LYMPHATIC FILARIASIS AT GOMOA OKYEREKO, AN IRRIGATION PROJECT COMMUNITY IN SOUTHERN GHANA: INFECTION, CLINICAL DISEASE AND VECTORS

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Degree of Master of Philosophy (M. Phil.)
Zoology

By

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SEPTEMBER, 1996

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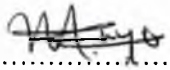
DECLARATION

I do hereby declare that except for other people's investigations which have been duly acknowledged, this work is the result of my own original research, and that this thesis, either in whole or in part, has not been presented elsewhere for another degree.



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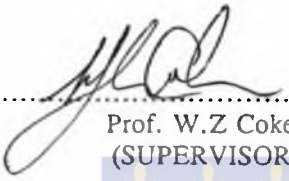

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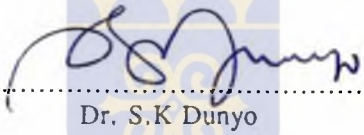

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DEDICATION

I dedicate this work to my parents, SETH and GRACE.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to the Director of Noguchi Memorial Institute for Medical Research (NMIMR) of University of Ghana, Prof. F.K Nkrumah and all his staff especially those of the Epidemiology and Parasitology Units for making available to me their excellent laboratory facilities to carry out this work. I am particularly grateful to the members of the general laboratories of these two units for their companionship and tolerance during my stay with them.

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SUMMARY

A parasitological, clinical, periodicity and entomological study of lymphatic filariasis was carried out at Gomoa Okyereko, an irrigation project community in Southern Ghana to provide baseline information on the infection and its transmission in the community. Out of a total of 636 inhabitants, fifty percent of the residents were selected from the compiled demographic data using computer generated random numbers to constitute the study population. Quantitative examination of night blood sample (100μl) between 21:00 and 01:00 hours from 296 (94.6%) selected persons using the counting chambers techniques (CCT) revealed high microfilarial prevalences and microfilarial geometric mean intensity (GMI) in the study group. Overall microfilarial prevalence of 26.4% was observed, and prevalence generally increased with age and was higher in females (27.6%) than in males (25%), though this was not statistically significant. Large variability was observed in individual mean intensities and an overall GMI among infected individuals was 819mf/ml of blood (1114.3mf/ml in males and 645.7mf/ml in females). There was however no statistical significance in GMI between these two sexes.

Hydrocoele was the most common clinical manifestation of lymphatic filariasis and 9.3% of the males examination had hydrocoeles of grades 1-IV The prevalence of hydrocoele increased with age and the highest prevalence of 33.3% was noted in 40-49 age group. Four female aged 13-55 had limb elephantiasis, two were grade I and the other two grade III.

Microfilarial periodic pattern of *Wuchereria bancrofti* was determined in eight microfilaraemic persons. From each individual, a 100μl finger prick blood was collected every two hours throughout one complete 24hours cycle. The peak hour (k) was calculated as 01:03 (i.e 3 minutes after 1 a.m) and the periodicity index (D) was 122.6, confirming the nocturnal periodicity of microfilariae of this parasite in Ghana.

Entomological studies using pyrethrum spray collections and landing catches revealed that the species of man-biting mosquitoes in the community include *Anopheles gambiae* s.1, *A. funestus*, *A. pharoensis*, *Culex quinquefasciatus* and *Mansonia* species. Their relative abundance showed seasonal variation influenced by the amount of rainfall and the availability of favourable breeding places such as the irrigation canals. In the rainy season, the most abundant species was *A. gambiae* s.1 while in the dry season, the most abundant species was *A. funestus*. During the period that the dam was opened, the most abundant species was *A. gambiae* s.1. Most of the mosquitoes were biting late in the night coinciding with the peak of microfilarial abundance in the peripheral blood. Overall infectivity rates of 7.8%, 3.9% and 9.1% were recorded for *A. gambiae* s.1, *A. funestus*, *A. pharoensis* respectively. *A. pharoensis* had a very high infectivity rate though very small numbers were collected. Other species collected in small numbers were *Culex quinquefasciatus* and *Mansonia* species and their role in the transmission of the disease was minimal. It was calculated that inhabitants in the area are at risk of getting 309.5 infective bites per man per year from *A. gambiae* s.1 while both *A. funestus* and *A. pharoensis* may give 24.1 infective bites each. The main vector responsible for the transmission of lymphatic filariasis in the community therefore is A. *gambiae* s.1 while A. *funestus* and A. *pharoensis* play minor roles.

CHAPTER 1

1.1 GENERAL INTRODUCTION

1.1.1 Prevalence and Distribution of Lymphatic Filariasis

Lymphatic filariasis is caused by infection with a group of slender elongated nematode worms which are called filarial worms. The disease in its various forms remains a public health problem of considerable magnitude in many tropical countries (WHO, 1992). According to WHO expert committee on filariasis, it is estimated that over 3 billion persons live in countries where the disease is endemic and some 757 million live in areas where transmission is known to occur (WHO, 1992). Its prevalence is increasing worldwide, largely because of rapid urbanisation in many endemic areas (Ottesen, 1995).

1.1.2 History and Classification of the Parasite

The microfilariae of the parasite *Wuchereria bancrofti* were first found by Wucherer in the urine of a patient suffering from chyluria in Bahia, Brazil in 1866 (Kettle, 1990). However, Nelson (1978) reported that the parasites were observed earlier on by Demarquay in 1863. Since then interest in filariasis grew worldwide and many workers such as Lewis in India, Manson in China and Bancroft in Australia detected microfilariae in the blood of patients in subsequent years. Three main filaria worms cause the disease in man namely, *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. The group of filaria worms that cause lymphatic filariasis belong to the superfamily Filarioidea, the order Spirurida and the class Nematoda. Of the estimated 90 million infections worldwide, *Wuchereria bancrofti* is responsible for over 80 million cases and is the only known aetiologic agent in the African Region where about 25.6 million people are

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affected (WHO, 1984). The adult worms are found in the lymph nodes and lymphatic channels of humans. Male worms are about 40mm in length and 0.1mm in width and are less than half the size of the females which measure 80-100 by 0.24-0.30mm. Both are creamy white, cylindrical, blunt tapering, threadlike nematodes with smooth cuticular surfaces and unarmed mouths.

1.1.3 Vectors and Transmission

The vectors responsible for the transmission of lymphatic filariasis vary in the different geographical regions of the world. Mosquitoes belonging to three genera, *Anopheles*, *Culex* and *Aedes* are known to be involved in transmission of the disease worldwide (WHO, 1987). In Africa, the mosquitoes, *Anopheles gambiae* sensu latum and *Anopheles funestus* are the principal vectors except along the coast of the Indian Ocean (ie in Kenya and Tanzania) where *Culex quinquefasciatus* is known to be involved in transmission (WHO, 1987). Transmission occurs when the mosquito in the process of feeding on the blood of the host deposits the infective larvae on the skin in a small fluid and successful penetration is known to depend on the relative humidity of the external environment (Lindsay *et al*, 1984).

1.1.4 Clinical Disease

Lymphatic filariasis produces a spectrum of infection and disease outcomes such that communities in endemic areas differ in the proportion of people who are microfilaraemic, in the mean densities of microfilariae that are detected and in the prevalence of clinical symptoms that suggest a difference in the intensity of transmission. The adult worms live in the lymphatic

system and obstruct the flow of the lymph, causing local inflammation of the lymphatic vessels (lymphangitis), swelling of the lymphatic glands (lymphadenitis) and oedema of the affected area, frequently the legs (Maegraith, 1980). Chronic obstruction of the lymphatic system leads to permanent oedema and the development of fibrous tissue resulting in permanent elephantiasis. Elephantiasis particularly affects the legs either below the knees or the whole limb, the arms, breasts, labia and scrotum. In males hydrocoele is a common complication. Obstructed abdominal lymphatic vessels may rupture leading to the excretion of lymph in the urine, a condition known as chyluria.

1.1.5 Social Impact of Lymphatic Filariasis Disease

Lymphatic filariasis persists as a major cause of clinical morbidity and thus a significant impediment to socioeconomic development in much of Asia, Africa and the Western pacific, as well as in certain regions of the Americas. The social impact of filariasis vary not only from place to place but also within the same communities and in general, the degree of stigma seems to be associated with the severity and visibility of the disease (Evans *et al*, 1993). Like many helminthic infections, filariasis is not considered to be a major cause of mortality, but filarial morbidity is devastating and is a crippling affliction that causes social and economic hardships at both the individual and community levels (Wamae, 1994). Hunter (1992) reported that the stigma attached to swollen limbs for instance, discourages young men from marrying girls from areas that are known for filariasis in North East Ghana. In the Philippines, Lu *et al* (1988) indicated that cases of hydrocoele were considered serious only when they reach the size of a sack but generally , people with hydrocoele were the subject of considerable teasing. Kessel,

cited by (Evans *et al*,1993) reported that in Polynesia, at least in the 1950s, people suffering from filariasis hid or retired to the background because they were the laughing stock of the community.

The distribution and transmission of the disease are closely related to socioeconomic and behavioural factors in the endemic populations (Muhodwa, 1983). For example, rural-urban migration and urbanization interact to facilitate the spread of *W. bancrofti* (Mak, 1986). This is believed to occur largely as a result of inadequate waste disposal and sanitation facilities providing suitable breeding sites for the vectors. The crowding of people living in urban areas in developing countries is also known to aid transmission. Other forms of migration in endemic regions such as occupational migration may also aid transmission and Laurence (1989) linked the historical spread of lymphatic filariasis throughout the world with large scale migrations of infected peoples.

1.1.6 Water Resources and Parasitic Diseases

Even though water resources development projects are essential for a wide range of human activities notably agriculture and energy production, it has long been recognized that inadvertent, unintended and adverse side effects of these projects can seriously threaten human health (Hunter, *et al*, 1993). Notable among the adverse side effects of such water development projects is the increase in certain parasitic diseases among the populations living nearby. In Ghana for instance, the Kpong Dam (which is about 25km below the Akosombo Dam), immediately after completion became infested with submerged and floating aquatic plants such

as *Ceratophyllum* and *Pistia* which served as suitable habitats for intermediate host snails of schistosomiasis (Odei, 1977). Similar events were reported in Egypt (Dawood, 1951) and in the Selingue dam in Mali (Traore, 1989). The Barekese reservoir, the main source of water supply in the Ashanti Region of Ghana was invaded by *Mansonia* and other mosquitoes soon after its completion in the early 1960s (Hunter *et al*, 1993).

1.1.7 Lymphatic Filariasis and Water Resources Development

The effect of water resources development projects on lymphatic filariasis transmission has received little attention. The incidence and prevalence of lymphatic filariasis is increased around small dams and the severe form of the disease may occur frequently because of the intensity of transmission (Hunter, 1992). In a reconnaissance survey of lymphatic filariasis in North East Ghana, Hunter (1992) reported that all communities reporting of filariasis as a public health problem were former beneficiaries of foreign assistance agricultural development projects such as small village dams which were constructed from 1958-1962. Notable among these dams are the Vea and Tono irrigation projects. The key understanding to this is that the irrigation canals provided additional habitats which are suitable for mosquito breeding leading to an increase in mosquito populations and therefore high intensity of mosquito-man contact which are necessary conditions for the transmission of lymphatic filariasis.

Little has been done on lymphatic filariasis in Ghana. However, in recent years, studies were carried out in the North (Gyapong *et al.* 1993) and along the coast of Ghana (Dunyo *et al.* in press). This study was therefore carried out at Gomoa Okyereko, a village situated very near to an irrigation project, the Okyereko Irrigation Project (OIP), which involved the construction of an earth dam across a small tributary of the River Ayensu. Since the advent of the dam however, it has been realized that there is an increase in mosquito populations (Hunter *et al*, 1993), and cases of clinical manifestations of lymphatic filariasis have been observed in the community (Ahorlu *et al,* personal communication 1995). This study therefore aims at providing baseline information on the prevalence and intensity of lymphatic filariasis in this irrigated community by determining the prevalence of microfilaraemia, clinical manifestations and also entomological observations. It also aims at establishing the periodicity pattern of the responsible filarial parasite, in order to document the appropriate time to collect blood sample for optimum microfilariae detection in Ghana.

CHAPTER 2

2.1 DESCRIPTION AND DEMOGRAPHIC CHARACTERISTICS OF THE STUDY VILLAGE

2.1.1 Location and Climatic Features

Gomoa Okyereko is a village located between latitudes 5°N 5½°N in the Central Region of Ghana. It is located about one and half kilometres off the Accra to Winneba road and is forty nine (49) kilometres from Accra. This village is in the dry equatorial climatic region of Ghana. It has two rainfall maxima and the average yearly rainfall varies between seventy-four and eighty nine centimetres. This region is the driest in Ghana (Dickson *et al*, 1977). The main rainy season lasts from May to July with a minor season of light showers between September and October. Daily temperatures average 26°C and many of the small streams dry up during the dry season. The vegetation is coastal savanna interspersed with very few trees. On the western margin of the village is the river Ayensu which is the main source of water supply to the village. This river normally has a large volume of water in the rainy season leading to periodic flooding but reduces in volume in the dry season. The people are mainly peasant farmers and to permit sustained agricultural activities during the dry season, the installation of irrigation facilities was necessary. The construction of an irrigation facility was therefore started in 1973 and completed in 1974. This involved the construction of an earth dam across a small tributary of the river Ayensu. The project thus envisages an increase in the production of rice and vegetables during the dry season in the Winneba area. It was also intended to minimize the drift of the youth to the urban areas by allocating plots to farmers where it is expected that the income would go a long way to raise the living standards of the people. The Okyereko settlement itself is situated

between the dam and the river Ayensu and most of the land surrounding the village is the irrigable land which is mainly used for the cultivation of cereals, mainly rice and maize and vegetables.

2.1.2 Demographic Characteristics

The houses at Okyereko are grouped together into one cluster and the general extended family system prevails with most households headed by men. In order to provide information on the population size of the village in relation to sex and age distribution in order to explain any variations in disease prevalence should the need arise, a population census was conducted. The census was also aimed at explaining any observed changes that might have occurred in the population and its effects on the population growth.

2.2 MATERIALS AND METHODS

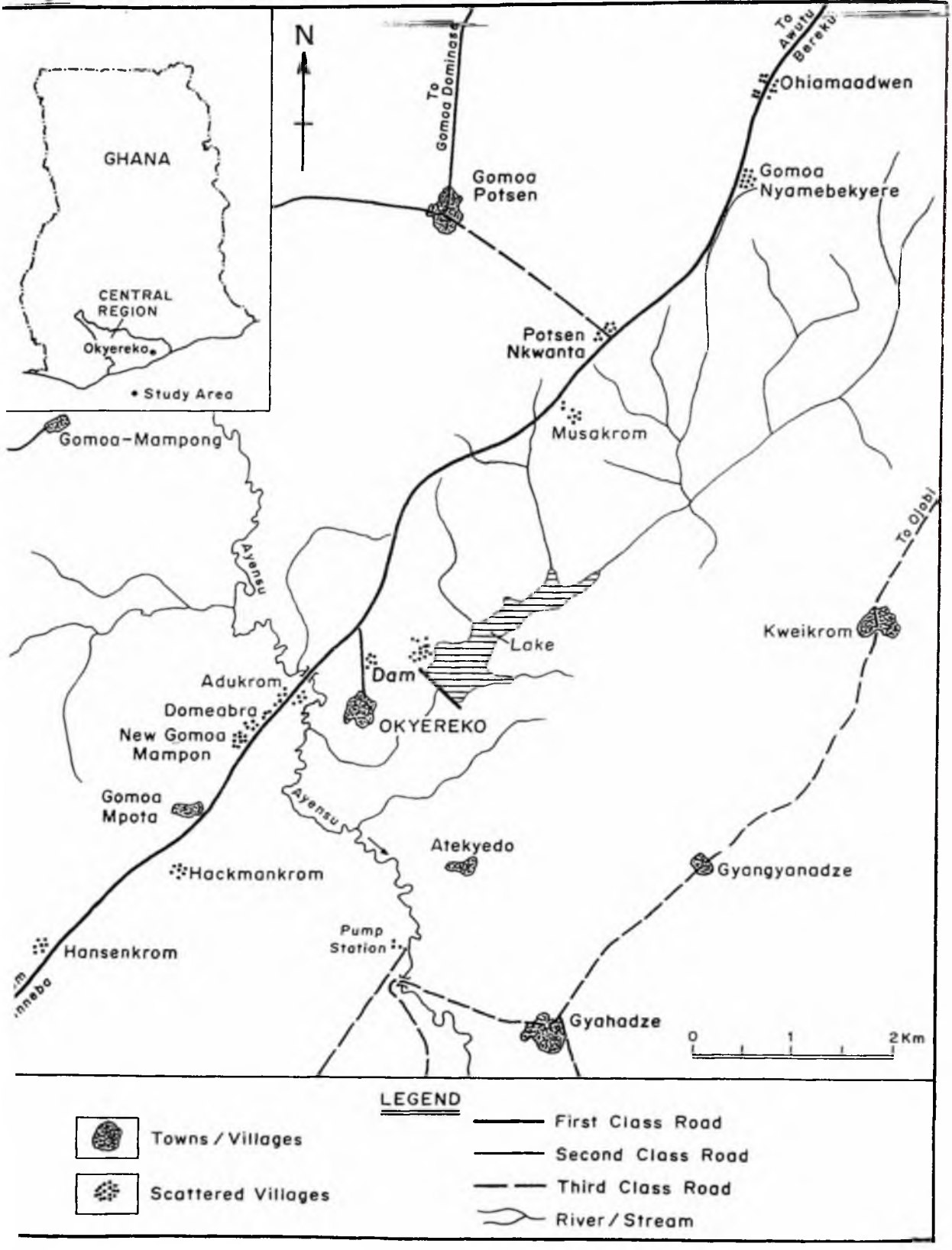
2.2.1 Village identification, community mobilization and ethical considerations

Prior to the start of the study, a map of the study area was collected from the Irrigation Development Authority of the Ministry of Agriculture. This was followed by a visit to the community to check on the existence of the village. After this has been confirmed, a series of meetings were held with the chiefs, elders and political leaders of the community to explain the essence of the study and seek their consent. A day was selected for a forum with all the residents of the community to describe and explain the procedures involved and answer questions to clarify any doubts and suspicions on the study. Their consent and participation was sought and the consent and participation of children was obtained from their parents.

2.2.2 Census

After approval for the study was given by the elders and responsible authorities of the Ministry of Health, the Irrigation Development Authority and the District Health Administration, all the houses in the village were numbered sequentially by the investigator with the assistance of five selected youth of the community nominated by the chief and elders. These youth were given prior training in simple demographic data collection such as house numbering and house to house registration of residents. A census was then conducted to register the usual residents of the community who are 1 year and above using the form in Appendix 1. During the census, all persons living in each house were registered by name, age, sex, and the duration of stay in the community. A map of the village was made depicting its location with reference to other landmarks such as water bodies, the irrigation dam and the streams flowing into it. Fig.1.

Fig. 1 MAP OF THE STUDY AREA — OKYEREKO, AND ITS ENVIRONS



2.2.3 Data Entry and Analysis

The data were entered into the computer using dbase program and age and sex compositions were analysed using SPSS. The individuals were divided into seven groups aged 1-9, 10-19, 20-29, 30-39, 40-49, 50-59 and ≥ 60. Data on age and sex compositions were used to construct a population pyramid.

2.3 Results

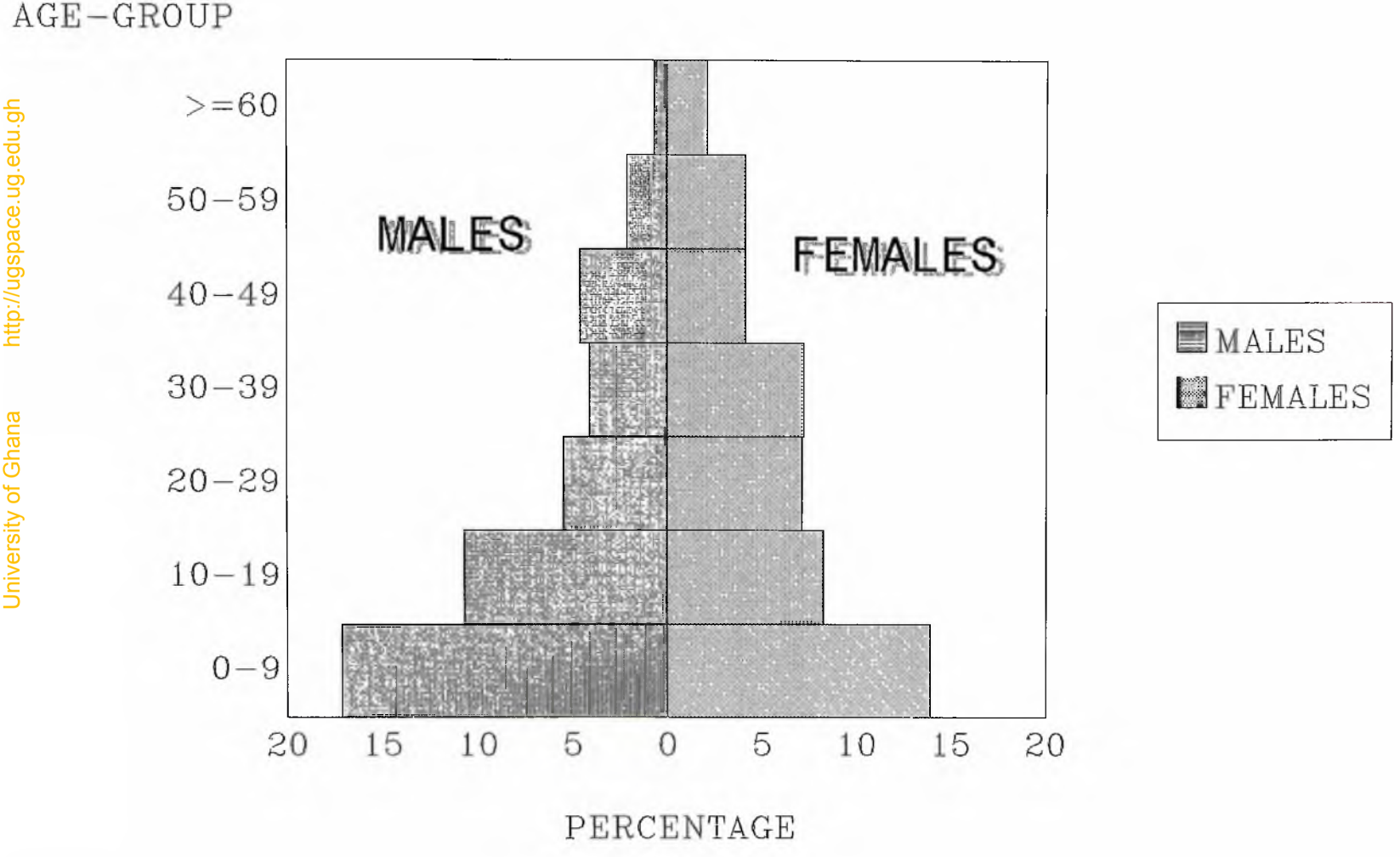
Table 1 shows the results of the census conducted in the study village. Overall, there were 636 inhabitants living in 99 houses. This was made up of 298 males (46.9%) and 338 females (53.1%). The age and sex distribution of the population is shown in a population pyramid in Figure 2. The most populous age group is the youngest age group (ie 1-9). The younger ages (0-19) form about 50% of the total population. Therefore the population pyramid is broadest at the base, with 31.1% in the (1-9) age group. This reduced to 18.9% in the (10-19) age group. The reduction continues with the (20-29) age group contributing 12.7%. The (30-39) group formed 11.3%, while (40-49) group forms 8.6%. The (50-59) year group are 6.1% while those above 60 years form 11.3% of the population.

Table 1. Population of inhabitants of Okyereko

	Males		Females		Males and Females	
Age-group (Years)	No.	% of total	No.	% of total	No.	% of total
1-9	109	17.1	89	13.9	198	31.1
10-19	68	10.7	52	8.2	120	18.9
20-29	35	5.5	45	7.1	80	12.7
30-39	26	4.1	46	7.2	72	11.3
40-49	29	4.6	26	4.1	55	8.6
50-59	13	2.1	26	4.1	39	6.1
≥60	18	2.8	54	8.5	72	11.3
Total	298	46.9	338	53.1	636	100.0

Fig. 2

POPULATION PYRAMID, OKYEREKO– 1995 CENSUS.



2.4 DISCUSSION

One important demographic fact about a population is its rate of growth. The population trend at Okyereko follows the "normal African population pyramid structure", which is broad at the base and narrowing at the apex with its consequent inbuilt dynamic growth. This follows also the age structure of Ghana's population in contrast to the population pyramid for an average developed country which is shaped more like a cylinder, reflecting that the distribution of the population is more even for all age groups. The implication is that countries with low population growth rates have a high percentage of their total population in the economically active age group (15-64). Looking at the trend of population growth in the various age groups, we realize that the population reduces as the age increases. This is normally the case in most developing countries putting a strain on the little resources available to them due to increasing rate of growth of the population. This concept is referred to as inbuilt dynamism (ie a situation where a large population base also grows to reproduce another large base, with consequent population problems due to constancy of available resources.

The rate at which a population is changing affects not only its size and numerical increase but also its composition. This is important in determining the provision of resources to match the population growth. In some populations, the number of males and females tends to be nearly equal and from the demographic viewpoint, a situation of near equality assures that each sex has an adequate supply of mates so that the population can replenish its losses from mortality. However, in most populations, the males outnumber females at the younger ages and females outnumbering males at the older ages.

An unique feature of the population of Okyereko is that at the base of the pyramid (ie 1-9 age group), males outnumber the females. It is, however, known that more male babies die between the ages of 0-5, making the female population within this group more often than not more than the males. This is because it is known that a disproportionately high percentage of male foetuses are miscarried or stillborn, so that a population in a poor nutritional state or in poor health may be expected to have a lower sex ratio (which is the number of males per a hundred or thousand females) at birth, other things being equal. Also in most populations, from the first instant of life onward, death rates for males are higher than those of females. This seeems to be due to constitutional factors and this is because males are more susceptible to most diseases and have higher death rates at every age. Also, within the reproductive age group (ie normally 15-44), it is expected that more females than males die resulting in higher male population than females. However, in the pyramid of Okyereko,the female population is higher than that of males. This point can be explained as due to migration of the male population. Most of the males in the economically active group move out of the community in search of greener pastures and most of the males in this age group at Okyereko are said to be involved in cocoa and other cash crop farming in some other parts of the Central Region and the Western Region. This observation in itself defeats the main purpose of the irrigation project which is aimed at allocating plots of land to the youth to indulge in agricultural activities in order to reduce the rural-urban drift.

Ghana's population, like any other country's is influenced by births, deaths and migration and the present high level of the population growth is the result of persistent high birth rates and declining mortality rates over the years (Benneh, 1990). Until recently, fertility has been

persistently high while mortality has consistently declined in the past three decades resulting in a population that is growing rapidly (Ghana Statistical Service, 1993). The fertility level of a country is the principal determinant of its rate of growth and rural women have a higher fertility rate of 6.4 compared to 4 in urban women. The women at Okyereko having a high fertility rate will apparently give birth to a lot of children resulting in a high population growth. For a variety of social and economic reasons, large families are attractive to many Ghanaians. The demographic data have therefore shown that at Okyereko there is the likelihood of a high rate of population growth due to the high fertility rate of the women, the broad base of the population pyramid with high numbers of infants.

CHAPTER 3

3.1 MICROFILARAEMIA AND CLINICAL MANIFESTATIONS IN LYMPHATIC FILARIASIS DUE TO *Wuchereria bancrofti*

3.1.1 The Life Cycle of *Wuchereria bancrofti*

The life cycle of *W. bancrofti* was first worked out by Manson in *Culex quinquefasciatus* in China in 1878 (Manson-Bahr *et al*, 1987). It involves both the human and the insect vector as definitive and intermediate hosts respectively. The female worm is viviparous liberating embryos into the lymphatic system in large numbers. The embryos or microfilariae are enclosed in a membrane and are therefore said to be sheathed. They ultimately escape from the lymphatics and appear in the peripheral blood. The microfilariae of *W. bancrofti* are long and slender measuring 260μm. They are taken up by the mosquito vector during feeding. Within an hour of entering the mosquito's stomach, they shed their sheaths and penetrate the midgut epithelium. They may however be damaged at this stage by the bucopharyngeal armature of the mosquito. Bryan *et al,* (1988) confirmed the damage of microfilariae by mosquito foregut but emphasized that the size of the mosquito does not seem to influence the amount of damage inflicted on the microfilariae. The damage explains the differing infection rates of the vectors responsible for transmission (Manson-Bahr *et al*, 1987).

At the end of an infective feed, the embryos collect at the anterior end of the stomach and then enter the anterior cylindrical portion of the midgut. Forward transportation from here is effected by reversed peristalsis until they are distributed over the whole cylinder. The proboscis of the mosquito exerts positive chemotaxis upon microfilariae therefore mosquitoes can abstract more

embryos than would be present in a similar quantity of circulating blood. The mosquitoes normally abstract 1mm^3 of blood at each feed and in so doing concentrates the embryos about tenfold (Manson-Bahr *et al*,1987). McGreevy *et al* (1982) demonstrated a "concentrating effect" of 8.6-12 fold of *W. bancrofti* in *Cx. quinquefasciatus*, *An. gambiae*, and *Aedes* in Tanzania. The parasites then enter the thorax where they lie between the fibrillar flight muscle of the thorax and the pharyngeal muscle of the head of the mosquito. Within 2 days they develop into thicker, shorter, "sausage" forms which undergo two moults before developing into elongated infective larvae measuring about 1.5 × 0.02mm. Mature third stage larvae leave the thoracic musculature and enter the haemocoele in which they move around actively and accumulate in the head. The complete life cycle in mosquito takes 10-14 days at high temperature and in moisture but is retarded to 6 weeks by cold (Manson-Bahr *et al*, 1987). They escape during the next feeding of the mosquito by entering the labium and rupturing the labella. They are deposited in a drop of haemolymph and enter the host through the puncture made by the feeding mosquito (McGreevy *et al,* 1974). The presence of the fluid prevents desiccation of the larvae on the surface of the skin (Lindsay *et al*, 1984). There is no asexual reproduction taking place between the infective larvae introduced and the adult stage. In man, the development of the worm takes at least seven to twelve months before becoming mature (Sasa, 1976).

3.1.2 Clinical manifestations

Clinical symptoms of lymphatic filariasis range from none to severe clinical manifestations such as elephantiasis and hydrocoele. The clinical manifestations may depend on host factors and parasite strain (Garcia *et al*, 1993). In a community where filariasis is endemic, individuals may be divided into the following clinical categories:

i) No microfilaraemia and no evidence of disease.

ii) Microfilaraemia with no evidence of disease.

iii) Acute filarial disease with or without microfilaraemia and

iv) Chronic filarial disease mostly amicrofilaraemic with or without acute attacks.

In most endemic communities, disease manifestation begins in late childhood or early adulthood, with acute attacks such as filarial fevers, adenolymphangitis and epididymo-orchitis.

3.1.2.1 Acute attacks

Early manifestations of filariasis is frequent high fevers, lymphangitis and lymphadenitis. Filarial fever usually begins with a high fever and chills that last one to five days before spontaneously subsiding and patients with filarial fevers in many cases do not have microfilariae. (Garcia *et al*, 1993). The attacks are often precipitated by hard labour in the fields but may occur without any apparent cause (Partono, 1984). The patient may be incapacitated for a few days, but occasionally may remain ambulatory. Acute episodes interrupt normal activities, rendering people unable to work, often confining them to bed. The events precipitating acute attacks are not known with certainty.

3.1.2.2 Chronic Disease

When the adult worms enter the lymphatic vessel, an inflammatory reaction occurs and there is an infiltration of plasma cells, eosinophils and macrophages in and around the affected vessel. Repeated inflammatory attacks produce hyperplasia of the endothelium resulting in an increase in hydrostatic pressure due to the damage to the lymph vessel and this in turn leads to an

increase in vessel wall permeability. The chronic leakage of high protein-containing fluid into the surrounding tissues results in a hard or brawny oedema, with thickening and varicous changes in the skin and this is known as elephantiasis. The onset of elephantiasis of the leg is frequently preceded by attacks of lymphangitis and lymphadenitis involving the inguinal glands draining the leg (Jordan, 1955). Lymphadenitis and lymphangitis develop in the lower extremities more commonly than in the upper, and apart from the limbs, there can be breast involvement.

Another chronic disease state frequently resulting from the infection with *W. bancrofti* is hydrocoele. The epitrochlear and femoral lymph nodes become firm, discrete and tend to remain enlarged. These conditions are preceded by severe pain in the scrotum (Garcia *et al*, 1993). The development of hydrocoele is usually characterized by an attack of epidymo-orchitis, epididymitis and funiculitis and these conditions are frequently associated with oedema of the scrotal skin and a small collection of inflammatory fluid in the tunica vaginalis (Jordan, 1955). There is swelling of the reflection of the peritoneal lining that surrounds each of the testicles and a clear hydrocoele fluid accumulates in this closed sac as a result of lymphatic blockage in draining lymphatics located in the retroperitoneal and sub diaphragmatic areas. Hydrocoele is the most common disease manifestation of *W. bancrofti* (WHO, 1992). Obstruction of the retroperitoneal lymphatics may cause the renal lymphatics to rupture into the urinary tract resulting in excretion of chyle in the urinary tract: a condition known as chyluria. Even though this condition is painless, large amounts of dietary lipids, proteins and possibly fat-soluble vitamins are excreted leading to weight loss.

The asymptomatic microfilaraemic state is not benign as was previously thought. Two sets of recent observations have revealed that this being clinically "asymptomatic" does not imply freedom from morbidity. It was recognized that most of the microfilaraemic individuals have haematuria and/or proteinuria which reflects low-grade renal damage which does not appear to be reversible after treatment (Dreyer *et al*, 1992). Secondly, using lymphoscintigraphy to visualize by radioisotope tracer techniques, it was found that almost all the microfilaraemic individuals even though asymptomatic, had markedly abnormal patterns of lymph flow (Freedman *et al*, 1994).

3.1.3 Mechanism of pathology

Experimental model systems have provided clear evidence that portion of the pathology resulting with bancroftian and brugian parasites is derived from the direct action of the parasites themselves or their released molecules on the lymphatic tissue, while the remainder results from the host's immune response to these parasites (Ottesen, 1991). It has also recently been observed that there is the danger of secondary bacterial and/or fungal infection in patients with lymph stasis and this may also contribute in part to the pathology in infected individuals (WHO, 1994).

Most of the pathology associated with bancroftian filariasis derives from damage to the lymphatics which ultimately leads to chronic lymphoedema, elephantiasis or chyluria. Normal lymph vessels are delicate endothelium-lined channels leading from an extensive network of

peripheral lymph capillaries through a series of collecting vessels and intermediate lymph nodes to the large cisterna chyli and thoracic duct that empties into the vena cava. In infected individuals, adult worms reside within these lymphatic vessels, generally in the afferent approaches or cortical sinuses of the lymph nodes. The parasite first causes dilatation of the lymphatic and then hypertrophy of the vessel wall. There is endothelial and connective tissue proliferation with polypoid growth protruding into the lumen, but so long as the worm remains alive, it appears the vessel stays patent. This patency, however, does not ensure normal lymphatic function and lymph stasis may occur while the worm is still alive. This may result in early lymphoedematous changes in the affected limb, genitals, kidney or breasts. Dreyer *et al* (1994) using ultrasonography demonstrated that adult worms located in the lymphatic vessels show marked stability in their location and did not appear to be disturbed by several potential sources of stress such as light, temperature change and manipulation of the lymphatic vessel of any kind. In ultrsonography, adult worms demonstrate a peculiar form of movement called " filarial dance sign"

Lymphatic pathology resulting from the presence of living worms include lymphatic proliferation, dilatation and oedema formation. Adult worms frequently invade the inguinal glands resulting in lymph stasis and this is the main factor in the formation of elephantiasis. There is the formation of extensive fibrosis in these nodes and this is an indication of lymphatic obstruction (Jordan, 1955). Obstructive obliterative reactions in the lymphatics results from dead parasites. When adult worms die, their death is associated with distinctive pathological changes. An area of necrosis develops around the dead parasite, followed by a granulomatous reaction

with the formation of cells and eosinophils. Collagen deposition results and the parasite is either resorbed or becomes partially calcified. It is during these inflamatory reactions that lymphatic obstruction occurs.

The manner in which an infected individual reacts immunologically to filarial antigen may determine whether or not lymphatic pathology will develop (WHO, 1992). The immune response to infection varies considerably, with antibody response the lowest in asymptomatic microfilaraemic patients and highest in amicrofilaraemic patients (Ottesen, 1982). In humans, IgG3 antibody response to filarial antigens are known to be made almost exclusively by patients who develop lymphatic pathology whereas IgG4 predominates in asymptomatic but microfilaraemic individuals (Hussain *et al*, 1987). Ottesen (1984) reported that in an endemic area, all exposed individuals mount cellular and humoral responses to the parasite and the measured levels of these responses vary among individuals but they tend to be similar and characteristic for those with similar clinical manifestation of infection and as such, they appear to be the determinants of clinical expression of filariasis. The different types of immune responses and thus pathological reactions are known to be genetic-based and are determined by differences in genetically restricted major histocompatibility complex-linked antigen processing in different individuals (Kwan-Lie *et al*, 1990). Tropical pulmonary eosinophilia (TPE) syndrome is the result of an immunological hyperresponsiveness on the part of the host to the parasite (WHO, 1984). Observations on the pathogenesis of TPE suggests that microfilariae released into the circulation from adult worms dwelling in the lymphatics are rapidly opsonized with antifilarial antibody and are then cleared in the pulmonary vasculature (Udwadia, 1975).

According to WHO (1992), patients with TPE have markedly increased numbers of inflammatory cells infiltrating the lungs and the majority of these cells are eosinophils. TPE is mediated by IgG antibody and the asthmatic symptoms resulting from the allergic responses is mediated through specific IgE antibodies bound to mast cells (Manson-Bahr *et al*, 1987). The acute phase is caused by an antibody-dependent mechanism while the chronic phase which is marked by pulmonary fibrosis may result from a type II delayed hypersensitivity response caused by an increase in or inadequately suppressed pulmonary lymphocyte reaction. This immunological hypersensitivity is found only in certain hosts and is probably of genetic origin. Prenatal conditioning could influence subsequent immunological and thus, pathogenic responses to filarial infection (Ottesen, 1991). Recent studies in Haiti have shown that maternal microfilaraemia predisposes to microfilaraemia in offsprings, and this is very important in explaining the differences in clinical manifestations of lymphatic filariasis among residents in endemic areas and those who migrate to such areas (WHO, 1992).

In many patients, there is the occurrence of intermittent episodes of lymphangitis or adenolymphangitis (ADL). This may be the only indication of filarial infection for many patients (Ottesen, 1982). The characteristic features of these episodes include the development of painful, tender lymph nodes with or without "retrograde" extension of the inflammation to involve the lymphatic tracks. This happens with a usual frequency of once or twice a year (but sometimes five or six times yearly.)

Recently, bacterial and fungal infections have been identified to be responsible for part of the pathology due to *W. bancrofti*. Recent evidence both from astute clinical observations and from immunohistological and bacteriological studies of tissues from lymphoedematous limbs of affected patients, has suggested that bacterial or fungal superinfections of limbs with compromised lymphatic function play the primary role in triggering most episodes of adenolymphangitis which, themselves, actually cause or exercebate the elephantiasis changes in affected patients (Addiss *et al*, 1994).

3.1.4 Diagnosis

Definitive diagnosis of lymphatic filariasis requires direct demonstration of the microfilariae since the adults worms are extremely difficult to detect. There are several methods available for diagnosis but the choice of method depends on its availability and the requirements of the particular study. The traditional or conventional methods of detecting microfilariae in the blood of infected persons involve the use of giemsa-stained smears, the counting chamber technique (CCT), the membrane filtration technique and the Knott's concentration method. However, diagnostic methods based on detection of microfilariae are often of low sensitivity because microfilariae can be present in very low numbers or sequestered in inaccessible sites, or they can be absent as in prepatent cases. Blod sampling may also be inconvenient particularly in those geographical regions where they exhibit nocturnal periodicity (Wamae, 1994). Alternatively, more recent techniques involving immunodiagnostic tools, lymphoscintigraphy and ultrasonography have been devised, even though they are not routinely employed for diagnosis.

3.1.4.1 Giemsa-stained thick smear: This method involves the collection of blood by finger prick unto a clean slide by using a sterile lancet, drying the blood and dehaemoglobinizing in clean tap water. The sample is dried again and fixed in methanol after which it is stained in Giemsa solution. The method is used to identify and quantitatively determine microfilarie. It is the most widely used method for field surveys. It is also a reliable method for qualitative and quantitative microfilarial studies and the processing of the blood slides need not be done on the spot. The method also provides permanent records. However, it is argued that some of the parasites could be lost during processing and staining of the blood films (Denham *et al*, 1971).

3.1.4.2 Counting Chamber Technique (CCT): This method involves the use of a counting cell which holds 100 cubic millimetres of liquid 1millimetre deep over an area of 50 X 20 millimetres. The base is divided into 1 millimetre squares. A cover glass traps liquid to the correct depth and a known volume of capillary blood (eg. 100μl, 60μl) washed into 3% acetic acid is poured into the chamber. By observing the liquid through a low magnification microscope, objects contained in each cubic millimetre can be identified and counted. The method allows fast and accurate counts of microfilariae to be made and it has been shown by (Denham *et al*, 1971) to be 30-40% more sensitive than the conventional stained blood smear. It also has a great advantage over other techniques due to the fact that positive results are demonstrable at the site of examination making it an excellent visual aid for education in the communities. However, this method cannot be used to distinguish species and should only be used in areas where the parasite species has previously been determined (Wamae, 1994).

3.1.4.3 Membrane (Nuclepore) Technique: This method involves the use of a nuclepore membrane filter with a pore size of 3-5μm into a filter holder and adding already collected anticoagulated venous blood by the use of a syringe and plunger. The blood is expressed through the filter to filter out microfilariae which are collected. The filter is then stained with Giemsa and examined under the microscope. This technique is sensitive and allows large volumes of blood to be examined and therefore enhances the detection of low level microfilaraemia (Wamae, 1994). McMahon *et al* (1979) working in Tanzania, compared this method with the CCT and the conventional thick smear and concluded that the use of the membrane filtration detected a proportion of infected individuals who would have been reported negative by the other methods. Membrane filters are however to be used in areas where low microfilaraemia is expected. The use of this method also involves venipuncture which is opposed by many communities.

3.1.4.4 Knott's Concentration Method: In this method, 1ml of blood is diluted in 9ml of distilled water and 1ml of formalin and centrifuged in a tube. After leaving the tube for some hours, the supernatant is poured off and the sediment which contains the microfilariae is examined. This method is however impractical where venipuncture is unacceptable (Wamae, 1994), and examination of the precipitate may be difficult since it may have a jelly-like consistency (WHO, 1994).

3.1.4.5 Immunodiagnosis: Immunodiagnostic procedures have become available for the diagnosis of filariasis for sometime now. Filarial nematodes are known to release antigenic products and elicit strong humoral antibody responses in human hosts (Ottesen, 1982). Both antigen and antibody detection assays have been developed as tools for the diagnosis of filarial infection. The importance of these assays lies in their ability to detect circulating antigens in both microfilaraemic infections (regardless of the time of day the blood is collected) and amicrofilaraemic cryptic infections. The antibody detction assays however have their drawbacks. Thus antibody responses appear to be persistent even after clinically defined cure has been achieved and they are therefore unable to discriminate between past and present infection (Lammie *et al* 1988). Also,the magnitude of the responses bear no relationship to parasite burden (Ottesen, 1984). More promising are recently developed assays for detection of filarial specific circulating antigens. Thus the Og4C3 antigen detection ELISA, which has recently become commercially available (Turner *et al*, 1993), appears to have a sensitivity and specificity close to 100%.

3.1.4.6 Lymphatic imaging: Recently, lymphoscintigraphy using radiolabelled colloid, albumin or dextran injected intradermally or subcutaneously and traced by a gamma-counter is being used to demonstrate the presence of lymphatic abnormalities in asymptomatic microfilaraemic individuals with no evidence of oedema. Their lymphatics are markedly dilated, with collateral channels. With this diagnostic method, it has now become possible to make a clear and precise analysis of the lymphatic system function in patients at risk (WHO, 1994).

3.1.4.7 DNA-detection assays: DNA-based technology can now be used for diagnosis of filarial infection both in humans and in the mosquito vectors by polymerase chain reaction (PCR-based) assays. Species-specific DNA probes developed so far detect DNA sequences that are highly repeated in the filarial genome and they are theoretically sensitive enough to detect DNA from a single filarial larva (WHO, 1992).

3.1.4.8 Ultrasonography: By using the method of ultrasonography, adult worms can be visualized in the lymphatics. Amaral *et al* (1994) used this method to describe adult worms performing " filaria dance" in the lymphatics. The visualization of adult worms by ultrasound is now available as a potential tool for direct assessment of the efficacy of adulticidal drugs.

3.2 MATERIALS AND METHODS

3.2.1 Study design

Every individual registered during the census was assigned a unique project number (PN). The PN of an individual indicated the village coded by two letters, followed by the house number, and a serial number within the community. For example, a man living in a house with house number OK045 who was registered as the 100th person in the community will have his PN as OK045100. This method is unique and has the added advantage of locating or tracing the person should the need arise. Fifty percent of the residents were selected from the compiled demographic data to constitute the study population. A simple record form which contained information on personal history, clinical examination and blood sampling time with the volume of blood collected was designed for each study individual. Refer to appendix 2.

3.2.2 Blood Collection for CCT:

Before each blood collection day, sampling tubes which are small 3ml test tubes were thoroughly cleaned with their lids and dried. Each was filled with 900μl of 3% acetic acid and stored in a rack. A central point was selected in the community where blood samples were collected. Blood collection was carried out between 21:00 and 01:00hrs. The pulp of the finger was cleaned with mediswab and the surface of the skin allowed to dry by allowing the excess alcohol to evaporate. A sterile lancet was used to make a perpendicular cut on the finger. The blood was allowed to flow freely and collected into a 100μl heparinized capillary tube and with a rubber bulb, the blood was transferred into the sampling tubes and mixed gently. It is stored well in the rack for examination at a later date. The tube was labelled with the date of blood examination and the PN of the individual. The volume of blood collected and the time of

collection were recorded on the record form. Occasionally if it became difficult to get the desired amount, the estimated volume taken was recorded and taken into consideration in the analysis.

3.2.3 Using the CCT:

For the examination and counting of the specimen, the fluid was transferred from the sampling tube to the counting chamber by pouring gently. A pasteur pipette was used to spread the fluid out to fill the whole well of the counting chamber, care taken not to damage any microfilariae. Any air bubble in the specimen was removed with a needle. The counting chamber with the specimen was placed under the microscope and left to stand for approximately 3-5 minutes, to enable the microfilariae settle at the bottom of the chamber. The specimens were then examined under the microscope with a magnification of 40X. The CCT enables speedy and accurate counts of microfilariae to be made.

3.2.4 Thick Blood Smear Preparation for Microfilariae Identification

Glass slides were cleaned in a watery solution of detergent and immersed in 96% alcohol. They were then dried and polished with a grease-free cloth, then stored in a slide box and taken to the field. To collect blood samples, the pulp of the finger was cleaned thoroughly with a mediswab, the excess alcohol allowed to evaporate and sterile lancet used to make a perpendicular cut on the finger which was held firmly between the collector's thumb and index finger. Care was taken not to squeeze the finger too much to dilute the blood sample with tissue fluid. The blood was allowed to flow and drawn into the 100μl heparinized capillary tube. Care

taken to avoid air bubbles in the tube which may result in underestimation of blood volume. The blood was expelled onto the cleaned microscope slide which has been already labelled with the individual's PN and date, using a rubber bulb. A rectangular thin smear of uniform thickness of blood was made and allowed to dry at room temperature in a horizontal position in a dust-free environment. Dry specimens were then sent to the laboratory for staining and examination later on. The blood samples for thick smear were collected at the same time blood was collected for CCT.

3.2.5 Giemsa-staining

Before staining, the smears were dehaemoglobinized in clean tap water for approximately 1-2 minutes, by immersing the slide with the dried blood in a vertical position in a staining jar in which tap water has been placed. The blood cells lyse and the pigment was seen falling into the water. This was done only for few minutes to avoid the likelihood of microfilarae falling off. The slides were removed and allowed to dry in the open air and fixed in methanol for approximately 1minute and allowed to dry again. The fixed smears were then stained in a diluted Giemsa solution (one part of Giemsa stock solution in 14 parts of buffered distilled water of pH 7.2) in a staining jar for half an hour. The slides were then rinsed briefly in clean water to remove excess stain and dried in upright position. They were then examined under the microscope with magnification of 40-100X under oil immersion.

3.2.6 Clinical Examination

A house was selected in the study village as a private examination room and all the selected persons were examined during the day by the clinician supervisor. All individuals aged ≥6 months were physically examined for evidence of acute and chronic signs and symptoms of bancroftian filariasis. Physical examination of males included the genitals, the legs and arms, and the lymph glands in the groin and axillae. Female examination was usually restricted to the legs and arms. The breast and genitals were to be included only when there was a history of their involvement. However, no such involvement was observed. Clinical manifestations were graded as described by Estambale *et al* (1994). Chronic manifestation of hydrocoele were graded into four stages. Grade I showed a swollen spermatic cord with either fluid accumulation somewhere along the cord, a lymphocoele above the testis or a testis larger than 6cm in longitudinal diameter without fluid accumulation. True hydrocoeles were graded as grades II-IV Grades II,III and IV were 6-10cm, 11-15cm and ≥15cm respectively, in longitudinal diameter with accumulation of fluid. Leg elephantiasis was graded as I when there is loss of contour and pitting oedema. Thickened skin with loss of elasticity and non-pitting oedema was graded as II, and grade III was evident elephantiasis with skin folds and papules. During the examination, males were asked if they had had a hydrocelectomy.

3.2.7 Data management and analysis

The number of parasites counted for each microfilaraemic individual were multiplied by a conversion factor and expressed as microfilariae per millilitre of blood. The geometric mean intensity (GMI) of microfilarial intensity was calculated as $[\Sigma \log (x+1)/n]$, where x is the

number of microfilariae per millilitre of blood in microfilaraemic subjects examined and n is the number of microfilaraemic subjects examined. Data on microfilariae prevalence, GMI, and the results of clinical manifestations resulting from infection of the parasite were entered into the computer using the dBase programme and analysed with SPSS. Microfilaraemia prevalence in different groups of subjects (sex and age groups) were compared statistically using the chi-squared test and microfilarial GMI in the different groups of subjects were compared with Student's t-test and ANOVA on log-transformed values. All P values of less than 0.05 were considered to indicate statistical significance.

3.3 RESULTS

3.3.1 Distribution of Selected Persons by Age and Sex

Table 2 shows the distribution of persons who were selected to participate in the study by age and sex and the proportion of those examined. Overall, 312 persons were selected and out of these, 296 making 94.4% of the selected population were examined for microfilaraemia and clinical manifestations of bancroftian filariasis. In most of the age groups, more females participated than males. Participation in the older age groups (ie $\geq$50 years) was high and in two age groups, 50-59 and $\geq$60, all the selected persons reported for examination, making 100% participation in these two age groups. The age group 40-49 however was poorly represented as only 57.1% of those selected came for examination. In the age group 10-19, 54 persons reported for the study out of the 60 selected, giving a coverage of 90%. However, during examination, 14 additional volunteers were examined resulting in 68 persons being examined.

Table 2. Distribution of study population by age and sex

Age-group (Years)	Total No. selected	No. examined (% of selected)		
		Males	Females	Total
1-9	99	54(54.5)	35(35.4)	89 (89.9)
*10-19	68	42(70.0)	26(43.3)	68(113.3)
20-29	40	15(37.5)	23(57.5)	38 (95.0)
30-39	36	7(19.4)	23(63.9)	30 (83.3)
40-49	28	9(32.1)	7(25.0)	16 (57.1)
50-59	19	7(36.8)	12(63.2)	19(100.0)
≥60	36	6(16.7)	30(83.3)	36(100.0)
Total	312	140(44.9)	156(50.0)	296 (94.4)

*: 14 additional volunteers participated in this age group.

3.3.2 Microfilariae identification

Microfilariae of Wuchereria bancrofti were identified after staining with Giemsa. In all, 50 samples were prepared and of these, 7 were identified as containing varied numbers of microfilariae. Identification was based on morphological characters such as pointed tail free of nuclei and body curve sinous, among other criteria.

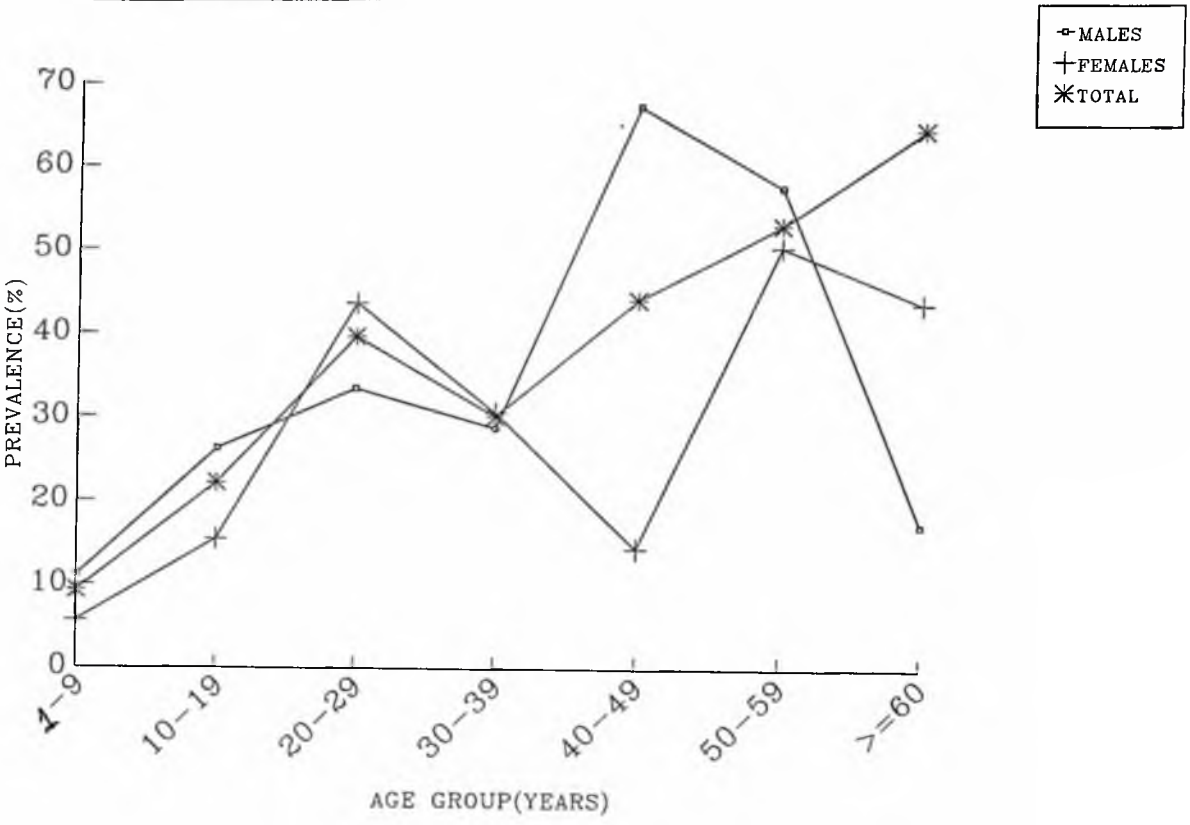
3.3.3 Microfilaraemia Prevalence

Results of microfilaraemia prevalence are presented in Table 3 and Figure 3. There were a total of 78 individuals infected with *W. bancrofti*, giving an overall prevalence rate of 26.4% in the community. Generally, prevalence was slightly higher in females than in males. However, the difference in prevalence between males and females was not statistically significant in any age group (χ^2 : $P > 0.05$) and in total prevalence in the two sexes (χ^2: $P > 0.05$). Of the 78 microfilaraemic individuals, 43(27.6%) were females while 35(25%) were males. In both sexes generally, microfilaraemia prevalence increased with age in the first 3 age groups after which it decreased in the 30-39 age group. After this, prevalence increased again until the 50-59 age group and reduced in persons above 60years. The same trend of increasing prevalence with increase in age is noted in the total prevalence of each age group until it again reduced in the 30-39 year group with subsequent increase thereof. The highest prevalence of 66.7% was in males in 40-49 age group while the lowest of 5.7% occurred in females 1-9 years. The youngest microfilaraemic person is a 3year-old girl with microfilarial intensity of 10mf/ml of blood.

able 3. Microfilaraemia prevalence by age and sex

Age-group Years)	No. examined	No.with microfilariae(prevalence in %) Males	Females	Total
1-9	89	6(11.1)	2(5.7)	8(9.3)
10-19	68	11(26.2)	4(15.4)	15(22.1)
20-29	38	5(33.3)	10(43.5)	15(39.5)
30-39	30	2(28.6)	7(30.4)	9(30.0)
40-49	16	6(66.7)	1(14.3)	7(43.8)
50-59	19	4(57.1)	6(50.0)	10(52.6)
≥60	36	1(17.1)	13(43.3)	14(63.9)
Total	296	35(25.0)	43(27.6)	78(26.4)

FIG. 3 PREVALENCE OF MICROFILARAEMIA IN THE DIFFERENT AGE GROUPS



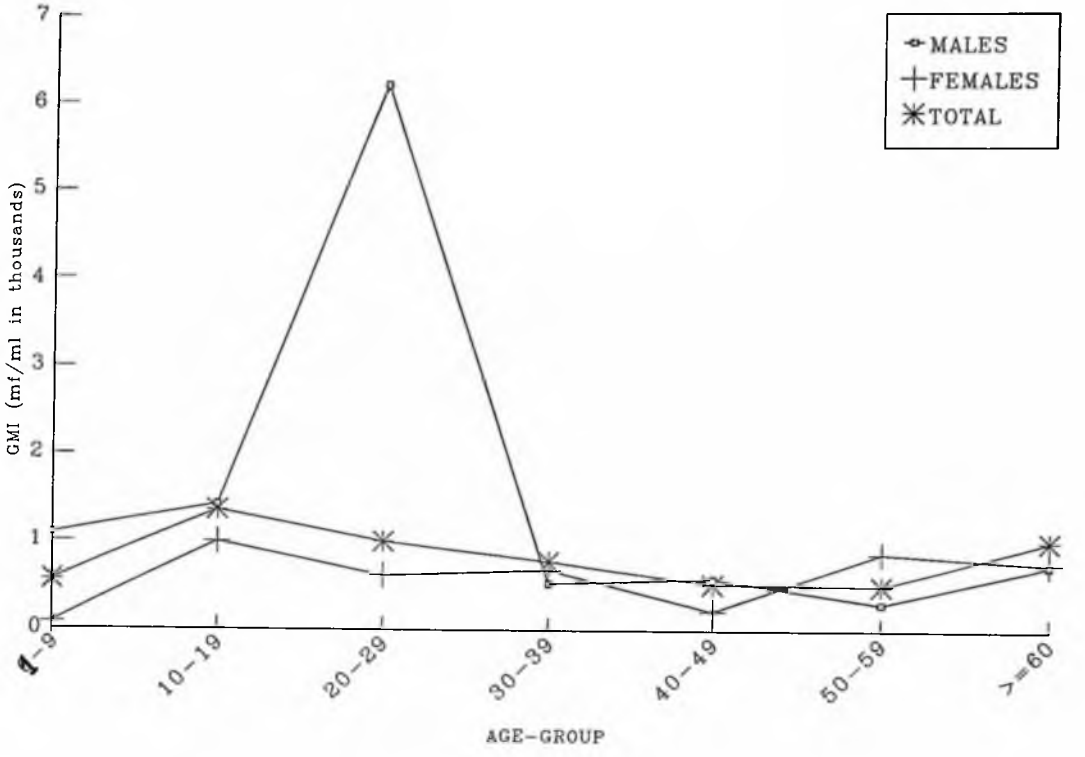
3.3.4 Microfilarial Intensities

Microfilarial intensities varied from 10-19240mf/ml of blood among micrifilaria positive individuals. For all microfilaraemic individuals in the community, the geometric mean intensity (GMI) was 819mf/ml of blood. It was higher in infected males (1114.3mf/ml) than in infected females (645.7mf/ml). However, microfilarial GMIs did not differ significantly between males and females in any age groups (two-way ANOVA; $P > 0.05$) or in total GMI between the two sexes (students' t-test; $P > 0.05$). In most age groups, the GMI was higher for males than for females. In males, except for the age groups 30-39 and 50-59, GMI increased with age. In females however, the variation of GMI with age does not follow a commendable order. Males in the age group, 20-29 had the highest GMI of 6194.4 compared to females in this age group who had 613.8. The lowest GMI of 218.8 ocurred in 40-49 age group (Table 4). The highest individual microfilarial intensity was 19270mf/ml.

Table 4. Geometric mean intensity of microfilaraemia by age and sex at Okyereko

Age-group	Geometric Mean Intensity		
(Years)	Males	Females	Total
1-9	1091.4	83.2	562.3
10-19	1422.3	995.4	1358.3
20-29	6194.4	613.8	1000.0
30-39	524.8	677.6	774.5
40-49	582.1	218.8	517.6
50-59	298.5	867.0	501.2
≥60	724.4	744.7	1000.0
Total	1114.3	645.7	819.0

GEOMETRIC MEAN INTENSITY (GMI) OF MICROFILARAEMIA BY AGE AND SEX AT OKYEREKO



3.3.5 Clinical Manifestations

3.3.5.1 Hydrocoele

Of the 140 males examined clinically for hydrocoele, 13 had hydrocoeles of grades I-IV giving a hydrocoele prevalence of 9.3%. All hydrocoele cases were however seen in males aged ≥18 years. There were 8 true hydrocoeles (ie grades II-IV) giving the prevalence of true hydrocoeles as 5.7% while 5 of the hydrocoele cases were grade I (ie 3.6% prevalence rate)(Table 5). The prevalence of hydrocoele increased with age and the highest prevalence of 33.3% was noted in the 40-49 age group. This was followed by 28.6% prevalence in the 50-59 age group. The commonest grades of hydrocoele were I and II occuring in 80% of hydrocoele cases. The only grade IV hydrocoele was in a 19 year old boy. Three males with hydrocoele were microfilaraemic including the case of the grade IV hydrocoele who had microfilarial intensity of 1130 mf/ml. This gives a prevalence of microfilaraemic hydrocoele cases as 23.1% The other two aged 42 and 56 and both with grade I hydrocoeles had microfilarial intensities of 20 and 70 mf/ml respectively.

Table 5. Prevalence of Hydrocoele in males with respect to age group.

	Hydrocoele			
Age-group (Years)	No. Examined	No. with Grade I	No. with Grades II-IV	Total no. (Prevalence in %)
0-9	54	0	0	0 (0.0)
10-19	42	1	2	3 (7.1)
20-29	15	1	2	3 (20.0)
30-39	7	1	0	1 (14.3)
40-49	9	1	2	3 (33.3)
50-59	7	1	1	2 (28.6)
≥60	6	0	1	1 (16.7)
Total	140	5	8	13 (9.3)

3.3.5.2 Limb Elephantiasis

The results of limb elephantiasis prevalence are shown in Table 6. Of the 296 persons examined clinically, 4 had elephantiasis and all 4 were females aged 13 to 55. This gives an overall prevalence of limb elephantiasis as 1.4%. Two were grade I and the other two grade III. There was no grade II elephantiasis. As in the case of hydrocoele, the prevalence of elephantiasis increased with age. The highest prevalence of 12.5% was in the 40-49 years age group and this formed 50% of the cases who were all grade III. Generally, elephantiasis was predominant in the older age groups (ie 40 years and above) but there was a case of grade I elephantiasis in a 13 year-old girl. One of the elephantiasis females was microfilaraemic with intensity of 220mf/ml and elephantiasis of grade III.

Table 6. Prevalence of elephantiasis in both sexes with respect to age group.

		Elephantiasis				
Age-group (Years)	No. Examined	Grade I	Grade II	Grade III	Total	Prevalence (%)
0-9	89	0	0	0	0	0
10-19	68	1	0	0	1	1.5
20-29	38	0	0	0	0	0
30-39	30	0	0	0	0	0
40-49	16	0	0	2	2	12.5
50-59	19	1	0	0	1	5.3
≥60	36	0	0	0	0	0
Total	296	2	0	2	4	1.4

3.4 DISCUSSION

Information on the prevalence and distribution of any disease is necessary for determining its public health importance and the subsequent planning of any control programme. This is because the evaluation of any control programme must have a reference point if it is to be meaningful. According to WHO (1992), data on the prevalence of lymphatic filariasis in sub-Saharan Africa is very limited. As a result therefore, the true picture of the disease burden is not known. This has made it difficult to assess the public health importance of the disease in this region.

This study has identified lymphatic filariasis as a major public health problem in the study community. Apart from earlier epidemiological surveys in the Kassena-Nankana District (Gyapong *et al*, 1994), a national filariasis survey (Gyapong *et al*, 1996) and reports of an epidemiological study along the coast of Ghana (Dunyo *et al*, 1996), a detailed information on the prevalence and distribution of the disease in an irrigation project community with conditions that favour the profuse breeding of the mosquito vectors involved in transmission have not been documented in Ghana. Earlier reports by Hunter (1992) identified small village dams in north east Ghana as potential transmission areas for lymphatic filariasis. In this report, Hunter pointed out that the closer a house is to the main canals in an irrigation community, the greater the frequency of filarial disease and the key to understanding this observation is the intensity of mosquitoes biting.

The overall microfilaraemia prevalence of 26.4% reported in this study indicates a high level of endemic filariasis, according to the World Heath Organisation (OMS, 1988). The prevalence of microfilaraemia is similar to those reported in Kassena-Nakana (Gyapong *et al*, 1994), the endemic areas on the coast of Western Region of Ghana (Dunyo *et al*, 1996) and Indian Ocean coast of Kenya and Tanzania (Estambale *et al*,1994, Wijers,1977, Meyrowitsch *et al,* 1995, and Simonsen *et al*, 1995). A lower prevalence rate of 9% had been reported in the Igwun Basin of Nigeria (Udonsi, 1988).

The slightly higher microfilaraemia prevalence in females than males does not conform with the trend in most reports (Estambale *et al*, 1994, Gyapong *et al*, 1994, Simonsen *et al*, 1995). Several investigators have suggested that females, especially those of childbearing age, are more resistant to infection of same age group and this was supported by serological studies which showed a higher antibody positivity to adult worm antigen in females (Brabin, 1990). However, observations by Brunhes (cited by Wijers, 1977) concluded that in high transmission areas, the differences in microfilariae rates generally observed between males and females tends to disappear because of the higher microfilarial densities in these areas. Jordan (1960) stated also that in many parts of Africa, the night-biting *A. gambiae* s.l and *A. funestus* were the main vectors and it was unlikely that one sex would be more exposed than the other. Specific physiological as well as behavioural and occupational differences between the two sexes therefore could be a likely predisposing factor affecting parasite establishment in the two sexes. However, very little differences between male and female occupations and behaviour patterns were observed in the community.

Microfilaraemia was generally rare in young children and prevalence generally increased with age and were high in the older age groups. If young children are equally exposed to moquito bites in the community, then the rare prevalence rate in them is surprising, especially when the pre-patent period of only 7 to 8 months is expected for the establishment of *W. bancrofti* (WHO,1992). However, the rare occurrence of microfilaraemia in young children might be related to the finding in transmission studies that high numbers of infective bites over a prolonged period of time are necessary before establishment of a patent infection with microfilaraemia will occur (Southgate, 1992). Parasite induced tolerance is also said to appear to play a role for survival of filariae in their hosts (Ottesen,1989) and long exposure period may be necessary for the parasites to induce a state of tolerance in the host before an infection can develop to patency. Steel *et al* (1994) in explaining the role of the immune system in the development of lymphatic pathology have shown that the microfilaraemic status of the mother during pregnancy influences the immune response of an individual to microfilarial antigens and thus children born of microfilaraemic mothers are much less capable of responding to microfilarial antigens than those of noninfected mothers even 17 years after birth.

The overall mean microfilarial intensity (GMI) was higher in males than in females. However, an immense variation was observed in individual microfilarial intensities. For most age groups, GMI was higher in males than in females. But this was not significant in any age group. The higher GMI in males than in females in most age groups is in accordance with reports by Bundy (1988) that males are more susceptible to parasitic infections than females. As most blood samples were obtained around the same time (ie between 21:00 and 02:00hrs), microfilarial

periodicity cannot be assumed to be resposible for these variations. However, the abnormally very high GMI of the five males in the 20-29 age group can be explained that all of them reported for blood sampling late (four of them were sampled at 01:25, 01:35, 01:45and 02:00hours) and this period happens to coincide with the peak hour from the periodicity study when maximum numbers of the parasite appear in the peripheral blood. Adult worms are only accidentally recovered from humans, and whether microfilarial intensity reflects adult worm burden is unknown. Studies on circulating antigens from adult *W. bancrofti* by Lammie *et al* (1994) have shown a significant, although weak correlation between antigen concentrations and microfilarial intensities. Therefore what actually determines the variations in microfilarial

intensities in infected individuals (whether adult worm burden, host regulation mechanisms or both) and the reasons for the great variability between individuals remain unclear (Meyrowitsch *et al*, 1995).

The GMI of all microfilaraemic persons were comparable with what has been found in other parts of Ghana and Africa. Gyapong *et al* (1994) recorded a GMI of 794mf/ml of blood in Kassena-Nankana. Along the coast of Ghana, Dunyo *et al* (1996) working in six villages and three urban communities recorded an overall mean intensity of 321-1172mf/ml of blood in microfilarial positive villages. In the Kwale District, Kenya, Estambale *et al* (1994) reported a lower GMI of 223mf/ml of blood. In North-eastern Tanzania, Meyrowitsch *et al* (1995) reported a range of 331-1202mf/ml.

Hydrocoele was the commonest chronic clinical manifestation of bancroftian filariasis in the community. A prevalence of 9.3% was seen in males aged ≥18 years. The prevalence of limb elephantiasis was 1.4% of those examined. It is considered that hydrocoeles may form more easily than limb lymphoedema because of the smaller increase in lymphatic pressure involved (Harinath, cited by Brabin, 1990). A higher prevalence of hydrocoele was reported in Kassena-Nankana (Gyapong *et al,* 1994) and (Dunyo *et al*, 1996). In other parts of Africa, higher prevalences were reported (Meyrowitsch *et al*, 1995, Wijers, 1977, Estambale *et al*, 1994). The prevalence of hydrocoele generally increased with age and the highest prevalence was noted in males above 40 years. All persons with elephantiasis were females and the fact that this condition has severe personal and social consequences cannot be underemphasized. Generally, the prevalence of elephantiasis increased with age and it is predominant in the older age group (ie above 40 years). This may have a detrimental effect on the mobility, working capacity and social acceptance of the affected individuals (Evans *et al*, 1993).

All the elephantiasis cases were seen only in females and similar higher prevalence in females than males was reported by Gyapong *et al* (1994) and Dunyo *et al* (1996) from other parts of Ghana. There was also a higher prevalence of microfilaraemia in females than males in the study community. However, with the very low numbers, this may be due to chance if not occupationaly-related. The study community is predominantly a farming community with majority of inhabitants being peasant farmers engaged actively in working on the rice farms on the irrigated plots with the two sexes being equally exposed to frequent lacerations or cuts at the lower extremities. Therefore, occupational differences could not be the likely factor.

There is similarity in the prevalence of microfilariae in persons with the two clinical outcomes of bancroftian filarial infection in the community (ie hydrocoele and limb elephantiasis). Three males with hydrocoele were microfilaraemic, and one of the elephantiasis cases was also microfilaraemic. A similar pattern of low microfilaraemia in elephantiasis patients has been reported by Simonsen *et al* (1995) in three endemic communities in Tanzania. A correlation between the severity of lymphatic lesions and host response, either cellular or humoral to parasite antigens has been described by Ottesen (1992) and if host responses are enhanced during reproductive years as proposed by Brabin (1990), then clinical disease might be expected in these groups of persons. However, in the study, there seems to be very little evidence that this occurs and overall, elephantiasis is more prominent in the older age groups.

CHAPTER 4

4.1 PERIODICITY STUDY OF LYMPHATIC FILARIASIS PARASITE

4.1.1 Introduction

Infection of humans with *Wuchereria bancrofti* begins with a bite by an infected mosquito which introduces the parasite into the host. The adult worms live in the lymph channels and paired adult worms produce millions of larval forms called microfilariae that circulate in the bloodstream. The density of microfilariae present in the peripheral blood varies throughout a 24-hour cycle. Depending on the time of the day that the maximum numbers appear in the peripheral blood in a 24-hour cycle, the periodic cycle may be referred to as nocturnal or diurnal (McMahon *et al*, 1996). Also, variations in completeness (ie the difference between the numbers of parasites present at the peak and the low point of the cycle) results in the cycle being referred to as periodic or subperiodic. In nocturnally periodic *W. bancrofti* for example, this difference is almost 100%, whereas in *Dirofilaria immitis* in the dog, although a definite peak occurs during the evening, approximately 30% of this number is present throughout the whole cycle and this state is defined as subperiodic (WHO, 1967). The nocturnally periodic type thus shows a marked peak of microfilarial density in the peripheral blood during the night hours, very few if any, during the day. In the nocturnally subperiodic and diurnally subperiodic types, microfilariae can be found in the peripheral blood at all hours but their density increases slightly during the night or day respectively. Manson first observed the phenomenon of periodicity in 1879 in Amoy (South China) where he found that the parasites were numerous in the blood of infected patients at night but became rare or absent from the blood during daytime. The periodicity occurs as the result of retention of large numbers of microfilariae in the lung

capillaries during certain hours of the day and their release into the circulating blood during other hours (Sasa, 1976). Various hypotheses or theories have been proposed as to the host or environmental factors responsible for such a circadian rhythm. In most instances, the pattern of periodicity of a form of microfilaria appears to be synchronized with the biting habits or activity of the mosquito vectors in the same area, thus ensuring a high level of transmission of the infection (Hawking, 1967, Sasa, 1976). Periodicity of microfilariae however can be easily converted by a change in the rhythm of the day (Manson-Bahr *et al*, 1987). Thus the periodic cycle is stable under "natural" conditions in a given locality but depends upon the activity of the host in relation to the day to night cycle. The numbers of microfilariae are influenced by sleeping and respond to waking and bodily activity. By reversing the hours of sleeping and waking, the periodicity is disturbed for 3 days and then reversed to diurnal periodicity. The parasite thus has the ability to adjust its cycle, after a brief delay, to changes in the host's activity cycle. This has been demonstrated many times for *W. bancrofti* and for other filariae in humans.

4.1.2 Mechanisms of Periodicity

Two mechanisms for periodicity have been suggested. These include the alteration in oxygen tension of the blood and phototaxis on the part of the microfilariae. The periodicity of *W. bancrofti* may depend on changes in the difference in oxygen tension between venous and arterial blood by day and night (Manson-Bahr *et al*, 1987). During daytime, the microfilariae accumulate in the lungs where the oxygen tension is high and they manage to hold themselves in the pulmonary capillaries by some force which is increased by the rise in oxygen tension and

decreased by its fall. This force seems to be switched on and off every 12 hours by an unknown mechanism inside the microfilariae (Manson-Bahr *et al*, 1987). It is presumed that microfilariae gather together in the capillaries and other vessels of the lung during their absence from the peripheral blood by the power of agglutination and thigmotaxis (which is the movement of the microfilarie in response to the stimulus of contact or touch to the lungs). By injection of an anticoagulant, heparin, to the drawn blood, a curious agglutinative phenomenon has been described. Intravenous injection of this subtance during daytime releases microfilariae into the peripheral blood for a short period. Hawking (1967) presents evidence that the migration of microfilariae is related to responses to circadial variations in host's physiological factors such as oxygen tension and temperature. For example, in man, the venous-arterial difference in oxygen tension is lower by night (40 mmHg) than by day (55 mmHg) with the result that microfilariae of *W. bancrofti* pass through the lungs by night but accumulate there by day. This pattern can thus be upset by causing a patient to breathe oxygen at night. A different mechanism for periodicity has been suggested in which the microfilariae are said to possess a photosensitive substance containing a vitamin A-like carotenoid similar to visual pigments in flourescent granules in the epidermis which causes them to leave the peripheral circulation in daylight and collect in the lungs. Periodic microfilariae therefore possess numerous granules in contrast to subperiodic forms which have few or none, Masaya, cited by (Manson-Bahr *et al*, 1987).

Studies by a number of workers in various regions of the world have revealed that there exists different forms even within the same species which differ in the pattern of microfilarial periodicity (Sasa, 1976). Thus differences in the nature of the periodic cycle may occur not

only between species of filariae but between "varieties" within a single species. *W. bancrofti* and *Brugia malayi* both occur in two forms within humans. Throughout the major part of its geographic range, *W. bancrofti* is nocturnally periodic, but in the South Pacific region, a diurnally subperiodic form exists (Sasa *et al*, 1972).

4.1.3 Implications of microfilarial Periodicity for Diagnosis

Diagnosis of bancroftian filariasis is generally dependent on the finding and identification of microfilariae in blood specimens (Wamae, 1994). To know the best time to take blood samples to detect microfilaraemia, there is the need to determine the periodicity of the parasite in the locality (WHO, 1987). Results of various surveys in many parts of the world have indicated that the different types of filarial parasites exhibit different periodic patterns in the diferent geographic regions. Results of surveys in Asia and the Pacific have indicated that at least five forms differing in type of microfilarial periodicity occur in the region. These include the nocturnally periodic *W. bancrofti* which is found widely in the tropical and subtropical zones, the diurnally subperiodic form in the South Pacific islands, the nocturnally subperiodic *W. bancrofti* recently discovered in West Thailand, the nocturnally periodic *Brugia malayi* widely distributed in South and East Asia and the nocturnally subperiodic *B. malayi* found in some rural areas in Malaysia, Indonesia and the Phillipines (Sasa *et al*, 1972). Ealier reports by many workers in these regions in which the periodicity index and peak hours were reported agreed with the above findings (Ramachandran *et al*, 1964, Rosen, 1955).

In Ghana, early works by Muirhead-Thomson (1954) reported of a plateau of microfilarial density rather than a peak when working on three subjects in Weija near Accra. A somewhat similar report was documented earlier on in Liberia where although a somewhat peak actually occurred between midnight and 02:00hours, a high microfilarial density near maximum was obtained between 21:00 and 04:00hours (Poindexter, 1950). These reports, although representative of the phenomenon, do not conform to the current acceptable mathematical analysis of periodicity data. The nocturnal periodicity of *W. bancrofti* microfilariae using the statistical method was confirmed recently in Kenya and Tanzania (Gatika *et al* 1994, Simonsen *et al*, 1996), There is however generally very little information on microfilarial periodicity in Africa as a whole. Surveys on *W. bancrofti* have been conducted at night in Ghana without documentary evidence of its periodicity and peak hour. This study therefore aimed at establishing the periodic pattern of the parasite since accurate information on the pattern of periodicity will be a useful guideline with respect to the optimal sampling time.

4.2 Materials and Methods

One month after blood samples were read and microfilaraemic individuals identified (see previous chapter), eight males with ages between 17 and 56 years were selected to participate in the periodicity study. The study participants were transported to Accra and accomodated at the Legon Hall of residence of the University of Ghana from the evening of the first study day, until the end of the following day. Each of the participants' age was noted and individual numbers were assigned to them.

Blood samples were collected from them every two hours starting from 18:00hours and ending at 16:00hours the next day. At each examination time, the pulp of the finger was cleaned thoroughly with mediswab, excess alcohol allowed to evaporate and a sterile lancet used to make a perpendicular cut on the finger which was held firmly between the examiner's thumb and index finger. The blood was allowed to flow and drawn into a 100μl heparinized capillary tube and expelled into the labelled test tube with the help of a rubber bulb. The tube was labelled with the date, time of collection and the individual number of the subject. During the daytime, subjects were made to perform simple excercises like walking around before blood was collected in order not to simulate night conditions. The blood samples were transferred from the sampling tubes into the counting chamber and the microfilariae for each individual counted for each hour.

4.2.1 Periodicity Data Analysis

Analysis of microfilarial periodicity was until recently done by just recognizing the presence or absence of microfilariae in blood examinations carried out both by day or by night. Such simple and non-quantitative methods obviously harbour the risk of causing various misunderstandings of the real nature of the phenomenon. Since the individual microfilaria counts are known to be subject to considerable errors of variation, efforts were made to develop mathematical methods for analysis of microfilarial data based on statistically most reliable measures such as the mean and the standard deviation. The basic idea employed for the statistical analysis of the microfilarial periodicity is that it is a biological phenomenon which can be interpreted simply as a wave of the microfilarial density in the circulating blood in relation to the hour of day. The time length required for the wave for one phase, or the wave period, is 24hours and is common

to all filarial forms. Therefore, the conventional terms "periodic", "subperiodic", and "nonperiodic" can be denoted by the relative amplitude of the wave and the terms "nocturnal" or "diurnal" can be defined more precisely by the time of day at which the peak density of microfilariae is encountered, ie peak hour (Sasa *et al*, 1972).

Generally, a wave formula is determined by various factors such as the amplitude, the wavelength, the period, the phase and the wave profile. In the case of microfilarial periodicity, the factors involved are simple because the wavelength and speed are fixed to 24-hour rhythms. Therefore the only three factors necessary to determine the character of such a wave are: the relative amplitude (the grade of difference between the maximum and minimum densities), the phase (the hour of day the density becomes maximum and minimum), and the wave profile.

In general, a wave profile such as the microfilarial periodicity can be expressed by the following simple formula:

$$y = m + a(fx) \qquad (1)$$

It was shown by Sasa (1976) that the microfilarial periodicity followed the simplest example of such a wave, namely the harmonic form (sine or cosine curve). The density of microfilariae y at the hour h can be expressed by the following simple formula:

$$y = m + a\cos x \; ; \qquad (2)$$

As 24 hours correspond to 360°, the hours 0 to 24 are multiplied by 15 to correspond to angles 0° to 360°.

Therefore, when $x = 15(h-k)$

$$y = m + a\cos 15(h-k) \qquad (3)$$

where a is the amplitude, m is the mean density and k the peak hour of microfilarial density in the blood.

By expanding (3) according to Aikat and Das (1977), we obtain

$$y = m + a\{\cos(15h - 15k)\}$$

$$= m + a\{\cos 15h \cos 15k + \sin 15h \sin 15k\}$$

$$= m + a\cos 15h \cos 15k + a\sin 15h \sin 15k$$

or $$y = m + b\cos 15h + c\sin 15h \qquad (4)$$

where $$b = a\cos 15k \qquad (5)$$

and $$c = a\sin 15k \qquad (6)$$

so that $$a^2 = b^2 + c^2 \qquad (7)$$

and $$\tan 15k = c/b. \qquad (8)$$

Thus the problem of estimating the parameters m, a and k of (3) is reduced to that of estimating the parameters of (4) and then application of relationships (7) and (8).

The least square estimates of m, b and c of equation (4) are as follows:

$$m = 1/n\ \Sigma y \qquad (9)$$

$$b = 2/n\ \Sigma y \cos 15h \text{ and} \qquad (10)$$

$$c = 2/n\ \Sigma y \sin 15h. \qquad (11)$$

In estimating these parameters, the following procedures were followed.

Microfilarial intensities at the hours of examination were recorded for each individual and the arithmetric mean microfilarial intensity calculated. Taking the mean intensity as the common denominator, microfilarial ratios were calculated for each blood sampling time as:

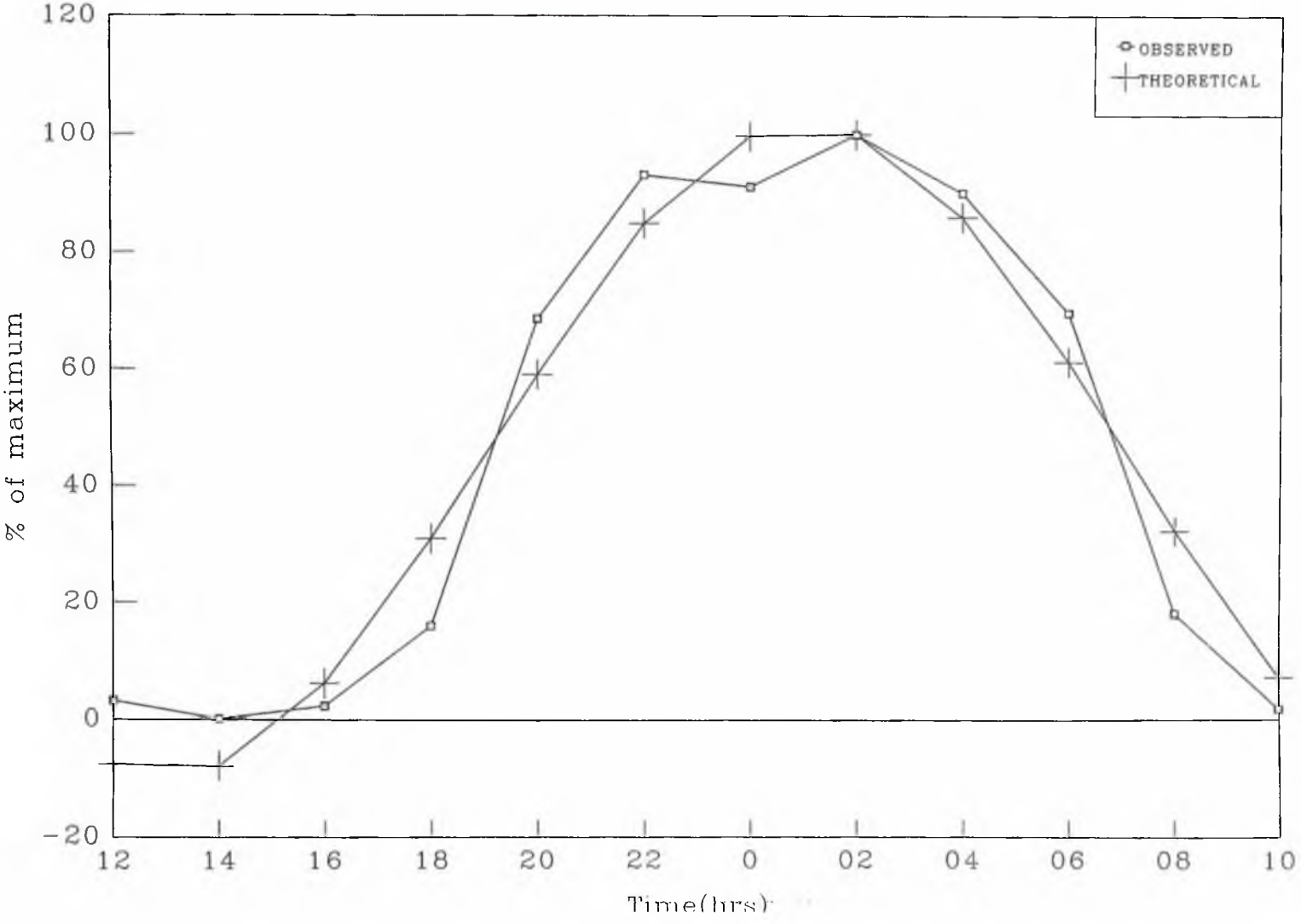
$$\text{Microfilarial ratio} = \frac{\text{microfilaria intensity}}{\text{mean density}} \times 100$$

The mean of the microfilarial ratios for the eight study individuals was calculated for each hour and were further expressed as a percentage of the maximum value. These represented the observed microfilarial ratios which were used to draw the observed microfilarial periodicity curve. Trigonometric analysis of the observed microfilarial ratios by the method of Aikat and Das (1977) making use of equations (7) and (8) and expressions (9), (10) and (11) enables the calulation of the m, b and c. These values were used to arrive at the harmonic wave equation for the microfilarial periodicity in the study area, which can be expressed as:

$$y = 100.2 + 116.7\cos 15h + 32.8\sin 15h$$

This represented the theoretical microfilarial equation which was used to calculate theoretical microfilarial ratios and the ratios were further expressed as a percentage of the maximum value. These were used to draw the theoretical microfilarial periodicity curve (Fig.5).

FIG 5 OBSERVED AND THEORETICAL MICROFILARIAL RATIOS-PERIODICITY CURVE



4.3 Results

Microfilarial intensities at the hours of examination in the periodicity study are presented in Table 7. Overall, the 2-hourly microfilarial intensities in the individuals ranged from 0-15740mf/ml of blood over the 24-hour period. Generally, except in a few samples, the mf intensities were low from the early hours of the morning (ie 06.00hrs) and increased gradually until maximum numbers from 22.00hours to 04.00hours. The individual mean microfilarial intensities ranged from 105.8 to 6150mf/ml.

Table 8 shows the results of microfilarial ratios calculated as the ratio of the two-hourly count to the mean 24-hour intensity for each individual. Microfilarial ratios ranged from 0 to 303. The means of the ratios were calculated and the results expressed as observed mean microfilarial ratio. The observed mean microfilarial ratios increased from low values during the day to high values in the night until a peak of 217.5 occurs at 02.00hrs. The mean microfilarial ratios were further expressed as percentage of the maximum value (217.5 which is the observed peak hour value) and these percentages have been used to draw the observed microfilarial periodicity curve shown in Figure 3. It is obvious that the observed and theoretical periodicity curves follow each other closely, especially between 20.00 and 10.00hours and the harmonic wave equation thus expresses rather accurately the relationship between microfilarial ratio and the time of day for the study area.

Table 9 is the trigonometric analyses of the microfilarial periodicity by the method of Aikat and Das (1977). From this, the periodicity index (D) has been calculated as 122.6 and the peak hour (k) is 1.03 (ie 01:03hrs)

Table 7. Two-hourly microfilarial intensities in the eight study individuals.

Case No.	Age and sex	Microfilaria intensity (mf/ml) at the hours of examination												Mean mf intensity
		18.00	20.00	22.00	0.00	02.00	04.00	06.00	08.00	10.00	12.00	14.00	16.00	
1	24M	60	870	2030	3640	3990	3430	3080	1450	40	10	0	10	1550.8
2	18M	50	310	310	460	560	440	110	0	10	90	0	0	195.0
3	20M	30	860	1610	2200	1610	2140	1970	610	40	0	0	0	922.5
4	18M	3210	6850	10480	9850	8270	10060	4130	670	100	120	0	700	4537.0
5	25M	4860	9560	15060	15740	8470	11800	6870	630	120	120	80	490	6150.0
6	56M	110	240	420	170	430	190	105	20	0	0	0	20	142.0
7	17M	40	260	470	330	480	330	585	150	10	10	0	0	222.0
8	19M	0	290	180	140	190	190	210	50	20	0	0	0	105.8

	Mf ratios at hours of examination											
Case No.	18.00	20.00	22.00	0.00	02.00	04.00	06.00	08.00	10.00	12.00	14.00	16.00
1	3.9	56.1	131.0	235.0	257.3	221.2	198.6	93.5	2.6	0.6	0	0.6
2	26.0	159.0	159.0	236.0	287.1	226.0	56.4	0	5.1	46.2	0	0
3	3.3	93.2	174.6	238.5	175.0	232.0	214.0	66.1	4.3	0	0	0
4	71.0	151.0	231.0	217.1	182.3	222.2	91.0	15.0	2.2	2.7	0	15.4
5	79.0	155.4	245.0	256.0	138.0	192.0	112.0	10.2	2.0	2.0	1.3	8.0
6	77.5	169.0	296.0	120.0	303.0	134.0	74.0	14.0	0	0	0	14.0
7	18.0	117.1	212.0	149.0	216.2	149.0	264.0	68.0	4.5	4.5	0	0
8	0	276.0	171.4	133.3	181.0	181.0	198.5	47.3	9.5	0	0	0
Observed mean mf ratio	35.0	147.1	202.5	198.1	217.5	195.0	151.1	39.3	4.0	7.0	0.2	5.0
% of maximum	16.1	68.5	93.1	91.1	100	90.0	69.5	18.1	1.8	3.2	0.1	2.3
Theoretical mf ratio	67.4	130.2	184.9	217.0	217.7	187.0	133.0	70.2	15.4	-16.5	-17.3	13.4
% of maximum	31.0	59.0	84.9	99.7	100	85.9	61.1	32.2	7.1	-7.6	-7.9	6.2

Table 9. Trigonometric analysis of the observed mf ratios, by the method of Aikat and Das (1977).

Examination hour(h)	y	y^2	y cos 15h	y sin 15h
12.00	7	49.0	-7.0	0.0
14.00	0.2	0.04	-0.17	-0.1
16.00	5.0	25.0	-2.5	-4.3
18.00	35.0	1225.0	0.0	-35.0
20.00	147.1	21638.4	73.6	-127.4
22.00	202.5	41006.3	175.4	-101.3
24.00	198.1	39243.6	198.1	0.0
02.00	217.5	47306.3	188.4	108.8
04.00	195.0	38025.0	97.5	168.9
06.00	151.1	22831.2	0.0	151.1
08.00	39.3	1544.5	-19.7	34.0
10.00	4.0	16.0	-3.5	2.0
Totals	1201.8	212910.3	700.1	196.7

$m = \Sigma y/n = 1201.8/12 = 100.2$

$b = 2\Sigma y\cos 15h/n = 2\times 700.1/12 = 116.7$

$c = 2\Sigma y\sin 15h/n = 2\times 196.7/12 = 32.8$

$a = \sqrt{b^2+c^2} = \sqrt{(116.7)^2+(38.2)^2} = 122.8$

$k = 1/15\tan^{-1}c/b = 1/15\tan^{-1}32.8/116.7 = 1.05$

$D = a/m \times 100 = 122.8/100.2 \times 100 = 122.6$

4.4 DISCUSSION

The appearance of microfilariae in the peripheral blood of the host follows a circadian rhythm. For an effective control programme for filariasis to be initiated in an endemic area, there is the need to determine accurately the periodicity of the responsible nematode in order to establish the ideal time to take blood samples for maximum microfilaria detection (WHO, 1987). In areas with nocturnal periodicity for instance, specimens are often sampled in the early evening, making the results obtained very sensitive to variations in sampling time. Also, the outcome of studies where microfilarial intensities are followed in some individuals over a period of time as in long-term epidemiological studies or in studies on drug efficacy, may be doubtful unless sampling time is taken into consideration when analysing the results. Again, it cannot be excluded that the commonly observed differences in age and sex-related mean microfilarial intensities from cross-sectional studies (Brabin, 1990) may partly be due to differences in sampling hours between different subgroups such as mothers and small children going earlier to bed than males, and therefore wishing to be examined earlier.

The modified statistical method for the analysis of microfilarial periodicity is accurate,more reliable and was used in this study to calculate the exact periodicity. The cosine and sine values corresponding to different hours and the observed microfilaria count are used to determine the amplitude (a) and the hour of highest microfilarial density (k). The least square method, which is based on the principle of minimisation of sum of squares of the deviations of the observed values from the expected values is used for the estimation of the various parameters. The proposal by Sasa and Tanaka (1974) that the appearance of microfilariae in the peripheral blood

follows a harmonic wave pattern, and thus can be characterized by the peak hour (k) and the periodicity index (D) has been utilised and found to be very useful in periodicity studies. The periodicity index of 122.6 and the peak hour of 01:03 observed in this study confirmed the nocturnal periodicity of *W. bancrofti* in the study area. Furthermore, the observed and theoretical periodicity curves followed each other closely, and thus justifying the use of the theoretical microfilarial periodicity equation to further examine and adjust the effect of sampling time on microfilarial intensity. Therefore, taking into account the curve shown in Figure 5, it can be seen that samples taken from the same individual at 19:00 and 22:00hours would on average show microfilarial intensities of 50% and 90% respectively. Therefore in order to obtain optimal microfilarial counts in individuals in Ghana during night blood examination, the blood samples should be collected between 24:00 and 02:00hours when microfilarial numbers in the peripheral blood of the individuals would be be almost 100%.

CHAPTER 5

5.1 ENTOMOLOGICAL STUDY OF THE VECTORS OF LYMPHATIC FILARIASIS

5.1.1 Introduction

Wuchereria bancrofti infection which results in lymphatic filariasis in man is transmitted by mosquitoes belonging to three genera; *Culex*, *Anopheles* and *Aedes* (WHO, 1987). The distribution and bionomics of each of these vectors varies from one geographical region to the other and also in terms of whether the transmission occurs in a rural or urban setting. Urban *W. bancrofti* for example, is transmitted by *Culex quinquefasciatus* in tropical regions while the rural form is mainly transmitted by several species of *Anopheles*, and occasionally by *Aedes* (WHO, 1987). There is also variation in the vector responsible for transmission depending on the periodic pattern of the nematode parasite (ie, which time of the day maximum numbers appear in the peripheral blood of the host for picking up by the vector). Thus while the nocturnally periodic rural and urban *W. bancrofti* is transmitted mainly by mosquitoes of the genus *Culex* and *Anopheles*, the diurnally subperiodic type is transmitted predominantly by several species of *Aedes* and the nocturnally subperiodic type by mosquitoes of the *Aedes niveus* group (WHO, 1987).

Differences in the ecology and breeding sites of mosquito species could partly be responsible for the type of mosquito that transmits the disease in urban and rural areas where different ecological habitats are provided. *Culex* has widespread distribution in tropical countries and breeds in polluted waters, sullage water, drains and pit latrines. *Anopheles* mosquitoes however breed in a wide variety of water bodies and the larval stages of *Mansonia* need to attach

themselves to aquatic host plants. The construction of water resource facilities like dams and the formation of reservoirs and irrigation systems also cause rapid environmental degradation and the changes in the ecology can result in changes in the surrounding areas and health risks may arise. Depending on their ecology therefore, certain mosquito species may disappear while others, finding more favourable physical, chemical or nutritional factors may proliferate enhancing their ability to transmit diseases.

Coluzzi (1964) noted that the adaptations most likely to be of importance epidemiologically in determining the relative abundance of a vector are those favoured by human activity. He pointed out that the rice field for example, is home to many vectors such as *An. gambiae, An. funestus,* and *An. culifacies*. The key understanding is that these species profit by the construction of poorly maintained irrigation schemes and impoundments. In Sri Lanka, (Ramasamy *et al*, 1994) has noted that recent irrigation development and other ecological changes, increase in human population and the development of insecticide resistance in several vectors, among others, have had a profound influence on the transmission of mosquito-borne diseases. Rapid unplanned urbanization is associated with problems of inadequate water supply, poor sewage disposal, insufficient solid waste management and poor water drainage all of which result in profusion of breeding habitats for mosquitoes such as *Cx. quinquefasciatus* and increased filariasis transmission (WHO, 1994).

5.1.2 Vector Abundance and Vectorial Capacity

It is obvious that, to be of any importance as a vector of a human disease, an insect must be relatively abundant and its habitat adjacent to human settlement. This will obviously lead to a high frequency of vector-man contact resulting in efficient transmission. Human activities have been known to favour the distribution and abundance of a vector in transmitting diseases (Gillies, 1988). The determination of population indices of a vector leads into assessment of its vectorial role in any target community and an important aspect of filariasis transmission is the evaluation of the relative importance of each proved vector, (White, 1971) and this will establish the relative vectorial role of each mosquito species. Species diversity of mosquitoes, among other important factors, has a vital role on the dynamics of filarial tranmission and it was reported that though very useful as a measure of intensity of transmission, the presence of a large number of different species of mosquitoes in an area could be an important cause in lowering the percentage of a particular species being incriminated as a filarial vector due to interspecific competition (Chandra, 1994).

There are several methods for determining vectorial importance such as collecting all mosquitoes in a series of representative quadrats or resting sites and estimating from this the total population of an area. This method however is fraught with problems arising from the contagious distribution of most insects. An alternative approach is the usual method of estimating absolute density by mark/release/recapture studies. Its reliability however depends in part on the accuracy of the estimates of emigration and immigration into the study area. Another approach to determining vectorial importance was used by Gillies (1955) in Tanzania. This is based on

a count of the total number of freshly fed mosquitoes resting in all the houses of an area, after collection from pyrethrum spray sheets. Based on earlier assessed population parameters, this method was used to calculate the number resting outside while still freshly fed and from this, the total number at each gonotrophic stage was determined. From this procedure the total populations of *A. gambiae* and *A. funestus* was estimated. The method involved a number of assumptions, nevertheless because it is a direct measurement, the results provide an approximate picture of the order of population density in an area.

5.1.3 Man-biting Rate and Vectorial Capacity

For a vector to be able to transmit any form of human disease, it must be physiologically susceptible to the infection and this is achieved successfully provided the vector is sufficiently abundant, and is man-biting and long-lived (Gillies, 1988). In other words, the vector must co-exist with the parasite and man for prolonged periods of time. Co-existence with the host apparently results in the vector becoming man-biting. The index of vectorial capacity is density-dependent and the parameter of density which can be measured is the man-biting rate or the incidence of bites per inhabitant per day. It may be estimated directly by means of captures throughout the night on human baits or indirectly from an index of the indoor-resting blood-fed females where the vector is known to be predominantly endophagic and endophilic in behaviour (Garrett-Jones *et al*, 1969).

Reduction in transmission through vector control can be achieved if the magnitude of the vector's contribution is known. To do this, there is the need to study the amount of transmission by assessing or studying both the numbers of infective larvae of the parasite in the mosquito in addition to measuring the prevalence and intensity of microfilaraemia in the human population. (WHO, 1987). Vector density and infectivity rate are therefore very useful in determining transmission in an area. Assessment of transmission depends on measuring the annual biting rate (ABR) of the vector which is the estimation of the number of bites received by one man per year and the number of infective larvae they are carrying. The incidence of infective mosquito caught biting man gives an indication of the" risk of infection " and thus the vector being regarded as having a vectorial role (Garrett-Jones *et al*, 1969). The biting density of the vector could be influenced by seasonal changes throughout the year and therefore the rate of infection in human populations. In a study conducted by McMahon *et al* (1981) in Tanzania, it was found that of the estimated bites by members of the *Anopheles gambiae* complex, 91.1% occurred during or following what was termed the "long rains" (i.e. April to early June) in contrast to the transmission by the *Culex quinquefasciatus* which occurred throughout the year.

However, transmission of *W. bancrofti* is an inefficient system in which only a small proportion of microfilariae gain ingress to a mosquito and many of those doing so even perish with or without the vector before reaching infectivity. Studies on *Culex* in Tanzania showed that only 2.7 to 13% of the ingested microfilariae developed into infective larvae (McGreevy *et al*, 1982). In vectors such as *A. gambiae* s.s, *A. arabiensis* and *A. farauti*, McGreevy *et al* (1982) have reported that the cibarial armature is well developed and damages microfilariae during feeding.

In that study, *A. gambiae* s.s was reported to have damaged 49% of ingested microfilariae of *W. bancrofti*, rendered 36% amotile and inflicted an even greater damage on *Brugia pahangi*. The failure of infective larvae to penetrate man, survive, contact a partner and reproduce is also prodigious. It has been estimated that an average of 15,500 bites by an "infective" mosquito is necessary to produce one case of microfilaraemia; thus very many bites by an infective mosquito are required to produce microfilaraemia (Hairston *et al*, 1968).

5.1.4 Vectors in Africa

In Africa, the principal vectors of *W. bancrofti* are *Cx. quinquefasciatus*, *A. gambiae* s.s, *A. funestus*, *A.arabiensis*, *A. merus*, and *A. melas* (Sasa, 1976). Brengues (1975) indicated that in West Africa, only *A. gambiae* and *A. funestus* are found to be natural vectors in most rural areas and reported that whereas foci of bancroftian filariasis are found throughout West Africa, it is the savanna areas (dry Sudan savanna woodland and moist Guinea savanna woodland) that harbour the most filariasis. The typical geographic pattern of spotty occurrence and localized variation of bancroftian filariasis is believed to arise from different epidemiologies of populations-mosquito contact. It is known that the flight range of *A. gambiae* for instance is only 1 or 2 km (Hunter, 1992) and thus essentially, it is infected people who spread filarial disease over distances not the mosquitoes.

In East Africa, three main vectors have been known as the most important vectors of filariasis and these include *A. funestus, A. gambiae* s.l and *Culex quinquefasciatus* (White, 1971, McMahon *et al*, 1981) and their importance depends on the extent to which climatic and other

environmental factors allowed each species to thrive seasonally. Sibling species of *A. gambiae* complex are regarded as the most efficient vectors of bancroftian filariasis in rural tropical Africa (White, 1974) and three species, *A. gambiae* s.s, *A. arabiensis* and *A. merus* have been recorded in Tanzania by (Mnzava *et al*, 1986).

In West Africa, the vectors responsible for transmission of filariasis have been reported by many workers. Brengues (1975) reviewed the transmission of filariasis in West African savanna. In Burkina Faso, he reported that *A. gambiae* s.l was responsible for about half the transmission and its transmission was mainly in the rainy season, *A. funestus* being responsible for dry season transmission. In Nigeria, Nwoke (1993) indicated that *Cx. quinquefasciatus,* among other mosquitoes, found breeding in septic tanks around human dwellings in urban areas could indicate the potentiality of it transmitting bancroftian filariasis. There was however, no documentary evidence of this claim. Also in Nigeria, Udonsi (1988) has reported that *Culex* species of mosquitoes which were involved in transmitting *W. bancrofti* were found in the Igwun basin, but their vectorial capacity was not investigated. Working in four villages in different bio-climatic zones in Liberia, Kuhlow *et al* (1978) reported that the most important vector was *A. gambiae*, on account of its higher biting density and higher infectivity rates while *A. funestus* and *A. nili* were also reported as vectors with lower vectorial roles. Earlier workers in that country had incriminated *A. gambiae, A. funestus,* and *A. melas* as natural vectors of *W. bancrofti* in Liberia (Gelfand, 1955, Maasch, 1973).

5.1.5 Vectors in Ghana

In Ghana, early reports by Muirhead-Thomson (1953,1954) indicated *A. gambiae* and *A. funestus* as perhaps the main vectors of *W. bancrofti* in West Africa. Since then, very little information had been reported of the main vectors and their vectorial roles in Ghana. There is a study currently on-going at the Noguchi Memorial Institute for Medical Research to identify the main vectors of lymphatic filariasis along the coast of Ghana. The results of this study however, are yet to be published. In order therefore to understand the epidemiology of bancroftian filariasis and also for vector control purposes, it is important that the species of vector responsible for transmission in a locality be reliably identified. The entomological study thus tries to identify the main species of the vectors in the locality and also to assess the risk of infection by documenting their infection and infectivity rates in order to propose an appropriate control strategy which could presumably be species-specific to control the disease in this irrigation community.

5.2 Materials and Methods

5.2.1 Adult mosquito collections

The study was conducted during three different periods. These were the wet months (April-May), the dry months (September-October) and the period when the dam was opened (November-December). It was intended to see if these periods could cause significant variations in vector populations and hence differences in transmission rates.

Two main collection methods were used to collect adult mosquitoes for dissection:

1. Pyrethrum spray collections.
2. Human landing catches.

5.2.1.1 Pyrethrum Spray Catches (PSC): Indoor resting mosquitoes were collected fortnightly from 15 randomly selected houses and in all 50 houses were examined during the study period. Adult mosquito collections were normally done during the early hours of the morning between 06.00-08.00hrs and at the convenience of the occupant who normally will be informed the night before. Houses were selected at random and the house numbers noted. Rooms in each house were designated as R1 (room number 1), R2 etc. Preferably, rooms with ceilings were selected for spraying. In each selected room, the number of sleepers was recorded. Before each spraying, all windows, doors, and any other possible exit points were closed and a white spreadsheet laid on the floor. Resting mosquitoes were knocked down by spraying the room with pyrethrum spray. Ten minutes later, the door was opened and the mosquitoes which fell on the spreadsheet were collected carefully using forceps starting at the door and moving to the interior of the room. They were kept on moist filter paper placed in petri dishes. Adult

mosquitoes collected were kept in this moist chamber and transferred immediately to the laboratory. Those which could not be worked on immediately were kept refrigerated at 4°C.

5.2.2.2 Human Landing Catches: This method involves collecting mosquitoes while they land on the collector to bite or while in the process of biting. This study was done during the period when the canals were opened (ie. Dec. to Jan.). This study was normally conducted between 18:00 and 06:00hrs. A paper cup covered with a mesh and an opening at the side just wide enough to allow a test tube to be pushed through was prepared for storing of live mosquitoes. For each collection site chosen, two people were made to sit outside while two others sat inside with their legs and arms exposed and each holding a torchlight. In all, a total of eight collectors were recruited for two collection sites. The torchlight was switched on anytime a bite was felt by the collector and mosquitoes were collected off the body by using a 100ml test tube whose mouth was applied perpendicularly over the wings of the mosquitoes in such a way that it will fly into the tube. The torchlight was also used to survey the other collector frequently in order to collect any mosquitoes that might land on him. After getting the mosquitoes in the test tube, they were transferred into the paper cups which had been labelled with the hour of collection and whether the collection was done indoor or outdoor. Mosquitoes were collected in each hour throughout a 12hr cycle. In order to keep the mosquitoes alive, cotton wool soaked in 2-5% sugar solution was placed on the mesh and a towel soaked in water was placed on the cups which were arranged in a wooden box, in order to maintain a high humidity. These were transported to the laboratory for identification and dissection. Three collections were done and they were all done in the period when the canals were opened.

5.2.3 Identification and Dissection of Adult Mosquitoes

In the laboratory, mosquitoes were killed with chloroform and transferred into petri dishes. Before dissection, the anopheline mosquitoes, *A. gambiae* s.l, *A. funestus* and *A. pharoensis* were identified morphologically using the keys described by Gillies and De Meillon (1968). Culicines were also identified by comparing them with collection preserved in the museum of entomology at the Department of Zoology. After identification, each female mosquito was placed on a clean glass slide. The wings and legs were removed and separated into three parts: the head, thorax and abdomen under approximately 20X magnification and each part placed separately on the slide. Normal saline was added and with fine needles, the labium was separated from the other parts of the proboscis and torn apart. The remainder of the head, thorax and abdomen was teased apart under high power of a dissecting microscope and the parts covered with cover slips. Careful examination was done under a compound microscope at 10X magnification for the presence of filarial larvae.

5.2.4 Definitions

Infection rate is defined as the percentage of mosquitoes found harbouring any of the larval stages (ie the first (L1), the second (L2) and the third (L3) stage of the parasite, *W. bancrofti* in them during dissection.

Infectivity rate is the percentage of mosquitoes found harbouring the third stage larva (L3) of the parasite *W. bancrofti* in them during dissection.

5.3 RESULTS

5.3.1 Mosquito abundance, infection and infectivity rates

The results of the total numbers (pyrethrum spray catch and human landing catch) of mosquitoes collected and their relative abundance during the study period are shown in Table 10. Mosquitoes belonging to three genera, *Anopheles*, *Culex* and *Mansonia* were collected. The results have indicated that the species which may possibly be involved in the transmission of lymphatic filariasis in the study community include *Anopheles gambiae* s.l, *Anopheles funestus*, *Anopheles pharoensis* and *Culex quinquefasciatus*. The most abundant species was *A. gambiae* s.l which contributed over half of the total number of mosquitoes collected (ie 58.2%). Close to this was *A.funestus* representing 33.2%. Apparently, these two species of Anophelines made up over 90% of the total number of mosquitoes collected. The other three species, *Anopheles pharoensis, Culex quinquefasciatus* and *Mansonia* species represented 8.6% of the totals collected.

Mosquito abundance, infection and infectivity rates of the indoor resting mosquitoes collected during the different periods indicate that there is seasonal variation in each of these parameters(Table 11). In the wet months, the most abundant species was *Anopheles gambiae* s.l, making a percentage of 61.1. This was followed by *A. funestus* constituting about half the population of *Anopheles gambiae* s.l. The other species, *A. pharoensis* and *Culex quinquefasciatus* occurred in low numbers of 5.4 and 3.2% respectively. In the dry months, a different scenario was observed in the relative abundance of the species. During this period, the most abundant species was *A. funestus* which was 81.5% of the total numbers collected. The

population of *A. gambiae* s.l fell and they were 12.1% of the total numbers, forming only a bit over a sixth of the total *A. funestus* population. The numbers of both *A. pharoensis* and *Culex quinquefasciatus* were still low (ie 2.4% and 4.0% respectively). Collections during the period the dam was opened have indicated that *A. gambiae* s.l again became the most abundant species (ie 76.6%) of the totals collected. *Anopheles funestus* was 21.7% (ie about a third of *A.gambiae* s.l. populations). *A. pharoensis* numbers were still low, contributing 1.6% while there were no *Cx. quinquefasciatus*.

The dissection of the indoor resting mosquitoes for filarial parasites have revealed developing larvae of *W. bancrofti* in all three *Anopheles* species during the different periods of collection. No filarial larvae were found in in indoor- resting *Cx. quinquefasciatus*. During the wet months, only about 50% of the total numbers of mosquitoes collected were dissected. Infection rate of 30.6% was recorded in *A. gambiae* s.l and this was followed by 22.2% in *A.funestus*. Infectivity rates of 9.7 and 5.6% were recorded in these two Anophelines. *A. pharoensis* had 50% infection rate when out of the 2 dissected, 1 was infected. In the dry months however, *A. funestus* had the highest infection rate of 31.6% compared to 6.7% in *A. gambiae* s.l. Third stage larvae of *W.bancrofti* were found only in *A. funestus* resulting in an infectivity rate of 4.1%. The infection rate of 33% was noted in *A. pharoensis* when 1 of the 3 mosquitoes dissected was infected. During the period that the dam was opened, there were 41 infected *A.gambiae* giving an infection rate of 29.1% and 10 of these were third-stage larvae giving an infectivity rate of 7.1%. In *A.funestus*, an infection rate of 17.5% was recorded for this period while two had third-stage larvae giving an infectivity rate of 5.0%. *A.pharoensis* had an

infection rate of 33.3% but none was infective. Overall, 487 mosquitoes were collected using PSC and 399 (representing 81.9%) were dissected for the presence of filarial larvae of all three stages. Out of these, 109, giving an infection rate of 22.4%, contained filarial larvae of all stages, while 24 mosquitoes had third stage larvae with different worm loads in them giving an overall infectivity rate of 4.9%. However, infection was observed only in anophelines mosquitoes, no culicines were found to harbor any form of larvae (Table 11.)

For the mosquitoes collected on human baits, a total of 260 mosquitoes were collected in 192 man-night hours (Table 12). The results indicate that even though there were variations in the biting pattern of each species, generally there was a gradual increase in biting activity of mosquitoes from the early hours of the evening until a peak around 24:00-01:00hours after which activity decreased until the early hours of the morning when minimum numbers were collected. The table shows the number of each species of mosquitoes coming to bite in each hour. Generally, in most species, peak biting activity occured around 23:00-02:00hrs. There is difference in biting peaks of *A.gambiae* s.l and *A. funestus*. *A. gambiae* s.l. had a peak at 23:00-24:00hrs and this reduced in the next hour followed by a second peak at 01:00-02:00. After this time, the numbers of *A. gambiae* s.l. decreased gradually till morning. In the case of *A.funestus*, a peak of biting activity occurred around 24:00 to 01:00 hrs and reduced in the next hour but peaked up again (even though to a lesser extent) at 02:00 to 03:00 hrs. The biting intensity of *Culex quinquefasciatus* rose gradually during the first five hours of the night and reached a peak at 24:00 to 01:00 hrs and there was no biting after 02:00hours. In *Mansonia*

species, a similar pattern existed for the first part of the night with a peak at 24:00 to 01:00 hrs and biting decreased thereafter (Refer to Fig. 8.)

Table 13 shows the results of infection and infectivity rates of the mosquitoes caught biting man. In all, 256 mosquitoes were dissected for the presence of filarial larvae. Of these, 57 representing 22.3% were infected with at least one of the three larval stages of *W. bancrofti* and *A. gambiae* s.l. accounted for about 79% of the total infections. Third-stage filarial larvae were found in 16 mosquitoes giving the infectivity rate of 6.3%. *A. gambiae* s.l had an infectivity rate of 8.3% and this was followed by 2.0% in *A. funestus*. Even though the infectivity rate of *A. pharoensis* was 33.3%, only very low numbers were collected. There was however no infective larvae in *Cx. quinquefasciatus* and *Mansonia* species.

5.3.2 Worm Load in the vectors

Table 14 shows the worm load at the different periods of collection of indoor- resting mosquitoes. The total number of larvae (ie first, second and third stages) found in all mosquitoes was 599, and there were variations in the different species at different times. *A. funestus* had the highest number of larvae (ie 279) in the dry months and consequently the highest average infection per infected mosquito of 9.0 during this same period. The total number of infective larvae recorded was 43. *A. gambiae* s.l had a total of 23 infective larvae during the period the dam was opened representing 53.5% of the total infective filarial larvae found. This was followed by *A. funestus* which had a total of 9 in the dry months. The total

average infective larvae per infected mosquito was 1.8 and both *A. funestus* and *A. gambiae* s.l had the highest values of 2.3 in the dry months and when the dam opened respectively.

For those caught off human bait, the total number of larvae found in all the five species was 544 and the average number per infected mosquito was 9.5 (Table 15). However, for the different vectors, the highest average infection was in *A. pharoensis* which was 28.0. This was followed by *A.gambiae* s.l with 9.8 and *Cx. quinquefasciatus* had 3.0. The total number of infective larvae was 42. Of these, *A. gambiae* s.l had 37, thus representing 88.0%. The total average larvae per infective mosquito was 2.6 (Table 16).

Table 16 shows the frequency distribution of infective larvae per mosquito. The frequency distribution is skewed with most infected mosquitoes having one worm. Out of a total of of 33 infective *A. gambiae* s.l, 48.5% had 1 infective larva, while 62.5% of *A. funestus*, had 1 infective larva. The highest number of infective larvae found in an individual mosquito was eleven (11) and this was in two *A. gambiae* s.l.

5.3.3 Intensity of Transmission

Table 17 shows the results of the estimated potential infective bites a person is likely to receive in the community in a year. Man- biting rates were calculated for each species monthly and yearly values were obtained by multiplying the monthly figures by twelve. An individual in the community would be exposed to 309.5 infective bites in a year from *Anopheles gambiae* s.l. *Anopheles funestus* and *Anopheles pharoensis* both had 24.1 while *Culex quinquefasciatus* and

Mansonia species may not give an infective bite during the year since they did not harbour infective stages of the parasite.

See appendix 3 for summary of results of dissection .

Table 10. Numbers of indoor resting and man biting mosquitoes collected in Okyereko (April 1995 to January 1996).

Vector species	Total numbers collected	Percentage (%)
Anopheles gambiae s.l.	438	58.2
Anopheles funestus	250	33.2
Anopheles pharoensis	19	2.5
Culex quinquefasciatus	29	3.8
Mansonia species	17	2.3
Total	753	100

Table 11. Infection and infectivity rates of indoor resting mosquitoes in Okyereko.

Period	Species	No.collected (% for period)	No. dissected	No. infected (infection rate in %)	No. infective (Infectivity rate in %)
Rainy					
	Anopheles gambiae s.l	113 (61.1)	72	22 (30.6)	7 (9.7)
	Anopheles funestus	56 (30.3)	18	4 (22.2)	1 (5.6)
	Anopheles pharoensis	10 (5.4)	2	1 (50.0)	0 (0.0)
	Culex quinquefasciatus	6 (3.2)	2	0 (0.0)	0 (0.0)
Dry					
	Anopheles gambiae s.l	15 (12.1)	15	1 (6.7)	0 (0.0)
	Anopheles funestus	101 (81.5)	98	31 (31.6)	4 (4.1)
	Anopheles pharoensis	3 (2.4)	3	1 (33.3)	0 (0.0)
	Culex quinquefasciatus	5 (4.0)	5	0 (0.0)	0 (0.0)
Canal Opened					
	Anopheles gambiae s.l	141 (76.6)	141	41 (29.1)	10 (7.1)
	Anopheles funestus	40 (21.7)	40	7 (17.5)	2 (5.0)
	Anopheles pharoensis	3 (1.6)	3	1 (33.3)	0 (0.0)
	Culex quinquefasciatus	0 (0.0)	0	0 (0.0)	0 (0.0)
	TOTAL	493	399	109 (22.4)	24 (4.9)

Table 12. **Hourly distribution of mosquitoes caught biting human baits in Okyereko.**

Species	20:00-21:0	21:00-22:0	22:00-23:0	23:00-24:0	24:00-01:0	01:00-02:0	02:00-03:0	03:00-04:0	04:00-05:0
Anopheles gambiae s.l.	1	8	20	33	28	34	21	14	10
Anopheles funestus	0	1	3	6	16	6	10	6	3
Anopheles pharoensis	1	0	0	0	1	0	1	0	0
Culex quinquefasciatus	1	2	3	4	7	1	0	0	0
Mansonia species	0	1	3	4	5	3	1	0	0
TOTAL	3	12	29	47	57	44	33	20	13

Table 13 **Infection and infectivity rates of mosquitoes caught on human baits in Okyereko.**

Species	No. Dissected	No. Infected	Infection Rate (%)	No.Infective	Infectivity Rate (%)
Anopheles gambiae s.l	168	45	26.8	14	8.3
Anopheles funestus	50	8	16.0	1	2.0
Anopheles pharoensis	3	2	66.7	1	33.3
Culex quinquefasciantus	18	1	5.6	0	0
Mansonia species	17	1	5.9	0	0
Total	256	57	22.3	16	6.3

Table 14. Filarial larvae recovered in indoor resting mosquitoes collected in Okyereko.

Period and vector species	Total no. of larvae (L1-L3)	Average infection per infected mosquito	Total no. of infective larvae(L3)	Average infective larvae per infected mosquito
Wet				
Anopheles gambiae s.l	68	3.1	7	1.0
Anopheles funestus	11	2.8	1	1.0
Anopheles pharoensis	4	4.0	0	0
Culex quinquefasciatus	0	0	0	0
Dry				
Anopheles gambiae s.l	1	1.0	0	0
Anopheles funestus	279	9.0	9	2.3
Anopheles pharoensis	8	8.0	0	0
Culex quinquefasciatus	0	0	0	0
Canal Opened				
Anopheles gambiae s.l	189	4.6	23	2.3
Anopheles funestus	30	4.2	3	1.5
Anopheles pharoensis	5	5.0	0	0
Culex quinquefasciatus	0	0	0	0
Total	599	5.5	43	1.8

Table 15. Filarial larvae recovered in mosquitoes caught biting human baits in Okyereko.

Vector species	Total no. of larvae (L1-L3)	Average infection per infected mosquito	Total number of infective larvae(L3)	Average infective larvae per infective mosquito
Anopheles gambiae s.l.	442	9.8	37	2.6
Anopheles funestus	37	4.6	1	1.0
Anopheles pharoensis	56	28.0	4	4.0
Culex quinquefasciatus	3	3.0	0	0
Mansonia species	6	6.0	0	0
Total	544	9.5	42	2.6

Table 16. Frequency distribution of infective filarial larvae in night biting and indoor resting mosquitoes in Okyereko

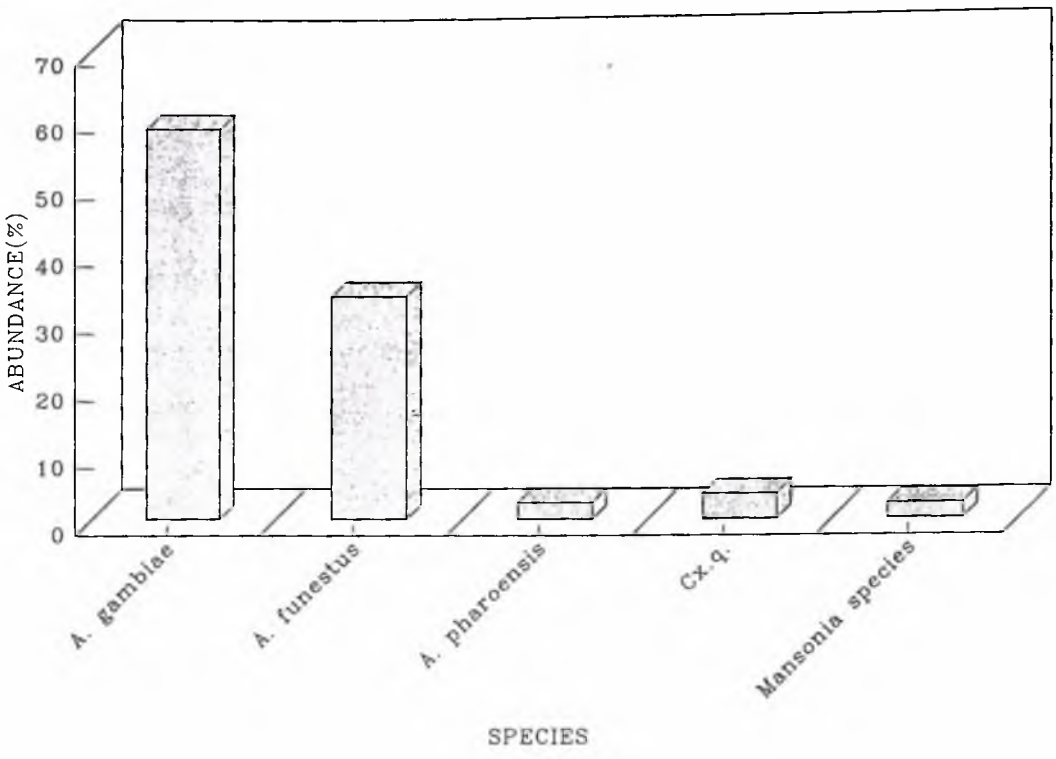
No. of infective larvae per mosquito	*Anopheles gambiae* s.l.	*Anopheles funestus*	*Anopheles pharoensis*	*Culex quinquefasciatus*	*Mansonia* species
1	16	5	-	-	-
2	6	2	-	-	-
3	4	1	-	-	-
4	2	-	1	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	3	-	-	-	-
10	-	-	-	-	-
11	2	-	-	-	-
Total	33	8	1	0	0

Table 17. Estimated number of yearly infective bites of the mosquitoes collected off human bait in Okyereko.

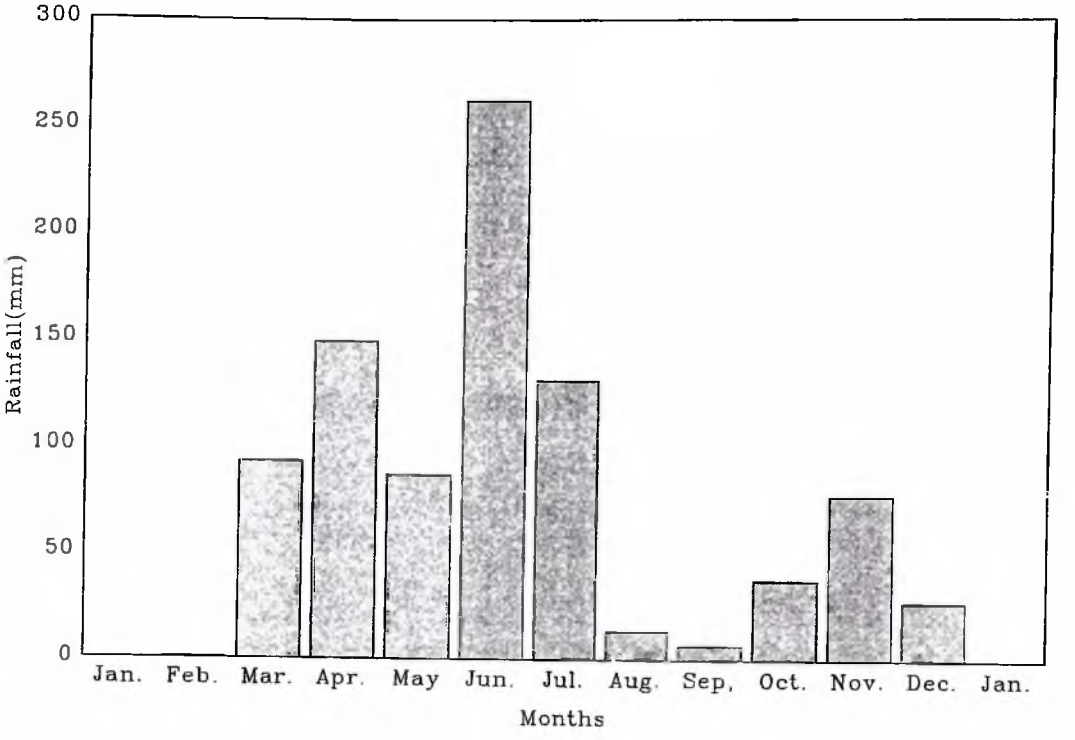
Vector species	No. collected	Man-biting rate[a](MBR) (Bites/Man/Night)	Infectivity rate	Potential infective bites/man/yr
A. gambie s.l.	169	10.6	0.08	309.5
A. funestus	53	3.3	0.02	24.1
A. pharoensis	3	0.2	0.33	24.1
C. quinquefasciatus	18	1.1	0	0
Mansonia species	17	1.0	0	0

[a] Calculated from MBR = $\frac{\text{No. of mosquitoes collected}}{\text{No. of catchers X No. of biting cathes}}$

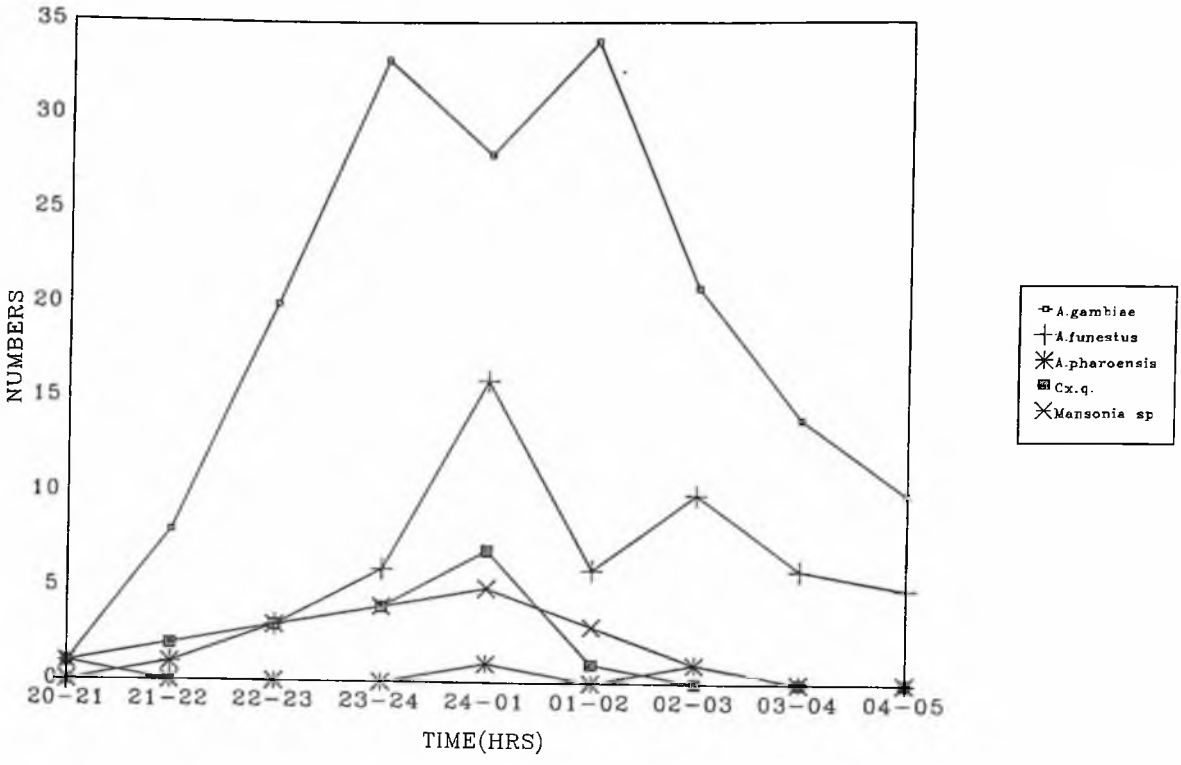
(Ref. MCmahon *et al* 1981)



RAINFALL PATTERN FOR OKYEREKO AREA (JAN.1995 – JAN.1996)
(Source: Ghana Meteorological Services Department, Accra.)



THE DIFFERENT SPECIES OF MOSQUITOES COLLECTED IN EACH HOUR



5.4 DISCUSSION

Vector studies are essential for the understanding and control of any vector-borne disease. Therefore, to understand the dynamics and transmission of a disease and for the proper monitoring of any control programme, it is essential that the the biology and transmission indices of the main vectors should be well understood. Even though filarial larvae were not identified to species, it is likely that the majority are *Wuchereria bancrofti* since the mosquitoes from which they were recovered were mainly man-biting. It can not be excluded, however, that a proportion of these larvae could be of animal origin. The fact that infective larval stages of the parasite have been found in the mosquitoes dissected may indicate that there is transmission of lymphatic filariasis in the community. In addition to this, the fact that infective stages of the parasite have been recovered in some mosquitoes indicates that the people in the community have been receiving infective bites from these vectors. Most of the buildings in the community are old with some having large cracks and eaves which predispose the people to high mosquito-man contact aiding transmission. According to WHO (1984), there is a correlation between the level of exposure to infective larvae and the prevalence and intensity of the disease.

The number of mosquitoes collected using the two standardized methods (ie PSC and LC) gives density indices. Such indices may obviously be affected by several factors such as the method and duration of collection method employed, and for instance in the case of PSC, the size of the room and the number of sleepers in each room. There was however very little variation in the sizes of the rooms and the number of sleepers in each room. The study has identified mosquitoes belonging to three genera, *Anopheles, Culex* and *Mansonia* in the study community.

Their populations vary and there was a variation also in their vectorial roles. The presence of the irrigation canals may favour their breeding and the activities of the inhabitants may increase the level of contact between them and these vectors.

Anopheles gambiae s.l and *Anopheles funestus* appear to be the two main vectors of bancroftian filariasis in the community and these incidentally are also the vectors of malaria in Africa (Service,1985). WHO (1992) has indicated that the genus *Anopheles* is important in the transmission of periodic *W. bancrofti* in Africa, Southern Asia and the island of New Guinea. Many workers reported similar findings in Africa. As far back as 1932, Gorden *et al* (cited by Muirhead-Thomson, 1954) recognized *A. gambiae* and *A. funestus* as important vectors, possibly the main ones of *W. bancrofti* in tropical Africa. *Culex quinquefasciatus* is an important vector of bancroftian filariasis in many parts of the world including East Africa (Mcmahon *et al*, 1981), but its importance as a vector in West Africa has been questioned on the basis of both field (Kuhlow *et al*, 1978, Brengues, 1975, Dunyo *et al*, in press) and experimental studies (Curtis *et al*, 1981). The results of this study have also not found any infective larval stages of *W. bancrofti* in *Culex quinquefasciatus* thus being a further support of the above findings. However, there is the need for further investigation into this claim in Ghana.

The small numbers of *Anopheles pharoensis* species collected may mean that it may be playing a minor role as a vector of the parasite in the community. This mosquito, though occurring in very low numbers seem to be a potential bancroftian filariasis vector. Of these low numbers collected, transforming stages as well as an infective stage were found in some of the mosquitoes. There have so far been no reports of its involvement in transmission in Ghana or

elsewhere. This study has therefore opened the way for a critical investigation into its vectorial role in lymphatic filariasis.

The three different seasons investigated showed differences in the vector populations and also in their infection and infectivity rates. In the wet months the mean rainfall in the area was 117.6mm and the maximum and minimum temperatures were 33°C and 24.4°C respectively (Refer to Fig. 7). These conditions favour the breeding of *A. gambiae* s.l resulting in an increase in their numbers. *Anopheles gambiae* breeds in shallow, open, sun-lit muddles and pools. The breeding places of this vector are often temporary and they correlate with the rainy season. In Nigeria, Smith (1980) reported that *A. gambiae* s.l. was present throughout the year with its lowest density occurring in the relatively dry months, and this builds up during the rainy season. Similar findings were reported by Bushrod (1981) in Tanzania. In the dry months, mean rainfall was 22mm, while maximum and minimum temperatures were 31.2°C and 23.2°C. The very little precipitation coupled with high temperatures provide suitable conditions for the breeding of *A. funestus* whose normal breeding places are those of a permanent nature such as clear fresh water slightly shaded with vegetation. *A. funestus* normally maintains transmission during the dry season when surface water dries up and permanent water partly covered with vegetation ideal for its breeding persists. At Okyereko, the presence of the river Ayensu at the outskirts of the town provides an important breeding place for *A. funestus* during the dry months.

Another important factor which determines vector populations in the study area apart from the amount of rainfall is the availability of stagnant water in the irrigation canals. The periodic

opening and closure of the spillways results in differences in vector numbers. The period when the canals were opened, November to December, the mean rainfall was 52.0mm with maximum and minimum temperatures being 32°C and 23°C and this period is a dry one but there was more of *A. gambiae* than *A. funestus* and this may be due to the opening of the dam for rice farming which results in the formation of pools and puddles of water which represents an important source for the prolific breeding of *A. gambiae* s.l. A review by Hamon *et al* (1963) summarizing observations of various workers in the African region stated that the breeding places of *A. gambiae* s.l are often temporary and are correlated with the rainy season, but waters in areas of rice cultivation provide residual breeding places during the dry season, at which period the peak of *A. gambiae* occurs. In this community therefore, mosquito density can be said to be controlled by the amount of rain and the opening or closure of the irrigation dam. These conditions, according to Hunter (1992), will result in an increase in the incidence and prevalence of the disease primarily because of the intensity of transmission due to high man-vector contact. The present study documented a high density of *A. gambiae* s.l. during the wet season in the study area. This combined with the high infectivity rate of this mosquito suggests that it is responsible for a higher level of transmission of filariasis than *A. funestus*. In the dry periods, there were more *A. funestus* than *A. gambiae* with an equally higher infectivity rate. In November to December, even though it was still in the dry season, the dams were opened resulting in availability of more breeding places that encouraged the breeding of *A. gambiae* resulting also in high infectivity rate of this species.

Transmission rate also depends on the proportion of vectors that are infective and not only on their density. It can be seen from the study that there is variation in the vector population depending on the time of the year and the risk of infection seem to correlate closely with vector density. Despite this however, the infection rate of the mosquitoes appears to be the same at the different seasons. Thus the most abundant vector at any period of collection have approximately similar infection and infectivity rates. The similarity in the infection and infectivity rates in the vector population may be explained by the high microfilaria reservoir in the human population and the high density of the vectors which maintain transmission throughout the year. For the study period, the overall infectivity rates were 7.8(31/396), 3.9(8/206) and 9.1(1/11)% for *A. gambiae* s.l, *A. funestus* and *A. pharoensis* respectively. These values seem high compared to reports from other endemic areas (MacMahon *et al*, 1981, Kuhlow *et al,* 1978, Dunyo *et al*, in press, Wijers *et al*, 1977). There is the need therefore for a comprehensive quantitative study, possibly throughout the whole year to validate this claim. The intensity of filariasis transmission at a given locality can be best estimated by calculating the average number of infective larvae transferred to a person per year. This depends largely on the density of the vectors and also on the prevalence of microfilaraemia in human population (Kuhlow *et al*, 1978). When landing catches are made and mosquitoes dissected, this parameter was estimated by the assumption that the same number of infective mosquitoes will be coming to bite each time collections are made throughout the year. The calculated numbers of infective larvae transmitted annually are to be considered as theoretical figures. They are most likely too high as only a fraction of the larvae encountered during dissection of mosquitoes would have been able to leave the vector and penetrate into the final host during feeding. In addition, some

species of mosquitoes such as *A. gambiae* possess cibarial armatures that are known to damage microfilariae (Bryan *et al*, 1988). Nevertheless the estimation of the number of larvae transmitted per person per year is a useful indication of the intensity of transmission in a community. The number of infective bites per person per year depends also on the density of a particular vector. Thus the most abundant vector *A. gambiae* s.l had the highest value of 309.5. This vector also had a high worm load indicated by the average infective larvae per infective mosquito which was 2.6. These findings have therefore indicated that the main vector of lymphatic filariasis in the study community is *A. gambiae* s.l. *A. funestus* ranks next to *A. gambiae* s.l in terms of its numbers, the estimated number of potential infective bites per person per year and the worm load. Though *A. pharoensis* had higher values of some transmission indices such as infection and infectivity rates as in the case for landing catches, low numbers recorded do not implicate it as a main vector involved in transmission of lymphatic filariasis in the community. Nevertheless, as stated earlier on, there is the need to further investigate the vectorial role of this species since this will contribute to the understanding of the epidemiology of the disease in the community. Similar findings have been reported in other endemic areas on the vectorial role of *A. gambiae* s.l and *A. funestus* (McMahon *et al*, 1981, Kuhlow *et al*, 1978).

Infective larvae are lost during feeding so that it would be expected that fewer mosquitoes from spray catches would be infective than from landing catches (McMahon *et al*, 1981). This was true for *A. gambiae* which had 8.3% infectivity rate for landing catches and 7.5% for spray

catches. However, for *A. funestus*, infectivity rate was higher in spray catch collections than for landing catches (ie. 4.5% and 2.0% respectively).

The biting patterns of the various vectors have shown that each of the mosquitoes has a definite biting pattern. Generally however, for most of them, there is little activity at sunset, increasing gradually to a peak around midnight and almost ceasing by daybreak. The biting activity seems to have a significance to the microfilarial periodicity. Many hypotheses or theories have been proposed to account for such a circadian rhythm in the microfilariae and in most instances, the periodicity of a species of microfilaria has been found to be closely related with the biting activity of the insect species which act as a vector (Sasa, 1976). Therefore the peak activity of the vectors seems to coincide with the time of the day the maximum numbers of the parasite appear in the peripheral blood of the host to be picked up by the vector. In addition, the risk of infection is greater during and after midnight when most of the hosts are asleep than in the period immediately following sunset. There was a slight difference in the peak of activity in *A.gambiae* and *A. funestus*. *A. gambiae* has shown two peaks of feeding in the night. The highest peak occured between 24:00 to 01:00hrs. Its activity however reduced in the next hour during which time the activity of *A. funestus* was at its peak. Since the two species occur in large numbers at the same time, the difference may be said to be due to avoidance of interspecific competition. However, the small sampling size of these two mosquito species may not give adequate information for the explanation of this phenomenon. The well known late-biting cycle of *A. gambiae* has been confirmed in a number of studies both in the savanna and forest regions of Liberia (Kuhlow *et al*, 1978). The biting pattern of *A. funestus* is similar to

that of *A. gambiae* except that very few bite during the early hours of the night while peak biting occurred around midnight. A similar report has been documented in the Garki project (Molineaux *et al*, 1980). *Culex quinquefasciatus* was active throughout the night and started biting early with a peak activity around midnight. Activity however ceased during the early hours of the day. This trend of activity was reported by other workers (Samarawickrema *et al*, 1992) who reported that biting commenced soon after dusk, and continued throughout the night with the highest level of activity during 22:00 to 03:00hrs.

CHAPTER 6

6.1 GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

6.1.1 General Discussion

The study has documented for the first time that lymphatic filariasis is an important public health problem in the irrigated community. The OIP completed over two decades ago was intended among other things to improve the economic life of the people and to restrain the rural-urban drift of the youth. Since lymphatic filariasis is a water-related vector-borne parasitic disease, its transmission would be enhanced by the availability and presence of surface water which serves as breeding place for the vectors. The presence of the dam will undoubtedly maintain and probably intensify its transmission. The dam is assumed to contribute immensely to very high mosquito populations and the canals have provided additional breeding habitats for these vectors and bilharzia snails.

The high prevalence of hydrocoele among the youth may result in increased out-migration of the residents from the area if they become aware that their plight is associated with the irrigation project thereby defeating the noble objectives of its installation. Also, personal communication with most of the villagers especially the young men who helped the investigator during the study indicated that some of them had haematuria. Dreyer *et al*, (1992) have reported that renal abnormalities (especially haematuria) are a regular, though rarely recognized, symptom of bancroftian filariasis. Whether the haematuria in this cummunity is due to infection with schistosomes of *W. bancrofti* is not known and, therefore, should be explored. Since there is no available information on the prevalence of lymphatic filariasis in this community prior to this

study, it is inappropriate to attribute the high prevalence rates of microfilaraemia in the population and the consequent clinical manifestations of the disease reported in this study to the presence of the dam.

6.1.2 Recommendations

Simple, safe and cost-effective methods to control and possibly eradicate lymphatic filariasis have recently become available (WHO,1994). For example, instead of the known and older 12-day regimens using the drug diethylcarbamazine (DEC), it has now become clear that much simpler treatment strategies using single yearly doses of DEC or in combination with other drugs such as ivermectin or the daily consumption of DEC as an additive to table/cooking salt is equally effective in control programmes. Therefore, the use of these drugs with concurrent vector control are very useful tools in the control of the diseases. Also, lymphatic filariasis has recently been identified by the International Task Force for Disease Eradication as one of the only six "eradicable" or potentially eradicable infectious diseases. This fact coupled with the recognition that appropriate control measures can be effectively and inexpensively linked with the existing national and local public health infrastructures, now provide strong impetus to initiate appropriate control measures such as chemotherapy with concurrent vector control to finally control this parasitic disease and the morbidity that it causes in all endemic areas.

As stated earlier, recent evidence has implicated bacterial and fungal superinfections of limbs with compromised lymphatic function as responsible in playing a primary role in triggering most episodes of adenolymphagitis (ADL). These themselves actually cause or exacerbate the elephantiasis changes in affected patients. Therefore in view of this new understanding, measures of hygiene coupled with local or in severe cases, systemic antibiotics given

prophylactically can have profound effects in preventing these damaging episodes of ADL and even allow the host to repair and recover from some or all of the overt damage caused by filarial infection and subsequent superinfections. The four cases of elephantiasis must be identified and managed with these simple leg-care measures which will eventually reduce the morbidity due to this disease.

To initiate an appropriate vector control programme, a thorough knowledge of the ecology of the local vectors is needed so that control methods would be adapted to local conditions. In view of the known differences in the vectorial roles of the different mosquito species collected from the community, appropriate vector control strategies aimed at controlling mainly the *Anophelines*, which have been found to be the vectors should be employed. An intensive study which should be extended to the surrounding areas of the village to define the species composition, their distribution and any temporal variation so as to determine their respective roles in the transmission of the disease should be carried out. Individuals in the community should be encouraged to practise measures that reduce man-vector contact including sleeping in bed nets (preferably impregnated ones), the use of mosquito repellents, screening of their doors and windows with mosquito nets, among other precautionary measures. Also, indoor house spraying with residual insecticides will be effective in reducing the vector density in rooms.

There is also the need for a socio-economic study in the community to assess the impact of the disease and to determine the knowledge, beliefs and practices associated with the disease in the community. This will help immensely in the design of appropriate control strategies for the community since it will reveal any hidden beliefs about the causes and perceived impressions of lymphatic filariasis among the residents.

6.1.3 Conclusion

The study has shown that lymphatic filariasis is a major public health problem at Gomoa Okyereko, an irrigation project community in Southern Ghana. However, since there is no documentary evidence to ascertain the presence or absence of this disease in this area prior to the construction of the irrigation facility, the high incidence of this disease reported in the study cannot be attributed entirely to the presence of the dam. Based on this observation, we recommend that prior to the establishment of any water development projects in a community, all water-related diseases must be investigated so as to ascertain the impact of the project on the emergence of any new disease and its contribution to an increase or decrease in its incidence. There should also be an intensive study, probably throughout the whole country to assess the contribution of irrigation or any other water development project on the health of the beneficiary communities.

APPENDIX 1

CENSUS REGISTRATION FORM -OKYEREKO, 1995

SERIAL NO.	HOUSE NO.	NAME	AGE	SEX	DURATION OF STAY IN VILLAGE

Village	OK
Serial no.	336 SELECTED:
House no.	057 ID_NO : OK057336
Name	EMELIA ANIBIL
Sex	F
Age	30.0
Length of stay in village	30.0

CLINICAL EXAMINATION Date:_____

CLINICAL MANIFESTATIONS	Grade	Remarks
Limb elephantiasis		
Hydrocoele		
Scrotal elephantiasis		
Other		

BLOOD SAMPLING Date:_____

COUNTING CHAMBER TECHNIQUE		
Time		
µl blood		
Species	Mf counted	mf/ml
W.b.		
M.p.		
L.l.		

BLOOD SMEAR OBTAINED	
Yes:	No:
Mf species identified	
W.b.	
M.p.	
L.l.	

Remarks:_____

Appendix 3 **Filarial worm infections of vectors caught off landing catches and pyrethrum spray catches.**

Vector Species	Pyrethrum spray catches			Landing catches			Total		
	No. dissected	Infection rate (%)	Infectivity rate (%)	No. dissected	Infection rate (%)	Infectivity rate (%)	No. dissected	Infection rate (%)	Infectivity rate (%)
A. gambiae s.l.	228	28.1	7.5	168	26.8	8.3	396	27.5	7.8
A. funestus	156	26.9	4.5	50	16.0	2.0	206	24.3	3.9
A. pharoensis	8	37.5	0	3	66.7	33.3	11	45.5	9.1
Cx. quinquefasciatus	7	0	0	18	5.6	0	25	4.0	0
Mansonia species	0	0	0	17	5.9	0	17	5.9	0
Total	399	27.3	6.0	256	22.3	6.3	655	25.3	6.1

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