VECTOR COMPETENCE OF THE *ANOPHELES* (DIPTERA: CULICIDAE) POPULATIONS FOR *WUCHERERIA BANCROFTI* (SPIRURIDA: FILARIIDAE), AFTER MASS DRUG ADMINISTRATION IN THE GOMOA DISTRICT OF GHANA

BY

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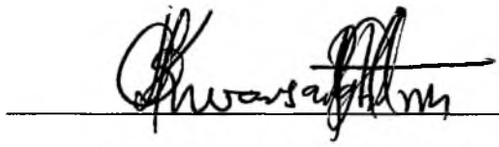
THIS THESIS IS SUBMITTED TO THE SCHOOL OF RESEARCH AND GRADUATE STUDIES, UNIVERSITY OF GHANA - LEGON, IN PARTIAL FULFILMENT FOR THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN ZOOLOGY (APPLIED PARASITOLOGY)

MAY 2005

DECLARATION

I hereby declare that except for references to other people's work, which have duly been acknowledged, this exercise is a result of my own research and this thesis neither in whole nor in part, had been presented for another degree elsewhere.



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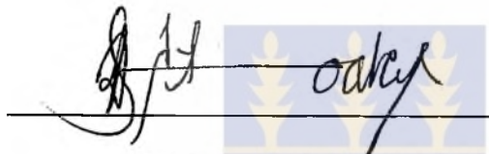

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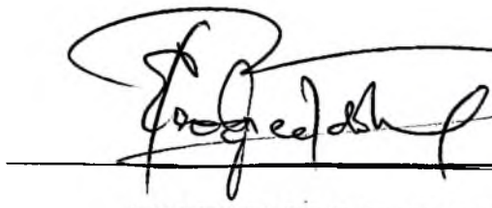

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DEDICATION

THIS WORK IS DEDICATED TO THE ANAA-BENTUM FAMILY FOR THEIR LOVE, SUPPORT, AND ENCOURAGEMENTS THAT HAVE BROUGHT MY EDUCATION THIS FAR

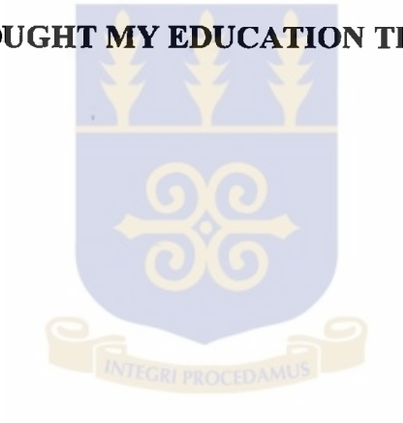

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TABLE OF CONTENTS

DECLARATION.....i

DEDICATION.....ii

ACKNOWLEDGEMENTS.....iii

TABLE OF CONTENTS.....v

LIST OF ILLUSTRATIONS.....x

LIST OF TABLES.....xii

LIST OF PLATES.....xiii

LIST OF APPENDICES.....xiv

LIST OF ABBREVIATIONS.....xv

ABSTRACT.....xvii

CHAPTER ONE.....1

GENERAL INTRODUCTION.....1

1.1 Introduction.....1

1.2 Rationale of Study.....3

1.2.1 Aim of study.....4

1.2.2 Specific objectives.....4

CHAPTER TWO.....5

LITERATURE REVIEW.....5

2.1 The Disease.....5

2.1.1 Global distribution of lymphatic filariasis 7

2.1.2 Pathogenesis and pathology 9

2.1.3 Clinical features of the disease 13

2.1.4 Asymptomatic presentations of the disease 15

2.1.5 Clinical diagnosis of *Wuchereria bancrofti* infection in humans 15

2.1.5.1 Immunological detection of microfilaria 16

2.1.5.2 Morphological detection of microfilaria 17

2.1.5.3 Molecular detection of microfilaria 18

2.1.5.4 Detection of lymphatic filarial infection by X-ray 20

2.1.6 Prevention of the disease 20

2.1.7 Treatment of the disease 22

2.1.8 Management of the disease 23

2.2 Biology and Life Cycle of *Wuchereria bancrofti* 24

2.3 Biology and Life Cycle of the Vectors 27

2.3.1 Biology of the vectors 27

2.3.2 *Anopheles gambiae* complex and *An. funestus* group 31

2.3.3 Life cycle of the vectors 32

2.4 Molecular Characterisation of *Anopheles* Population 36

2.4.1 PCR-RFLP identification of *Anopheles funestus* and *An. gambiae* species complex 37

2.5 Detection of *Wuchereria bancrofti* in Mosquito Vectors 39

2.5.1 Microscopy 39

2.5.2 Polymerase Chain Reaction (PCR) 40

2.6 Factors Affecting the Transmission of *Wuchereria bancrofti* 40

2.6.1 Infection in the human population 40

2.6.2 Vectorial capacity 41

2.6.2.1 Vector density relative to man and human feeding habit of vector 41

2.6.2.2 Vector survival and time from infection to infectivity 42

2.6.3 Vectorial competence 42

2.6.3.1 Parasite uptake 43

2.6.3.2 Parasite development to infective larvae (L_3) 44

2.6.3.3 Density dependent processes in the vector 44

2.6.3.4 Parasite induced vector mortality 45

2.6.4 Climatic factors 46

2.6.4.1 Temperature 47

2.6.4.2 Sunshine 47

2.6.4.3 Rainfall 48

2.6.4.4 Relative humidity 48

CHAPTER THREE 49

MATERIALS AND METHODS 49

3.1 Chemicals, Reagents and Equipments 49

3.2 Study Site 49

3.3 Ethical Considerations 52

3.4 Mass Screening for Microfilaria and Feeding Experiment 52

3.5 Laboratory Studies 55

3.5.1 Morphological identification, maintenance and dissection of mosquitoes 55

3.5.2 Molecular Studies 58

3.5.2.1 Chemicals, reagents and solutions 58

3.5.2.2 PCR-RFLP species identification of molecular forms of the *Anopheles gambiae* Giles complex 58

3.5.2.2.1 Genomic DNA extraction 58

3.5.2.2.2 PCR amplification 59

3.5.2.2.3 Molecular forms of *Anopheles gambiae* s.s. identification 60

3.5.2.3 Identification of members of the *Anopheles funestus* group 60

3.5.2.3.1 Genomic DNA extraction 60

3.5.2.3.2 PCR amplification 61

3.5.2.4 Species identification of *Wuchereria bancrofti* 61

3.5.2.4.1 Genomic DNA extraction 61

3.5.2.4.2 PCR identification of *Wuchereria bancrofti* 62

3.5.2.5 Observation and analyses of amplified PCR-RFLP products 63

3.6 Sampling Method and Data Analysis 64

CHAPTER FOUR 65

RESULTS 65

4.1 Microfilaraemia Load in the Study Area 65

4.2 Collection and Dissection of Mosquitoes 68

4.3 Molecular Identification of *Anopheles funestus* and *An. gambiae* Species Complexes 75

4.4 PCR Identification of *Wuchereria bancrofti* 75

CHAPTER FIVE 80

DISCUSSION 80

CONCLUSION AND RECOMMENDATIONS 85

REFERENCES **86**

APPENDICES **107**

LIST OF ILLUSTRATIONS

Figure 1: Global distribution of lymphatic filariasis **8**

Figure 2: Clinical presentations of bancroftian filariasis in adult populations living in filariasis endemic areas. 12

Figure 3: Schematic diagram of the life cycle of *Wuchereria bancrofti* 26

Figure 4: Eggs of mosquitoes 28

Figure 5: Mosquito egg rafts and clusters attached to underside of a floating leaf 28

Figure 7: Map of Ghana showing the study site, Gomoa District 51

Figure 8: Generic composition of of the mosquitoes caught during the feeding experiment 69

Figure 9: Variation of *Anopheles* species and the geometric mean intensity (geomean) of microfilaraemia among the twelve consented volunteers observed during the collection of mosquitoes 70

Figure 10: Photograph of an example of ethidium bromide-stained agarose gel (2%) electrophoregram of amplified PCR products for the identification of *Anopheles gambiae* s.l. species..... 76

Figure 11: Photograph of an example ethidium bromide-stained agarose gel (2%) electrophoregram of PCR amplified products for the identification of *Anopheles funestus* s.l. species. 77

Figure 12: Photograph of ethidium bromide-stained agarose gel (2%) electrophoregram of *Hha* I restriction enzyme digest for the identification of molecular forms of *Anopheles gambiae* s.s..... 78

Figure 13: Photograph of an example of ethidium bromide-stained agarose gel (2%) electrophoregram analysis of a PCR diagnostic test for detection of *Wuchereria bancrofti*..... 79

LIST OF TABLES

Table 1: The numbers of individuals examined and number of cases in the nine study sites 66

Table 2: The geometric mean intensities of *Wuchereria bancrofti* infections in the nine study sites** 67

Table 4: Distribution of infected mosquitoes and number of mf ingested by mosquitoes 71

Table 5: Distribution of infected and infective *Anopheles* mosquitoes, and number of mf ingested by female *Anopheles* mosquitoes caught during the study..... 72

Table 6: Distribution of infection with *Wuchereria bancrofti* in the various mosquito species caught after 13 days of maintenance..... 73

Table 7: Distribution of infection with *Wuchereria bancrofti* in the *Anopheles* mosquitoes caught after 13 days of maintenance..... 74

LIST OF PLATES

Plate 1: Finger prick blood being taken from a volunteer sleeping under mosquito net. 54

Plate 2: Boxes with netted paper-cups containing the collected mosquitoes at the insectary 58

LIST OF APPENDICES

Appendix I: Preparation of standard solutions 107

Appendix II: Sequence of the synthetic oligonucleotide primers used in the molecular studies 110

Appendix III: Constituents of a 20µL PCR reaction mix used in the molecular studies.... 113

Appendix IV: Information and consent form 116

Appendix V: The hourly examinations of blood for *Wuchereria bancrofti* among the twelve consented volunteers during the mosquito collection..... 121

Appendix VI: The number of subjects examined, number of positive and mf/ ml intensity of blood among the age groupings during mass screening for *Wuchereria bancrofti* in the individual communities..... 122

Appendix VIIa: Entomological survey sheet 131

Appendix VIIb: Mosquito dissection entry sheet 132

LIST OF ABBREVIATIONS

ADLA	Acute Dermatolymphangioadenitis
AFL	Acute Filarial Lymphangitis
ANOVA	One-way analysis of variance
bp	base pair
CFA	Circulating Filarial Antigen
CIOMS	Council for Internationaal Organisations of Medical Science
DEC	Diethylcarbamazine
DNA	Deoxyribonucleic acid
DNTPs	Deoxyribonucleotide triphosphates
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
GMI	Geometric Mean Intensity
GPELF	Global Programme to Eliminate Lymphatic Filariasis
IgE, IgG4	Immunoglobins E, G4
IGS	Intergenic Spacer
IL-4, IL-5, IL-10	Interleukins-4, 5, 10
ITS	Internal Transcribed Spacer
L_1	First stage larvae of mf
L_2	Second stage larvae of mf
L_3	Human infective third-stage larvae
L_4	Fourth stage larvae of mf
mf	microfilariae

OCP	Onchocerciasis Control Programme
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
rDNA	Ribosomal DNA
RFLP	Restriction Fragment Length Polymorphisms
RNAse	Ribonuclease
rpm	Revolution per minute
s.l.	*sensu latu*
s.s.	*sensu stricto*
SDS	Sodium deodecylsulphate
SSCP	Single-Stranded Conformation Polymorphism
TAE	Tris-acetate EDTA
Tm	Melting temperature
TPE	Tropical Pulmonary Eosinophilia
Tris	2-amino-1-hydroxyl-1,3-propanediol
VNTR	Variable Number of Tandem Repeats
WHO	World Health Organisation

ABSTRACT

Ability of mosquitoes to ingest microfilariae (mf), promote their maturation to the infective stage, and their survival rate to parasite maturation for transmission to humans seems to differ according to geographic mosquito strains. The proportion of ingested mf that develops successfully into L_3 may decrease (limitation) or increase (facilitation) with higher mf uptake. Transmission intensity depends on a number of factors such as the level of infection in the human population, vectorial capacity, vectorial competence, and climatic factors. Results obtained from a preliminary study after three years of mass drug administration (MDA) showed a decrease in annual transmission potential (ATP) of *Anopheles funestus* but no change in *An. gambiae* s.s. This study was conducted to determine the vector competence of these two *Anopheles* species in the transmission of *Wuchereria bancrofti* at low mf levels. Mass screening for mf was done using 100µl finger-prick blood and consented positive individuals who volunteered and slept under mosquito nets that had one side opened. Wild mosquitoes that fed on them were collected hourly from 21:00 to 06:00 hours GMT. Approximately half of the mosquitoes were killed immediately and dissected to count the number of mf ingested. The remaining mosquitoes were maintained for 13 days to observe parasite maturation after which they were dissected. Along side the mosquito collection, 100µl finger-prick blood was collected hourly to observe mf level in peripheral blood. The overall prevalence of mf in the study community (N = 1083) was 1.6%. The levels of mf varied from 0 to 59 mf/ 100μl blood, with a geometric mean intensity of 1.1 mf/ ml. Some variation in intensity with age-group was observed, however neither the intensities in age group (P = 0.40) nor the intensities in the male and female subjects (P = 0.91) were significant. Out of the 564 mosquitoes collected, 62.1% were *Anopheles* species, 32.3% *Mansonia* species, 5% *Aedes* species and 0.7% *Culex* species. *Anopheles funestus* and *An.*

gambiae formed 88.6% and 9.1% of *Anopheles* caught respectively. Both mf level in peripheral blood and biting rates of the *Anopheles* mosquitoes peaked between 00:00 and 03:00 hours. Six mosquitoes each of *Anopheles* (1.7%) and *Mansonia* (3.3%) were found infected but none was infective after day 13 of maintenance. Molecular studies showed all *Anopheles gambiae* s.l. to be *An. gambiae* s.s. out of which 70% were M form. All infected *Anopheles gambiae* were M forms. A total of 86% of the *An. funestus* were identified as *An. funestus* s.s. with 6% being *An. leesoni*. Although these *Anopheles* species were not competent in promoting the maturation of the parasites when mf is low, a repeat of this study targeting larger mosquito numbers is required to ascertain the role played especially by M forms of *An. gambiae* in the transmission of lymphatic filariasis when parasite levels in the community are low. Considering the fact that the study was conducted in the natural setting, this finding will help as to whether the combination therapy with ivermectin and albendazole is enough to eliminate the disease or vector management has to be integrated for the success of the GPELF in areas like Ghana where *Anopheles gambiae* and *An. funestus* are the main vectors.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

The relationship between ingestion of microfilariae (mf), production of infective larvae (L_3) and mf density in human blood has been suggested as an important determinant in the transmission dynamics of lymphatic filariasis (Albuquerque *et al.,* 1999). Understanding vector-parasite interactions is thus, essential for assessing the prospects of elimination and rational development of control strategies. This is particularly important, considering that vectorial competence (the ability of mosquitoes to ingest mf and to promote their maturation until the infective stage), and the rate of mosquito survival until parasite maturation (Failloux *et al.*, 1995; Bryan *et al.*, 1990), seems to differ according to geographic mosquito strains (Crans, 1973; McGreevy *et al.*, 1982; Wharton, 1960). Variation in density of mf in blood and parasite behaviour also influences vector-parasite relationships (Failloux *et al.*, 1995; Southgate and Bryan, 1992; Tabachnick *et al.,* 1985).

Studies on the factors that affect transmission of *Wuchereria bancrofti* by anopheline mosquitoes include the uptake of mf by mosquitoes, which depend on the density and distribution of mf in the human host (Bryan and Southgate, 1988; Samarawickrema *et al.*, 1985). The ratio of numbers of ingested mf to numbers of infective larvae, which subsequently develop is another important effect on the transmission dynamics of *W. bancrofti*. Vector competence involves three processes; the uptake of mf from the human

host, the development of mf to the infective-stage larvae (L_3) and the transmission of L_3 to human (Subramanian *et al.*, 1997).

Changes in climatic factors such as temperature, rainfall and sunshine affect human health and disease vector populations in various parts of the globe in very different ways, often through complex changes in ecological systems. It is the interaction among these factors in combination with other non-climatic factors that will determine the timing of infectious disease outbreaks. The female mosquito becomes infected with *Wuchereria bancrofti* if it sucks blood from an infected person, and may then infect the next person it bites. The spread of the disease is thus limited by conditions that favour the vector and the parasite growth.

For the Global Programme to Eliminate Lymphatic Filariasis (GPELF) to be successful, the ability of various mosquito vectors to pick the mf (especially when the community mf load is low), support the development of the ingested mf into L_3, and to transmit those L_3 to humans has to be understood. Some species of mosquito (those exhibiting the phenomenon of facilitation) are unable to transmit parasites from humans with low levels of microfilaraemia whereas other species (exhibiting limitation) can effectively transmit the parasites even when the mf in their blood meal source is at a very low level.

The quantitative relations of transmission intensity and mf reservoir such as the proportion (40 60%) of ingested mfs which are damaged by the pharyngeal foregut armature of *Anopheles* mosquitoes, percentage of mosquitoes ingesting mf and host mf density, also the percentage of mosquitoes infected or mf density per mosquito and numbers of mf per

millilitre of host blood have been found to vary among members of the *Anopheles gambiae* complex and *An. funestus* (Bryan and Southgate, 1988; McGreevy *et al.*, 1982; Bryan *et al.*, 1990).

1.2 Rationale of Study

Several sympatric *Anopheles* species that are vectors of lymphatic filariasis in Ghana might differ in vectorial role and capacity to transmit low-density mf. Dzodzomenyo *et al.*, (1999) identified *An. gambiae* and *An. funestus* as the most important vectors of the disease along the coast of Ghana. The parent project of which this work is a sub-component seeks to investigate the trends in levels of transmission and infection with *W. bancrofti* during mass treatment with ivermectin and albendazole in some communities of Gomoa District of the Central Region of Ghana. The preliminary results seem to indicate that although transmission potential of *An. funestus* has decreased significantly after mass chemotherapy with ivermectin and albendazole, there appears to be no change in that for *An. gambiae* s.s. in the area. A similar study in the Bongo District of Northern Ghana showed a probable relationship of limitation between *W. bancrofti* and *An. gambiae* s.l., *An. funestus* or both taxa (Boakye *et al.*, 2004). This work therefore sets out to determine the roles of the two different *Anopheles* species in transmission of *W. bancrofti* at low mf levels since this information is crucial to the success of the Global Lymphatic Filariasis Elimination Programme.

1.2.1 Aim of study

The main aim of the study is to look at the competence of *Anopheles gambiae* s.s. and *An. funestus* in the transmission of lymphatic filariasis after mass drug treatment with ivermectin and albendazole in an endemic area of the Gomoa District of Ghana.

1.2.2 Specific objectives

The specific objectives of this study are:

1. To determine the importance of low density microfilaraemia in the transmission of *Wuchereria bancrofti.*
2. To ascertain the intensity of lymphatic filariasis transmission in the study area.
3. To evaluate the vector competence of *W. bancrofti* in *Anopheles gambiae* and *An. funestus* after feeding on humans with varying densities of mf.
4. To identify by polymerase chain reaction (PCR) the sibling species of *An. gambiae* s.s. and *An. funestus* collected.
5. To identify and determine the distribution of the M and S forms of *An. gambiae* s.s. by restriction fragment length polymorphism (RFLP).
6. To confirm the *W. bancrofti* in the human population and the mosquitoes using PCR.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Disease

Lymphatic filariasis (LF) commonly known as elephantiasis is caused by the mosquito-borne parasitic nematodes *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* that live almost exclusively in humans of which *W. bancrofti* makes up about 90% of the cases (Michael *et al.*, 1996). These worms lodge in the human lymphatic system, the network of nodes and vessels that maintain the delicate fluid balance between the tissues and blood, which is an essential component for the body's immune defence system. The adult filarial worms live for 4-6 years in the vessels of the lymphatic system causing the vessels to dilate leading to dysfunction due to the slow movement of lymph fluid (WHO, 2000) due to decrease in pressure of lymph flow. Large numbers of bacteria build up that are not filtered away in acute stage leading to blockade of the vessels. The adult worms produce millions of immature mf (minute larvae) that circulate in the blood, which may be picked up by mosquitoes, therefore spreading the infection to others. About 20-50 % of men and up to 10% of women in endemic communities can be affected (WHO, 2000).

Lymphatic filariasis causes enlargement of the entire leg or arm, the genitals (vulva, scrotum) and breast in its most obvious manifestations. The adult worms cause internal damage to the kidneys, lymphatic system and the disabilities caused by the disease have considerable economic impact on affected communities (WHO, 2000). It is a major cause of poverty and people afflicted do not live normal working and social life. The psychological and social

stigmata associated with these aspects of the disease are immense, including reduced marital prospects (Ramaiah *et al*., 1997; Ahorlu *et al* 2001).

The disease was generally thought to occur once a while in children although recognized as an infection of adults (Witt and Ottesen, 2001). New highly sensitive diagnostic tests such as antigen detection and ultrasound examination, now reveal the disease is primarily acquired in childhood, often before age 5 (Witt and Ottesen, 2001). Individual case reports and numerous community-based epidemiological studies attest both to the existence of lymphatic filariasis infection in children and the occurrence of clinically evident disease. Microfilaraemia prevalence in children from different populations is related significantly to the level of endemicity seen in their respective adult populations (WHO, 2000). Initial damage to the lymphatic system by the parasites generally remains hidden for years or gives rise to presentations of adenitis (adenopathy), but especially after puberty lymphoedema (elephantiasis) and hydrocoele which are the characteristic clinical features begin to develop. Recognizing lymphatic filariasis as a disease of childhood has immediate practical implications both for management and prevention in individual patients and for broader public health efforts to overcome the crippling, debilitating diseases of childhood. Protecting children from lymphatic filariasis infection and disease should therefore be a primary goal of national elimination programmes. Recognizing this means children will not only be the principal benefactors of lymphatic filariasis elimination but also a population particularly important to target in order to achieve the Global Programme to Eliminate Lymphatic Filariasis twin goals of interrupting transmission and preventing disease (WHO, 2000).

2.1.1 Global distribution of lymphatic filariasis

The disease affects 120 million people in more than 83 countries worldwide with 1.2 billion (20% of the world's population) people at risk of acquiring infection (WHO, 2000). One third of these infected persons live in India, one-third in Africa (with prevalence rates exceeding 10% in 17 of 34 endemic countries). Most of the remainder occurs in Asia, the Pacific and the Americas. *Wuchereria bancrofti* cause ninety percent of these infections and most of the remainder by *Brugia malayi* (WHO, 2000). Even though certain strains of *B. malayi* can also infect some feline and monkey species, humans are the exclusive host for *W. bancrofti*. The life cycles in humans and in these animals remain epidemiologically distinct that overlap little (WHO, 2000). In most urban and semi-urban areas, the major vectors for *W. bancrofti* are *Culex* mosquitoes. *Anopheles* species is the major vector in rural areas of Africa and elsewhere, while *Aedes* mosquitoes in many of the endemic Pacific Islands. For the Brugian parasites, *Mansonia* species serve as the major vector, but in some areas anopheline mosquitoes are responsible for transmitting the infection. Brugian parasites are confined to areas of east and south Asia, especially India, Malaysia, Indonesia, the Philippines, and China. In Ghana, prevalence of lymphatic filariasis is between 9.2-25.4 % along the coast (Dunyo *et al.*, 1996) and 20-40 % in the northern regions (Gyapong *et al.*, 1996).

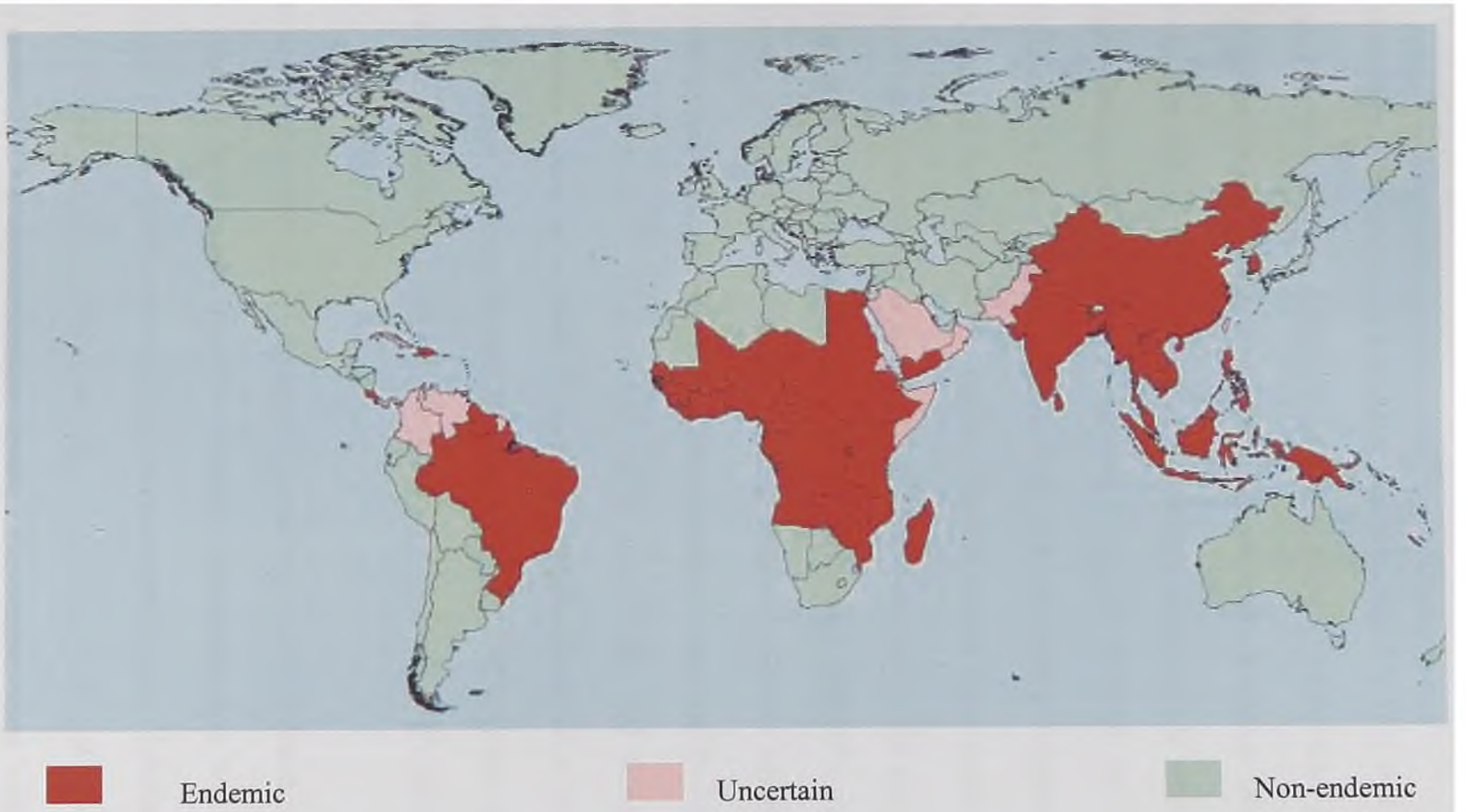


Figure 1: Global distribution of lymphatic filariasis

2.1.2 Pathogenesis and pathology

The pathology associated with lymphatic filariasis results from a complex interplay of the pathogenic potential of the parasite, the immune response of the host, and external bacterial and fungal infections (WHO, 2000). In the absence of such overt inflammatory responses some changes that can lead to both lymphoedema and hydrocoele formation occurs. Genital damage particularly hydrocoele (collection of serous fluid in the cavity of the tunica vaginalis caused by lymphatic dysfunction) and chylocoele (collection of white fat-rich lymph fluid in the cavity of the tunica vaginalis caused by a ruptured dilated lymphatic vessel) occur. Others include chyluria (milky fluid caused by the presence of white lymph fluid that is rich in fat, resulting from a ruptured dilated lymphatic vessel in the excretory urinary tract) and lymph scrotum (superficial dilated lymphatic vessels of the scrotal skin with intermittent discharge of white or straw-coloured lymph fluid). The rest are disfiguring clinical presentation of lymphoedema (with hypertrophy and fibrosis of the skin and subcutaneous tissues as a result of long-term lymphoedema after recurrent skin bacterial episodes) known as acute dermatolymphangioadenitis (ADLA) are the most recognizable clinical entities associated with lymphatic filarial infections (WHO, 2000).

There are much earlier stages of lymphatic pathology and dysfunction whose recognition has only recently been made possible through ultrasonographic and lymphoscintigraphic techniques (WHO, 2000). For example, ultrasonography has identified massive lymphatic dilation around and for several centimetres beyond adult filarial worms, which though in continuous vigorous motion, remain 'fixed' at characteristic sites within lymphatic vessels. Dilation and proliferation of lymphatic endothelium can be identified histologically, and the

abnormal lymphatic function associated with these changes can be readily documented by lymphoscintigraphy (WHO, 2000).

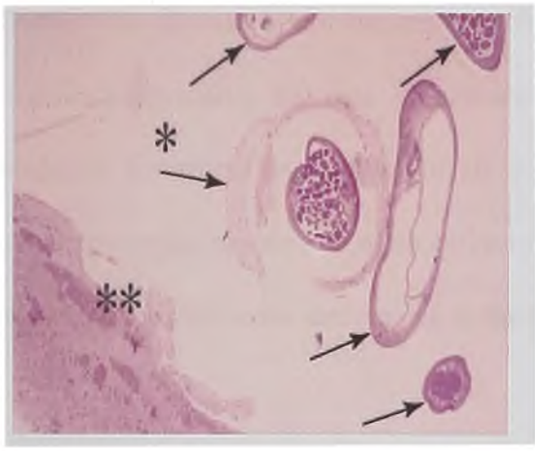
The immune system keeps itself 'down-regulated' through the production of contra-inflammatory immune molecules during the development of 'non-inflammatory pathology'. These are the characteristic mediators of Th2-type T-cell responses (IL-4, IL-5, IL-10) and antibodies of the IgG4 (non-complement-fixing) subclass that serve as "blocking antibodies" (WHO, 2000). Such adaptations serve to promote the biological principle of parasitism in which a satisfactory balance between parasite 'aggressiveness' and host responsiveness must evolve to maintain this special relationship. This response can be initiated by immune reactivity (clinically expressed as the characteristic adenitis and retrograde lymphangitis earlier described as 'filarial fevers') or by bacterial and fungal super infections of tissues with compromised lymphatic function originating from filarial infection (WHO, 2000). Recognition of the importance of these secondary infections in causing much of the progression and physical destruction associated with elephantiasis has had a major impact on improving the care, management and prospects for affected patients.

Immune-mediated pathology in lymphatic filariasis most commonly derives from the lymphatic obstructive consequences of the responses to dead or dying worms in the lymphatics. However, tropical pulmonary eosinophilia (TPE) syndrome pathogenesis is distinctly different. Indeed, it is this syndrome that demonstrates most dramatically what happens when the immune system's response to the parasite goes unchecked (i.e., escapes the down-regulating mechanisms usually seen during patent infection). In TPE, there is

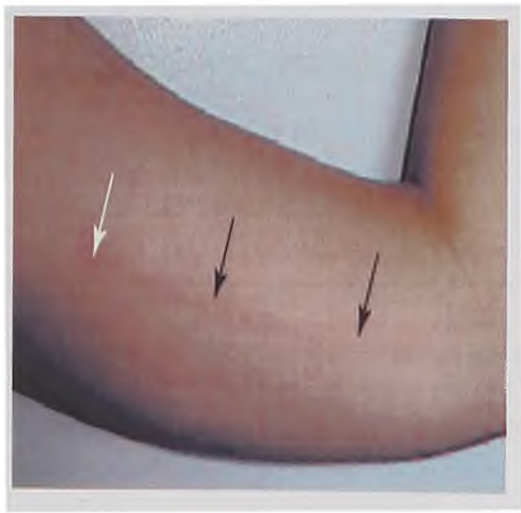
enormous immunologic hyper-responsiveness especially of IgE and other pro-inflammatory molecules directed against mf. This results in massive hyper-eosinophilia, allergic and other immunologic responses to those mf stage parasites causing them to be rapidly opsonized and cleared from the blood immediately as they pass through the lungs. The consequence of these unchecked, un-modulated responses and consequent inflammation is severe pulmonary functional compromise and tissue destruction that leads to crippling and permanent lung disease. Other clinical presentations include lymphangiectasia and acute filarial lymphangitis (AFL) and are shown in Figure 2.

Figure 2: Clinical presentations of bancroftian filariasis in adult populations living in filariasis endemic areas.

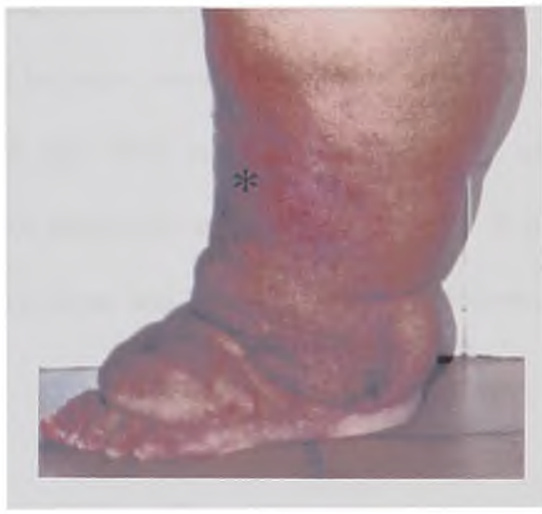
(a): Lymphangiectasia: dilation of lymphatic vessels (*) not caused by obstruction but by 'toxins' released by living filarial adult worms (arrows) in this context. No inflammatory reaction is found in the lymphatic vessel wall (**). Hematocyclin and eosin stained.



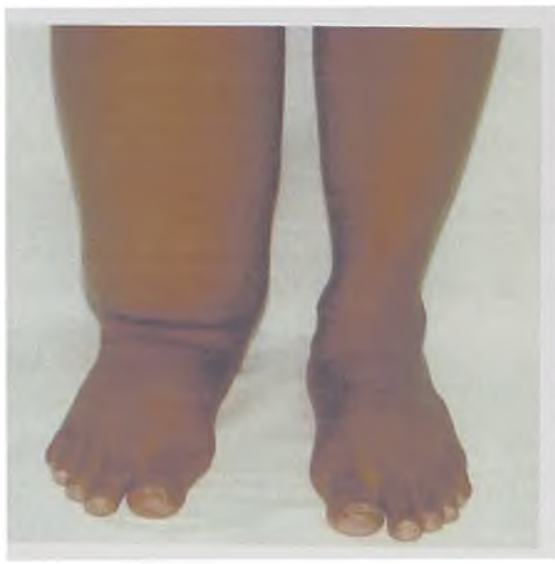
(b): Acute filarial lymphangitis (AFL) (arrows): caused by the death of adult worms



(c): Acute skin bacterial episode: a reticular lymphangitis (*) caused by bacterial infection, currently named acute dermatolymphangioadenitis (ADLA)



(d): Lymphoedema: swelling of the skin, as a result of accumulation of interstitial fluid after recurrent bacterial infections, predisposed in its turn by lymphatic dysfunction



2.1.3 Clinical features of the disease

There are chronic, acute and 'asymptomatic' presentations of lymphatic filarial disease, as well as a number of syndromes associated with these infections that may or not be caused by the parasites. Hydrocoele is found only with *W. bancrofti* infections (i.e. not *Brugia* infections) yet it is the most common clinical manifestation of lymphatic filariasis (WHO, 2000). The disease is not common in childhood but seen more frequently post-puberty and with a progressive increase in prevalence with age (Witt and Ottesen, 2001). In many endemic communities 40-60% of all adult males have hydrocoele (WHO, 2000). It often develops in the absence of overt inflammatory reactions, and many patients with hydrocoele also have microfilariae circulating in the blood.

The mechanism of fluid accumulation in the tunica vaginalis is still unknown, but direct ultrasonographic evidence indicates that in bancroftian filariasis the scrotal lymphatic are the preferred site for localization of the adult worms, and their presence may stimulate not only the proliferation of lymphatic endothelium but also a transudation of 'hydrocoele fluid' whose chemical constituents are similar to those of serum (WHO, 2000). The localization of adult worms in the lymphatic of the spermatic cord leads to a thickening of the cord that is palpable on physical examination of most patients. The hydrocoele can become massive but still occur without lymphoedema or elephantiasis developing in the penis and scrotum, since the lymphatic drainage of these tissues is separate and more superficial.

Recently filarial syndrome has been described as one of clinical and immunologic hyper-responsiveness found in expatriate visitors to regions endemic for loasis (WHO, 2000). This has also been described in patients with onchocerciasis, lymphatic filariasis, and other filarial infections (WHO, 2000). Persons who grow up outside endemic regions and then move to these regions and acquire filarial infection manifest prominent signs and symptoms of inflammatory (including allergic) reactions to the mature or maturing parasites instead of developing the commonly described chronic clinical manifestations. In loasis, manifestations primarily include Calabar swellings, hives, rashes and occasionally asthma whereas in bancroftian filariasis (migrants to endemic areas who acquire the infection), lymphangitis, lymphadenitis, genital pain (from inflammation of the associated lymphatic), along with hives, rashes and other 'allergic-like' manifestations, including blood eosinophilia are the symptoms. The different immunoregulatory responses to filarial antigens between those with long (including prenatal) exposure to these antigens and those meeting them for the first time leads to these different clinical presentations

Other syndromes co-existing with filariasis are found in filarial endemic regions, and because they show some evidence of therapeutic response to diethylcarbamazine (DEC), they have been suggested as possible manifestations of lymphatic filariasis (WHO, 2000). These include arthritis (typically monoarticular), endomyocardial fibrosis, tenosynovitis, thrombophlebitis, glomerulonephritis, lateral popliteal nerve palsy, and others. While future studies may strengthen the relationships, such syndromes at present cannot confidently be attributed to filarial infection (WHO, 2000).

2.1.4 Asymptomatic presentations of the disease

Though patients of lymphatic filariasis have mf circulating in their blood and essentially all have hidden damage to their lymphatic and/ or renal systems (microscopic heamaturia and/ or proteinuria), at least half appear clinically asymptomatic (WHO, 2000). This state of asymptomatic microfilaraemia is associated with high down-regulated immune system, but it is unclear how, when or whether these persons will progress to develop one of the more overt clinical manifestations of filarial disease (WHO, 2000). Another asymptomatic 'presentation' previously termed 'endemic normals' also exists. Their infections are not defined by microfilaraemia instead by the presence of parasite antigen in the blood (which will disappear after appropriate treatment) (WHO, 2000). This group of patients were recognised recently and both their clinical features and consequence remain to be defined (WHO, 2000).

2.1.5 Clinical diagnosis of *Wuchereria bancrofti* infection in humans

Diagnosis of filarial infection until recently depended on the direct demonstration of the parasite in blood or skin specimens. Alternative methods based on detection of antibodies by immunodiagnostic tests (WHO, 2000) did not prove satisfactory since they both failed to distinguish between active and past infections and had problems with specificity owing to their cross-reactivity with common gastrointestinal parasites and other organisms. Circulating filarial antigen (CFA) detection is now the standard for diagnosing *W. bancrofti* infections (WHO, 2000).

The specificity of CFA assays is near complete, and the sensitivity is greater than that achievable by the earlier parasite-detection assays. All individuals with microfilaraemia have

detectable circulating antigen, as well as do a proportion of those individuals with clinical manifestations of filariasis (e.g. lymphoedema or elephantiasis) but with no circulating mf. In addition, some individuals who appear normal also have detectable circulating antigen that disappears after effective treatment with DEC for these cryptic infections (WHO, 2000). Two methods, one based on ELISA yielding semi-quantitative results, and the other based on a simple card (immunochromatographic) test, giving only qualitative (positive/ negative) answers are available (WHO, 2000).

2.1.5.1 Immunological detection of microfilaria

Many lymphatic filariasis patients are amicrofilaraemic, and because no serologic test other than detecting CFA is specific, in the absence of antigen testing the diagnoses of these infections must be made clinically with support from antibody or other laboratory assays. The tropical eosinophilia syndrome is the most secure of these clinical diagnoses. In addition to its distinctive clinical presentation such patients have extraordinarily high levels of total serum IgG and IgE depending on the specific tests used. For other amicrofilaraemic syndromes serologic findings based on detecting IgG4 antibodies have proven helpful, since this subclass has greater diagnostic specificity and is stimulated by the presence of active infection (WHO, 2000). Such antibody analyses are also especially helpful in diagnosing the expatriate syndrome' where 'background (i.e. pre-exposure) levels' of IgG and especially IgG4 antibodies to filarial antigens will be very low, so that elevated levels have significant diagnostic implications in association with the clinical presentations. Eosinophilia is a frequent concomitant of all filarial syndromes, but they are diagnostically helpful only when the levels are extremely high.

2.1.5.2 Morphological detection of microfilaria

Prior to the development of the CFA assay, detection of mf in blood was the standard approach to diagnosing lymphatic filarial infection. It is still the one required today for situations where antigen detection test is not available for bancroftian filariasis. The simplest technique for examining blood or other fluids (including hydrocoele fluid, articular effusions and urine) is to spread 20µl evenly over a clean slide, dried and then stained with Giemsa or a similar stain (Mak, 1989). A wet smear may also be made by diluting 20-40 µl of anti-coagulated blood with water or 2% saponin, which lyses the red blood cells but allow the mf to remain motile and thus more readily identifiable (Mak, 1989). One must take into account the parasites' possible nocturnal periodicity in selecting the optimal blood drawing time (2200-0200 hrs) for such assessments.

The larger the blood volumes examined, the likelihood of detecting low parasitaemia will be greater. Knott's concentration technique has been used to examine 1ml volumes of anti-coagulated blood by mixing the blood with 10ml of 2% formalin, centrifuging the preparation and examining the sediment either unstained or fixed and stained (Mak, 1989). The mfs are non-motile, generally straight and can be easily missed if the viscous sediment is not searched diligently. Membrane filtration has been advanced as the most sensitive technique for detecting and quantifying mf in blood, urine or other body fluids (Mak, 1989). Polycarbonate (Nuclepore) filters with a 3µm pore size has proved most satisfactory. A known volume of anti-coagulated blood or other fluid is passed through a Swinnex holder containing the filter, followed by a large volume (about 35ml) of pre-filtered water that lyses the red blood cells. A volume of air then follows the water, and the filter is removed, placed

on a slide and stained. Morphology of the parasite is much more difficult to assess than when specimens are prepared initially on slides, but detection and quantification are very straightforward.

2.1.5.3 Molecular detection of microfilaria

The advent of new molecular biological techniques such as DNA probes, PCR have provided the opportunity for improved diagnosis of lymphatic filarial parasites. The PCR assay is very sensitive even in cases of low-level infections because amplification process is exponential. It may also be possible with PCR to detect circulating parasite DNA liberated from host-destroyed mf or from adult worms.

Zhong *et al.* (1996) reported that the *Ssp*I PCR assay for *W. bancrofti* DNA detection was developed and was first tested on blood samples collected in French Polynesia (Williams *et al.*, 1996), India (McCarthy *et al.*, 1996) and Egypt (Ramzy *et al.*, 1997). This was after the first PCR-based assay designed to detect DNA from a human filarial parasite (*B. malayi*) was developed (Lizotte *et al.*, 1994). This *Ssp*I PCR assay was adopted, improved and field-tested on pools of field-collected mosquitoes (Ramzy *et al.*, 1997). Other laboratories have been successful in adapting the PCR-based assay for mosquitoes, in a number of different field situations since then (Nicolas and Plichart, 1997; Fischer *et al.*, 1999; Bockarie *et al.*, 2000; Farid *et al.*, 2001; Hoti *et al.*, 2001; Kamal *et al.*, 2001). The specific protocols used by these investigators somewhat differed and standardization was clearly needed to move this technique from the realm of research into a routine monitoring tool for LF control efforts. The feasibility of this goal was supported by the highly successful screening of blackflies in

the countries covered by the Onchocerciasis Control Programme (OCP) in West Africa, where the PCR-based detection of onchocercal larvae is now a routinely used and has been found to be a very reliable monitoring tool (Yameogo *et al.*, 1999). The detection of *W. bancrofti* in mosquitoes with this PCR-based assay has two principal roles; the xenomonitoring of microfilaraemia during LF-elimination programme and determining the absence of infection in a defined region or country (particularly for certifying that a country had successfully eliminated LF) (WHO, 2000).

With respect to xenomonitoring, the PCR-based approach to identifying infection in a community has the particular advantage of a 'real-time' assessment of the transmission of infection. Antigen and antibody tests only give a positive result many months post-infection and therefore the results of such tests reflect the state of filarial transmission at a much earlier time point (Helmy, *et al.*, 2004). Compared with mosquito dissection, the potential of the PCR-based assays to screen pools up to 40 mosquitoes/ tube and 30 tubes/ run (i.e. up to 1200 mosquitoes/ run) will prove particularly valuable when the prevalence of infection in the mosquitoes falls to levels below 1%. The ability to screen such large numbers of mosquitoes rapidly is also clearly advantageous in determining the reductions to levels below 1% and the absence of infection in a defined region or country, following the completion of an LF-elimination programme. Dissection is an inexpensive method but requires dedicated personnel trained in the identification of larvae in dissected mosquitoes, and becomes increasingly inefficient as prevalence of infection in the vector population decreases. Both dissection and PCR-based methods are employed as surveillance tools at the beginning of

eradication programme, with PCR taking over as the primary screening tool as transmission level declines (Helmy *et al.*, 2004).

2.1.5.4 Detection of lymphatic filarial infection by X-ray

Conventional X-rays are rarely helpful in diagnosing lymphatic filarial infection, except in the case of tropical eosinophilia where the picture can vary but characteristically includes interstitial thickening and diffuse nodular mottling in the lung fields (Fox *et al.*, 2005). Ultrasound examination of the lymphatic (especially scrotal lymphatic in men, and the breast and retro-peritoneal lymphatic in women) can reveal rapidly moving adult worms, and though not diagnostic of filarial infection lymphoscintigraphy can identify lymphatic functional and gross anatomical abnormalities (Fox *et al.*, 2005).

2.1.6 Prevention of the disease

Filarial infection is acquired only from vector-borne infective larvae. Prevention of infection can therefore be achieved either by decreasing contact between humans and vectors or by decreasing the amount of infection the vector can acquire, by treating the human host. Individually, contact with infected mosquitoes can be decreased through the use of personal repellents, bednets or insecticide-impregnated materials. Alternatively, suggestive evidence from animal models and some limited experience in human populations indicate that a prophylactic regimen of DEC (6 mg/ kg per day x 2 days each month) could be effective in preventing the acquisition of infection (Shenoy *et al.*, 1998; Ramaiah *et al.*, 2003).

Efforts at filariasis control through reducing the numbers of mosquito vectors have been difficult as mosquitoes have high fecundity rate and large range of breeding sites. Even when good mosquito control are put in place, the long life-span of the parasite (4-8 years) means that the infection remains in the community for a long period of time, generally longer than intensive vector control efforts can be sustained. More recently, with the advent of extremely effective single-dose, once-yearly, 2-drug treatment regimens (selecting among albendazole and either ivermectin or DEC). An initiative has been launched through the World Health Organization to utilize a strategy of yearly mass treatment to all population at risk by decreasing mf load in endemic communities thereby interrupting transmission and preventing infection permanently, particularly if the vectors are anopheline mosquitoes to eliminate lymphatic filariasis as a public health problem (Webber, 1991; Southgate and Bryan, 1992; Bockarie *et al.*, 1998).

This strategy is based on the assumption that if mf reservoir in the human host can be reduced to below a certain threshold, the transmission of *W. bancrofti* by anopheline vectors will be interrupted. Southgate and Bryan (1992) reported that *Anopheles* appears to produce infection and disease much more effectively than *Culex* and *Aedes* transmitting yet observed that *Mansonia*, *Culex* and *Aedes* species vectors ingest and develop low-density mf readily as against *Anopheles* species because they exhibit limitation or proportionality. Facilitation has been advocated as being responsible for the possible elimination of anopheline-transmitted filariasis but Southgate and Bryan (1992) observed facilitation in *An. gambiae* s.s and *An. arabiensis* and not in *An. melas* in Gambia or *An. merus* in Tanzania.

2.1.7 Treatment of the disease

Advances in treating lymphatic filariasis have been achieved, but most of these have focussed not on the individual but rather on the community with infection. Thus, the goal has been to reduce microfilaraemia in a community to levels below which successful transmission of infection will not occur. Few clinical trials, however, have focussed on optimizing treatment of the individual patient, so there is little new data arguing for or against a change from the earlier recommended treatment regimens of DEC (6 mg/ kg per day) for 12 days in bancroftian filariasis and for six days in brugian filariasis. These regimens repeated at 1-6 monthly intervals if necessary, or even the administration of DEC (6-8 mg/kg per day) for 2 days each month for over a period of about 5–6 years is appropriate for treating lymphatic filariasis (Shenoy *et al.*, 1998; Ramaiah *et al.*, 2003).

Although very effective in decreasing microfilaraemia, ivermectin appears not to kill adult worms (i.e. not macrofilaricidal) and so does not completely cure infection (Dreyer *et al.*, 1995; Plaisier *et al.*, 1999). Albendazole on the other hand can be macrofilaricidal for *W. bancrofti* if given daily for 2-3 weeks, but optimisation of its usage has not been attempted (Simonsen *et al.*, 2004). Thus, for treating infection in individual patients single or repeated courses of DEC are still recommended. Since the use of DEC in patients with either onchocerciasis or loasis can be unsafe, it is however important that patients with bancroftian filariasis who live in areas endemic for these other infections be examined for co-infection with these parasites before being treated with DEC.

2.1.8 Management of the disease

While it is important to cure the infection itself, management of the infection (particularly the lymphoedema, elephantiasis and hydrocoele) is what is often of greatest concern to the patient. It has now been shown repeatedly that treatment of hydrocoele in communities with either intermittent (monthly, 6-monthly, yearly) drug administration or steady use of DEC-fortified table/ cooking salt, leads to clinical improvement with decreases in both hydrocoele size and prevalence (Nanda and Ramaiah, 2003). It is also common to find early lymphoedema regressing completely after treatment of an affected patient with DEC (Nanda and Ramaiah, 2003). Larger hydrocoele that do not regress spontaneously in more chronic states or after treatment must be subjected to surgical procedures to drain the fluid and render the tunica vaginalis incapable of trapping and retaining it again. The sclerosing effects of lymphangiography or, often time alone can lead to the cessation of the lymphatic leakage into the renal pelvis collecting system and the urine.

Management regimens include twice-daily washing of the affected parts with soap and water, raising the affected limb at night, regularly exercising the affected limb to promote lymph flow, keeping the nails clean, wearing shoes, use of antiseptic or antibiotic creams to treat small wounds or abrasions. These same intensive hygiene efforts and antibiotic ointments can also decrease the frequency of recurrent infection episodes in patients with elephantiasis of the penis or scrotum, but principles of management have not yet been developed for successfully reversing the anatomic distortions caused by the infection (WHO, 2000). Non-invasive management of chyluria relies on nutritional support, especially substitution of fat-

rich foods by high protein, high fluid diets supplemented where possible with medium chain triglycerides.

2.2 Biology and Life Cycle of *Wuchereria bancrofti*

The life cycle of *W. bancrofti* is shown in Figure 3. *Wuchereria bancrofti* belongs to the class Nematoda, subclass Secernentea, superfamily Filarioidea and family Onchocercidae (Anderson, 1992). When an infective mosquito takes a blood meal, some or all of the L_3 enter human host through the surface of the labella on to the skin surface. The L_3 enter the human host through the puncture made by the mosquito, as they are unable to penetrate intact skin, and those left on the skin surface in a drop of haemolymph have to enter before it dries out (McGreevy *et al.*, 1974). Only a few L_3 manage to enter the skin after a blood meal (Denham and McGreevy, 1977). The L_3 migrate to the lymphatic system in human and transform into L_4 between 9-14 days after entry. Within 6-12 months they grow into mature adults, which can live in the human host for 4 to 8 years. Female worms measure 80 to 100 mm in length and 0.24 to 0.30 mm in diameter, while the males measure about 40 by 1mm.

Adults reside in lymphatic vessels, mate and the viviparous females produce thousands of sheathed mf (L_1) measuring 244 to 296 μm by 7.5 to 10 μm, into lymph circulation. The mf migrates from lymphatic system to circulatory system and has nocturnal periodicity, except in the South Pacific there is absence of marked periodicity (Lardeux and Cheffort, 2001; Hawking *et al.*, 1966; Denham and McGreevy, 1977). Microfilaria numbers fluctuate in the peripheral blood over 24 hours. Nocturnal, periodic worms peak in the peripheral blood from

2200 to 0200 hours, corresponding with peak mosquito biting. Microfilariae are concentrated in the micro-vessels of deep tissues, mainly the lungs during the day and one of the many theories put forward to account for this interesting behavioural pattern is that oxygen tension plays a role (Edeson *et al.*, 1957; Hawking *et al.*, 1966; Hawking and Gammage, 1968; Denham and McGreevy, 1977; Mossinger, 1991). If a person is given extra pure oxygen during the night, the microfilariae stay in deep tissues other than accumulating in peripheral circulation. Another suggestion has been that mfs peak in the peripheral blood when humans are inactive (Hawking *et al.*, 1966; Denham and McGreevy, 1977).

A mosquito picks mf, during a blood meal and ingested L_1 stage of mf move to the stomach of the mosquito, lose their sheaths 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 flight muscles (Christensen *et al.*, 1984). Within 6-10 days of entering the mosquito, L_1 mf develop into the L_2 "sausage" stage in the thoracic muscles. The L_2 larvae develop between 11-13 days and moult into third-stage infective larvae (L_3) and reach a length of 1.2-1.6 mm. The L_3 larvae migrate through the haemocoel to the mosquito's proboscis and when blood meal is taken from another human, the infective L_3 emerge and enters the skin via the bite wound to continue the cycle. Unlike malaria parasites that are injected with saliva into the host, infective stage filarial worms actively break out of the proboscis within a drop of haemolymph and must find and enter the puncture wound made by the mosquito, hair follicles, or other abrasions. The transmission of filarial worms is highly inefficient and filarial worm development in the mosquito is not a benign process and may even disable or kill their mosquito host.

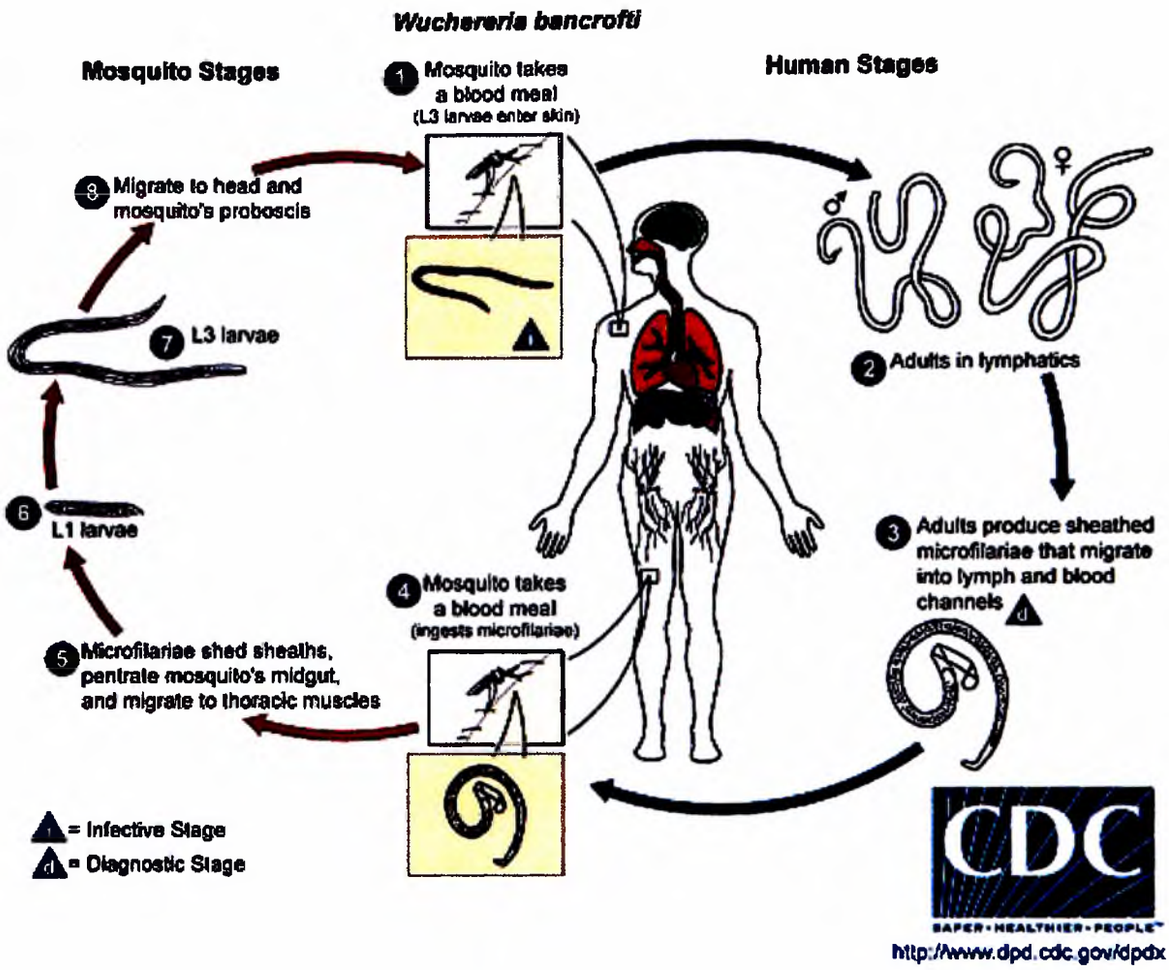


Figure 3: Schematic diagram of the life cycle of *Wuchereria bancrofti*

2.3 Biology and Life Cycle of the Vectors

Depending on the geographical location, different species of the following genera of mosquitoes are vectors of bancroftian filariasis. Among them are: *Anopheles* (*An. arabiensis*, *An. bancroftii*, *An. farauti*, *An. funestus*, *An. gambiae*, *An. koliensis*, *An. melas*, *An. merus*, *An. punctulatus* and *An. wellcomei*); *Culex* (*Cx. annulirostris*, *Cx. bitaeniorhynchus*, *Cx. quinquefasciatus*, and *Cx. pipiens*); *Aedes* (*A. aegypti*, *A. aquasalis*, *A. bellator*, *A. cooki*, *A. darlingi*, *A. kochi*, *A. polynesiensis*, *A. pseudoscutellaris*, *A. rotumae*, *A. scapularis*, and *A. vigilax*); *Mansonia* (*M. pseudotitillans*, *M. uniformis*); *Coquillettidia* (*C. juxtamansonia*) These different species, however, have a similar life cycle that is completed in four distinct developmental stages (complete metamorphosis): egg, larva, pupa and adult.

2.3.1 Biology of the vectors

The female mosquitoes lay about 50 to 500 eggs at a time that are deposited on water or site that is liable to flood. The eggs of most mosquitoes are elongate, ovoid, or spindle-shaped with a few being spherical or rhomboid. Each egg is protected by an eggshell or chorion, which in many species is highly sculptured. The chorion of *Anopheles* species have unique, transparent, air-filled compartments flanking the egg that serve as floats. Whereas the eggs of certain mosquito species are laid individually (e.g. *Anopheles, Aedes, Ochlerotatus, Psorophora, Toxorhynchites, Wyeomyia* and *Haemagogus* species), others attach their eggs together in a single clump forming a floating egg raft (*Culex* and *Culiseta*) or a submerged cluster (*Coquillettidia* and *Mansonia*). Viable eggs take 2-3 days after oviposition to hatch into larvae. Adult male and female mosquitoes feed on plant nectar but only the latter needs blood to nourish and mature the eggs. Mating could be once in the life time of a female

mosquito. This is as a result of a sac-like compartment called spermatheaca, which stores sperm during mating and it is stimulated to release sperm at every oviposition.

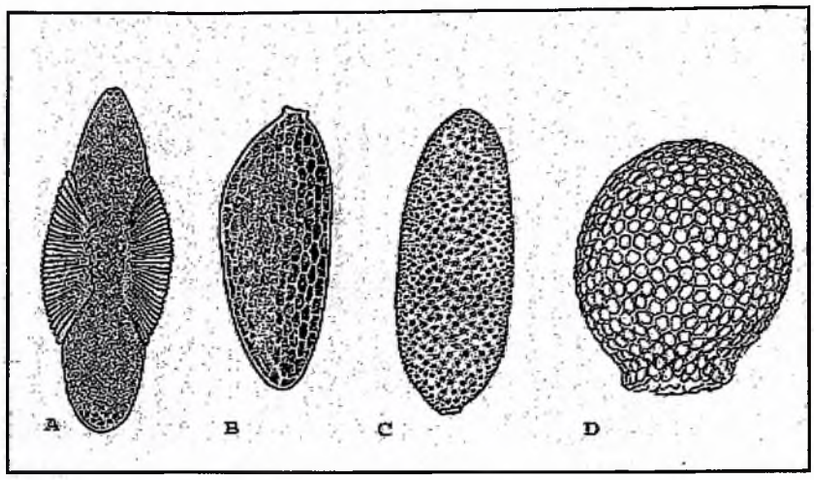


Figure 4: Eggs of mosquitoes: A-*Anopheles*; B-*Culex*; C-*Aedes aegypti*; D-*Toxorhynchites*

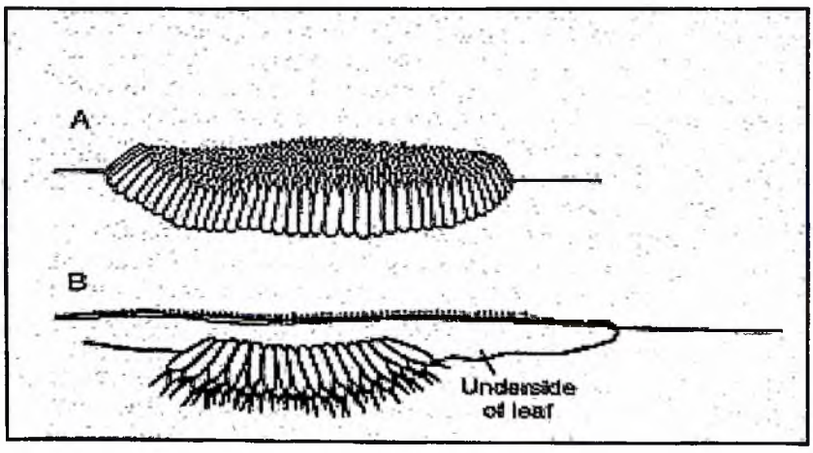


Figure 5: Mosquito egg rafts and clusters. A–*Culex*; B–*Mansonia* attached to underside of a floating leaf

Mosquito larvae, commonly known as wrigglers pass through four instars which closely resemble one another except for their size. When it hatches from the egg, the young mosquito larva is fully adapted for living in water. It uses atmospheric oxygen for respiration and water-borne particles as food. The air-breathing habit requires mosquito larvae either to live more or less permanently at the air/ water interface, or to make frequent visits to the water surface. The pair of spiracles at the end of the abdomen or at the end of a tube called siphon, is the only respiratory openings from which air-filled trachea extends to all parts of the body. The position of the spiracles determines the larval position in relation to the water surface. Larvae of two genera are able to force saw-like tips of their respiratory siphons into the air-filled stems or roots of certain aquatic plants, and remain permanently submerged. Within 6-9 days the larvae moult and reach pupa stage.

The pupae also commonly known as tumblers are comma-shaped with the head and thorax fused to form a cephalothorax. The abdomen is curled beneath with two broad paddles at the end, which propel the pupa through the water when it is disturbed. An air bubble, which is enclosed between the appendages, provides buoyancy that allows the pupa to float with the top of its thorax in contact with the water surface. Located at the top of the cephalothorax are air trumpets, which are used to obtain oxygen. Certain larval organs are destroyed during the pupal stage while others are carried over to the adult stage. When the adult is fully formed the pupa starts to swallow air and the consequent increase in internal pressure forces a split along the midline of the pupal thoracic cuticle, with the adult slowly expanding out of the pupal cuticle and steps on to the water surface. The pupa which does not feed reaches adult stage within 2-3 days.

Adult mosquitoes are slender, have thin legs and narrow, elongate wings. The body surface is covered with scales, setae, and fine pile, creating the characteristic markings and colors of each species. Like other Diptera, mosquitoes are fluid feeders with mouthparts, which have evolved into an elongate composite proboscis, half as long as the body, suitable for probing for nectars and for piercing skin, and imbibing blood from peripheral blood vessels. The saliva that is injected as the mouthparts penetrate the skin contains a substance that prevents blood coagulation. The saliva is also the source of pathogens; such is mf of *W. bancrofti* and immunogens that are responsible for the characteristic skin reactions to mosquito bites. Within a few minutes, engorging mosquitoes can imbibe blood up to four times their own weight. This inflicts upon the mosquito a water load, and potentially toxic amounts of sodium and potassium. The mosquito excretory system is capable of rapid elimination of water and salts, and diuresis commences while the female is still feeding. An adult mosquito can live for about 3 weeks.

The resting position of adult *Anopheles* is usually at an angle of 45° to the surface with the proboscis and abdomen in a straight line. Some species such as *An. culicifacies* rests at angles much smaller than right angles, while others are at almost that angle to the surface (Kettle, 1992). A distinctive morphological feature of *Anopheles* mosquitoes are the dark and pale scales on their wing veins arranged in blocks to form a spotted pattern. The spots vary between species. Female *Anopheles* mosquitoes have non-plumose antennae while males have plumose antennae. Adult anopheline females have palps, which are almost as long as the proboscis and usually lie closely alongside it and may be marked, especially the apical half with broad and narrow rings of pale scales. The palps of the males may also have apical rings of pale scales besides being distinctly swollen at the ends.

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2.3.2 *Anopheles gambiae* complex and *An. funestus* group

Several techniques are employed in identifying the members of *Anopheles gambiae* s.l. with varying degrees of specificity (Collins *et al.,* 2000). These are largely based on universal characters that cut across geographical zones for particular sibling species members and are applicable in field situations. Gillies and De Meillon (1968) as well as Gillies and Coetzee (1987) provide established identification techniques, by using morphological characters, for the anopheline mosquitoes. *An. gambiae* adult females are identified by their smooth palps with 3 pale bands on the 3rd, 4th and 5th segments; the wing field is pale with yellowish or creamy markings and has pale fairly long costal spots. The femora, tibia and 1st tarsal segment speckled to a variable degree. The abdomen is pale brown and hairy with scales on the 8th tergite and scales on the cerci.

Adult female *An. funestus* are identified by three pale bands on the 2nd, 3rd and 5th segments of the palps. The dark wings also bears characteristic pale scales, with the costa having four pale spots usually shorter than the intervening dark areas. The abdomen is dark brown and lack scale including the cerci, while the legs are usually dark with a small apical white spot on the tibia.

Anopheles gambiae s.l. lives in sympatry with *An. funestus* and adapts to changing environments in many parts of Africa (Coluzzi, 1984). During rainy seasons, *An. gambiae* s.l. populations dominate while populations of the *An. funestus* become more abundant in the dry seasons (Coluzzi, 1984). In African urban centres where proportions of anopheline mosquitoes are largely made of *An. gambiae* s.l., *An. funestus* is very rare. The former colonizes well-lit, clean temporary pools of rainwater, shallow slow moving streams, bore

holes and burrow pits, roadside puddles formed by tire tracks, irrigation ditches and other artificial bodies of water (Gillies and De Meillon, 1968; Gimnig *et al.,* 2001).

Anopheles funestus s.l. on the other hand preferentially breeds in semi-permanent water pools and are often associated with rice fields (Gillies and De Meillon, 1968). These two species have narrow host ranges and exhibit preferentially anthropophagic and endophilic characters that make humans their main source of blood meal (Burkot, 1988). The processes involved in host selection are nonetheless well coordinated and the choice of an individual from a pool of those available is influenced mainly by the degree of defensive behaviour exhibited by the selected host (Burkot, 1988). Parasitized and morbid potential hosts exhibit less defensive activity and are therefore more prone to being prime sources of vector blood meal (Burkot, 1988), perhaps an adaptive modification by the parasites for their optimal and uninterrupted uptake during mosquito blood meal.

2.3.3 Life cycle of the vectors

The life cycle of *Anopheles* mosquitoes (Figure 8) takes about 12-14 days. There is a wide range of habitats for breeding, varying from mostly permanent and large collections of water such as fresh water swamps, marshes, rice fields and borrow pits to smaller collections of temporary water such as small pools, puddles, water filled car tracks, ditches, drains, gullies, hoof prints, etc. The most preferred breeding sites are the shallow open sun lit pools (Service, 1993). Wells and manmade container habitats such as clay pots, motor vehicle tyres, water storage jars and tin cans may also be ideal for breeding (Chinery, 1984). *Anopheles gambiae* s.s however, prefers small and undisturbed temporary pools of water exposed to sunshine as such their predominance in irrigated and forested areas (Muirhead-Thomson, 1945).

Heavy mortality among larvae leading to the drastic reduction in number of eggs that develop into adults is a result of predation, disease, drought, flood and desiccation. Notonectidae and other larvivorous fishes such as *Gambusia affinis* and *Lebister reticulatus* which prey on the larvae of *Anopheles* spp, and *Poecilia reticulata* and *Oreochromis mossambicus* on *Culex spp* are being used as biological control tools (Cech and Linden, 1987; Blaustein, 1989; Saliternik, 1977; Mahmoud, 1985). It may be noted that the vagility often displayed by *An. gambiae* larvae, in contrast to species like *An. funestus* would tend to increase their vulnerability to attack by predators (Service, 1980). In some instances, *Culex tigripes* colonizes the same pools as *An. gambiae*, causing a dramatic reduction in larval density due to competition pressure (Haddow, 1942).

Adult females lay in singles 50-200 small (1mm long and 2-5mm wide) brown or blackish boat shaped eggs with lateral floats scattered on the water surface. Viable eggs hatch 2-3 days after oviposition, but may take 4-7 days or longer to hatch in the temperate regions (Service, 1980). The larva is a filter feeder and forages for microscopic organisms and plants even as it is lying parallel to the water surface. The larvae reach pupa stage after four moults, within 6-9 days depending on the availability of nutrients, optimum temperature (Gimnig *et al.,* 2002) and other competing organisms within the habitat. Under the same conditions the pupa, which does not feed, remains in the water for 2-3 days. The pupal case splits dorsally and the adult emerges. The duration from an egg to the adult stage is thus in a range of 7-14 days and after emerging the adult inflates its wings, separates and grooms its head appendages before flying away (Kettle, 1992).

Gimnig *et al.* (2002) reported that in most cases prolonged larval developmental stages might result in emergence of small females due to high population densities of developing *An. gambiae* s.l larva. The males in any egg batch emerge first as adults and become ready for mating within 24 hours before the females emerge. The females on the other hand, require vertebrate blood meal for ovarian development, followed by the maturation and oviposition of a batch of eggs (Gillies, 1955). The feeding of the females on a vertebrate is however stimulated by a combination of carbon dioxide, temperature, moisture, smell, colour and host movement (Service, 1980). Adult males feed on plant nectar and do not bite, however most of the male mosquitoes usually die after a single mating.

Anopheles are mostly nocturnal in their activities, thus emergence from the pupae, mating, blood feeding and oviposition normally occur in the evenings, at night or early in the morning around sunrise. Some species may bite man mainly outdoors (exophagic) from about sunset to 2100 hours whereas others bite mainly after 2100 hours and mostly indoors (endophagic). Whereas some species rest outside (exophilic) in a variety of natural shelters like vegetation, rodent burrows, cracks and crevices in trees, under bridges, termite mounds, and other cracks in the ground in between feeding, others rest in houses (endophilic). Mixtures of these extremes of behaviour are exhibited by most *Anopheles* species that are exophagic and endophagic, exophilic and endophilic. Few of this species exclusively feed on humans though they are predominantly zoophilic. Most feed on both human and animals but the degree of anthropophilism and zoophilism varies according to species and host availability.

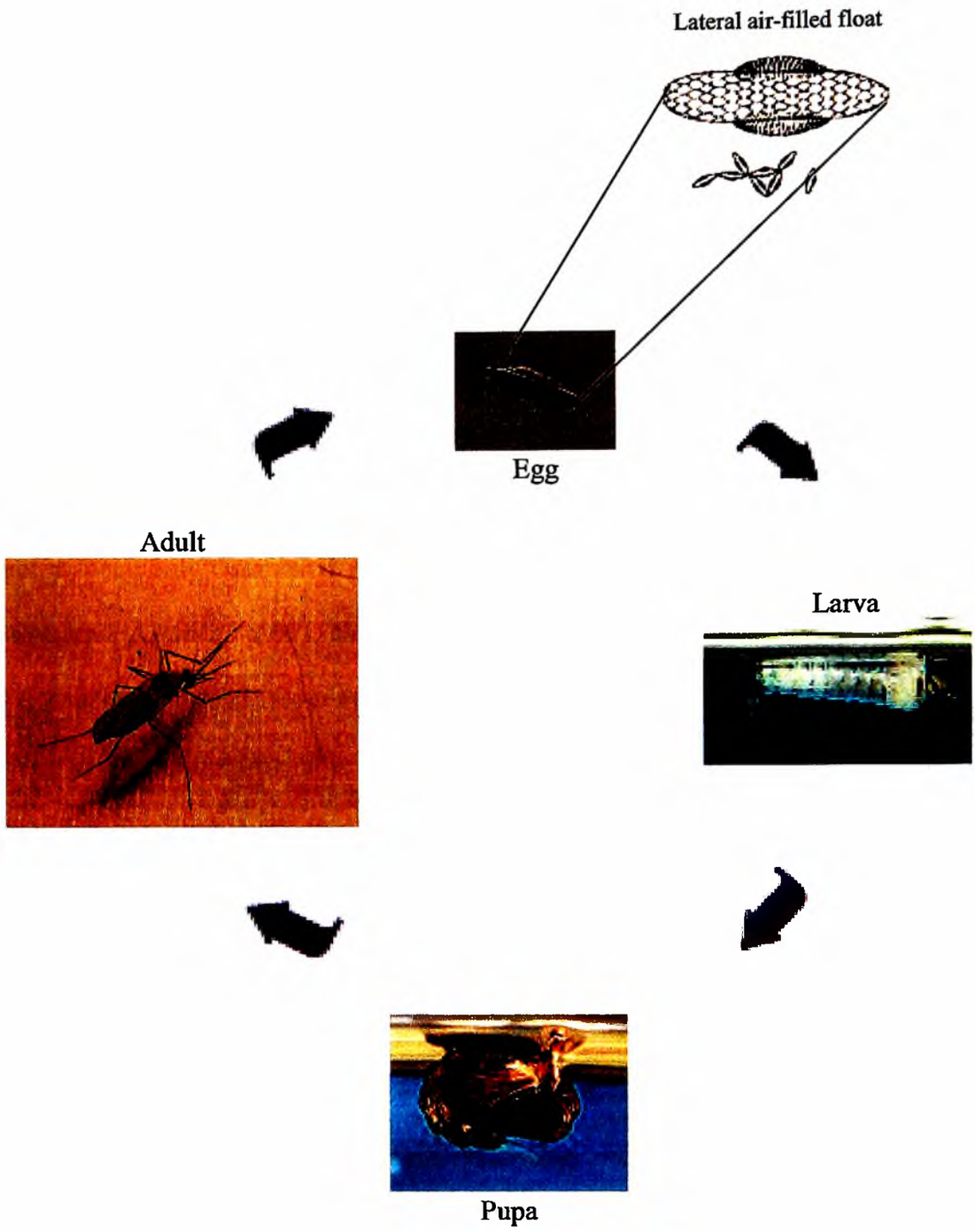

Figure 6: A typical life cycle of *Anopheles* mosquito

2.4 Molecular Characterisation of *Anopheles* Population

The theory and practise of classifying organisms, called Taxonomy dates back over two centuries and draws from extensive accumulated information on morphology, biogeography, and habitat distributions of a large proportion of extant species. Species are identified on the basis of one or several consistently distinguishing morphological characters. As knowledge of species however became more detailed, more examples appeared where morphologically identical organisms differed greatly in behaviour, physiology and genetics suggesting that these identical organisms might belong to different species. For these reasons morphological species concept came to be replaced by the more stringent biological species concept in which species were only considered valid when they exist as reproductively isolated gene pools (Mayr and Ashlock, 1991). With the biological species concept, morphologically identical organisms, sympatric (overlapping in geographical range) are classified as separate species when they can be shown to be reproductively isolated. Conversely, morphologically distinct organisms can be a single species, especially when allopatric (distributed in different geographic ranges), or when the morphological features constitute intraspecies genetic polymorphisms.

Many closely related vector species are difficult or impossible to identify using morphological characters. In these cases, researchers have turned to the molecular phylogenetic and concordant species concept. Molecular taxonomy is the classification and identification of organisms based on either protein or nucleic acid characters rather than morphological characters, and the vector species composition in a geographical region is often well known and correct identifications are obtained without the need of highly trained taxonomists. Once a correct identification has been made, control programs can interrupt

disease transmission by accessing critical information on adult and larval habitats, host preference, ecology, vector competence, histories of earlier epidemics and ultimately, apply the control strategy that are appropriate for that species. Accurate species identification is critical to medical entomologists during control of disease outbreaks and also in associated epidemiological and ecological studies for monitoring the vectors population. The polymerase chain reaction (PCR) is a molecular technique, which currently widely employed for species identification. The technique can amplify a DNA sequence from a minute amount of template DNA, which can then be analysed by various methods to determine species identity. The technique also permits the investigator to retain most of the specimen that can then be examined for pathogens, parasites, biological control agents, gut contents, age or other epidemiological informative characters.

2.4.1 PCR-RFLP identification of *Anopheles funestus* and *An. gambiae* species complex

There are presently nine recognised members of the *Anopheles funestus* group; these are *An. funestus* s.s., *An. vaneedeni* Gilles and Coetzee, *An. leesoni* Evans, *An. rivulorum* Leeson, *An. parensis* Gilles, *An. fuseivenosus* Leeson, *An. aruni* Subii, *An. brucei* Service and *An. confuses* Evans and Leeson. The PCR-based method for identification of *Anopheles funestus* group is based on sequence differences of a variable 3D domain in the 28S gene (Koekemoer *et al.,* 1999). Primers are designed from conserve regions of the 18S, 5.8S or 28S that contains amplify regions of the internal transcribed spacer (ITS) 1 and 2. This PCR method then uses cocktail primers that are species-specific for the identification of these species. See Appendix II for sequence details of the primers.

There are seven recognised sibling species of the *Anopheles gambiae* complex which are morphologically indistinguishable. *Anopheles gambiae* s.s., *An. arabiensis*, *An. quadriannulatus* A and B, *An. merus*, *An. melas*, *An. bwambae* (White, 1985; Scott *et al.*, 1993). There is a further subdivision of the *Anopheles gambiae* s.s. based on gene arrangements of the 2R chromosomal arm into five chromosomal forms; Mopti, Bamako, Forest, Savanna and Bissau (Coluzzi, *et al.*, 2002; Toure, *et al.*, 1994; Powell *et al.*, 1999). Molecular analysis of these forms has shown the existence of genetic discontinuity in this species. Two molecular forms; M and S have been identified based on diagnostic difference in the intergenic sequence (IGS) and internal transcribed sequence (ITS) regions of rDNA on the X chromosome (della Torre *et al.*, 2002; Favia *et al.*, 1997; Favia *et al.*, 2001). Even though breeding between M and S forms yields fertile progenyin the laboratory, M-S hybrids are rarely observed in nature (della Torre *et al.*, 2002; Favia *et al.*, 2001).

The complexity of *Anopheles gambiae* Giles s.s., a sibling species of the *An. gambiae* complex has been shown by the extent of chromosomal inversion polymorphisms and more recently, by divergence at the molecular level (Masendu *et al.*, 2004). Within the amplified rDNA, there are two nucleotide substitutions; AT for M but GC for S population forms. The molecular M and S forms of *An. gambiae* have been found by Yawson *et al.* (2004) to occur in sympatry in southern Ghana. The S form is predominant throughout its distribution in the coastal savannah except at one location in the strand and mangrove zone where there is rice cultivation. The M form was the only form collected in northern Ghana. For the identification of the M and S molecular forms, the PCR products obtained using the method of Scott *et al.* (1993) is restricted further, using *Hha* I or *Tru* I which cuts the S form at one site but not the M form.

2.5 Detection of *Wuchereria bancrofti* in Mosquito Vectors

The limition in mass screening of human population for microfilaria is that not every body will be willing to be screened. This means that one is likely to miss some positive individuals. Observations of the various stages of the parasite in mosquito vectors are used as a xenomonitory procedure to add up to the parasitological monitoring.

2.5.1 Microscopy

The dissection of mosquitoes for presence of the parasites larval stages and identification of the larvae based on morphology continues to be the most reliable detection method. For this the head, thorax and abdomen of each mosquito are separated from each other and placed into separate drops of saline on a microscope slide. The labium is separated from the other parts of the proboscis and if infective filarial worms are present they emerge from the labium into the saline. The remaining part of the head, thorax, and abdomen are carefully macerated under a dissecting microscope and examined for both immature and infective filarial worms. Careful examination of the slides for the parasite at 100x magnification is carried out using a compound microscope.

Microfilariae in mosquitoes can be morphologically identified by their characteristic smooth silvery outline, rounded at the anterior end and pointed at the posterior and their size, and 250 to 300μm long x 7.5 to 10μm wide (Sasa, 1976). The L_3s of *W. bancrofti* have the characteristic three equal caudal papilla or protuberances, which are bubble-like. The infective larvae which measure 1100 μm in length can be separated from the second stage larvae (L_2) that are 500 - 600μm long and 25 - 30μm wide, and the lack of an anal plug (protrusion), which is present in the 3rd stage larvae.

2.5.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) assay for *W. bancrofti* infection in mosquitoes has been developed and tested in the field (Farid *et al.*, 2001). This detects a repeated sequence of *W. bancrofti* DNA and is very sensitive although expensive and requires highly trained technical staff.

2.6 Factors Affecting the Transmission of *Wuchereria bancrofti*

Transmission of the parasite involves three processes; the uptake of mf from the human host, the development of mf to the infective-stage larvae (L_3) and the transmission of L_3 to human (Subramanian *et al.*, 1997). Transmission intensity depends on a number of factors such as the level of infection in the human population, vectorial capacity, vectorial competence, and climatic factors.

2.6.1 Infection in the human population

It has been demonstrated experimentally that the uptake of mf by mosquitoes depends on the density and distribution of mf in the human host (Bryan and Southgate, 1988). Microfilaria density in human population differs from one endemic community to the other. This may be due to variation in human exposure to mosquitoes, susceptibility in tha human population to infection and their response to treatment. Such heterogeneneities are often overlooked leading to overestimation of the effectiveness of population-based control measures and the probability of elimination (Duerr *et al.*, 2005). Individual mosquitoes on the average pick up more in a human population that has high mf prevalence and mean intensity compared to low prevalence and mean intensity (Southgate and Bryan, 1992). The overall load of mf picked

up by the vectors however depends on vector density and human-vector contact in a given locality.

Some vectors ingest mf at high human microfilaraemia density and not all may develop to the L_3 stage. Others ingest mf at low-density human microfilaraemia much more readily than others and almost all develop to the infective L_3 stage. Microfilariae may also be ingested not at high-density human microfilaraemia as in facilitation and not at very low-density human microfilaraemia as in limitation, which is proportionality.

2.6.2 Vectorial capacity

The capacity of vector concept in the transmission of parasites was introduced by Garret-Jones (1964) based on work done by MacDonald (1952) as an attempt to quantify the capacity of a given mosquito population to transmit malaria under natural conditions, which is also very useful in the transmission of lymphatic filariasis. Vectorial capacity (C) is defined by the formula:

$$C = (ma^2p^n)/ -\ln p$$

Where C is the estimated number of new infections that arise from a single infection per day; 'm' is vector density relative to man, 'a' is human feeding habit of the vector, 'p' is the daily vector survivor and 'n' is the time from infection to infectivity.

2.6.2.1 Vector density relative to man and human feeding habit of vector

The estimated human biting rate (ma) is the product of the vector density relative to man (m) and the human feeding habit of the vector (a), which is expressed in bites per man per night. Vector density relative to man or vector abundance depends largely on the availability of

suitable breeding sites (Gillies, 1988). White (1969) has shown that inhabitants living close to abundant mosquito breeding sites were more infected compared to those far away. This index is the main component of the entomological indices used in the estimation of exposure to filariasis infection [i.e. Annual Biting Rate (ABR) and Annual Transmission Potential (ATP)].

The human feeding habit of the vector is determined by two factors; the frequency at which a female mosquito takes blood meal and the proportion of the meals that is taken on humans. A mosquito species that takes 90% of its blood meal on humans has 81% probability of taking human blood meal twice, i.e. at the time of ingesting mf and time of releasing infective larvae (Sasa, 1976). Other species on the contrary that have 5% rate of feeding on human have 0.25% probability. This means the former species is 324 times more efficient as a vector than the latter.

2.6.2.2 Vector survival and time from infection to infectivity

The time it takes from when a mosquito picks mf in a blood meal until the parasites develop to infective larvae and migrate to the head of the mosquito takes 10 to 12 days depending on temperature and other tropical conditions. This means only adult mosquitoes that live longer than this are able to transmit. The daily vector survivor that is linked to vector longevity is an important condition for a vector.

2.6.3 Vectorial competence

This takes into account the physiological ability of a vector to acquire and maintain the parasite development to the infective stage and transmit it to the definitive host (Reisen,

1989). He therefore agued that vector competence (v) should be included in the above equation to give the one below.

$$C = (ma^2vp^n)/ -\ln p$$

The factors that influence the proportion of mf development to infective larvae include parasite uptake, parasite development, density dependent processes in the vector and parasite induced vector mortality.

2.6.3.1 Parasite uptake

Bryan and Southgate (1976) found that wild caught *A. polynesiensis* feeding on a carrier with low mf density of 6 parasites/ ml took up about 12 times the expected number of mf when compared to the concentration in the venous blood of the carrier. Comparison between mf concentration in mosquito blood meal and human blood reveals higher concentration in the former. This trend has been attributed partly to the excretion of blood especially by *Anopheles* mosquitoes during feeding (Brengues, 1975; Reid, 1982), as well as variation in mf concentration in the vascular system of the host whereby mosquitoes ingest more mf than expected simply because they draw blood from superficial vessels where mf are concentrated.

Barriers in the oesophagus of mosquitoes are also known to affect mf uptake by vectors. The mf during ingestion has to pass the cibarial and bucco-pharyngeal armatures, which are sclerotized teeth protruding into the oesophagus lumen. The armatures, which are well developed in *An. gambiae, An. arabiensis, An. melas*, and *An. funestus* but poorly in *Cx. quinquefasciatus* can inflict lethal damage on the mf (Bryan and Southgate, 1988b).

2.6.3.2 Parasite development to infective larvae (L_3)

When mosquito takes a blood meal, the stomach wall dilates and this initiates the formation of a chitinous structure called peritrophic membrane (Pedersen, 1977). Ingested blood starts to coagulate when it reaches the mid gut at a rate, which varies with mosquito species and mf can get trapped in the clot or by the peritrophic membrane (Deham and McGreevy, 1977). Although the effect of the peritrophic membrane as a mid gut barrier is not well known (Townson and Chaitong, 1991), coagulation has been reported to have a substantial effect on mf migration in *Cx. quinquefasciatus* by retaining 97% of ingested mf in blood clot (Ewert, 1971). Townson and Chaitong (1991) found that addition of anticoagulants such as heparin to the blood increases the migration of *B. pahangi* in refractory mosquitoes to virtually the same level as that found in susceptible strains.

The mf that passes across the stomach wall can be trapped in the haemocoele and be melanized (Townson and Chaitong, 1991). In *Cx. pipiens fatigans* the defence mechanism against *W. bancrofti* is evident after the parasite has entered the thoracic muscle and reached the sausage stage (Omar and Zielke, 1978). Larvae that are not trapped and melanized in the haemocoele develop to L_3, which then escape from the muscles and migrate to the haemocoele of the head and mouthparts (Lindsay and Denham, 1986).

2.6.3.3 Density dependent processes in the vector

Three types of parasite-vector relationships have been described for *W. bancrofti* and its vectors. These are *limitation, facilitation* and *proportionality*. Limitation is when the parasite success rate or yield in a mosquito decreases as the mf intake increases. The converse is facilitation, when the parasite yield increases as the mf intake increases and proportionality

occurs when the parasite yield is a constant ratio, neither increasing nor decreasing as mf intake increases. In all three types of host-parasite relationship, the value of the parasite yield can never exceed unity, as there is no multiplication of filarial larvae in mosquitoes, i.e. one mf can never yield more than one infective larva (Bain, 1971; Bain and Brengues, 1972).

Facilitation has been observed in *An. gambiae* (Southgate and Bryan, 1992). Vectors exhibiting such a vector-parasite relationship appear to produce infection and disease much more effectively than those exhibiting limitation and proportionality. With limitation as in *Cx. quinquefasciatus* (Southgate and Bryan, 1992), the vector ingests mf at low-density human microfilaraemia and almost all develop to the infective L_3 stage. In proportionality, mf may be ingested not at high-density human microfilaraemia as in facilitation and not at very low-density human microfilaraemia as in limitation. The argument is that, in the case of limitation, it would be difficult to totally interrupt transmission even when control programmes reduce mf prevalence and intensity to very low levels. On the other hand, it would be relatively easier to block transmission and eradicate the parasite from the human population in the case of facilitation (Subramanian *et al.*, 1997).

2.6.3.4 Parasite induced vector mortality

Bahr (1912) was the first to find out that large numbers of ingested mfs result in mortality of vectors during parasite maturation. High filarial intensities have been shown to kill a considerable number of mosquitoes in laboratory experiments (Pedersen, 1977; Lindsay and Denham, 1986). Vector mortality begins within a few days after ingestion of the infected blood meal and is associated with the migration of mf from the gut to the thorax (Krishnamoorthy *et al.*, 2004) and when the L_3s start to migrate from the thorax to the

labium. It is also thought that the rate of mortality in a population of infected mosquitoes at this time is dependent on the intrinsic activity of the L_3, which varies from one species to another. A third period occurs after another blood meal, possibly due to the damage or by the emergence of the L_3, loss of haemolymph from the mosquito or increased chance of bacterial infection through the wound caused by the escaping larvae.

This means the use of mass drug administrations (MDA) to reduce the prevalence and intensity of microfilaraemia may increase the mean lifespan of some of the local *Anopheles* species (Pichon 2002). If these same species also act as vectors of malarial parasites, effective drug-based control of *W. bancrofti* may worsen the problem posed by malaria. Therefore, wherever malaria and bancroftian filariasis are co-endemic and caused by parasites transmitted by the same species of mosquito, MDA should be augmented by vector management such use of bednets or house-spraying against adult *Anopheles* mosquitoes (Pichon 2002).

2.6.4 Climatic factors

Changes in climatic factors such as temperature, rainfall and sunshine affect human health in very different ways, often through complex changes in ecological systems. It is the interaction among these factors, in combination with other non-climatic factors that determine the timing of infectious disease outbreaks. The female mosquito becomes infected with *W. bancrofti* if it sucks blood from an infected person, and may then infect the next person it bites. The spread of the disease is thus limited by conditions that favour the vector and the parasite growth.

2.6.4.1 Temperature

Temperature is known to affect mosquito larval development, thereby influencing the density of mosquitoes. The range of temperatures suitable for the development of aquatic stages and maturation of the gonads in adults differ between vector species but the optimal range is 20-30 ^{0}C (Molineaux, 1988). An increasing temperature within this range tends to increase the growth rate of vector populations by shortening the intervals between successive oviposition. Gillies and DeMeillon (1968) found that *An. gambiae* s.l., which has a generation time of two to three weeks at lower temperatures shortened to 10-11 days at high temperatures. The rate of aquatic development at a given temperature varies between species and the high rates attained by certain vectors (e.g. *An. gambiae* and *An. arabiensis*) allow them to breed in temporary pools and to get ahead of vegetation, pollution, competitors and predators.

High temperatures on the other hand can reduce the longevity of adult mosquitoes and thus reduce population growth and density. The optimal temperatures for the vectors ranges between 25 and 28^{0}C (rarely exceeding 30^{0}C) also favour the growth of filarial larvae inside the vector. Although a few, *An. gambiae* and *An. funestus* are found in areas with temperatures that exceed 30^{0}C.

2.6.4.2 Sunshine

Duration of sunshine has a direct influence on green plants, and the effects of changing intensities and temperature on photosynthesis are enormous. Increasing intensity of light accelerates photosynthesis but increasing temperature does not (Zaltsman and Feder, 2005; Rozema *et al.*, 2002). At high light intensities, however, an increase in temperature greatly accelerates photosynthesis. Plants provide nectar that are fed on by mosquitoes, and also

serve as hiding places of mosquitoes especially during the day. During summer, people spend more time outdoors and together with warmth, rain and sunshine favourable for mosquito production, thereby increasing human-vector contact.

2.6.4.3 Rainfall

Rainfall is important for creating breeding sites, thereby influencing vector abundance. Excessive rainfall (floods) may cause destruction of breeding sites and a temporary reduction in numbers of vectors, but never eliminates the vector (Craig *et al.*, 1999). A number of studies carried out in different areas of Africa showed negative or positive relationship between rainfall and the peak breeding periods of mosquitoes (Dzodzomenyo *et al.*, 1999). Subra (1973) recorded in urban areas of Burkina Faso that mosquito densities peak in the rainy season and at the beginning of the dry season.

2.6.4.4 Relative humidity

High relative humidity is favourable for mosquitoes by increasing their life span. Mosquito vectors normally prefer a relative humidity above 60% (Molineaux, 1988). High humidity has been reported to increase longevity for adult anopheline mosquitoes and hence their vectorial capacity (Muir, 1988). The infective larvae in a drop of haemolymph left on the skin surface of man (from an infective mosquito bite) have to enter the body before the drop dries out and therefore high humidity also favours a successful transmission (Lindsay and Denham, 1986).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chemicals, Reagents and Equipments

The sources and manufacturers of reagents, buffers, solutions and equipments used in the study are shown in Appendix I.

3.2 Study Site

The study was part of an onging larger programme (GPELF) aimed at eliminating the disease. For the past 4 years MDA with ivermectin and albendazole are administered to the population at risk. This study was used as a monitoring programme to determine the effect of the combination treatment on ransmission of bancroftian filariasis by *Anopheles* vectors in Ghana. The study was conducted in Gomoa District of Central Region of Ghana (Figure 9). Nine communities were selected based on available data on population, endemicity of lymphatic filariasis and logistic considerations. These areas have been earmarked for treatment and are Gomoa Mampong, Gomoa Kyeren, Gomoa Fawomanyo, Gomoa Amanful and Gomoa Obiri. The rest are Gomoa Hwida, Gomoa Dago, Gomoa Ayesuano and Gomoa Okyereko. After mass screening for microfilareamia, four (Gomoa Hwida, Gomoa Dago, Gomoa Ayesuano and Gomoa Okyereko) had positive cases. The study was however concentrated in Gomoa Okyereko due to vector species distribution; thus only *Culex* and *Aedes* species were collected from the other three communities after the first round of mosquito collection, whereas *Anopheles, Mansonia, Culex* and *Aedes* species were caught in Gomoa Okyereko.

Gomoa Okyereko is located about 50 km west of Accra and 10 km northeast of Winneba on the western extension of the coastal savannah zone of Ghana. It has an average annual rainfall range between 760 and 1000 mm, a mean temperature range of 26 to 30 ^{0}C, and has a natural vegetation of strand and mangrove swamp with patchy growth tolerant of salt water. Its geographical coordinate is between Latitudes 5^{0} 24' to 35'N and Longitudes 0^{0} 25' to 36'W.

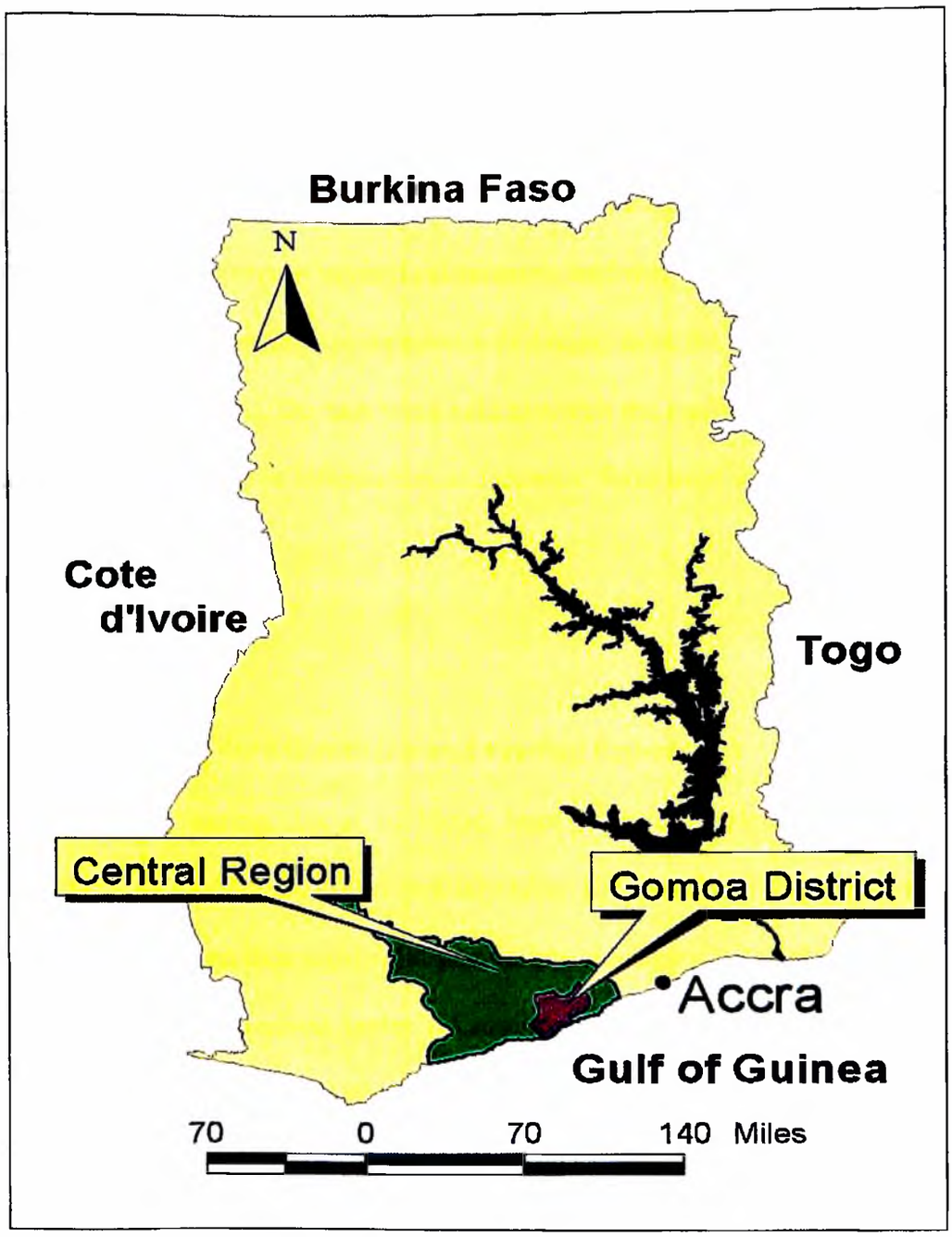

Figure 7: Map of Ghana showing the study site, Gomoa District

3.3 Ethical Considerations

Mass screening of the population for mfs was carried out and those found positive were informed on the importance, purpose and procedures of the study. Individual consent was, however, obtained at the time of subject recruitment. This study was conducted in accordance with good clinical practice with the latest edition of the Helsinki declaration on the use of human subjects in biomedical research, and with local laws and customs (CIOMS, 2002). Prior to the screening, permission was sought from the divisional chiefs and opinion leaders of the study area. Durbars were held at which the study was thoroughly explained to the people. A copy of the information and consent form used in the study can be found in Appendix IV.

3.4 Mass Screening for Microfilaria and Feeding Experiment

For the mass screening, 100µl of blood from a finger prick was collected from each individual using heparinized 100µl capillary tube. The blood was then transferred into 900µl 3% acetic acid, mixed thoroughly and poured into a Sedgwick-Rafter counting chamber and the number of mfs observed under microscope countered (McMahon *et al.*, 1979). The geometric mean intensity (GMI) of microfilaraemia in the community was calculated as Antilog [Σlog (x+1)/n], where x is number of mf/ ml blood in the mf individuals, and n is number of people examined.

From these, 12 consented volunteers with different levels of microfilaraemia were then made to sleep under a mosquito net with one side partly open (Plate 1) for mosquito entry. Mosquitoes that fed on each subject during the night were collected hourly from 2100 hours

to 0600 hours the next day (Subramanian *et al.*, 1998). At the end of each hour, the opened part of the net was let down briefly as spotlight was used to locate the host-seeking mosquito and any mosquitoes trapped were collected using an aspirator. The trapped mosquitoes were released into paper cups covered with mosquito netting and labelled to reflect the hour, subject and site of collection. The mosquitoes were fed on 10% sucrose solution soaked in cotton wool that was placed on the netting cover. This was repeated the following night for more mosquitoes but no blood sample was taken this time.

At the mid-point of each collection hour, a finger prick sample of blood was collected from each of the 12 volunteers using 100µl heparinized capillary tube. The blood was mixed with 900µl 3% acetic acid and number of mf determined by microscopy. The blood count of mf at each hour was paired with the results of the corresponding mosquito dissection.

Plate 1: Finger prick blood being taken from a volunteer sleeping under a mosquito net with one side partly open for mosquito entry.

3.5 Laboratory Studies

Mosquito samples were transported to the laboratory of the Parasitology Department of the Noguchi Memorial Institute for Medical Research for identification, maintenance and dissection.

3.5.1 Morphological identification, maintenance and dissection of mosquitoes

About half of the mosquitoes collected each hour from each subject were killed immediately after feeding by putting them into the fridge and stored for less than 18 hours at 4^0C until ready to use. They were then sorted out according to species, kept in labelled petri dishes on damp filter paper at -20^0 C until ready for dissection. They were dissected and the number of mf in the blood meal of each mosquito counted.

The remaining 50% of mosquitoes were maintained in cups and kept in boxes and transported under cool condition to the insectary at Noguchi (Plate 2). They were maintained at approximately 26 - 28^0C and 70 - 80% relative humidity and were fed on 20% sucrose solution until day 12 post collection when they were killed, dissected and examined for L_3 (Janousek and Lowrie, 1989). Any mosquito that died before day 12 was also dissected and any larval stages of *W. bancrofti* if harboured recorded.

Each specimen was placed in a drop of normal saline (0.9 NaCl solution) on a labelled slide, under a dissecting microscope (OLYMPUS SZ 11, Japan) at 20X magnification. The legs and wings were removed and the head, thorax and abdomen were then separated and each part transferred into separate drops of saline on the same slide. The labium was separated from other parts of the proboscis. If L_3s were present they emerged from the labium into the

saline. The remaining part of the head, thorax, and abdomen were teased apart under the high power of the dissecting microscope and examined for mfs. Careful examination of the slides at 100X magnification was done under a compound microscope (OLYMPUS B50, Japan) to ensure no parasite was missed. The mosquitoes positive for filarial infection were recorded and positive slides as well as negatives were placed in racks and kept at – 4^0C until ready for molecular identification of the mosquitoes and *W. bancrofti*.

Plate 2: Boxes with netted paper-cups containing the collected mosquitoes at the insectary.

Each cup has 20% of sucrose-soaked cotton wool placed on it to feed them.

3.5.2 Molecular Studies

The molecular identifications of *Anopheles gambiae*, *An. funestus* and *Wuchereria bancrofti* were done as described below.

3.5.2.1 Chemicals, reagents and solutions

Preparations of standard solutions for the molecular studies are described in Appendix I.

3.5.2.2 PCR-RFLP species identification of molecular forms of the *Anopheles gambiae* Giles complex

The genomic DNA extraction and DNA amplification for identification were carried out as described below.

3.5.2.2.1 Genomic DNA extraction

The carcass of dissected *An. gambiae* s.l. mosquito was placed in a 1.5 ml eppendorf tube and homogenized with sterile Konte's plastic pestles in 100µl bender buffer. The homogenate was then incubated at 65^0C for 30 minutes followed by the addition of 125µl of phenol. It was vortexed and span at 14,000rpm for 10 minutes. The supernatant was transferred into a fresh tube and 250µl of pre-chilled absolute ethanol and 10µl of 8M potassium acetate were added. It was then incubated at -40^0C for one hour, span at 10,000rpm for 10 minutes and supernatant poured off. The pellet was then rinsed with 200µl of 70% ethanol, span at 10,000rpm for 5 minutes and supernatant poured off. The pellet was dried and redissolved in 50µl TE + RNAse and then kept at 4% until required for PCR. One micro-litre was used for PCR.

3.5.2.2.2 PCR amplification

Five sets of oligonucleotide primers designed from the DNA sequences of the intergenic spacer region of *An. gambiae* complex ribosomal DNA (rDNA) were used in PCR for the identifications (Scott *et al.*, 1993). The sequence details of these primers abbreviated UN, GA, ME, AR and QD (expected sizes of the PCR products are given in Appendix 2). The UN primer anneals to the same position on the rDNA sequences of all five species and GA anneals specifically to *An. gambiae* s.s., ME to both *An. merus* and *melas*, AR to *An. arabiensis*, and QD to *An. quadriannulatus*.

The PCR reaction mix of 25µl contained 1X PCR buffer supplied by the manufacturer (Sigma, USA), 200µM of each of the four deoxynucleotide triphosphates (dNTPs), 10µM of each oligonucleotide primers and 0.125 units of *Taq* Polymerase enzyme (Sigma, USA). One micro litre of the genomic DNA was used as template for the amplification reaction and sterile double distilled water added to make up the volume. The reaction mix was centrifuged briefly and overlaid with mineral oil to avoid evaporation and refluxing during thermo cycling. The amplification was carried out using a PTC 100 thermal cycler (MJ Research Inc., USA). The cycling parameters for the reactions were as follows: 93° C for 3min (initial denaturation), followed by 35 cycles of 93^{0}C for 30 sec, 50^{0}C for 30 sec, 72^{0}C for 1min (annealing) and ended with a cycle of 93^0 C for 30 sec, 50° C for 30 sec and 72^{0}C for 10 min. For each reaction a positive control containing PCR product of *Anopheles* of the same primer set, as a template and a negative control that contained no DNA template were included.

3.5.2.2.3 Molecular forms of *Anopheles gambiae* s.s. identification

Members of the *Anopheles gambiae* complex were identified using PCR and *An. gambiae* s.s. specimens were further analyzed by amplification of 1.3kb rDNA followed by Restriction Fragment Length Polymorphism (RFLP) with restriction enzyme *Hha* I to determine the M and S population forms.

The *Hha* I enzyme digestions were carried out using the recommended protocol of the manufacturers (Sigma-Aldrich, USA). The final reaction volume of 20µl contained 10µl of amplified product, 0.3µl of 1U *Hha* I, 2µl of 1x Buffer C (Promega, USA), made up to the final volume by adding sterile double distilled water and then digested at 37^0C for three hours. Electrophoresis was done on the digested fragments through 2% agarose gel stained with ethidium bromide and photographed under UV light illumination as described in Section 3.5.2.5.

3.5.2.3 Identification of members of the *Anopheles funestus* group

The genomic DNA extraction and amplification were done as shown below for *An. funestus* mosquitoes.

3.5.2.3.1 Genomic DNA extraction

The carcass of dissected *An. funestus* s.l. mosquito was placed in a 1.5 ml eppendorf tube and homogenized with sterile Konte's plastic pestles in 100µl bender buffer. The homogenate was then incubated at 65^0C for 30 minutes followed by the addition of 125µl of phenol. It was vortexed and span at 14,000rpm for 10 minutes. The supernatant was transferred into fresh tube and 250µl of pre-chilled absolute ethanol and 10µl of 8M potassium acetate were

added. It was then incubated at -40^{0}C for one hour, span at 10,000rpm for 10 minutes and supernatant poured off. The pellet was then rinsed with 200µl of 70% ethanol, span at 10,000rpm for 5 minutes and supernatant poured off. The pellet was dried and redissolved in 50µl TE + RNAse and then kept at 4% until required for PCR. One micro-litre was used for PCR.

3.5.2.3.2 PCR amplification

The PCR mix contained the following; 2.5µl of 10x reaction buffer (500mM KCl, 100mM Tris-HCl pH 8.3), 1.5 mM $MgCl_2$, 1 µM of each primer, 200µM of each dNTP, 2U Taq DNA polymerase, 1µl of the DNA template and overlaid by 25µl mineral oil. Amplification conditions were: 30 cycles of denaturation at 94^{0}C for 30 seconds, annealing at 40^{0}C for 30 seconds, extension at 72^{0}C for 30 seconds. There was a final extension at 72^{0}C for 10 minutes. Electrophoresis was done on the digested fragments using 2% agarose gel stained with ethidium bromide and photographed under UV light illumination as described in Section 3.5.2.5.

3.5.2.4 Species identification of *Wuchereria bancrofti*

Molecular characterisation of *W. bancrofti* was also carried out with dissected mosquitoes, which had the larvae of the parasite in them.

3.5.2.4.1 Genomic DNA extraction

DNeasy Tissue Kit (QIAGEN Inc., USA) was used in the extraction of the parasite's genomic DNA from animal tissues following the manufacturer's recommended protocol. The

dried *W. bancrofti* larvae on slides were scrapped into 1.5ml eppendorf tube, and were homogenised in 90µl buffer ATL using sterile pestles.

Then 10µl proteinase K was added to each tube, vortexed and incubated at 55^0C in a water bath for 3 hours with intermittent vortexing. The homogenates were vortexed finally for 15 seconds and then 100µl of buffer ATL added. The tubes were then incubated at 70^0C for 10 minutes and 100µl ethanol (96 to 100%) added and vortex to mix thoroughly. Each mixture was transferred into a DNeasy mini column and centrifuged at 8000 rpm for 1 minute after which the collection tubes with contents were discarded. The DNeasy mini column was then placed in a fresh 2 ml collection tube and 500µl buffer AW1 added and centrifuged at 8000 rpm for 1 minute to wash the extracts. The flow trough and collection tubes were again discarded. The samples were washed again by placing the DNeasy mini columns in fresh 2 ml collection tubes and 500µl buffer AW2 added, and centrifuged at 8000 rpm for 3 minutes. The DNeasy mini columns were placed in new 1.5 ml microcentrifuge tubes and 200 ml buffer AE added directly onto the membrane. Samples were incubated at room temperature for 1 minute and then centrifuged for 1 minute at 8000 rpm to elute the DNA. Aliquots of 5µl of the filarial DNA extract from the mosquitoes were used as templates for the amplification reaction.

3.5.2.4.2 PCR identification of *Wuchereria bancrofti*

The PCR assay was performed using two published specific oligonucleotide primers, NV-1 and NV-2 (Ramzy *et al.*, 1997). The contents of the reaction mix and their concentrations are given in Appendix III. The 25µl reaction mix was centrifuged briefly and overlaid with mineral oil to prevent evaporation and refluxing.

The thermal cycling profile was as follows; an initial denaturation at 94^{0}C for 3 minutes, 35 cycles of denaturation at 94^{0}C for 1 minute, annealing at 55^{0}C for 1 minute and extension at 72^{0}C for 2 minutes, and ended with a final cycle of 94^{0}C for 1 minute, annealing at 55^{0}C for 1 minute and extension at 72^{0}C for 10 minutes. For each reaction a positive control containing PCR product of *W. bancrofti* of the same primer set, as a template and a negative control that contained no DNA template were included. Electrophoresis was done on the digested fragments through 2% agarose gel stained with ethidium bromide and photographed under UV light illumination as described in Section 3.5.2.5.

3.5.2.5 Observation and analyses of amplified PCR-RFLP products

The amplified products of *An. gambiae*, *An. funestus* and *W. bancrofti* were electrophoresed separately on 2% agarose gel stained with 0.5µg/ml Ethidium Bromide. Eight microlitres of each sample was added to 1µl of orange G (5X) gel loading dye for the electrophoresis. The gel was prepared and electrophoresed in 1X TAE buffer using a mini gel system (BIORAD USA) at 100 volts for one hour and the gel photographed over a UV trans-illuminator (UPC, USA) at short wavelength using a Polaroid camera and film type 667 (Polaroid, USA). The sizes of the PCR products were estimated by comparison with the mobility of a 100 base pair molecular marker (Sigma p-1473, see Appendix I) and the expected diagnostic sizes of the PCR amplified fragments of the different species are in Appendix II.

3.6 Sampling Method and Data Analysis

This work was part of an ongoing larger longitudinal intervention study; a community based rather than individuals randomised to receive intervention. Therefore cluster sampling method was used for participants and the mosquitoes as well. The data was analysed to determine the vector competency of *Anopheles* in transmitting *W. bancrofti* and to quantify the relationship between the level of microfilaraemia in the blood meal source and the mf uptake by the mosquitoes. One-way analysis of variance (ANOVA) was used to test for the significance of the age- and gender-specific variations found in the initial screening of the total number of people screened. The geometric mean intensity of microfilaria in the population was used to estimate the intensity of the parasite in the study communities.

CHAPTER FOUR

RESULTS

4.1 Microfilaraemia Load in the Study Area

Four out of the nine communities (Gomoa Okyereko, Gomoa Hwida, Gomoa Dago and Gomoa Ayesuano) recorded positive cases. Among these communities Gomoa Okyereko recorded the highest number of cases with a geometric mean intensity of 1.2 mf/ ml, whilst Gomoa Dago recorded the lowest of 1.0 mf/ ml. Among these positive cases, Gomoa Okyereko recorded 155.6 mf/ ml whilst Gomoa Dago recorded 15.3 mf/ ml.

The overall prevalence of microfilaraemia in the study community ($N = 1083$) was 1.6%. The prevalence among males and females (2.05% and 1.10% respectively) was not significant ($P = 0.39$). The youngest individual found microfilaraemic was a girl of 10 years. Prevalence was observed to generally increase with age (Table 2). The levels of microfilaraemia in the 1083 individuals varied from 0 to 59 mf/ 100μl blood, with a geometric mean intensity of 1.1 mf/ ml. Although some variation in intensity with age-group was observed, no significant differences were observed between age groups ($P = 0.40$) nor the intensities between sexes ($P = 0.91$).

Table 1: The numbers of individuals examined and number of cases in the nine study sites

	Number of individuals examined			Number of mf positive individuals (%)		
Age Group (years)	Females	Males	Total	Females	Males	Total
1 – 14	242	252	494	2(0.83)	3(1.19)	5(1.01)
15 – 24	114	137	251	0	5(3.65)	5(1.99)
25 – 34	57	44	101	0	0	0
35 – 44	42	30	72	1(2.38)	0	1(1.39)
45+	92	73	165	3(3.26)	3(4.11)	6(3.64)
Total	**547**	**536**	**1083**	**6(1.10)**	**11(2.05)**	**17(1.57)**

Table 2: The geometric mean intensities of *Wuchereria bancrofti* infections in the nine study sites**

Age Group (years)	Microfilariae mean intensity*			
	Females	Males	Total	mf positives
1 – 14	1.05	1.05	1.05	127.85
15 – 24	0	1.18	1.05	86.40
25 – 34	0	0	0	0
35 – 44	1.10	0	1.06	51
45+	1.10	1.23	1.16	53.66
Total	**1.05**	**1.10**	**1.07**	**79.45**

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

**See Appendix 4 for individual tables of the various study communities

4.2 Collection and Dissection of Mosquitoes

The 564 mosquitoes collected were mostly *Anopheles* species (62.1%) followed by *Mansonia* species (32.3%), a few *Aedes* species (5%) and *Culex* species (0.7%) (Figure 8). The *Anopheles* caught comprised of 88.6% *An. funestus*, 9.1% *An. gambiae* and 2.3% *An. pharoensis* (Figure 8). The 12 volunteers who participated in the feeding experiments carried between 1 and 59 mf/ 100μl blood, with a geometric mean intensity of 83.3 mf/ ml blood. It was observed that the peak period of the parasites in peripheral blood coincided with the peak biting rates of the *Anopheles* mosquitoes around 00:00 and 03:00 hours GMT (Figure 9). In all, 32% of *Anopheles*, 50% *Culex*, 7% *Aedes* and 41.8% *Mansonia* were engorged with blood (Tables 4 and 5). Six mosquitoes each of *Anopheles* and *Mansonia* dissected immediately were found infected.

There were a total of 47 L_1 out of which 35 (74.5%) and 12 (25.5%) were found in *Anopheles* and *Mansonia* respectively. All the infected mosquitoes were among those dissected within five days after feeding, and as such no L_3 was found. Among the *Anopheles* infected four (1.3%) were *An. funestus* and two (6.3%) were *An. gambiae*, while no infection was found in *An. pharoensis* with 31.6%, 37.5% and 25% engorged with blood respectively. Entomological parameters (Table 6) such as infection rates were found to be 0.02 and 0.03 for *Anopheles* and *Mansonia* respectively. Biting rates were 70, 0.8, 5.6 and 36.4 for *Anopheles*, *Culex*, *Aedes* and *Mansonia* respectively. Survival rates were 0.48, 5.00 and 0.13 for *Anopheles*, *Aedes* and *Mansonia* respectively. Among the *Anopheles*, infection rates were 0.06 and 0.01 for *An. gambiae* and *An. funestus* respectively (Table 7). Biting rates were 6.4, 62 and 1.6 while survival rates were 0.5, 0.47 and 1 respectively for *An. gambiae, An. funestus* and *An. pharoensis.*

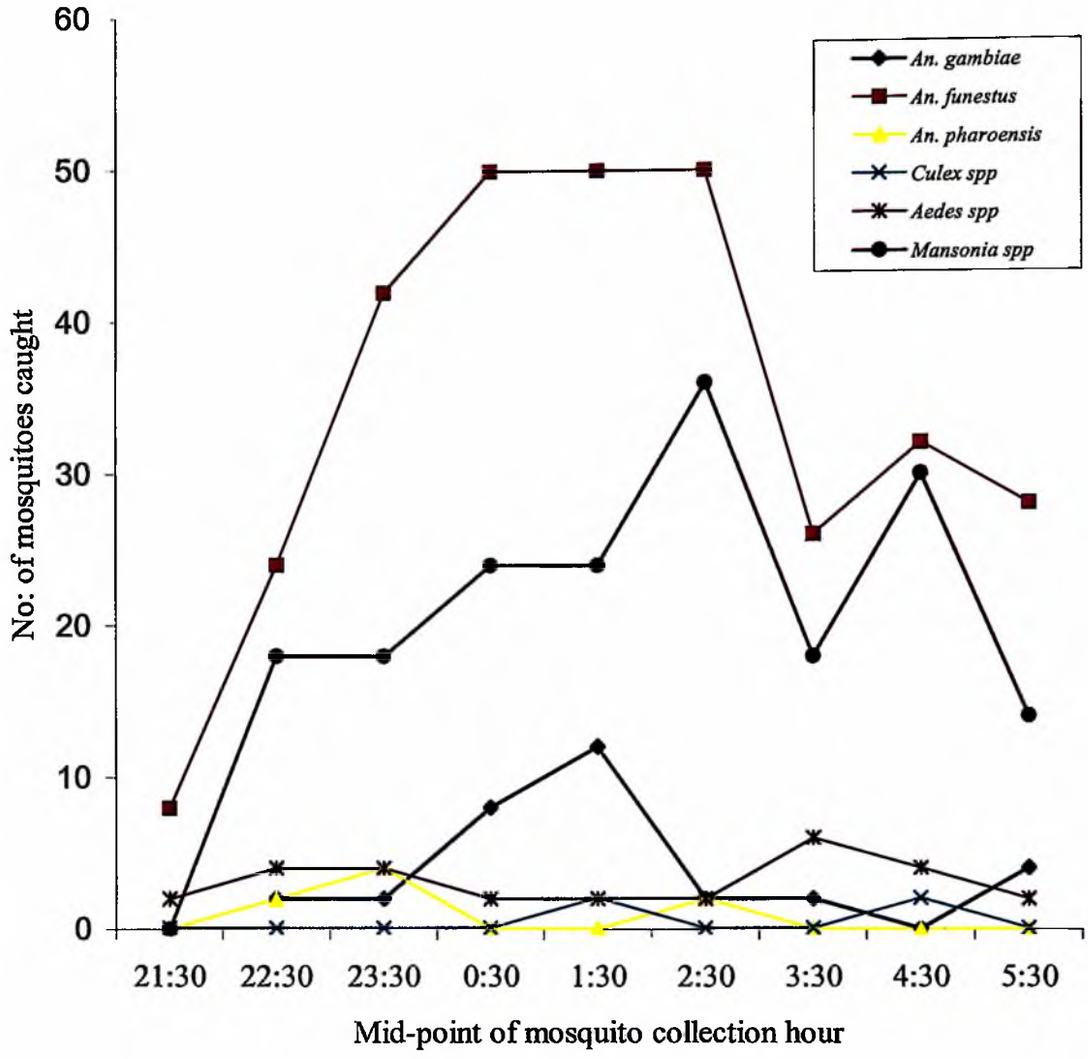

Figure 8: Generic composition of of the mosquitoes caught during the feeding experiment

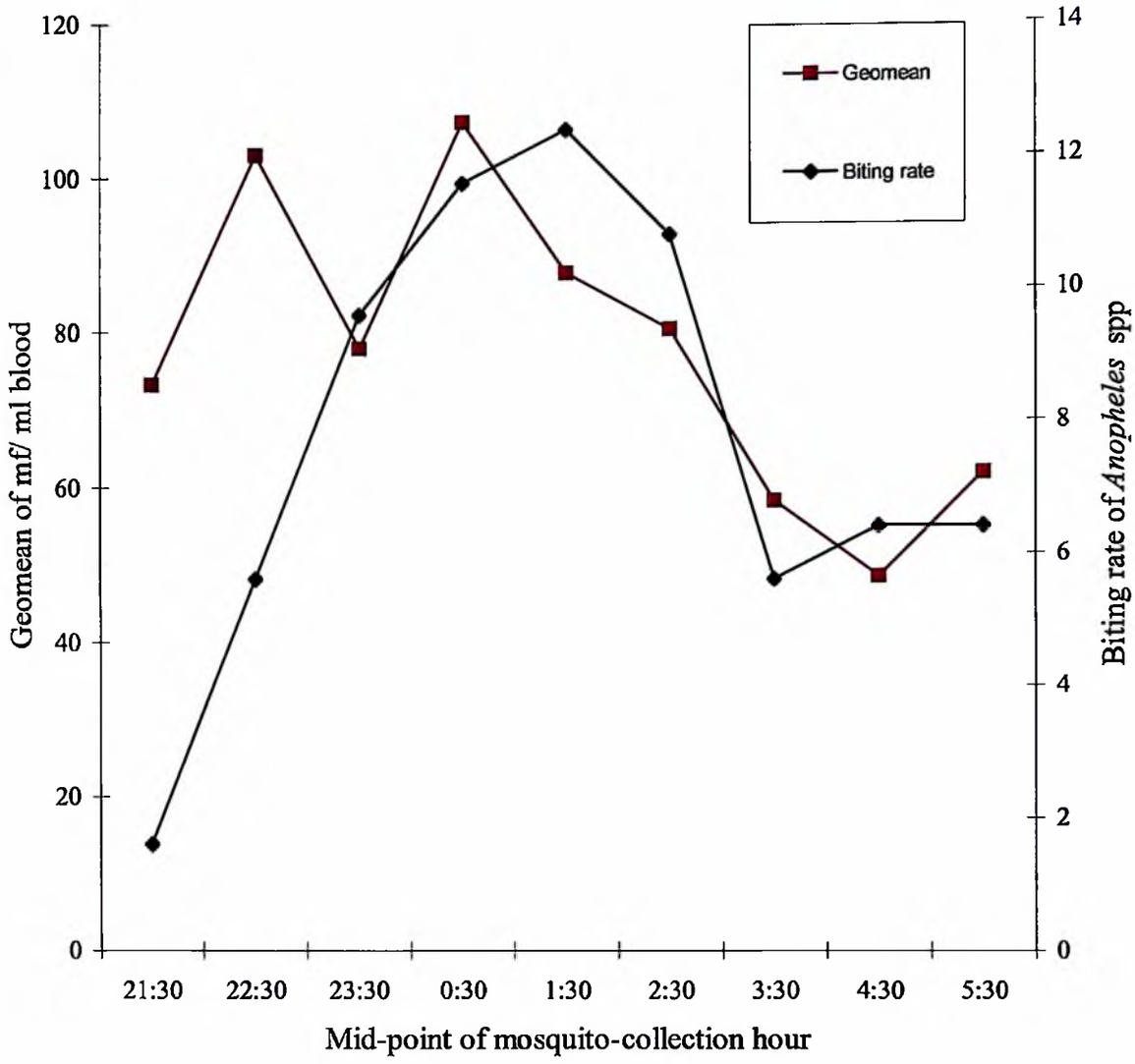

Figure 9: Variation of *Anopheles* species and the geometric mean intensity (geomean) of microfilaraemia among the twelve consented volunteers observed during the collection of mosquitoes

Table 4: Distribution of infected mosquitoes and number of mf ingested by mosquitoes

Mosquito spp	Number of mosquito			Number of mosquito examined immediately after feeding		Mosquito examined from the 1st to 10th day after feeding	Mosquito with L_3 between 11th and 13th day after feeding
	Caught	Dissected	Engorged	Infected mosquito/ dissected	Mf/ mosquito dissected	Number of infected mosquito dissected	L_3/ dissected mosquito
Anopheles spp	350	350	112	6	35	6	0
Culex sp	4	4	2	0	0	0	0
Aedes sp	28	28	2	0	0	0	0
Mansonia sp.	182	182	76	6	12	6	0
Total	**564**	**564**	**192**	**12**	**47**	**12**	**0**

Table 5: Distribution of infected and infective *Anopheles* mosquitoes, and number of mf ingested by female *Anopheles* mosquitoes caught during the study

Mosquito spp	Number of mosquito			Number of mosquito examined immediately after feeding		Mosquito examined from the 1st to 10th day after feeding	Mosquito with L_3 between 11th and 13th day after feeding
	Caught	Dissected	Engorged	Infected mosquito/ dissected	Mf/ mosquito dissected	Number of infected mosquito dissected	L_3/ dissected mosquito
An. ga.[a]	32	32	12	2	2	2	0
An. fu.[b]	310	310	98	4	33	4	0
An. ph.[c]	8	8	2	0	0	0	0
Total	**350**	**350**	**112**	**6**	**35**	**6**	**0**

[a]*Anopheles gambiae*, [b]*Anopheles funestus*, [c]*Anopheles pharoensis*

Table 6: Distribution of infection with *Wuchereria bancrofti* in the various mosquito species caught after 13 days of maintenance

Mosquito species	Indices (%)					
	Infection rates[a]	Infectivity rate[b]	Intensity of infection[c]	Biting rate[d]	Survival rate[e]	Vector efficiency VE (%)
Anopheles	0.02	0	0	70	0.48	0
Culex sp	0	0	0	0.8	0	0
Aedes sp	0	0	0	5.6	5	0
Mansonia sp	0.03	0	0	36.4	0.13	0

[a]Number of mosquitoes infected/ number of mosquitoes dissected; [b]L_3 in the head and proboscis/ number of surviving mosquitoes; [c]Number of mosquitoes with L_3 in the head and proboscis/ number of mosquitoes with L_3; [d]Number of mosquitoes caught/ number of collectors x number of captures; [e]Number of surviving mosquitoes/ number of engorged mosquitoes. VE (%) X number of L3 x 100/ X number of mf ingested among dissected mosquitoes in accordance to Kartman (1953, 1954), Ramachandran (1970), Brito *et al.*, (1997)

Table 7: Distribution of infection with *Wuchereria bancrofti* in the *Anopheles* mosquitoes caught after 13 days of maintenance

Mosquito species	Indices (%)					
	Infection rates[a]	Infectivity rate[b]	Intensity of infection[c]	Biting rate[d]	Survival rate[e]	Vector efficiency VE (%)
Anopheles gambiae	0.06	0	0	6.4	0.5	0
Anopheles funestus	0.01	0	0	62	0.47	0
Anopheles pharoensis	0	0	0	1.6	1	0

[a]Number of mosquitoes infected/ number of mosquitoes dissected; [b]L_3 in the head and proboscis/ number of surviving mosquitoes; [c]Number of mosquitoes with L_3 in the head and proboscis/ number of mosquitoes with L_3; [d]Number of mosquitoes caught/ number of collectors x number of captures; [e]Number of surviving mosquitoes/ number of engorged mosquitoes. VE (%) X number of L3 x 100/ X number of mf ingested among dissected mosquitoes in accordance to Kartman (1953, 1954), Ramachandran (1970), Brito *et al.*, (1997)

4.3 Molecular Identification of *Anopheles funestus* and *An. gambiae* Species Complexes

All the anophelines captured in the study were identified at the molecular level with the exception of *Anopheles pharoensis*. Out of the 32 *An. gambiae* s.l. collected, 30 (93.8%) were identified by PCR to be *An. gambiae* s.s. (Figure 10) and of the 310 *An. funestus* s.l. identified, 286 were successfully identified by PCR for which 267 (86%) were *An. funestus* s.s. and 19 (6%) were *An. leesoni* (Figure 11). Twenty-four (7.7%) were not successfully amplified by PCR. PCR-RFLP also identified 21 (70%) M and 9 (30%) S forms of the *An. gambiae* s.s. (Figure 12). All infected *Anopheles gambiae* were M forms.

4.4 PCR Identification of *Wuchereria bancrofti*

Thirty-five parasites were identified in 6 *Anopheles gambiae*. All 6 mosquitoes were used for PCR identification of the parasites and 4 were successfully identified to harbour *Wuchereria bancrofti* (Figure 13). Ten positive blood samples of volunteers were also used for PCR identification of parasites. All were successfully identified as *W. bancrofti*.

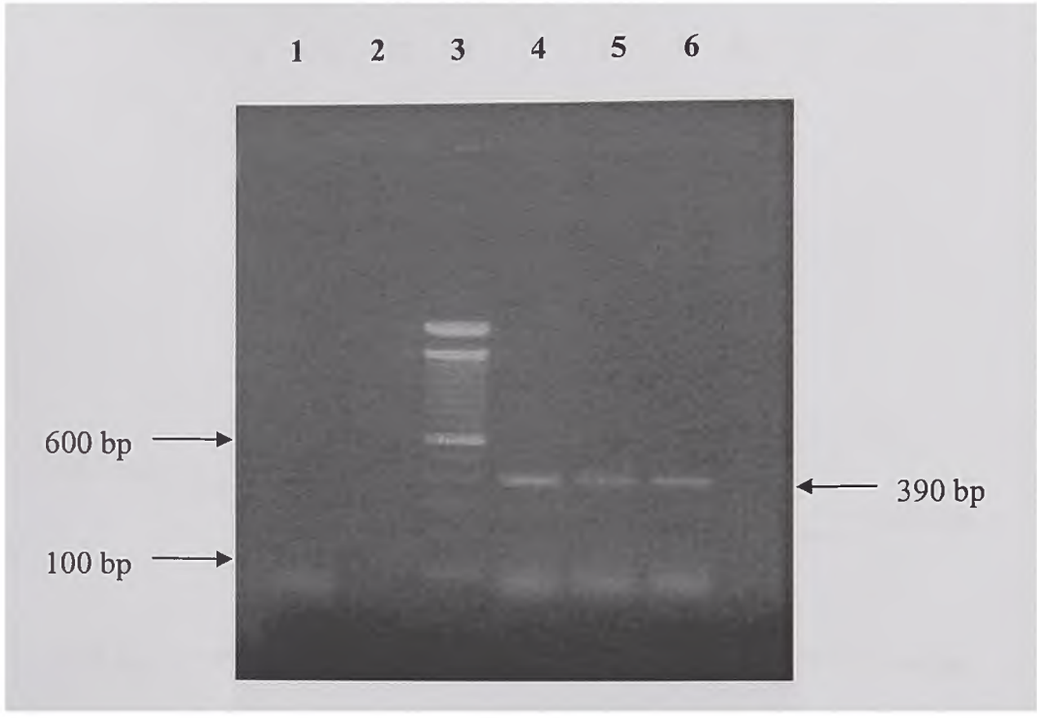

Figure 10: Photograph of an example of ethidium bromide-stained agarose gel (2%) electrophoregram of amplified PCR products for the identification of *Anopheles gambiae* s.l. species. Lane 1 is negative control; Lane 3 is molecular weight marker (100-bp ladder); Lane 4 is *An. gambiae* s.s. positive control; Lanes 5 and 6 are *An. gambiae* s.s. Also indicated is the diagnostic band of 390bp for *An. gambiae* s.s.

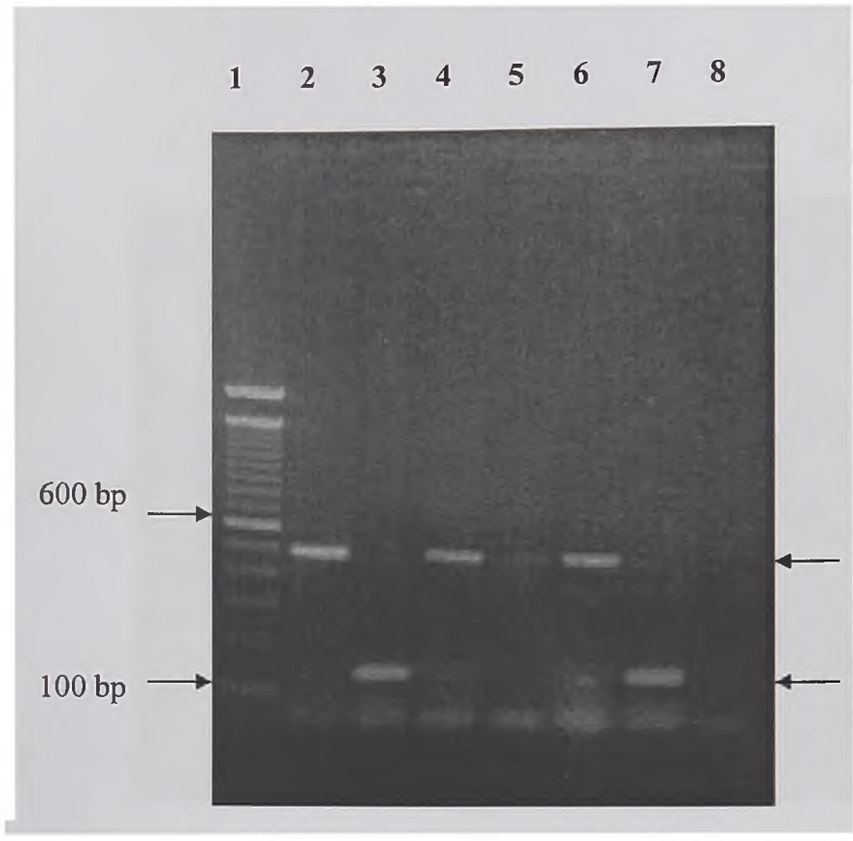

Figure 11: Photograph of an example ethidium bromide-stained agarose gel (2%) electrophoregram of PCR amplified products for the identification of *Anopheles funestus* s.l. species. Lane 1 is molecular base pair marker (100-bp ladder); Lane 2 is *An. funestus* s.s. positive sample; Lanes 3 and 7 are An. leesoni; Lanes 4, 5 and 6 are *An. funestus* s.s.; Lane 8 is a negative control which contained no DNA; Indicated are diagnostic bands of 146bp for *An. leesoni* and 460bp for *An. funestus*.

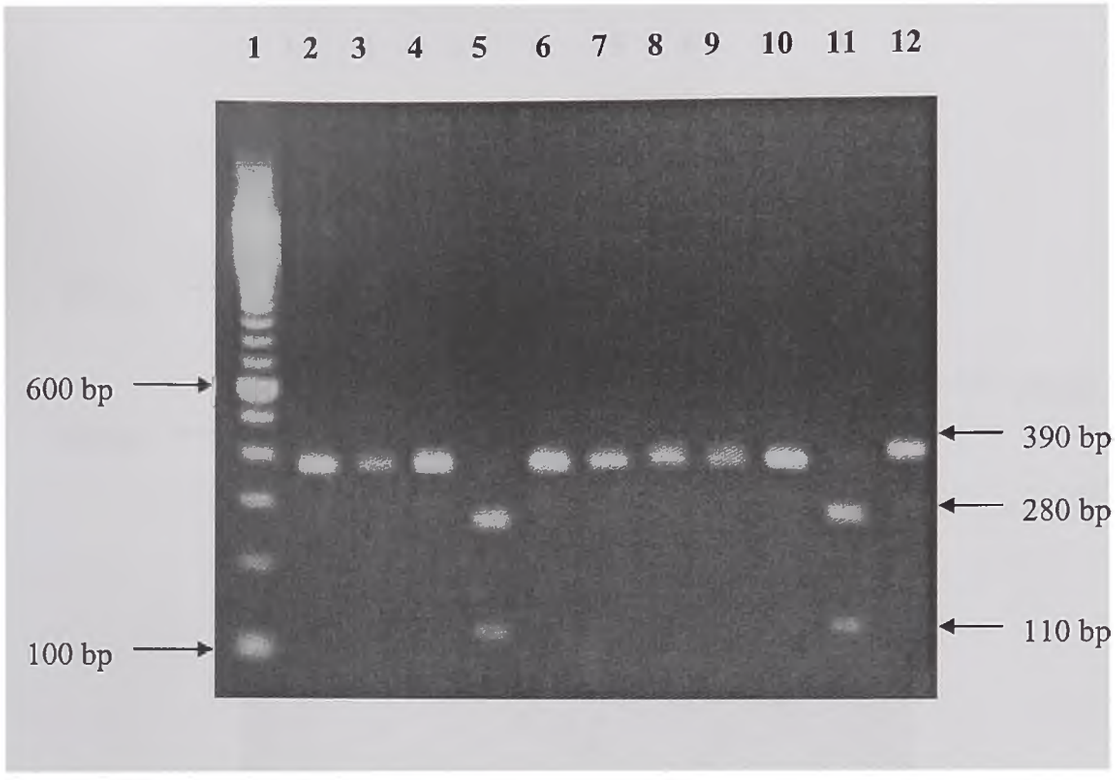

Figure 12: Photograph of ethidium bromide-stained agarose gel (2%) electrophoregram of Hha I restriction enzyme digest for the identification of molecular forms of *Anopheles gambiae* s.s. Lane 1 is molecular weight marker (100-bp ladder); Lanes 2-4 and 6-10 are M forms; Lanes 5 and 11 are S forms; Lane 12 is undigested *An. gambiae* s.s. PCR product; also indicated are the sizes of the restricted products.

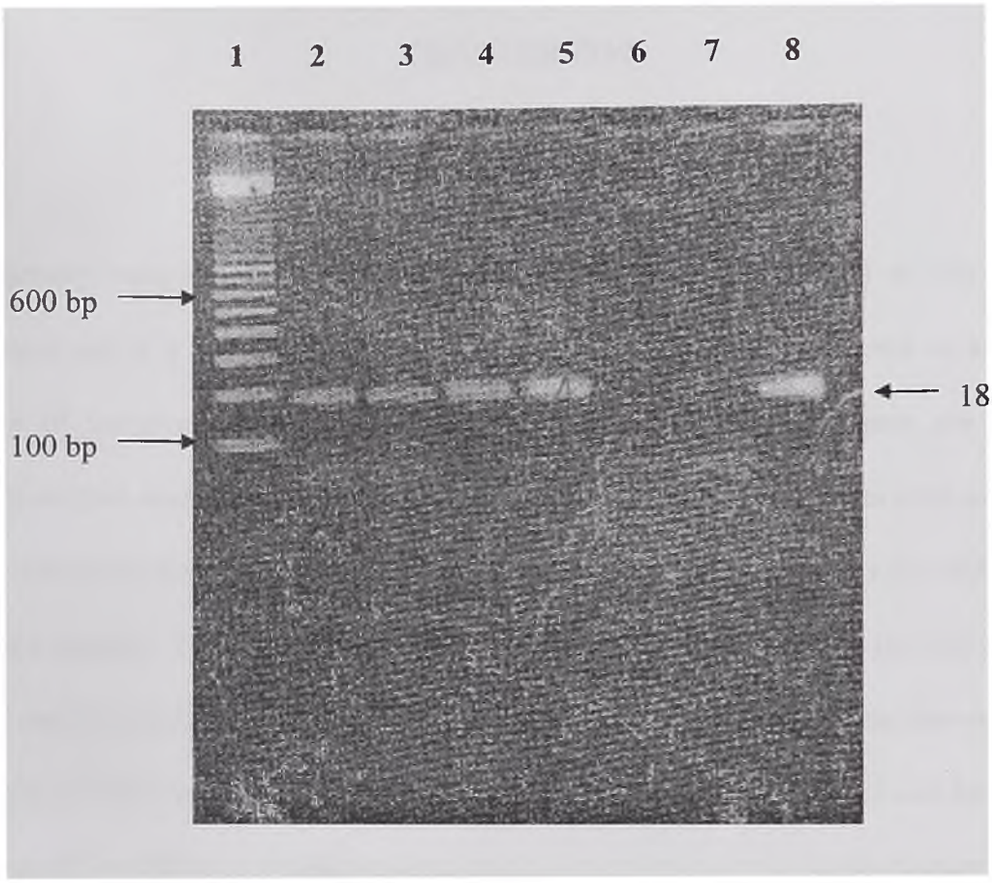

Figure 13: Photograph of an example of ethidium bromide-stained agarose gel (2%) electrophoregram analysis of a PCR diagnostic test for detection of *Wuchereria bancrofti*. Lane 1 is molecular base pair marker (100-bp ladder); Lane 2 is *W. bancrofti* positive control; Lanes 3-5 and 8 are *W. bancrofti*; Lane 6 is a negative control. Also indicated is the diagnostic band size of 188bp for *W. bancrofti*.

CHAPTER FIVE

DISCUSSION

Low density microfilaraemia has been defined by Southgate (1992) as the density of circulating mf in a specified blood compartment that cannot be detected in a significant number of instances when commonly used blood sampling techniques are applied in epidemiological studies. This, however, depends on a number of variables such as volume of blood examined, source of blood sampled (usually venous or capillary) and method of mf detection adopted. The author gave the limit of a value of 4mf per 20µl (200mf per ml) and less of capillary blood to denote low microfilaraemia. Southgate (1974) has also reported that increases in blood volume will reduce the proportion of mf zeros reported and the theoretical analyses of Grenfell *et al.* (1990) indicate increase in sampling volume has greater effect than increase in mf detection efficiency. In this study, 100µl of finger prick blood was used and read in counting chamber, which is an appreciable amount of blood compared to the popular technique for mf detection in routine public health practice of 20µl finger-prick blood films (Southgate, 1992).

With this background it can be concluded that the mf mean intensity of 1.07 and 79.45 mf per ml of capillary blood among the entire district and microfilaraemic individuals respectively that the densities were low in the studied site. This may be due to the fact that the area has been receiving mass chemotherapy with ivermectin and albendazole for the past 4 years aimed at elimination of the disease. This is supported by the fact that Gomoa

Okyereko, which recorded the highest intensity of 155.6 mf/ ml of capillary blood among the microfilaraemic individuals in this study, was recording 819 mf/ ml of blood in 1999 (Dzodzomenyo *et al*, 1999). In their study, Dzodzomenyo *et al.* (1999) observed no significant difference in prevalence among males and females as well as variation in intentisities between age-groups. It was no surprise a similar result was observed in this study although Boakye *et al.* (2004) observed prevalence to be significantly higher in males than females. Dunyo *et al.* (1996) also observed that more females than males are affected by elephantiasis in Ghana unlike the coastal part of east Africa. These inconsistent observations may be attributed to the occupational activities of the inhabitants of the study areas and the biting pattern of the anopheline vectors.

Anopheles are mostly nocturnal in their activities, thus emergence from the pupae, mating, blood feeding and oviposition normally occur in the evenings, at night or early in the morning around sunrise. Some species may bite man mainly outdoors (exophagic) from about sunset to 21:00 hours whereas others bite mainly after 21:00 hours and mostly indoors (endophagic). Whereas some species rest outside (exophilic) in a variety of natural shelters like vegetation, rodent burrows, cracks and crevices in trees, under bridges, termite mounds, and other cracks in the ground in between feeding, others rest in houses (endophilic). Mixtures of these extremes of behaviour are exhibited by most *Anopheles* species that are exophagic and endophagic, exophilic and endophilic. Few of this species exclusively feed on humans though they are predominantly zoophilic. Most feed on both human and animals but the degree of anthropophilism and zoophilism varies according to species and host availability.

The inhabitants of Gomoa Okyereko are mainly peasant farmers and although they will not be on the farm in the night, they may be either indoors or outside in the early hours of the night. The disease manifests itself in adulthood, eventhough it is contracted in childhood as long as one is exposed to infective bites of the mosquitoes (WHO, 2000).

Muirhead-Thompson (1954) in a small survey near Accra in Ghana demonstrated that mf of *W. bancrofti* were present in highest concentration in the peripheral blood during night time. Apart from Dzodzomenyo *et al.* (1999) no detailed study had described the pattern of circadian mf periodicity in Ghana. Based on hourly examination of twelve volunteers for nine hours, it was found that mf concentration in peripheral blood followed a wave-like concentration peaking around 01:00 hours. The observed periodicity then is similar to those reported from other parts of Africa (Tanaka, 1981; Gatika *et al.*, 1994) probably because night biting species of mosquitoes serve as vectors throughout the continent. Many other explanations have been given to account for this interesting behavioural pattern of mf. One suggestion is that oxygen tension plays a part and that if a person is given oxygen during the night, the mfs will stay in deep tissues other than accumulating in peripheral circulation (Hawking *et al.*, 1966; Denham and McGreevy, 1977). Another suggestion is that periodicity is related to human activity because if a person reverses his/ her working hours (resting during the day and working at night) after a few days, there will be reversal in periodicity (Hawking *et al.*, 1966; Denham and McGreevy, 1977).

Dzodzomenyo M. *et al.* (1999) in a study carried out at the same site observed *An. funestus* to be the most abundant species of mosquito in early dry season while *An. gambiae* was

predominant in the wet season with a few culicines. The authors also observed a remarkable increase in abundance of *An. gambiae* and reduction in abundance of *An. funestus* during the wet season. This pattern was similarly observed in this work. March is in the peak of the dry month in Ghana and this may contribute to the low number of *An. gambiae* captured. Even though 1.7% *Anopheles* and 3.3% *Mansonia* were found infected with the L_1 stage of *W. bancrofti*, there was no recovery of L_3 from any of the mosquitoes after 12 days of maintenance. Each of the infected *An. gambiae* had one L_1 stage of the parasite whiles on the average the infected *Mansonia* species had two each of the parasite. On the other hand, however, each of the infected *An. funestus* had $8L_1$ stage of the parasite. Survival rate was 0.5 for both *An. gambiae* and *An. funestus* but 0.1 for *Mansonia* species. *Mansonia* transmits *W. bancrofti* in parts of Asia but has not been identified as a vector in Africa (Sasa, 1976; Service, 1990). It was therefore not surprising to find no infective stage larvae of the parasite in the *Mansonia* species.

In their work, Southgate and Bryan (1992) reported that although many of the mf ingested by *Anopheles* vectors is damaged by the mosquito's foregut armature, the proportion of mf destroyed is independent on the number of mf ingested and varies between members of the *An. gambiae* complex and *An. funestus*. Studies on the relationship between the level of microfilaraemia in the blood meal source and the percentage of feeding mosquitoes that ingest mf or the number of mf ingested per mosquito have not provided consistent results for *Anopheles* species (McGreevy *et al.*, 1982; Bryan *et al.*, 1990; Bryan and Southgate, 1988).

Some species of *Anopheles* have been reported by Southgate and Bryan (1992) to exhibit facilitation in their relationship with *W. bancrofti* unlike *An. melas* in Gambia and *An. merus* in Tanzania, which exhibit limitation. In view of the observed variation with mosquito species and geographical region, it will be unreasonable to extrapolate the results of one study on one *Anopheles* species in a region to the same species in another region or to a different species in the same or different geographical area. A similar study conducted in the Bongo District of Ghana found limitation to be more probable mechanism for *W. bancrofti* and *An. gambiae* s.l. and *An. funestus* or both of these taxa (Boakye *et al.,* 2004).

The significance of distinctly different host-parasite relationships lies in the importance of low-density mf in sustaining transmission in various endemic areas with different genera of mosquito vectors (Southgate, 1992). In theory, low level proportionality and limitation will allow transmission to occur and build-up when most infected human hosts have low mf densities, whereas situations of well marked proportionality or facilitation will give rise to transmission thresholds below which the ultimate transmission will cease and the parasites eliminated from the human population. However predictions of parasite extinction or parasite resurgence can be made with confidence when characteristics of the local vector/ filarial relationship are known (Webber, 1991).

Yawson *et al.* (2004) found the M and S forms of *Anopheles gambiae* s.s. to occur in sympatry in southern Ghana. Studies in this coastal community have shown the abundance of the M form. It was therefore not surprising to find most of the *Anopheles gambiae* s.s. being M which has a remarkable ecological flexibility and is known to prevail in inundated areas

where dry season breeding opportunities exist (Appawu *et al.*, 1994). A reduction in the mf level of the human population following drug distribution is likely to lead to a decrease in transmission of *W. bancrofti* by anophelines because they show facilitation pattern in infections with filaria (Southgate & Bryan, 1992). This seems to be true for the M form of *Anopheles gambiae* s.s.

CONCLUSION AND RECOMMENDATIONS

The main vector species encountered in this study were the M form of *Anopheles gambiae* s.s. and *An. funestus* s.s. Considering the fact that the study was conducted in the natural setting, this finding shows that the yearly combination therapy with ivermectin and albendazole for 5 to 6 years will help eliminate the disease in areas like Ghana where *Anopheles gambiae* and *An. funestus* are the main vectors. Although these *Anopheles* species were not competent in promoting the maturation of the parasites when mf is low, a repeat of this study targeting larger mosquito numbers is required to ascertain the role played especially by M forms of *An. gambiae* in the transmission of lymphatic filariasis when parasite levels in the community are low.

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APPENDICES

APPENDIX I

PREPARATION OF STANDARD SOLUTIONS

With standard sterile double distilled water (sdd H_2O), the following standard solutions were prepared and autoclaved at 121 lb/ sq for 15 minutes in an Eyela autoclave (Rikikkaki, Tokyo).

0.5 M EDTA (pH 8.0)

186 g of EDTA, dissolved in 800 ml double distilled water, pH adjusted with NaOH pellets, the volume made to 1000 ml with ssd H_2O and stored at room temperature

10ml of TE (pH 8.0)

20µl of 0.5 M EDTA, 50µl of 2.0M Tris HCl, 9.93ml of sddH_2O and stored at room temperature.

TE-RNAse

995µl TE in 5µl RNAse

Primers for PCR

Primers used in PCR reaction were reconstituted and diluted according to the manufacturers recommendations (Oswell Laboratories).

Bender Buffer

0.1M NaCl, 0.2M Sucrose, 0.1M Tris HCl (pH 7.5), 0.05M EDTA (pH 9.1), 0.5% SDS.

Buffer C for PCR mixture

300mM Tris HCl, 75mM $(NH_4)_2SO_4$, 2.5mM $MgCl_2$

10X TAE buffer

242g Tris base, 57.1 ml glacial acetic acid, 100 ml 0.5 M EDTA, pH adjusted to 7.7 (with glacial acetic acid) and the volume made to 1000 ml with double distilled water.

5X Orange G (Gel loading buffer)

20% (w/ v) Ficoll, 25 mM EDTA, 2.5% (w/ v) orange G and stored at room temperature.

DNA molecular weight size markers

100bp DNA molecular weight size marker obtained from the manufacturers (Sigma) was diluted according to recommendations and used; 10µl of DNA ladder, 30µl of loading dye (orange G), 60µl of sddH_2O. For the 100bp ladder, the first band size is 100bp, subsequent ones are read 200, 300, 400, ...1000bp.

2% Agarose gel

First 1.0g of agarose powder was put into flask and TAE was added to make a volume of 50ml. It was heated in microwave oven (230V, 50Hz, 2660W, 12.0A) for 1minute to dissolve solute. The mixture was cooled under running water and 2µl of Ethidium Bromide was added. The gel was cast to set in a chamber with comb to make the wells.

APPENDIX II

SEQUENCE OF THE SYNTHETIC OLIGONUCLEOTIDE PRIMERS USED IN THE MOLECULAR STUDIES OF:

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

Species	Name of primer	Sequence (5' - 3')	Melting temperature T_m (^{0}C)	Expected amplified DNA size (base pair)
Universal forward primer	UN	GTG TGC CCC TTC CTC GAT GT	58.3	468
Anopheles gambiae s.s.	GA	CTG GTT TGG TCG GCA CGT TT	59.3	390
Anopheles merus/ melax	ME	TGA CCA ACC CAC TCC CTT GA	57.2	464
Anopheles arabiensis	AR	AAG TGT CCT TCT CCA TCC TA	47.4	315
Anopheles quadrianulatus	QD	CAG ACC AAG ATG GTT AGT AT	42.7	153

Anopheles funestus s.l. species

Species	Name of primer	Sequence (5' - 3')	Melting temperature T_m (^{0}C)	Expected amplified DNA size (base pair)
Universal forward primer	UV	TGT GAA CTG CAG GAC ACA T	55.3	
Anopheles funestus s.s.	FUN	GCA TCG ATG GGT TAA TCA TG	52.4	460
Anopheles vaneedeni	VAN	TGT CGA CTT GGT AGC CGA AC	58	555
Anopheles rivulorum	RIV	CAA GCC GTT CGA CCC TGA TT	58.8	400
Anopheles parensis	PAR	TGC GGT CCC AAG CTA GGT TC	60.5	235
Anopheles leesoni	LEES	TAC ACG GGC GCC ATG TAG TT	60.2	146

Wuchereria bancrofti

Species	Name of primer	Sequence (5' - 3')	Expected amplified DNA size (base pair)
Wuchereria bancrofti	NV-1	CGT GAT GGC ATC AAA GTA GCG	188
	NV-2	CCC TCA CTT ACC ATA AGA CAA C	188

APPENDIX III

CONSTITUENTS OF A 20µl PCR REACTION MIX USED IN THE MOLECULAR STUDIES OF:

***Anopheles gambiae* DNA**

Reagent	Volume	Final concentration
Sterile distilled water	14.3µl	-
10 x PCR buffer	2.0µl	1x
20µM of each dNTP (dATP, dCTP, dGTP, dTTP)	0.2µl	20µM
20 µM of each primer (UN, GA, ME, AR, QD)	1.5µl	20µM
5U Taq polymerase	0.1µl	0.625 µM
DNA template	1.0µl	1 ng-1µg

***Anopheles funestus* DNA**

Reagent	Volume	Final concentration
Sterile distilled water	14.9µl	-
10 x PCR buffer (plus 25mM MgCl)	2.0µl	1x
20µM of each dNTP (dATP, dCTP, dGTP, dTTP)	0.2µl	200µM each
20 µM of each primer (UN, FUN, VAN, RIV, PAR, LEES)	1.8µl	20µM
5U Taq polymerase	0.1µl	0.625U/ µl
DNA template	1.0µl	1 ng-1µg

***Wuchereria bancrofti* DNA**

Reagent	Volume	Final concentration
Sterile distilled water	11.7µl	
10 x PCR buffer	2.0µl	1x
20µM of each dNTP (dATP, dGTP, dCTP, dTTP)	0.2µl	200µM each
20 µM of each primer (NV-1, NV-2)	1.0µl	200nM
5U Taq polymerase	0.1µl	0.025U/ µl
DNA template	5.0µl	1 ng-1µg

APPENDIX IV

INFORMATION AND CONSENT FORM

Invitation to participate in the study

We invite you to take part in a research study by the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon. The research study is called: Vector competence of the *Anopheles* (Diptera: Culicidae) populations for *Wuchereria bancrofti* (Spirurida: Filariidae) in the Gomoa District of Ghana after mass drug administration. The primary investigator for the study is Dr. Daniel Adjei Boakye, NMIMR.

Introduction

This consent form informs you about the background, aims and the method of this study. In addition it explains the anticipated benefits, potential risk of the study and the discomfort it may entail. Finally it informs you of entrance criteria and your rights regarding participating in this study.

Background and purpose of this study

The relationship between ingestion of microfilariae (mf), production of infective larvae (L_3) and mf density in human blood has been suggested as an important determinant in the transmission dynamics of lymphatic filariasis. Thus understanding vector-parasite interactions is essential for rational development of filariasis control strategies. This is particularly important, considering that vectorial competence (the ability of mosquitoes to ingest mf and to promote their maturation until the infective stage), and the rate of mosquito

survival until parasite maturation, seems to differ according to geographic mosquito strains. Variation in the density of mf in the blood and parasite behaviour also influences vector-parasite relationships.

For the Global Programme to Eliminate Lymphatic Filariasis (GPELF) to be successful, the competence of various mosquito vectors to pick the mf (especially when the community mf load is low), support the development of the ingested mf into the human-infective third-stage larvae (L_3), and to transmit those L_3 to humans has to be understood. Some species of mosquito (those exhibiting the phenomenon of facilitation) are unable to transmit parasites from humans with low levels of microfilaraemia whereas other species (exhibiting limitation) can effectively transmit the parasites even when the mf in their blood meal source is at a very low level. The quantitative relations of transmission intensity and mf reservoir such as the proportion of microfilaria (mf) ingested by *Anopheles* mosquitoes, which are damaged by their foregut armature, percentage of mosquitoes ingesting mf, host mf density, the percentage of mosquitoes infected or mf density per mosquito and numbers of mf per millilitre of host blood have been found to vary among members of the *Anopheles gambiae* complex and *An. funestus*.

In Ghana several sympatric *Anopheles* species are vectors of lymphatic filariasis that might differ in vectorial role and capacity to transmit low-density microfilaraemia. *An. gambiae* and *An. funestus* have been identified as the most important vectors of the disease in earlier studies conducted along the coast of Ghana. Results obtained from a preliminary study after three years of MDA showed decrease in ATP of *Anopheles funestus* but no change in *An.*

gambiae s.s. in eight communities of the Gomoa District. This study was conducted to determine the vector competence of these *Anopheles* species in transmission of *Wuchereria bancrofti* at low mf levels.

Procedure

There will be mass screening for microfilaraemia in the population, using 100µl blood/ subject and standard techniques. From these 20 adults with different levels of microfilaraemia (all of whom will provide their informed consent) will be selected as blood meal sources. The volunteers will sleep under a mosquito net with one side partly open for mosquito entry. Hourly collection of mosquitoes feeding on each subject during the night will be done from 2100 to 0600 hours the next. At the mid-point of each collection hour, a finger prick sample of blood will be collected from each subject. Part of each sample will be screened for mf as a blood smear, while 100µl will be mixed with 900µl 3% acetic acid so that the number of mf could be determined by microscopy.

About 50% of the mosquitoes from each hour's collection from each subject will be killed immediately after feeding, stored for less than 18 hours at 4^0C, and then dissected so that the number of mf in the blood meal of each mosquito could be counted. The remaining mosquitoes will be maintained at 26-28^0C and 70%-80% relative humidity, with access to 10% sucrose solution until day 12 post collection, when they will be killed, dissected and checked for L_3. Any mosquito that will die before day 12 will also be dissected so that larval

stages of *W. bancrofti* they harboured could be recorded. Molecular identification of *W. bancrofti, An. gambiae* s.l. and *An. funestus* will be done. The data will be analysed to determine vector competency of *Anopheles* in transmitting *W. bancrofti* and to quantify the relationship between the level of microfilaraemia in the blood meal source and the mf uptake by the mosquitoes.

Benefit

There will be no direct benefit for subjects' participation in this study as such but they will receive drugs for the ailment. The main benefit of your participation is indirect as you will help us understand the vector competence in the transmission of the parasite.

No major risks will be seen from your participation in this study apart from the bite one may get from the mosquitoes and a small discomfort as a result of pain from the drawing of blood.

Confidentiality

Your records will be kept in a secure location at NMIMR. A study ID number will be assigned to secure confidentiality. It is likely that data obtained from tests done on you may be published in medical journals, however, your identity will not be disclosed.

Your Right

Your participation is solely voluntary and you may change your mind at any time. Refusal to participate in or withdraw from this study will not have any penalties or loss of benefits that you may be entitled to.

Contact information

For any further information you may contact the primary investigator, Dr. Daniel Adjei Boakye, Noguchi Memorial Institute for Medical Research, University of Ghana, Box LG581, Legon. Tel: 021 500374.

Your Statement as a volunteer

I have read this consent form. I have received satisfactory answers to my questions. I understand that my participation is voluntary. I know about the purpose, methods, risks and possible benefits of the research study to judge that I want to participate, and I know that I can call my study assistant if I have any questions or concerns. I understand that I will be given a copy of the consent form so that the above information is available to me.

Informed consent signature

Name _____ Signature or thumb print _____

Witness Name _____ Witness signature _____

Primary Investigator Signature _____

Date

(Study ID number assigned to this participation)

APPENDIX V

The hourly examinations of blood for *Wuchereria bancrofti* among the twelve consented volunteers during the mosquito collection

			Mf intensities (mf/ ml) at the mid-hours of examination								
Volunteer	Sex	Age	21:30	22:30	23:30	0:30	1:30	2:30	3:30	4:30	5:30
1	M	21	110	350	190	220	150	110	60	40	70
2	M	20	20	50	110	80	20	90	90	40	60
3	F	13	250	500	190	430	450	590	320	280	260
4	M	16	150	250	150	190	130	70	100	140	200
5	M	47	100	80	100	180	100	50	160	130	80
6	M	50	220	240	200	380	320	310	190	250	240
7	M	11	200	250	270	170	190	210	260	110	110
8	F	55	70	20	20	20	40	20	0	0	0
9	F	10	270	270	230	350	210	260	250	530	270
10	M	11	100	50	50	40	60	20	100	40	50
11	F	65	0	10	0	10	10	10	0	0	10
12	M	15	30	40	60	40	30	40	40	30	40

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

APPENDIX VI

(A) The number of subjects examined, number of positive and mf/ ml intensity of blood among the age groupings during mass screening for *Wuchereria bancrofti* in Gomoa Okyereko

Age Group (years)	Number examined			Number mf positive (%)			mf mean intensity*			
	Females	Males	Total	Females	Males	Total	Females	Males	Total	mf positives
1 - 14	70	71	141	1(1.43)	2(2.86)	3(2.13)	1.10	1.13	1.11	146.97
15 - 24	29	20	49	0	3(15)	3(6.12)	0	2.10	1.33	105.13
25 - 34	14	6	20	0	0	0	0	0	0	0
35 - 44	14	4	18	1(7.14)	0	1(5.56)	1.32	0	1.24	51
45+	28	21	49	1(3.57)	2(9.52)	3(6.12)	1.25	1.64	1.41	261.76
All	**155**	**122**	**277**	**3(1.94)**	**7(5.74)**	**10(3.61)**	**1.11**	**1.32**	**1.20**	**155.59**

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

APPENDIX VI

(B) Table showing the number of subjects examined, number of positive and mf/ ml intensity of blood among the age groupings during mass screening for *Wuchereria bancrofti* in Gomoa Mampong

Age Group (years)	Number examined			Number mf positive (%)			mf mean intensity*			
	Females	Males	Total	Females	Males	Total	Females	Males	Total	mf positives
1 - 14	7	5	12	0	0	0	0	0	0	0
15 - 24	10	19	29	0	0	0	0	0	0	0
25 - 34	8	4	12	0	0	0	0	0	0	0
35 - 44	3	1	4	0	0	0	0	0	0	0
45+	4	2	6	0	0	0	0	0	0	0
All	**32**	**31**	**63**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

APPENDIX VI

(C) Table showing the number of subjects examined, number of positive and mf/ ml intensity of blood among the age groupings during mass screening for *Wuchereria bancrofti* in Gomoa Kyeren

Age Group (years)	Number examined			Number mf positive (%)			mf mean intensity*			
	Females	Males	Total	Females	Males	Total	Females	Males	Total	mf positives
1 - 14	55	43	98	0	0	0	0	0	0	0
15 - 24	10	5	15	0	0	0	0	0	0	0
25 - 34	10	4	14	0	0	0	0	0	0	0
35 - 44	3	5	8	0	0	0	0	0	0	0
45+	19	7	26	0	0	0	0	0	0	0
All	**97**	**64**	**161**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

APPENDIX VI

(D) Table showing the number of subjects examined, number of positive and mf/ ml intensity of blood among the age groupings during mass screening for *Wuchereria bancrofti* in Gomoa Fawomanyo

Age Group	Number examined			Number mf positive (%)			mf mean intensity*			
(years)	Females	Males	Total	Females	Males	Total	Females	Males	Total	mf positives
1 - 14	20	14	34	0	0	0	0	0	0	0
15 - 24	4	6	10	0	0	0	0	0	0	0
25 - 34	3	4	7	0	0	0	0	0	0	0
35 - 44	3	3	6	0	0	0	0	0	0	0
45+	2	7	9	0	0	0	0	0	0	0
All	**32**	**34**	**66**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

APPENDIX VI

(E) Table showing the number of subjects examined, number of positive and mf/ ml intensity of blood among the age groupings during mass screening for *Wuchereria bancrofti* in Gomoa Amanful

Age Group	Number examined			Number mf positive (%)			mf mean intensity*			
(years)	Females	Males	Total	Females	Males	Total	Females	Males	Total	mf positives
1 - 14	10	10	20	0	0	0	0	0	0	0
15 - 24	6	5	11	0	0	0	0	0	0	0
25 - 34	5	3	8	0	0	0	0	0	0	0
35 - 44	7	3	10	0	0	0	0	0	0	0
45+	11	1	12	0	0	0	0	0	0	0
All	**39**	**22**	**61**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

APPENDIX VI

(F) Table showing the number of subjects examined, number of positive and mf/ ml intensity of blood among the age groupings during mass screening for *Wuchereria bancrofti* in Gomoa Obiri

Age Group (years)	Number examined			Number mf positive (%)			mf mean intensity*			
	Females	Males	Total	Females	Males	Total	Females	Males	Total	mf positives
1 - 14	4	14	18	0	0	0	0	0	0	0
15 - 24	8	15	23	0	0	0	0	0	0	0
25 - 34	8	7	15	0	0	0	0	0	0	0
35 - 44	1	3	4	0	0	0	0	0	0	0
45+	5	5	10	0	0	0	0	0	0	0
All	**26**	**44**	**70**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

APPENDIX VI

(G) Table showing the number of subjects examined, number of positive and mf/ ml intensity of blood among the age groupings during mass screening for *Wuchereria bancrofti* in Gomoa Hwida

Age Group (years)	Number examined			Number mf positive (%)			mf mean intensity*			
	Females	Males	Total	Females	Males	Total	Females	Males	Total	mf positives
1 - 14	14	15	29	1(7.14)	1(6.67)	2(6.90)	1.47	1.28	1.38	103.74
15 - 24	13	21	34	0	0	0	0	0	0	0
25 - 34	4	6	10	0	0	0	0	0	0	0
35 - 44	1	3	4	0	0	0	0	0	0	0
45+	4	7	11	0	0	0	0	0	0	0
All	**36**	**52**	**88**	**1(2.78)**	**1(1.92)**	**2(2.27)**	**1.16**	**1.07**	**1.11**	**103.74**

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

APPENDIX VI

(H) Table showing the number of subjects examined, number of positive and mf/ ml intensity of blood among the age groupings during mass screening for *Wuchereria bancrofti* in Gomoa Dago

Age Group	Number examined			Number mf positive (%)			mf mean intensity*			
(years)	Females	Males	Total	Females	Males	Total	Females	Males	Total	mf positives
1 - 14	50	69	119	0	0	0	0	0	0	0
15 - 24	16	31	47	0	1(3.23)	1(2.13)	0	1.13	1.08	41
25 - 34	4	5	9	0	0	0	0	0	0	0
35 - 44	7	7	14	0	0	0	0	0	0	0
45+	18	21	39	1(5.56)	2(9.52)	3(7.69)	1.14	1.26	2.40	11
All	**95**	**133**	**228**	**1(1.05)**	**3(2.26)**	**2(2.27)**	**1.03**	**1.07**	**1.05**	**15.28**

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

APPENDIX VI

(I) Table showing the number of subjects examined, number of positive and mf/ ml intensity of blood among the age groupings during mass screening for *Wuchereria bancrofti* in Gomoa Ayesuano

Age Group (years)	Number examined			Number mf positive (%)			mf mean intensity*			
	Females	Males	Total	Females	Males	Total	Females	Males	Total	mf positives
1 - 14	12	11	23	0	0	0	0	0	0	0
15 - 24	18	15	33	0	1(6.67)	1(3.03)	0	1.28	1.12	41
25 - 34	1	5	6	0	0	0	0	0	0	0
35 - 44	3	1	4	0	0	0	0	0	0	0
45+	1	2	3	0	0	0	0	0	0	0
All	**35**	**34**	**69**	**0**	**1(2.94)**	**1(1.45)**	**0**	**1.12**	**1.06**	**41**

*Antilog [Σ log (x+1)/ n], where x is the number of mf/ ml blood in mf individuals and n is the number of people examined

APPENDIX VII

(A) ENTOMOLOGICAL SURVEY SHEET

Morphological species identification

Date of collection..... Date of identification..... Subject code..... Village of subject.....

Time (GMT)	Parasitaemia mf/ 100µl	No. mosquito collected	Mosquito species captured					
			An. ga.	An. fu.	Cu. sp	Mans	Ae. sp	Others
2100-2200								
2200-2300								
2300-0000								
0000-0100								
0100-0200								
0200-0300								
0300-0400								
0400-0500								
0500-0600								

APPENDIX VII

(B) MOSQUITO DISSECTION ENTRY SHEET

No:	Time	Loc.	Spp.	Stage of mf found in head			Stage of mf found in thorax			Stage of mf found in abdomen			Number of days old after capture	Subject code
				L1	L2	L3	L1	L2	L3	L1	L2	L3		
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														