018) at 95% confidence interval.

**Figure 4.1: Mosquito species obtained by each of the collection methods**



51
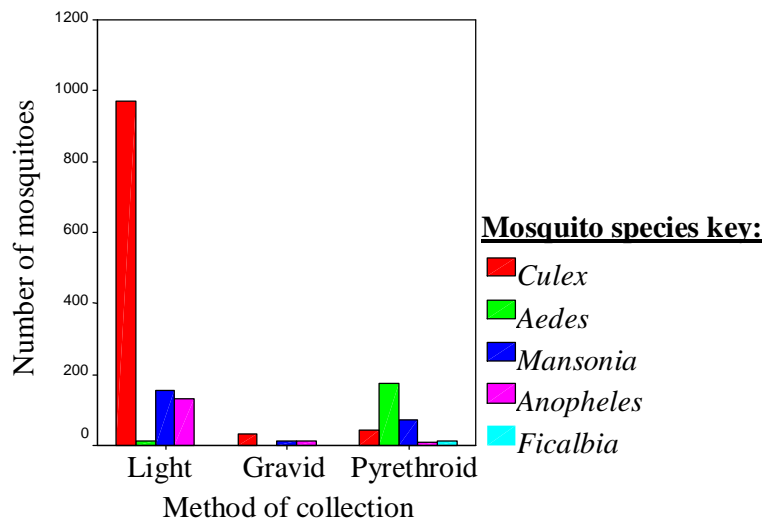
### 4.2.2 Mosquito distribution in the study area

The number of mosquitoes obtained from each village were as follows; Kilelengwani village had the highest catch (951= 58.3%), Kisiwani farm (320= 19.6 %,), Chakamba (225= 13.8 %), Onindo (105 =6.4%), Idsowe (19= 1.2%) and Hewani had the lowest catch (12= 0.7%) (Fig.4.2).The villages had a significant effect on the mosquito density caught (df = 5, f = 263.416, p= <0.0001), due to different ecological factors. Kilelengwani village was significantly different from all the other villages with the highest number of mosquitoes collected.

**Figure 4.2: Mosquitoes obtained from each village in the study area**
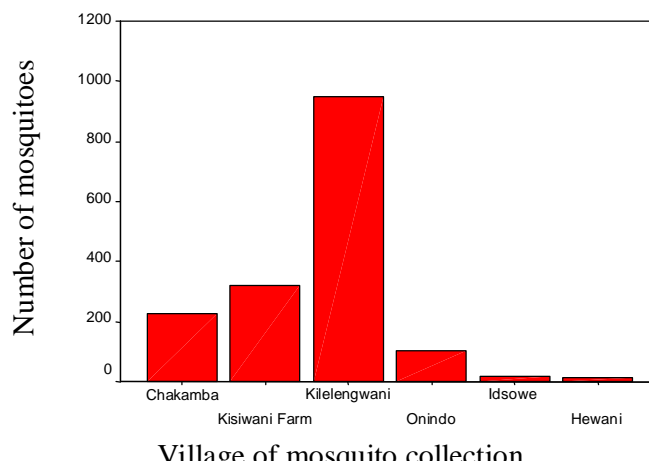


### 4.2.3 Mosquito species composition in each of the villages of collection

There were five mosquito genera collected from the study area (Table 4.4). *Culex* species was the most prevalent species, with the highest number (542) obtained from Kilelengwani village and the least (7) from Hewani (Table 4.4). There were no *Aedes, Anopheles* and *Ficalbia* species obtained from Idsowe village. Hewani village

had no *Mansonia* and *Ficalbia* species. *Ficalbia* species mosquitoes were obtained from Kisiwani farm (1) and Kilelengwani village (11). The villages had a significant effect on mosquito species (df =5, f= 62.113, p= <0.0001).

**Table 4.4: Mosquito species obtained in each of the collection villages in the study area**

| Collection Villages | Mosquito species | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | *Culex* | *Aedes* | *Mansonia* | *Anopheles* | *Ficalbia* | Total |
| Chakamba | 142 | 2 | 61 | 20 | | 225 |
| Kisiwani Farm | 259 | 6 | 45 | 9 | 1 | 320 |
| Kilelengwani | 542 | 177 | 120 | 101 | 11 | 951 |
| Onindo | 84 | 2 | 5 | 14 | | 105 |
| Idsowe | 14 | | 5 | | | 19 |
| Hewani | 7 | 1 | | 4 | | 12 |
| Total | 1048 | 188 | 236 | 148 | 12 | 1632 |

**4.3 Abdominal status of the obtained mosquitoes**

Out of the total number of mosquitoes collected, 1460 (89.5%) had not taken a blood meal (unfed). Eighty two (5%) were blood fed, 68 (4.2%) gravid and 22 (1.3%) half gravid (Table 4.5). Seventy five blood fed mosquitoes were obtained by light traps, 6 fed mosquitoes by pyrethrum spray and 1 fed mosquito by gravid traps (Table 4.5). Gravid traps obtained the largest number of the gravid mosquitoes (54), 13 gravid mosquitoes were by light traps and 1 gravid mosquito by pyrethrum spray (Table 4.5). Light traps obtained 14 half gravid mosquitoes, pyrethrum spray had 7 half gravid and Gravid traps with 1 half gravid mosquito (Table 4.5). The abdominal status of the mosquitoes obtained by different methods were significantly different (df =3, f= 0.841, p=< 0.526).

**Table 4.5: Abdominal status of the mosquitoes collected by the different methods**

| Collection method | Blood fed | | | | Total |
|---|---|---|---|---|---|
| | Fed | Unfed | Gravid | Half gravid | |
| Light traps | 75 | 1163 | 13 | 14 | 1265 |
| Gravid traps | 1 | | 54 | 1 | 56 |
| Pyrethroid spray | 6 | 297 | 1 | 7 | 311 |
| Total | 82 | 1460 | 68 | 22 | 1632 |

## 4.4 Larvae status of the dissected mosquitoes

Out of 50 *Mansonia* mosquito species dissected, only 2 of them had stage II larvae ($L_2$) which were taxonomically identified on morphology as of Brugian filariasis species.

## 4.5 Polymerase chain reaction and gel electrophoresis

Deoxyribonucleic acid (DNA) was extracted form 100 individual mosquitoes of *Mansonia* species and from the 2 filarial larvae obtained from mosquito dissections. Neither the mosquitoes nor the larvae tested positive for *W. bancrofti* DNA by the conventional PCR method (Plate 4.1).

**Plate 4.1: Agarose Gel electrophoresis for PCR product analysis to detect the presence of *W. bancrofti* DNA in *Mansonia* mosquito species**
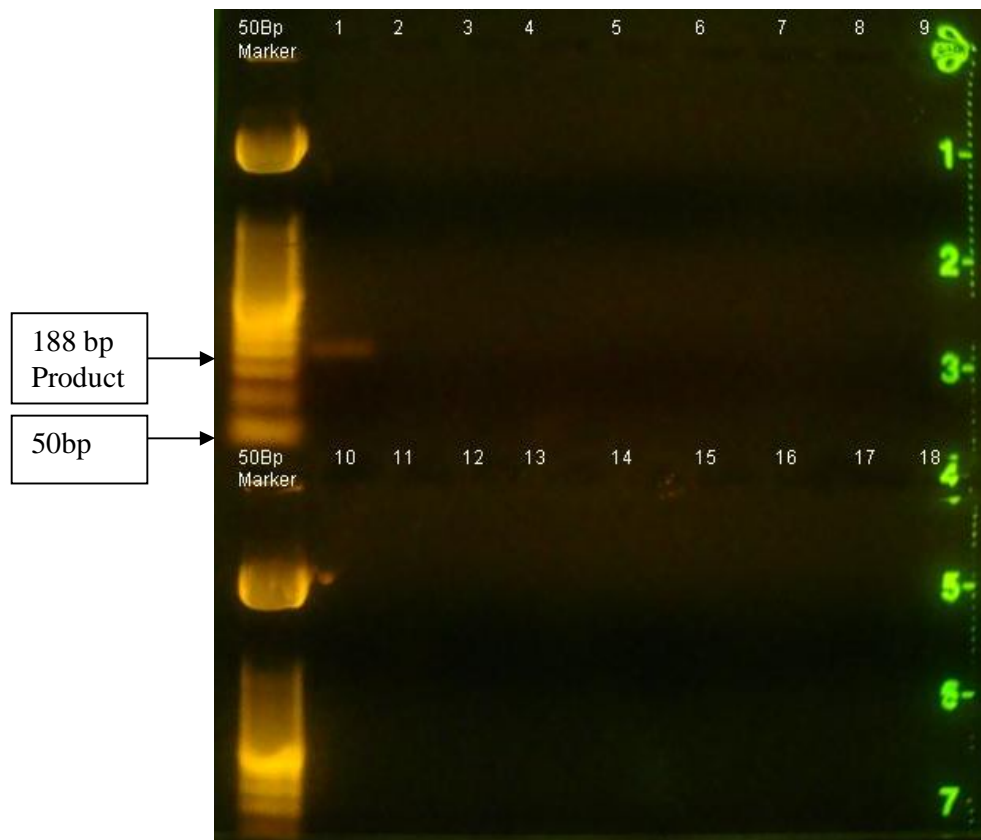


**Label:** 50Bp marker = Molecular weight ladder, well 1 = Positive control, well 2 = Negative control, wells 3-9 and wells 10-18 = Mosquito specimens.

   **NB**; No band was seen on the negative control and on all the test specimen wells.

## CHAPTER FIVE

## 5.0 DISCUSSION, CONCLUSIONS AND RECOMMENTATIONS

## 5.1 Evaluation of mosquito collection methods

The field evaluation of light traps, gravid traps and pyrethrum sprays in the same ecological settings enabled the efficient comparison of these sampling methods in mosquito collection. In this study the three methods were found to be significantly different (P= <0.0001) at 95% confidence interval (Table 4.1). Light traps were able to obtain large number of mosquitoes in areas where there were large or small numbers of mosquitoes, followed by pyrethrum sprays and then gravid traps (Table 4.1). This suggested that in terms of getting large numbers of mosquitoes, light traps would be the most suitable.

The results of this study are in agreement with those from a study conducted in Tanzania by Mboera *et al.* (1998), who found that light traps collect a lot of mosquitoes because light from the bulb could attract mosquitoes from a distance. The traps in this study were set in sleeping area/rooms to use humans as baits for attracting mosquitoes. This was done in reference to observations of Mboera *et al.* (1998) who reported that human baits are the most efficient in attracting mosquitoes as compared to other attractants used in traps. Different odours and carbon dioxide produced by humans have attractant effect on the mosquitoes (Mboera *et al.*, 1998). Light traps were able to obtain mosquitoes of different abdominal status; unfed, fed, half gravid and gravid mosquitoes (Table 4.5). This difference in abdominal status reflected the number of mosquitoes seeking for blood meal, those which have taken

blood and resting for blood digestion and egg development and those seeking oviposition sites (WHO, 1975). In addition to large numbers of mosquitoes obtained by light traps, several other unwanted insects were caught, for example moths, flies and black ants (Table 4.2). Light traps also trapped male mosquitoes which were not blood feeders and hence not transmitters of disease. Hay infusions (oviposition medium) used in gravid traps only attracted gravid mosquitoes (Reiter, 1983) and this limited the captured mosquitoes only to female gravid mosquitoes which were attracted for oviposition as in Table 4.1. Selective collection of gravid mosquitoes reduces the collection of other unwanted insects and male mosquitoes. New attractant media was used each day as reported by Reiter (1983), hay infusions when rotten produce an odour which acts as a repellent to the mosquitoes. Bad odour was also a problem to people sleeping in the rooms where the traps were set. This was observed in this study during mosquito collection.  Pyrethrum sprays were capable of obtaining unfed, fed, half gravid and gravid mosquitoes as long as they are in reach of the sprays (Table 4.5). The number of blood fed mosquitoes trapped was different for each collection method, with gravid traps having the least number (1 fed mosquito) (Table 4.5). Gravid traps attract only gravid mosquitoes for oviposition and thus the blood meal had been digested for egg development. This means that mosquitoes obtained by gravid traps had a higher chance of being infected since they had taken at least one blood meal as reported by Reiter (1983). Most of the mosquitoes obtained by light traps and pyrethrum sprays were unfed. This suggests that, most of the mosquitoes obtained by these two methods were uninfected. There were no unfed or male mosquitoes obtained by gravid traps. This suggests that mosquitoes obtained by gravid traps have a higher chance of being infected as

compared to light traps and pyrethrum sprays hence are suitable for assessing disease dynamics in the vectors as it has been suggested by Reiter (1983). Different sampling methods have shown varying ability in collecting mosquitoes of different abdominal conditions which can be more informative in disease epidemiology (WHO, 1975). The abdominal status of the mosquitoes obtained by different methods were different (P =< 0.526).

## 5.2 Number of mosquitoes from each village

Different numbers of mosquitoes were obtained from each village. This suggested that there were differences in ecological factors of the different villages sampled. For example, in villages near swampy and marshy areas, more mosquitoes were obtained compared to villages not surrounded by water bodies. For instant, Kilelengwani village being near swamps, had the highest catch (951 mosquitoes representing 58.3% of all the caught mosquitoes) and Hewani village had the lowest catch (12 mosquitoes representing 0.7% of the caught mosquitoes) since there were no water bodies around the homesteads in Hewani (Table 4.4). The villages where the sampling of mosquitoes was carried out had significant effect on the number of mosquitoes obtained (P = <0.0001) due to differences in ecological factors. These findings suggest that there are more attractive ecologic niches that favour breeding of filarial vectors in villages with highest mosquito catches than the villages with few catches. This means that understanding the ecological requirements of the mosquito is important as it helps in vector control and human-vector contact control efforts of the GPELF. For example *Mansonia* species are found in submerged vegetation and the larvae attach themselves to plants (Rajendran *et al.,* 1989). Removal of such

plants through mechanical, biological or chemical control would effectively prevent breeding of *Mansonia* species. The numbers of mosquitoes obtained by the different sampling methods were also significantly different between houses in the same villages. This suggested that the nature of houses and housing materials influenced the mosquito density. The houses were made of makuti/grass thatched both on the roof and walls, mud walled and grass/makuti thatched house and block walled and galvanise iron sheet roofs with window screens. There were few mosquitoes obtained from well build houses, for example, in Hewani village, most of the houses were well build with window screens and thus few mosquitoes were obtained in these houses. More mosquitoes were obtained from grass thatched houses, most of which had open windows and many other openings (holes) into the houses.

### 5.3 Mosquito species collected

Five mosquito genera were obtained from the study area (Table 4.3). The species obtained depended on the prevalence of the species in that particular area. There was a significant different on the mosquito species obtained from the collection villages (df= 4, f =2.430, p= 0.046). *Culex* species had the highest number (1048 mosquitoes representing 64.2% of all the obtained mosquitoes) and *Ficalbia* species was the least (12 mosquitoes representing 0.7%) (Table 4.3). According to this study, the most prevalent mosquito species in Tana Delta district were *Culex* species (Table 4.3). This is in agreement with reports by Mwandawiro *et al. (*1997), *Culex* species is known to be the main LF vectors in both urban and rural areas. Mosquito population increases during the wet season due to increase of mosquito breeding areas (Evans *et al.*, 1993). Increasing urbanization, inadequate disposal and sanitation facilities lead

to increased breeding sites for LF vectors. *Culex* species breed in the foulest waters especially in wet pit latrines (Mwandawiro *et al.,* 1997), which are common in the study area. *Mansonia* species breed in submerged vegetations which are common around swampy and marshy areas (Rajendran *et al.,* 1989) and most of *Mansonia* species were caught in the houses near the swamps. This suggests that different species of mosquitoes are found in different areas attributed to the availability of the breeding sites. Increase in mosquito breeding coincides with a high transmission rates (Kasili *et al.,* 2009) especially during and after the long and short rain seasons. Few mosquitoes are found during the dry season with very low transmission rates (Kasili *et al.,* 2009).

## 5.4 Mosquito species collected by each method

Different numbers of each mosquito genera were obtained by different methods. Out of 1048 *culex* mosquitoes obtained, 970 (92.56%) were obtained by light traps (Fig. 4.1). One hundred and seventy six (93.61%) mosquitoes of *Aedes* species were caught through the spraying method. There were no *Aedes* and *Ficalbia* mosquito species obtained by gravid traps. Most of the *Mansonia* mosquito species 153 (64.83%) were by light traps. One hundred and thirty *Anopheles* mosquito species were obtained by the light trap. All the *Ficalbia* mosquitoes were caught through spraying (Fig. 4.1). The results of the mosquito species obtained by each method suggest that, different methods are suitable for different mosquito species. The mosquito species obtained by each method were significantly different (df= 4, f=8.617, p= <0.018) at 95% confidence interval. This information is more suitable for guiding people working on mosquito vectors for different infections on which

method(s) to use in obtaining different mosquito species according to the required species. For example, from this study spraying method would be more suitable for *Aedes* and *Ficalbia* species (Fig. 4.1). Light traps would be suitable for all the species except for *Ficalbia* species.  Gravid traps are not suitable for *Aedes* and *Ficalbia* species.

## 5.5 The role of *Mansonia* species in the transmission of *W. bancrofti*

*Mansonia africanus* and *Ma. uniformis* were not found to play any role in transmission of *W. bancrofti*. The two filarial larvae ($L_2$) obtained from dissection were not of *W. bancrofti*. In addition, *W. bancrofti* DNA could not be amplified from *Mansonia* mosquito species (Plate 4.1). These findings compare well with those by Kasili *et al*. (2009) who found no *W. bancrofti* larvae in all the *Mansonia* species dissected in studies of seasonal variation of filariasis transmission in Coastal Kenya. These  results were also similar to those of Onapa *et al*. (2007) who found that *Mansonia uniformis* had a limited potential to support development of *W. bancrofti* to the infective stages and they did not appear to play any role as vectors of *W. bancrofti* under natural conditions. These results suggest that there were no *W. bancrofti* larvae development in *Mansonia* species.  The PCR product analysis suggested that *Mansonia* species were negative for *W. bancrofti* infection. Experimental infections of *Mansonia* species of mosquitoes were found to show some larvae development (Onapa *et al.,* 2007). The larvae were seen to show microfilariae ex-sheath and first stage larvae ($L_1$) were seen to accumulate in the thorax and only few developed to second stage ($L_2$) and further to infective stages

(L$_3$) ( Onapa *et al.*, 2007). Other studies have found *Mansonia africanus* and *Ma. uniformis* containing infective larvae of animal origin (Nelson and Heisch, 1962). Previous records incriminating *Mansonia* species as vectors of *W. bancrofti* in Africa were probably due to misidentification (Nelson and Heisch, 1962).

The extremely low levels of transmissions of LF in the vectors are likely to be linked to the use of different vector control measures by the community. It was for example observed that the use of Insecticide Treated bed Nets (ITN), which was implemented by the malaria control programme, is widespread in the area. Also people practice traditional methods in controlling mosquitoes such as use of firewood smoke outside and inside the houses as a means of preventing mosquitoes from getting in the houses. Although Mass Drug Administration (MDA) has not yet started in Tana Delta district, some individuals had the opportunity of getting drugs from other areas where MDA had begun like Malindi and Kilifi districts (Personal communication).

**5.6 Advantages of the mosquito collection methods**

- Gravid traps are very selective and obtain only gravid mosquitoes which have high chance of having microfilaria because they have had atleast one blood meal.

- Light traps can collect large numbers of mosquitoes of different abdominal statuses.

- Setting traps over the night gives them a chance to obtain mosquitoes coming in the house at any time of the night, thus they are able to collect different species of mosquitoes.

- Pyrethrum spray is easy to apply and one can cover large area of collection within a short period of time.

## 5.7 Limitations of the collection methods

- Knockdown spray catch collections are not suitable where live mosquitoes are required especially for dissection work where larvae need to be observed live for stages determination microscopically and other experimental infections.

- With spray method the late night and early morning, mosquitoes in the houses were missed since the collection was done at dusk between 7.00pm and 10.00pm.

- Gravid traps are not capable of obtaining large numbers of mosquitoes because of the gravid selectivity.

- Light traps collect male mosquitoes and many other unwanted insects in addition to female mosquitoes, making the work of mosquito sorting difficult.

- In general, the collection was limited to time and funds because comparison of mosquito collection methods in different rainy seasons and dry seasons needed to be done for fair comparison.

## 5.8 Conclusions

- In this study, light traps, were found to be appropriate for mosquito collection since they were capable of obtaining fed, half gravid and gravid mosquitoes which have a high chance of having microfilariae. Indoor collection of mosquitoes by light traps using humans or carbon dioxide as baits are sufficient for collecting mosquitoes needed for accurate estimation of disease transmission indices.

- In this study, there was no evidence that *Mansonia* species play any role in transmission of *W. bancrofti* in Tana Delta district, Kenya. The study provides relevant information to ongoing control efforts and to support future campaigns aimed at eliminating filariasis.

## 5.9 Recommendations

- According to the results of this study, it is recommended that light traps are the best methods in mosquito collection since they are capable of obtaining mosquitoes of different abdominal status (fed, half gravid and gravid), and in large numbers. Gravid traps are recommended when only gravid mosquitoes are required for disease dynamics evaluation. Pyrethrum sprays can be used when live mosquitoes are not required.

- The ecological characteristics of an area should be used to apply the appropriate sampling technique for existing vectors. In this study, factors such as proximity of the houses to water bodies, nature of the house and collection methods were found to influence the number and the species of mosquitoes obtained.

- Since *Mansonia* species from the natural habitant/wild were found not to be infected with *W. bancrofti,* further studies should be carried out experimentally by infecting *Mansonia* species with *W. bancrofti* microfilaria to assess whether *Mansonia* species can support development of microfilaria in the mosquitoes to the infective stages.

# REFERENCES

**Abbasi I., Githure J., Ochola J.J., Agure R., Koech D.K., Ramzy R.M., Williams S.A. & Hamburger J. (1999).** Diagnosis of *W. bancrofti* infection by the polymerase chain reaction employing patient's sputum. *Parasitology,* **85**: 844-849.

**Abu Hassn A. & Narayanan V**. **(1992).** Effectiveness of a soap repellent against *Mansonia* mosquitoes in a fresh water swamp forest in Northwest Malaysia. *Bulletin of the Society for Vector Ecology,* **17:** 83-83.

**Addiss D.G., Eberhard M.L. & Lammie P.L. (1994).** Filarial adenolymphangitis without filarial infection (letter). *Lancet,* **343**: 597.

**Ahorlu C .K., Dunyo S. K., Koram K.A., Nkrumah F.K., Aagaard-Hansen J. & Simonsen P. E. (1999).** Lymphatic filariasis related perceptions and practices on the coast of Ghana: Implications for prevention and control. *Acta Tropica*, **73:** 251-261.

**Babu S., Blauvelt C.P., Kumaraswami V. & Nutman T.B. (2005**). Chemokine receptors of T cells and of B cells in lymphatic filarial infection: a role for CCR9 in pathogenesis. *Journal of Infectious Disease*, **191**: 1018-1026.

**Bockarie M.J., Fischer P., Williams S.A., Zimmerman P.A., Griffin L., Alpers M.P. & Kazura J.W. (2000).** Application of a polymerase chain reaction-ELISA to detect *Wuchereria bancrofti* in pools of wild-caught *Anopheles punctulatus* in a filariasis control area in Papua New Guinea. *American Journal of Tropical Medicine and Hygiene,* **62(3):**363-367.

**Bogh C., Pedersen E.M., Mukoko D.A. & Ouma J.H. (1998).** Permethrin impregnated bednet effects on resting and feeding behaviour of lymphatic filariasis vector mosquitoes in Kenya. *Medical Veterinary Entomology*, **12(1):**52.9.

**Brabin L. (1990).** Sex differentials in susceptibility to lymphatic filariasis and implications for maternal child immunity. *Epidemiology and Infection*, **105**: 335-353.

**CDC. (1993).** Recommendations of the International Task Force for Disease Eradication. *Morbidity and Mortality Weekly Report*. **42**: 1-38.

**Chandler A. C. & Read A.C**. **(1969).** Introduction to Parasitology. Tokyo, Japan; *Japan company limited*, Pg 473-479.

**Chanteau S., Glaziou P., Luquiaud P., Plicahrt C., Moulia-Pelat J. P. & Cartel J.L**. **(1994b).** Og4C3 circulating antigen, anti-B malayi IgG and IgG4 titres in *W. bancrofti* infected patients, according to their parasitological status. *Journal of Tropical Medicine and Parasitology,* **45**: 255-257.

**Chanteau S., Glaziou P., Luquiaud P., Plicahrt C., Moulia-Pelat J. P. & Cartel J.L**. **(1995).** *Wuchereria bancrofti* in French Polynesia : Age specific patterns of microfilaraemia, circulating filarial antigen and specific IgG and LgG4 responses according to transmission levels. *International Journal of Parasitology*, **25**:81-85.

**Chanteau S., Luquiaud P., Failloux A.B. & Williams S.A. (2002)**. 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.

**Cheong W.C. & Yap H.H. (1985).** Bioassay of *Bacillus sphaericus* (1953) against mosquitoes of public health importance in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health*, **16**: 54-58.

**Chodakewitz J**. **(1995)**. Ivermectin and lymphatic filariasis: a clinical update. *Parasitology Today,* **11***: 233–235.

**Dreyer G., Countinho A., Miranda D., Noroes J., Rizzo J. A., Galdino E., Rocha A., Medeiros Z., Andrade L.D. & Santos A**. **(1995a).** Treatment of bancroftian

filariasis in Rife, Brazil: a two year comparison study of the efficacy of single treatment with Ivermectin or Diethylcarbamazine. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **89:** 98-103.

**Dreyer G., Amaral F., Noroes J., Medeiros Z. & Addiss D. (1995b).** A new tool to assess the adulticidal efficacy in vivo of antifilarial drugs for bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **89**: 225-226.

**Dreyer G., Brandao A.C., Amaral F., Medeiros Z. & Addiss D. (1996c).** Detection by ultrasound of living adult *Wuchereria bancrofti* in the female breast. *Memorias do Instituto Oswaldo Cruz,* **91**: 95-96.

**Dreyer G., Noroes J. & Addiss D**. **(1997).** The silent burden of sexual disability associated with lymphatic filariasis. *Acta Tropica,* **63***: 57–60.

**Dreyer G., Figueredo-Silva J., Neafie R.C. & Addiss D.G. (1998).** Lymphatic filariasis. In Pathology of Emerging Infections 2 (Ed. Nelson AM & Horsburgh CR Jr.). *American Society for Microbiology, Washington DC,* pp 317-342.

**Dreyer G. & Piessens W. (2000).** Worms and micro-organisms can cause Lymphatic disease   in residents of Filariasis Endemic areas. In: Lymphatic disease. 1st edn. (ed. TB Nutman) *Imperial College Press, London*, pp 245-264.

**Estambale B.B.A., Simonsen P. E., Knight R. & Bwayo J.J. (1994a**). Bancroftian filariasis in Kwale District of Kenya. 1. Clinical and parasitological survey in an endemic community. *Annals of Tropical Medicine and Parasitology,* **88**: 145-151.

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

**Fischer P., Boakye D. & Hamburger J. (2003).** Polymerase chain reaction-based detection of lymphatic filariasis. *Medical Microbiology and Immunology,* **192(1)**: 3-7.

**Freedman D.O., Almeida-Filho P.J., De Besh S., Maia-e-silva M.C., Braga C. & Maciel A. (1994).** Lymphoscintigraphic analysis of lymphatic abnormalities in symptomatic and asymptomatic human filariasis. *Journal of Infectious Diseases*, **170**: 927-933.

**Gilles M.T. & Coetzee M. (1987).** A supplement to the Anophelenae of Africa South of Sahara. Johannesburg. *South African Institute of Medical Research*.

**Grenfell B.T. & Michael E. (1992)**. Infection and disease in lymphatic filariasis: an epidemiological approach. *Parasitology*, **104**: S81-S90.

**Goodman D.S., Orelus J.N., Roberts J.M., Lammie P.J. & Streit T.G. (2003).** PCR and mosquito dissection as tools to monitor filarial infection levels following mass treatment. *Filaria Journal*, **2**: 11.

**Gyapong J. O., Gyapong M. & Adjei S. (1996a)**. The epidemiology of acute Adenolymphangitis due to lymphatic filariasis in Northern Ghana. *American Journal of Tropical Medicine and Hygiene,* **54**: 591-595.

**Gyapong J. O., Gyapong M., Evans D.B., Alkins M. K. & Adjei S. (1996b).** The economic burden of lymphatic filariasis in Northern Ghana. *Annals of Tropical Medicine and Parasitology*, **90**:39-48.

**Haddix A.C., Carter S., Michael E. & Benton B. (1999).** Cost effectiveness of integrating lymphatic filariasis elimination into APOC onchocerciasis control programs. *Report prepared for the Joint Action Forum/Joint Program Committee*, *December 1999, Den Haag*.

**Helmy H., Fischer P., Farid H. A., Bradley M. H. & Ramzy R.M. (2004).**Test strip detection of *Wuchereria bancrofti* amplified DNA in wild-caught *Culex pipiens* and

estimation of infection rate by a Pool Screen algorithm. *Tropical Medicine of International Health,* **9**: 158–163.

**Heisch R.B., Goiny H.H. & Ikata M. (1956)**. A new vector of filariasis in east Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **50:** 421-422.

**Hougard J.M., Mbentengam R., Lochouarn L., Escaffre H., Darriet F., Barbazan P. & Quillevere D**. **(1993).** Campaign against *Culex* q*uinquefasciatus* using *Bacillus sphaericus:* Result of a pilot project in a large urban area of equatorial Africa. *Bulletin of the World Health Organization*, **71**: 367-375.

**Jones C.G., Heathcote O.H. & Magayuka S.A. (1972)**. Epidemiological study of infections in the mosquito: selective trapping of unfed malaria-filariasis vectors seeking a blood meal in bedrooms. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **66**:24.

**Kagai J. M., Mpoke S., Muli F., Hamburger J. & Kenya E.U. (2008).** Molecular technique utilizing sputum for detecting *Wuchereria bancrofti* infections in Malindi, Kenya. *East Africa Medical Journal*, **85 (3)**: 28-33.

**Kasili S., Oyieke F., Wamae C. & Obogo C**. **(2009)**. Seasonal changes of infectivity rates of bancroftian filariasis vectors in Coast province Kenya. *Journal of vector borne Disease,* **46**: pg 219-224.

**Kazura J. W., Spark R., Forsyth K., Brown G., Heywood P., Peters P. & Alpers M. (1984).** Parasitological and clinical features of bancroftian filariasis in a community in East Sepik Province, Papua New Guinea. *American Journal of Tropical Medicine and Hygiene,* **33**: 1119-1123.

**Kazura J. W., Bockaria M., Alexander N., Perry R., Bockaria F., Dagoro H., Dimber Z., Hyun P. & Alpers M**. **(1997).** Transmission intensity and its

relationships to infection and disease due to bancroftian filariasis in Papua New Guinea. *Journal of Infectious Disease,* **176:** 242-246.

**Lammie P. J., Hightower A.W. & Eberhard M.L**. **(1994).** Age- specific prevalence of antigenaemia in a *W. bancrofti-* exposed population. *American Journal of Tropical Medicine and Hygiene,* **51**: 348-355.

**Lammie P., Weil G., Rahmah N., Kaliraj P., Steel C., Goodman D., Lakshmikanthan V. & Ottesen E**. **(2004)**. Recombinant antigen based assays for the diagnosis and surveillance of lymphatic filariasis- *a multicenter trial. Filarial Journal*, **3**:9.

**Leeminsawat S., Deesin T., Vutikes S., Sucharit S. & Supavej S**. **(1987).** Determination of filariae in mosquitoes.  Practical entomology, malaria and filariasis. *Bangkok: Siriyod Printing,* 42-46.

**Lu A.G., Valencia. L.B., Llafas L., Aballa L. & Postrado L. (1988).** Filariasis: a study of Knowledge, attitudes and practices of the People of Sorsogon. *Social and Economic Research Project Reports No 1, Geneva*: World Health Organisation.

**Maizels R. M. & Denham D. A. (1992)**. Diethylcarbamazine (DEC): immuno- pharmacological interactions of an anti-filarial drug. *Parasitology*, **105** Supp: S49- S60.

**Maxwell C. A., Curtis C.F., Haji H., Kisumku S., Thalib A. I. & Yahya S. A**. **(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,* **84**: 709-714.

**Mboera L.E., Kihonda J., Braks M.A. & Knols B.G. (1998).** Short report: Influence of centers for disease control light trap position, relative to a human-baited bed net,

on catches of *Anopheles gambiae* and *Culex quinquefasciatus* in Tanzania. *American Journal of Tropical Medicine and Hygiene*, **59**:595-596.

**Mboera L. E. G., Mdira K. Y., Salum F. M., Takken W. & Pickett J. A. (1999).** The influence of synthetic oviposition pheromone and volatiles from soakage pits and grass infusions upon oviposition site-selection of *Culex* mosquitoes in Tanzania. *Journal of Chemical Ecology,* **35:**1855–1865.

**McMahon J. E. & Simonsen P. E. (1979)**. *in Manson's Tropical Disease*. **12**[th] edn. *Wuchereria bancrofti* Saunders. London, 1336-1360.

**McMahon J.E., Magayuka S.A., Kolstrup N., Mosha F.W., Bushrod F.M., Abaru D.E & Bryan J.H. (1981).** Studies on the transmission and prevalence of bancroftian filariasis in four coastal villages of coastal Tanzania. *Annals of Tropical Medicine and Parasitology,* **75:** 415-431.

**McMahon J. E. & Simonsen P. E. (1996)**. *In Mansons Tropical Diseases*. 20th edn. (Ed. Cook GC) *WB Saunders, London*, pp 1321-1368.

**Meyrowitsch D.W. & Simonsen P.E. (1998).** Long term effect of mass diethylcarbamazine chemotherapy on Bancroftian filariasis results at four years after start of treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **92**: 98-103.

**Michael E. & Bundy D.A.P. (1997).** Global mapping of lymphatic filariasis. *Parasitology Today, 1***3:** 472–476.

**Miranda J., Maciel A., Souza R.M., Furtado A.F. & Malagueno E. (2005) .** Proteic profile and antigenic recognition of extracts from *Wuchereria bancrofti* $L_3$ infective larvae. *Royal  Society of Brazil Tropical Medicine*, **38**: 27-32.

**Molyneux D.H. & Zagaria N**. (**2002**). Lymphatic filariasis elimination: Progress in global programme development. *Annals of Tropical Medicine and Parasitology*, **96** Suppl **2**: S15-S40.

**Molyneux D. (2003).** Lymphatic filariasis (elephantiasis) elimination: a public health success and development opportunity. *Filaria Journal, 2:* 13.

**Musa M. T., Fahal A. H., & El Arabi Y. E**. **(1995).** Aspiration Sclerotherapy for hydroceles in the tropics. *British Journal of Urology*, **76**: 488-490.

**Mwandawiro C. S., Fujimaki Y., Mitsui Y. & Katsivo M. (1997).** Mosquito vectors of Bancroftian filariasis in Kwale District, Kenya. *East African Medical Journal, volume*, **74:** No. 5.

**Mwobobia I. K., Muniu E. M., Kombe Y. & Wamae C. N. (2000).** Hydrocelectomy: a proxy for hydrocele prevalence in coastal Kenya. *Annals of Tropical Medicine and Parasitology*, **94**: 479-484.

**Nelson G. S., Heisch R.B. & Furlong M. (1962).** Studies on filarial in east Africa.11. Filariasis infections in man, animals and mosquitoes on the Kenyan coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **56:** 202-217.

**Njenga S.M. and Wamae C. N. (2001).** Evaluation of ICT filariasis Card Test using whole blood; Comparison with Knott's concentration and counting chamber methods. *Journal of Parasitology*, **87(5):** 1140-1143.

**Noroes J., Addiss D., Santos A., Mendes V.G., Medeiros Z., Countinho A. & Dreyer G. (1996)**. Ultrasonographic evidence of abnormal lymphatic vessels in young men with adult *Wuchereria bancrofti* infection in the scrotal area. *Journal of urology*, **156**: 409-412.

**Noroes J., Dreyer G.**, **Santos A., Mendes V.G., Medeiros Z., Countinho A. & Addiss D. (1997).** Assessment of the efficacy of diethylcarbamazine on adult *W. bancrofti* in vivo. *Transactions of Tropical Medicine and Hygiene,* **91**: 71-81.

**Onapa A.W., Pedersen E.M., Reimert C.M. & Simonsen P.E. (2007).** A role for *Mansonia Uniformis* in the transmission of LF in Uganda. *Acta Tropica*, Volume **101** Issue 2: page159-168.

**Ottesen E.A. (1980).** Immunopathology of lymphatic filariasis in man. *Springer semin Immunopathology,* **2:** *373-385.*

**Ottesen E.A (1984).** Immunological aspects of lymphatic filariasis and onchocerciasis in man. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **78;** 9-17.

**Ottesen E. A. (1990)**. The filariasis and Tropical eosinophilia. In. Tropical and geographical Medicine **2<sup>nd</sup> Edn. 9 Ed**. *Warren K S & Mahmoud AAF) Mcgraw Hill Information Services* **.**

**Ottesen E. A. (1992).** Infections and disease in lymphatic filariasis. An Immunological perspective. *Parasitology*: **104**; S71-S79.

**Ottesen E.A. & Campbell W.C. (1994)**. Ivermectin in human medicine. *Journal of Antimicrobial Chemotherapy,* **34**: 195-203.

**Ottesen E.A. & Ramachandran C.P**. **(1995).** Lymphatic filariasis infection and disease: control strategies. *Parasitology Today,* **11***:*129–131.

**Ottesen E.A., Ismail M.M. & Horton J. (1997)**. The role of albendazole in programs to eliminate lymphatic filariasis. *Parasitology Today, 15:* 382–386.

**Ottesen E.A., Ismail M.M. & Horton J**. **(1999)**. The role of albendazole in programs to eliminate lymphatic filariasis. *Parasitology Today, 15(9): 382-6.*

**Ottesen E.A. (2000).** The global programme to eliminate lymphatic filariasis. *Tropical Medicine of International Health,* **5**: 591–594. *Co., New York*, pp 407-429.

**Ottesen E.A., Hoopers P.J., Bradley M. & Biswas G. (2008).** The Global Programme for Elimination of Lymphatic Filariasis: Health impact after 8 years. *Plots of Neglected Tropical Disease,* **2** : e317.

**Pani, S.P., Yuvaraj J., Vanamail P., Dhanda V., Michael E., Grenfell B.T. & Bundy D.A P. (1995)**. Episodic Adenolymphangitis and lymphoedema in patients with bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **85**: 260-264.

**Panicker K.N., Jayasree M. & Krishnamoorthy K. (1992).** A cost benefits analysis of fish culture strategy towards the control of Mansonioides in Shertallai, Kerala state. *Indian Journal of Medical Research***, 95**: 157-160.

**Peyton E.L.** & **Scanlon J.E. (1966)**. Illustrated key to the female *Anopheles* mosquitoes of Thailand. Bangkok: *United States Army Medical Component, Southeast Asia Treaty Organization*.

**Plichart C., Sechan Y., Davies N. & Legrand A.M**. **(2006).** PCR and dissection as tools to monitor filarial infection in *Ae. polynesiensis* in French Polynesia**.** *Filarial Journal*, **5**: 2.

**Ramaiah K.D., Ramu K., Kumar K.N. & Guyatt H**. **(1996).** Epidemiology of acute filarial episodes caused by *Wuchereria bancrofti* infection in two rural villages in Tamil Nadu, South India. *Transactions of Royal Society of Tropical Medicine and Hygiene*, **90**: 639-643.

**Ramaiah K. D., Das P.K., Michael E. & Guyatt H**. **(2000).** The economic burden of lymphatic filariasis in India. *Parasitology Today*, **16**: 251-253.

**Ramzy R.M., Farid H.A., Kamal I.H., Ibrahim G.H., Morsy Z.S., Faris R., Weil G.J., Williams S.A. & Gad A.M. (1997).** A polymerase chain reaction assay for detection of *Wuchereria bancrofti* in human blood and *Culex pipiens***. *Trans Royal Society of Tropical Medicine & Hygiene*, **91:**156-160.

**Ramzy R.M. (2002).** Field application of PCR-based assays for monitoring *Wuchereria bancrofti* infection in Africa**. *Annals of Tropical Medicine & Parasitology*, **96 (Suppl 2)**): S55-S59.

**Rajendran G., Krishnamoorthy K., Sabesan S., & Panicker K. N. (1989).** Attachment behaviour of immatures of *Mansonia annulifera* (Theobald). *Indian Journal of Medical Royal Society,* **89**:192-197.

**Rattanarithikul R. (1982).** A guide to the genera of mosquitoes (Diptera: Culicidae) of Thailand with illustrated keys, biological notes and preservation and mounting techniques. *Mosquito System*, **14**: 139-208.

**Reiter P. (1983**). A portable, battery powered trap for collecting gravid *Culex* mosquitoes. *Mosquito News*, **43**: 496-498.

**Rozendaal J.A**. (**1989).** Impregnated mosquito nets and curtains for self-protection and vector control. *Tropical Disease Bulletin*, **86**: 1-41.

**Scott A.L. (2000).** lymphatic-dwelling filariae. In lymphatic filariasis.1st edn ( ed. Nutman TB) *Imperial College Press, London,* Pp 5-39.

**Simonsen P.E., Meyrowitsch D.W., Makunde W. H. & Magnussen P**. (**1995a).** Bancroftian filariasis. The pattern of microfilaremia, and clinical manifestations in three endemic communities of northern Tanzania. *Acta Tropica*, **60**: 179-185.

**Simonsen P.E., Niemanm L. & Meyrowitsch D.W. (1997b).** *Wuchereria bancrofti* in Tanzania: microfilarial periodicity and effect of blood sampling time on microfilaria intensities. *Tropical Medicine and International Health*, **2**: 153-158.

**Southgate B.A, (1984).** Recent advances in the epidemiology and control of filarial infections including entomological aspects of transmission. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **78 (suppl.):** 19-28.

**Subra R. and Hebrard G. (1975).** Ecology of *Culex pipiens fatigans* larvae in an area of high endemicity of Bancroftian filariasis. *Tropical Medicine of Parasitology,* **26**:48-59.

**Thanomsub B .W., Chansiri K., Sarataphan N. & Phantana S. (2000).** Differential diagnosis of human lymphatic filariasis using PCR-RFLP. *Molecular and Cellular Probes,* **14:**41-46.

**United Nations Information Centre. (2007)** Nairobi.

**Vickery A.C., Albertine A. C., Nayar J.K. & Kwa B.H. (1991).** Histopathology of *Brugia malayi* infected nude mice after immune-reconstitution. *Acta Tropica,* **49**: 45-55.

**Walsh J.F., Davies J.B. & Le Berre R. (1978).** Standardisation of criteria for assessing the effect of Simulium control on Onchocerciasis control programmes. *Transactions of Royal Society of Tropical of Medical Hygiene,* **72**: 675-676.

**Wamae C.N., Lammie P.J. & Mutunga J.N. (1994).** Advances in the diagnosis of Lymphatic filariasis: a review. *East African Medical Journal*, **71**: 171-182.

**Wamae C.N., Gatika S.M., Roberts J.M. & Lammie P.J. (1998).** *Wuchereria bancrofti* in Kwale District, Coastal Kenya: Patterns of focal distribution of infection,

clinical manifestations and anti-filarial IgG responsiveness. *Parasitology*, **166**: 173-182.

**Wamae C.N., Mwandawiro C., Wambayi E., Njenga S. & Kiliku F. (2001)**. Lymphatic filariasis since 1910, and the prospects for its Elimination; *East African Medical Journal,* **78:** 595-603.

**Wamae C., Njenga S., Kisingu W., Muthigani P. & Kiiru K**. **(2006).** Community-directed treatment of lymphatic filariasis in Kenya and its role in the national programmes for elimination of lymphatic filariasis. *African Journal of Health Sciences,* **13:** *1-2.*

**Webb J.K.G., Job C.K. & Gault E.W**. **(1960).** Tropical eosinophilia: Demonstration of microfilariae in lung, liver and Lymph nodes. *The Lancet,* **1**: 835-842.

**Weil G.J., Ramzy R.M., El- Setouhy M., Kandil A. M., Lowrie R.C. Jr & Faris R. (1996)**. Parasite antigenaemia without microfilaraemia in bancroftian filariasis. *American Journal of Tropical Medicine and Hygiene*, **61**: 53-58.

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

**Witte M.H., Jamal S., Williams W.H., Witte C.L., Kumaraswami V., McNeil G., Case T. & Panicker T.R. (1993).** Lymphatic abnormalities in human filariasis as depicted by lymphoangioscintigraphy. *Archives of International Medicine*, **153**: 737-744.

**Wharton R.H. (1962).** The biology of *Mansonia* mosquitoes in relation to the transmission of filariasis in Malaya. *Bull Institutes of Medicine of Royal Society (Malaysia),* **11**: 114.

**WHO. (1964).**The human blood index of malaria vectors in relation to epidemiological assessment. *Bull World Health Organization*. 30: 241-261.

**WHO (1975).** Manual on Practical Entomological in Malaria. Part II. Methods and Techniques. *W.H.O Offset Publication* **13.** Geneva: Switzerland.

**WHO. (1984)** Technical report series. Lymphatic filariasis: *fourth report of the WHO Expert Committee on Filariasis*. **No.702.**

**WHO. (1987).** Control of lymphatic filariasis. A manual of Health personnel. World Health Organization, Geneva.

**WHO**. **(1992).** Lymphatic filariasis. The disease and its control. *WHO Technical Report series* **821.** World health Organization, Geneva.

**WHO. (1994)**. Lymphatic filariasis infection and disease: Control Strategies. *World Health Organization,* Geneva.

**WHO. (1995).**The world Health Report. Report of the Director General. *World Health Organisation,* Geneva.

**WHO. (1997).** Elimination of lymphatic filariasis as a public health problem. *Document WHA50/1997/REC/1. World Health Organisation, Geneva.*

**WHO**. **(2000).** Preparing and Implementing a National Plan to Eliminate Lymphatic Filariasis: *A guideline for Programme Managers*. Geneva.

**WHO. (2001).** Lymphatic filariasis. *Weekly Epidemiology Record,* **76***:* 149–154.

**WHO (WHO/CDS/CPE/CEE/2000.16**). **(2002).** Global programme to eliminate lymphatic filariasis. *Weekly Epidemiology of Royal society* **80**: 202–212.

**WHO. (2004).** Annual report on lymphatic filariasis. 2003. 2004 Geneva.

**Wijers D.J.B. (1977a).** Bancrofti filariasis in Kenya. Prevalence survey among adult males in the coast province**.** *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **71**:313-331.

**Wijers D.J.B. and Kinyanjui H**. **(1977).** Bancroftian filariasis in Kenya, 11. Clinical and parasitological investigations in Mambrui, a small coastal town, and in Jaribuni, a rural area more inland coast province. *Annals of Tropical Medicine and Parasitology*, **71**, 347-359.

**Wijers D.J.B. and Kiilu G. (1977).** Bancrofti filariasis in Kenya 111. Entomological Investigations in Mambrui, a small coastal town and Jaribuni a rural area more inland (Coast province). *Annals of Tropical Medicine Parasitology.* **71**:347-359.

**Williams S., Laney S., Bierwert L., Saunders L., Boakye D., Fischer P., Goodman D., Helmy H., Hoti S., Vasuki V., Lammie P., Plichart C., Ramzy R. & Ottesen E. (2002).** Development and standardization of a rapid PCR-based method for the detection of *Wuchereria bancrofti* in mosquitoes for xenomonitoring the human prevalence of bancroftian filariasis. *Annals of Tropical Medicine of Parasitology,* 96: S41–S46.

**Zhong M., McCarthy J., Bierwert L., Lizotte-Waniewski M., Chanteau S., Nutman T.B., Ottesen E.A. & 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.

**Appendix 1:     INFORMED CONSENT DOCUMENT (ICD)**

TITLE*:* **VECTORIAL POTENTIAL OF** *MANSONIA* **SPECIES IN THE TRANSMISSION OF** *WUCHERERIA BANCROFTI* **AND EVALUATION OF MOSQUITO COLLECTION METHODS AND DETERMINATION IN TANA-DELTA DISTRICT, COASTAL-KENYA.**

Participants Information

Name of the Household head: ---------------------------------------------

Date of birth: ---------------Age---------------Sex--------------

Address: ----------------------------------------------------------

**Investigators**

Nancy M. Kinyatta[1, 2] (PI)

Prof. Zipporah Ng'ang'a[2] (Co-Investigator)

Dr. Luna Kamau[1] (Co-Investigator)

**Institutional affiliations.**

1.  Kenya Medical Research Institute (KEMRI)

2.  Jomo Kenyatta Institute of Agriculture and Technology (JKUAT)

**1. Introduction**.

This is a research on lymphatic filariasis transmission in the vector mosquitoes. This study will be carried out by Nancy Kinyatta who is a member of staff of KEMRI and a student at Institute of Tropical Medicine and Infectious Disease. There is no commercial interest in this study but the aim is to add knowledge on LF vectors and raise awareness to you as a community on the transmission of LF and vector control. The participation is voluntary and you have the right to refuse or to withdraw from the study without loss of benefits.

**2. Purpose of the study.**

The overall aim of this study is to collect mosquitoes inside your houses for the determination of the vectorial potential of *Mansonia* species in the transmission of *W. bancrofti* in Tana Delta district, Coastal-Kenya. This will involve indoor collection of mosquitoes using CDC light traps, gravid traps and pyrethrum spray methods. Thirty six house hold heads (participants) will be required. There will be a day for traps setting and another day for pyrethrum spraying for each of the selected house and this will take 10-12 days during the month of May, 2010.

**3. Procedures**

In this study you will be required to allow me and the field workers inside your houses especially the sleeping rooms for setting of traps and latter in another day allow us for spraying. You will also be asked not to interfere with the traps and during the day of spraying you will be requested to take foodstuff, water and young children outside or to another house.

**4. Benefits**

You and your community as a whole will benefit from free bednet which is one of the strategies used in vector control.

Through National Programme for Elimination of Lymphatic Filariasis you will benefit from free annual Mass Drug Administration using DEC, Albendazole or Ivermectin drugs for LF treatment.

**5. Risks**

The risks involved here are minimal. Care will be taken during spraying to ensure that no sprays get into food stuff or in contact with young children by taking them outside or to another house prior spraying.

**6. Confidentiality**

Any personal details will be maintained in confidentiality. The mosquitoes will be coded in such a way that the infectivity rates of mosquitoes from each household of collection are not exposed.

**7. Contact of Principal Investigator**

This study is under the direction of NANCY MUTANU KINYATTA ID no 22090982 and in case of any problem, you (the participant) are requested to contact me at KEMRI, box 54840, Tel, 2722541 (Weekday, daytime) or on cell, 0721598040 (all-time).

**8. Contact of KEMRI/ National Ethical Review Committee**

Incase you need to enquire about your security and right to participate in this study, the involved institute will be KEMRI, box 54840, Tel, 2722541 (Weekday, daytime)

**9. Compensation**

There will be no losses or major risks involved in this study so no compensation you should expect.

**10. Sample storage exportation and further studies**

Mosquitoes from the traps will be exposed to chloroform for killing. Mosquitoes both from traps and spray methods will be taken to the field laboratory setting for sorting, identification and dissection. The mosquitoes will be preserved under silica gel or 70% alcohol for transportation to KEMRI Radio Isotope laboratory-Nairobi where *W. bancrofti* DNA extraction will take place. DNA amplification (PCR) and PCR product analysis (Electrophoresis) will also be done.

**11. Consent and signature options**
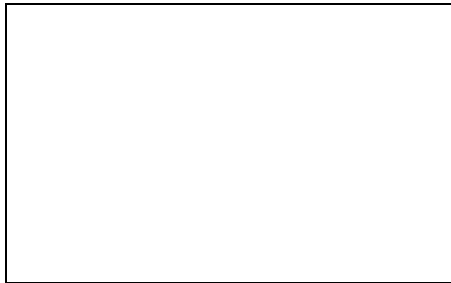
Name of the Household head --------------------------------------------

Date of birth: ---------------Age---------------Sex--------------

Address: ------------------------------------------------------------

I---------------------------------------------- have fully capacity to consent. I have been informed about the study in details. Having read the information explained to me and understood it, I give consent to have traps be set in my house and spraying done in my house.

**PARTICIPANT'S SIGNATURE OR LEFT THUMB PRINT**

_____

Witness:  I have witnessed that the above named participant whose thumb print is as above has given full consent to take part in this study.

Name.………………………………………..

SIGNATURE……………………..

**Appendix 2: DATA COLLECTION FORM**

**Table 11**

| Date | Serial no. | House hold | village | Method of collection |
|------|-----------|-----------|---------|---------------------|
|      |           |           |         |                     |
|      |           |           |         |                     |
|      |           |           |         |                     |

**Appendix 3: RESULTS FORM**

**Table 12**

| Date | serial no. | House hold | village | Method of collection | Blood fed | Mosquito Species | PCR | Dissection |
|------|-----------|-----------|---------|---------------------|-----------|-----------------|-----|-----------|
|      |           |           |         |                     |           |                 |     |           |
|      |           |           |         |                     |           |                 |     |           |
|      |           |           |         |                     |           |                 |     |           |
|      |           |           |         |                     |           |                 |     |           |
|      |           |           |         |                     |           |                 |     |           |
|      |           |           |         |                     |           |                 |     |           |

**Appendix 4:** *Wuchereria bancrofti* **DNA extraction procedures**

Ethanol precipitation method of DNA extraction was used as described by Abbasi *et al.* (1999) and Ramzy *et al. (*2002) with few modifications. Mosquitoes were ground in grinding buffer which was prepared by mixing homogenising and lysis buffer in the ratio of 4:1 respectively. The homogenization buffer was prepared by dissolving the following reagents in 100ml of sterile double distilled water and adjusting the PH to 8.0. 0.10M; Sodium Chloride  0.59g, 0.20M Sucrose 6.84g, 0.01 EDTA 0.37g, 0.03 Trizma Base  0.36g.  Lysis buffer was prepared with the following reagents; 0.25 EDTA 9.28g, 2.5% (w/v) SDS, 1.88g**,** 0.5 Trizma Base 6.03g dissolved in 100ml of sterile water and adjusting the PH to 9.2. Using mortar and pestle, individual mosquitoes were ground in 100µl of grinding buffer in well labeled tubes. Making sure no large particles of mosquito remained. Separate mortar and pestle were used for each mosquito and they were soaked in 10% JIK and cleaned well. They were sterilized by autoclaving before re-use to avoid cross contaminations. The ground mosquitoes were heated at 65$^{o}$c for 30 minutes in a thermo mixture and 14µl of potassium acetate (58.89g in 75ml of sterile water) added and vortexed. This was cooled on ice for 30 minutes and the pH was adjusted to 8-9 by adding either 1M NaOH if the mixture was acidic or 1: 4 HCl if the mixture was basic then centrifuged at 14000rpm for 10 minutes. The supernatant was transferred to new labeled vials. 200µl of absolute ethanol was added and vortexed. This was incubated at -80$^{o}$C for 2 hours or overnight. After incubation, it was centrifuged at 14000rpm for 20 minutes. The supernatant was removed and discarded leaving behind small volume not to disturb the DNA pellet. The pellet was washed twice with 200µl of 70% ethanol. For each wash, the mixture was centrifuged for 10 minutes at 14000rpm. It was rinsed

once with 100µl of absolute ethanol. The DNA pellet was dried in micro concentrator for 30 minutes. The pellet was suspended in 50µl of TE    PH 8.0 and Store at -80°C for PCR.

**Appendix 5:** *Wuchereria bancrofti* **DNA amplification (PCR)**

**Master Mix preparation**

Several reagents are required in the preparation of master mix including buffer, primers, dNTPs mix and PCR water. Buffers are used to regulate the pH and salt concentration in the master mix for enzyme activity. Deoxynucleotides mix made of dATP, dCTP, dGTP and dTTP provides bases for new DNA strand formation. The bases are linked together by Taq to synthesis new strands. The primers are short DNA sequences with base pairs between 20-25 which are complimentary to sequences flanking the target region. They provide an initiation site for DNA elongation. Taq Polymerase is the enzyme responsible for linking the free nucleotides, isolated from heat-stable Thermus aquaticus bacteria. Template DNA is the DNA sequence of interest to be amplified.

The above reagents were mixed in the preparation of premix for PCR amplification per reaction as shown in table 1.  5µl Taq DNA polymerase buffer, 0.5µl dNTPs, 0.5µl primer I (direct primer), 0.5 µl primer II (reverse primer), 43.5µl PCR water to top up to total reaction volume of 50µl. Vortex the mixture, Pipette 45µl of the mixture to each of the labeled tubes. Add 5µl of the samples to each of the tubes. Add 0.5µl of Taq polymerase to each of the reaction tube and vortex. Place the tubes in a thermocycler with the programs set and run PCR (Zhong *et al.,* 1996).

**Appendix 6: PCR Product analysis**

Target gene amplification was confirmed by analysis using agarose gel electrophoresis (Photo 5). 2% (W/V) of agarose gel was prepared by dissolving 3.0g of molecular grade agarose (Sigma) in 150ml of 1 X TAE buffer. The mixture was heated at $90^oC$ in a water bath or microwave until it dissolved completely and then Cooled at $50^oC$ in water bath. 5µl/ml of ethidium bromide (Promega) was added for DNA product visualization. The gel tank was set with the combs on a flat leveled bench. The gel was poured in the gel tank and let to set for 30-60 minutes. The gel was put in an electrophoresis tank. The combs were removed carefully to form wells to load the samples. 1X TAE buffer was added to cover the gel completely. 2µl of loading buffer (Promega) were added to 10µl of the amplified product of each sample and were loaded in the wells and the gel run for 30-60 minutes at 70-100V electrode potential on horizontal electrophoresis tank (Bio Rad[®]). A marker (DNA ladder), positive and negative controls were included in the gel. DNA amplified products were visualized as bands under ultraviolet (UV) light on a transilluminator and the results were recorded and photographed using Polaroid camera and Polaroid films instant films were taken for reference.