Lymphatic Filariasis in Malindi District, Kenya: Epidemiology and Impact of Annual Mass Chemotherapy

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by

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ABSTRACT

The present study is divided into two areas: a pre-treatment study focusing on epidemiology of lymphatic filariasis and the monitoring of the impact of annual single dose mass drug administration (MDA) of a combination of diethylcarbamazine (DEC) and albendazole against *Wuchereria bancrofti*. This study is the first to provide epidemiological data on lymphatic filariasis in the northern coast (north of Mombasa Island) since earlier surveys were conducted in the 1970s. There was a need for a study from this area particularly because of the current mass treatment campaigns under the Global Programme to Eliminate Lymphatic Filariasis (GPELF). The overall baseline prevalence of microfilaraemia and antigenaemia (by the Og4C3 ELISA assay) was > 20% and > 40%, respectively. Similarly, the prevalence of chronic disease was relatively high: leg lymphoedema in individuals aged > 14 years was 8.5% and the prevalence of hydrocele in males aged > 40 years was more than 55%. The high prevalence of antifilarial IgG1 (86%) and IgG4 (91%) responses indicates that most people living in this setting were exposed to *W. bancrofti* infection. Taken together, these results show that lymphatic filariasis is a major public health problem in Malindi District, Kenya and the disease extends beyond coastal villages. This study is the first detailed evaluation of the available battery of parasitological and immunological methods in monitoring impact of DEC/albendazole against *W. bancrofti* in an *Anopheles* transmission setting. The level of antifilarial IgG1 in antigen-positive and –negative individuals was similar and higher in children compared with adults. This finding argues for the use of antifilarial IgG1 as a marker of exposure to infection rather than a marker of active infection. This is an important finding because it indicates that antifilarial IgG1 could be used as a proxy for monitoring the impact of mass treatment on transmission of *W. bancrofti* infection during elimination programmes and post-MDA surveillance of resurgence of transmission. Comparative evaluation of the immunochromatographic (ICT) test and the Og4C3 ELISA assay showed that the ICT test had significantly lower sensitivity after two rounds of MDA suggesting that the ICT test will be less reliable in monitoring of elimination programmes. The administration of two rounds of mass chemotherapy resulted in a significant decline in the intensity of microfilaraemia (> 95%), antigenaemia (> 90%), antifilarial IgG responses (> 70%) and the proportion of infected mosquitoes (65%). In addition, there was only one incident case of antigenaemia after the two rounds of mass treatment. These results led to the conclusion that mass treatment strategy using a combination of DEC and albendazole had a dramatic impact on the transmission of lymphatic filariasis. The study also provided important information on the evolution of chronic disease during mass treatment. Although there was no significant change in the prevalence of leg lymphoedema, resolution of hydrocele was observed in 26.5% of the individuals who had this clinical manifestation during the pre-treatment survey. The finding indicates that mass treatment with DEC/albendazole under GPELF might result in significant clinical benefits in addition to the decline in levels of transmission.

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DEDICATION

This thesis is dedicated to

My wife Eunice W. Michugu

Our children Caroline Watiri, Kevin Njenga and Faith Murugi

Who endured numerous long periods of my absence from home during this study

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CHAPTER 1 – INTRODUCTION

Lymphatic filariasis is a mosquito-transmitted disease that is estimated to infect over 120 million people worldwide making it one of the most prevalent tropical diseases (Michael *et al.*, 1996; Zagaria and Savioli, 2002). In addition, more than 40 million of those infected are suffering from one or more of the overt manifestations caused by the infection (Ottesen *et al.*, 1997; Molyneux and Zagaria, 2002). More than one billion people are living in endemic areas where they are at risk of infection with lymphatic filariasis by virtue of continuous exposure to infected mosquito vectors (WHO, 2002c). Thus the incidence and prevalence of lymphatic filariasis will increase if efforts to control transmission of the infection are not put into place.

Lymphatic filariasis is one of the so called "neglected" tropical diseases, which usually affect the poorest people in rural areas of low-income countries. Other neglected diseases in sub-Saharan Africa include, onchocerciasis, schistosomiasis, intestinal helminths (hookworm, ascariasis, trichuriasis), leprosy, sleeping sickness (African trypanosomiasis), leishmaniasis, dracunculiasis, trachoma and Buruli ulcer (Hotez *et al.*, 2005). In aggregate, the neglected tropical diseases are responsible for approximately 500,000 deaths annually, and using the DALY (Disease-Adjusted Life Years) as a metric the burden of neglected tropical diseases is equivalent to approximately one-quarter of the disease burden from HIV/AIDS and one-half that of malaria (Hotez *et al.*, 2005). While neglected diseases cause immense suffering and often life-long disabilities, the mortality rate associated with these diseases is relatively low and therefore do not receive the attention and funding of high-mortality diseases such as the so-called "big three", HIV/AIDS, tuberculosis, and malaria (Molyneux, 2004). Nonetheless, the high morbidity of neglected tropical diseases

affects school attendance, cognitive development, growth and overall productivity. Figure 1.1 is a pie chart showing the burden of neglected diseases (including diarrhoeal diseases) compared with HIV/TB/malaria (Engels D & Savioli L, pers. commun.). Most patients with neglected diseases live in developing countries and are too poor to pay for drugs. Hence, the pharmaceutical industry has traditionally ignored these diseases because they do not promise good return on investment (Yamey, 2002).

Over the past two decades, however, there have been significant achievements in the control of some of the tropical infections through vertical interventions. This includes the substantive reduction in the prevalence and incidence of lymphatic filariasis, onchocerciasis, chagas disease, leprosy and trachoma (Hotez *et al.*, 2004) and the successful progress towards eradication of Guinea-worm disease (WHO, 2004). These successes have given optimism that some neglected diseases could eventually be controlled to a point of elimination as public health problems in some areas of endemicity.

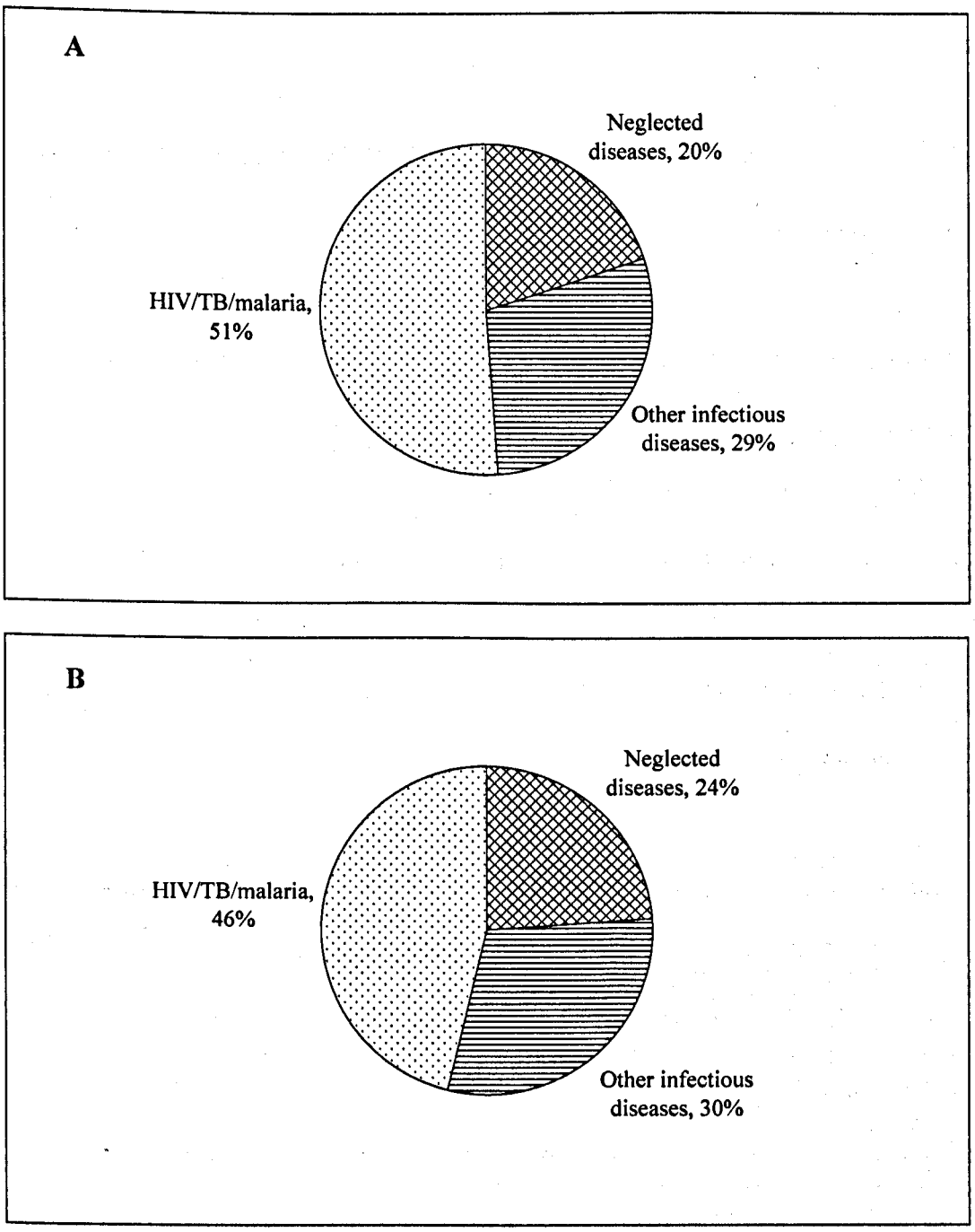

Figure 1.1: The burden of neglected diseases (including diarrhoeal diseases) compared with HIV/TB/Malaria in terms of death (A) and Disability Adjusted Life Years - DALYs (B) (Englels D & Savioli L, Pers. Commun.)

1.1 Global geographical distribution of lymphatic filariasis

In 1993 a World Bank Global Disease Burden (GDB) study was conducted in an effort to develop global burden of diseases. For lymphatic filariasis, the estimates were based on national prevalence rates of overall filariasis cases (both infection and chronic disease cases combined) for 96 endemic countries (World Bank, 1993). The methods used involved aggregating and projecting prevalence data from over 120 published papers, and documents held by the World Health Organization (WHO), to the national level. The highest regional prevalence for filariasis before the current Global Programme to Eliminate Lymphatic Filariasis (GPELF) was in the Pacific region (29%), with the three highest prevalence rates in Tonga (48%), Papua New Guinea (39%) and the Cook Islands (39%) (Michael and Bundy, 1997). However, the Indian sub-continent has the largest number of cases of both *Wuchereria bancrofti* and *Brugia malayi* infections (Michael *et al.*, 1996) and therefore remains an important endemic area for filariasis (WHO, 1984; WHO, 1992). In terms of populations living in areas at risk of infection with *W. bancrofti*, South-east Asia has the greatest number of people at risk. India alone has 454 million people at risk and ranks first worldwide (WHO, 2005). Africa represents the second largest number of people at risk (at least 478 million) and 39 (almost 47%) of the 83 lymphatic filariasis-endemic countries are in this region (WHO, 2005). Nigeria has the largest population at risk (80 million people) on the African continent, and ranks second worldwide (WHO, 2002c).

1.2 Earlier studies on bancroftian filariasis in Kenya

The first published report on lymphatic filariasis in Kenya is from the annual medical report for Lamu Island for the period 1911-1912 which reported *W. bancrofti*

infection in 42 (35.6%) of 118 persons examined (Dunderdale, 1921). A survey conducted to examine for microfilariae in filariasis endemic villages in Pate Island and the Tana River area found 112 (35.3%) persons out of 317 persons examined to be microfilaria-positive (Dunderdale, 1921). An epidemiological survey, also in Pate Island, recorded a human infection (microfilaria) rate of 32% and an "elephantiasis" (lymphoedema) rate of 11% in 142 males examined (Heisch *et al.*, 1959).

A major survey involving 4566 persons in 36 villages representing the entire Kenyan coast from Vanga on the southern coast near the Tanzanian border to Pate Island on the northern coast was conducted in 1962 (Nelson *et al.*, 1962). In general, microfilaria rates among the villages were found to vary from 25% in the north to 10% in the south. Among 89 males examined in Faza Island in the north coast the prevalence rates of microfilaria, "elephantiasis" and hydrocele were 40.6%, 16.8% and 39.3% respectively. However, the survey was mainly confined to the 10 km wide "coastal strip" and the areas along the Tana and Sabaki rivers.

Between 1971 and 1973, a cluster sample survey was conducted among adult males over 14 years of age in 73 sites to gain an insight in the prevalence of the disease in the Coast Province and to assess its public health importance by determining the proportion with clinical signs and symptoms of lymphatic filariasis (Wijers, 1977). A total of 5004 males were examined in the study and 28.4% were found to be microfilaria-positive. Physical examinations for clinical signs and symptoms of chronic disease identified 30.2% to have either hydrocele or elephantiasis of the genitalia or limbs. The overall prevalence of hydrocele alone was 29.9%. The highest microfilaria rate observed was 56% and the highest clinical signs and symptoms rate 64%.

Two areas Mambrui, a small coastal town, and Jaribuni, a rural area, were chosen for further investigations on the epidemiology of the disease (Wijers and Kiilu, 1977; Wijers and Kinyanjui, 1977). Microfilaria rate in Mambrui was 21.7% and the hydrocele rate 15.4% and in Jaribuni, the corresponding rates were 22.0% and 17.0%, respectively.

1.3 Recent epidemiological studies in Kenya

Although bancroftian filariasis was demonstrated to be highly endemic in Kenya through studies conducted in the 1960s and 1970s there was paucity in collection of epidemiological data in this region for more than 20 years. It was not until the mid-1990s that different groups started to conduct bancroftian filariasis epidemiological studies in the country. The first study was conducted in a community, near Vanga at the south-eastern part of Kwale District near the border with Tanzania (Estambale *et al.*, 1994). Microfilaria prevalence was 13.7% and hydrocele (males 15 years or more old) and elephantiasis prevalence rates were 16.5% and 2.4%, respectively.

A more detailed study was conducted in two adjacent communities in Muhaka area, Kwale District (Wamae *et al.*, 1998). In addition to microfilaraemia, the study also tested individuals for antigenaemia and antifilarial antibody responses and underscored the highly focal distribution of bancroftian filariasis in the area. Although the two communities are very close to one another, the transmission intensities were significantly different.

A cross-sectional survey in three villages designated for filariasis control in Kinango Location, Kwale District reported 16.4% microfilaria prevalence and 10.4% hydrocele (males 5 years or more old) prevalence (Njenga *et al.*, 2000). An

epidemiological survey in 12 villages selected for a study to determine the effects of permethrin-impregnated bednets (Mukoko *et al.*, 2004) found the overall microfilaria prevalence to be 16.0% (8.1%-27.4%). This study re-emphasized the highly focal nature of filariasis.

It is interesting to note that all the recent studies were conducted in Kwale District in the south coast region of the Coast Province. The south and north coast are defined as south and north of Mombasa Island, respectively in the present study. The present study is the first to be reported since the earlier studies conducted in north coast region in the 1970s. In addition, this study was conducted in more inland communities unlike previous studies that focused on predominantly coastal villages. The present study was designed to elucidate the current epidemiological picture of bancroftian filariasis in the north coast region, which was previously shown to be an important endemic area for bancroftian filariasis (Heisch *et al.*, 1959; Nelson *et al.*, 1962; Wijers, 1977; Wijers and Kinyanjui, 1977). The setting of the present study is a highly *W. bancrofti*-endemic foci which was selected for filariasis elimination programme using mass treatment. This study was designed to take advantage of the opportunity presented by the elimination programme to compare effectiveness of different tools proposed for monitoring the impact of mass treatment.

1.4 Global Programme to Eliminate Lymphatic Filariasis (GPELF)

Of all the international eradication or elimination initiatives launched against human pathogens, only the smallpox eradication programme was ultimately successful, with the last case occurring in 1977 in Somalia (Fenner *et al.*, 1998). In 1988, the International Task Force for Disease Eradication (ITFDE) was formed to evaluate the potential for eradication of candidate diseases and to identify specific

barriers to eradication or elimination (Molyneux *et al.*, 2004). Three broad criteria have been proposed for use to assess feasibility of disease eradication programmes. These include social and political considerations, biological and technical feasibility, and a full understanding of cost and benefit issues (Aylward *et al.*, 2000). These criteria were used to review more than 80 diseases and the ITFDE in 1993 concluded that only six diseases could probably be eradicated using existing technology, namely, dracunculiasis, rubella, poliomyelitis, mumps, lymphatic filariasis and cysticercosis (Molyneux *et al.*, 2004).

In theory if the right tools were available, some of the infectious diseases would be eradicable, but in reality there are distinct biological features of the organisms and technical factors that make potential eradicability more or less likely. The Dahlem Workshop on the Eradication of Infectious Diseases held in Berlin in March 1997 (Dowdle and Hopkins, 1998) focused on the science of eradication and addressed four questions: 1) How is eradication to be defined and what are the biological criteria? 2) What are the criteria for estimating the costs and benefits of disease eradication? 3) What are the societal and political criteria for eradication? 4) When and how should eradication programmes be implemented? Eradication was defined as permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts; i.e. interventions are no longer needed. Elimination refers to local reduction to zero of the incidence of infection as a result of deliberate efforts. Since infection can be imported from other areas that are still endemic permanent intervention is required (Dowdle, 1998; Molyneux *et al.*, 2004).

The Dahlem Workshop considered three indicators to be of primary importance in assessing eradicability of infectious diseases: an effective intervention is available to interrupt transmission of the agent; practical diagnostic tools with sufficient sensitivity and specificity are available to detect levels of infection that can lead to cessation of transmission; and humans are essential for the life-cycle of the agent, which has no other vertebrate reservoir and does not amplify in the environment (Dowdle, 1998).

In most endemic areas of *W. bancrofti*, notably India and Africa, the levels of lymphatic filariasis infection have remained high. The major reason for persistence of the disease has been the lack of diagnostic and control tools and strategies that are cost-effective and appropriate for the endemic countries (Ottesen *et al.*, 1997). However, recent research advances in lymphatic filariasis have led to a new understanding about the severity and impact of the disease, new diagnostic and monitoring tools have been developed, and, most importantly, new treatment and control strategies (Ottesen *et al.*, 1997). Three available drugs, diethylcarbamazine (DEC), ivermectin and albendazole have been shown by intensive investigation to be safe and effective anti-filaricides for mass treatment to control the transmission of lymphatic filariasis.

The recognition that two-drug single dose treatment strategies are significantly more effective than treatment with either drug alone has been a major advance in the development of control regimens for lymphatic filariasis (Moulia-Pelat *et al.*, 1995; Ottesen and Ramachandran, 1995; Addiss *et al.*, 1997; Ismail *et al.*, 1998; Bockarie *et al.*, 2002). The introduction of effective treatment regimens to control transmission by reducing microfilaraemia has been primarily responsible for identification of

lymphatic filariasis by an International Task Force for Disease Eradication as an eradicable, or potentially eradicable, infectious disease (CDC, 1993). Lymphatic filariasis was previously eliminated from several areas including Quemoy (Kinmen) Islands in China by use of DEC-fortified salt (Fan, 1990), Solomon Islands by use of residual insecticide spraying for vector control (Webber, 1979) and small foci in Santa Catarina state, Brazil by use of DEC chemotherapy (Schlemper *et al.*, 2000). On the basis of these advances, in 1997, the Fiftieth World Health Assembly (WHA) passed a resolution to eliminate lymphatic filariasis globally as a public health problem (WHO, 1997). However, it is important to note that elimination of filariasis was not achieved in some areas despite long-term control programmes (Esterre *et al.*, 2001).

Following the WHA resolution on lymphatic filariasis elimination, the WHO initiated the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000 (Molyneux and Zagaria, 2002). The principal goals of GPELF are to interrupt transmission of infection and to alleviate and prevent the suffering and disability caused by the disease. The main strategy employed to interrupt transmission of lymphatic filariasis is mass drug administration (MDA) of antifilarial drugs to all persons living in endemic areas (at risk population) to decrease the reservoir of microfilariae. The two-drug once-yearly regimen administered as the mainstay of national lymphatic filariasis elimination programmes is the combination of albendazole plus either ivermectin (Mectizan®) or DEC (Ottesen *et al.*, 1997; Molyneux *et al.*, 2000). In many areas of sub-Saharan Africa where lymphatic filariasis and onchocerciasis are co-endemic DEC cannot be used because it was previously shown to cause severe side effects in individuals with *Onchocerca volvulus* microfilariae (Greene *et al.*, 1985).

Early support in the task of eliminating lymphatic filariasis came from ministries of health of the endemic countries and a number of international organizations, including the Arab Fund for Economic and Social Development (AFESD), the United States Centers for Disease Control and Prevention (CDC) and the United Kingdom Department for International Development (DFID). In January 1998, the coalition was given a powerful boost when GlaxoSmithKline (GSK) announced its commitment to collaborate with WHO in a partnership between the private and public sector to support the global programme to eliminate lymphatic filariasis by donating albendazole free of charge for as long as necessary. Later that year, a further boost was given by Merck & Co., Inc. by pledging to expand its Mectizan® Donation Programme for onchocerciasis to cover treatment of lymphatic filariasis with ivermectin in all African countries where the two diseases occur together (Molyneux *et al.*, 2000; WHO, 2002a). The GPELF has rapidly upscaled and by 2004, 38 of the 83 countries and territories classified as lymphatic filariasis-endemic had started elimination programmes. It is estimated that more than 76.5 million people were treated with the two-drug combinations of ivermectin/albendazole or DEC/albendazole in 2004, while 171.6 million in Brazil, Guyana and India were treated with DEC alone or DEC-fortified salt (WHO, 2005).

It is important to collect detailed epidemiological baseline data on the prevalence and intensity of infection, disease rates and other indicators of transmission and infection before implementation of a mass treatment programme to eliminate lymphatic filariasis. The baseline data would make it possible to assess the impact of the mass chemotherapy and enable stakeholders to understand the progress of the programme. Once the elimination programme is implemented epidemiological approaches are required for longitudinal monitoring of the impact of the mass

treatment. Before stopping the mass treatment, data will be required to demonstrate that transmission has been interrupted and that elimination can be certified. Longitudinal follow-up data using different epidemiological tools may help to identify appropriate indicators of interruption of transmission. There is also need to determine sensitive and specific methods that could be used for post-mass treatment surveillance of resurgence of transmission.

1.5 Hypothesis for the current study

To obtain information about the effectiveness of the filariasis elimination programmes in different countries longterm monitoring of the impact of the annual mass drug administration will be required. Monitoring of the impact of mass chemotherapy in filariasis elimination programmes under GPELF is also a crucial activity in deciding when to stop the mass drug administration campaigns. Microfilaria detection and the immunochromatographic (ICT) card test are the standard methods proposed for evaluation and subsequent longitudinal monitoring of infection during mass treatment of lymphatic filariasis (WHO, 2000). However, their usefulness in assessing residual infection after several rounds of MDA is questionable due to low sensitivity when the intensity of infection decreases (Washington *et al.*, 2004). We hypothesize that methods based on antifilarial antibody responses, quantitative circulating filarial antigen (CFA) assays, and detection of parasite deoxyribonucleic acid (DNA) in vectors by polymerase chain reaction (PCR)-based assays may be more sensitive and useful than microfilaria detection and the ICT test when the intensity of infection is reduced by mass chemotherapy. The present study was undertaken to compare the usefulness of different diagnostic strategies in parallel.

The information obtained in this study will enable programme managers to choose a rational strategy for monitoring of impact of their national filariasis programmes.

1.6 General objectives

1. To determine the baseline epidemiology of lymphatic filariasis caused by *W. bancrofti* in communities living in a highly endemic focus along River Sabaki (also called River Galana) in Malindi District, Coast Province of Kenya.

2. To assess the impact of annual mass chemotherapy with diethylcarbazine (DEC) and albendazole on transmission of bancroftian filariasis.

3. To evaluate the usefulness of different tools in monitoring the impact of annual mass drug administration for elimination of lymphatic filariasis.

1.7 Specific objectives

1.7.1 Baseline epidemiological study

1. To determine the baseline prevalence and intensity of infection due to *W. bancrofti* and specific antifilarial antibodies.

2. To determine baseline clinical manifestations due to bancroftian filariasis.

3. To determine *W. bancrofti* infection rates in vector mosquitoes using PCR-based assay xenomonitoring.

4. To determine the species of vector mosquitoes involved in transmission of bancroftian filariasis in the area along River Sabaki.

1.7.2 Monitoring of impact of mass treatment using different tools

1. To monitor the impact of annual single dose mass drug administration of DEC/albendazole on the prevalence and intensity of active infection due to *W. bancrofti* and antifilarial antibody responses.

2. To monitor the impact of annual single dose mass drug administration of DEC/albendazole on clinical manifestations due to bancroftian filariasis.

3. To compare in parallel the usefulness of different tools, namely, microfilaria detection, ICT test, Og4C3 ELISA assay, antifilarial antibody assays, and PCR-based xenomonitoring for longitudinal monitoring of the impact of mass treatment on lymphatic filariasis.

CHAPTER 2 - LITERATURE REVIEW

2.1 Filarial worms

Filarial worms are nematode parasites belonging to the class Secernentia, order Spirurida and superfamily Filarioidea that live in tissues and body cavities of a vertebrate host. Within the superfamily Filarioidea, two families, Filariidea and Onchocercidae are recognized. All filarial parasites infecting man belong to the family Onchocercidae (Chabaud, 1974; Anderson and Bain, 1976).

There are several hundreds of described species of filarial worms from many different taxa of avian and mammalian hosts. However, the species that are important parasites of humans are *Wuchereria bancrofti*, *Brugia malayi*, *B. timori*, *Onchocerca volvulus*, *Loa loa*, *Mansonella* (syn. *Dipetalonema*) *perstans*, *Mansonella streptocerca*, and *Mansonella ozzardi*. *Wuchereria bancrofti*, *B. malayi*, and *B. timori* are the causative agents of human lymphatic filariasis (Beaver *et al.*, 1984). More than 90% of lymphatic filariasis is caused by *W. bancrofti*, while the remaining 10% is caused by *B. malayi* and *B. timori* (WHO, 1984).

The other important filarial infections of humans are onchocerciasis (river blindness) and loiasis. Onchocerciasis caused by *O. volvulus* is transmitted by blackflies of the genus *Simulium* and is predominantly found in sub-Saharan Africa with a few foci in Yemen and in south and central American countries including, Brazil, Colombia, Ecuador, Guatemala, Mexico and Venezuela (Molyneux *et al.*, 2003). An estimated 50 million individuals remain at risk of onchocerciasis with 18 million infected with *O. volvulus* and over 95% of them living in 22 countries in sub-Saharan Africa (Molyneux *et al.*, 2003).

The principal clinical manifestations of onchocerciasis are ocular lesions, resulting in visual impairment and blindness and onchocercal skin disease. Significant success has been achieved in the control of onchocerciasis in Africa first by vector control under Onchocerciasis Control Programme (OCP) and more recently by mass drug administration of ivermectin under African Programme for Onchocerciasis Control (APOC). *Loa loa*, transmitted by a tabanid fly of the genus *Chrysops* occurs in west and central Africa and manifests itself as tropical eye worm or calabar swelling (transient subcutaneous oedema) when adult worms migrate through the eye and cutaneous tissues. A particular constraint of the APOC is severe adverse effects associated with ivermectin use in areas where *L. loa* is co-endemic. Ivermectin causes encephalopathy in individuals with high levels of *L. loa* microfilariae (Gardon *et al.*, 1997; Boussinesq *et al.*, 2003).

Guinea-worm disease (dracunculiasis) is caused by the nematode *Dracunculus medinensis*. Humans become infected by drinking water containing copepods which are infected with larvae of *D. medinensis*. There has been substantial success in eradication of dracunculiasis in many previously endemic foci but 14 countries in sub-Saharan Africa still reported incident cases in 2000 (WHO, 2001). Nonetheless, there has been a remarkable decrease in the number of cases of dracunculiasis, with a decline of 99% from 1989 to 2003. Currently, the transmission is confined to only 12 African countries while 168 countries and territories are already certified free of transmission (WHO, 2004).

2.2 Life cycle of filarial worms

Filarial worms have unique features in their life cycle. They require an arthropod vector for both maturation of their larvae and transmission from one

vertebrate host to another (Schacher, 1973; Sasa, 1976). After maturation, the lymphatic-dwelling female nematodes produce live prelarval forms called microfilariae. In some species, the egg membrane becomes elongated to accommodate the developing embryo as it grows and develops in the uterus, resulting in "sheathed" microfilariae being released by the females. In some species, the membrane splits allowing the naked embryo to escape as an "unsheathed" microfilaria (Rogers *et al.*, 1976).

Once released by the females, microfilariae enter the blood or lymphatic vessels. While circulating in the peripheral blood or moving about in cutaneous tissue the microfilariae are ingested by blood-sucking arthropods. In an appropriate arthropod the microfilariae migrate through the wall of the digestive tract into haemocoele then into specific suitable locations (usually the thoracic muscles, malpighian tubules or fat bodies) for development to first stage larvae (L1), second stage larvae (L2), and infective third stage larvae (L3). The L3 then migrates to the mouthparts and escapes into or onto the vertebrate host's skin when the arthropod takes a blood meal (Beaver *et al.*, 1984). The L3 enters the lymphatic vessels under the skin and migrates to the deeper lymphatic vessels and lymph nodes. In an appropriate host, the L3 undergo moulting between 9 and 14 days to become fourth stage larva (L4), and after approximately 30 days post infection the L4 moults into adult worm. The fecund life span of *W. bancrofti* in an endemic area is estimated to be 5 years (Vanamail *et al.*, 1996) whereas the mean life-span of the parasite is estimated at about 10 years (Subramanian *et al.*, 2004).

2.3 Microfilarial periodicity

The epidemiology of lymphatic filariasis is dominated by the concept of periodicity. Microfilariae appear in the peripheral blood in large numbers at a specific period of the 24-hour cycle. When the largest density of microfilariae is found in the peripheral blood at night they are said to have a nocturnal periodicity. In the nocturnally periodic forms of lymphatic filariasis, microfilariae are found in the peripheral blood in greatest density between 9 pm and 2 am. This form of lymphatic filariasis is transmitted by mosquitoes that bite at night. *Wuchereria bancrofti* is the only known aetiologic agent of lymphatic filariasis in East African region and the microfilariae show nocturnal periodicity. In Kenya, a study to determine the periodicity of microfilariae showed that microfilariae reached peak density in peripheral blood at 00:56 hrs (Gatika *et al.*, 1994), confirming nocturnal periodicity in the setting of the present study.

In sub-periodic filariasis, significant levels of the microfilariae are found in peripheral blood throughout the 24 hour period, but numbers increase at a specific period. In the diurnally sub-periodic type, the concentration of microfilariae in peripheral blood increases in the daytime. This type of filariasis is found in Polynesia and Fiji in the Pacific region and is transmitted primarily by day-biting mosquitoes, typically *Aedes* species.

2.4 Immunology of lymphatic filariasis

As in many parasitic diseases, antifilarial immune responsiveness is correlated with filarial infection in humans (Ottesen, 1992). Serologic assays conducted on samples collected from areas where lymphatic filariasis is endemic provide strong evidence that nearly all individuals in such areas have been exposed to filarial

parasites (Piessens *et al.*, 1980b; Bailey *et al.*, 1995). There is however a large group of individuals, who despite lifelong exposure to infection have no detectable microfilaraemia and/or antigenaemia and clinical history or evidence of infection (Freedman *et al.*, 1989; Day, 1991; King and Nutman, 1991; Mahanty *et al.*, 1992). These individuals, variously termed "endemic normal" or "putatively immune", exhibit a greater degree of increased immune responsiveness than that of microfilaraemic individuals (Ottesen *et al.*, 1977; Piessens *et al.*, 1980a; Piessens *et al.*, 1980b; Ottesen *et al.*, 1982; Ottesen, 1984; King and Nutman, 1991; Dimock *et al.*, 1994). The presence or absence of specific antibody and cellular responses actively generated by the host are important in determining the outcome of infection (Ottesen, 1984).

2.4.1 Cellular immune responsiveness

Previous work has demonstrated that the hallmark of infection with lymphatic filariasis is cellular immune hypo-responsiveness limited almost exclusively to filarial antigens (Ottesen *et al.*, 1977; Piessens *et al.*, 1980a; Piessens *et al.*, 1980b). The only exception to the generalized hypo-responsiveness to filarial antigens occurs in patients with tropical pulmonary eosinophilia. These patients demonstrate a hyper-responsiveness to filarial antigens especially those derived from the microfilarial stage of the parasite, a feature that is not seen in other filariasis patients (Ottesen, 1984). This observation led to the speculation that tropical pulmonary eosinophilia is a form of occult filariasis in which the absence of microfilariae reflects an immunologic hyper-responsiveness on the part of the host which results in effective clearance of the stage of the parasite from the blood (Ottesen, 1984).

The existence of specific cellular immune unresponsiveness in human filariasis is presumably important for the successful persistence of the parasite within the host (Ottesen, 1984). It is recognized that the unresponsiveness occurs not because the patients fail to become sensitised to filarial antigens, but because various modulating mechanisms develop that can specifically suppress responses to these antigens (Ottesen, 1984). The mechanisms involved in modulation of the cellular immune responses to filarial antigens include, serum suppressor factors, suppressive adherent cells that are probably monocytes and T-lymphocyte suppressor cells (Piessens *et al.*, 1980c; Piessens *et al.*, 1982).

Cellular immune responses can be defined on the basis of cytokine production by T helper cells, with T helper 1 (Th1) and Th2 representing proposed subgroups (Mosmann and Coffman, 1989). Stimulation of peripheral blood mononuclear cells (PBMC) from individuals with active filarial infection using parasite antigen has been shown to result in increased Th2 cytokine production (IL-4, IL-5 and IL-10 responses) suggesting an active immunological cross-regulation response that inhibits proinflammatory responses to the parasite. In contrast, antigen-negative individuals characteristically have strong Th1 (IFN-γ responses) (Steel *et al.*, 1994; de Almeida *et al.*, 1996; Dimock *et al.*, 1996).

2.4.2 Humoral immune responsiveness

Hyperglobulinaemia with elevated levels of specific antibody has long been recognized in filariasis, with only microfilaraemic patients having relatively deficient antibody responses, which could reflect an element of specific humoral immunosuppression (Ottesen, 1984). Humoral immune response in human filariasis is generally dominated by the IgG4 isotype, when measured by enzyme-linked

immunosorbet assay (ELISA) against somatic adult worm antigen (Ottesen *et al.*, 1985; Kwan-Lim *et al.*, 1990; Egwang *et al.*, 1993) and individuals harbouring adult worms have been found to have higher antifilarial IgG4 levels than adult worm-free individuals (Nicolas *et al.*, 1999).

For children living in filariasis endemic areas, antifilarial IgG4 responses are higher in individuals with antigenaemia than in those without antigenaemia and increases in antifilarial IgG4 are associated with acquisition of infection, as defined in antigen status. In contrast, antifilarial IgG1 and IgG2 increase to the same extent among children who acquire infection (antigenaemia) and among those who remain antigen negative suggesting that these responses are driven by exposure to filarial larvae rather than infection (Lammie *et al.*, 1998). Other investigators have postulated that IgG1 levels are more related to microfilaria status than to infection status (Simonsen *et al.*, 1996). However, in adults, antifilarial IgG2 responses of antigen-negative persons are similar to those of antigen-positive (microfilaria-negative) persons, but are significantly higher than those of microfilaria-positive individuals (Addiss *et al.*, 1995; Dimock *et al.*, 1996). The reason for decreased antifilarial IgG2 responses in microfilaraemic persons is not clear, but may be related to shifts in cytokine production (King *et al.*, 1990).

2.5 Clinical manifestations of lymphatic filariasis

The clinical manifestations of lymphatic filariasis vary from one endemic area to another and also differ to some extent on the species of the parasite that is involved (Sasa, 1976). Lymphatic filariasis caused by *W. bancrofti* is characterized by a wide spectrum of clinical manifestations from clinically asymptomatic microfilaria-positive

individuals to patients with disfiguring chronic filarial disease. The individuals living in endemic areas are generally categorized into five broad groups.

2.5.1 Endemic "normals" group

There is a proportion of individuals living in endemic areas that exhibit no clinical or parasitological evidence of infection. This group is generally referred to as "endemic normals" (WHO, 1992). Previously, this group consisted of persons who are negative for microfilariae and without clinical symptoms of filariasis. However, the development of sensitive and specific tests has led to a refinement of this group to include persons who are also negative for circulating filarial antigen.

2.5.2 Asymptomatic microfilaraemic group

A large group of individuals in endemic areas have circulating filarial antigen with or without microfilariae, but clinically without symptoms of disease. A large proportion of these individuals remain asymptomatic for many years (Ottesen, 1992; WHO, 1992) whereas some may develop clinical symptoms. However, studies have shown that individuals in this group have subclinical lymphatic pathology (Amaral *et al.*, 1994; Freedman *et al.*, 1994). In addition to lymphatic pathology it was observed that up to 45% of microfilaraemic patients have renal pathology manifested as haematuria and/or proteinuria (Dreyer *et al.*, 2000). The specific mechanisms underlying these renal abnormalities have not been defined but it has been suggested that immunologic damage induced by immune complexes deposited in renal glomeruli is likely to be the cause. Such complexes have been detected circulating in the blood of some clinically asymptomatic individuals with bancroftian filariasis (Prasad *et al.*, 1983; Lunde *et al.*, 1988; Kobayashi *et al.*, 1997). In terms of humoral immune responsiveness, individuals with microfilaraemia but who are asymptomatic

and without acute or chronic lymphatic disease form the group that is immunologically least reactive (Ottesen *et al.*, 1977; Piessens *et al.*, 1980a).

2.5.3 Acute symptomatic group

In filariasis endemic areas, some infected individuals develop periodic episodes of acute filarial disease characterized by fever and accompanied by inflammation of the lymphatic vessels (lymphangitis) and lymph nodes (lymphadenitis). This clinical manifestation is generally referred to as adenolymphangitis (ADL) and is thought to be induced by adult worms located in the lymphatics (Ottesen, 1992). The recurrent episodes of ADL are believed to be a major risk factor for the development of lymphoedema of the legs (Pani *et al.*, 1990; Das *et al.*, 1994).

Two distinct acute clinical syndromes accompanied by lymphangitis have been suggested to occur in residents of filariasis endemic areas (Dreyer *et al.*, 1999). One syndrome referred to as acute filarial lymphangitis (AFL) is caused by death of adult worms. Indeed studies have shown that death of the worms either spontaneously or as a result of treatment could trigger ADL (Noroes *et al.*, 1997; Dreyer *et al.*, 1998a). Acute filarial lymphangitis has a mild clinical course and rarely causes residual lymphoedema. The syndrome is characterized by lymphangitis that progresses distally or in a retrograde fashion along the lymphatic vessel. The second syndrome is referred to as acute dermatolymphangitis (ADLA) and is suggested to be a result of secondary bacterial infections. This syndrome is characterized by plaque-like areas of relatively diffuse subcutaneous inflammation with or without retrograde lymphangitis. Acute dermatolymphangitis is often accompanied or followed by distal oedema of the affected leg. The resolution of the oedema may be accompanied by

extensive exfoliation of the skin in the affected limb (Dunyo *et al.*, 1998). The syndrome is a common cause of lymphoedema both within and outside filariasis endemic areas.

2.5.4 Chronic lymphatic pathology group

There is a group of individuals in endemic areas that develop chronic lymphatic pathology. Hydrocele, lymphoedema/elephantiasis and chyluria are the main clinical manifestations of chronic lymphatic filariasis (WHO, 1992). Hydrocele is characterized by accumulation of clear, straw-coloured fluid in the cavity of tunica vaginalis as a result of blockage of lymphatics draining the retroperitoneal and subdiaphragmatic areas. Tunica vaginalis is a serous membrane surrounding each of the testes. Chylocele is a chronic condition that resembles hydrocele but occurs when dilated lymphatic vessels in the spermatic cord rupture and cause leakage of milky coloured lymph fluid (rich in fat) into the cavity of tunica vaginalis (Dreyer *et al.*, 1998b).

Chyluria is a less common manifestation defined as the excretion of chyle (intestinal lymph) into the urinary tract. The basic pathophysiology is related to blockage of the retroperitoneal lymph nodes below the cisterna chyli with consequent reflux and flow of the lymph directly into the renal lymphatic vessels, which may rupture and permit flow of chyle into the urinary tract (WHO, 1992). The urine often appears milky white in colour.

Lymphoedema occurs due to accumulation of lymph fluid in the tissues following damage of the lymphatic vessels (Ottesen, 1990). Lymphoedema is common in the lower limbs but can affect the arms, breasts, scrotum, penis and sometimes vulva. Lymphoedema develops gradually starting as pitting oedema that is

reversible to later become persistent swelling accompanied by fibrotic changes of the skin. These changes can ultimately lead to gross pathology generally referred to as elephantiasis. Elephantiasis is characterized by profound fibrosis as well as profoundly thickened and leathery skin sometimes with wart-like growths and secondary microbial infections (Ottesen, 1990).

2.5.5 Tropical pulmonary eosinophilia

There are individuals who comprise less than 1% of all patients with lymphatic filariasis who present with a clinical syndrome known as Tropical pulmonary eosinophilia (TPE). Tropical pulmonary eosinophilia presents a clinical picture of paroxysmal nocturnal cough (and sometimes wheezing) similar to asthmatic symptoms (Ottesen, 1992). Visualization of chest radiographs indicates nodular or diffuse pulmonary lesions. Other clinical features of TPE include elevated peripheral blood eosinophilia (> 3000/mm^3), extraordinary high levels of serum IgE and very high levels of specific antifilarial antibodies. Unlike all other forms of lymphatic filariasis, patients with TPE are extremely hyper-responsive to filarial antigens, especially those derived from the microfilarial stage of the parasite (Ottesen, 1984).

2.6 Pathogenesis of lymphatic disease

The pathogenesis of filarial disease remains poorly understood and has been a subject of controversial debate. Unlike most human diseases, human filariasis suffers from lack of an appropriate animal model for research into the basic pathophysiology of the disease (Nelson *et al.*, 1991). The most widely investigated animal models are the Mongolian jird (*Meriones unguiculatus*) (Ash and Riley, 1970) and the ferret (*Mustela putorius furo*) (Crandall *et al.*, 1982). The major deficiency of these animal models, however, is that the infection does not mimic the human disease in the

anatomic localization of adult worms, in the symptomatology, or in the immune effector mechanisms that may be involved (Nelson *et al.*, 1991).

Abnormalities in the lymphatic vessels have been shown to occur in a variety of hosts with experimental filariasis (Schacher and Sahyoun, 1967; Denham and Rogers, 1975; Rogers *et al.*, 1975; Vincent *et al.*, 1980; Hines *et al.*, 1985). In cats (*Felis catus*) infected with *B. pahangi* for example, lymphatic vessels begin to dilate as early as 10 days after larval inoculation (Denham and Rogers, 1975). Detailed examination of the nature and distribution of lymphatic and blood vascular abnormalities in ferrets infected with *B. malayi* demonstrated generalized vascular abnormalities (Case *et al.*, 1991).

Dilatation of the lymphatic vessels (lymphangiectasia) is the most investigated of the lymphatic abnormalities. Investigations employing videomicroscopy (Case *et al.*, 1991; Case *et al.*, 1992) and ultrasonography (Noroes *et al.*, 1996) demonstrated nests of wriggling live adult worms, which typically stretched and dilated the lymphatic vessel walls. Histological examination of tissues from infected ferrets revealed that dilated lymphatics, most prominent near living or dead adult worms, exhibited plump endothelium and thickened walls and valves with perilymphangitis and adjacent tissue fibrosis was frequently present. Histological examination of tissues obtained from patients with filariasis found that lymphatic vascular lesions parallel those seen in infected ferrets (Case *et al.*, 1991).

Examination of clinically asymptomatic microfilaraemic persons using radionuclide lymphoscintigraphy revealed varying degrees of structural lymphatic damage including lymph vessel dilatation (Freedman *et al.*, 1995). In addition to dilatation of lymphatic filariasis, flow studies reported an enhanced pattern of rapid,

increased lymph flow in asymptomatic microfilaraemic persons, which is thought to be due to increased lymph production in distal tissue (Freedman *et al.*, 1995). Studies on the B and T lymphocyte immunodeficient mutant mouse called SCID (severely combined immunodeficiency) demonstrated that the presence of adult worms in the lymphatic vessels was associated with lymphangitis and lymphangiectasia and in some cases there were significant inflammatory changes and retention of lymph fluid (Nelson *et al.*, 1991). Thus, in the absence of any immune response the parasite or its excretory/secretory products is able to induce lymphatic pathology.

The aetiology of abnormally increased lymph production seem to result from either structural damage to lymph vessel walls caused by the constantly motile adult worms (Case *et al.*, 1992; Witte *et al.*, 1993) or by the effect of unidentified parasite secretory products that alter endothelial cell function (Kaiser *et al.*, 1990; Mupanomunda *et al.*, 1997).

Earlier studies attribute pathogenesis to obstructed afferent lymph flow with truncal back pressure from nodal congestion and inflammatory or granulomatous responses to released products or calcified filariae. It was postulated that perilymphatic inflammation, such as that resulting from reaction to a dead adult worm might generate fibrosis and narrowing of lymphatics with gradual distal dilatation (Manson-Bahr, 1959). Formation of lymphatic granulomatous nodules and development of acute filarial lymphangitis as a result of death of the adult worms is observed in some patients, e.g. after administration of treatment (Noroes *et al.*, 1997; Dreyer *et al.*, 1998a). This has made some investigators to propose that lymphangiectasia and inflammatory reactions are two independent components of

lymphatic pathology that are triggered by 'toxins' of living adult worms and by host reactions to damaged or dead worms, respectively (Dreyer *et al.*, 2000).

Studies in animal models have shown that secondary infections with bacteria and fungi may play an important role in the development of chronic lymphatic pathology. For example, secondary infection with *Streptococcus* bacteria in cats infected with *B. malayi* resulted in more severe lymphatic pathology than in control animals (Bosworth and Ewert, 1975). Similar results were obtained when *B. malayi* infected cats were exposed to the yeast phase of the fungus *Sporothrix schenkii* (Barbee *et al.*, 1977). Oedema and fibrosis tended to appear earlier, more consistently and progressed more rapidly in cats with dual infections than in controls.

Bacteriological investigations of patients with filarial lymphoedema found bacteria in skin, tissue and lymph fluids, and lymph nodes of patients with chronic lymphoedema without recent episodes of filarial ADL (Olszewski *et al.*, 1999). Further, 47% of patients with ADL or a recent episode (within the last 2 weeks) had positive blood cultures. Taken together, these results suggest that secondary infections may be responsible for filarial ADL and exacerbation of lymphatic pathology. Improved foot care hygiene combined with appropriate use of topical antibiotics or antifungals reduces the number of ADL attacks (Shenoy *et al.*, 1998) thus providing further evidence that secondary infections have a role to play in lymphatic pathology. The quality of life of lymphoedema patients after education on appropriate hygiene, skin care techniques and simple exercises that encourage lymph drainage was previously shown to improve using a Dermatology Life Quality Index (DLQI) questionnaire (McPherson, 2003).

2.7 Principal vectors of lymphatic filariasis

The identification of a mosquito as the vector for *W. bancrofti* by Sir Patrick Manson in 1878 was the first demonstration that arthropods were vectors of parasitic organisms (Scott, 2000). However, not all mosquitoes serve as vectors of lymphatic filariasis. The principal mosquito species that transmit the lymphatic filariae of humans are found in four genera: *Anopheles* (*W. bancrofti, B. malayi, B. timori*), *Aedes* (*W. bancrofti, B. malayi*), *Culex* (*W. bancrofti*) and *Mansonia* (*W. bancrofti, B. malayi*) (Sasa, 1976; Scott, 2000). However, compared with other infectious agents transmitted by arthropods this is an extremely wide range of genera and species with a capacity to transmit *W. bancrofti*. For example, malaria and dengue haemorrhagic fever are transmitted by mosquitoes of the genus *Anopheles* and *Aedes*, respectively.

2.7.1 Vectors of Wuchereria bancrofti

Mosquitoes belonging to the *Culex pipiens* complex principally transmit the nocturnally periodic form of *W. bancrofti*. The major vector in the group is *Culex quinquefasciatus*. However, anophelines of the subgenus Myzomyia e.g. *An. gambiae* and *An. punctulatus* and members of the subgenus *Stegomyia* e.g. *Aedes pseudoscutellaris* group are also involved in transmission (Sasa, 1976; Scott, 2000). Table 2.1 is a summary of the major vectors of *W. bancrofti* in different geographical regions.

Geographical region	Vector species	References
The Americas and Carribean	Primary vector is *Culex quinquefasciatus.* Other vectors include members of the subgenus *Nyssorhynchus* (*Anopheles darlingi, An. aquasalis, An. Albitarsis*).	(Raghavan, 1961; Raccurt *et al.*, 1988)
East Mediterranean (Egypt)	Members of *Culex pipiens* group are the principal vectors.	(Raghavan, 1961; Southgate, 1979)
South and East Asia	Nocturnally periodic *W. bancrofti* is mainly transmitted by *Culex pipiens* group. Other vectors include members of subgenus *Anopheles (An. barbirostris, An. nigerrimus)* and subgenus *Myzomyia (An. Philippinensis, An. Stephensi, An. varuna)*	(Raghavan, 1961)
Pacific	The major vectors include *Aedes (Stegomyia) pseudoscutellaris* group (e.g., *Ae. polynesiensis*), *Anopheles (Myzomyia) punctulatus* group, *Cx. quinquefasciatus, Ochlerotatus vigilax*	(Raghavan, 1961; Kimura *et al.*, 1992; Burkot and Ichimori, 2002)
Africa	*Cx. quinquefasciatus, An. gambiae s.l, An. funestus*	(Mansfield-Aders, 1927; White, 1971; Hawking, 1977; Wijers and Kiilu, 1977; McMahon *et al.*, 1981)

Table 2.1: Major vectors of *Wuchereria bancrofti* in different geographical regions.

Earlier entomological investigations in East Africa reported that *Anopheles gambiae sensu lato, An. funestus* and *Culex quinquefasciatus* are the important vectors of filariasis in East Africa (Nelson *et al.*, 1962; White, 1971; Wijers and Kiilu, 1977). The observation was confirmed in relatively recent entomological studies (Mwandawiro *et al.*, 1997; Pedersen and Mukoko, 2002). In a study conducted in Mambrui, a coastal town in Malindi District, and Jaribuni, a rural area in Kilifi

District, in the Coast Province of Kenya (Wijers and Kiilu, 1977), *Cx. quinquefasciatus* was found to be the main vector in Mambrui, whereas *An. funestus* was the main vector in Jaribuni. An entomological survey conducted in four coastal villages near Tanga, Tanzania, identified the principal vectors of lymphatic filariasis, in order of importance, as *An. gambiae s.l.*, *Cx. quinquefasciatus* and *An. funestus* (McMahon *et al.*, 1981). The conclusion drawn from these studies is that *Cx. quinquefasciatus* was the main vector in the coastal towns and villages, while *An. gambiae* and *An. funestus* were more important in inland areas.

The importance of the three mosquito vectors in transmission appears to depend on the prevailing ecological and socioeconomic factors. In rural areas, where there are no pit latrines, the two anophelines are often the only vectors of *W. bancrofti* present, whereas in urban areas *Cx. quinquefasciatus* is the principal vector (Mansfield-Aders, 1927; White, 1971; Hawking, 1977; Maxwell *et al.*, 1999). Availability of breeding grounds in urbanized areas leads to continuous transmission by *Cx. quinquefasciatus* mosquitoes throughout the year whereas transmission is interrupted in the dry season in rural areas due to unavailability of standing water bodies (Wijers and Kiilu, 1977; McMahon *et al.*, 1981). The *Cx. quinquefasciatus* mosquitoes are a major biting nuisance in many urban areas and their abundance appears to be increasing. This is mainly due to rapid urbanization without provision of proper sanitation leading to increased numbers of breeding sites in the form of wet pit latrines, cess pits and blocked open drains (Curtis and Feachem, 1981).

The *Anopheles gambiae* complex is a group of six morphologically indistinguishable yet genetically and behaviourally distinct mosquito species (Coluzzi *et al.*, 1979; Pock Tsy *et al.*, 2003), and as many as three and possibly four species

may be sympatric in some regions (Scott *et al.*, 1993). Studies using the polymerase chain reaction (PCR) based on the intergenic spacer (IGS) region of ribosomal DNA (rDNA) have been used to successfully identify mosquito species of the *Anopheles gambiae* complex (Paskewitz and Collins, 1990; Scott *et al.*, 1993). Previous work using PCR-based assay identified *Anopheles gambiae sensu stricto, An. arabiensis* and *An. merus* along the Kenyan coastal areas (Mbogo *et al.*, 2003). *Anopheles gambiae s.s.* and *An. arabiensis* are recognized to be the most important vectors of malaria due to their close association with human habitats, their anthropophilic nature and their efficiency in transmission of malaria parasites (Gentile *et al.*, 2001). Characterization of *An. gambiae s.s.* using nucleotide polymorphisms in the IGS of rDNA of the X chromosome has shown that the species is subdivided into two partially isolated molecular forms known as the M and S forms (Favia *et al.*, 2001; Gentile *et al.*, 2001).

2.7.2 Vectors of Brugia malayi

Brugia malayi occurs in Asia and the major vectors are primarily species of *Mansonia*, subgenus *Mansonioides*, and secondarily mosquitoes of the subgenus *Anopheles* e.g. *An. barbirostris* (Raghavan, 1961). In India, brugian filariasis is mostly endemic in Kerala state but many smaller foci occur in parts of Andhra Pradesh, Tamil Nadu, Orissa, West Bengal, Assam and Madhya Pradesh (WHO, 1992). The vectors of *B. malayi* in these areas include *Mansonia annulifera, M. uniformis* and *M. indiana*, but *M. annulifera* is the major vector (Sabesan *et al.*, 1991). However, there is some evidence that the prevalence of *B. malayi* infection in India has declined naturally in many areas while in some areas the species may have

disappeared, become co-endemic with *W. bancrofti*, or have been replaced by bancroftian filariasis (Sabesan *et al.*, 2000).

Surveys conducted in Sri Lanka in the 1930s established that *B. malayi* was transmitted by *Mansonia* mosquitoes (namely *M. uniformis, M. annulifera* and *M. indiana*) and that these species of mosquitoes were found in association with the water plant *Pistia stratiotes* (water lettuce). Based on the findings, campaigns for removal and destruction of *P. stratiotes*, initially by manual methods and later using herbicides, were undertaken (Dassanayake and Chow, 1954). A comprehensive survey of *B. malayi* infection carried out in 21 previously endemic foci in Sri Lanka concluded that the parasite was eradicated mainly through destruction of aquatic vegetation, particularly *P. stratiotes*, through the use of a weedicide (Phenoxylene 30) and selective treatment of microfilaria-positive individuals with diethylcarbamazine (Gautamadasa, 1986).

2.8 Patterns of vector-parasite relationship

The ability of mosquitoes to ingest microfilariae of *W. bancrofti* and successful development of the ingested microfilariae to infective larvae (L3) are important determinants of transmission of infection. Studies on vector-parasite relationship have identified two different patterns of infection that have important implications on the epidemiology and success of interruption of lymphatic filariasis. The relationship between culicine species and *W. bancrofti* is known as limitation. As the number of ingested microfilariae increases, the success rate of the ingested microfilariae to yield of infective filarial larvae decreases in limitation (Southgate and Bryan, 1992; Pichon, 2002). Previous entomologic studies have shown that a considerable proportion of ingested microfilariae are damaged during uptake by the

foregut (pharyngeal) armatures of the mosquitoes (Bryan *et al.*, 1974; McGreevy *et al.*, 1978). However, in culicine species such as *Aedes* and *Culex*, the pharyngeal armatures are less well developed and thus inflict less damage on ingested microfilariae (McGreevy *et al.*, 1978). Thus, even at very low microfilarial numbers parasite uptake by culicine mosquitoes occurs. It has been proposed that in areas where limitation occurs the total interruption of transmission will be difficult to achieve (Pichon, 2002).

The relationship between anopheline species and *W. bancrofti* is known as facilitation (Pichon, 2002). Investigations on *An. gambiae* and *An. arabiensis* revealed that the percentage of mosquitoes ingesting *W. bancrofti* microfilariae was strongly associated with microfilariae density in the host blood (Bryan and Southgate, 1988b). Conversely, the probability of microfilariae successfully penetrating the gut wall in *Anopheles* mosquitoes diminishes as the number of microfilariae ingested diminishes due to damage inflicted on the microfilariae. The pharyngeal armature is well developed in anopheline mosquitoes so that microfilariae are damaged when they are ingested and at low densities there are very few undamaged microfilariae left to infect the mosquito (Bryan and Southgate, 1988a). In facilitation there is a critical threshold point below which the parasite population will die out spontaneously (Webber, 1991; Pichon, 2002). These findings have led to the conclusion that interruption of filariasis is relatively easier to achieve when the local vectors are anopheline and not culicine mosquitoes (Bryan and Southgate, 1988b).

2.9 Methods for diagnosis and monitoring of lymphatic filariasis

Despite the widely held belief that transmission of lymphatic filariasis will cease once the microfilaria prevalence is reduced to 1%, there is little scientific evidence to support this view (Melrose *et al.*, 2004). *Aedes polynesiensis*, a major vector in the Pacific, can transmit parasites even when microfilaria prevalence and density are extremely low (Esterre *et al.*, 2001). An important factor in the long-term success of the Global Programme to Eliminate Lymphatic Filariasis is the availability of effective tools for diagnosis and monitoring of transmission (Melrose *et al.*, 2004), even when microfilaria prevalence is below 1% level by thick smear.

2.9.1 Parasitologic methods

Many filariasis epidemiological surveys and control programmes employ microfilaria detection methods to determine the prevalence of infection and assess effect of intervention. Measurement of microfilaraemia by examination and counting of microfilariae in blood samples is the gold standard for determination of active infection in lymphatic filariasis because the parasite itself is seen under a microscope. Various versions of microfilaria examination techniques are available. Examination of Giemsa-stained thick blood films is widely used for detection of microfilaraemia (Eberhard and Lammie, 1991). A thick blood film is made on a microscope glass slide and dried. The blood film is dehaemoglobinized before fixing and staining in Giemsa stain. The film is then examined for the presence of microfilaria under a microscope. Loss of microfilariae during fixation and staining can lead to erroneous results (Sabesan *et al.*, 1991).

Other methods are based on concentration of the microfilariae in the blood specimen to increase sensitivity. In the membrane (Nuclepore) filtration technique

(Chulerek and Desowitz, 1970), a measured amount of blood (usually 1 ml) is hemolysed and filtered through a filter membrane mounted on special holders. After a washing step, the membrane is carefully placed onto a glass slide and examined for the presence of microfilaria under a microscope. Another concentration technique is modified Knott's concentration method (Knott, 1935). In this method, 1 ml of blood is mixed with 9 ml of 2% formalin and centrifuged to sediment the microfilariae at the bottom of the tube. The supernatant is removed and a drop of 1% methylene blue is added to the sediment. The sediment is transferred to a glass slide and examined for microfilariae. However, this method is not favourable because the resulting precipitate makes examination of microfilaria difficult thus reducing its sensitivity.

Many studies in East Africa use the counting chamber technique (McMahon *et al.*, 1979) for examination and counting of microfilariae. In the counting chamber method, 100 µl of blood is mixed with 900 µl of 3% acetic acid and then transferred into a special chamber called "counting chamber" and microfilaria examination and counting done under a microscope.

Although bancroftian filariasis accounts for more than 90% of lymphatic filariasis in the world (Michael *et al.*, 1996), the resulting microfilaraemia has a nocturnal periodicity in many endemic areas (Sasa, 1976). This requires that blood collection be done at night, a situation that is inconvenient for both the affected communities and the research team. It is recognized that measurement of microfilaraemia is a relatively insensitive method especially if microfilaria density is very low (Eberhard and Lammie, 1991; Melrose *et al.*, 2004). Additionally, a significant proportion of the population with active infection has no circulating microfilariae (Lammie *et al.*, 1994) and although these people do not contribute to

transmission, basing prevalence on microfilaria detection alone underestimates the burden of infection (Turner *et al.*, 1993).

2.9.2 Methods based on detection of circulating antigen

For bancroftian filariasis, the development of a monoclonal antibody, Og4C3, for detecting circulating filarial antigen (CFA) by ELISA in early 1990 (More and Copeman, 1990) offered the convenience of daytime testing and greater sensitivity than testing for microfilaria (Turner *et al.*, 1993; Simonsen and Dunyo, 1999). Animal studies have shown that circulating filarial antigen levels correlate with the number of adult worms in the host (Weil *et al.*, 1985; Weil *et al.*, 1990) and the same is believed to be true in bancroftian filariasis (Ismail *et al.*, 1998). Almost 50% of infected children are not diagnosed using measurement of microfilaraemia alone, indicating the advantage of CFA assay for detecting infection in this group of people (Steel *et al.*, 2001). In a study to determine the suitability of the Og4C3 assay for field studies, the assay was found to be 100% sensitive for patent infection as determined using Giemsa-stained thick blood films (Lammie *et al.*, 1994).

A rapid-format immunochromatographic (ICT) card test based on the monoclonal antibody AD12.1 was recently developed (Weil *et al.*, 1997). The ICT test has greater sensitivity and specificity in detecting patent infection than most parasitological tests in many different geographical regions (Simonsen and Dunyo, 1999; Pani *et al.*, 2000; Njenga and Wamae, 2001; Chandrasena *et al.*, 2002). The ICT test has revolutionized diagnosis of bancroftian filariasis because of its applicability in field setting and has become the preferred method for mapping of lymphatic filariasis (Gyapong *et al.*, 2002; Onapa *et al.*, 2005). However, the ICT

card test is only qualitative, providing negative/positive results and is not suitable when measurement of the effect of intervention is required in quantitative terms.

Although the available tests for CFA can identify infection as early as the first month of patency (Weil *et al.*, 1996), both microfilaraemia and antigenaemia develop from months to years after exposure, reducing their utility for detection of low levels of infection or recrudescence of transmission (Weil *et al.*, 1987; More and Copeman, 1990; Weil *et al.*, 1997; Lammie *et al.*, 1998; Witt and Ottesen, 2001).

2.9.3 Methods based on antibody detection

Methods for filarial antibody detection have been available since 1960s, mostly based upon crude filarial antigens (Harnett *et al.*, 1998; Melrose *et al.*, 2004). Assays based on detection of filarial-specific antibodies are usually used for epidemiological and diagnostic purposes. By providing a cumulative measure of exposure to filarial infection, antibody assays may circumvent many of the limitations of methods based on direct detection of the parasite or its antigens (Lammie *et al.*, 2004). In terms of the immunological response to lymphatic filarial infections, among populations living in endemic areas, isotype-specific antifilarial antibody responses against parasite antigens are characteristically correlated with infection status (Ottesen, 1992) and this feature has been exploited to develop antibody assays. For example, filarial-specific IgG4 levels are more related to antigenaemia (Addiss *et al.*, 1995). Therefore, measurement of antifilarial IgG4 levels may be a useful strategy for assessing the impact of mass chemotherapy on filariasis infection. A method was also developed for the detection of antifilarial IgG4 antibody in urine (Itoh *et al.*, 2001). The method is desirable in certain situations e.g. young children because use of urine is less invasive than blood sampling. Although assays based on antifilarial IgG4

antibody reduces cross-reactions with non-filarial helminths (Melrose *et al.*, 2004), they are limited by cross-reactivity with antibodies from other nematodes, especially with *Strongyloides* (Muck *et al.*, 2003).

For brugian filariasis, a recently devised dipstick test called "Brugia Rapid" demonstrated high sensitivity (97%) and specificity (99%). Unlike the ICT card test, the test detect antifilarial IgG4 antibody, not CFA (Kumari *et al.*, 1994). However, performance of the test requires several steps that make it not as convenient as the ICT card test (Melrose *et al.*, 2004).

A number of recombinant filarial antigens have been developed for use in antibody assays for filariasis (Wang *et al.*, 1999; Rahmah *et al.*, 2001). Recombinant filarial antigens should, in principle, be more useful as the basis of diagnostic or exposure assays because of their greater specificity (Lammie *et al.*, 2004). Assays conducted to examine the performance of antibody assays using 3 recombinant antigens, Bm14, WbSXP and BmR1 demonstrated good sensitivity (>90%) for field use and none of the assays demonstrated cross-reactivity with specimens from persons with non-filarial helminth infections. The Bm14 ELISA assay, however, demonstrated some antibody reactivity with sera from patients with *W. bancrofti*, *B. malayi*, *L. loa* and *O. volvulus*. The BmR1 assays are sensitive for *B. malayi* infection but relatively insensitive for *W. bancrofti* infection, making the assays suitable for areas with brugian filariasis (Lammie *et al.*, 2004). The Bm14 ELISA assay may be useful in areas with exclusively one type of filariasis.

Antibody responses develop in the absence of demonstrable infection, and detecting incident antibody responses should provide a more sensitive measure of transmission than microfilaria or CFA. Children born after the cessation of

transmission should be antibody-negative, while older adults may have evidence of residual antibody reactivity (Gao *et al.*, 1994; Rodriguez-Perez *et al.*, 1999; Weil *et al.*, 2000). Since antibody responses provide an early indicator of infection, assays for antifilarial antibodies should be useful for surveillance following initiation of lymphatic filariasis elimination programmes (Lammie *et al.*, 2004).

2.9.4 Entomologic methods for monitoring of lymphatic filariasis

Traditionally, collection of mosquitoes which are dissected and examined for infective larvae has been used for assessment of active transmission of lymphatic filariasis. Detection of *W. bancrofti* in mosquitoes requires time-consuming dissection and microscopic examination of individual mosquitoes. In addition, speciation of filarial larvae requires additional technical expertise. In terms of monitoring filariasis elimination programmes, dissection is the most ideal means for detecting infections in vector populations but becomes increasingly costly and laborious when the prevalence of infection in the mosquito population drops below 1% (Ramzy, 2002; Goodman *et al.*, 2003). The number of mosquitoes that can be processed using this technique was estimated to be about 35 per person-hour and is slower if mosquitoes are preserved in alcohol (Bockarie *et al.*, 2000). Thus, the use of mosquito dissection for monitoring filariasis elimination programmes may be inappropriate when the prevalence of infection is low because of the need to collect and dissect thousands of mosquitoes. However, this technique may be more useful as a tool for baseline assessment of transmission.

In recent years, however, a PCR assay based on the amplification of a highly repeated DNA sequence found in *W. bancrofti* (the 188 bp *Ssp*I repeat) has been developed to address the shortcomings of traditional diagnostic methods (McCarthy *et*

al., 1996; Nicolas *et al.*, 1996; Williams *et al.*, 1996; Zhong *et al.*, 1996; Ramzy *et al.*, 1997). Detection of parasite DNA in human blood and mosquitoes by PCR has been shown to be a sensitive and specific method for determining infection rates in endemic areas and thus a powerful new tool for evaluation and monitoring of community-based filariasis control programmes (Fischer *et al.*, 1999; Farid *et al.*, 2001). Screening of pools of mosquitoes by PCR has been proposed to be a rapid noninvasive tool to monitor the success of elimination programs and to detect reestablishment of transmission in post-intervention period (Bockarie *et al.*, 2000). A method for detection and quantification of the *Ssp*I repeat PCR amplification products by ELISA (PCR-ELISA) has been developed (Fischer *et al.*, 1999). However, the PCR-ELISA assay is too laborious and expensive and offers few significant advantages over the standard *Ssp*I PCR assay (Ramzy, 2002).

The *W. bancrofti Ssp*I PCR assay, however, does not differentiate infective larvae (L3) from the other stages of the parasite (microfilariae, L1 and L2) in the mosquito. The presence of *W. bancrofti* infective larvae in the vector population is a direct measure of transmission because only mosquitoes carrying the infective stage of the parasite are capable of contributing to transmission. Therefore, an ideal PCR assay for monitoring the level of transmission during a filariasis control programme would be one based on L3 specific primers.

The collection of wild-caught, blood-engorged vector mosquitoes to detect infection in the human population is defined as xenomonitoring (WHO, 2002b). The bloodmeals are taken to represent human blood samples in the population. For standardised sampling of mosquitoes to be used for xenomonitoring, indoor-resting collection of blood-fed females is considered most appropriate with the bloodmeals

representing the human population (WHO, 2002b). A major advantage of xenomonitoring is that it indirectly gives a 'real-time' assessment of the relative levels of infection in the human population (Williams *et al.*, 2002)

2.9.5 Ultrasonographic techniques

Ultrasonographic examination of the scrotal area of males has been shown to be a non-invasive method with which to directly visualize living adult *W. bancrofti in vivo* (Amaral *et al.*, 1994). Worm nests are detected by the typical movements of adult worms (known as filarial-dance signs). The sensitivity of detecting the adult worm nests by ultrasound has been shown to be approximately 80% in men who are microfilaria-positive (Noroes *et al.*, 1996). Inactivation of the worm nests has been used as a novel technique to monitor the effects of treatment on adult filarial worms.

A previous study by Brazilian investigators demonstrated that up to 50% of adult worm nests are completely inactivated by a single-dose combination of ivermectin and diethylcarbamazine (Dreyer *et al.*, 1998a). A recent study of individuals with microfilaraemia in Egypt reported that 90% of the worm nests were inactivated 12 months after co-administration of DEC (6 mg/kg) and albendazole (400 mg) (Hussein *et al.*, 2004). Another recent field trial of a novel approach to the treatment of *W. bancrofti* infection using the antibiotic doxycycline applied ultrasonography to assess the effect of the treatment on adult worms (Taylor *et al.*, 2005). However, the usefulness of ultrasonography in detecting worm nests in females is rarely reported.

2.10 Impact of mass treatment on chronic disease

There is limited information on the longterm effects of MDA on clinical manifestations due to lymphatic filariasis infection. A study conducted in Papua New Guinea reported resolution of hydrocele in 91 of 105 males (87%) after four annual MDAs with DEC alone or DEC plus ivermectin (Bockarie *et al.*, 2002). The study also reported that resolution of lymphoedema of the legs occurred in 62 of 90 adults (69%) and resolution was not correlated with the drug regimen. Another study comparing 4 mass treatment strategies for control of bancroftian filariasis using DEC in north-eastern Tanzania reported complete disappearance and reduction in size of hydrocele one year after start of treatment (Meyrowitsch *et al.*, 1996a; Meyrowitsch *et al.*, 1996b). The study reported complete resolution of hydrocele in 25.0%, 36.8%, 45.5% and 46.2% of males treated using DEC-fortified salt (0.33% w/w), standard 12-day course of DEC (6 mg/kg body weight), semi-annual DEC (2 single doses of 6 mg/kg body weight given with an interval of 6 months) and monthly low-dose DEC (50 mg to children aged < 14 years and 100 mg to individuals aged > 14 years), respectively. However, the study reported that there were no clear effects of DEC treatment on leg lymphoedema.

As the GPELF up-scales to include all areas of filariasis endemicity opportunities to study the natural history of filariasis morbidity become fewer. In the present study we took advantage of the opportunity to examine communities living along River Sabaki a highly endemic area for bancroftian filariasis that were selected for MDA using DEC plus albendazole. The communities had never received antifilarial treatment before introduction of MDA under GPELF. The study has allowed us to examine paired observations before and after two rounds of the MDA in

persons with and without chronic disease which is a useful way to accurately document both resolution and incidence of clinical manifestations. Assessing the public health impact of mass treatment with antifilarial drugs is a critically important issue for programme advocacy and for planning morbidity control strategies (Addiss and Mackenzie, 2004).

CHAPTER 3 - MATERIALS AND METHODS

3.1 Description of the study district

3.1.1 Malindi District

The study area is located in Malindi District, which is one of the seven administrative districts in the Coast Province of Kenya. Malindi town, the administrative headquarters of the district, is located on the shores of the Indian Ocean 120 km north of Mombasa Island (Figure 3.1). The district is the newest among the seven districts having been carved out of Kilifi District in 1996. Malindi District borders Kilifi District to the south, Tana River District to the north and northwest and Indian Ocean to the east. Malindi District covers an area of 7,605 km^2 and is divided into three administrative Divisions namely Malindi, Marafa and Magarini.

3.1.2 Topography and climate

The district has four major topographic features namely, Coastal Plains, Foot Plateau, Coastal Range and Nyika Plateau. The climate of the district is generally hot and humid in areas near the ocean and hot and dry in further inland areas.

3.1.3 Coastal Plains

The Coastal Plains run along the Indian Ocean and the width of the plains range from 3 km to 20 km. The Coastal Plains highest altitude is about 30 m above sea level. Along the Coastal Plains are several creeks characterized by mangrove vegetation. A monsoon type of climate with hot and humid conditions characterizes

the Coastal Plains area. The average rainfall is between 1000 mm and 1200 mm per annum.

3.1.4 The Foot Plateau

The Foot Plateau is characterized by slightly undulating terrain sloping towards the sea with an altitude of 60 m to 136 m above sea level. The average rainfall in this area is between 900 mm and 1000 mm per annum and is characterized by grassland and medium size trees.

3.1.5 The Coastal Range

The Coastal Range is characterized by low range sandstone hills of 130 m to 420 m above sea level. This area has an annual rainfall of about 1200 mm per annum and has fertile soils. The coastal range is relatively densely populated compared to the rest of the district because of the higher annual rainfall.

3.1.6 The Nyika Plateau

The Nyika plateau is a lowland ranching zone characterized by hot and dry climate most of the year with low fertility sandy soils, low grassland and thorny bush. The annual rainfall for this area is between 500 mm and 700 mm and is sparsely populated. Most of the human settlements in the Nyika Plateau are found along River Sabaki (Figure 3.2). The communities in the present study are located in this geographic zone.

3.1.7 Socio-economic activities

According to data contained in the Malindi District Poverty Assessment Report 2000, the district has an estimated 198,120 persons (66%) considered to be in

absolute poverty (Ministry of Planning and National Development, 2002). These are defined as persons who cannot meet their basic food and non-food requirements. Most parts of the district are characterized by poor state of housing and sanitary conditions that is exacerbated by lack of clean water. There was a long dry spell between 2002 and 2004 that caused crops and livestock failure thus forcing many families to depend on famine relief food supply. During this period, many men in the drier Nyika Plateau, turned to charcoal burning for a source of income thus causing severe destruction of the vegetation.

The major ethnic group living in Malindi District is the Giriama. The Giriama form the major sub-group among the group known as Miji Kenda occupying most of the Coast Province. There are also smaller groups of Arabic descent mostly living in urban settings. A large proportion of the inhabitants of Malindi town, the administrative headquarters of the district, are immigrants from other parts of Kenya.

The Coastal Plains area has beaches and coupled with the warm climate serves as a favourite tourist attraction. It is particularly popular with Italian and German tourists some of whom have bought homes in and around Malindi town. Many young men have migrated to the town in search of jobs in the hotels and other tourist related businesses. There is also fishing in the ocean using small boats.

The Foot Plateau and Coast Range have fertile dark soils which make these areas suitable for crop farming thus encouraging relatively high human settlement. The main crops grown in these areas are maize and cassava. Mango trees are also found in these areas and mangoes provide a source of income from October to January. Another crop in this zone is cashew nut. However, cashew nut farming is

faced by problems mainly due to the collapse of a local processing factory and low yields due to old cashew nut trees and poor farming methods.

Most areas in Nyika Plateau, however, are characterized by brown sandy soils which are poor for crop farming. The main economic activities in the Nyika plateau area include livestock keeping and growing of drought resistant crops such as cassava and maize. The livestock include East African Zebu cattle, sheep and goats. Since the area is semi-arid, there is an acute water problem. However, in the low lying areas near R. Sabaki, where rich soils from upcountry are deposited the communities engage in small-scale crop farming. The main crops grown include coconut trees, maize, pawpaws, cowpeas (kunde) and tomatoes.

Coconut farming is found in many parts across Malindi District especially in low-lying areas and near R. Sabaki. The coconut tree is used as a source of both coconut fruit, and palm wine (locally known as "mnazi"). Many young men ferry the "mnazi" to Malindi town everyday using bicycles. The coconut leaves are also a popular roofing material and provide a source of income for some people in the area.

3.1.8 Health care

There is a general inadequacy of health facilities in Malindi District especially in the rural areas. There are three main types of health facilities that are mainly involved in provision of health care in rural areas of Malindi District. These include government dispensaries, "Bamako" community pharmacies and private clinics. The "Bamako" community pharmacies are a part of an earlier initiative designed to make basic health care available to the communities. The initial stock of essential medicines is provided by the district health office and the community is supposed to pay to enable re-stocking. Prices for the medicines are set by the community pharmacy

committee with guidance from the district health staff. The drugs are dispensed by a community health worker (CHW) who is trained and approved by the district health staff. The CHW is only allowed to dispense simple drugs such as antimalarial drugs, antihelminthics, multivitamins, cough syrups and paracetamol. The only district hospital is located in Malindi town. However, the health facilities are few and far (more than 5 km) from many of the communities and thus are difficult to access. All the health facilities charge a fee, which discourages many people from visiting them to seek medical care.

3.2 Study communities

In 2001 the *W. bancrofti*-endemic area along River Sabaki in Malindi District in the northern coast, Kenya was selected for a filariasis elimination project using single-dose annual mass treatment with diethylcarbamazine plus albendazole under the GPELF. The present study was carried out in four sentinel communities namely, Jilore, Marikano, Magongoloni and Mkondoni, selected for a detailed baseline epidemiological study of bancroftian filariasis and monitoring of the impact of mass treatment. The communities are typically rural and situated along River Sabaki in the Nyika Plateau between 40 and 60 kilometres west of Malindi town on the shores of Indian Ocean (Figure 3.1).

The communities live in dispersed households and homesteads with each community being headed by a Village Chairman. The houses are made of mud walls and thatched with coconut leaves or grass and have open eaves (Figure 3.3). The Village Chairman could be a male or female and is usually appointed by the local government administrator - the Chief or Assistant Chief. In the bigger communities, two or more village chairmen could be appointed each in-charge of a specific area

within the village. The Village Chairmen are useful in solving disputes within their communities and are regarded as the 'eyes' of the government at the local level. The Chiefs, Assistant Chiefs and village Chairmen are essential in mobilization of the communities during different types of community activities.

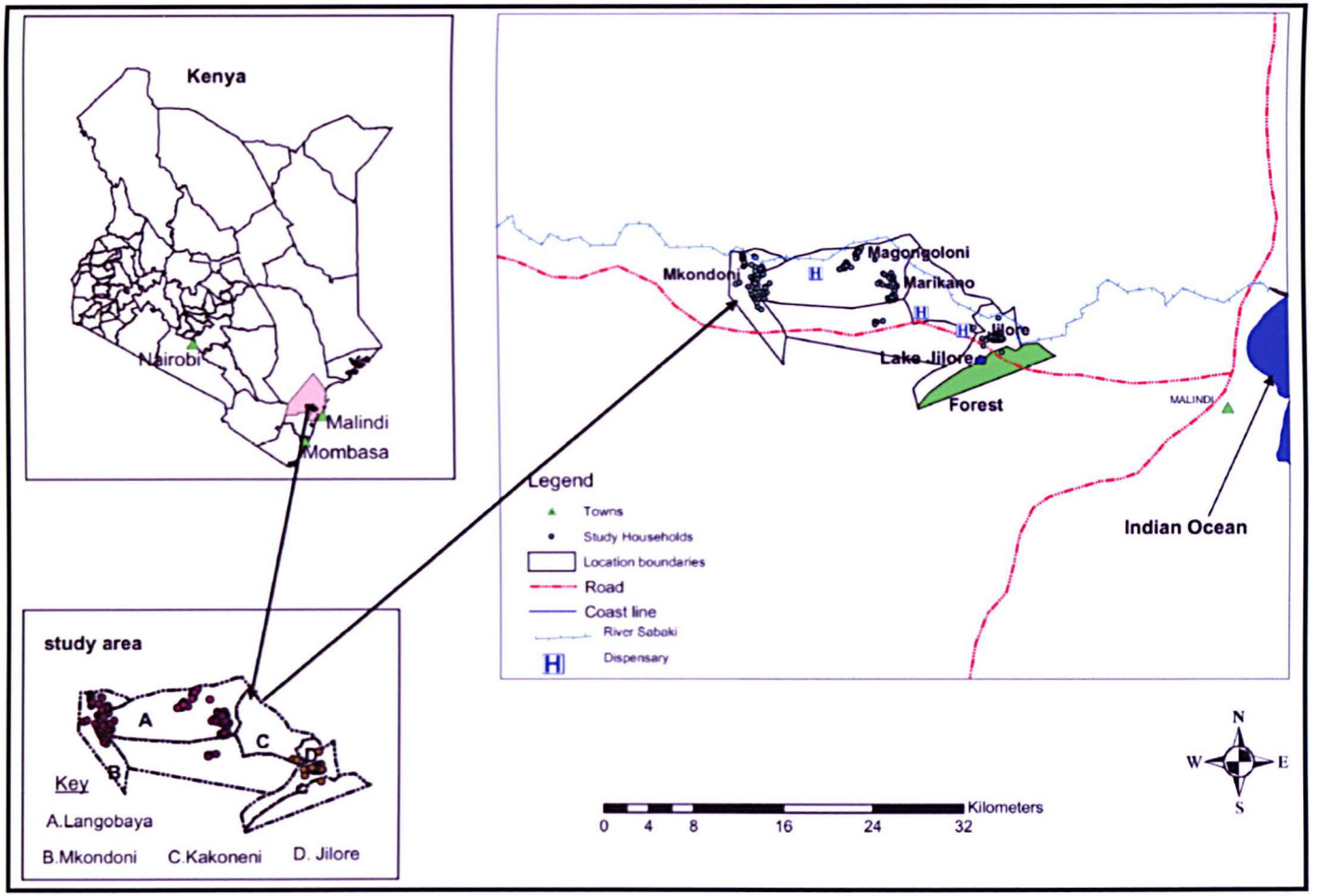

Fig 3.1: Map of study area showing the study villages. The map also shows GPS locations of the study households.

Figure 3.2: River Sabaki. Note the coconut trees growing along the river.

Figure 3.3: A typical house made of mud walls and thatched with coconut leaves.

3.3 Census

Baseline demographic information was collected for all members of each household in the 4 communities in house-to-house visits. Census data collected included name, gender, year of birth, ethnicity and duration of residency in the area. Due to low literacy rates in the area there were many people who did not know their own age or that of the children. In such instances the age was estimated by asking specific questions on historical events.

3.4 Study population

The purpose of the present study was explained to the communities in Swahili and Giriama languages during public meetings conducted in each village. In each village, a convenience sample of approximately 200 individuals was recruited for clinical examinations and blood sampling at baseline and post-treatment. Individuals in the follow-up group were registered after a consent was read and translated to them in the local language (Appendices A and B). All the houses from which the members of the follow-up group belonged were mapped using a Global Positioning System (GPS, Garmin® eTrex™, Garmin International Inc., Kansas, USA). The protocol for this study was reviewed and approved by the Scientific Steering Committee of the Kenya Medical Research Institute, Kenya and the Research Ethics Committee of Liverpool School of Tropical Medicine, UK.

3.5 Mass drug administration

In Kenya co-administration of diethylcarbamazine and albendazole is recommended for elimination of lymphatic filariasis. Kenya is the only sub-Saharan African country currently using DEC/albendazole because of the absence of

onchocerciasis or loiasis, which are co-endemic with bancroftian filariasis in many other countries in Africa. The National Programme to Eliminate Lymphatic Filariasis (NPELF) in Kenya had not expanded its activities to include Malindi district at the time the present study was being implemented.

The first MDA was conducted in April 2002 by health staff of the district hospital and local dispensaries assisted by the research team. Members of the communities received treatment in designated sites previously selected during public meetings. Treatment was given to persons aged 5 years and above (age recommended by Ministry of Health), but pregnant women and severely ill individuals were not treated. A female nurse determined pregnancy by asking females about menses. Diethylcarbamazine was given by weight at 6 mg/kg body weight whereas albendazole was given at 400 mg dose. Swallowing of the drug tablets was done in the presence of the medical team.

The second MDA was conducted in September 2003 by the Kenya National Programme to Eliminate Lymphatic Filariasis which had up-scaled its activities to include Malindi district. The treatment was given by trained community drug distributors (CDD) using community-directed treatment (Com-DT) strategy. Records on treatment in the second MDA were not available at the time of data analysis for the present study. Thus, to estimate coverage interviews were conducted in the follow-up group. The interviews were conducted on household heads or adult members of the household where the household head was not available. At the time of the interview, the respondents were also asked to provide information on treatment status in 2002.

3.6 Clinical examinations

All consenting individuals aged 5 years and above were examined clinically for chronic manifestations of bancroftian filariasis. The clinical examinations were performed during daytime in selected buildings within the study villages. In one village (Jilore) the clinical examinations were conducted in a spare observation room in a dispensary located within the village, while in Marikano and Magongoloni examinations were done in churches, and in Mkondoni a spare room in a local shop was used.

A female nurse conducted examinations of females, while a male Clinical Officer conducted examinations of males. In addition to examination for lymphoedema of the limbs (Figure 3.4), males were also examined for filarial-related lesions in the genitalia including hydrocele and scrotal or penile lymphoedema. Hydrocele and scrotal lymphoedema were graded as previously described in a study conducted in this setting (Wijers and Kinyanjui, 1977) (see Appendix C) with few modifications. In the present study true hydrocele (Figure 3.5) was defined as a swelling of the scrotal sac at least 6 cm in longitudinal axis due to fluid accumulation. Also, oedema of the scrotal skin without hardening was not classified as true scrotal lymphoedema in the present study. Females were examined for lymphoedema of the limbs and breasts, but unlike in males, examination of the female genitalia was not conducted. Lymphoedema of the legs was graded as previously described (Dreyer *et al.*, 2002b). During the physical examinations the individuals were also examined for inguinal lymphadenopathy which was graded as previously described (Wijers and Kinyanjui, 1977).

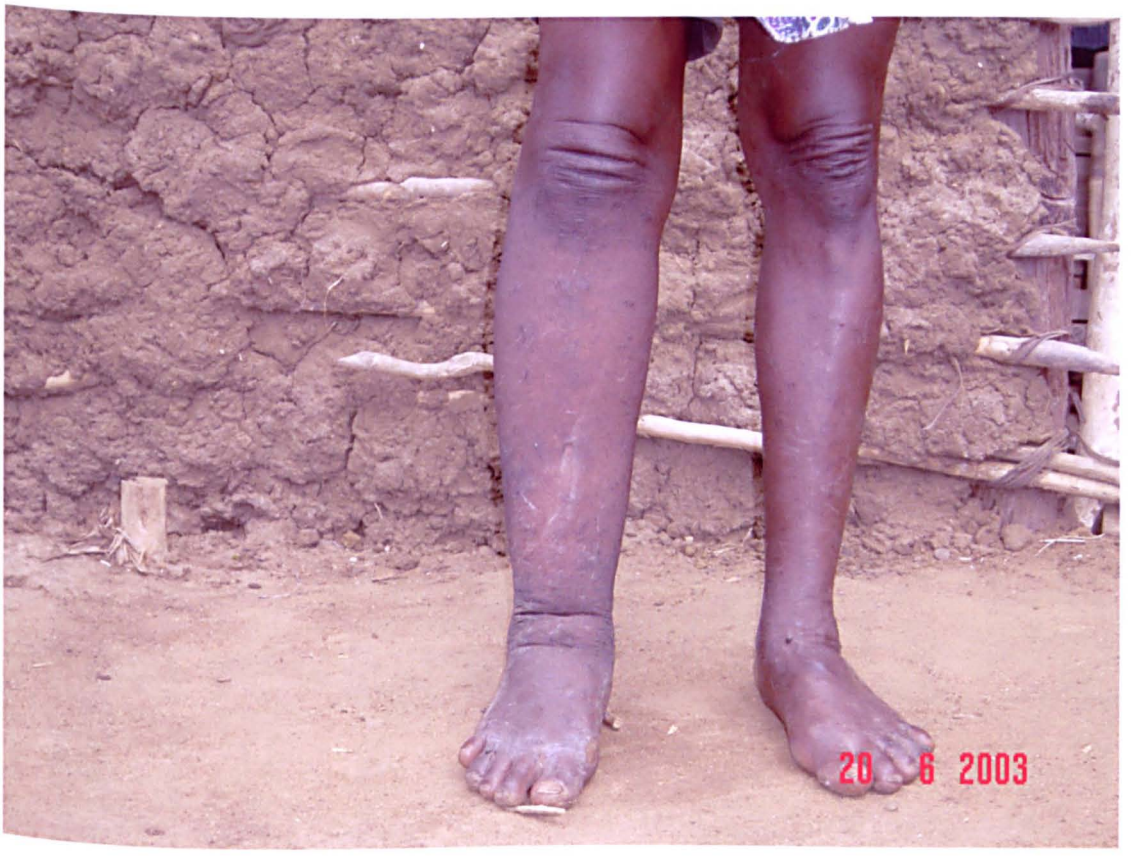

Figure 3.4: Leg lymphoedema.

Figure 3.5: Hydrocele with scrotal skin thickening and inguinal lymphadenopathy.

3.7 Collection of blood samples

Blood samples were collected from the follow-up group between 2030h and 2400h, when the numbers of microfilariae approach peak levels in the peripheral blood circulation in this setting (Gatika *et al.*, 1994). Finger prick blood samples for microfilaria counting and the ICT test were collected annually before each round of mass drug administration. In addition, venous blood samples for plasma were collected at baseline and after two rounds of mass treatment.

Finger prick blood was obtained by cleaning the tip of the middle finger with cotton swab containing 70% isopropyl alcohol and pricking with a sterile lancet. Two100-µl blood samples, for microfilaria detection and the ICT test, were collected from the prick into heparinized capillary tubes (Figure 3.6).

For collection of venous blood samples, the puncture site was cleaned with cotton swabs containing 70% isopropyl alcohol. Two millilitres of venous blood sample was collected from the vein using a needle and 4.5 ml vacutainer bottle containing EDTA (BD Vacutainer® Brand). The blood was gently mixed with EDTA and kept at room temperature until the following morning when plasma samples were collected. The plasma samples were initially stored at 4 °C in the field and then at -20 °C in the central laboratory in Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi.

3.8 Microfilaria detection

One finger prick blood sample was transferred into a tube containing 0.9 ml of 3% acetic acid solution and mixed gently. The acetic acid solution serves as a fixative/preservative as well as a lysing solution for the red blood cells. The samples

were kept at room temperature until the following day when microfilaria examination and counting was done under a microscope at the local Langobaya National Water Corporation (NAWACO) dispensary laboratory, using the counting-chamber method (McMahon *et al.*, 1979).

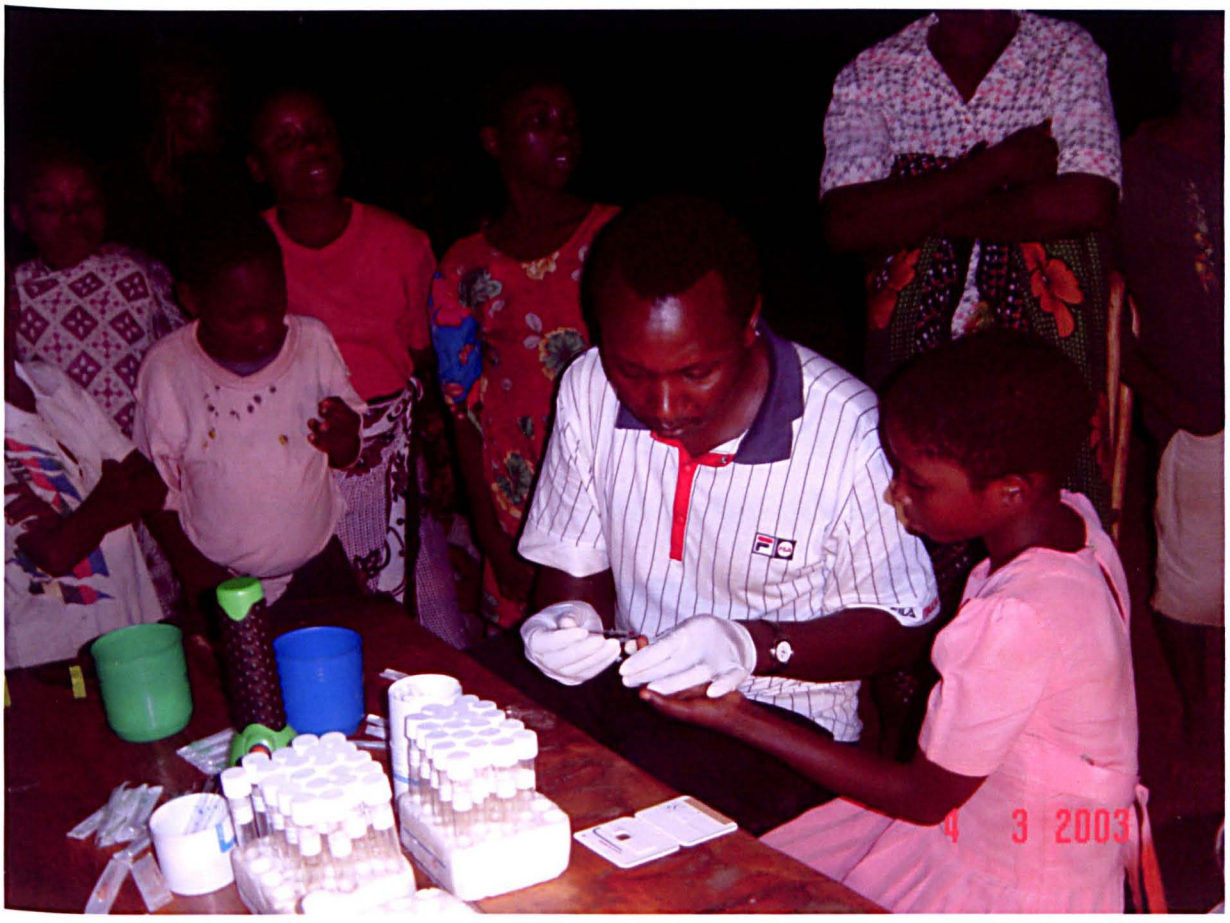

Figure 3.6: Night time blood collection. Fingerprick blood specimens were used for microfilaria detection and the ICT test. Venous blood was also collected for plasma samples.

3.9 Immunochromatographic (ICT) card test

The second finger prick blood was added to the sample pad of the ICT test card. The pad contains dried colloidal gold-labelled polyclonal antifilarial antibodies that bind to adult worm antigen in the blood. When the card is closed, the antibody-antigen complexes, as well as unbound antigen, flow across a nitrocellulose strip and are trapped by a monoclonal antibody (AD12.1) in the strip's coating. The results were read after 5 minutes. The resulting gold-labelled conjugates yield a pink line next to a control pink line that appears in all valid cards. Thus blood samples from antigen-negative persons exhibit one pink line, whereas those from antigen-positive individuals display two pink lines. Note that the ICT cards used at pre-treatment were from ICT Diagnostics/AMRAD ICT (New South Wales, Australia) while at the two post-treatment surveys the cards were from Binax Inc. (Portland, ME, USA) due to the change of location of manufacture and company ownership.

3.10 Og4C3 circulating antigen ELISA assay

Paired plasma samples from a cohort of individuals (n = 463) that provided venous blood specimens at baseline and after two rounds of mass treatment were tested for circulating filarial antigen (CFA) using a commercial ELISA kit based on the monoclonal antibody Og4C3 (TropBio Pty Ltd, Queensland, Australia). Using sample diluent provided in the kit, a 1:10 dilution of the plasma samples was made in 0.5 ml centrifuge tubes. The diluted specimens were boiled at 100 °C (in a boiling water bath) for 5 minutes. The boiled samples were centrifuged for 5 minutes at 10000 g in a microcentrifuge (IEC Micromax, International Equipment Company, Needham Heights, MA, USA). The clear supernatant contains the heat stable antigen in positive specimens.

For the ELISA assay, 50 µl of the boiled plasma samples were added to the U-bottom polystyrene microtitre plates pre-coated with Og4C3 monoclonal antibody in duplicates. Each ELISA plate included *Onchocerca gibsoni* antigen standards (provided) and a blank (sample diluent only). The plates were incubated overnight at room temperature in a humid container. After washing 3 times using freshly made washing buffer, 50 µl /well of rabbit anti-*Onchocerca* antibody diluted 1:120 using the antibody/conjugate diluent was added. The plates were incubated at room temperature for 1 hr and 50 µl/well of anti-rabbit horse radish peroxidase (HRPO) conjugate at 1:120 dilution was added, after a second washing step. The plates were incubated at room temperature for 1 hr and 100 µl/well of ABTS (2,2'-azino-bis-(3-ethyl benzthiazoline-6-sulfonic acid)) substrate chromogen added after a final wash. The optical density (OD) was read at 405 nm after 1 hr incubation at room temperature. Specimens with ≥ 128 antigen units (titre group 4 and above) were considered positive for CFA.

3.11 Antifilarial immunoglobulin G1 (IgG1) and IgG4 ELISA

The plasma specimens from the cohort that provided venous blood samples at pre-treatment and after two rounds of mass treatment were also tested for antifilarial immunoglobulin G1 (IgG1) and IgG4 using previously described methods (Hitch *et al.*, 1991). Briefly, 96-well flat-bottom Immulon® 2 HB polystyrene microtitre plates (Dynex Technologies, Chantily,VA, USA) were coated with 50 µl/well of 2 µg/ml of *Brugia pahangi* antigen in 0.1 M sodium bicarbonate ($NaHCO_3$) buffer, pH 9.6. The plates were incubated overnight at +4 °C. The following morning the bicarbonate buffer was poured out and excess solution removed by tapping the plates onto a stack of laboratory paper towels. The uncoated sites were blocked for 1 hour at +4 °C with

100 µl/well of 0.01 M phosphate buffered saline (PBS), pH 7.2 containing 0.3% Tween 20. After a washing step using PBS/0.05% Tween 20, 50 µl/well serum specimens diluted 1:50 in PBS/0.05% Tween 20 were added in duplicate. Serial dilutions of a standard serum sample with known amounts of antifilarial IgG1 and IgG4 were included on each plate to generate a standard curve. In order to compute the amounts of antibody in the test samples, the OD values were compared to the standard curve. The plates were incubated at room temperature for 2 hr to allow complete binding of antifilarial immunoglobulins to the *B. pahangi* antigen. After washing the plates 4 times with PBS/0.05% Tween to remove unbound material, 50 µl/well of biotin conjugated mouse anti-human IgG1 (for IgG1 ELISA) or IgG4 (for IgG4 ELISA) subclass monoclonal antibody (Zymed Laboratories, Inc., South San Francisco, CA, USA) diluted 1:1000 in PBS/0.05% Tween was added. The plate was incubated at room temperature for 1 hr and after another washing step, streptavidin conjugated to alkaline phosphatase was added. Following incubation for one hour at room temperature and a final washing step, the plate was developed by addition of 50 µl/well of 1 mg/ml p-nitrophenyl phosphate (p-NPP) (Sigma, St. Louis, Missouri, USA) in 10% diethanolamine (DEA), pH 9.8 containing 3 mM $MgCl_2$. The optical density (OD) was monitored at 405 nm using an ELISA reader (UVmax, Molecular Devices, USA) and the readings saved when the OD of the highest standard dilution reached 1.2.

All the washing steps were done using a manual microtitre plate washer (Flow Laboratories, Titertek™ Microtitration equipment). Control serum samples from a filariasis non-endemic area in Kenya were generously provided by Dr. W. Evan Secor, Centers for Disease Control and Prevention (CDC), Atlanta, USA. The cut-off values were established from the mean value of 20 negative controls plus two

standard deviations. The cut-off values for antifilarial IgG1 and IgG4 were 9.9 μg/ml and 2.7 μg/ml, respectively. The immunologic assays were conducted at the CDC laboratories. All the materials required for the antifilarial antibody assays were generously provided by Dr. Patrick J. Lammie, CDC, Atlanta, USA.

3.12 Mosquito collection

Mosquitoes were sampled using a pyrethroid knock down (PKD) catch method in randomly selected houses. Oral consent was sought from the house owners prior to registration of the selected house. Since many households have several houses, the houses in which mosquitoes were collected were chosen on the basis that at least some of the family members slept in the selected house. In the study area, it is common to find one house used as a kitchen and separate houses as bedrooms for parents and older children. On the day before mosquitoes were collected from each house, the house owner was informed so as to minimize any inconvenience caused.

Mosquito collections were conducted early in the morning between 0600h and 0800h. All persons, animals and food were removed to a safe distance outside or in a different house during the spraying. Floors and horizontal surfaces such as tables and beds were covered with white sheets. A domestic class pyrethroid-based aerosol insecticide commonly known as 'Doom' (Mortein™ Doom® containing d-phenothrin 1.0 g/kg, imiprothrin 0.4 g/kg; Reckitt Benckiser East Africa Ltd., Nairobi, Kenya), was used. The insecticide was first sprayed around the eaves and other openings from the outside to prevent mosquitoes from escaping. Spraying was then applied to the inside of the house and the door closed. After 15 minutes, the sheets were carefully removed for inspection. Female mosquitoes were collected using forceps and kept in

labelled petri dishes and sealed with masking tape for transportation to Langobaya dispensary laboratory.

In the dispensary laboratory, the mosquitoes were observed under a dissecting microscope and sorted according to species using morphological keys (Gillies and Coetzee, 1987). Female vector mosquitoes were stored in pools of 1-20 in tubes containing silica gel (Sigma, St. Louis, Missouri, USA) covered by a plug of cotton wool. Another cotton wool plug was loosely placed above the mosquitoes to prevent movement which could lead to breaking of the insects when dry. The tubes were labelled with species of mosquitoes, date of collection, village name and house number. The tubes were then placed in plastic bags and kept at ambient temperature until DNA extraction was done for PCR assays in March-April 2005.

3.13 Extraction of DNA from mosquitoes

The extraction of DNA from the preserved mosquitoes was done using a standard protocol (Collins *et al.*, 1987; Cornel and Collins, 1996). An individual mosquito was transferred into sterile 1.5-ml centrifuge tube and 50 µl of grind buffer containing 80 mM sodium chloride (NaCl), 60 mM ethylenediamine tetracetic acid (EDTA), 100 mM tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl), 160 mM sucrose and 0.5% sodium dodecyl sulfate (SDS) added to the tube. The mosquito was homogenised using a plastic pestle until no identifiable body parts were seen. The pestle was rinsed with 50 µl of grind buffer. The homogenate was centrifuged for 30 sec at maximum speed (14000g) to collect all the material at the bottom of the tube. After centrifugation, the homogenate was heated at 65 °C in a dry bath incubator for 20 minutes to inactivate nucleases released after grinding. While still hot, 18 µl of 5 M potassium acetate (KAc) was added and mixed by tapping the tube. This step

helped to precipitate proteins and lipids. The tube was then placed in ice for at least 30 minutes and then centrifuged at 14000g for 15 minutes at room temperature. The supernatant was transferred into a clean labelled tube.

To precipitate the DNA, 200 µl of ice-cold 100% ethanol was added and mixed thoroughly by inverting the tube 3 times. The solution was incubated at room temperature for 5 minutes and then centrifuged at 14000g for 20 minutes at 4 °C. In many specimens the DNA formed a purple pellet at the bottom of the tube. The supernatant was discarded and the DNA pellet washed with 200 µl of ice-cold 70% ethanol. The DNA was re-pelleted by centrifugation for 5 minutes and washed again with 200 µl of 100% ethanol. The 100% ethanol supernatant was carefully discarded and the DNA sample dried in a speedVac machine in the alcohol mode. The DNA was resuspended in 100 µl of 10 mM Tris EDTA (TE) buffer, pH 7.4 and incubated at 4 °C for at least 12 hours (overnight) to allow the DNA to dissolve fully.

3.14 *Wuchereria bancrofti Ssp*I PCR assay

Polymerase chain reaction was performed in a DNA thermal cycler (iCycler®, Bio-Rad Laboratories, CA, USA) using two oligonucleotide primers: 21-mer NV1 (5'-CGT GAT GGC ATC AAA GTA GCG-3') and 22-mer NV2 (5'-CCC TCA CTT ACC ATA AGA CAA C-3'). The primers allow the amplification of a 188-bp *Ssp*I repeat DNA sequence which is specific for *W. bancrofti* (Williams *et al.*, 1996). The PCR was carried out in a 25 µl reaction mixture containing 0.6 µl of template DNA, 10 pmol of each primer, 25 µM of each dNTP, 1.5 mM $MgCl_2$, 1 unit of *Taq* DNA polymerase and 1x *Taq* DNA polymerase buffer (Promega, WI, USA). The PCR assay was performed using the following thermal cycling profile: an initial denaturation step at 94 °C for 5 minutes followed by 36 cycles each at 94 °C for 30

sec, 55 °C for 30 sec and 72 °C for 30 sec. The profile ended with a final extension at 72 °C for 5 minutes and 4 °C hold. A positive control was included in each PCR assay.

Following amplification, 5 µl of 6x loading dye was added to each reaction and 20 µl electrophoresed through a 2% agarose gel at 120 volts for 40 minutes. The agarose gel was stained with ethidium bromide and visualized in an UV transilluminator to confirm amplification before a photograph was taken. The DNA extraction and PCR assays were conducted in CDC, Atlanta, USA.

3.15 Statistical analyses

Statistical analyses were done using SPSS statistical software version 12.0.1 (SPSS Inc., Chicago, IL, USA) and Epi Info version 2002 (Centers for Disease Control and Prevention, Atlanta, USA). Tests for normal distribution for continuous variables were performed using one-sample Kolmogorov-Smirnov Z test and a variable was considered to have normal distribution if the P value was more than 0.05. Homogeneity of variances among groups was determined using Levene's test (Glantz, 2002).

Parametric tests were used for observations that were normally distributed with equal variances. For comparisons between two means the Student's t test was applied whereas one-way analysis of variance (ANOVA) was used to analyse 3 or more groups. The Pearson Chi-square (χ^2) test was used to compare proportions in two-by-two contingency tables. Where data were not normally distributed and/or with unequal variances, nonparametric tests were used to compare groups for differences. The Mann-Whitney rank-sum test was used to determine differences between two

independent groups, while the Kruskal-Wallis test was employed to determine differences among multiple groups (Glantz, 2002).

When differences were shown to exist among group means, post hoc pairwise multiple comparison tests were used to determine which means differed. Where variances were equal, Tukey HSD test was used for multiple comparisons; otherwise Dunnett's T3 test was used. Variables with data that had large standard deviations were log transformed so as to minimize the variance.

Statistical association between variables was measured using correlation coefficients. Pearson product-moment correlation coefficient (r) was used to measure the strength of association between normally distributed quantitative variables. Spearman rank correlation coefficient (r_s) was used to measure the strength of association between variables on an ordinal scale or when the variables were not normally distributed.

Changes in overall prevalence rates were measured using the Pearson Chi-square test. Fisher's exact test (2-tailed) was applied where at least one of the cells in the 2 x 2 contingency table had an expected value less than 5. Paired comparisons of data collected at baseline and after MDA were used to test for change. McNemar's test was used to test for change in paired data that were measured on dichotomous variables. The Wilcoxon signed-ranks test was used where the data consisted of quantitative variables, but not normally distributed. When the data were normally distributed the paired t test was used to test for change (Glantz, 2002).

CHAPTER 4 – IMMUNOPARASITOLOGY OF *WUCHERERIA BANCROFTI* IN RIVER SABAKI AREA, MALINDI DISTRICT, KENYA

Microfilaria detection is the gold standard method for determination of active infection in lymphatic filariasis. For filariasis caused by *W. bancrofti* in most of the world, however, microfilaraemia has a nocturnal periodicity (Sasa, 1976) and blood collection has to be done at night. This is inconvenient for the affected communities, the research teams and those responsible for programme evaluation. Examination of Giemsa-stained thick blood films (20 – 60 µl blood) is widely used for detection of microfilaraemia (Eberhard and Lammie, 1991). However, the sensitivity of the Giemsa-stained thick blood film technique is highly compromised when the microfilaria count is low. Screening of larger volumes of blood using concentration techniques can improve sensitivity. Many studies in East Africa use the counting chamber technique (McMahon *et al.*, 1979) for microfilaria detection in 100 µl of blood. The most sensitive parasitological method is the membrane (Nuclepore) filtration technique (Chulerek and Desowitz, 1970) which requires 1 ml of blood. However, a significant proportion of the population with active infection has no circulating microfilariae (Lammie *et al.*, 1994), which results in underestimation of the true prevalence of lymphatic filariasis when using parasitological methods.

To facilitate diagnosis, evaluation, monitoring and surveillance, a monoclonal antibody, Og4C3, has been produced for use in a sandwich ELISA assay to detect circulating antigen of *W. bancrofti*, in human sera (More and Copeman, 1990). Animal studies have shown that circulating filarial antigen levels correlate with the number of adult worms in the host (Weil *et al.*, 1985; Weil *et al.*, 1990), and the same is believed to be true in bancroftian filariasis (Ismail *et al.*, 1998). In addition, a rapid

immunochromatographic (ICT) card test that is suitable for field setting has also been developed (Weil *et al.*, 1997). In the ICT test, a monoclonal antifilarial antibody, AD12.1, bound to a nitrocellulose strip traps free antigen and antigen-antibody complexes in whole blood or plasma and concentrates colloidal gold-labeled conjugate in the card to form a visible pink line. Both the Og4C3 ELISA assay and ICT test are commercially available but the ICT test is only qualitative, providing negative/positive results. However, the ICT test is relatively easy to use and has been widely used for rapid mapping of lymphatic filariasis before the implementation of elimination programmes under the GPELF.

In terms of the immunological response to lymphatic filarial infections, among populations living in areas of endemicity, isotype-specific antibody responses against parasite antigens are characteristically correlated with clinical status. Previous work has provided evidence that antifilarial IgG1 responses serve as a marker of filarial exposure, while antifilarial IgG4 response is associated with presence of circulating filarial antigen (Kwan-Lim *et al.*, 1990; Lammie *et al.*, 1998; Wamae *et al.*, 1998). It was previously found that individuals harbouring adult worms have higher filarial-specific IgG4 than adult worm-free individuals independently of the presence of microfilariae (Nicolas *et al.*, 1999). Thus, filarial-specific antibodies may be a useful marker of the impact of mass chemotherapy on community adult worm loads.

Traditionally, detection of *W. bancrofti* in mosquitoes requires time-consuming dissection and microscopic examination of individual mosquitoes. The number of mosquitoes that can be processed using this technique is about 35 per person-hour and is slower if mosquitoes are preserved in alcohol (Bockarie *et al.*, 2000). In recent years, however, a PCR assay based on a highly repeated DNA

sequence found in *W. bancrofti* (the *Ssp*I repeat) has been developed to address the shortcomings of traditional diagnostic methods (McCarthy *et al.*, 1996; Nicolas *et al.*, 1996; Williams *et al.*, 1996; Zhong *et al.*, 1996; Ramzy *et al.*, 1997). Detection of parasite DNA in human blood and mosquitoes by PCR is a sensitive and specific method for determining infection rates in endemic areas and thus a powerful new tool for evaluation and monitoring of community-based filariasis control programmes (Fischer *et al.*, 1999; Farid *et al.*, 2001). Screening of pools of mosquitoes by PCR has been shown to be similar to traditional dissection approaches in terms of its ability to detect infection rates (Fischer *et al.*, 1999; Bockarie *et al.*, 2000).

Advances in the diagnostic and treatment tools for lymphatic filariasis have led to the development of a Global Programme to Eliminate Lymphatic Filariasis (GPELF). The major goal of GPELF is to interrupt transmission of infection by reducing the levels of microfilariae in endemic communities through annual mass treatment using DEC or ivermectin plus albendazole (Molyneux and Zagaria, 2002). The GPELF recommends assessment of microfilaraemia and antigenaemia by the ICT test to measure the impact of lymphatic filariasis elimination programmes (WHO, 2000).

Although bancroftian filariasis was demonstrated to be highly endemic in the coastal areas of Kenya through studies conducted in the 1960s and 1970s there was a paucity of work to collect epidemiological data in Kenya for more than 20 years. However, since the mid-1990s, different groups have conducted epidemiological studies in the coastal area, but the present study is the first to be reported since the earlier studies conducted in north coast region in 1970s as the recent studies have been conducted in Kwale District in the south coast (south of Mombasa town) of the

Coast Province (Estambale *et al.*, 1994; Wamae *et al.*, 1998; Njenga *et al.*, 2000; Mukoko *et al.*, 2004).

In a study conducted in clusters of males in the Kenyan coast between 1971 and 1973 (Wijers, 1977), the area along R. Sabaki was shown to be one of four major foci for bancroftian filariasis in the coastal areas of Kenya. In the present study, baseline parasitologic indicators of infection and exposure to *W. bancrofti* were evaluated in 4 communities living along R. Sabaki. This area was selected for a pilot lymphatic filariasis elimination programme using annual single dose mass treatment with a combination of DEC and albendazole. A map of the study area is shown in Figure 3.1.

4.1 Demographic characteristics of the study area

The total number of people living in the 4 selected communities in 2002 was 2611. Table 4.1 shows the number of females and males living in the study villages in 2002. The number of females (1341) was higher than that of males (1270), but this difference was not statistically significant (P = 0.564).

Village	Males (%)	Females (%)	Total
Jilore	291 (49.3)	299 (50.7)	590
Marikano	312 (46.4)	361 (53.6)	673
Magongoloni	280 (48.9)	293 (51.1)	573
Mkondoni	387 (49.9)	388 (50.1)	775
Total	1270 (48.6)	1341 (51.4)	2611

Table 4.1: Total population of four study villages in Malindi District, Kenya in 2002. The number of females was higher compared to males but the difference was not significant. The four communities live in a filariasis focus along R. Sabaki, Malindi District, Kenya.

Figure 4.1 shows distribution of the population in terms of age. The overall mean age of inhabitants of the study villages was 20.2 years. Comparison of mean age between males (19.6 years) and females (20.8 years) showed that there was no statistical difference between the two sexes (P = 0.056). Mean age among the 4 villages was between 18.1-22.1 years. Mkondoni village had the lowest mean age (18.1 years) whereas Jilore village had the highest mean age (22.1 years). Comparison of mean age among the 4 villages revealed that there was a difference between Jilore and Mkondoni villages (P = 0.005). However, information on the age of 503 persons was not recorded and this may have influenced the analysis.

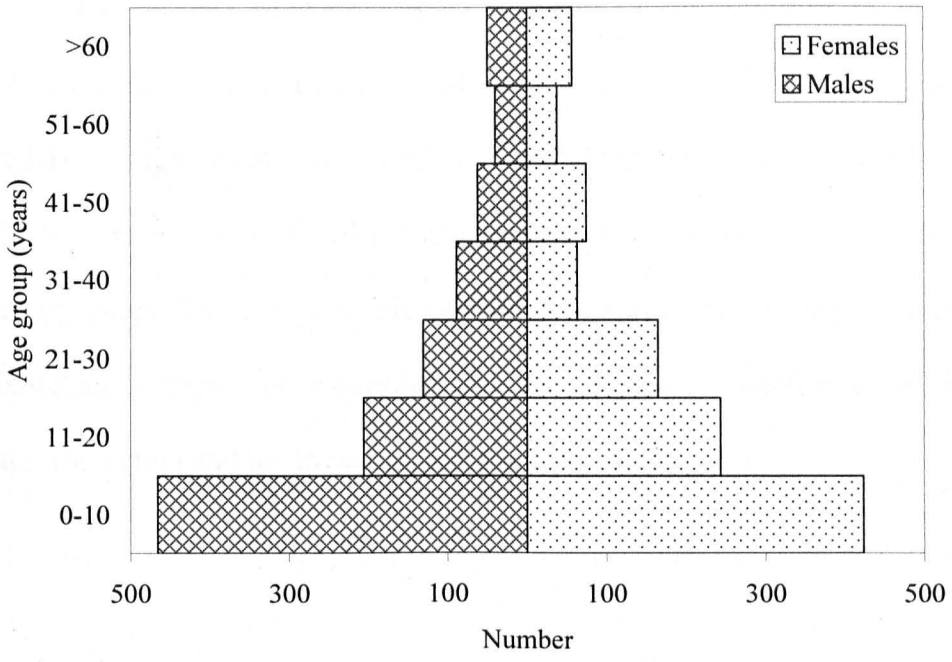

Figure 4.1: Age-specific distribution of the population in study area during baseline survey in 2002. The population is predominantly young.

4.2 Prevalence and intensity of microfilaraemia

In each village, a group of willing individuals was recruited to participate in annual blood sampling for determination of *W. bancrofti* infection and antifilarial antibodies. A total of 808 individuals were registered in the 4 study communities. Of these, 740 persons (325 males and 415 females) were examined for microfilariae during a baseline survey conducted in February 2002. The overall prevalence of microfilaraemia among the 740 individuals that participated in the baseline survey was 20.5%. The prevalence of microfilaraemia among the 4 villages ranged from 17.7-22.9%, but did not differ significantly by village (P = 0.465). Table 4.2 summarizes the prevalence and geometric mean intensity of microfilaraemia in the four study villages.

The overall geometric mean intensity of microfilariae in microfilaraemic persons in the study area was 526 mf/ml. The intensity of microfilaraemia among the 4 villages ranged from 274-739 mf/ml, but did not differ significantly by village (P = 0.089). The intensity of microfilaraemia in microfilaria positive persons was not directly proportional to the microfilaria prevalence rate in the respective village. Mkondoni village, for example, had the highest geometric mean intensity of microfilaraemia and the lowest microfilaria prevalence rate.

	No. mf positive/No. examined (%)			GMI (mf/ml)*		
Village	Males	Females	All	Males	Females	All
Jilore	17/81 (20.9)	15/92 (16.3)	32/173 (18.5)	516	675	585
Marikano	15/81 (18.5)	29/111 (26.1)	44/192 (22.9)	277	273	274
Magongoloni	19/80 (23.8)	25/114 (21.9)	44/194 (22.7)	655	788	728
Mkondoni	17/83 (20.5)	15/92 (15.3)	32/181 (17.7)	842	637	739
All	68/325 (20.9)	84/415 (20.2)	152/740 (20.5)	543	512	526

Table 4.2: Prevalence and intensity of microfilaraemia in four study villages during baseline survey in 2002. There was not significant difference in the prevalence and intensity of microfilaraemia among the study communities.

*Geometric mean intensity in microfilaria-positive persons

4.2.1 Microfilaraemia by gender and age

Figure 4.2 shows the prevalence of microfilaraemia by age and gender in the study area. The prevalence of microfilaraemia was similar between males (20.9%) and females (20.2%). The geometric mean intensity of microfilaraemia for 68 males and 84 females that were microfilaria positive was 543 mf/ml and 512 mf/ml, respectively. In general, the prevalence of microfilaraemia increased with age in both sexes. There was a marked increase in prevalence of microfilaraemia in males between 11-20 and 21-30 year age groups whereas there was a decline between the two age groups in females. Further analyses were performed for the population categorized as children (<16 years), young adults (16-30 years) and mature adults (>30 years). The prevalence of microfilaraemia in children (10.8%) was significantly lower than in young adults (22.2%; $P < 0.001$) and mature adults (31.3%; $P < 0.001$). Also, the microfilaria prevalence was significantly higher in mature adults than in young adults ($P = 0.034$).

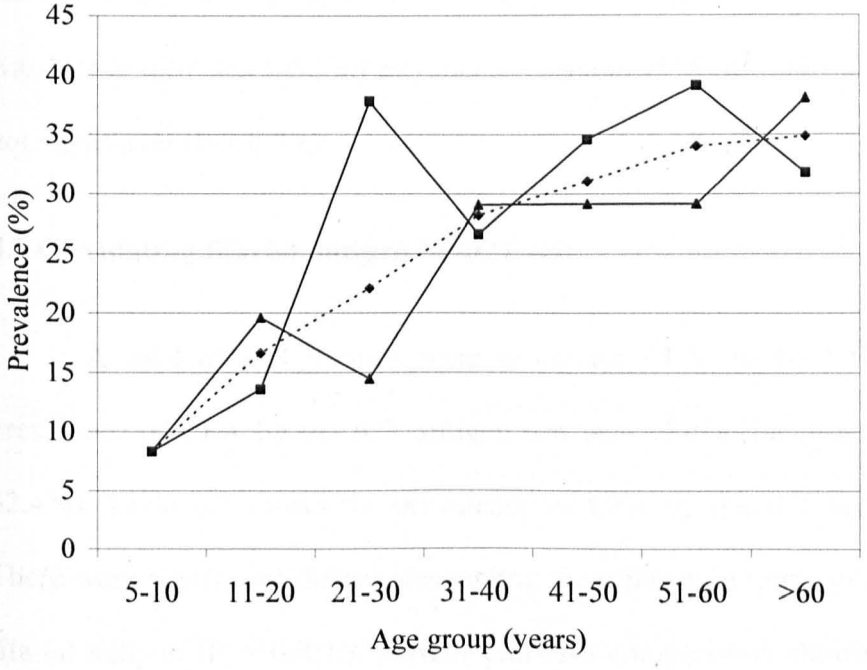

Figure 4.2: Prevalence of microfilaraemia by age and gender during baseline survey in 2002: males (■); females (▲); all (◆). The prevalence of microfilaraemia increased with age and was similar between males and females.

The intensity of microfilariae was higher in children (426 mf/ml) than in young adults (263 mf/ml), but the difference was not significant (P = 0.251). The intensity of microfilariae in mature adults (826 mf/ml) was higher than in children, but the difference was not significant (P = 0.095). The intensity of microfilariae in young adults, however, was significantly lower than in mature adults (P =0.002).

It was previously reported that females in the reproductive age have significantly lower prevalence of microfilaraemia than males in the same age group (Brabin, 1990). Of the 740 individuals tested for microfilariae there were 129 males and 212 females belonging to the 15-45 year age group. The prevalence of

microfilaraemia in this group was 26.4% for males and 21.7% for females, but the difference was not significant (P = 0.324). Similarly, the intensity of microfilaraemia was higher in males (552 mf/ml) than in females (518 mf/ml), but the difference was not significant (P = 0.796).

4.3 Circulating filarial antigen by ICT test

A total of 748 persons were tested for CFA by the ICT test. The overall prevalence of CFA by the ICT antigen test was 35.4% (range among villages 27.5-42.4%). Table 4.3 shows the prevalence of CFA by the ICT test in the 4 villages. There were significant differences among the villages in terms of prevalence rates of filarial antigen (P = 0.016). Further pairwise comparisons showed that there was a significant difference in prevalence of CFA between Magongoloni (42.4%) and Mkondoni (27.5%) villages (P = 0.002).

Village	No. tested	No. positive	Prevalence (%)
Jilore	172	66	38.4
Marikano	196	65	33.2
Magongoloni	198	84	42.4
Mkondoni	182	50	27.4
All	748	265	35.4

Table 4.3: Prevalence of circulating filarial antigen (by ICT test) during baseline survey in 2002. The prevalence of antigenaemia was significantly different among the four villages.

Figure 4.3 shows the baseline prevalence rates of filarial antigen by age and gender in the study area. The prevalence of CFA (by ICT test) in males (37.9%) was higher than in females (33.5%), but the difference was not significant (P = 0.213). Like microfilaraemia, the prevalence of antigenaemia increased steadily with age. The prevalence rate of parasite antigenaemia was significantly lower in children aged < 16 years (23.6%) than in young adults (34.2%; P = 0.011) and mature adults (50.8%; P < 0.001). Similarly, prevalence rate of antigenaemia was significantly lower in young adults compared to mature adults (P = 0.001).

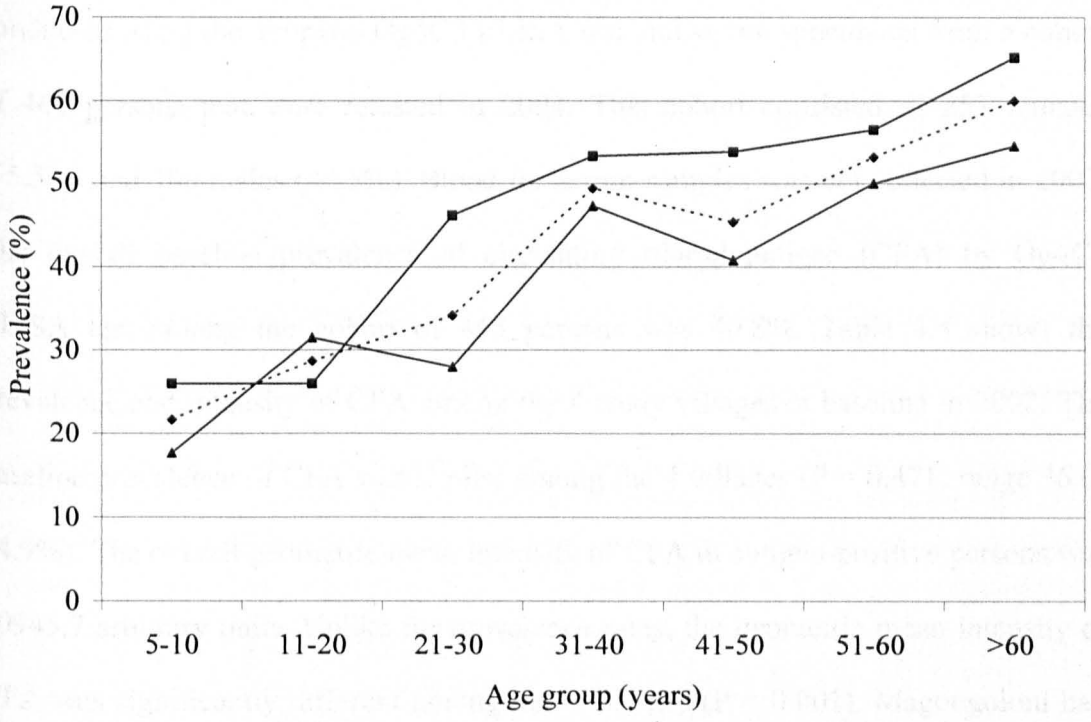

Figure 4.3: Prevalence of filarial antigenaemia (ICT test) by age and gender during baseline survey in 2002: males (■); females (▲); all (◆). The prevalence of antigenaemia increased steadily with age.

Among the persons tested for microfilaraemia and antigenaemia, 729 individuals were tested using both microfilaria detection and the ICT test. There were 151 and 256 individuals positive for CFA and microfilaraemia, respectively in this group. Thus, the ICT test detected 105 (14.4%) more infections than microfilaria detection. Of the 151 persons that were microfilaria positive, 136 (90.1%) individuals were antigen positive by the ICT test. Twelve of the 15 microfilaraemic persons that were negative by the ICT test were also tested by Og4C3 ELISA test and only two were antigen positive. This result did not change when the cut-off for the Og4C3 ELISA test was lowered to 32 or 64 units.

4.4 Circulating filarial antigen by Og4C3 ELISA

In addition to testing of CFA by the ICT test, further CFA measurement was conducted using the TropBio Og4C3 ELISA test and serum specimens from a cohort of 463 persons that were retested in 2004. This cohort consisted of 257 females (55.5%) and 206 males (44.5%). Blood for serum samples was not collected in 2003. The overall baseline prevalence of circulating filarial antigen (CFA) by Og4C3 ELISA test among the cohort of 463 persons was 40.8%. Table 4.4 shows the prevalence and intensity of CFA among the 4 study villages at baseline in 2002. The baseline prevalence of CFA was similar among the 4 villages (P = 0.471; range 36.0-44.9%). The overall geometric mean intensity of CFA in antigen-positive persons was 20945.7 arbitrary units. Unlike for prevalence rates, the geometric mean intensity of CFA was significantly different among the 4 villages (P = 0.001). Magongoloni had significantly higher intensity of CFA than both Jilore and Marikano (P = 0.006).

The prevalence of CFA in males (43.0%) was higher than in females (39.1%), but the difference was not significant (P = 0.392). Similarly, the intensity of CFA in

males (24895.2 units) was higher than in females (17960.8 units), but the difference was not significant (P = 0.335). As for microfilaraemia, the prevalence and intensity of CFA in individuals in the 15-45 year age group (reproductive age) were higher in males than in females, but the differences were not significant.

Village	No. tested	No. positive	Prevalence (%)	GMI (AU)[1]
Jilore	104	40	38.5	11150.2
Marikano	107	46	43.0	11025.6
Magongoloni	138	62	44.9	52917.9
Mkondoni	114	41	36.0	19598.9
Total	463	189	40.8	20945.7
P value			0.471[a]	0.001*

Table 4.4: Prevalence and intensity of circulating filarial antigen (by Og4C3 ELISA) during baseline survey in 2002. The prevalence of antigenaemia was similar among the four villages whereas the intensity was significantly different.

[a]Chi-square test, *Kruskal-Wallis test

[1]Geometric mean intensity in antigen-positive specimens

The age-specific prevalence of CFA is shown in Figure 4.4. The prevalence of CFA increased with age and there was a sharp rise after 21-30 year age group. The prevalence and intensity of CFA in children, young adults and mature adults are shown in Table 4.5. The prevalence rates of CFA in children, young adults and mature adults were significantly different (P < 0.001). Further analyses showed that

the prevalence of CFA was similar in children (28.6%) and young adults (40.0%) (P = 0.100), but significantly higher in mature adults (56.0%) than in children (P < 0.001) and young adults (P = 0.037). However, unlike prevalence rates, the intensity of CFA in children, young adults and mature adults were similar.

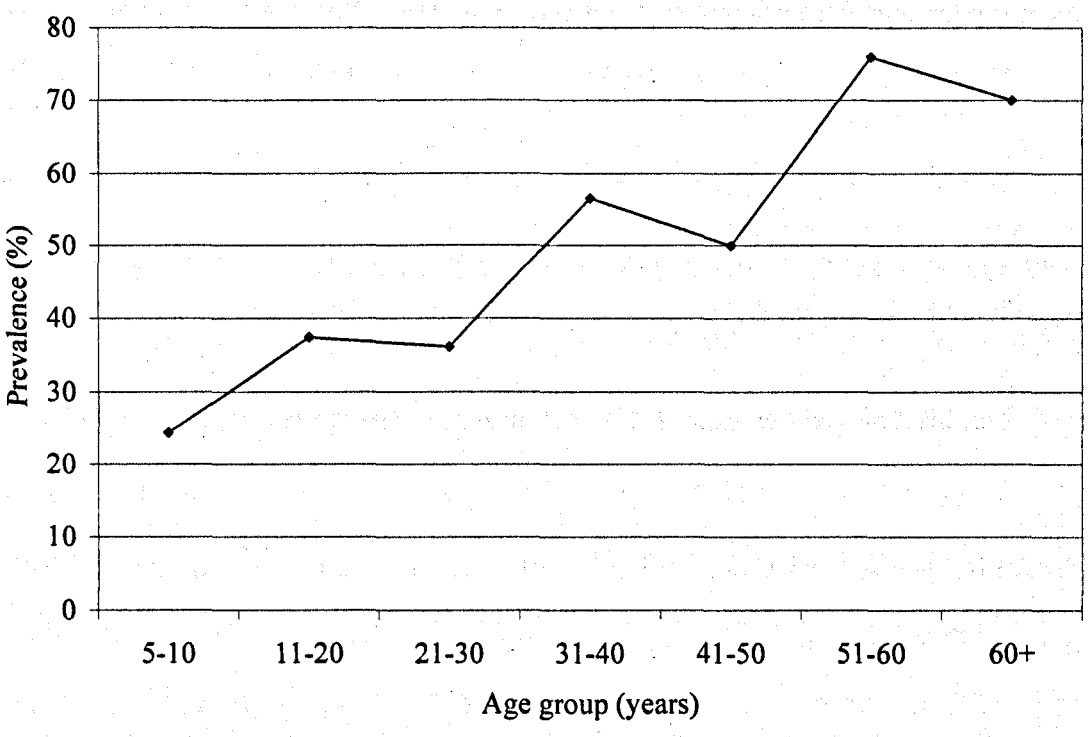

Figure 4.4: Age-specific prevalence of circulating filarial antigen (by Og4C3 ELISA) during baseline survey in 2002. The prevalence of antigenaemia increased steadily with age.

Age group (years)	No. tested	No. positive	Prevalence (%)	GMI (units)[1]
< 16	224	64	28.6	15716.6
16-30	87	35	40.2	12350.4
> 30	152	90	59.2	31550.8
All	463	189	40.8	20945.7

Table 4.5: Age-specific prevalence and intensity of circulating filarial antigen (by Og4C3 ELISA) during baseline survey in 2002. The prevalence of antigenaemia was significantly higher in mature adults compared to children and young adults. However, the intensity of antigenaemia was similar among the three age categories.
[1]Geometric mean intensity in antigen-positive specimens

The prevalence of CFA by ICT test in the cohort of 463 persons was 37.1% and not significantly different from prevalence by Og4C3 ELISA test (P = 0.245). Analysis of the association between the two CFA tests among individuals in the cohort group showed a strong correlation (r = 0.912, P < 0.001). Of 104 microfilaraemic persons that were tested for CFA by Og4C3 ELISA test, 94 (90.4%) were antigen positive. However, of 345 microfilaria negative persons, 89 (25.8%) had antigenaemia by Og4C3 ELISA test. Interestingly, even when the cut-off was lowered to 64 or 32 units still 94 of 104 (90.4%) microfilaraemic individuals had antigenaemia. However, of 345 microfilaria negative persons, 146 (42.3%) and 96 (27.8%) individuals were CFA positive based on 32 and 64 units cut-off, respectively. The absolute median microfilaria count of the 94 microfilaraemic individuals that were also CFA positive was 69 mf/100 µl (range 2-2471 mf/100 µl). For the remaining 10 microfilaraemic individuals that were antigen negative the median microfilaria count was 3 mf/100 µl (range 2-97 mf/100 µl). The median microfilaria

count of antigen positive persons was significantly higher than that of antigen negative individuals ($P < 0.001$).

4.5 Antifilarial antibody responses to crude filarial antigen

Antifilarial antibody assays to *B. pahangi* antigen were conducted for the cohort of 463 persons who provided serum samples both at pre-treatment (2002) and after two rounds of MDA (2004). The overall prevalence of antifilarial IgG1 and IgG4 responses in the study area was 86.2% and 90.9%, respectively. The prevalence rates of antifilarial IgG1 and IgG4 responses among the 4 study villages are shown in Table 4.6. The prevalence of antifilarial IgG1 levels among the 4 villages ranged from 82.2-96.8% and were statistically similar ($P = 0.280$). The overall prevalence of antifilarial IgG4 levels ranged from 84.1-97.1%. Marikano village had significantly lower prevalence of antifilarial IgG4 (84.1%) than Magongoloni (97.1%, $P < 0.01$).

Village	No. tested	Antifilarial IgG1		Antifilarial IgG4	
		No. positive	Prevalence (%)	No. positive	Prevalence (%)
Jilore	104	95	91.3	92	88.5
Marikano	107	88	82.2	90	84.1
Magongoloni	138	119	86.2	134	97.1
Mkondoni	114	97	85.1	105	92.1
All	463	399	86.2	421	90.9

Table 4.6: Prevalence of antifilarial IgG1 and IgG4 responses by village during baseline survey in 2002. Marikano village had significantly lower prevalence of antifilarial IgG4 compared to Magongoloni.

The overall geometric mean intensity of antifilarial IgG1 and IgG4 in the study population was 31.6 μg/ml and 24.2 μg/ml, respectively. There was a positive correlation between antifilarial IgG1 and IgG4 antibody responses ($r = 0.463$, $P < 0.01$). The geometric mean intensity of antifilarial IgG1 and IgG4 in the study villages ranged from 22.3-38.0 μg/ml and 16.1-31.8 μg/ml, respectively. Figure 4.5 shows the overall geometric mean intensity of antifilarial IgG1 and IgG4 responses in the four study villages. Marikano village had significantly lower intensity of antifilarial IgG1 responses compared to the other three villages ($P < 0.05$). Similarly, comparison of geometric mean intensity of antifilarial IgG4 responses among the 4 villages showed that Marikano and Jilore villages had significantly lower intensity than Magongoloni and Mkondoni ($P < 0.05$).

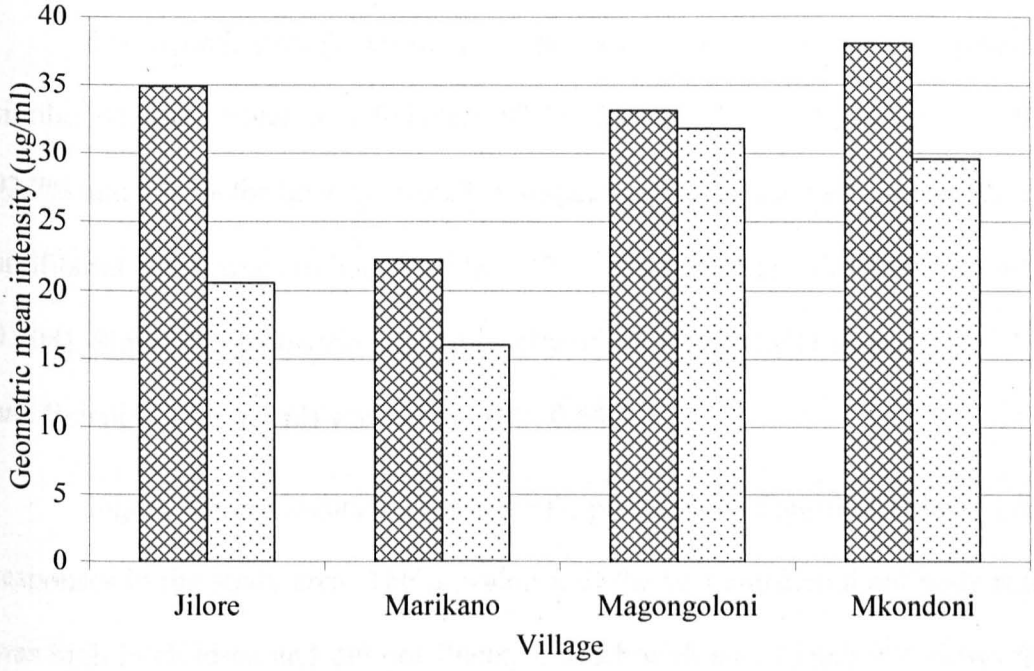

Figure 4.5: Levels of antifilarial IgG1 (▨) and IgG4 (□) antibodies in study villages, Malindi, Kenya during baseline survey in 2002. Marikano village had significantly lower intensity of antifilarial IgG1 responses compared to the other three villages. Marikano and Jilore villages had significantly lower intensity of antifilarial IgG4 compared to Magongoloni and Mkondoni.

4.5.1 Antifilarial antibody responses by gender and age

The overall prevalence rates of the two antifilarial antibody responses were similar between males and females; 87.4% and 85.2% for IgG1 (P = 0.502) and 92.7% and 89.5% for IgG4 (P = 0.230), respectively. The geometric mean intensity of antifilarial IgG1 was similar in males (32.3 μg/ml) and females (31.1 μg/ml), (P = 0.708). Similarly, geometric mean intensity of antifilarial IgG4 in males (25.2 μg/ml) and females (23.5 μg/ml) was similar (P = 0.636).

Figure 4.6 represents the age-specific prevalence of antifilarial IgG1 and IgG4 responses in the study area. The prevalence of the two antifilarial antibody responses was high in children and did not fluctuate much with age. Figure 4.7 shows the age-specific geometric mean intensity of antifilarial IgG1 and IgG4 responses. The trend of geometric mean intensities was similar to that observed for prevalence rates. The intensity of antifilarial IgG1 reached peak levels in the 11-20 year age group. Thereafter, the level of antifilarial IgG1 decreased steadily to reach a minimum in the 51-60 year age group. The geometric mean intensity of antifilarial IgG4 in the study population was slightly lower in persons below 11 years of age than in older persons. The peak intensity of antifilarial IgG4 increased gradually with age and levelled off at the 31-40 year age group.

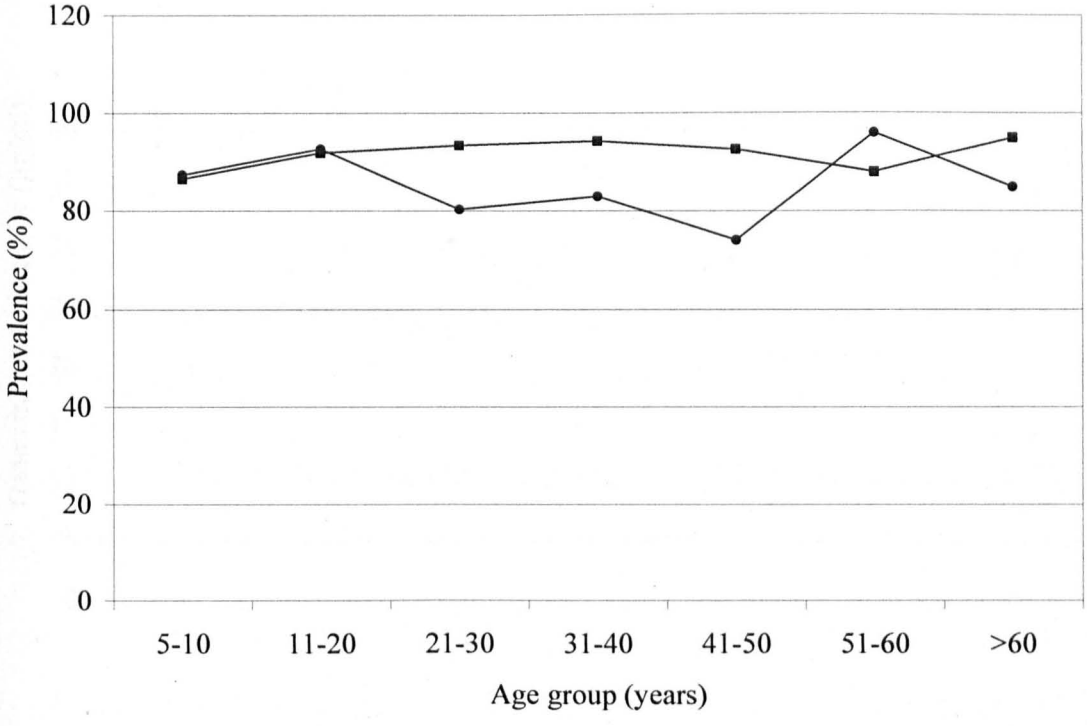

Figure 4.6: Age-specific prevalence of antifilarial IgG1 (●) and IgG4 (■) antibodies during baseline survey in 2002. The prevalence of the two antifilarial antibody responses was high in children and did not fluctuate much with age.

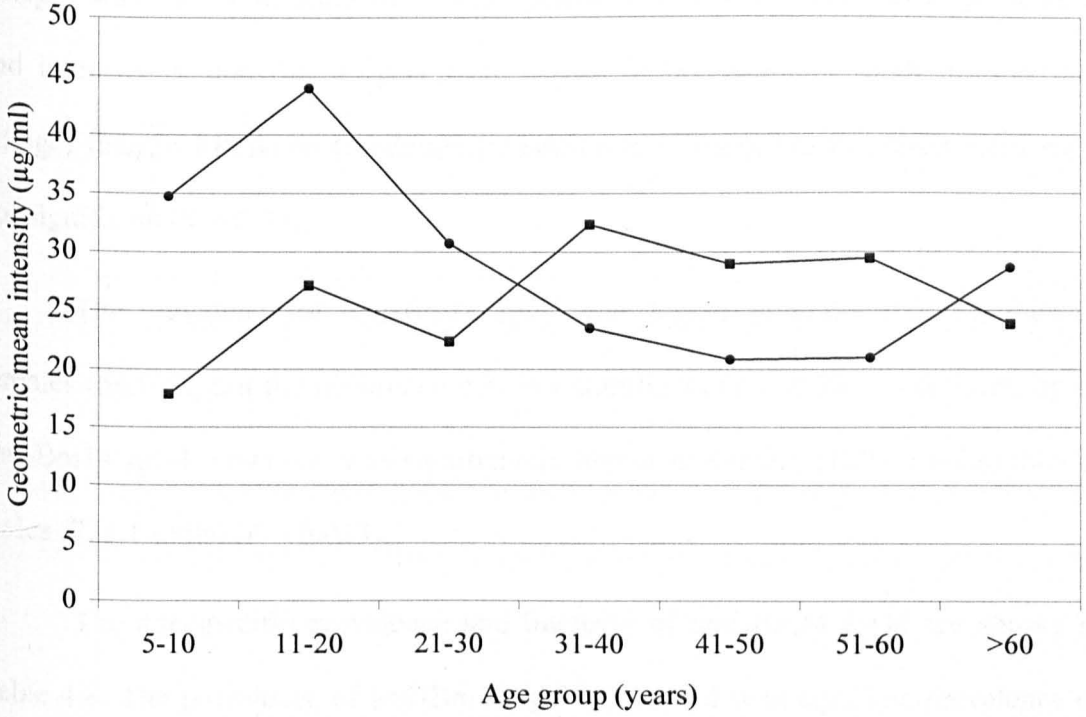

Figure 4.7: Age-specific geometric mean intensity of antifilarial IgG1 (●) and IgG4 (■) responses during baseline survey (2002). The intensity of antifilarial IgG1 was relatively high in children compared to adults.

4.6 Baseline IgG4 antibody responses to Bm14 recombinant antigen

Serum specimens from individuals in two communities (Magongoloni and Marikano) were used to determine IgG4 antibody responses to the recombinant antigen Bm14 (anti-Bm14 IgG4). The two communities were selected to represent areas of high (Magongoloni) and moderate (Marikano) transmission of infection based on intensities of CFA and microfilaraemia. A total of 245 individuals (104 males and 141 females) were tested for anti-Bm14 IgG4.

The baseline prevalence and intensity of anti-Bm14 IgG4 in the two villages are shown in Table 4.7. The overall prevalence of anti-Bm14 IgG4 between the two

villages was 58.8% whereas the overall intensity was 957.3 units. Both prevalence and intensity of anti-Bm14 IgG4 were higher in Magongoloni (high transmission village) than in Marikano (moderate transmission village), but the differences were not significant ($P > 0.05$).

The prevalence of anti-Bm14 IgG4 was higher in males (62.5%) than in females (56.0%), but the difference was not significant ($P = 0.309$). The intensity of anti-Bm14 IgG4, however, was significantly higher in females (1203.1 units) than in males (725.1 units) ($P = 0.023$).

The age-specific prevalence and intensity of anti-Bm14 IgG4 are shown in Table 4.8. The prevalence of anti-Bm14 IgG4 increased with age. The prevalence of anti-Bm14 IgG4 in children aged < 16 years (50.9%) was significantly lower than in mature adults aged > 30 years (68.8%) ($P = 0.013$). The prevalence of anti-Bm14 IgG4 in young adults aged 16-30 years (61.2%) was higher than in children and lower than in mature adults, but the differences were not significant ($P > 0.05$). The intensity of anti-Bm14 IgG4 was higher in children (1062.4 units) than in young adults (877.4 units) and mature adults (897.7 units), but the differences were not significant ($P > 0.05$).

The sensitivity of the anti-Bm14 IgG4 ELISA test, however, appeared to be lower than that of the Og4C3 ELISA test. Of 61 microfilaraemic persons, 48 (78.7%) individuals were positive for anti-Bm14 IgG4. Further, of 108 CFA positive (by Og4C3 ELISA) persons, 93 (86.1%) individuals were positive for anti-Bm14 IgG4.

Village	No. tested	No. positive	% positive	GMI (units)[1]
Marikano	107	59	55.1	865.6
Magongoloni	138	85	61.6	1026.6
All	245	144	58.8	957.3

Table 4.7: Prevalence and intensity of anti-Bm14 IgG4 by village during baseline survey in 2002. Both the prevalence and intensity of anti-Bm14 IgG4 were higher in Magongoloni (high transmission village) than in Marikano (moderate transmission village), but the differences were not significant.

[1]Geometric mean intensity was computed in antibody-positive persons only

Age category (years)	No. tested	No. positive	% positive	GMI[1]
< 16	116	59	50.9	1062.4
16-30	49	30	61.2	877.4
> 30	80	55	68.8	897.7
All	245	144	58.8	957.3
*P value			0.041[a]	0.682*

Table 4.8: Age-specific prevalence and intensity of anti-Bm14 IgG4 during baseline survey in 2002. The prevalence of anti-Bm14 IgG4 increased with age. However, there was no significant difference in the intensity of anti-Bm14 IgG4 by age.

[1]Geometric mean intensity (GMI) in anti-Bm14 IgG4-positive persons

[a]Chi square, *Kruskal-Wallis test

4.7 Association between antifilarial antibody responses and circulating filarial antigen

4.7.1 Antifilarial antibody levels by circulating filarial antigen status

Correlations between logarithmic transformed antifilarial antibody responses and circulating antigen measurements were performed to determine whether there was an association between these variables. There was no significant association between antifilarial IgG1 and CFA status ($r = 0.049$, $P = 0.290$). Figure 4.8 is a boxplot showing the relationship between logarithmic transformed antifilarial IgG1 and CFA status. There was no significant difference in antifilarial IgG1 levels between filarial antigen (Og4C3 ELISA test) negative (34.2 μg/ml) and positive persons (30.0 μg/ml). The intensity of antifilarial IgG4 in CFA positive individuals (60.0 μg/ml) was more than four-fold higher compared to CFA negative persons (13.0 μg/ml) ($P < 0.001$). The level of antifilarial IgG4 was significantly associated with CFA status ($r = 0.469$, $P < 0.001$). Figure 4.9 is a boxplot showing the relationship between logarithmic transformed antifilarial IgG4 and CFA status.

Similarly, anti-Bm14 IgG4 was significantly correlated with CFA ($r = 0.484$; $P < 0.001$). Figure 4.10 is a boxplot showing the relationship between logarithmic transformed anti-Bm14 IgG4 and CFA. The geometric mean intensity of anti-Bm14 IgG4 was more than 16-fold higher in antigen-positive persons (748.4 units) compared to antigen-negative persons (46.4 units).

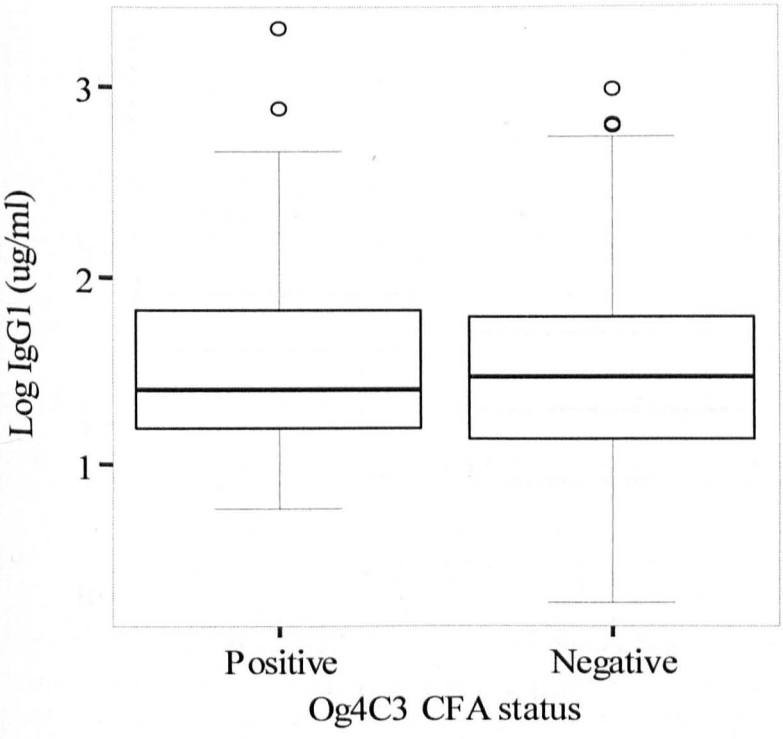

Figure 4.8: Association between antifilarial IgG1 and circulating filarial antigen (by Og4C3 ELISA assay). There was no significant difference in antifilarial IgG1 levels between filarial antigen-negative and -positive persons.

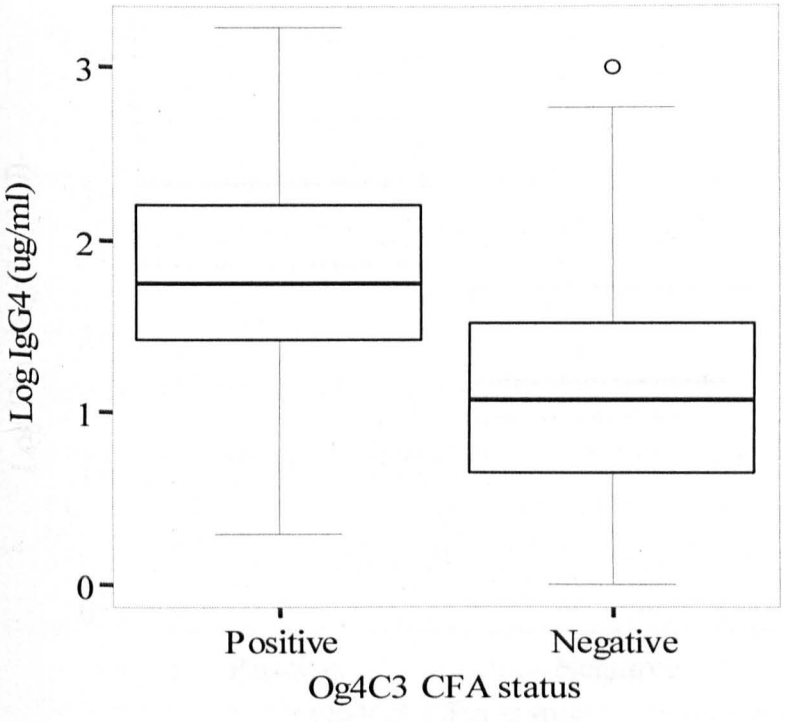

Figure 4.9: Association between antifilarial IgG4 and circulating filarial antigen (by Og4C3 ELISA assay). The intensity of antifilarial IgG4 in antigen-positive individuals was more than four-fold higher compared to antigen-negative persons.

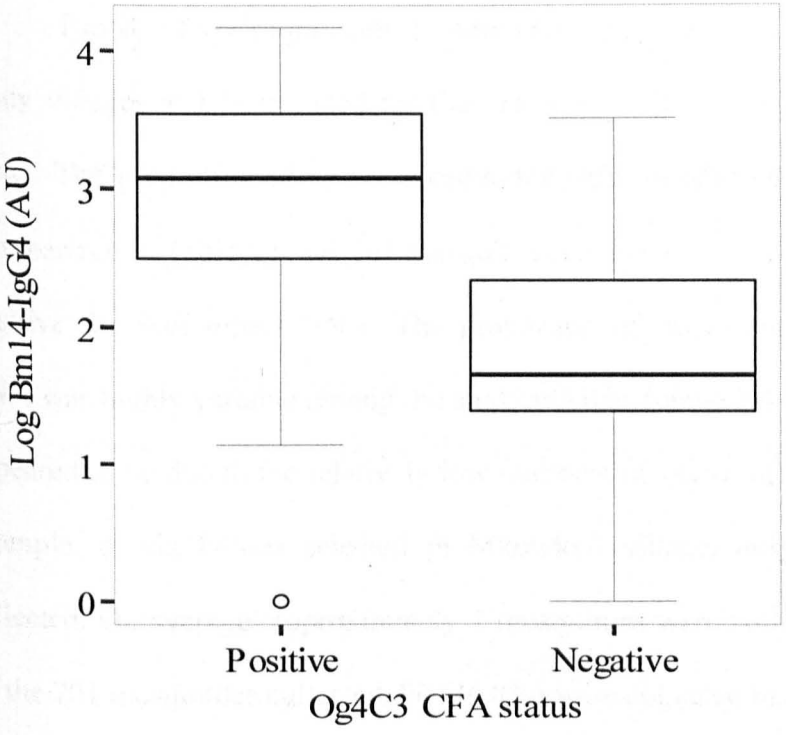

Figure 4.10: Association between anti-Bm14 IgG4 and circulating filarial antigen (by Og4C3 ELISA assay). The geometric mean intensity of anti-Bm14 IgG4 in antigen-positive persons was more than 16-fold higher compared to antigen-negative individuals.

4.8 Xenomonitoring of vector mosquitoes for *W. bancrofti* DNA

Blood fed vector mosquitoes were collected in selected houses in each of the 4 study villages and later tested for the presence of *W. bancrofti Ssp*I DNA by PCR assay. The proportion of vector mosquitoes with parasite DNA in the 4 villages is summarized in Table 4.9. Of 201 mosquitoes collected in 27 houses, 50 (24.9%) were positive for *Ssp*I repeat DNA. The proportion of vector mosquitoes with parasite DNA was highly variable among the study villages (range 2.4-41.1%). This variation appeared to be due to the relatively low numbers of vector mosquitoes collected. For example, of six houses selected in Mkondoni village, only 25 mosquitoes were collected. On average, approximately 7 mosquitoes were collected from each house. Of the 201 mosquitoes collected, 90 (44.8%) were collected in Jilore village.

Village	No. tested	No. positive	% positive
Jilore	90	37	41.1
Marikano	42	1	2.4
Magongoloni	44	10	22.7
Mkondoni	25	2	8.0
All	201	50	24.9

Table 4.9: Prevalence of *W. bancrofti Ssp*I DNA in vector mosquitoes during baseline survey in 2002. The proportion of vector mosquitoes with parasite DNA was highly variable among the study villages due to the relatively low numbers of vector mosquitoes collected.

Three species of vector mosquitoes, namely, *Anopheles gambiae s.l.*, *An. funestus* and *Culex quinquefasciatus* were collected in the area. However, parasite DNA was detected in *An. gambiae s.l.* and *An. funestus* mosquitoes. Only one *Cx. quinquefasciatus* mosquito was collected and this mosquito was negative for parasite DNA. *Anopheles gambiae s.l.* mosquitoes appeared to be the principal vectors of bancroftian filariasis in the area. Of 127 *An. gambiae s.l.* mosquitoes collected, 45 (35.4%) were positive for parasite DNA. For *An. funestus,* of 73 mosquitoes collected, 5 (6.8%) were positive for parasite DNA.

Anopheles gambiae s.l. was the dominant vector in Jilore and Mkondoni villages. Of 90 mosquitoes collected in Jilore village, 87 (96.7%) were *An. gambiae s.l.* Similarly, of 25 mosquitoes collected in Mkondoni village, 22 (88.0%) were *An. gambiae s.l. Anopheles funestus* was the predominant vector in Marikano village. Of 42 mosquitoes collected in this village, 37 (88.1%) were *An. funestus*. The one *Cx. quinquefasciatus* mosquito collected was from Mkondoni village. In Magongoloni village, 13 (29.5%) and 31 (70.5%) of 44 vector mosquitoes collected were *An. gambiae s.l.* and *An. funestus*, respectively.

CHAPTER 5 – INVESTIGATIONS OF CLINICAL MANIFESTATIONS OF BANCROFTIAN FILARIASIS IN MALINDI DISTRICT, KENYA

The Global Programme to Eliminate Lymphatic Filariasis has two major objectives: to interrupt transmission of infection by mass administration of antifilarial drugs and reduce suffering for those already with chronic disease (lymphoedema and hydrocele). Some 38 endemic countries, through the support of the GPELF, have started national filariasis elimination programmes and millions of people living in endemic areas are treated annually with antifilarial drugs. It is essential to aim not just for transmission control but also for morbidity control because even when microfilariae have been eliminated, the adult worms, as well as external microbial pathogens, may continue to induce lymphatic pathology and consequent morbidity (Ottesen *et al.*, 1997). However, before morbidity control programmes can be initiated it is necessary to obtain baseline information on prevalence and severity of disease. Such information would help programme managers to institute appropriate morbidity control programmes. The present study was conducted to establish the baseline clinical manifestations due to bancroftian filariasis in an area selected for mass drug administration using DEC and albendazole.

5.1 Hydrocele

A total of 348 males were examined for hydrocele during an epidemiological survey conducted in 2002. Hydrocele was detected in 66 males with two cases found in boys below 15 years old (12 and 14 years old). The prevalence rates of hydrocele in males above 14 years old are summarized in Table 5.1. Of 186 males above 14 years old examined for hydrocele during the baseline survey 64 individuals (34.4%) had hydrocele. The prevalence rates of hydrocele among the 4 study villages ranged from

28.3-41.0% in males above 14 years old, but were not significantly different (P = 0.543).

village	No. examined	No. (%) with hydrocele	No. (%) with scrotal lymphoedema
Jilore	42	17 (40.5)	4 (9.5)
Marikano	52	16 (30.8)	1 (1.9)
Magongoloni	39	16 (41.0)	3 (7.7)
Mkondoni	53	15 (28.3)	2 (3.8)
All	186	64 (34.4)	10 (5.4)
P value*		0.543	0.345

Table 5.1: Prevalence rates of hydrocele and scrotal lymphoedema in males aged 15 years and above during baseline survey in 2002.
*Chi-square test

The age-specific prevalence of hydrocele is summarized in Table 5.2 (and Figure 5.1). The prevalence of hydrocele increased steadily with increase in age. Males above 40 years old had a significantly higher prevalence of hydrocele than those in the 15-20 year ($P < 0.001$) and 21-40 year ($P = 0.003$) age groups. Similarly, males in the 21-40 year age group had a higher prevalence rate of hydrocele than those in the 15-20 year age group ($P = 0.017$).

Age group (years)	No. examined	No. (%) with hydrocele	No. (%) with scrotal lymphoedema
15-20	50	5 (10.0)	0 (0.0)
21-40	60	17 (28.3)	1 (1.7)
> 40	76	42 (55.3)	9 (11.8)
All	186	64 (34.4)	10 (5.4)

Table 5.2: Age-specific prevalence rates of hydrocele and scrotal lymphoedema in males aged 15 years and above during baseline survey in 2002. Both the prevalence rates of hydrocele and scrotal lymphoedema increased with age.

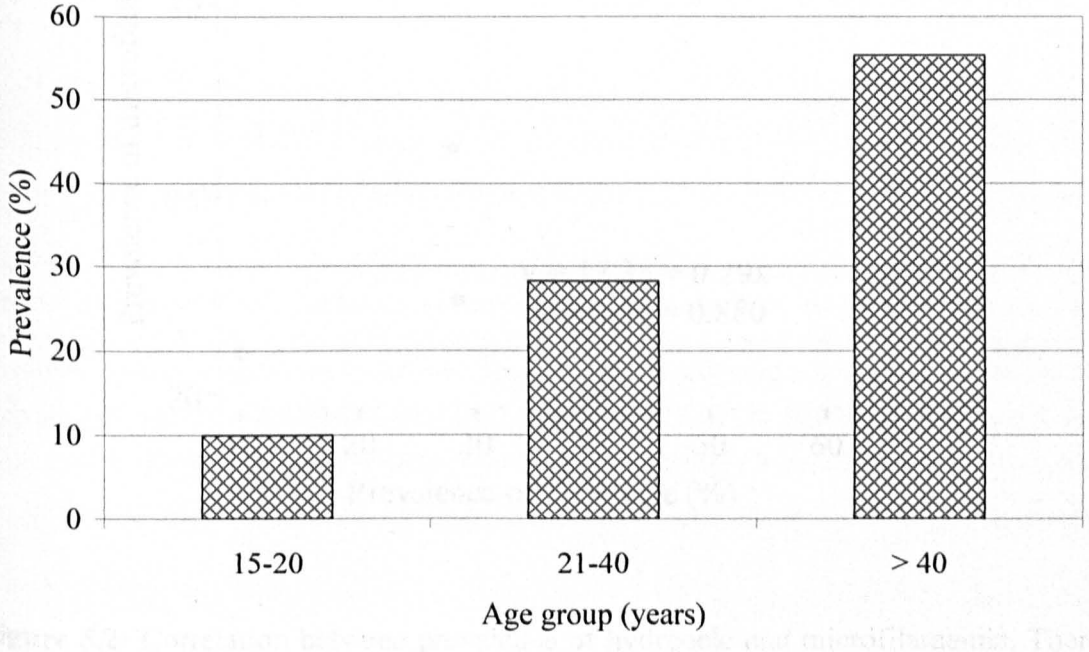

Figure 5.1: Age-specific prevalence of hydrocele in males aged > 14 years during baseline survey in 2002. The prevalence of hydrocele increased steadily with age.

The association between the prevalence of hydrocele and microfilaraemia was determined in persons aged > 14 years stratified into age groups to generate enough data to allow statistical analysis. Figure 5.2 is a scatterplot showing the association between prevalence of hydrocele and microfilaraemia. There was a significant correlation between prevalence of hydrocele and microfilaraemia ($r = 0.941$, $r^2 = 0.880$, $P = 0.005$).

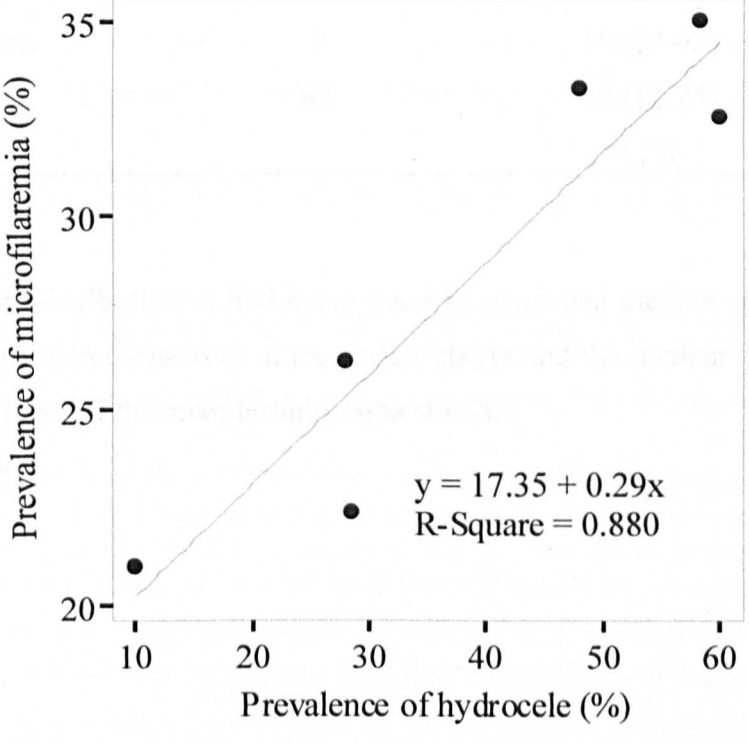

Figure 5.2: Correlation between prevalence of hydrocele and microfilaraemia. There was a significant correlation between prevalence of hydrocele and microfilaraemia ($r = 0.941$, $r^2 = 0.880$).

The frequencies of hydrocele by stage are summarized in Table 5.3. The majority of cases (59.1%) were in stage 1 (6-8 cm, longitudinal axis). In general there were fewer hydroceles in the more advanced stages. The median age of persons with different stages of hydrocele were similar (P = 0.540).

Stage of hydrocele	N	Median age (range), years
1 (6-8 cm)	37	49 (16-85)
2 (8-11 cm)	14	45 (18-75)
3 (11-15 cm)	11	52 (25-75)
4 (> 15 cm)	2	58 (53-62)
All	66	49 (12-85)
P value*		0.246

Table 5.3: Distribution of hydrocele cases by stage and median age of patients. The majority of hydroceles were in the earlier stages and the median age of persons with different stages of this manifestation was similar.

*Median test

5.1.1 Relationship between hydrocele and parasitological status

Table 5.4 summarizes the filarial infection and antifilarial antibody status of males aged > 14 years with and without hydrocele using different tests. Detection of microfilaria and CFA are specific tests for the presence of active infection. The prevalence of microfilaraemia in males with and without hydrocele was 35.2% and 24.7%, respectively, but the difference was not significant (P = 0.284). Similarly, the prevalence of CFA in males with and without hydrocele was 64.7% and 46.7%, respectively, but the difference was not significant (P = 0.164).

In addition to the presence of active infection, males with and without hydrocele were compared for the presence of antifilarial antibodies. All individuals that were tested for CFA were also tested for antifilarial IgG1 and IgG4 antibody to crude *B. pahangi* antigen. The prevalence of antifilarial antibodies was similar between the two groups. For example, the prevalence rates of antifilarial IgG4 in males with (n = 34) and without (n = 45) hydrocele were 97.1% and 97.8%, respectively (P = 0.811). Some of the individuals with and without hydrocele were also tested for IgG4 response to a recombinant Bm14 antigen (anti-Bm14 IgG4). The prevalence rates of anti-Bm14 IgG4 in individuals with (n = 18) and without (n = 17) hydrocele were 61.1% and 70.6%, respectively (P = 0.404).

In addition to comparing individuals with and without hydrocele for the presence of active infection, analyses were also done for intensity of infection. The geometric mean intensity of microfilariae in 18 microfilaraemic males with hydrocele was 828 mf/ml. For 23 microfilaraemic males without hydrocele, the intensity of microfilaraemia was 325 mf/ml. However, the difference in intensity of microfilaraemia in microfilaraemic individuals with and without hydrocele was not

significant (P = 0.109). Among antigenaemic males, the intensity of CFA in 22 with hydrocele was 95,449.0 units and had a tendency to be higher compared to 30,266.9 units in 21 without hydrocele (P = 0.058).

Table 5.5 summarizes the infection and antifilarial antibody status of persons with hydrocele by stage using different tests. Active filarial infection was detected in all stages of hydrocele using microfilaria detection or Og4C3 ELISA test for CFA. Two cases with hydrocele stage 4 (scrotal sac > 15 cm in longitudinal axis) that were tested for microfilaraemia and CFA were positive by both tests. Similarly, antifilarial antibody responses were detected in all the stages of hydrocele.

Indicator of infection/exposure	*Hydrocele status:* Present	Absent	Total	P value*
Microfilaraemia				
No. positive/ No. examined	18/51	23/93	41/144	
% positive	35.2	24.7	28.5	0.284
Circulating antigen (Og4C3)				
No. positive/ No. examined	22/34	21/45	43/79	
% positive	64.7	46.7	54.4	0.164
Antifilarial IgG1				
No. positive/ No. examined	30/34	39/45	69/79	
% positive	88.2	86.7	87.3	0.903
Antifilarial IgG4				
No. positive/ No. examined	33/34	44/45	77/79	
% positive	97.1	97.8	97.5	0.811
Anti-Bm14 IgG4				
No. positive/ No. examined	11/18	12/17	23/35	
% positive	61.1	70.6	65.7	0.404

Table 5.4: Indicators of infection/exposure in males above 14 years with and without hydrocele.
The prevalence of active infection (microfilaraemia and antigenaemia) and antifilarial antibodies was similar between males with and without hydrocele.
*Chi-square test

Indicator of infection/exposure	Stage of hydrocele				Total	P value*
	1	2	3	4		
Microfilaraemia						
No. positive/ No. examined	11/31	2/9	3/10	2/2	18/52	
% positive	35.5	22.2	30.0	100.0	34.6	0.208
Circulating antigen (Og4C3)						
No. positive/ No. examined	13/22	2/5	5/6	2/2	22/35	
% positive	59.1	40.0	83.3	100.0	62.9	0.306
Antifilarial IgG1						
No. positive/ No. examined	20/22	4/5	5/6	2/2	31/35	
% positive	90.9	80.0	83.3	100.0	88.6	0.841
Antifilarial IgG4						
No. positive/ No. examined	21/22	5/5	6/6	2/2	34/35	
% positive	95.5	100.0	100.0	100.0	97.1	0.888
Anti-Bm14 IgG4						
No. positive/ No. examined	6/10	2/4	2/3	1/1	11/18	
% positive	60.0	50.0	66.7	100.0	61.1	0.815

Table 5.5: Baseline (2002) infection and antifilarial antibody status of persons with hydrocele by stage using different tests. Active *W. bancrofti* infection and antifilarial antibody responses were detected in all the stages of hydrocele.

*Chi-square test

5.2 Scrotal lymphoedema

During examination for hydrocele males were also examined for scrotal lymphoedema. Of the 348 males examined 10 individuals had scrotal lymphoedema. Of these 10 individuals of scrotal lymphoedema 4 also had hydrocele. The prevalence rates of scrotal lymphoedema in males above 14 years old among the 4 study villages are summarized together with prevalence rates of hydrocele in Table 5.1. The overall prevalence rate of scrotal lymphoedema in males above 14 years old was 5.4%. The prevalence rates by village ranged from 1.9 to 9.5%, but there was no significant difference among the villages (P = 0.345).

The prevalence of scrotal lymphoedema, like hydrocele, increased with age (Table 5.2). There was no case of scrotal lymphoedema in the 15-20 year age group and only one case was in the 21-40 year age group. The age of males with scrotal lymphoedema ranged from 39 to 80 years. Of the 10 cases of scrotal lymphoedema, 4 (40%) were in stage 1 (scrotal skin hard and thick) and 6 (60%) in stage 2 (fully developed scrotal elephantiasis). The median ages of individuals with scrotal lymphoedema stage 1 and stage 2 were 50 years (range 47-79 years) and 55 years (range 39-80 years), respectively, but the difference was not significant (P = 0.221).

5.2.1 Relationship between scrotal lymphoedema and infection status

Low numbers of individuals with scrotal lymphoedema restricted statistical analyses. However, there was a tendency for higher proportion of individuals with scrotal lymphoedema to have active infection and antifilarial antibody than those without. For example, 5 of 6 (83.3%) persons with scrotal lymphoedema that were tested for CFA were

positive compared to 38 of 73 (52.1%) persons without the manifestation. Similarly, 4 of 7 (57.1%) individuals with scrotal lymphoedema that were tested for microfilaraemia were positive compared to 37 of 137 (27.0%) individuals without the manifestation. Active filarial infection was detected in both stages of scrotal lymphoedema. Of the 4 cases with microfilariae, 3 were in stage 1 and of the 5 cases with parasite antigenaemia 3 were in stage one.

5.3 Lymphoedema of the legs

Lymphoedema of the legs was found in 42 of 782 persons who were examined. Two individuals were below 15 years of age (an 8 year-old male and a 13 year-old female). The prevalence rates of lymphoedema of the legs in persons above 14 years old among the 4 study villages are summarized in Table 5.6. The overall prevalence rate of lymphoedema of the legs in 469 persons above 14 years old was 8.5%. The prevalence rates in the 4 communities ranged from 6.3 to 10.7%, but there was no significant difference among the villages (P = 0.661). Of the 469 persons above 14 years examined 186 (39.7%) and 283 (60.3%) were males and females, respectively. The prevalence rate of lymphoedema in males (12.9%) was higher than in females (5.7%) (P = 0.006). In addition, the prevalence rates in males were higher than in females for all the 4 villages.

The age-specific prevalence of leg lymphoedema is summarized in Table 5.7 (and Figure 5.3). The prevalence rate of lymphoedema of the legs increased with age in both males and females. Of 50 males and 49 females in the 15-20 year age group examined, lymphoedema of the leg was detected in 3 males and no female. Among males, the prevalence of lymphoedema in the 15-20 year (6.0%) and 21-40 year (11.7%) age groups was similar (P = 0.400). However, the prevalence rate in males > 40 years old was significantly

higher (14.8%) than in males 15-20 (P = 0.002) and 21-40 (P = 0.005) years old. Similarly, females > 40 years old had a significantly higher (11.8%) prevalence of lymphoedema of the leg than those 21-40 years old (3.5%) (P = 0.014).

As for hydrocele, the association between the prevalence of leg lymphoedema and microfilaraemia was determined in persons aged > 14 years stratified into age groups to generate enough data to allow statistical analysis. The association between prevalence of leg lymphoedema and microfilaraemia was not significant (r = 0.662, r^2 =0.440, P = 0.152).

Table 5.8 shows the distribution of lymphoedema of the leg by stage and median age of affected individuals. The majority of lymphoedema (47.6%) was in stage 1, which is mild and reversible at night. Of 11 persons with lymphoedema stage 3 and greater, 8 (72.7%) were female. The prevalence of lymphoedema of the leg stage 3 and greater was higher in females (2.8%) than in males (1.6%), but the difference was not significant (P = 0.476). The median age was similar for individuals with different stages of lymphoedema of the leg, both in males (P = 0.287) and females (P = 0.863).

The leg affected by lymphoedema was recorded for 38 cases. Of these 38 cases, 22 (57.9%) and 16 (42.1%) individuals had bilateral and unilateral leg lymphoedema, respectively. In terms of stage, 27 individuals (71.1%) had lymphoedema below stage 3 whereas 11 persons (28.9%) had stage 3 and above. Of the 11 individuals with lymphoedema stage 3 and above, 9 (81.8%) persons had bilateral lymphoedema. Of the 27 persons with leg lymphoedema < stage 3, 13 (48.1%) had bilateral involvement. For lymphoedema < stage 3, there was no significant difference in proportion of persons with bilateral and those with unilateral involvement (P = 0.785).

Village	All		Males		Females	
	No. examined	No. (%) with lymphoedema	No. examined	No. (%) with lymphoedema	No. examined	No. (%) with lymphoedema
Jilore	95	6 (6.3)	42	4 (9.5)	53	2 (3.8)
Marikano	128	12 (9.4)	52	6 (11.5)	76	6 (7.9)
Magongoloni	112	12 (10.7)	39	8 (20.5)	73	4 (5.5)
Mkondoni	134	10 (7.5)	53	6 (11.3)	81	4 (4.9)
All	469	40 (8.5)	186	24 (12.9)	283	16 (5.7)

Table 5.6: Baseline (2002) prevalence rates of leg lymphoedema in persons aged 15 years and above. The prevalence rate of leg lymphoedema in males was significantly higher than in females.

Age category (years)	*All*		*Males*		*Females*	
	No. examined	No. (%) with lymphoedema	No. examined	No. (%) with lymphoedema	No. examined	No. (%) with lymphoedema
15-20	99	3 (3.0)	50	3 (6.0)	49	0 (0.0)
21-40	201	12 (6.0)	60	7 (11.7)	141	5 (3.5)
> 40	169	25 (14.8)	76	14 (18.4)	93	11 (11.8)
All	469	40 (8.5)	186	24 (12.9)	283	16 (5.7)

Table 5.7: Prevalence rates of leg lymphoedema in persons aged > 14 years during baseline survey in 2002. The prevalence rate of lymphoedema of the legs increased with age in both males and females.

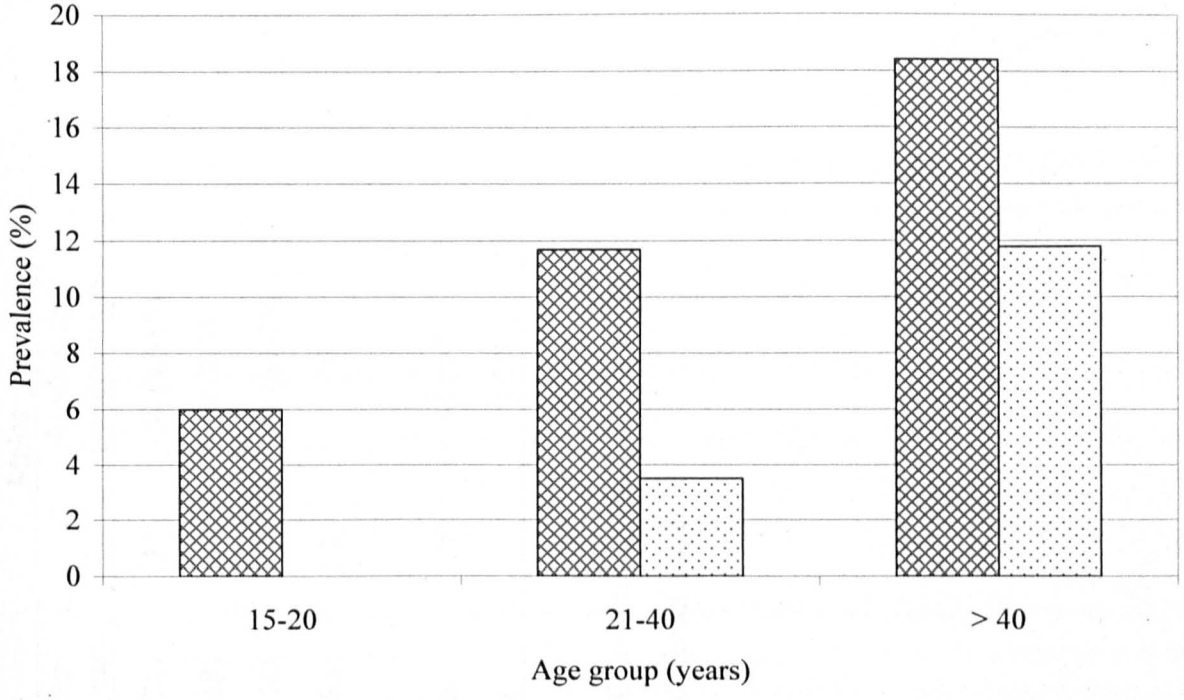

Figure 5.3: Prevalence of leg lymphoedema in males (⊠) and females (□) aged > 14 years during baseline survey in 2002. The prevalence rate of lymphoedema of the legs increased with age in both males and females.

Stage of leg lymphoedema	All persons		Females		Males	
	n	Median age (range), years	n	Median age (range), years	N	Median age (range), years
1-Swelling reversible at night	20	43 (13-85)	7	45 (13-76)	13	42 (17-85)
2-Swelling does not disappear	11	45 (8-75)	2	57 (45-68)	9	39 (8-75)
3-Skin has shallow folds	7	57 (25-73)	5	51 (25-70)	2	71 (69-73)
4-Skin has irregular growths	3	79 (30-83)	2	57 (30-85)	1	79
6-Skin has moss-like growth	1	66	1	66	–	–
All	42	48 (8-85)	17	51 (13-83)	25	44 (8-85)
P value*		0.476		0.863		0.287

Table 5.8: Distribution of leg lymphoedema by stage, age and gender during baseline survey in 2002. The majority of lymphoedema was in stage 1. The median age was similar for individuals with different stages of lymphoedema of the leg.

*Median test

5.3.1 Relationship between lymphoedema of the legs and infection status

Table 5.9 summarizes the infection and antifilarial antibody status of persons aged > 14 years with and without lymphoedema of the leg. The prevalence of microfilaraemia in individuals with and without leg lymphoedema was 31.0% and 25.5%, respectively, but the difference was not significant (P = 0.515). Similarly, the prevalence of CFA in persons with and without leg lymphoedema was 41.2% and 47.7%, respectively (P = 0.607).

As with microfilaraemia and CFA, the prevalence of antifilarial antibodies was similar for persons with and without leg lymphoedema. The prevalence rates of antifilarial IgG1 in persons with and without leg lymphoedema were 94.1% and 82.9%, respectively (P = 0.229). Similarly, the prevalence rates of antifilarial IgG4 in persons with and without leg lymphoedema were 100% and 93.3%, respectively (P = 0.269). The prevalence of anti-Bm14 IgG4 had a tendency to be higher in individuals with leg lymphoedema (84.6%) than in those without lymphoedema (61.9%), but the difference was not significant (P = 0.107).

The geometric mean intensity of microfilariae of 9 individuals with leg lymphoedema and 87 persons without leg lymphoedema was 357 mf/ml and 526 mf/ml, respectively (P = 0.530). The intensity of CFA, however, was higher in persons with leg lymphoedema (122,580.4 units) than in those without lymphoedema (33,401.7 units), but the difference was not significant (P = 0.088).

The infection and antifilarial antibody status of individuals with leg lymphoedema by stage of the manifestation is summarized in Table 5.10. The infection and antifilarial antibody patterns were similar for the different stages of

lymphoedema of the legs. For example, all the stages had a proportion of individuals infected based on either microfilaria detection or CFA testing using Og4C3 ELISA assay. Similarly, all the stages of leg lymphoedema were associated with antifilarial antibody responses.

Indicator of infection/exposure	Leg lymphoedema status: Present	Absent	Total	P value*
Microfilaraemia				
No. positive/ No. examined	9/29	87/341	96/370	
% positive	31.0	25.5	25.9	0.515
Circulating filarial antigen (Og4C3)				
No. positive/ No. examined	7/17	92/193	99/210	
% positive	41.2	47.7	47.1	0.607
Antifilarial IgG1				
No. positive/ No. examined	16/17	160/193	176/210	
% positive	94.1	82.9	83.8	0.229
Antifilarial IgG4				
No. positive/ No. examined	17/17	180/193	197/210	
% positive	100.0	93.3	93.8	0.269
Anti-Bm14 IgG4				
No. positive/ No. examined	11/13	60/97	71/110	
% positive	84.6	61.9	64.5	0.107

Table 5.9: Prevalence of active infection (microfilaraemia and antigenaemia) and antifilarial antibodies in persons above 14 years old with and without leg lymphoedema. The prevalence of active infection and antifilarial antibodies in individuals with and without leg lymphoedema was similar.

*Chi-square test

Indicator of infection/exposure	Stage of leg lymphoedema					Total	P value*
	1	2	3	4	6		
Microfilaraemia							
No. positive/ No. examined	5/14	3/9	0/5	1/2	0/1	9/31	
% positive	35.7	33.3	0.0	50.0	0.0	29.0	0.514
Circulating antigen (Og4C3)							
No. positive/ No. examined	2/7	3/5	1/5	0/1	1/1	7/19	
% positive	28.6	60.0	20.0	0.0	100.0	36.8	0.371
Antifilarial IgG1							
No. positive/ No. examined	7/7	5/5	4/5	1/1	1/1	18/19	
% positive	100.0	100.0	80.0	100.0	100.0	94.7	0.565
Antifilarial IgG4							
No. positive/ No. examined	7/7	5/5	5/5	1/1	1/1	19/19	1.000
% positive	100.0	100.0	100.0	100.0	100.0	100.0	
Anti-Bm14 IgG4							
No. positive/ No. examined	3/4	4/5	3/3	0/1	1/1	11/14	
% positive	75.0	80.0	100.0	100.0	100.0	78.6	0.309

Table 5.10: Baseline infection status of persons with leg lymphoedema by stage of disease. The infection and antifilarial antibody patterns were similar for the different stages of lymphoedema of the legs.

*Chi-square test

5.4 Inguinal adenopathy

Of 782 individuals examined for clinical manifestations of bancroftian filariasis 67 cases of inguinal adenopathy were detected. Of these, 45 (67.2%) and 22 (32.8%) were in males and females, respectively. The prevalence rates of inguinal adenopathy among the 4 study communities are summarized in Table 5.11. The overall prevalence rate of inguinal adenopathy was 8.6% and ranged from 7.0 to 10.3% among the 4 villages. Males had a significantly higher (12.9%) prevalence rate of adenopathy than females (5.1%) ($P < 0.001$).

Table 5.12 shows the age-specific prevalence of inguinal adenopathy in the study area. The prevalence of inguinal adenopathy increased with age in males and females. The age of persons with inguinal adenopathy ranged from 5 to 80 years old. In general, the prevalence rate of adenopathy was lower in 5-10 year age group (3.7%) and similar in 11-20 (8.1%) and 21-40 (8.0%) year age groups. The prevalence of inguinal adenopathy then increased by two-fold in the > 40 years age group (16.0%). However, males had higher prevalence of adenopathy than females in all the age groups. There were very few females aged below 21 years with inguinal adenopathy. Of 107 females in the 5-10 year age group, 3 (2.8%) had inguinal adenopathy, but of 93 females in the 11-20 year age group none had adenopathy.

As for hydrocele and leg lymphoedema, the association between the prevalence of inguinal adenopathy and microfilaraemia was determined in persons aged > 14 years stratified into age groups to generate enough data to allow statistical analysis. There was a tendency toward association between prevalence of inguinal adenopathy and microfilaraemia ($r = 0.748$, $r^2 = 0.560$, $P = 0.087$).

Table 5.13 shows the frequencies of different stages of inguinal adenopathy and the median ages of the individuals having the different stages. In general, most cases of adenopathy were stage 1 (56.7%) and stage 2 (35.8%). However, there was a sharp difference in the distribution of adenopathy between males and females. Of the 45 males with inguinal adenopathy 18 (40.0%) and 23 (51.1%) were in stages 1 and 2, respectively. In contrast, of 22 females with adenopathy, 20 (90.9%) were in stage 1 and one in stage 2. Further, 4 (80.0%) of the 5 cases of stage 3 adenopathy were in males. The median age was similar for individuals with different stages of adenopathy, both in males ($P = 0.885$) and females ($P = 0.333$).

village	All		Males		Females	
	No. examined	No. (%)with adenopathy	No. examined	No. (%)with adenopathy	No. examined	No. (%) with adenopathy
Jilore	174	18 (10.3)	83	12 (14.5)	91	6 (6.6)
Marikano	193	15 (7.8)	87	9 (10.3)	106	6 (5.7)
Magongoloni	201	19 (9.5)	82	11 (13.4)	119	8 (6.7)
Mkondoni	214	15 (7.0)	96	13 (13.5)	118	2 (1.7)
All	782	67 (8.6)	348	45 (12.9)	434	22 (5.1)

Table 5.11: Baseline prevalence rates of inguinal adenopathy in all persons examined by village. Males had a significantly higher prevalence rate of adenopathy than females.

*Chi-square test

Age category (years)	All		Males		Females	
	No. examined	No. (%)with adenopathy	No. examined	No. (%)with adenopathy	No. examined	No. (%) with adenopathy
5-10	214	8 (3.7)	107	5 (4.7)	107	3 (2.8)
11-20	198	16 (8.1)	105	16 (15.2)	93	0 (0.0)
21-40	201	16 (8.0)	60	8 (13.3)	141	8 (5.7)
> 40	169	27 (16.0)	76	16 (21.1)	93	11 (11.8)
All	782	67 (8.6)	348	45 (12.9)	434	22 (5.1)

Table 5.12: Age-specific prevalence rates of inguinal adenopathy during baseline survey in 2002.

The prevalence of inguinal adenopathy increased with age in males and females.

Stage of inguinal adenopathy	All persons		Males		Females	
	n	Median age (range), years	n	Median age (range), years	n	Median age (range), years
1-Visible, ≤ 3 cm	38	28 (5-80)	18	23 (7-69)	20	44 (5-80)
2-More than 3 cm	24	23 (6-79)	23	25 (9-79)	1	6
3-"Hanging groin"	5	38 (9-80)	4	44 (9-80)	1	38
All	67	28 (5-80)	45	23 (7-80)	22	41 (5-80)
P value*		0.844		0.885		0.333

Table 5.13: Distribution of inguinal adenopathy cases by stage, age and gender. Most cases of inguinal adenopathy in females were stage 1. The median age was similar for individuals with different stages of adenopathy.

*Median test

5.4.1 Relationship between inguinal adenopathy and infection status

Table 5.14 summarizes the infection and antifilarial antibody status of persons with and without inguinal adenopathy by different tests. There was a tendency toward higher prevalence rates of active infection in individuals with inguinal adenopathy than in those without. For example, microfilariae were detected in 30.2% and 19.8% of individuals with and without adenopathy, respectively (P = 0.077). Similarly, CFA was detected in 51.3% and 36.3% of persons with and without inguinal adenopathy, respectively (P = 0.067). However, the prevalence of anti-Bm14 IgG4 was significantly higher in persons with inguinal adenopathy (88.2%) than in those without (59.7%) (P = 0.014). The prevalence of antifilarial IgG1 and IgG4, however, was similar between persons with and without inguinal adenopathy.

The proportion of persons with inguinal adenopathy who had evidence of filarial infection and antifilarial antibody response is summarized by stage of adenopathy in Table 5.15. This proportion differed among the different indicators of infection and antifilarial antibody. Of 53 persons with adenopathy tested for microfilaraemia, 16 (30.2%) had detectable microfilaraemia. Similarly, of 39 persons with adenopathy tested for CFA, 51.3% had detectable parasite antigenaemia. In addition, infection was detected in the three stages of inguinal adenopathy in similar frequencies.

Indicator of infection/exposure	Inguinal adenopathy status: Present	Absent	Total	P value*
Microfilaraemia				
No. positive/ No. examined	16/53	105/529	121/582	
% positive	30.2	19.8	20.8	0.077
Circulating filarial antigen (Og4C3)				
No. positive/ No. examined	20/39	123/339	143/378	
% positive	51.3	36.3	37.8	0.067
Antifilarial IgG1				
No. positive/ No. examined	36/39	293/339	329/378	
% positive	92.3	86.4	87.0	0.301
Antifilarial IgG4				
No. positive/ No. examined	38/39	306/339	344/378	
% positive	97.4	90.3	91.0	0.138
Anti-Bm14 IgG4				
No. positive/ No. examined	15/17	103/178	118/195	
% positive	88.2	57.9	60.5	0.014

Table 5.14: Infection and antifilarial antibody status of persons with and without inguinal adenopathy. There was a tendency toward higher prevalence rates of active infection in individuals with inguinal adenopathy compared to those without.

*Chi-square test

Indicator of infection/exposure	Stage of inguinal adenopathy 1	2	3	Total	P value*
Microfilaraemia					
No. positive/ No. examined	9/29	7/19	0/5	16/53	
% positive	31.0	36.8	0.0	30.2	0.276
Circulating antigen (Og4C3)					
No. positive/ No. examined	11/23	8/13	1/3	20/39	
% positive	47.8	61.5	33.3	51.3	0.593
Antifilarial IgG1					
No. positive/ No. examined	21/23	12/13	3/3	36/39	
% positive	91.3	92.3	100.0	92.3	0.868
Antifilarial IgG4					
No. positive/ No. examined	22/23	13/13	3/3	38/39	
% positive	95.7	100.0	100.0	97.4	0.700
Anti-Bm14 IgG4					
No. positive/ No. examined	8/10	6/6	1/1	15/17	
% positive	80.0	100.0	100.0	88.2	0.452

Table 5.15: Infection and antifilarial antibody status for persons with inguinal adenopathy by stage of disease. Infection was detected in the three stages of inguinal adenopathy in similar frequencies.

*Chi-square test

5.5 Other clinical manifestations

Two males (39 years and 44 years) had penile lymphoedema. Lymphoedema of the arms was detected in 6 individuals, with two of the cases being bilateral. Of the 6 cases of lymphoedema of the arm, 5 were in males. All the individuals with penile or arm lymphoedema also had other clinical manifestations of bancroftian filariasis. The youngest person with lymphoedema of the arm was a 28 years old male who had bilateral involvement. The two males with penile lymphoedema also had scrotal and leg lymphoedema. Among the 6 individuals with lymphoedema of the arm, 5 had leg lymphoedema and the one without leg lymphoedema had hydrocele.

CHAPTER 6 - IMPACT OF MASS DRUG ADMINISTRATION ON IMMUNOPARASITOLOGIC INDICATORS OF BANCROFTIAN FILARIASIS

Based on models that estimate the reproductive lifespan of *W. bancrofti* to be approximately five years it was predicted that four to six annual rounds of MDA with treatment coverage of > 80% might reduce microfilaraemia to levels that could interrupt transmission (Plaisier *et al.*, 1998; Norman *et al.*, 2000). The GPELF recommends the use of microfilaraemia and CFA detection by the ICT test to monitor the impact of MDA on infection (WHO, 2000). However, both microfilaraemia and antigenaemia develop from months to years after exposure, thus reducing their utility to detect low levels of infection or recrudescence of transmission (Weil *et al.*, 1987; More and Copeman, 1990; Weil *et al.*, 1997). Detection of microfilaraemia particularly at low levels after implementation of intervention programmes is of limited sensitivity. Therefore, it is important to explore the suitability of other tests in monitoring of filariasis elimination programmes.

Development of antifilarial antibody responses is a characteristic feature of infection with filarial parasites that can be exploited to develop tools to monitor the progress of filariasis elimination programmes under the GPELF. By providing cumulative measure of exposure to filarial infection, antibody assays may circumvent many of the limitations of microfilaria detection and the ICT test (Lammie *et al.*, 2004). Antifilarial IgG1 responses have been proposed to be related to exposure to filarial larvae (Lammie *et al.*, 1998), while antifilarial IgG4 responses have been shown to correlate with active filarial infection (Kwan-Lim *et al.*, 1990; Lammie *et al.*, 1998; Wamae *et al.*, 1998). These findings suggest that antifilarial IgG1 and IgG4

may be used as additional tools when monitoring elimination programmes to collect important information regarding exposure and active infection.

Evaluation of the impact of intervention programmes on the intensity of transmission of lymphatic filariasis has traditionally been based on dissection of vector mosquitoes to determine the presence of infective filarial larvae (Ramaiah *et al.*, 2003; Richards *et al.*, 2005). When the prevalence of microfilaraemia is greatly reduced after mass treatment, however, the use of mosquito dissection for monitoring of elimination programmes becomes too laborious and expensive because thousands of mosquitoes have to be collected and dissected in order to demonstrate a significant decline the prevalence of infection (Burkot and Ichimori, 2002). Since the interruption of transmission of filarial parasites is the primary goal of the filariasis elimination programmes, the availability of tools to monitor the presence or absence of the parasites in the vector mosquitoes is a vital requirement (Chadee *et al.*, 2002). Previous studies have demonstrated that detection of parasite DNA in mosquitoes by PCR assay is a sensitive and specific test to detect mosquitoes infected with *W. bancrofti* and thus is a potentially powerful new tool for monitoring of filariasis control programmes (Fischer *et al.*, 1999; Farid *et al.*, 2001). Further, screening of pools of mosquitoes by PCR assay has been shown to be a rapid tool that could be used to monitor the success of elimination programs (Bockarie *et al.*, 2000; Farid *et al.*, 2001; Goodman *et al.*, 2003). The term xenomonitoring is used when the PCR assay is applied to test blood-engorged vector mosquitoes for parasite DNA (WHO, 2002b).

The GPELF has two principal goals: to interrupt transmission of infection, and to alleviate and prevent both the suffering and the disability caused by the disease

(Ottesen, 2000). The interruption of transmission may be achieved by reducing the levels of microfilariae in endemic communities through annual mass treatment using DEC or ivermectin plus albendazole (Molyneux and Zagaria, 2002). Efficient monitoring of *W. bancrofti* infection is especially important as the prevalence and levels of the infection decline due to the effect of widespread mass treatment with antifilarial drugs under GPELF. The present study was designed to assess the impact of two annual rounds of MDA of DEC and albendazole on bancroftian filariasis in an endemic area in the northern coast of Kenya. In an effort to gain indepth information on the impact of mass treatment on lymphatic filariasis different epidemiological indicators of infection and exposure were used. The different parameters determined include, microfilaraemia, antigenaemia, antifilarial antibody responses and xenomonitoring of vector mosquitoes for presence of parasite DNA.

Kenya is unique from the rest of sub-Saharan Africa because DEC/albendazole is the treatment regimen being used for elimination of lymphatic filariasis as onchocerciasis and loiasis are not endemic. Diethylcarbamazine is not recommended for use in areas which lymphatic filariasis is co-endemic with onchocerciasis and loiasis because it can cause severe (sometimes fatal) side effects. Successful interruption of transmission of *Onchocerca volvulus* by *Simulium* flies in Kenya through vector control by means of dichloro-diphenyl-trichloroethane (DDT) applications was demonstrated in 1949 (Roberts *et al.*, 1967). In addition to clearance of microfilariae, DEC has some activity against adult worms (Ottesen, 1985; Noroes *et al.*, 1997), which are the source of microfilariae.

6.1 Treatment coverage

Figure 6.1 shows treatment coverage in 2002 in persons aged > 4 years in the general population (n = 2428) and the registered follow-up group (n = 797). In all the villages treatment coverage was higher in the follow-up group than in the general population. Overall, treatment coverage was significantly higher in the follow-up group (83.4%) than in the general population (64.4%; $P < 0.001$). The treatment coverage ranged from 51.9% to 76.3% and 64.9% to 92.7% in the general population and the follow-up group, respectively.

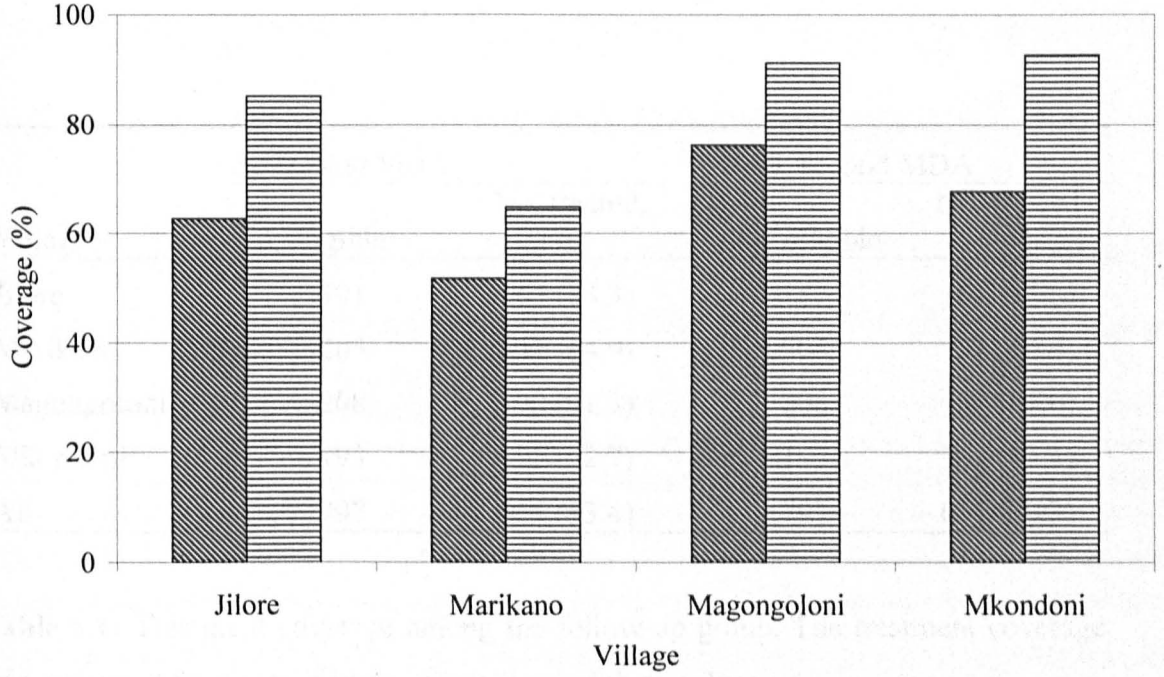

Figure 6.1: Treatment coverage in general population (▨) and follow-up group (▤). The treatment coverage was significantly higher in the follow-up group compared to the general population.

Table 6.1 summarizes treatment coverage among eligible persons in the follow-up group in 2002 and 2003 in the 4 study communities. The overall coverage in 2002 and 2003 in the follow-up group was > 80.0% for 3 of the 4 villages. Marikano village, however, had treatment coverage < 80.0% during the two MDAs.

The pattern of treatment among the follow-up group in the 4 villages is summarized in Table 6.2. Of 797 persons in the follow-up group, 581 individuals (72.9%) received treatment during the two MDAs. A total of 140 persons (17.6%) received treatment once during the two MDAs. However, 90.5% of the individuals in the follow-up group received at least one treatment. Of the 797 individuals, however, 76 (9.5%) persons did not get treatment at all during the two MDAs.

	2002, first MDA		2003, second MDA	
Village	No. eligible	No. treated (%)	No. eligible	No. treated (%)
Jilore	191	163 (85.3)	187	162 (86.6)
Marikano	205	133 (64.9)	205	133 (64.9)
Magongoloni	208	190 (91.3)	208	181 (87.0)
Mkondoni	193	179 (92.7)	193	161 (83.4)
All	797	665 (83.4)	793	637 (80.3)

Table 6.1: Treatment coverage among the follow-up group. The treatment coverage was estimated from interviews conducted on adult members.

Village	N	No. of treatments received during two MDAs			
		none	Once	Twice	At least once
Jilore	191	12 (6.3)	33 (17.3)	146 (76.4)	179 (93.7)
Marikano	205	47 (22.9)	50 (24.4)	108 (52.7)	158 (77.1)
Magongoloni	208	8 (3.8)	29 (13.9)	171 (82.2)	200 (96.2)
Mkondoni	193	9 (4.7)	28 (14.5)	156 (80.8)	184 (95.3)
All	797	76 (9.5)	140 (17.6)	581 (72.9)	721 (90.5)

Table 6.2: Treatment patterns among the follow-up group during two MDAs. Evaluation of treatment patterns is based on individuals aged > 4 years eligible for treatment. Numbers in parentheses indicate percent in group.

6.2 Impact of MDA on microfilaraemia

6.2.1 Changes in prevalence and intensity of microfilaraemia

Post-treatment night blood surveys to assess the impact of MDA on microfilaraemia were conducted one year after each mass drug administration (MDA). A total of 740, 468 and 493 individuals were tested for microfilaraemia in 2002 (baseline survey), 2003 (one year after first MDA) and 2004 (one year after second MDA), respectively. The overall prevalence of microfilaraemia in 2002, 2003 and 2004 was 20.5% (range 17.7-22.9%), 11.3% (range 8.6-13.6%) and 7.1% (range 4.2-9.0%), respectively. The changes in overall prevalence of microfilaraemia in the 4 study communities are shown in Figure 6.2. In 2003, one year after the first MDA, the overall prevalence of microfilaraemia decreased by 44.9% and was significantly

lower than at baseline ($P < 0.001$). The overall prevalence of microfilaraemia had decreased significantly by 65.4% in 2004 after two rounds of MDA ($P < 0.001$).

The change in prevalence of microfilaraemia among persons that were tested at baseline was similar to the change in overall prevalence. Of the 740 persons tested for microfilaraemia in 2002 there were 439 (59.3%) and 456 (61.6%) individuals that were retested in 2003 and 2004, respectively. The prevalence of microfilaria among the 439 and 456 persons that had been tested at baseline decreased by 44.3% (from 20.5% to 11.4%) and 62.4% (from 20.5% to 7.7%) in 2003 and 2004, respectively.

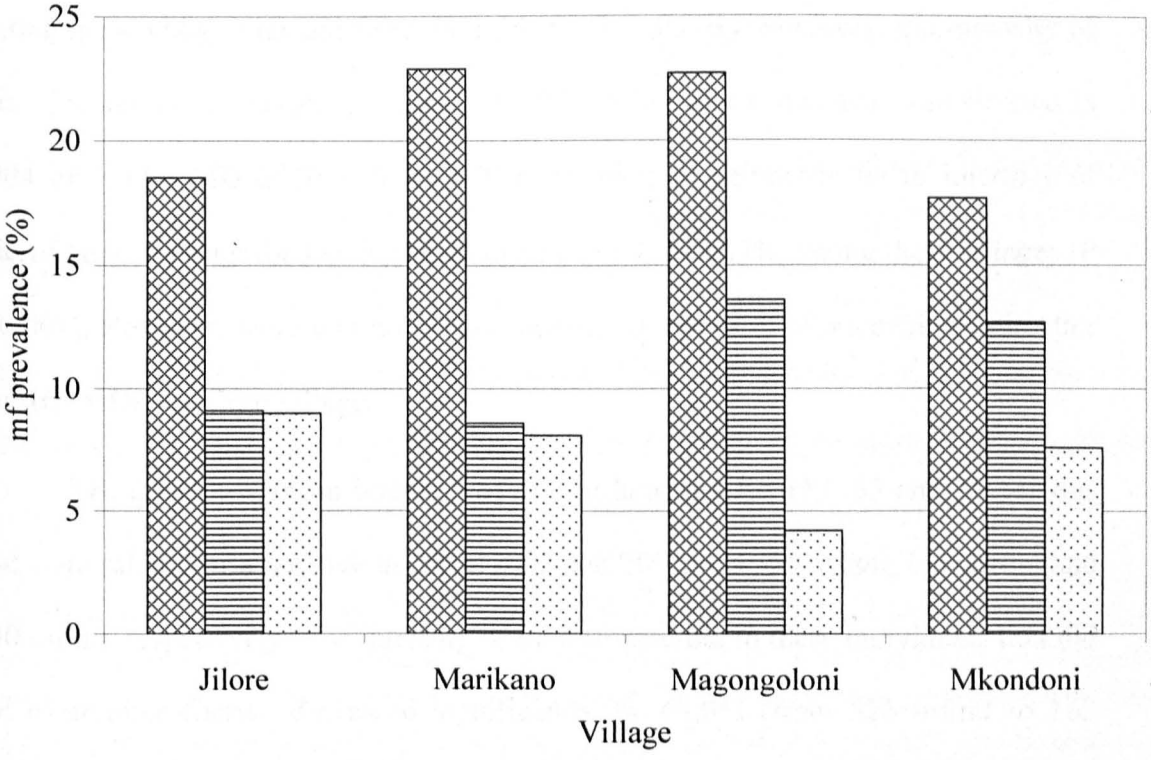

Figure 6.2: Changes in prevalence of microfilaraemia by village after two MDAs. The bars represent microfilaria (mf) prevalence during pre-treatment in 2002 (▨), after one MDA in 2003 (▤) and after two MDAs in 2004 (□). The prevalence of microfilaraemia decreased significantly after mass treatment.

Of 152 persons that were microfilaria positive at the baseline survey in 2002 there were 90 and 104 individuals retested for microfilaraemia in 2003 and 2004, respectively. The change in intensity of microfilaraemia among these individuals that were microfilaria positive at baseline survey is summarized in Figure 6.3. The geometric mean intensity of microfilaraemia in the 90 microfilaria positive persons at baseline that were retested in 2003 decreased significantly by 91.8% ($P < 0.001$; from

526 mf/ml to 43 mf/ml). The decrease in geometric mean intensity of microfilaraemia among the 4 villages ranged from 89.4-94.9% ($P < 0.001$). Similarly, the intensity of microfilaraemia decreased significantly by 95.8% in 104 persons that were retested in 2004 after two MDAs ($P < 0.001$). The decrease in geometric mean intensity of microfilaraemia after the two MDAs ranged from 92.3-97.7% among the 4 villages ($P < 0.001$). However, there was no further decrease in intensity of microfilaraemia after the first MDA in Jilore village.

The geometric mean intensity of microfilaraemia for 152, 53 and 35 persons that were microfilaria positive in 2002, 2003 and 2004 was 526 mf/ml, 163 mf/ml and 130 mf/ml, respectively. The intensity of microfilaraemia in these individuals that did not clear microfilariae decreased significantly by 69.0% (from 526 mf/ml to 163 mf/ml) after the first round of MDA ($P < 0.001$). The administration of the second round of treatment further decreased the geometric mean intensity of microfilaraemia in 35 microfilaraemic individuals by 75.3% compared to baseline ($P < 0.001$; from 526 mf/ml to 130 mf/ml). Of the 35 persons that were still microfilaria positive in 2004, 23 and 10 individuals had received treatment twice and once, respectively. Two microfilaraemic persons in 2004 had not received treatment during the two MDAs.

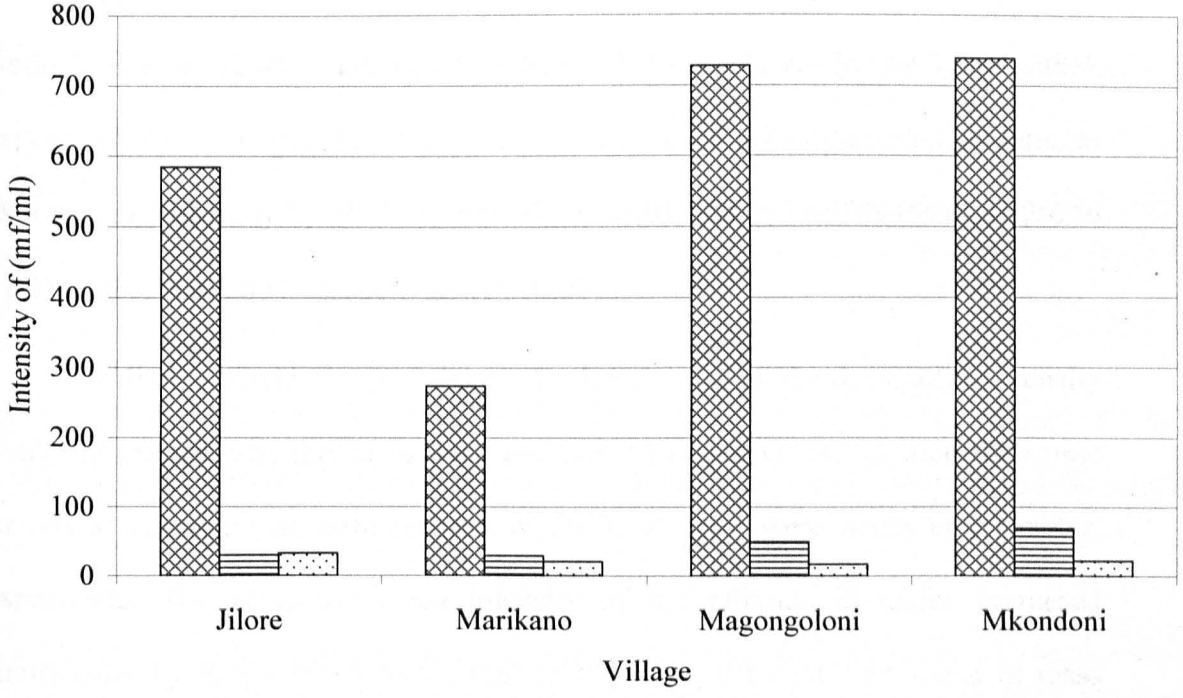

Figure 6.3: Changes in geometric mean intensity of microfilaraemia (mf/ml) after two MDAs. The bars represent microfilaria density during pre-treatment in 2002 (▨), after one MDA in 2003 (▤), after two MDAs in 2004 (□). The intensity of microfilaraemia decreased significantly after mass treatment.

6.2.2 Changes in microfilaraemia by gender and age

Similarly, as during the baseline survey, there were more females than males tested for microfilaraemia in 2003 and 2004. Of 468 persons tested for microfilaraemia in 2003 there were 210 (44.9%) males and 258 (55.1%) females. Similarly, of 493 individuals tested for microfilaraemia in 2004 there were 226 (45.8%) males and 267 (54.2%) females. The change in overall prevalence of microfilaraemia showed a similar trend between males and females over the study

period. The prevalence of microfilaraemia in males decreased significantly by 40.7% (from 20.9% to 12.4%) and 66.0% (from 20.9% to 7.1%) in 2003 and 2004, respectively ($P < 0.001$). Similarly, the prevalence of microfilaraemia in females decreased significantly by 48.0% (from 20.2% to 10.5%) and 64.9% (from 20.2% to 7.1%) in 2003 and 2004, respectively ($P < 0.001$).

Similarly, as with the prevalence of microfilaraemia, the decrease in intensity of microfilaraemia was similar between males and females. Of the 90 microfilaraemic persons at baseline that were retested in 2003, 45 each were males and females, respectively. The geometric mean intensity of microfilariae in males decreased significantly by 92.8% (from 543 mf/ml to 39 mf/ml) after the first round of mass treatment ($P < 0.001$). Similarly, the intensity of microfilariae in females also decreased significantly by 90.8% (from 512 mf/ml to 47 mf/ml) after the first MDA. Of 104 microfilaraemic individuals at baseline that were retested in 2004, 49 and 55 were males and females, respectively. In 2004, the geometric mean intensity of microfilariae had decreased significantly to 21 mf/ml (96.1% decrease) and 23 mf/ml (95.5% decrease) in males and females, respectively ($P < 0.001$).

Figure 6.4 summarizes changes in overall prevalence of microfilaraemia among children (aged < 16 years), young adults (aged 16-30 years) and mature adults (aged > 30 years). Children had lower prevalence of microfilaraemia compared to adults at all the 3 surveys. The overall prevalence of microfilaraemia in children, young adults and mature adults decreased significantly by 45.8% (from 10.7% to 5.8%), 40.5% (from 22.2% to 13.2%) and 36.1% (31.3% to 20.0%), respectively after the first round of MDA ($P < 0.001$). The overall decline in prevalence of microfilaraemia in children, young adults and mature adults in 2004, after two MDAs,

was 77.6% (from 10.7% to 2.4%), 60.4% (from 22.2% to 8.8%) and 56.9% (from 31.3% to 13.5%), respectively ($P < 0.001$). The prevalence of microfilaraemia after the two MDAs was significantly lower in children than in young ($P = 0.025$) and mature adults ($P < 0.001$). The prevalence rates of microfilaraemia, however, were similar in young and mature adults ($P = 0.264$).

Age-specific changes in geometric mean intensity of microfilaraemia among persons that were microfilaria positive at baseline are summarized in Figure 6.5. The intensity of microfilariae decreased significantly by 90.8% (426 mf/ml to 39 mf/ml), 85.9% (263 mf/ml to 37 mf/ml) and 94.1% (826 mf/ml to 49 mf/ml) in children, young adults and mature adults, respectively after the first MDA ($P < 0.001$). There was further decrease in geometric mean intensity of microfilaraemia in all the 3 age groups in 2004 after two MDAs. The decrease in intensity of microfilaraemia in 2004 was 96.0% (from 426 mf/ml to 17 mf/ml), 91.3% (from 263 to 23 mf/ml) and 97.0% (from 826 mf/ml to 25 mf/ml) in children, young adults and mature adults, respectively ($P < 0.001$).

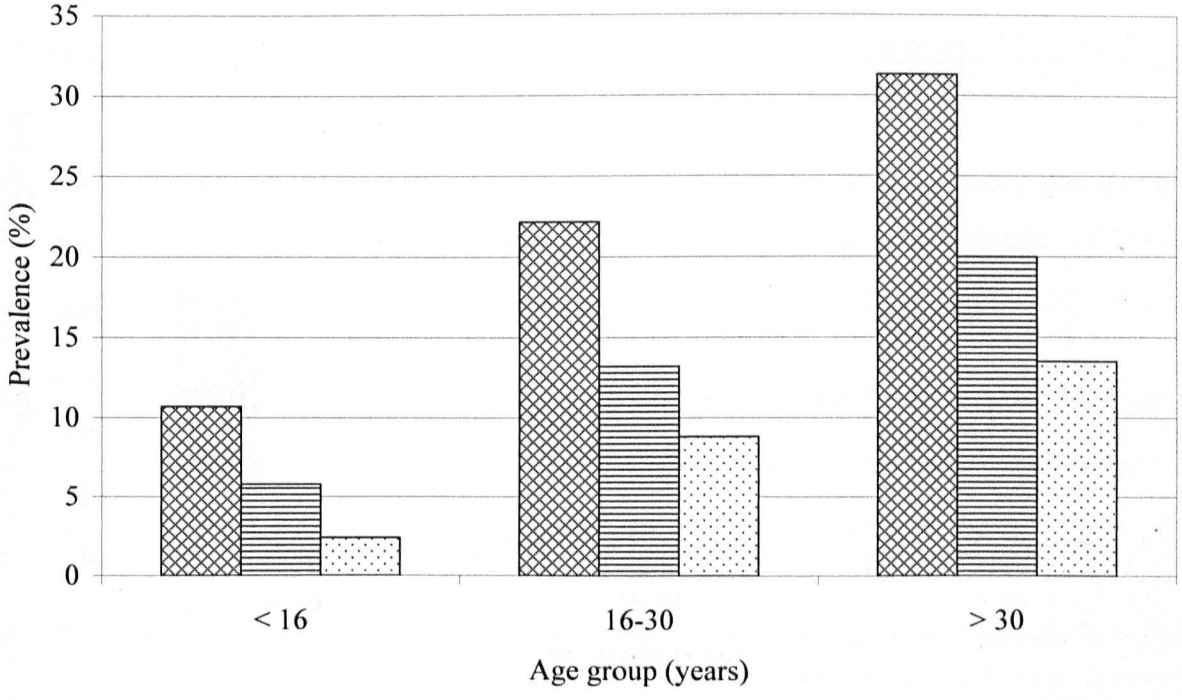

Figure 6.4: Age-specific changes in prevalence of microfilaraemia. The bars represent microfilaria density during pre-treatment in 2002 (▨), after one MDA in 2003 (▤), after two MDAs in 2004 (▫). The prevalence of microfilaraemia decreased significantly in all age groups after mass treatment.

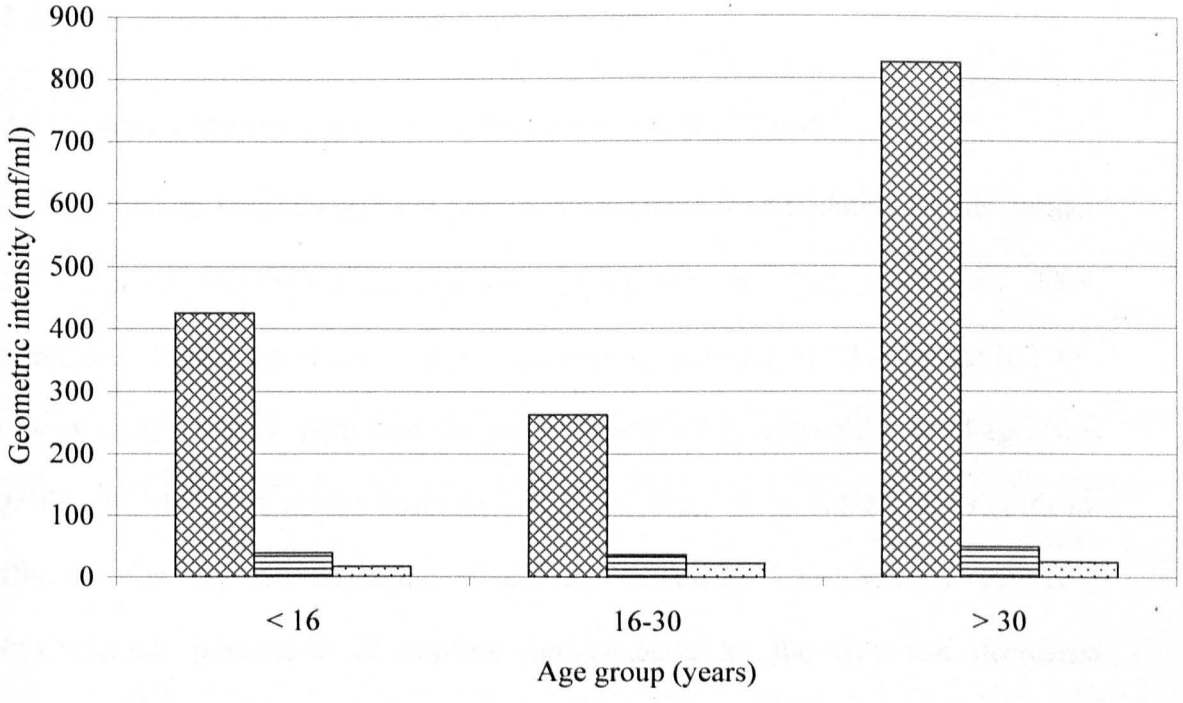

Figure 6.5: Age-specific changes in intensity of microfilaraemia during MDA. The bars represent microfilaria density during pre-treatment in 2002 (▨), after one MDA in 2003 (▤), after two MDAs in 2004 (□). The intensity of microfilaraemia decreased significantly in all age groups after mass treatment.

6.3 Impact of MDA on circulating filarial antigen

6.3.1 Changes in overall prevalence of antigenaemia by ICT test

A total of 748, 469 and 495 persons were tested for circulating filarial antigen (CFA) by the immunochromatographic (ICT) test in 2002, 2003 and 2004, respectively. Figure 6.6 shows changes in overall prevalence of CFA by the ICT test in the 4 study villages. Although the prevalence of CFA among the 4 villages was significantly different at the baseline survey in 2002 ($P = 0.016$), no significant difference was observed among the villages in 2003 and 2004 post-MDA surveys ($P > 0.05$). Overall prevalence of parasite antigenaemia by the ICT test decreased significantly by 22.3% from 35.4% (range 27.5-42.4%) in 2002 to 27.5% (range 25.0-31.8%) in 2003, one year after the first MDA ($P = 0.004$). There was further decline in overall prevalence of CFA by 43.5% from 35.4% in 2002 to 20.0% (range 16.9-21.7%) in 2004 one year after the second MDA ($P < 0.001$).

The change in prevalence of CFA among persons that were tested at baseline was similar to change in overall prevalence. Of the 748 persons tested for CFA (by ICT test) in 2002 there were 446 (59.6%) and 467 (62.4%) individuals that were retested in 2003 and 2004, respectively. The prevalence of CFA among the 446 and 467 persons that had been tested at baseline decreased by 18.4% ($P = 0.021$; from 35.4% to 28.9%) and 40.1% ($P < 0.001$; from 35.4% to 21.2%) in 2003 and 2004, respectively.

6.3.2 Changes in antigenaemia (by ICT test) by gender and age

The prevalence of CFA by the ICT test decreased by 20.1% (P = 0.073; from 37.9% to 30.3%) and 24.8% (P = 0.023; 33.5% to 25.2%) in males and females, respectively, after the first MDA. After the second MDA, the prevalence rates of CFA by the ICT test in males and females decreased by 38.0% (P < 0.001; from 37.9% to 23.5%) and 49.0% (P < 0.001; from 33.5% to 17.1%), respectively. Further, the prevalence of CFA was slightly lower in females than in males at three time points.

Age-specific changes in overall prevalence of CFA by the ICT test are shown in Figure 6.7. After the first round of MDA, the prevalence of CFA by the ICT test in children, young adults and mature adults decreased by 26.7% (P = 0.069; from 23.6% to 17.3%), 16.4% (P = 0.345; 34.2% to 28.6%) and 11.0% (P = 0.293; from 50.8% to 45.2%), respectively. After the second MDA the prevalence rates of circulating filarial antigen among children, young adults and mature adults declined by 53.8% (P < 0.001; from 23.6% to 10.9%), 38.9% (P = 0.023; from 34.2% to 20.9%) and 33.1% (P = 0.001; from 50.8% to 34.0%), respectively. The prevalence of antigenaemia was significantly lower in children than in young adults and mature adults at the three time points (P < 0.05). Similarly, the prevalence of antigenaemia in young adults was significantly lower than in mature adults at the three time points (P < 0.05).

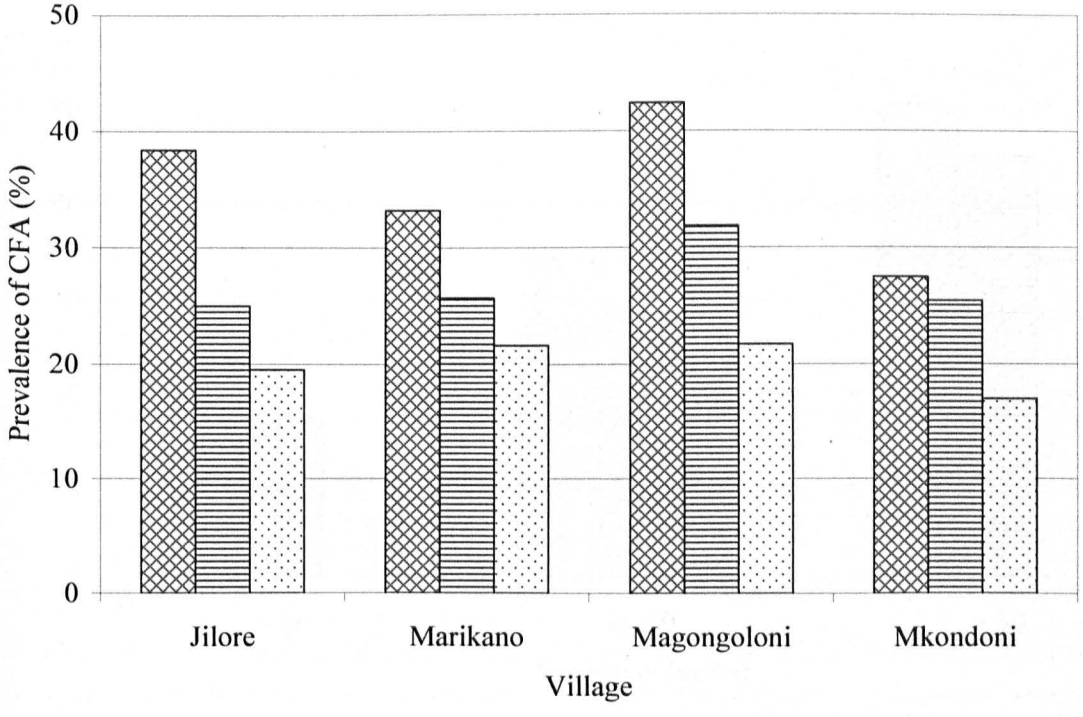

Figure 6.6: Changes in overall prevalence of circulating filarial antigen (by the ICT test). The bars represent prevalence of antigenaemia during pre-treatment in 2002 (▨), after one MDA in 2003 (▤), after two MDAs in 2004 (▫). The prevalence of antigenaemia decreased significantly in all villages after mass treatment.

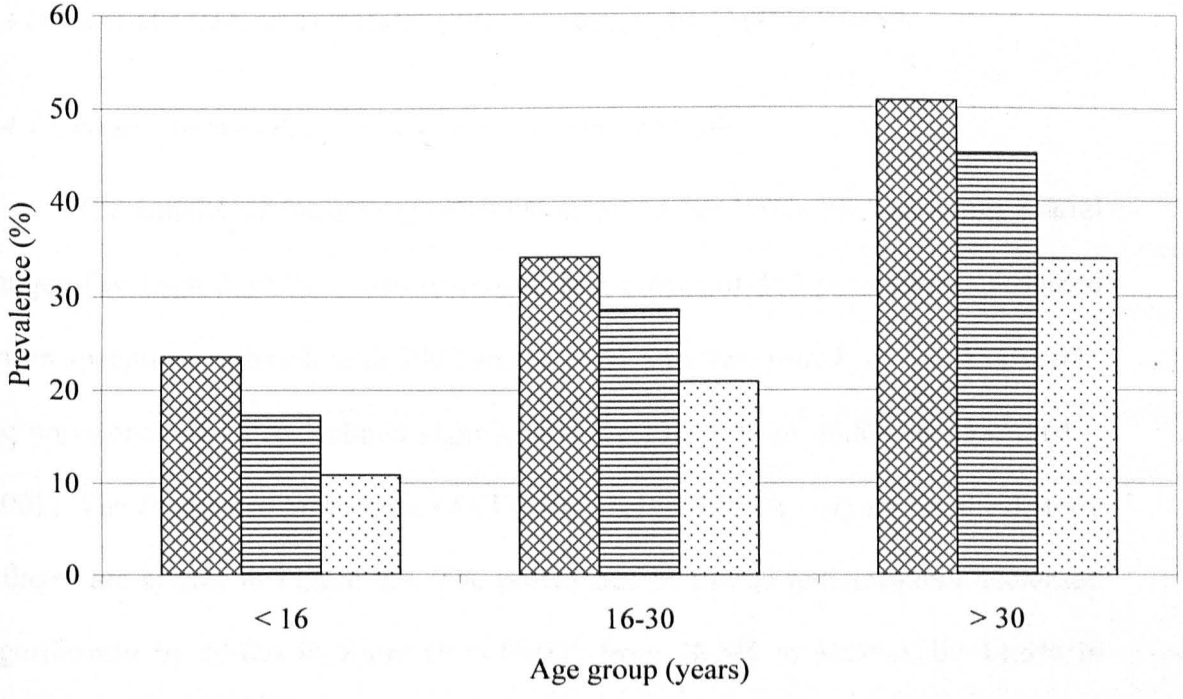

Figure 6.7: Age-specific changes in prevalence of CFA by the ICT test. The bars represent prevalence of CFA during pre-treatment in 2002 (▨), after one MDA in 2003 (▤) and after two MDAs in 2004 (□). The prevalence of CFA decreased significantly in all age groups after mass treatment.

6.4 Impact of MDA on circulating filarial antigen by Og4C3 ELISA

6.4.1 Changes in prevalence and intensity of antigenaemia

The impact of mass drug administration on the levels of circulating filarial antigen (by Og4C3 ELISA) was evaluated in a cohort of 463 persons that provided serum specimens at baseline in 2002 and in 2004 after two rounds of MDA. Overall, the prevalence of CFA declined significantly by 13.2% from 40.8% to 35.4% ($P < 0.001$). The changes in prevalence of CFA (by Og4C3 ELISA test) among the 4 study villages are shown in Figure 6.8. The prevalence of filarial antigenaemia decreased significantly by 15.0% in Jilore ($P = 0.031$; from 38.5% to 32.7%), by 14.5% in Magongoloni ($P = 0.012$; 44.9% to 38.4%) and by 14.6% in Mkondoni ($P = 0.031$; 36.0% to 30.7%). However, the decrease in prevalence of CFA in Marikano village (10.9%) was not significant ($P = 0.063$; from 43.0% to 38.3%). Of 75 persons that had changed from antigen-positive to antigen-negative by 2004 when assessed by the ICT test, 61 (81.3%) were still positive by the Og4C3 ELISA test.

The geometric mean intensity of CFA in a group of 189 persons that were antigen-positive at baseline decreased significantly by 90.3% from 20,945.7 units in 2002 to 2,026.4 units in 2004 ($P < 0.001$). The changes in GMI of parasite antigenaemia in this group among the 4 study villages are summarized in Figure 6.9. The decrease in intensity of CFA among the 4 villages ranged from 80.5% to 95.9% and was significant in all the villages ($P < 0.001$). The decrease in CFA was most dramatic in Magongoloni village (95.9%; from 52,917.9 units to 2,163.9 units), which had the highest intensity in 2002.

6.4.2 Changes in antigenaemia (by Og4C3 ELISA) by gender and age

Of the 463 individuals tested for CFA by the Og4C3 test in 2002 and 2004 there were 206 males and 257 females. The prevalence of CFA in males decreased significantly by 14.6% from 43.0% to 36.7% (P = 0.001). Similarly, the prevalence of CFA decreased significantly in females by 12.0% from 39.1% to 34.4% (P = 0.002). The intensity of CFA in males decreased significantly by 93.1% from 24,895.2 units to 1,708.1 units (P < 0.001). Similarly, the intensity of CFA in females decreased significantly by 86.9% from 17,960.8 units to 2,359.3 units (P < 0.001).

Age-specific changes in prevalence of CFA by the Og4C3 ELISA test are summarized in Figure 6.10. The decrease in prevalence of CFA was greater in children aged < 16 years than in young and mature adults. In children, the prevalence of CFA decreased significantly by 21.8% from 28.5% to 22.3% (P < 0.001). The prevalence of CFA in young adults decreased by 14.2% from 40.2% to 34.5%, but the decrease was not significant due to the small number of positive individuals in this group (P = 0.125). The lowest decrease in prevalence of CFA was observed in mature adults (7.8%; from 59.2% to 54.6%), but the decline was significant (P = 0.016).

Figure 6.11 represents age-specific geometric mean intensities of CFA by the Og4C3 ELISA. The intensity of CFA decreased significantly in children (94.2%, 15,716.6 units to 917.8 units), young adults (84.7%, 12,350.4 units to 1,888.3 units) and mature adults (88.4%, 31,550.8 units to 3,658.2 units) (P < 0.001). In summary, there was a dramatic decrease in the intensity of parasite antigenaemia after the two rounds of mass treatment. However, the impact of mass treatment on prevalence of parasite antigenaemia was less pronounced.

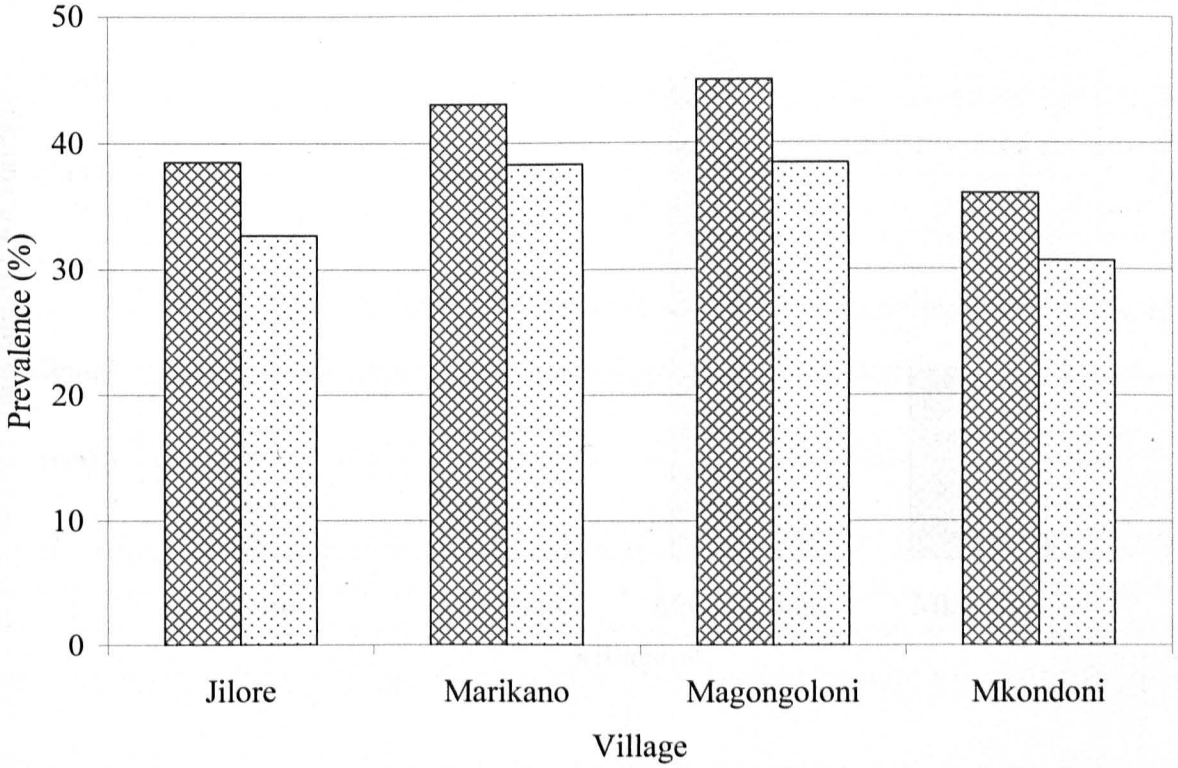

Figure 6.8: Changes in prevalence of circulating filarial antigen (CFA, by Og4C3 ELISA assay) by village. The bars represent CFA prevalence during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The decrease in prevalence of CFA was not significant in Marikano village.

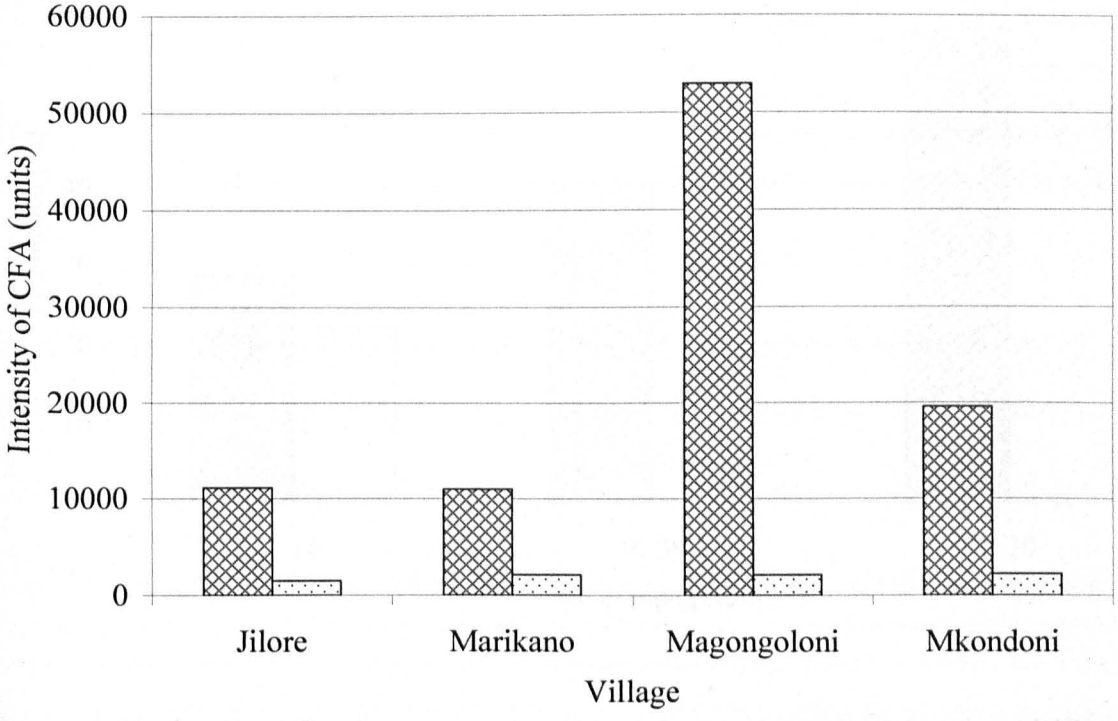

Figure 6.9: Changes in geometric intensity of circulating filarial antigen (CFA, by Og4C3 ELISA assay) among individuals that were positive at baseline by village. The bars represent intensity of antigenaemia during pre-treatment in 2002 (▨) and after two MDAs in 2004 (▫). The decrease in intensity of antigenaemia was significant in all villages.

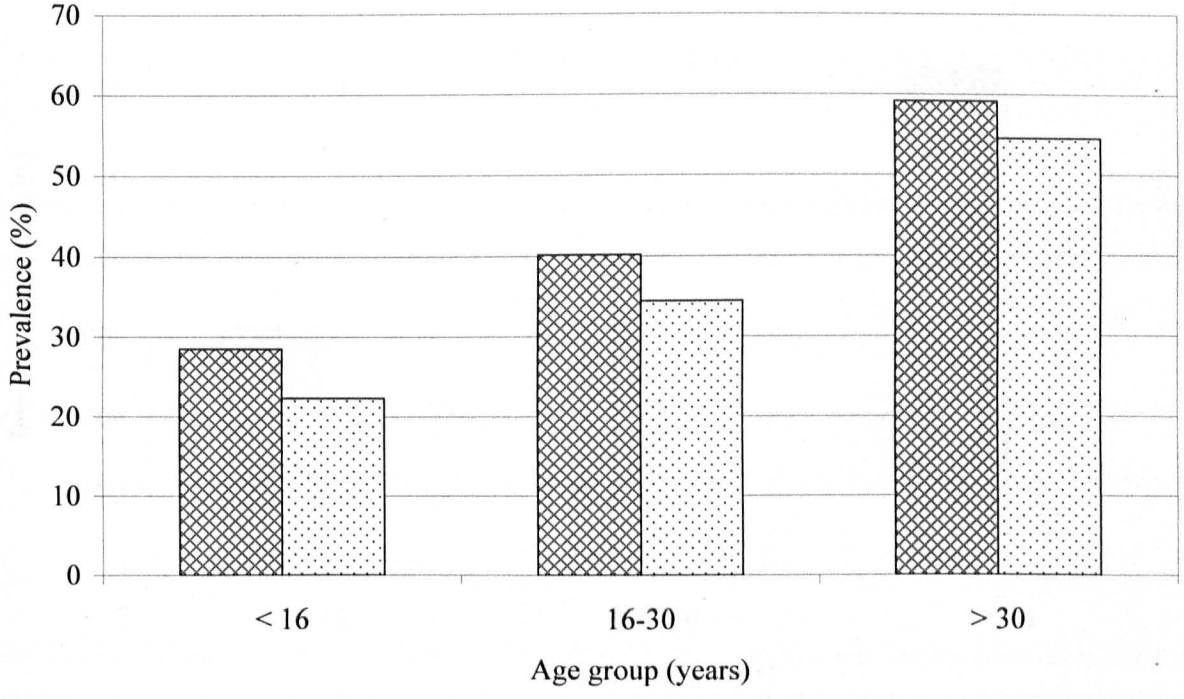

Figure 6.10: Age-specific changes in prevalence of circulating filarial antigen (CFA, by Og4C3 ELISA assay). The bars represent the prevalence of antigenaemia during pre-treatment in 2002 (⊠) and after two MDAs in 2004 (⊡). The decrease in prevalence of antigenaemia was significant in all age groups.

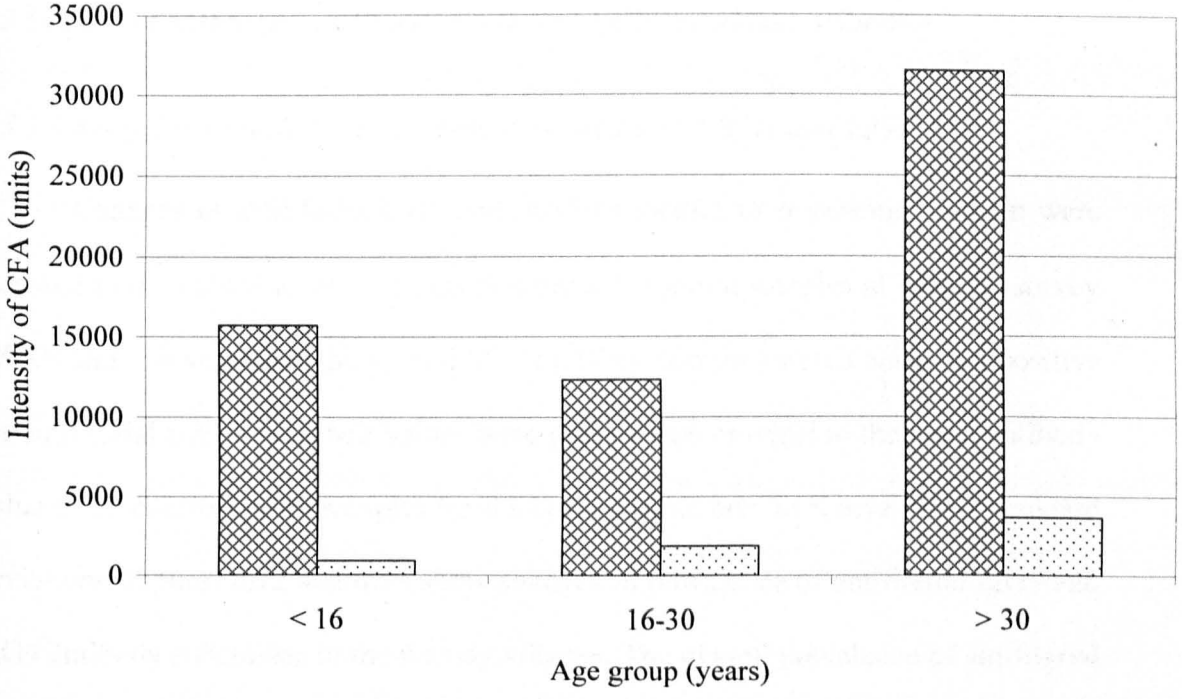

Figure 6.11: Age-specific changes in intensity of circulating filarial antigen (CFA, by Og4C3 ELISA assay) in individuals that were antigen-positive at baseline. The bars represent intensity of antigenaemia during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The decrease in intensity of antigenaemia was significant in all age groups.

6.5 Impact of MDA on antifilarial IgG1 and IgG4 subclass antibodies

6.5.1 Changes in prevalence and intensity of antifilarial IgG1 and IgG4

Changes in antifilarial IgG1 and IgG4 antibodies to *B. pahangi* antigen were assessed in the cohort of 463 persons that provided serum samples at baseline survey (2002) and one year after the second MDA (2004). Samples were considered positive for antifilarial antibody if their values were greater than or equal to the mean antibody value of 20 control serum samples from a non-endemic area in Kenya plus 2 standard deviations. Figures 6.12 and 6.13 show changes in prevalence of antifilarial IgG1 and IgG4 antibody subclasses in the 4 study villages. The overall prevalence of antifilarial IgG1 decreased significantly by 44.7% (from 86.2% to 47.7%) after the two MDAs ($P < 0.001$). The decrease in prevalence of antifilarial IgG1 was significant in all 4 villages ($P < 0.001$) and ranged from 32.6% to 58.8% among the 4 communities. The overall prevalence of antifilarial IgG4 declined by 13.3% (from 90.9% to 78.8%) after the two MDAs. The decrease in prevalence of antifilarial IgG4 ranged from 8.6% to 20.0% among the four villages and was significant in all villages ($P < 0.05$).

A total of 399 and 421 individuals were positive for antifilarial IgG1 and IgG4 antibodies, respectively at baseline. Data from these groups were used to assess the impact of mass treatment on levels of antifilarial antibodies. Figures 6.14 and 6.15 represent changes in geometric mean intensities of antifilarial IgG1 and IgG4 subclass antibodies, respectively in the 4 study villages. The geometric mean intensity of antifilarial IgG1 decreased significantly by 70.4% from 40.9 μg/ml in 2002 to 12.1 μg/ml in 2004 ($P < 0.001$). The decrease in intensity of antifilarial IgG1 ranged from 57.5% to 78.7% and was significant in all villages ($P < 0.001$). Similarly, the

geometric mean intensity of antifilarial IgG4 decreased significantly by 76.4% from 31.3 μg/ml in 2002 to 7.4 μg/ml in 2004 ($P < 0.001$). There was a significant decrease in geometric mean intensity of antifilarial IgG4 in the 4 villages ($P < 0.001$; range 70.0-81.5%).

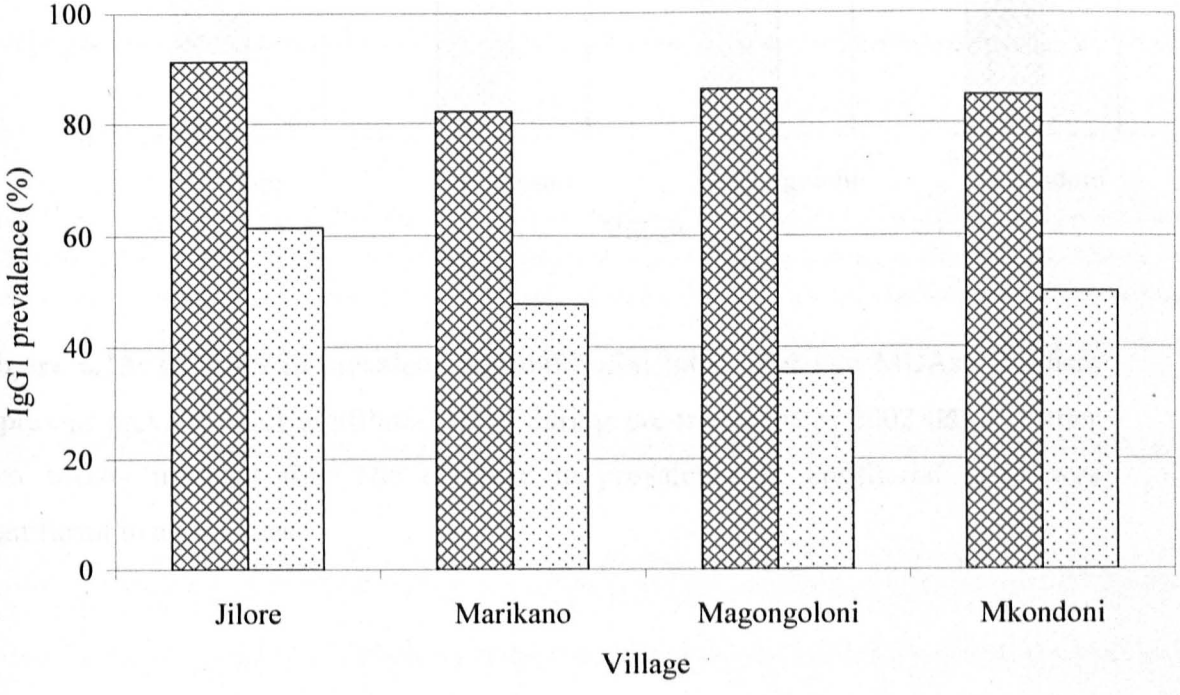

Figure 6.12: Changes in prevalence of antifilarial IgG1 after two MDAs. The bars represent prevalence of antifilarial IgG1 during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The decrease in prevalence of antifilarial IgG1 was significant in all villages.

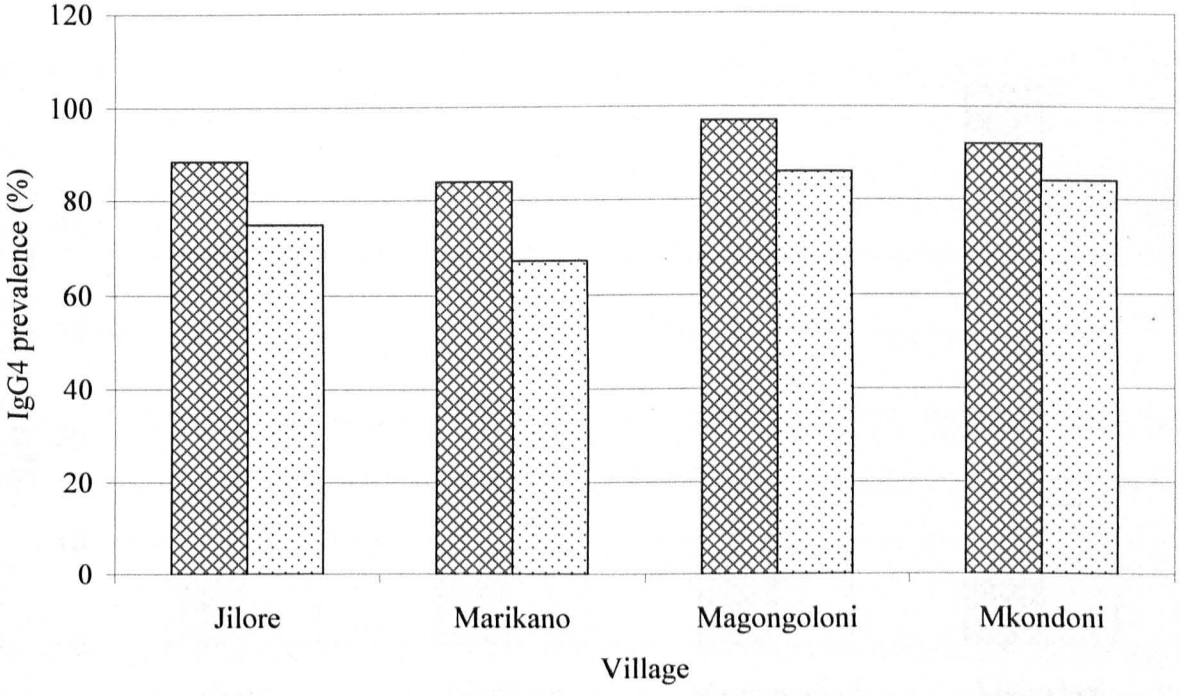

Figure 6.13: Changes in prevalence of antifilarial IgG4 after two MDAs. The bars represent prevalence of antifilarial IgG4 during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The decrease in prevalence of antifilarial IgG4 was significant in all villages.

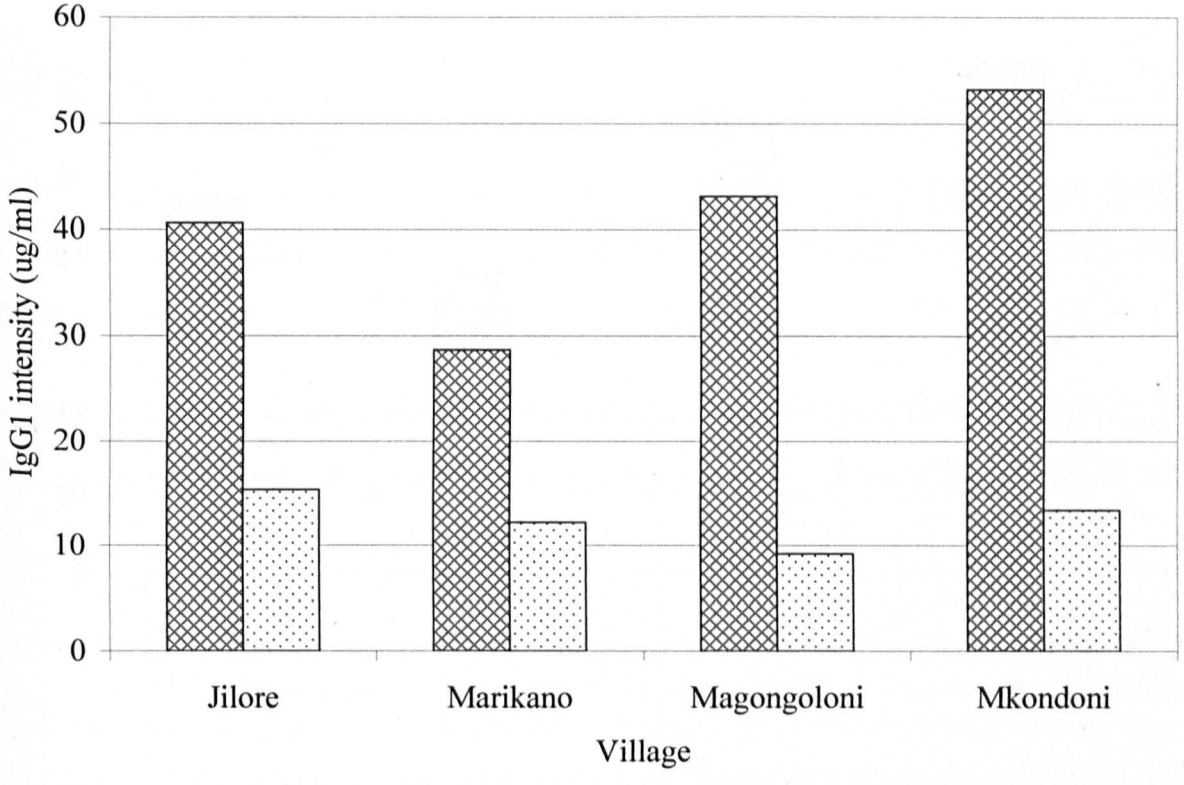

Figure 6.14: Changes in intensity of antifilarial IgG1 in persons that were positive at baseline by village. The bars represent intensity of antifilarial IgG1 during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The decrease in intensity of antifilarial IgG1 was significant in all villages.

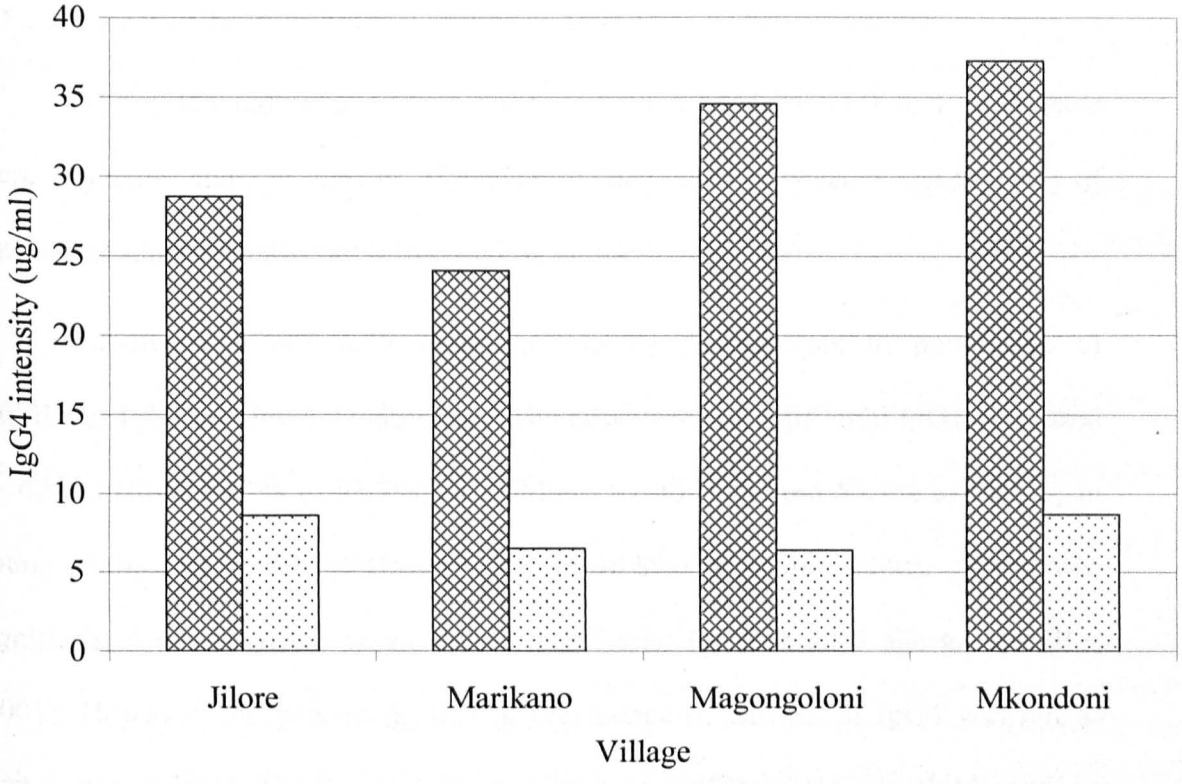

Figure 6.15: Changes in intensity of antifilarial IgG4 in persons that were positive at baseline by village. The bars represent intensity of antifilarial IgG4 during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The decrease in intensity of antifilarial IgG4 was significant in all villages.

6.5.2 Changes in antifilarial IgG1 and IgG4 antibodies by gender and age

Of the 463 individuals that were tested for antifilarial IgG1 and IgG4 there were 206 males and 257 females. However, the decline in prevalence and intensity of antifilarial antibodies was not influenced by gender.

Figures 6.16 and 6.17 represent age-specific changes in prevalence of antifilarial IgG1 and IgG4, respectively. The prevalence of antifilarial IgG1 decreased by 42.6% (from 89.3% to 51.3%) in children, by 40.5% (from 85.1% to 50.6%) in young adults and by 50.4% (from 82.2% to 40.8%) in mature adults. There was significant decrease in the prevalence of antifilarial IgG1 in all 3 age groups ($P < 0.001$). However, the percent decline in prevalence of antifilarial IgG4 was not as high as that of IgG1. The decrease in prevalence of antifilarial IgG4 in children (from 88.8% to 72.8%), young adults (93.1% to 82.8%) and mature adults (92.8% to 85.5%) was 18.0% ($P < 0.001$), 11.1% ($P = 0.012$) and 7.9% ($P = 0.013$), respectively. Although the prevalence of antifilarial IgG4 was similar among the 3 age groups at baseline, the prevalence was significantly lower in children aged < 16 years (72.8%) than in individuals aged > 30 years (85.5%) after the two MDAs ($P = 0.003$).

Figures 6.18 and 6.19 represent the age-specific changes in geometric mean intensities of antifilarial IgG1 and IgG4, respectively. There was significant decline in geometric mean intensities of antifilarial IgG1 and IgG4 antibodies across the 3 age categories. The decrease in geometric mean intensity of antifilarial IgG1 in children, young and mature adults was 73.9% (from 48.3 μg/ml to 12.6 μg/ml), 67.8% (from 43.8 μg/ml to 14.1 μg/ml) and 65.1% (from 30.1 μg/ml to 10.5 μg/ml), respectively ($P < 0.001$). As in the case of antifilarial IgG1, there was dramatic decrease in the

intensity of antifilarial IgG4. The decrease in geometric mean intensity of antifilarial IgG4 in children, young adults and adults was 79.8% (from 27.7 μg/ml to 5.6 μg/ml), 70.8% (from 32.2 μg/ml to 9.4 μg/ml) and 74.5% (from 36.8 μg/ml to 9.4 μg/ml), respectively ($P < 0.001$).

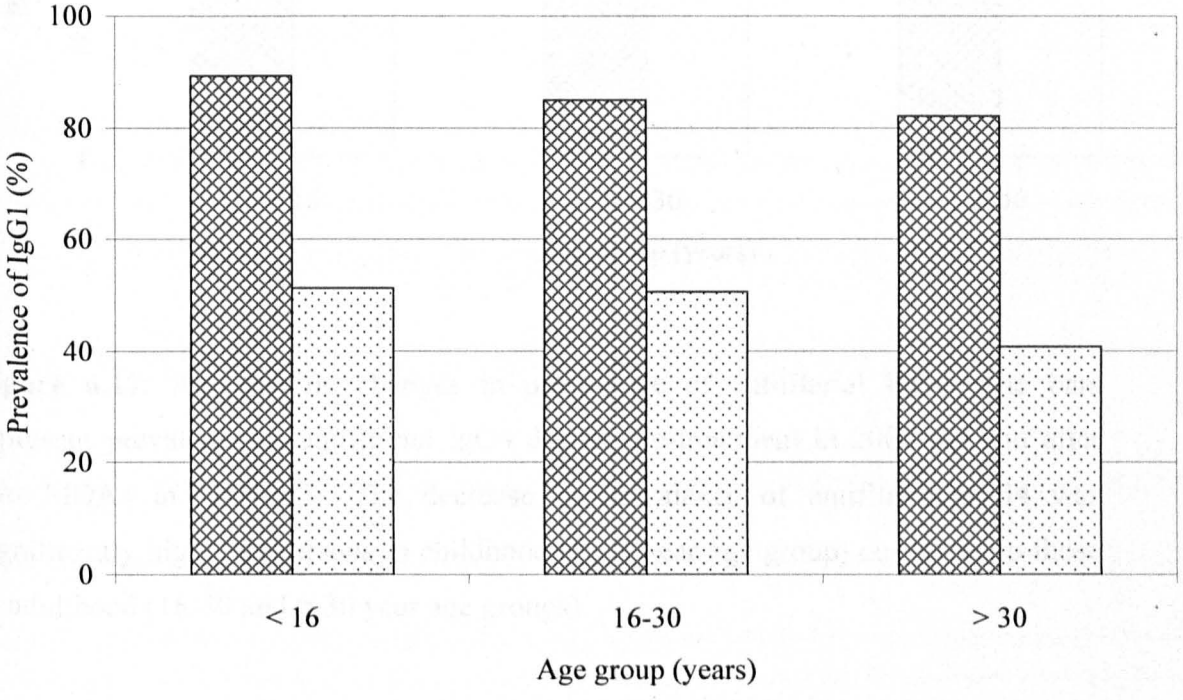

Figure 6.16: Age-specific changes in prevalence of antifilarial IgG1. The bars represent prevalence of antifilarial IgG1 during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The decrease in prevalence of antifilarial IgG1 was significant in all age groups.

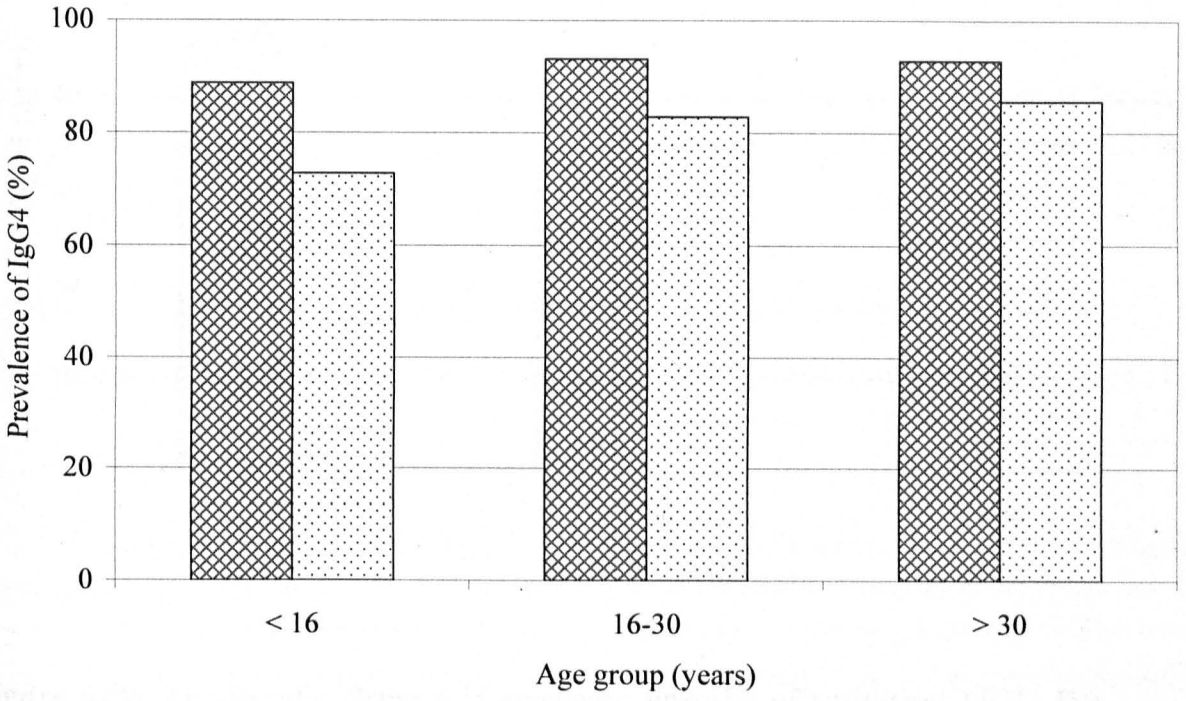

Figure 6.17: Age-specific changes in prevalence of antifilarial IgG4. The bars represent prevalence of antifilarial IgG4 during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The decrease in prevalence of antifilarial IgG4 was significantly higher in persons in childhood (< 16 year age group) compared to those in adulthood (16-30 and > 30 year age groups).

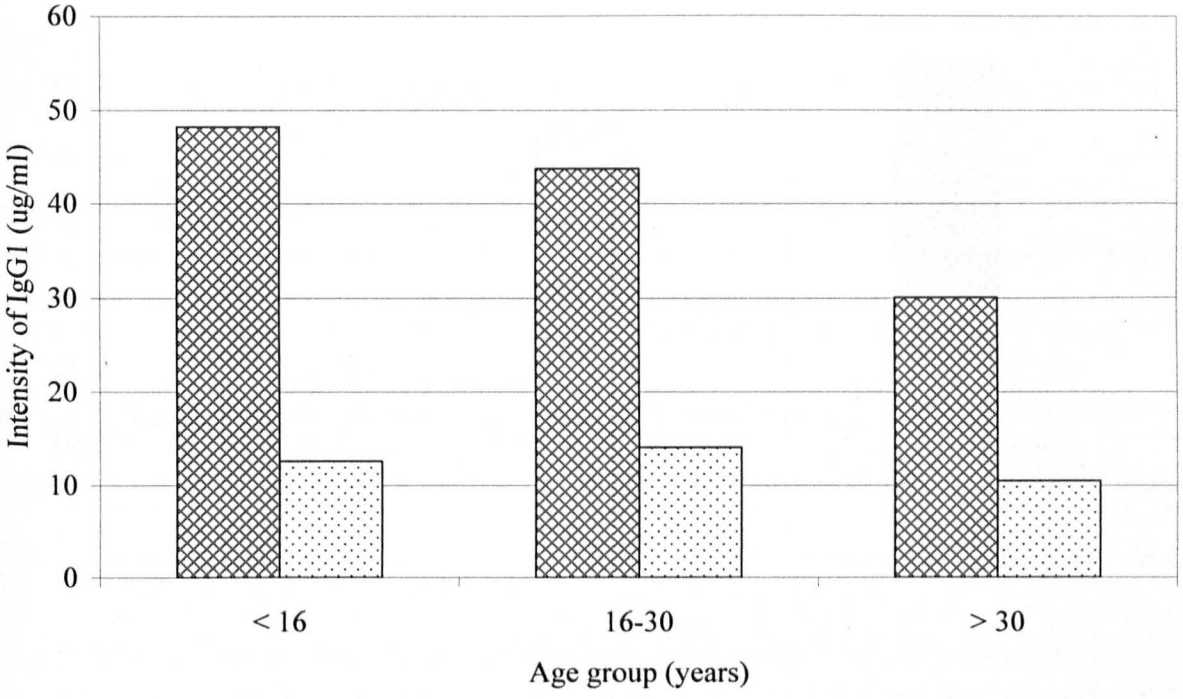

Figure 6.18: Age-specific changes in geometric intensity of antifilarial IgG1. The bars represent intensity of antifilarial IgG1 during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The decrease in intensity of antifilarial IgG1 was significant in all age groups.

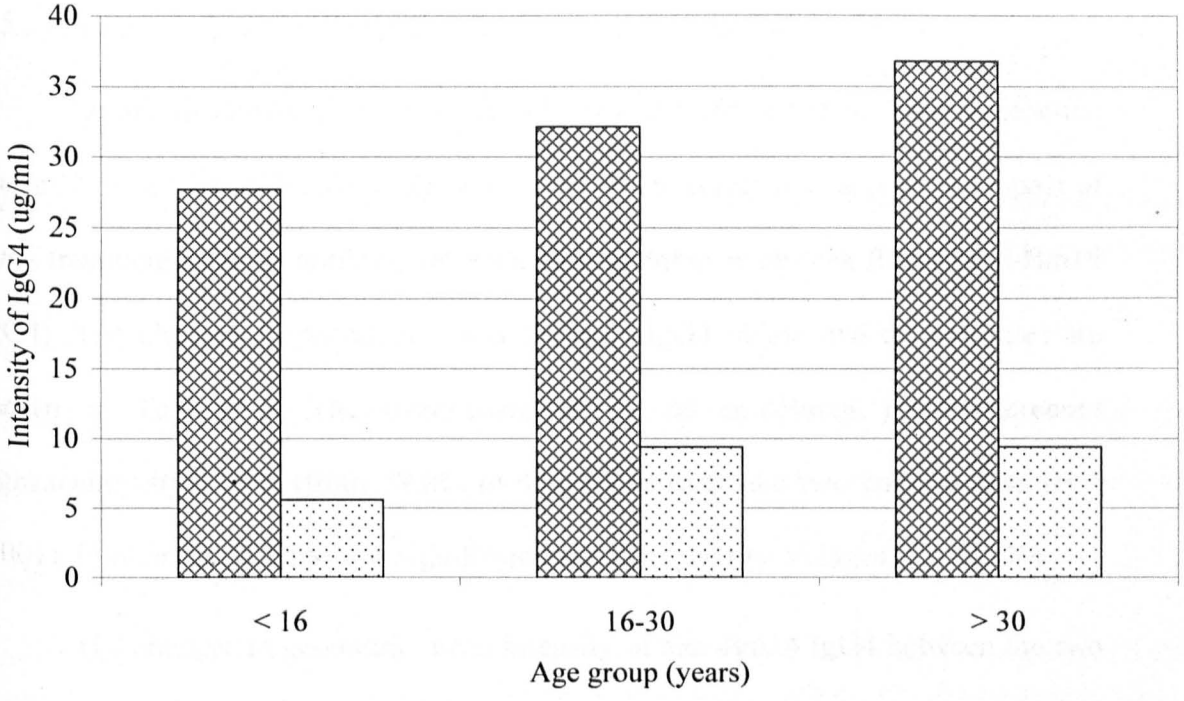

Figure 6.19: Age-specific changes in geometric mean intensity of antifilarial IgG4. The bars represent intensity of antifilarial IgG4 during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The decrease in intensity of antifilarial IgG4 was significant in all age groups.

6.5.3 Changes in IgG4 antibody responses to recombinant antigen Bm14

Serum specimens from 245 individuals that were tested in 2002 at baseline and in 2004 after two MDAs from two villages were selected to assess the impact of mass treatment on IgG4 antibody responses to recombinant antigen Bm14 (anti-Bm14 IgG4). The changes in prevalence of anti-Bm14 IgG4 in the two communities are shown in Table 6.3. The overall prevalence of anti-Bm14 IgG4 decreased significantly by 32.0% (from 58.8% to 40.0%) between the two communities ($P < 0.001$). Further, the decline was significant in each of the two villages ($P < 0.001$).

The changes in geometric mean intensity of anti-Bm14 IgG4 between the two villages are summarized in Table 6.4. Overall, the intensity of anti-Bm14 IgG4 decreased significantly by 84.9% (from 957.3 units to 144.5 units; $P < 0.001$). The decrease in intensity of anti-Bm14 IgG4 was significant in each of the two villages (82.8% in Marikano and 86.2% in Magongoloni; $P < 0.001$).

The decrease in prevalence of anti-Bm14 IgG4 was highest in children aged < 16 years (52.7%; $P < 0.001$) followed by individuals in the group aged 16-30 years (23.4%; $P = 0.016$) and lowest in mature adults group aged > 30 years (14.5%; $P = 0.039$). As with prevalence rates, the decrease in intensity of anti-Bm14 IgG4 was highest in children (92.6%) followed by young adults (76.3%) and lowest in mature adults (84.9%). The decline was significant in all 3 age groups ($P < 0.001$).

	2002, baseline survey		2004, one year after 2nd MDA		
Village	No. pos/ No. tested	% positive	No. pos/ No. tested	% positive[1]	P value*
Marikano	59/107	55.1	40/107	37.4 (32.1)	< 0.001
Magongoloni	85/138	61.6	58/138	42.0 (31.8)	< 0.001
All	144/245	58.8	98/245	40.0 (32.0)	< 0.001

Table 6.3: Changes in overall prevalence rates of anti-Bm14 IgG4 by village. There was significant decrease in anti-Bm14 IgG4 in both villages.

[1]Numbers in parentheses indicate percent decrease compared to baseline

*McNemar's test

	2002, baseline survey		2004, one year after 2nd MDA		
Village	No. positive	GMI	No. retested	GMI[1]	P value*
Marikano	59	865.6	59	148.5 (82.8)	< 0.001
Magongoloni	85	1026.6	85	141.8 (86.2)	< 0.001
All	144	957.3	144	144.5 (84.9)	< 0.001

Table 6.4: Changes in geometric mean intensity (GMI) of anti-Bm14 IgG4 in 144 positive persons during baseline survey. There was significant decline in intensity of anti-Bm14 IgG4 in both villages.

[1]Numbers in parentheses indicate percent decrease compared to baseline

*Wilcoxon signed-ranks test

6.6 Changes in parasitological status by treatment compliance

6.6.1 Changes in prevalence of active infection and antifilarial antibodies

Table 6.5 summarizes changes in the prevalence of active infection (microfilaraemia and CFA) and antifilarial antibodies in groups of individuals that received two treatments, one treatment or no treatment during the two MDAs. Most people that were tested for active infection or antifilarial antibodies, however, had received the two treatments offered during the MDAs. For example, treatment data from 456 persons tested for microfilaraemia at baseline and in 2004 indicated that 380 (83.3%) and 60 (13.2%) individuals had received two and one treatments, respectively. Sixteen individuals (3.5%) reported that they had not received treatment during either of the two MDAs.

The decrease in prevalence of microfilaraemia in the group that received two treatments decreased significantly by 71.1% ($P < 0.001$). There was also significant decrease in prevalence of microfilaraemia in the group that received one treatment (52.3%; $P = 0.003$). However, of 3 microfilaraemic individuals at baseline in the group that did not receive any treatment one individual had no microfilariae in 2004.

Treatment data were available for 463 and 467 persons tested for CFA at baseline and in 2004 by the Og4C3 ELISA assay and the ICT tests, respectively. Surprisingly, the decrease in prevalence of CFA by the Og4C3 ELISA test in the group that received one treatment was higher (18.3%) than in the group that received two treatments (12.7%). However, the decrease of CFA by the ICT test was higher in the group that received two treatments (47.4%) than in the group that received one treatment (30.1%). There was no significant change in prevalence of CFA either by

the Og4C3 ELISA assay or the ICT test in the group that did not receive treatment (P = 1.000).

The decrease in prevalence of antifilarial IgG1 in the group that received two treatments was higher (46.4%) than in the group that received one treatment (35.1%). However, although the decrease in prevalence of antifilarial IgG4 was higher in the group that received two treatments (14.1%) than in the group that received one treatment (12.0%), the difference between the two groups was not as pronounced as for antifilarial IgG1. For anti-Bm14 IgG4, however, the decrease in prevalence was more than three-fold in the group that received two treatments (38.9%) compared to the group that received one treatment (10.7%).

Indicator	No. times treated	N	No. (%) positive in 2002	No. (%) positive in 2004	% Reduction	P value*
Microfilaraemia	2	380	80 (21.1)	23 (6.1)	71.1	< 0.001
	1	60	21 (35.0)	10 (16.7)	52.3	0.003
	0	16	3 (18.8)	2 (12.5)	33.5	1.000
Antigenaemia (Og4C3)	2	383	148 (38.6)	129 (33.7)	12.7	< 0.001
	1	65	33 (50.8)	33 (41.5)	18.5	0.031
	0	15	8 (53.3)	7 (46.7)	12.4	1.000
Antigenaemia (ICT)	2	386	135 (35.0)	71 (18.4)	47.4	< 0.001
	1	66	30 (45.5)	21 (31.8)	30.1	0.004
	0	15	7 (46.7)	7 (46.7)	0	1.000
Antifilarial IgG1	2	383	329 (85.9)	176 (46.0)	46.4	< 0.001
	1	65	57 (87.7)	37 (56.9)	35.1	< 0.001
	0	15	13 (86.7)	8 (53.3)	38.5	0.063
Antifilarial IgG4	2	383	352 (91.9)	302 (78.9)	14.1	< 0.001
	1	65	58 (89.2)	51 (78.5)	12	0.039
	0	15	11 (73.3)	12 (80.0)	-9.1	1.000
Anti-Bm14 IgG4	2	186	108 (58.1)	66 (35.5)	38.9	< 0.001
	1	44	28 (63.6)	25 (56.8)	10.7	0.250
	0	15	8 (53.3)	7 (46.7)	12.4	1.000

Table 6.5: Changes in prevalence of active infection and antifilarial antibodies by treatment compliance. Overall, decline in prevalence of active infection and antifilarial antibodies was higher in individuals that received two annual treatments.
*McNemar's test

6.6.2 Changes in intensity of active infection and antifilarial antibodies

Table 6.6 shows changes in intensity of active infection and antifilarial antibodies in groups of individuals that received two treatments, one treatment or no treatment during the two MDAs. The intensity of microfilaraemia in persons that received two and one treatments decreased significantly by 97.1% and 85.4%, respectively ($P < 0.001$). However, the intensity of microfilaraemia in 3 microfilaraemic individuals during baseline survey in a group of 16 persons that did not receive treatment in either of the two MDAs decreased by 79.3%, but the difference was not significant because of the small numbers in the group. Similarly, the decrease in intensity of CFA was 91.9% in the group that received two treatments and 82.1% in the group that received one treatment. However, the intensity of CFA in 8 persons that had parasite antigenaemia at baseline decreased significantly by 79.0% although they reported that they had not received treatment during the two MDAs ($P = 0.036$).

As with the intensity of CFA, the decrease in intensity of antifilarial IgG1 and IgG4 was higher in individuals that received two treatments than in those that received one treatment, but the decrease was significant in both groups. There was also significant decrease in intensity of antifilarial IgG1 and IgG4 in individuals that reported not being treated in either of the two MDAs. For example, the decrease in intensity of antifilarial IgG4 was 77.8% in the group that received two treatments and 70.0% in the group that received one treatment. However, the intensity of antifilarial IgG4 in 11 persons that were positive at baseline decreased significantly by 55.4% although they had not been treated during the two MDAs ($P = 0.033$).

Indicator	No. times treated	No. positive in 2002	GMI in 2002	GMI in 2004	% Reduction	P value*
Microfilaraemia	2	80	655	19	97.1	< 0.001
	1	21	254	37	85.4	< 0.001
	0	3	188	39	79.3	0.109
Antigenaemia (Og4C3)	2	148	23221.4	1879.2	91.9	< 0.001
	1	33	13439.4	2401.4	82.1	< 0.001
	0	8	19379.8	4063.2	79.0	0.036
Antifilarial IgG1	2	329	41.8	11.6	72.2	< 0.001
	1	57	41.1	15.5	62.3	< 0.001
	0	13	23.2	13.7	40.9	0.025
Antifilarial IgG4	2	352	30.2	6.7	77.8	< 0.001
	1	58	40.0	12.0	70.0	< 0.001
	0	11	27.8	12.4	55.4	0.033
Anti-Bm14 IgG4	2	108	845.4	105.9	87.5	< 0.001
	1	28	1485.4	421.3	71.6	< 0.001
	0	8	1101.9	227.5	79.4	0.017

Table 6.6: Changes in intensity of active infection and antifilarial antibodies by treatment compliance. Generally, there was a significant decline in the intensity of infection and antifilarial antibodies, but the decrease was higher in individuals that received two treatments.

*Wilcoxon signed-ranks test; GMI = geometric mean intensity

6.7 Incidence of infection during MDA

6.7.1 Incidence of microfilaraemia

Of 740 persons that were tested for microfilaraemia at baseline in 2002 there were 456 individuals retested in 2004. Of these 456 individuals, 352 persons were microfilaria negative at baseline. Of the 352 microfilaria-negative individuals at baseline, 4 individuals (1.1%) had microfilariae in 2004. The characteristics of the 4 incident cases are summarized in Table 6.7. All the 4 individuals, however, were positive for CFA by Og4C3 ELISA at baseline and in 2004. Using the ICT test, 3 of the 4 individuals were positive at baseline and all 4 were positive in 2004. All the 4 individuals were positive for antifilarial IgG4 to *B. pahangi* antigen. Also, both cases that were tested were positive for anti-Bm14 IgG4. Of the 4 incident microfilaraemia cases two reported that they had received treatment during the two MDAs, one had received one treatment whereas the fourth had not received any treatment.

				Infection status in 2002 by:				
Case #	Age	Gender	mf count*	ICT	Og4C3	IgG4	Anti-Bm14 IgG4	Tx status
1	27	M	9	+	+	+	+	None
2	9	F	27	+	+	+	ND	Twice
3	38	F	4	+	+	+	ND	Once
4	28	F	2	-	+	+	+	Twice

Table 6.7: Characteristics of four incident cases of microfilaraemia after two MDAs.
*Microfilaria (mf) count is expressed as mf/100 µl night time blood.
ND = not done; Tx status = treatments received during the two MDAs

6.7.2 Incidence of antigenaemia

Of 748 persons tested for CFA by the ICT test in 2002 there were 467 individuals retested in 2004. Of the 467 persons that were tested during the two surveys 295 individuals were CFA negative by the ICT test in 2002. Of these 295 CFA negative persons at baseline, there were 3 individuals (1.0%) that were ICT positive in 2004. Two of the 3 incident cases, however, were negative for CFA using the more sensitive Og4C3 ELISA test in 2004. Further, the one individual that was positive by Og4C3 test in 2004 was also positive at baseline using the test (Og4C3). The Og4C3 ELISA test identified one individual of 274 individuals (0.7%) that were negative by this test at baseline. This individual (a female aged 24 years) was negative by the ICT test. The one individual with incident parasite antigenaemia had received treatment during the two MDAs.

6.7.3 Incidence of antifilarial IgG antibodies

In the cohort of 463 individuals tested for antifilarial IgG1 and IgG4 in 2002 and 2004 there were 64 individuals that were negative for antifilarial IgG1. Of the 64 persons two individuals (3.1%) had a positive antifilarial IgG1 test in 2004. However, the two individuals were negative for microfilariae and CFA. Further, the two persons had received treatment during the two MDAs. For antifilarial IgG4, however, 7 of 42 persons (16.7%) that were negative at baseline were positive in 2004. All 7 persons were negative for CFA by the Og4C3 ELISA and the ICT tests in 2002 and 2004. One individual had not received treatment during the two MDAs whereas another had received one treatment only.

Of 245 persons tested for anti-Bm14 IgG4 in 2002 and 2004 there were 101 individuals that were negative at baseline. Of these 101 persons negative at baseline, 5 individuals (5.0%) were positive for anti-Bm14 IgG4 in 2004. However, only one individual was positive for CFA by Og4C3 and ICT tests. One individual had not received treatment during the two MDAs whereas the other 4 had received the two treatments.

6.8 Assessment of the impact of mass treatment by xenomonitoring

In addition to blood sampling, collection of blood fed vector mosquitoes was also done every year before the administration of mass treatment. Figure 6.20 represents overall changes in proportion of vector mosquitoes with *W. bancrofti Ssp*I repeat DNA. The proportion of mosquitoes with parasite DNA decreased by 29.7% from 24.9% at baseline in 2002 to 17.5% in 2003 after the first mass treatment ($P = 0.068$). There was a significant decrease in the prevalence of parasite DNA in vector mosquitoes after the second round of MDA; the prevalence decreased by 64.7% from 24.9% in 2002 to 8.8% in 2004 ($P < 0.001$).

Except for Jilore village, the change in prevalence of parasite DNA in vector mosquitoes was quite variable within the villages (Table 6.8). This variation was associated with the low numbers of mosquitoes collected in Marikano, Magongoloni and Mkondoni villages in 2002 and 2003. In Marikano village for example, 42 and 25 mosquitoes were collected in 2002 and 2003, respectively. Only one mosquito in each of the collections was positive for parasite DNA making the proportion of mosquitoes with parasite DNA to be 2.4% and 4.8% in 2002 and 2003, respectively. In 2004, however, a total of 164 mosquitoes were collected in Marikano village and 16 (9.8%)

had parasite DNA. In Jilore village, however, 90, 97 and 83 mosquitoes were collected in 2002, 2003 and 2004, respectively. The proportion of mosquitoes with parasite DNA in Jilore in 2002, 2003 and 2004 was 41.1%, 12.4% and 6.0%, respectively.

Figure 6.21 shows the changes in prevalence of parasite DNA in *An. gambiae s.l.* and *An. funestus* mosquitoes. The number of *Cx. quinquefasciatus* mosquitoes collected in 2002, 2003 and 2004 was one, 5 and one, respectively and none had parasite DNA. The total number of *An. gambiae s.l.* mosquitoes collected in 2002, 2003 and 2004 was 127, 193 and 168, respectively. The proportion of *An. gambiae s.l.* with parasite DNA was significantly higher in 2002 (35.4%) than in 2003 (19.2%) representing a 45.8% decrease ($P = 0.001$). In 2004, there was a significant decrease (78.2%) in proportion of *An. gambiae s.l.* mosquitoes with parasite DNA; from 35.4% in 2002 to 7.7% in 2004 ($P < 0.001$). There were only 13 *An. funestus* mosquitoes collected in 2003 and none had parasite DNA. In 2004, however, 151 *An. funestus* mosquitoes were collected. The proportion of *An. funestus* mosquitoes with parasite DNA was slightly higher in 2004 (9.9%) than in 2002 (6.8%), but the difference was not significant ($P = 0.448$).

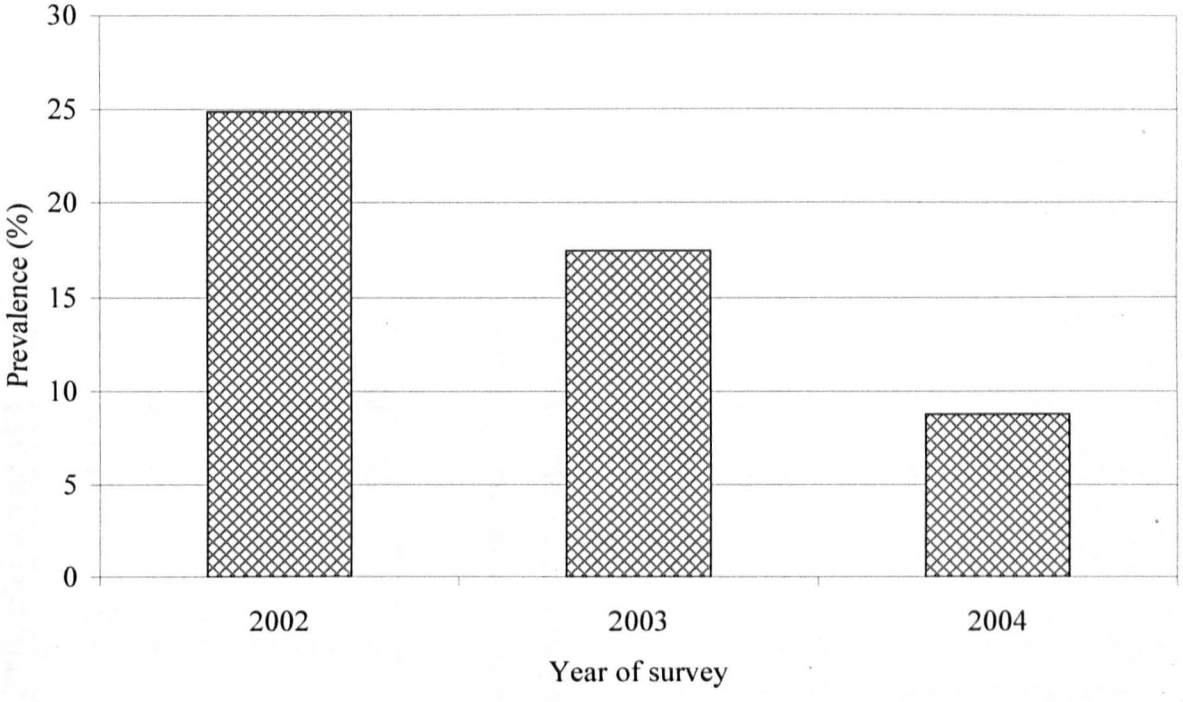

Figure 6.20: Changes in prevalence of *W. bancrofti Ssp*I repeat DNA in vector mosquitoes. The proportion of vector mosquitoes with parasite DNA decreased significantly after each round of mass treatment.

Village	2002, baseline survey		2003, after first MDA		2004, after second MDA	
	No. tested	No. (%) positive	No. tested	No. (%) positive	No. tested	No. (%) positive
Jilore	90	37 (41.1)	97	12 (12.4)	83	5 (6.0)
Marikano	42	1 (2.4)	25	1 (4.0)	164	16 (9.8)
Magongoloni	44	10 (22.7)	34	4 (11.8)	20	0 (0.0)
Mkondoni	25	2 (8.0)	55	20 (36.4)	53	7 (13.2)
All	201	50 (24.9)	211	37 (17.5)	320	28 (8.8)

Table 6.8: Changes in prevalence of *W. bancrofti Ssp*I repeat DNA in vector mosquitoes. Note that except for Jilore village, the change in proportion of vector mosquitoes with parasite DNA in the other 3 villages was quite variable due to low numbers of mosquitoes collected in these villages.

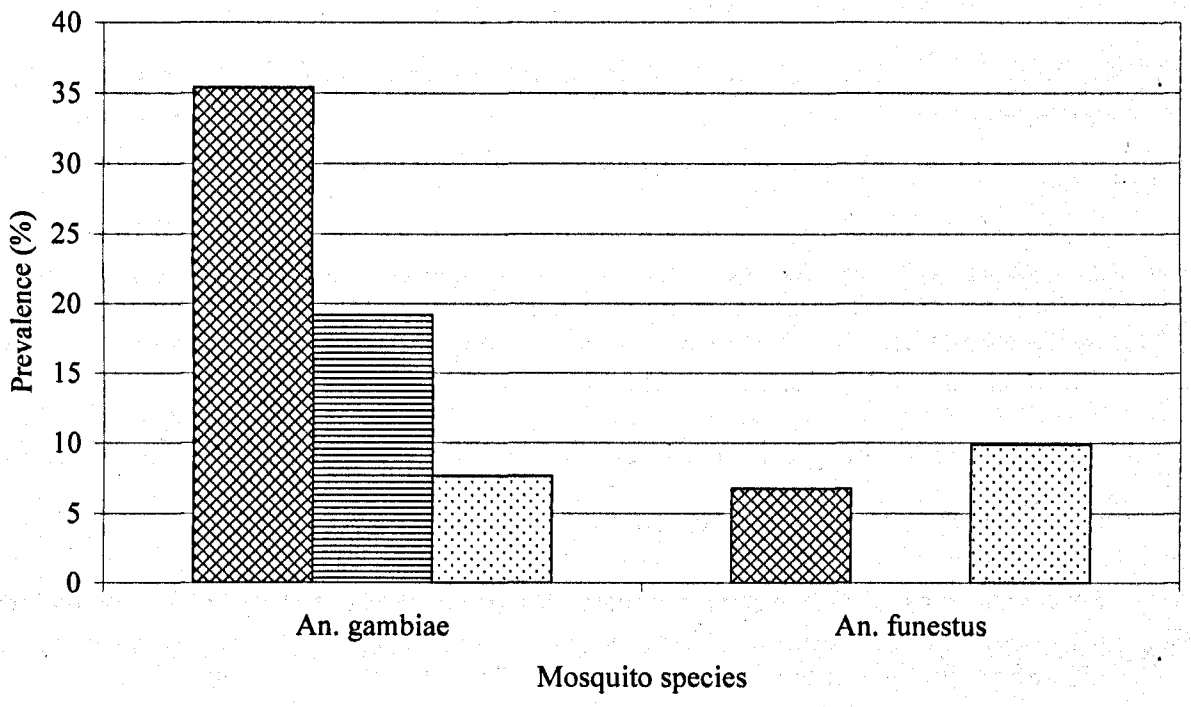

Figure 6.21: Changes in proportion of mosquitoes with parasite DNA. There was significant decrease in the proportion of *An. gambiae s.l.* mosquitoes with parasite DNA after each round of mass treatment. The proportion of *An. funestus* mosquitoes with parasite DNA was higher in 2004 than in 2002, but the difference was not significant.

CHAPTER 7 - IMPACT OF MASS TREATMENT ON CLINICAL MANIFESTATIONS OF BANCROFTIAN FILARIASIS

Approximately 40 million people living in lymphatic filariasis endemic areas have clinical disease resulting primarily from damaged lymphatic vessels (Molyneux and Zagaria, 2002). Many countries have started annual mass drug administration under GPELF in an effort to interrupt transmission of *W. bancrofti* infection. However, there is limited information on the longterm effects of MDA on clinical manifestations due to lymphatic filariasis infection. Assessing the public health impact of mass treatment with antifilarial drugs is a critically important issue for programme advocacy and for planning morbidity control strategies (Addiss and Mackenzie, 2004). Studies conducted in Tanzania indicated that DEC administered in different regimens results in complete resolution or reduction of hydrocele (Meyrowitsch *et al.*, 1996a; Meyrowitsch *et al.*, 1996b). Complete resolution of hydrocele was seen in 25.0%, 36.8%, 45.5% and 46.2% of males treated using DEC-fortified salt, 12-day course of DEC, semi-annual DEC and monthly low-dose DEC, respectively. However, these studies reported that were was no significant effect of DEC treatment on leg lymphoedema. Another study conducted in Papua New Guinea reported resolution of hydrocele in 91 of 105 males (87%) after four annual MDAs with DEC alone or DEC plus ivermectin (Bockarie *et al.*, 2002). The Papua New Guinea study also reported that resolution of lymphoedema of the legs occurred in 62 of 90 adults (69%) and that resolution was not correlated with the drug regimen. Previous investigations have shown that all cases of pitting lymphoedema could resolve within one year after treatment with DEC, but that advanced lymphoedema required two to four years to resolve (Partono, 1985).

As the GPELF up-scales to include all areas of filariasis endemicity opportunities to study the natural history of filariasis morbidity during mass treatment become fewer. Four communities in an area selected for filariasis elimination using mass treatment with a combination of DEC and albendazole were examined for clinical manifestations of bancroftian filariasis during a pre-treatment survey in 2002 and after two rounds of MDA in 2004. The communities had never received antifilarial treatment before introduction of MDA under GPELF. In the current chapter, the impact of the two rounds of MDA on clinical manifestations of bancroftian filariasis was assessed in individuals that were examined at baseline in 2002 and after two rounds of mass treatment in 2004. The study has allowed us to examine paired observations before and after two rounds of MDA in persons with and without chronic disease which is a useful way to accurately document both resolution and incidence of clinical manifestations thus giving us an insight into the dynamics of filarial disease during mass treatment.

7.1 Impact of MDA on hydrocele prevalence

A total of 159 males were examined both at baseline in 2002 and after two MDAs in 2004. However, since hydrocele was not a common manifestation in children below 15 years of age analyses for the impact of mass treatment on hydrocele were confined to males above 14 years of age. Table 7.1 shows the prevalence rates of hydrocele in a group of 72 males aged above 14 years that were examined in 2002 and 2004. There was a decrease in prevalence of hydrocele in three villages, but the decrease did not give rise to significant differences ($P > 0.05$). In one village (Magongoloni), however, the prevalence of hydrocele in 2002 and 2004 was constant

at 42.9% (P = 1.000). Overall, the prevalence of hydrocele in this group decreased by 17.6% from 47.2% in 2002 to 38.9% in 2004, but the difference was not significant (P = 0.267).

To investigate the age-specific impact of mass treatment, individuals were grouped into three broad age groups, namely, 15-20 year, 21-40 year and > 40 year. The age-specific prevalence rates of hydrocele in the 72 males aged > 14 years who were examined in 2002 and 2004 are summarized in Table 7.2. There was a reduction in the number of males with hydrocele in 15-20 year and 21-40 year age groups, but the difference was not significant because of small numbers. Of 15 males in the 15-20 year age group who were examined in 2002 and 2004, 3 had hydrocele at baseline. After two rounds of MDA in 2004, only one individual had hydrocele in this age group. Similarly, of 24 males in the 21-40 year age group, 7 had hydrocele at baseline, compared with 3 after two rounds of MDA. Of 33 males > 40 years old who were examined at baseline and after two MDAs, the prevalence of hydrocele remained unchanged. Twenty four males (72.7%) had hydrocele both in 2002 and 2004 in this age group.

7.1.1 Resolution and incidence of hydrocele

Resolution and incidence of hydrocele was assessed in 159 males aged > 4 years examined at pre-treatment in 2002 and after two rounds of MDA in 2004. Among 35 males who had hydrocele in 2002, resolution was observed in 10 individuals of whom one was a boy aged 14 years. Among the 72 males aged > 14 years re-examined for hydrocele after two rounds of MDA (Table 7.1), there were 34 and 38 individuals with and without hydrocele in 2002, respectively. Thus, for 34

males aged > 14 years with hydrocele in 2002 that were re-examined in 2004 resolution was observed in 9 individuals (26.5%). Of 38 males aged > 14 years without hydrocele in 2002 there were 3 (7.9%) incident cases observed in 2004. The characteristics of the individuals that resolved and developed hydrocele are summarized in Table 7.3. All individuals that resolved or developed hydrocele claimed to have received treatment during the two MDAs. Resolution of hydrocele did not appear to be associated with clearance of infection. Of the 9 men that resolved hydrocele, 4 individuals that had CFA in 2002 were still positive for parasite antigenaemia in 2004. Similarly, incidence of hydrocele did not appear to be associated with infection status. Of the 3 males with incident hydrocele, one had cleared all signs of infection by 2004; the infection status of one did not change, and the third developed parasite antigenaemia.

When stage of hydrocele was stratified into ≤ stage 2 and ≥ stage 3, resolution was found to be significantly associated with stage (P = 0.034). All the cases of hydrocele that resolved were ≤ stage 2. Among the 34 males aged > 14 years with hydrocele in 2002 that were re-examined in 2004, 9 had hydrocele ≥ stage 3 but none of these resolved. Eight of these 9 individuals received two treatments whereas the remaining one person received one treatment. There was no incidence of hydrocele in 86 boys aged < 15 years (in 2002) that were examined in both 2002 and 2004.

Village	2002, baseline survey No. examined	2002, baseline survey No. (%) with hydrocele	2004, after two MDAs No. examined	2004, after two MDAs No. (%) with hydrocele	P value*
Jilore	21	10 (47.6)	21	8 (38.1)	0.500
Marikano	12	6 (50.0)	12	4 (33.3)	0.500
Magongoloni	21	9 (42.9)	21	9 (42.9)	1.000
Mkondoni	18	9 (50.0)	18	7 (38.9)	0.625
Total	72	34 (47.2)	72	28 (38.9)	0.146

Table 7.1: Changes in prevalence of hydrocele by village in a cohort of 72 males aged above 14 years examined in 2002 and 2004. The overall decline in prevalence of hydrocele in this group was not significant. However, there were 9 and 3 resolutions and incident cases, respectively observed in 2004.

*McNemar's test

Age group (years)	2002, baseline survey No. examined	2002, baseline survey No. (%) with hydrocele	2004, after two MDAs No. examined	2004, after two MDAs No. (%) with hydrocele	P value*
15-20	15	3 (20.0)	15	1 (6.7)	0.625
21-40	24	7 (29.2)	24	3 (12.5)	0.125
> 40	33	24 (72.7)	33	24 (72.7)	1.000
All	72	34 (47.2)	72	28 (38.9)	0.146

Table 7.2: Age-specific changes in prevalence of hydrocele in a cohort of 72 males aged above 14 years examined in 2002 and 2004. There was a reduction in the number of males with hydrocele in 15-20 year and 21-40 year age groups, but the difference was not significant because of small numbers.

*McNemar's test

Case #	Age	Stage*	Tx status	Infection status in 2002 by: MF	CFA	IgG4	Infection status in 2004 by: MF	CFA	IgG4
Resolution of hydrocele:									
1	14	1	Twice	-	-	+	-	-	+
2	18	1	Twice	+	+	+	+	+	+
3	18	2	Twice	-	-	+	-	-	+
4	19	1	Twice	+	+	+	+	+	+
5	23	1	Twice	-	-	-	-	-	-
6	30	1	Twice	-	-	+	-	-	+
7	32	1	Twice	+	+	+	-	+	+
8	33	2	Twice	-	-	+	-	-	+
9	42	2	Twice	+	+	+	+	+	+
10	68	2	Twice	-	ND	ND	ND	ND	ND
Incidence of hydrocele:									
1	15	1	Twice	-	-	+	-	-	-
2	73	2	Twice	-	+	+	-	+	+
3	44	4	Twice	-	-	+	-	+	+

Table 7.3: Characteristics of males that had resolution or incidence of hydrocele after two MDAs. Resolution or incidence of hydrocele did not appear to be associated with clearance of infection.

*Stage: 1 = scrotal sac 6-8 cm; 2 = scrotal sac 8-11 cm; 4 = scrotal sac > 15 cm

MF = microfilaraemia; CFA = circulating filarial antigen (by Og4C3 ELISA);

IgG4 = antifilarial IgG4 to *B. pahangi* antigen; Tx status = treatment status;

ND = not done

7.2 Impact of MDA on scrotal lymphoedema

All the 72 males aged > 14 years that were re-examined for hydrocele were also re-examined for scrotal lymphoedema. Of these 72 men, 6 (8.3%) had scrotal lymphoedema at baseline in 2002 compared with 7 (9.7%) after two rounds of MDA in 2004. This increase in prevalence of scrotal lymphoedema, however, was not significant (P = 1.000). The number of males with scrotal lymphoedema changed in one village (Jilore) only. Of 21 males aged > 14 years re-examined for scrotal lymphoedema in this village, there were two and 3 individuals with this clinical manifestation in 2002 and 2004, respectively. But the difference was not significant (P = 1.000).

All the cases of scrotal lymphoedema in males aged > 14 years that were re-examined in 2004 were in individuals aged > 40 years old. Thus, of 33 persons in the > 40 year age group there were 6 (18.2%) and 7 (21.2%) individuals with scrotal lymphoedema in 2002 and 2004, respectively, but the difference was not significant (P = 1.000).

7.2.1 Resolution and incidence of scrotal lymphoedema

Of 6 men with scrotal lymphoedema at baseline resolution was observed in one individual. However, this individual had hydrocele in 2002 and 2004. For 66 persons without scrotal lymphoedema at baseline 2 individuals (3.0%) had this manifestation in 2004. The two individuals also had hydrocele in 2002 and 2004.

7.3 Impact of MDA on leg lymphoedema

A total of 379 persons were re-examined for leg lymphoedema in 2004 after two rounds of MDA. Children aged < 15 years were excluded from analyses of impact of MDA on leg lymphoedema because this manifestation was not common in this group. There were 202 persons aged > 14 years examined for lymphoedema of the legs in 2002 and 2004. The prevalence rates of lymphoedema in this group were similar in 2002 (8.9%) and 2004 (7.9%) (P = 0.687). Table 7.4 summarizes the prevalence of leg lymphoedema in the 202 individuals aged > 14 years that were re-examined after two MDAs among the 4 study communities. In one village (Magongoloni), the prevalence of leg lymphoedema dropped slightly from 12.7% to 9.5%, but the decrease was not significant (P = 0.500). In general, the prevalence rates of lymphoedema were similar in 2002 and 2004 in all the villages (P > 0.05).

For 36 persons in the 15-20 year age group there was one individual with leg lymphoedema in 2002 and 2004. Of 86 persons in the 21-40 year age group, there were 4 (4.7%) and 5 (5.8%) persons with leg lymphoedema in 2002 and 2004 respectively, but the increase was not significant (P = 1.000). However, of 80 persons in the > 40 year age group, there were 13 (16.3%) and 10 (12.5%) individuals with leg lymphoedema in 2002 and 2004, respectively, but the decrease was not significant (P = 0.375). Thus, there was no significant change in prevalence rates of leg lymphoedema in all 3 age groups.

The group of 202 persons aged > 14 years that were re-examined for leg lymphoedema in 2004 comprised of 72 males and 130 females. For the 72 males, there were 9 (12.5%) and 5 (6.9%) individuals with leg lymphoedema in 2002 and

2004, respectively, but the decrease was not significant (P = 0.125). Among the 130 females, there were 9 (6.9%) and 11 (8.5%) individuals with leg lymphoedema in 2002 and 2004, respectively, but the increase was not significant (P = 0.500).

	2002, baseline survey		2004, after two MDAs		
Village	No. examined	No. (%) with lymphoedema	No. examined	No. (%) with lymphoedema	P value*
Jilore	46	2 (4.3)	46	2 (4.3)	1.000
Marikano	46	4 (8.7)	46	4 (8.7)	1.000
Magongoloni	63	8 (12.7)	63	6 (9.5)	0.500
Mkondoni	47	4 (8.5)	47	4 (8.5)	1.000
Total	202	18 (8.9)	202	16 (7.9)	0.687

Table 7.4: Changes in prevalence of leg lymphoedema by village in a cohort of 202 persons above 14 years old examined in 2002 and 2004. In general, the prevalence rates of lymphoedema were similar in 2002 and 2004 in all the villages.
*McNemar's test

7.3.1 Resolution and incidence of leg lymphoedema

Of the 379 persons that were re-examined for leg lymphoedema in 2004, there were 20 individuals with lymphoedema in 2002 and two were children aged below 15 years. In the group of 202 persons aged > 14 years, resolution of leg lymphoedema was observed in 4 of 18 individuals (22.2%) that had lymphoedema at baseline. For 184 persons aged > 14 years without lymphoedema at baseline, 2 individuals (1.1%) had developed lymphoedema by 2004. In children < 15 years, there was one case each of resolution and incidence of leg lymphoedema. Thus, overall there were 5 cases of resolution and 3 cases of incidence of leg lymphoedema in the 379 individuals re-examined in 2004, respectively. The characteristics of individuals that resolved and developed leg lymphoedema are summarized in Table 7.5. The leg(s) affected by lymphoedema was recorded for 4 of the 5 individuals in whom resolution of leg lymphoedema was observed. Two of these persons had bilateral lymphoedema whereas the other two had unilateral lymphoedema (right leg).

Case #	Age	Gender	Stage	Tx status	Infection status in 2002 by: MF	CFA	IgG4	Infection status in 2004 by: MF	CFA	IgG4
Resolution of leg lymphoedema:										
1	13	F	1	twice	-	-	+	-	-	+
2	42	M	1	twice	+	+	+	+	+	+
3	44	M	1	twice	-	-	+	-	-	+
4	68	M	2	twice	-	ND	ND	ND	ND	ND
5	75	M	2	twice	+	+	+	-	+	+
Incidence of leg lymphoedema:										
1	14	M	1	twice	-	-	+	-	-	+
2	34	F	1	twice	+	+	+	-	+	+
3	58	F	2	twice	+	+	+	-	+	+

Table 7.5: Characteristics of individuals that had resolution or incidence of leg lymphoedema after two MDAs.

MF = microfilaraemia; CFA = circulating filarial antigen (by Og4C3 ELISA);

IgG4 = antifilarial IgG4 to *B. pahangi* antigen; Tx status = treatment status; ND = not done

7.4 Impact of MDA on inguinal adenopathy

A group of 379 persons aged 5 years or more were re-examined for inguinal adenopathy in 2004 after two rounds of MDA. The prevalence rates of adenopathy in this group among the 4 study communities are shown in Table 7.6. Overall, the prevalence rate of adenopathy was significantly higher in 2004 (23.0%) than in 2002 (10.0%) which represented a 130% increase ($P < 0.001$). However, the changes in prevalence rates of adenopathy were variable among the 4 villages. In two villages (Marikano and Magongoloni) the increase in prevalence rates of adenopathy was significant ($P < 0.001$). In the other two villages (Jilore and Mkondoni), however, the increase was not significant ($P > 0.05$).

The age-specific prevalence rates of inguinal adenopathy in the 379 persons examined in 2002 and 2004 are shown in Table 7.7 and Figure 7.1. The prevalence rates of adenopathy increased significantly by more than 100% between 2002 and 2004 in all age groups ($P < 0.05$). The prevalence rates of adenopathy in males and females in 2002 and 2004 are shown in Table 7.8. The prevalence rates increased significantly by 145% and 100% in males ($P < 0.001$) and females ($P = 0.021$), respectively.

Village	2002, baseline survey No. examined	2002, baseline survey No. (%) with adenopathy	2004, after two MDAs No. examined	2004, after two MDAs No. (%) with adenopathy	*P value
Jilore	94	14 (14.9)	94	20 (21.3)	0.210
Marikano	73	3 (4.1)	73	22 (30.1)	< 0.001
Magongoloni	122	11 (9.0)	122	28 (23.0)	0.001
Mkondoni	90	10 (11.1)	90	17 (18.9)	0.167
Total	379	38 (10.0)	379	87 (23.0)	< 0.001

Table 7.6: Changes in prevalence of inguinal adenopathy by village in a cohort of 379 persons examined in 2002 and 2004. The prevalence of inguinal adenopathy increased significantly after treatment.

*McNemar's test

Age category (years)	2002, baseline survey No. examined	2002, baseline survey No. (%) with adenopathy	2004, after two MDAs No. examined	2004, after two MDAs No. (%) with adenopathy	P value*
5-10	119	6 (5.0)	119	17 (14.3)	0.019
11-20	94	8 (8.5)	94	17 (18.1)	0.035
21-40	86	10 (11.6)	86	21 (24.4)	0.035
> 40	80	14 (17.5)	80	32 (40.0)	< 0.001
All	379	38 (10.0)	379	87 (23.0)	< 0.001

Table 7.7: Age-specific changes in prevalence of inguinal adenopathy in a cohort of 379 persons examined in 2002 and 2004. The prevalence of inguinal adenopathy increased significantly in all age groups after mass treatment.

*McNemar's test

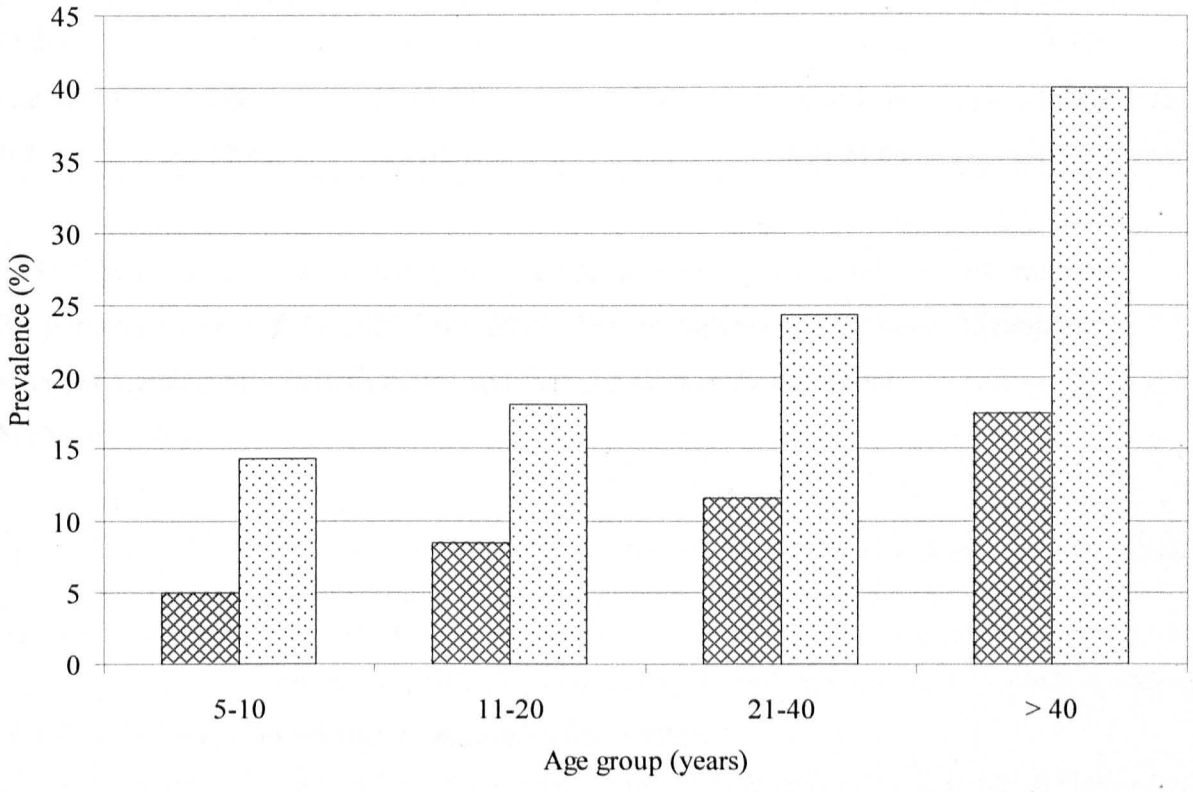

Figure 7.1: Age-specific prevalence of inguinal adenopathy in a group of 379 individuals examined during pre-treatment in 2002 (▨) and after two MDAs in 2004 (□). The prevalence rates of adenopathy increased significantly by more than 100% between 2002 and 2004 in all age groups.

Gender	2002, baseline survey No. examined	2002, baseline survey No. (%) with adenopathy	2004, after two MDAs No. examined	2004, after two MDAs No. (%) with adenopathy	P value*
Males	159	25 (15.7)	159	61 (38.4)	< 0.001
Females	220	13 (5.9)	220	26 (11.8)	0.021
All	379	38 (10.0)	379	87 (23.0)	< 0.001

Table 7.8: Changes in prevalence of inguinal adenopathy by gender in a cohort of 379 persons examined in 2002 and 2004. The prevalence of inguinal adenopathy increased significantly in both males and females after mass treatment.
*McNemar's test

7.4.1 Resolution and incidence of inguinal adenopathy

Resolution of inguinal adenopathy was observed in 16 (42.1%) of 38 individuals in the group of 379 persons examined in 2002 and 2004. There were 65 (19.1%) new cases of inguinal adenopathy among 341 persons that did not have this manifestation in 2002. The baseline characteristics of individuals that resolved and developed inguinal adenopathy are summarized in Table 7.9. Neither resolution nor incidence of adenopathy was associated with clearance of infection. For example, for 3 individuals with microfilaraemia in 2002, there was resolution of adenopathy but microfilariae were still detected in two of the individuals in 2004. Similarly, for 6 persons that had parasite antigenaemia in 2002, resolution of adenopathy was observed even though parasite antigenaemia was still detected in 4 individuals in

2004. Further, among the 65 new cases of adenopathy, 33 individuals had CFA in 2002 and parasite antigenaemia was still detected in 25 (75.8%) persons in 2004.

				Infection status in 2002 as assessed by:					
				MF		CFA (Og4C3)		IgG4	
Stage*	No.	M/F	Age range (yr)	No. pos	No. neg	No. pos	No. neg	No. pos	No. neg
Resolution of adenopathy:									
1	12	5/7	7-60	2	10	5	7	12	0
2	3	3/0	13-68	1	2	1	1	2	0
3	1	1/0	9	0	1	0	1	1	0
Total	16	9/7		3	13	6	9	15	0
Incidence of adenopathy:									
1	48	35/13	5-77	12	34	22	21	39	4
2	17	10/7	5-83	6	9	11	4	15	0
Total	65	45/20	5-83	18	43	33	25	54	4

Table 7.9: Characteristics of individuals that had resolution or incidence of inguinal adenopathy after two MDAs. Neither resolution nor incidence of adenopathy was associated with clearance of infection.

*Stage adenopathy: 1, approx. 3 cm; 2, > 3cm; 3, skin folded under gland (hanging groin)

CHAPTER 8 - DISCUSSION

The World Health Organization (WHO) has targeted lymphatic filariasis for elimination by the year 2020. To achieve this objective the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was established to interrupt transmission of infection and reduce suffering in those already with chronic disease. The major strategy employed by the GPELF to interrupt transmission of infection is mass administration of antifilarial drugs to reduce the reservoir of microfilariae available to the mosquito vectors. The GPELF recommends the use of microfilaria detection and the ICT test to monitor the impact of mass treatment on *W. bancrofti* infection (WHO, 2000) in routine post-MDA surveillance of sentinel sites.

The collection of baseline data is usually the first step before the implementation of any disease control programme. Such data provides epidemiological information that is essential in understanding the distribution, prevalence and intensity of the problem targeted for control or elimination as a public health problem. For lymphatic filariasis, baseline data collection normally includes demographic information, prevalence and intensity of infection (microfilaraemia and antigenaemia) and transmission indices from entomologic surveys. Other indicators of infection and transmission include antifilarial antibody responses. The baseline data forms a basis for longitudinal monitoring of the impact of the intervention strategy employed in the control programme.

Mass treatment of endemic communities with antifilarial drugs under the GPELF is expected to result in significant reduction in filarial parasite prevalence rates and levels. Monitoring the impact of elimination programmes is an important

activity which will provide information required to be used to make changes and improvements that would ensure that the objectives of the GPELF are achieved. Monitoring the impact of lymphatic filariasis elimination programmes requires that samples be collected at regular intervals from sentinel populations during the period of the elimination programme. Such data would be helpful in making critical judgements such as when to stop mass treatment or to change the intervention strategy.

Although the GPELF recommends the use of microfilaraemia and filarial antigen detection by the ICT test to monitor the impact of MDA (WHO, 2000), concern has been raised about the usefulness these tests during the later stages of elimination programmes when the levels of infection decreases (Washington *et al.*, 2004). Alternative tools for monitoring the impact of lymphatic filariasis elimination programmes include antifilarial antibody detection and xenomonitoring of vector mosquitoes using PCR-based assays. Comparative evaluation of the different tools proposed for monitoring of filariasis elimination programmes would be helpful in deciding which tools are most informative.

8.1 Baseline prevalence and intensity of infection

Earlier surveys had shown that bancroftian filariasis is highly endemic in the coastal areas of East Africa (Heisch *et al.*, 1959; Nelson *et al.*, 1962; Wijers, 1977; Abaru *et al.*, 1980; McMahon *et al.*, 1981). Two major surveys representing the entire Kenyan coast from Vanga on the southern coast near the border with Tanzania to Pate Island in the northern coast found the prevalence and intensity of microfilaraemia to vary from place to place but generally greater in the northern than in the southern

coastal areas (Nelson *et al.*, 1962; Wijers, 1977). Four major foci of bancroftian filariasis in the Coast Province of Kenya were identified: the area bordering Tanzania, an area West of Mombasa town, just inland from Kilifi town and along River Sabaki in Malindi District (Wijers, 1977). The present study was conducted in the focus along R. Sabaki.

The available epidemiological data on bancroftian filariasis in the northern coast of Kenya was collected more than 30 years ago (Wijers and Kinyanjui, 1977). It was clear that there was a need for a study from this area particularly because of the current mass treatment campaigns under the GPELF. Baseline epidemiological data is a prerequisite for longitudinal monitoring of filariasis elimination programmes.

Different groups have collected some epidemiological data in coastal Kenya during studies conducted since mid-1990s, but most recent studies were conducted in Kwale District in the south coast region of the Coast Province (Estambale *et al.*, 1994; Wamae *et al.*, 1998; Njenga *et al.*, 2000; Mukoko *et al.*, 2004). The current study is the first to be reported since the studies conducted in the north coast region in early 1970s (Wijers, 1977; Wijers and Kiilu, 1977; Wijers and Kinyanjui, 1977). In addition, the present study was conducted in more inland rural communities living 40 to 60 kilometres from the coast line. The prevalence and intensity of microfilaraemia observed in the present study (20.5% and 526 mf/ml, respectively) are higher than those reported in most areas in Kwale District in the southern coast (Estambale *et al.*, 1994; Wamae *et al.*, 1998; Njenga *et al.*, 2000; Mukoko *et al.*, 2004). Interestingly, the prevalence of microfilaremia in the present study was similar to the figures reported for the district more than 30 years ago (Wijers and Kinyanjui, 1977).

The overall baseline prevalence of circulating filarial antigenaemia by the ICT test was 35.4%. Among 729 individuals tested by both the ICT test and microfilaria detection, 151 and 256 were microfilaraemic and antigenaemic, respectively. Thus, the ICT test detected infection in 105 (14.4%) persons that were not detected using microfilaria detection. Many studies have previously shown that filarial antigen detection is superior to microfilaria detection for identifying infections in endemic areas (Lammie *et al.*, 1994; Wamae *et al.*, 1998). These studies demonstrate that the prevalence of circulating antigen is approximately twice the prevalence of microfilaraemia. Since antigenaemia is a good indicator of active infection the proportion of microfilaria-negative individuals who are antigen-positive is likely due to presence of adult worms without microfilaraemia or low sensitivity of microfilaria detection tests. The reasons why some individuals harbour adult worms in the absence of microfilariae are unknown. Possible reasons include lack of mating or fecundity of adult worms, or an efficient host immune response against microfilariae (Nicolas *et al.*, 1999).

The prevalence of both microfilaraemia and antigenaemia increased with age. Since circulating filarial antigen is an indirect indicator of adult worm loads (Weil *et al.*, 1985; Weil *et al.*, 1990) the age-related increase in prevalence of antigenaemia means that acquisition of infection is also age-related. It is recognised that transmission of lymphatic filariasis by mosquitoes is relatively inefficient and infection generally develops only after prolonged or intense exposure to infective larvae (Hairston and de Meillon, 1968; Gubler and Bhattacharya, 1974; Southgate, 1992). Thus, in areas where the transmission of filariasis is seasonal it may take several years of exposure for patent infection to develop.

The TropBio Og4C3 ELISA test was used to determine antigenaemia in specimens from a cohort that was tested at baseline and after the two rounds of annual mass treatment. The baseline prevalence of antigenaemia in this cohort using the Og4C3 ELISA test and using the ICT test was 40.8% and 37.1%, respectively, but the difference was not significant. There was a very strong correlation between the ICT test and Og4C3 ELISA test ($r = 0.912$). Further, when using microfilaria detection as a gold standard, the sensitivity of the ICT test and Og4C3 ELISA assay was 90.1% and 90.4%, respectively.

The sensitivity of the Og4C3 ELISA test in the present study is lower than has been reported in many studies (Chanteau *et al.*, 1994; Lammie *et al.*, 1994; Wamae *et al.*, 1998; Simonsen and Dunyo, 1999). Interestingly, lowering the cut-off value to 32 units did not change the results of the present study. The sensitivity of Og4C3 ELISA assay has previously been shown to be less than 100% in individuals with low level infections and may be as low as 72 – 75% (Chanteau *et al.*, 1994; Rocha *et al.*, 1996). In the present study, the median count of microfilariae in individuals that were antigen-negative using Og4C3 ELISA test was significantly lower (3 mf/100 µl; range 2-97 mf/100 µl) than in individuals that were antigen-positive (69 mf/100 µl; range 2-2471 mf/100 µl). The implication of this finding is that when the level of infection is very low both antigen tests may not be 100% reliable.

In the present study, the prevalence of antigenaemia and microfilaraemia increased steadily with age but the increase was less pronounced after 50 years of age. Similar observations have been made in other studies conducted in the Kenyan coast (Wijers, 1977; Estambale *et al.*, 1994; Wamae *et al.*, 1998). It is recognized that the dynamics of acquisition of bancroftian filariasis in humans are not well understood

and are probably related to intensity and the duration of exposure (Chanteau *et al.*, 1995). A longitudinal study in a cohort of children in Haiti concluded that filarial infections are acquired early in life (Lammie *et al.*, 1998). The Haitian study led to the hypothesis that initial intensity of infection is low and require some time to build up to detectable levels and that most early infections are characterized by the presence of few unmated adult worms or a single sex of worms. The intensity of microfilaraemia in the present study, however, was higher in children aged 5-16 years than in young adults aged 16-30 years, but the difference was not significant.

It has previously been hypothesized that the adult and microfilarial stages of the parasite provide a form of concomitant immunity by protecting the infected host from establishment of new infections when additional infective larvae are introduced (Day *et al.*, 1991; Maizels and Lawrence, 1991). Previous work on laboratory animal models has shown that animals repeatedly infected with infective larvae develop concomitant immunity that prevents the establishment of later cohorts of infective larvae (Grenfell *et al.*, 1991). The hypothesis on concomitant immunity has been used to explain the convex-shaped prevalence of infection/age curves reported in some studies (Grenfell and Michael, 1992). However, a recent meta-analysis using published studies from India and sub-Saharan Africa concluded that there is no evidence of a general age-prevalence pattern that would correspond to acquired immunity models (Stolk *et al.*, 2004). The age-prevalence patterns in the present study were not truly convex.

The prevalence of infection has been reported to be higher in males than females in most studies. It was hypothesized that this gender-specific difference is hormonal related and that females in the reproductive age have markedly lower levels

of infection (Brabin, 1990). In the present study females in the reproductive age had slightly lower prevalence and intensity of microfilaraemia and antigenaemia than age matched males, but the difference was not significant. In areas where *W. bancrofti* is transmitted by day-biting mosquitoes, such as *Aedes* species, the significantly higher prevalence of infection in males than in females has been attributed to males being more exposed to infection during outdoor activities (Chanteau *et al.*, 1995). Thus based on the results of the present study, we have no reason to conclude that significant gender-differences in the prevalence of infection exist.

8.2 Baseline antifilarial antibody responses

The high prevalence of antifilarial IgG1 (86%) and IgG4 (91%) responses indicates that most people living in endemic areas are exposed to infection. There are conflicting conclusions on whether antifilarial IgG1 is a marker of exposure to infective larvae or a marker of infection. Some studies have reported that active infection is positively correlated with elevated IgG1 (Simonsen *et al.*, 1996), while data from other studies have suggested that antifilarial IgG1 is a marker of exposure to infective larvae (Lammie *et al.*, 1998). The finding that the levels of antifilarial IgG1 were similar between antigen-negative (34.2 μg/ml) and antigen-positive (30.0 μg/ml) persons lends support to the hypothesis that antifilarial IgG1 response is not induced by active infection, but most likely by exposure to infective larvae.

The dominant isotype of antifilarial antibody in infected humans is IgG4, which can represent up to 90% of total IgG, when measured by ELISA against adult worm antigen (Kwan-Lim *et al.*, 1990; Kurniawan *et al.*, 1993). The intensity of antifilarial IgG4 was more than four-fold in antigen-positive individuals (60.0 μg/ml)

compared to antigen-negative persons (13.0 μg/ml). This finding supports the hypothesis that antifilarial IgG4 levels are related to active infection (Lal and Ottesen, 1988; Kwan-Lim *et al.*, 1990; Hitch *et al.*, 1991). Individuals harbouring adult worms have higher filarial-specific IgG4 than adult worm-free individuals independently of the presence of microfilariae (Nicolas *et al.*, 1999). The finding that 91% of the population had positive antifilarial IgG4 response indicates an overestimation of the prevalence of infection when compared with the Og4C3 ELISA test. This may be due to persistence of antibody in individuals in whom pre-adult stages fail to reach adult stage or in those where early infections are spontaneously cleared by the host innate immune response.

Children had elevated levels of antifilarial IgG1 compared to adults. Conversely, the levels of antifilarial IgG4 were higher in adults than in children. The intensity of IgG4 increased gradually with age and reached peak level at around 30 years. It appears that initial exposure to filarial infective larvae results in a heightened antifilarial IgG1 response. The shift in ratio of antifilarial IgG1 to IgG4 with age may be reflecting changes in infection status or some form of immune regulation. It was previously suggested that the antifilarial IgG1 is down-regulated by antifilarial IgG4 (Lammie *et al.*, 1998), which appears to build up at a much slower rate.

Intestinal helminth infections are a potential source of IgG antibodies that cross-react with filarial antigens (Muck *et al.*, 2003). In addition, antibody assays based on crude filarial extracts have been shown to cross-react with most other filarial infections, such as, liaises and onchocerciasis (Maizels *et al.*, 1985). An assay based on detection of IgG4 antibody responses to the Bm14 recombinant antigen has been proposed to reduce cross-reactivity with intestinal helminths. In a study to compare

the performance of antibody assays based on recombinant antigens, the anti-Bm14 IgG4 ELISA assay was shown to have good sensitivity (91%) for *W. bancrofti* infection (Lammie *et al.*, 2004). However, the anti-Bm14 IgG4 assay acted as a 'pan-filaria' assay, having cross-reactivity with *W. bancrofti*, *B. malayi*, *L. loa* and *O. volvulus*.

When using microfilaraemia as the gold standard, the sensitivity of anti-Bm14 IgG4 ELISA assay in the present study (79%) was lower than that reported in the validation study (Lammie *et al.*, 2004). Nonetheless, the observation that the prevalence of IgG4 responses to Bm14 (58%) was higher than prevalence of antigenaemia by Og4C3 ELISA test (41%) suggests that the proportion of persons with active infection could even be higher than estimated by the Og4C3 ELISA assay. It is likely that individuals living in endemic areas acquire infective larvae at one time point or another, but most infections are spontaneously cleared before the parasites reach adult stage. The finding that some persons were anti-Bm14 IgG4 positive and antigen-negative indicates that anti-Bm14 IgG4 responses in some persons may reflect intense exposure to infective filarial larvae. Whether the infective larvae or other pre-adult stages are involved in development of immunity in some individuals is a matter of speculation.

8.3 Baseline xenomonitoring

Xenomonitoring is defined as the collection of wild-caught, blood-engorged vector mosquitoes to detect infection in the human population (WHO, 2002b). The bloodmeal is taken to represent human blood samples in the population. Mosquitoes usually rest on surfaces close to their bloodmeal source after feeding, while they

remove excess fluid from the bloodmeal by diuresis, prior to flying away to secure resting sites for vitellogenesis and subsequent oviposition (Clements, 1999). Thus xenomonitoring is best suited to areas where mosquito vectors rest indoors after bloodmeal. *Wuchereria bancrofti* infections in the blood-engorged mosquitoes that are collected while they rest can be detected either by dissection or by a PCR-based assay for the parasite DNA (Chadee *et al.*, 2002). It was previously shown that detection of parasite DNA in human blood and mosquitoes by PCR is a sensitive and specific method for determining infection rates in endemic areas and thus a powerful new epidemiological tool for evaluation and monitoring of community-based filariasis control programmes (Fischer *et al.*, 1999; Farid *et al.*, 2001).

In the present study, the overall proportion of blood fed vector mosquitoes with parasite DNA (24.9%) was comparable to the overall prevalence of microfilaraemia (20.5%), although there was marked variability among the 4 communities. This observation suggests that prevalence data obtained from blood-engorged mosquitoes could at least be used to identify areas where there is transmission of infection. For standardised sampling of mosquitoes to be used for xenomonitoring, indoor-resting collection of blood-fed females is considered most appropriate. Such samples of *Anopheles*, *Culex* or *Mansonia* can be readily obtained in most situations, with bloodmeals representing the human population in the majority of epidemiological settings (WHO, 2002b).

8.4 Baseline clinical manifestations

Hydrocele and lymphoedema are the most widely recognized clinical manifestations of lymphatic filariasis because of the gross disfigurement that they

cause in affected patients. The prevalence of hydrocele and leg lymphoedema reported in the present study conducted in the north coast area of Kenya is higher than reported in south coast areas (Estambale *et al.*, 1994; Wamae *et al.*, 1998; Njenga *et al.*, 2000; Mukoko *et al.*, 2004). An earlier survey covering the whole of the coastal area indicated that the prevalence rates of infection and chronic disease were higher in communities in the northern than southern coast areas (Wijers, 1977). Hydrocele was the most common clinical manifestation of lymphatic filariasis in this setting. The finding that hydrocele prevalence was more than 55% in males aged > 40 years indicates that bancroftian filariasis is a major cause of morbidity in adult males in the northern coastal areas of Kenya. It appears that the parasite species, host genetic factors and environmental factors may be important in the outcome of chronic filarial disease in an endemic area. In Alor Island, Indonesia, where *Brugia timori* is the aetiologic agent, for example, lymphoedema is the most common clinical manifestation of lymphatic filariasis (Supali *et al.*, 2002), while hydrocele is not a feature of *Brugia* infections.

The prevalence of lymphoedema ≤ stage 2 (non-severe pitting lymphoedema) was higher in males than in females while prevalence of fully developed non-pitting lymphoedema (> stage 2) was higher in females than in males. Although this finding may suggest that females had higher prevalence of advanced disease than males, it is also possible that the difference observed in early form of lymphoedema may due to observer differences because different examiners examined females and males. However, the finding that more males than females had lymphoedema of the arms indicates that the higher prevalence of leg lymphoedema in males than in females may not be an artifact. A study involving 12 communities in Kwale District also reported

higher prevalence of lymphoedema in males than females (Mukoko *et al.*, 2004). In many areas, however, the prevalence of lymphoedema is significantly higher in females than in males (Lammie *et al.*, 1993; Addiss *et al.*, 1995; Njenga *et al.*, 2000; Simonsen *et al.*, 2002), but in other areas there is no significant difference between males and females (Supali *et al.*, 2002). The reason for the differences in prevalence of lymphoedema between males and females among different studies is unclear. Host or parasite factors or both could play a role.

Hydrocele and lymphoedema were mostly prevalent in persons aged > 14 years and the prevalence increased with age. This observation suggests that lymphatic pathology develops due to cumulative damage to the lymphatic vessels. The pathogenesis of lymphatic filariasis is not well understood, but it is thought that damaged lymphatic vessels result in compromised lymphatic function, which may be expressed clinically as lymphoedema or hydrocele (Ottesen, 1992). The application of ultrasonography has demonstrated lymphatic dilatation in virtually all persons with adult filarial worms examined (Dreyer *et al.*, 2000). Further ultrasonographic investigation has shown that dilatation of lymphatic vessels progresses in the presence living *W. bancrofti* adult worms (Dreyer *et al.*, 2002a).

The prevalence and intensity of infection was similar in persons with and without lymphoedema in the present study. Previous reports on the association of lymphoedema and infection, however, have often been conflicting. Some studies have reported that persons with lymphoedema frequently have no detectable microfilaraemia (Lammie *et al.*, 1993; Estambale *et al.*, 1994; Addiss *et al.*, 1995), whereas other studies have shown infection among individuals with lymphoedema, although not as high as seen in hydrocele (Alexander, 2000; Steel *et al.*, 2001;

Simonsen *et al.*, 2002). In the present study, there was a tendency toward higher prevalence and intensity of infection (microfilaraemia and CFA) in persons with hydrocele than those without hydrocele. There seems to be a general agreement that hydrocele is usually associated with infection from studies conducted in other parts of East Africa (Meyrowitsch *et al.*, 1995; Wamae *et al.*, 1998; Simonsen *et al.*, 2002) and other regions (Lammie *et al.*, 1993; Addiss *et al.*, 1995; Steel *et al.*, 2001). Overall, the results of the present study support a meta-analysis that showed no evidence for a negative association between infection and disease (Michael *et al.*, 1994).

Two alternative pathways to the development of lymphatic pathology have previously been suggested: one dependent on the induction of inflammatory reactions by the host immune response and the other entirely independent of the immune system reflecting the direct actions of the parasite or its products on the lymphatic vessels (Ottesen, 1992). The finding that there was no significant difference in prevalence of active infection between persons with and without lymphoedema suggests that filarial parasites may be primarily involved in the development of lymphoedema. The development of lymphoedema, however, has been suggested to be due to a complex interplay of different factors including adult worm death, inflammatory responses to endosymbiotic *Wolbachia* and host immune responsiveness (Lammie *et al.*, 2002).

The finding that persons with hydrocele had a tendency to have higher levels of infection compared to persons without hydrocele seems suggest that the mechanism that leads to development of hydrocele does not cause clearance of infection. This observation lends support to the hypothesis that development of

hydrocele may be due to active infection independent of host immune response. Thus the results of the present study suggest that both hydrocele and leg lymphoedema have similar pathophysiologic basis and the difference in clinical presentation may be related to the location of the parasites.

The finding that leg lymphoedema ≤ stage 2 (mild disease) was higher in males than in females while stage > 2 (advanced disease) was higher in females than males is interesting. The simplest explanation of this finding is that factors that are involved in development of lymphoedema may be different from those involved in its progression to "elephantiasis". Nonetheless, previous work has demonstrated that secondary microbial pathogens play an important role in the progression of lymphoedema (Dreyer *et al.*, 2000). Simple improved hygiene practices using soap and water and skin care have been shown to result in significant reduction in lymphoedema (Addiss and Dreyer, 2000; McPherson, 2003). Thus, the implementation of chronic disease control programmes under GPELF might result in more clinical benefits which could lead to increased motivation of the communities to participate in the elimination programme.

The prevalence of hydrocele has previously been shown to be a good indicator of the prevalence of microfilaraemia (Gyapong *et al.*, 1998). However, a study conducted in 12 communities in south coast of Kenya did not find significant correlation between prevalence of microfilaraemia and hydrocele (Mukoko *et al.*, 2004). In the present study, the prevalence of hydrocele and microfilaraemia in males aged > 14 years were stratified into age groups to generate enough data-points for correlation analyses. There was a significant correlation between prevalence of hydrocele and microfilaraemia ($r^2 = 0.880$, $P < 0.05$). This finding suggests that

examination of hydrocele in males aged > 14 years can augment ICT testing in rapid baseline epidemiological assessment for filariasis especially when determining areas to be included for mass treatment under GPELF.

A relatively high prevalence of inguinal adenopathy was observed in the present study. A previous study involving two adjacent communities in south coast Kenya reported that the prevalence of inguinal adenopathy was significantly higher in a community with high prevalence of microfilaraemia than in the community with low prevalence of microfilaraemia (Wamae *et al.*, 1998). It is possible that inguinal adenopathy is an important clinical manifestation of lymphatic filariasis in endemic areas, but it is currently under appreciated. Although inguinal adenopathy is correlated with prevalence of infection it is considered to be non-specific because other infections such as sexually transmitted diseases also cause similar clinical presentation. In addition, for communities in which the majority of people walk bare footed, a significant number of lymphadenopathies may result from secondary infections obtained through injuries on the feet. This may explain why many studies have not previously reported data on prevalence rates of inguinal adenopathy. Nevertheless, the finding that there was a tendency toward higher prevalence of active infection in individuals with inguinal adenopathy compared to those without in the present study leads to the speculation that the manifestation in this setting was due to *W. bancrofti*.

Histopathological studies seem to suggest that death of adult worms may provoke formation of granulomas with consequent development of lymphadenopathy. A detailed histopathological study of inguinal adenopathy in 58 specimens from patients from an endemic area in Brazil identified a wide range of tissue reactions to

adult worms in the glands (Jungmann *et al.*, 1991). Also, a case report of histological examination of a biopsy from an Indian male patient presenting with inguinal adenopathy demonstrated tissue reaction to degenerating intravascular adult filarial worms (Abdel-Hameed *et al.*, 2004). These histopathological studies suggest that the inflammatory host response that follows adult worm death as seen in the biopsies is a risk factor for the development of adenopathy.

The observation that inguinal adenopathy was also present in young children suggests that lymphatic pathology starts early in life. The recent application of ultrasonography to identify living adult worms presented the opportunity to localize the worms non-invasively in infected patients (Noroes *et al.*, 1996). Ultrasonographic examination of amicrofilaraemic children (two girls aged 6 and 12 years) presenting with chronic inguinal adenopathy in Brazil revealed the presence of living adult worms in their enlarged inguinal nodes (Dreyer *et al.*, 2001). In addition to demonstrating that lymphatic pathology occurs early in life, this study provided evidence that living adult worms (or their products) in the inguinal glands may be involved in the development of adenopathy. The results of the present study together with the observations made in the Brazilian study raise questions as to whether inguinal adenopathy is an important marker of lymphatic filariasis particularly in children.

Like other clinical manifestations of lymphatic filariasis the prevalence of inguinal adenopathy increased with age. In addition, like lymphoedema, the prevalence of adenopathy was higher in males than females. Examination of autopsy materials has previously shown that the lymphatics of the male genitalia and the inguinal region are commonly affected in filariasis (Lichtenberg, 1957; Lichtenberg

and Medina, 1957; Galindo *et al.*, 1962). This observation might explain why the prevalence of inguinal adenopathy was higher in males than females.

8.5 Impact of mass treatment on microfilaraemia

The decrease in prevalence of microfilaraemia after one round of mass treatment in the present study (44.9%) is similar to that in a community treated with a standard 12-day regimen of DEC in Tanzania (Meyrowitsch *et al.*, 1996a). A two-arm filariasis elimination trial in Tamil Nadu State, India reported that annual mass treatment with DEC/albendazole resulted in two times higher reduction of the prevalence of microfilaraemia compared to treatment with DEC alone (Rajendran *et al.*, 2004). The results of the present study seem to support the conclusion that single-dose annual mass treatment with DEC/albendazole is as effective as a standard 12-day DEC regimen (Ottesen *et al.*, 1999). This was one of the major advancements in filariasis research that together with development of newer sensitive and specific diagnostic tools resulted in the realization that lymphatic filariasis is potentially eliminable as a public health problem. Logistically, a single dose is easier to administer during mass treatment compared to a 12-day regimen. Two rounds of MDA of DEC/albendazole decreased the prevalence of microfilaraemia by 65.4% in the present study. The high reduction of microfilaria prevalence after each round of treatment indicates that complete clearance of microfilaraemia might be achieved in the next two or three rounds of mass treatment.

One round of mass treatment resulted in dramatic decrease (91.8%) in intensity of microfilaraemia in persons who were positive at pre-treatment. The administration of the second round of mass treatment decreased the intensity of

microfilaraemia by 95.8% of the pre-treatment level. It appears that the first round of mass treatment results in drastic decrease in levels of microfilaraemia while further rounds of MDA are necessary to achieve sustained reduction. In a study in Papua New Guinea (Bockarie *et al.*, 2002), for example, the first round of mass treatment with DEC/ivermectin decreased intensity of microfilaraemia by 91%, but interestingly three additional annual rounds of MDA decreased microfilaria level by only 4% to 95%. Even in individuals that did not clear microfilariae, significant decrease in intensity of microfilaraemia (75.3%) was observed after the two rounds of mass treatment in the present study. This finding indicates that the microfilaria load of persons who remained microfilaraemic was greatly reduced. The primary goal of the GPELF is to reduce the reservoir of microfilariae in the human population to levels that will result in the interruption of transmission by vector mosquitoes (Molyneux and Zagaria, 2002). The significant decline in levels of microfilaraemia as seen in the present study shows that interruption of lymphatic filariasis in this setting may be feasible.

The effectiveness of DEC and albendazole against adult *W. bancrofti* worms is not well understood. Ultrasonographic visualization of adult worms in scrotal lymphatics after treatment with DEC has shown that at least some adult worms are killed by DEC (Noroes *et al.*, 1997). The recovery of dead and degenerating adult worms in excised nodules appearing after DEC treatment further suggests that DEC has some macrofilaricidal effect (Ottesen, 1985; Figueredo-Silva *et al.*, 1996). Previous studies in laboratory animals have shown that parasite antigenaemia correlates with the number of adult worms in the host (Weil *et al.*, 1985; Weil *et al.*, 1990), and the same is believed to be true in bancroftian filariasis (Ismail *et al.*, 1998).

Thus, the evolution of parasite antigenaemia could be used as a good indicator for monitoring the effect of mass treatment on the adult worm population during lymphatic filariasis elimination programmes.

8.6 Impact of mass treatment on antigenaemia

The effect of mass treatment using DEC/albendazole on overall prevalence of antigenaemia was assessed annually using the ICT test. In addition, the Og4C3 ELISA assay was used to determine the impact of the two rounds of MDA on prevalence and intensity of antigenaemia in a cohort of the study population. The ICT test is a rapid card test that gives a positive/negative result whereas the Og4C3 ELISA assay gives both qualitative and quantitative results. The decline in prevalence of antigenaemia, by ICT test, was 22.3% and 43.5% after one and two rounds of MDA, respectively.

In contrast, the decline in prevalence of antigenaemia after the two rounds of MDA, by Og4C3 ELISA assay, was 13.2%. The relatively low decline in prevalence of antigenaemia suggests survival of some adult worms in many infected persons. This finding emphasizes the importance of repeated mass treatment if elimination of filariasis is to be achieved. Nonetheless, the observation that there was complete clearance of CFA in some individuals in the present study indicates that DEC/albendazole has some effect on *W. bancrofti* adult worms in addition to being highly microfilaricidal.

Of 75 antigen-positive individuals by ICT test at baseline that were antigen-negative by 2004, 61 (81.3%) were still antigen-positive by the Og4C3 ELISA assay. This observation indicates that the sensitivity of the ICT test is lower than that of

Og4C3 ELISA assay when the level of CFA decreases. This observation raises concern about the reliability of the ICT test in determining presence or absence of infection when making decision on whether to stop mass treatment in an area.

The level of circulating filarial antigen decreased significantly by 90.3% in persons that were antigen-positive at baseline by the Og4C3 ELISA assay. Since circulating filarial antigen is an indicator of adult worm burden these observations indicate that there was significant reduction in adult *W. bancrofti* loads, which are the source of microfilariae in the community, after the two rounds of treatment. This finding supports the hypothesis above that DEC/albendazole might be highly effective against adult worms, but complete clearance may require several rounds of treatment. A clinical trial study conducted in Egypt to assess the impact of DEC/albendazole on adult worms *in vivo* using ultrasound reported that 97% of the pre-treatment worm nests were inactive by two years follow-up (Hussein *et al.*, 2004). The decline in levels of antigenaemia observed in the present study is also comparable to the 91.1% reduction by two years post-treatment reported in the clinical study in Egypt.

If transmission is to be interrupted, mass treatment must cover the whole of an area at risk. In many areas of sub-Saharan Africa where onchocerciasis is also endemic, a combination of ivermectin and albendazole is recommended, while in regions where onchocerciasis is not endemic, the co-administration of a single dose of DEC and albendazole or DEC-medicated salt is used (WHO, 2002c). There seems to be conflicting views on the role of albendazole in interruption of transmission in filariasis elimination programmes. A recent review designed to assess the effectiveness of albendazole alone or in combination with either DEC or ivermectin concluded that there is not sufficient evidence to confirm or refute that albendazole

alone, or co-administered with DEC or ivermectin, has an effect on lymphatic filariasis (Addiss *et al.*, 2004). Another recent review reported that the addition of albendazole to DEC or ivermectin does not appear to improve the effectiveness of either drug alone, and therefore may not directly benefit the transmission elimination aspect of the GPELF (Tisch *et al.*, 2005). The review concluded that combination treatments containing DEC (or DEC-medicated salt) are the most effective means of lowering microfilaraemia, but combination treatment with DEC and ivermectin, now not utilised in the GPELF, exhibited the greatest effect on microfilaraemia.

Contrary conclusions have been made in another review designed to assess the efficacy and safety of two-drug regimens used in filariasis elimination programmes (Gyapong *et al.*, 2005). This review concluded that the suppression of microfilaraemia is significantly enhanced by the addition of albendazole to DEC or ivermectin compared to either drug alone. In support of enhanced effectiveness of DEC after addition of albendazole, a community-based study in India reported significantly greater decline in microfilaraemia and antigenaemia in communities treated with DEC/albendazole combination compared to those treated with DEC alone (Rajendran *et al.*, 2004). The results of the present study indicate a relatively high effectiveness of mass chemotherapy using DEC/albendazole combination against *W. bancrofti* infection.

8.7 Impact of mass treatment on antifilarial antibody responses

The length of time required for antifilarial antibodies to clear after implementation of programmes to eliminate lymphatic filariasis is currently unknown. In the present study, the prevalence of antifilarial IgG1 and IgG4 responses decreased

by 44.7% and 13.3%, respectively after two years. Clearance of antifilarial antibody responses has been suggested to be a useful marker of the absence of transmission following implementation of control programmes (Ottesen, 1984; Gao *et al.*, 1994; Rodriguez-Perez *et al.*, 1999). As mentioned above, antifilarial IgG1 appears to be a marker of exposure to infective filarial larvae. Previous work has shown that antifilarial antibody responses develop before onset of antigenaemia thus making them early markers of exposure to filarial larvae and infection (Eberhard and Lammie, 1991; Lammie *et al.*, 1998; Weil *et al.*, 1999), a feature that can be exploited to develop tools for surveillance after cessation of mass drug administration. The decrease in prevalence of antifilarial IgG1 (44.7%) seen in the present study suggests that the antibody may become a useful tool for surveillance after cessation of MDA.

The absence of antibody responses in an appropriately selected population, such as children, would strongly suggest that transmission has been interrupted. Children born after successful interruption of transmission should be antibody negative, while older children and adults may still have evidence of residual antibody reactivity (Lammie *et al.*, 2004). However, it appears that the prevalence of antifilarial IgG4 (a marker of active infection) may take longer time to drop because of the relatively longer period required for complete clearance of adult worms. Thus, monitoring the trends of the prevalence of antifilarial IgG4 may provide an insight on decline in prevalence of *W. bancrofti* adult worm infections.

There is limited information on changes in the intensity of antifilarial antibody responses after implementation of mass treatment under the GPELF. The levels of antifilarial IgG1 and IgG4 decreased significantly by more than 70% in the present study. Although this is an encouraging finding, assays based on antifilarial antibody

responses to crude filarial antigen are limited by cross-reactions with antibody responses to other nematode infections (Muck *et al.*, 2003), especially intestinal helminths. The decline in antifilarial antibody responses to crude worm extract observed in the present study, however, may be a true reflection of the impact of mass treatment on exposure and infection levels because it parallels the decline in intensity of antigenaemia. The decrease in the level of anti-Bm14 IgG4 (85%), for example, was very similar to that for CFA (90%). This is an important finding because anti-Bm14 IgG4 does not suffer from cross-reactivity to intestinal helminth infections (Lammie *et al.*, 2004). Thus measurement of antifilarial antibody levels could be a useful strategy in longterm monitoring of the impact of mass treatment programmes in sentinel sites under GPELF.

8.8 Incident active infections and antifilarial antibodies

Although four incident cases of microfilaraemia were detected in 2004, after two rounds of mass treatment, all were antigen-positive (by Og4C3 ELISA assay) both at pre-treatment and after two rounds of MDA in 2004. This finding highlights concern about the relatively low sensitivity of microfilaria detection by microscopy. Of 274 antigen-negative individuals at baseline that were retested after two rounds of MDA using the more sensitive Og4C3 ELISA assay, only one incident case of antigenaemia was identified. Thus, it appears that the two rounds of mass treatment resulted in almost complete prevention of acquisition of new infections in the study community. This finding provides evidence for the high effectiveness of mass treatment with DEC/albendazole in reducing the transmission of *W. bancrofti*.

Since antibody responses can develop in the absence of demonstrable active infection, detecting incident antibody responses should provide a more sensitive measure of transmission than microfilaria or antigen detection (Lammie *et al.*, 2004). Testing of carefully selected sentinel populations for incident antifilarial IgG1 responses, for example children born after MDA has stopped, is one strategy that could be used for surveillance post-mass treatment.

Despite the very low level of incidence of active infection after two rounds of mass treatment, there was a relatively higher rate of incidence of antifilarial antibody responses. This was particularly interesting for antifilarial IgG4 where 7 of 42 (16.7%) individuals had developed a positive response in 2004. The meaning of these observations is unclear. In contrast to antifilarial IgG4, of 64 antifilarial IgG1-negative persons only 2 (3.1%) had developed a positive antibody response by 2004. Since antifilarial IgG1 is correlated with exposure to infective larvae, this finding provides further evidence that the mass treatment had a high effect on reduction of transmission in the study community.

8.9 Treatment coverage

The treatment coverage in 2002 was higher in the follow-up group (83.4%) than in the whole population (64.4%). This observation underscores the importance of intensive mobilization campaigns before MDA. It is likely that interaction of the follow-up group with the research team during registration resulted in higher mobilization among individuals in this group. Alternatively, persons that agreed to be registered for follow-up studies may be naturally compliant and more likely to take treatment than the rest of the population. More than 90% of the eligible individuals in

the follow-up group received at least one treatment during the two rounds of MDA. However, 9.5% of the individuals did not receive treatment during the two MDAs. Although it is likely that such individuals will be covered in subsequent MDAs, it is important to identify these persons as early as possible so that arrangements can be made to reach them. Three patterns of compliance with MDA namely random, systematic and semi-systematic have been considered possible (Plaisier *et al.*, 2000). Microfilaraemic persons that might systematically remain untreated after the elimination programme could be a reservoir of microfilariae, which can cause resurgence of infection.

8.10 Changes in prevalence of infection and antibody responses by treatment compliance

More than 80% of the individuals in the follow-up group received the two treatments offered during both MDAs compared to 13% that received only one treatment and 4% that were not treated during both MDAs. In general, persons that received two treatments had lower prevalence of infection (microfilaraemia and antigenaemia) and antifilarial antibody responses on follow-up than those who received one treatment. Similarly, decline in the levels of infection and antifilarial antibody responses were higher for persons that received two treatments than for those that received one treatment. These findings indicate that treatment coverage is an important factor in the outcome of mass treatment under GPELF. Simulation models have estimated that four to six annual rounds of MDA with treatment coverage of > 80% are required to reduce microfilaraemia to levels that could interrupt transmission (Plaisier *et al.*, 1998; Norman *et al.*, 2000). Increased social

mobilization is required so that treatment coverage can be increased to at least 80%. It is likely that more than six rounds of mass treatment will be required to interrupt interruption of *W. bancrofti* infection in areas where coverage is lower than 80%. Indeed a recent review on mathematical modelling work suggested that the control of filariasis by mass chemotherapy alone is likely to be considerably difficult to achieve, or would require a substantially longer intervention period, in areas with higher microfilaria prevalence and especially where ivermectin-based treatments are to be administered (Michael *et al.*, 2004). A key conclusion of the study was that in such areas (defined as 2.5% and 5% microfilaria prevalence for the ivermectin/albendazole and DEC/albendazole regimens, respectively), effective elimination or reduction of parasitic infection within a reasonable timeframe would require inclusion of vector-control strategies and increasing the intensity of treatment by increasing the cycles of treatment.

There were no significant changes in the prevalence of infection and antifilarial antibody responses in persons that did not receive treatment. However, there was an unexpected decline in the intensity of infection and antifilarial antibody response in persons that did not receive treatment during the two MDAs. The decrease in levels of infection and antifilarial antibody responses in persons that were not treated is interesting. Although this finding most likely reflects a dramatic decline of *W. bancrofti* transmission, it may also indicate that sustained transmission is required for persistence of high infection levels. A study in Egypt reported that 21% of untreated individuals with CFA were antigen-negative one year later and many subjects with microfilaraemia also spontaneously cleared their infections (Weil *et al.*,

1999). The investigators suggested that clearance of microfilariae and antigenaemia in untreated subjects is likely to be immune-mediated.

8.11 Assessment of impact of mass treatment by xenomonitoring

The proportion of vector mosquitoes containing parasite DNA decreased significantly by 64.7% after the two rounds of mass treatment. This finding supports the view that xenomonitoring could be used for monitoring of filariasis elimination programmes. Since the interruption of transmission is a principal goal of lymphatic filariasis elimination programmes, the availability of tools to monitor the presence or absence of infection in the vector mosquitoes is particularly useful (Williams *et al.*, 2002). Traditionally, mosquito dissection is used to monitor the prevalence of infection in vector populations. Dissection is the most suitable means for monitoring infections in vector populations but becomes increasingly costly and laborious when the prevalence of infection in the mosquito population drops below 1% (Ramzy, 2002; Goodman *et al.*, 2003).

A weakness of the xenomonitoring data in the present study is the relatively low numbers of mosquitoes collected which necessitated replacement of some of the houses selected at pre-treatment when mosquitoes were not found during the post-treatment surveys. This explains the high variability in decline in prevalence of infection in mosquito vectors observed among villages. This finding suggests that the sampling strategy used should be improved to enable collection of sufficient numbers of vector mosquitoes. Previous work has shown that there is a marked seasonal variation in the abundance of mosquito vectors and transmission of *W. bancrofti* in rural areas of East Africa, with a peak occurring during and shortly after the rainy

season (Wijers and Kiilu, 1977; McMahon *et al.*, 1981). For xenomonitoring to become a useful epidemiological tool, it could be important to time mosquito collection activities to coincide with peak mosquito abundance and transmission seasons.

A major advantage of xenomonitoring is that it indirectly gives a 'real-time' assessment of the relative levels of infection in the human population (Williams *et al.*, 2002). Human populations in most settings are opposed to repetitive blood sampling, which makes the use of xenomonitoring an alternative non-invasive epidemiological tool for assessing the impact of mass treatment programmes. The major disadvantage of *Ssp*I PCR assay is the requirement of a central laboratory with a thermocycler, a facility that may be lacking in some endemic areas.

The *W. bancrofti SsP*I PCR assay does not differentiate infective larvae (L3) from the other stages of the parasite (microfilariae, L1 and L2) in the mosquito. The presence of *W. bancrofti* infective larvae in the vector population is a direct measure of transmission because only mosquitoes carrying the infective stage of the parasite are capable of contributing to transmission. Therefore, an ideal PCR assay for monitoring the level of transmission would be one based on L3 specific primers. To circumvent this problem, some studies in onchocerciasis have applied a method that allows mass separation of heads and bodies of vector flies (Yameogo *et al.*, 1999; Guevara *et al.*, 2003). However, *W. bancrofti* infective larvae have been shown to wander between thorax and head and using heads alone for extraction of DNA would lead to an underestimation of infection rates (Hoti *et al.*, 2001). Hence, most studies use whole mosquitoes for estimation of infection rates.

The finding that *An. gambiae* and *An. funestus* are the vectors of bancroftian filariasis has important implications on the potential for successful elimination of infection in the area along R. Sabaki. Anopheline mosquitoes exhibit a vector-parasite relationship known as facilitation where the success rate of ingested microfilariae to yield infective larvae increases as the number of ingested microfilariae increases. However, anopheline mosquitoes, unlike culicines, have well developed pharyngeal armatures so that at low microfilarial densities there are very few undamaged microfilariae left to infect the mosquito (Bryan and Southgate, 1988a). These findings have led to the conclusion that interruption of transmission of *W. bancrofti* is relatively easier to achieve when the local vectors are *Anopheles* and not *Culex* or *Aedes* mosquitoes. The results on decline in levels of infection observed in the present study appear to support this hypothesis.

8.12 Impact of mass treatment on clinical manifestations

Although DEC has been used for mass control of lymphatic filariasis for many years, its effect on clinical manifestations has not been widely investigated. In most control programmes attention has focused on effect of the treatment on microfilaraemia and more recently antigenaemia. The present study evaluated the impact of mass treatment using DEC plus albendazole in a group of people examined at baseline and after two rounds of MDA.

There was an overall 17.6% decrease in the prevalence of hydrocele in this group. Resolution of hydrocele was observed in 26.5% of individuals who had hydrocele at baseline and there were only 3 incident cases. The percent resolution observed in the present study is similar to that observed in a community treated for

one year with DEC-fortified salt, but lower than for communities treated with 12-day, monthly or semi-annually DEC regimens (Meyrowitsch *et al.*, 1996a; Meyrowitsch *et al.*, 1996b).

All the cases of hydrocele that resolved in the present study were in stages 1 and 2. Of the 3 incident cases there was one each in stages 1, 2 and 4, respectively. This finding suggests that early stages of hydrocele are more likely to resolve after treatment than advanced stages. The finding that one incident case was already at stage 4 in 2004 suggests that once hydrocele has developed it can rapidly progress to a more severe stage. It is likely that earlier stages of hydrocele occur as a result of reversible obstruction of the scrotal lymphatics perhaps as a result of inflammatory reaction induced by death of worms or worm products.

The prevalence of leg lymphoedema in a group of individuals examined at baseline and after two rounds of MDA was similar at the two time points. However, there were 5 and 3 cases of resolution and incidence of leg lymphoedema, respectively. Thus, resolution was approximately balanced by incidence resulting in a steady state of leg lymphoedema. All the cases of lymphoedema that resolved and developed were in stages 1 and 2 in the present study. This observation suggests that some of the early stages of leg lymphoedema, as for hydrocele, are likely to resolve after treatment. Previous work has shown that all cases of pitting lymphoedema (stages 1 and 2) could resolve within one year after treatment with DEC, but that lymphoedema greater than stage 2 (variously referred to as elephantiasis) required two to four years to resolve (Partono, 1985). The progression of incident cases of lymphoedema, however, could be much slower than in hydrocele. The finding that DEC/albendazole treatment can result in resolution of hydrocele means that mass

treatment under GPELF might result in significant reduction of morbidity in addition to dramatic decrease in levels of microfilariae.

It is important to continue longitudinal monitoring of the impact of subsequent rounds of MDA in the communities reported in the present study to assess whether additional treatment will result in further decrease in hydrocele and lymphoedema rates. It is also important to determine whether the later stages of disease could also resolve after further rounds of mass treatment. A study conducted in Papua New Guinea reported resolution in 87% and 69% of hydrocele and leg lymphoedema cases, respectively after four rounds of MDA with DEC alone or DEC plus ivermectin (Bockarie *et al.*, 2002), which are the highest reported so far. It will be interesting to find out whether four rounds of mass treatment with DEC/albendazole in the setting of the present study could result in similar results to those obtained in Papua New Guinea.

Resolution of hydrocele or lymphoedema does not appear to be related to clearance of infection. Of 10 cases of hydrocele that resolved, 4 individuals had CFA both at baseline and in 2004 (Table 7.3). Similarly, two of 5 cases of lymphoedema that resolved had CFA at baseline and in 2004. In addition, resolution was observed in some cases that had no signs of active infection. This finding makes it difficult to hypothesize the mechanism by which resolution occurs, but it is likely that DEC has properties that directly or indirectly leads to restoration of the previously damaged lymphatic vessels and improved flow of the lymph. Although DEC has been used for many years in the treatment of lymphatic filariasis, its mode of action is not well understood although previous work has shown the drug to have anti-inflammatory properties (Maizels and Denham, 1992). It has also been speculated that mass

treatment decreases exposure to infective larvae and that this, in turn, diminishes the intensity of inflammatory responses to newly inoculated *W. bancrofti* or their endosymbiotic *Wolbachia* bacteria (Bockarie *et al.*, 2002).

An unexpected result in the present study is the significant increase in the prevalence of inguinal adenopathy after the two rounds of mass treatment. This observation suggest that inguinal adenopathy is most likely related to death and, or injury of the adult worms due to treatment. It is most likely that the dead or dying worms in the lymph nodes were involved in the observed adenopathy. A previous review (Ottesen, 1985) concluded that DEC causes death of adult *W. bancrofti*. Using ultrasonography, a single dose of 6 mg/kg DEC has been estimated to kill up to 50% of adult *W. bancrofti* (Dreyer *et al.*, 1994). Albendazole was first shown to be effective against lymphatic filariasis by a study conducted in laboratory animals infected with *B. malayi* (Mak *et al.*, 1984). The first formal study to establish the effectiveness of albendazole in *W. bancrofti* infections in humans used high doses (400 mg twice daily) given for three weeks to 15 microfilaraemic males (Jayakody *et al.*, 1993). This long-term high-dose albendazole regimen caused significant severe adverse reactions that discouraged further investigations. It is possible that co-administration of DEC and albendazole results in increased macrofilaricidal effects than when either drug is given alone and the increase in inguinal adenopathy could be due to increased worm death.

The present study has provided critical data on the epidemiology of clinical manifestations of bancroftian filariasis in an area targeted for mass treatment. In addition, the present work provided knowledge on dynamics of clinical manifestations during MDA. The clinical benefits observed in the present study mainly as a result of

resolution of hydrocele provide useful information that can be used in programme advocacy.

CHAPTER 9 - SUMMARY AND RECOMMENDATIONS

Development of new diagnostic tools and treatment strategies for lymphatic filariasis has resulted in the realization that the disease can be eliminated as a public health problem. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was established in 2000 following a World Health Assembly (WHA) resolution in May 1997 to eliminate lymphatic filariasis as a public health problem by 2020. Soon after its formation, the GPELF encouraged many countries to initiate pilot scale or national level filariasis elimination programmes. A major boost for the GPELF came in the form of free donations of two antifilarial drugs by major pharmaceutical companies: albendazole (GlaxoSmithKline) and ivermectin (Merck and Co. Inc.). Currently, the biggest challenge for the GPELF is the need to implement mass treatment in as many endemic countries as possible and to upscale efforts in those countries where treatment activities have begun.

With the global implementation of filariasis elimination programmes it is crucial to gather information on the impact of the different treatment strategies in different endemic regions. The outcome of the elimination programmes might be affected by many factors including, efficiency of the local vectors, host and parasite factors, efficacy of treatment strategy applied, treatment coverage and the impact of social mobilisation. Currently, there is limited practical experience of monitoring of the impact of annual mass drug administration under the GPELF. To be able to make decisions about the effectiveness of the treatment strategies employed in GPELF, it is important to identify epidemiological tools that could be used to evaluate the long-term effects of the elimination programmes.

The present study has contributed knowledge in the epidemiology of lymphatic filariasis in a highly endemic area in Malindi, Kenya. A pilot scale elimination programme based on annual mass chemotherapy of DEC and albendazole was implemented in the area in 2002 and the impact of two rounds of treatment has been assessed using different monitoring strategies.

9.1 Baseline epidemiological data

The current estimates of the number of people already suffering from chronic disease are based on limited information, especially for many areas in sub-Saharan Africa where knowledge on the distribution of the disease is less than adequate. Chapter 5 describes pre-treatment investigations of the epidemiology of chronic disease due to *W. bancrofti* in the R. Sabaki area, Malindi District, Kenya. The finding that hydrocele prevalence was more than 55% in males aged > 40 years (Table 5.2) indicates that bancroftian filariasis is a major cause of morbidity in adult males in the northern coastal areas of Kenya. Also, the prevalence of lymphoedema (8.5%) in individuals aged > 14 years (Table 5.6) was relatively high. The clinical results of the present study indicate that lymphatic filariasis is a major public health burden in Malindi District, Kenya. Patients with chronic clinical manifestations especially the advanced stages of hydrocele and lymphoedema suffer a wide range of physical, psychological and social problems as a result of the disfigurement. These patients have limited access to health care facilities and cannot afford cost of surgery for hydrocele repair (hydrocelectomy).

It is important for filariasis elimination programmes to establish the disease rates during collection of baseline data on prevalence and intensity of infection before

implementation of mass treatment so that appropriate morbidity control can be incorporated into the programmes. The district hospitals have the capacity to perform hydrocelectomy but the communities in many filariasis endemic foci are often very poor and cannot afford to pay for the cost of the operation. Arrangements could be made to provide the district hospital with materials required for the surgical operations. Those with lymphoedema and members of their families should be taught on improved hygiene regimens and skin care techniques that have previously been shown to significantly alleviate the suffering and progression of the manifestation.

Chapter 4 presents detailed immunoparasitologic investigations of *W. bancrofti* infection in the R. Sabaki area. The present study is the first to describe the prevalence and intensity of antigenaemia in the north coast of Coast Province, Kenya. The pre-treatment prevalence and intensity of microfilaraemia in the present study (Table 4.2) is higher than previously reported in many areas in the south coast. The high levels of infection indicate that the area along R. Sabaki is still an important focus of bancroftian filariasis. The high baseline prevalence of antifilarial IgG1 (86%) and IgG4 (91%) responses (Table 4.6) indicates that most people living in this endemic area had been exposed to infection or already infected. These findings corroborate the conclusion that the endemicity of bancroftian filariasis in the area is high and that a significant proportion of individuals in Malindi District, Kenya are infected with *W. bancrofti* or already suffering from chronic filarial disease. The epidemiological study provided a baseline platform to be used for longitudinal assessment of mass treatment with DEC and albendazole during a filariasis elimination programme implemented in the area in 2002.

9.2 Impact of mass treatment on transmission of infection

A comprehensive assessment of the impact of two rounds of annual single dose mass chemotherapy with a combination DEC (6 mg/kg) plus albendazole (400 mg) on immunoparasitologic indicators of *W. bancrofti* infection is presented in Chapter 6. The results of the present study lead to the conclusion that the two rounds of treatment had a dramatic impact on transmission of lymphatic filariasis. This conclusion is supported by the following findings; 1) the intensity of microfilaraemia decreased by 95.8% of the pre-treatment level after two rounds of annual mass chemotherapy (Figure 6.3). Also, there was significant decline in the intensity of microfilaraemia in persons that did not clear microfilaraemia 2) the level of circulating filarial antigen in persons that were antigen-positive at baseline decreased significantly by 90% of the pre-treatment level (Figure 6.9) 3) the levels of antifilarial IgG1 and IgG4 decreased significantly by more than 70% (Figures 6.14 and 6.15). Similarly, the level of anti-Bm14 IgG4 declined significantly by 85% (Table 6.4) 4) only one individual had incident infection (antigenaemia) after the two rounds of mass treatment 5) there was significant decline in the intensity of infection and antifilarial antibody responses in individuals that were not treated (Table 6.6) 6) there was a decline in the proportion of mosquitoes positive for *W. bancrofti Ssp*I by PCR after each round of mass treatment (Figure 6.20). Collectively, these findings indicate that the two rounds of mass chemotherapy with DEC and albendazole resulted in a dramatic reduction of the transmission of *W. bancrofti* infection.

The significant decline in the intensity of circulating antigen is an encouraging finding indicating that mass treatment with DEC/albendazole might also be capable of

reducing adult *W. bancrofti* levels, the source of microfilariae in the community. The decline in the prevalence of circulating filarial antigen, however, was relatively low which indicates that complete clearance of adult worms may take several rounds of treatment. The length of time required for circulating filarial antigen to clear after mass treatment with antifilarial drugs is unknown. Longitudinal monitoring of the sentinel communities in the present study may provide important knowledge on the dynamics of adult worm loads during mass treatment with DEC/albendazole.

Individuals that received two treatments had lower prevalence of infection (microfilaraemia and antigenaemia) and antifilarial antibody responses after two years than those who received one treatment (Table 6.5). Similarly, decline in the intensity of infection and antifilarial antibody responses was higher in persons that received two treatments compared to those that received one treatment (Table 6.6). These findings indicate that compliance to treatment is a key factor in the outcome of mass treatment under GPELF. It will not be surprising that some areas will report a significant decline in levels of infection while other areas report lower decline mainly due to significant differences in compliance to treatment. Increased social mobilisation is required to ensure that treatment coverage is increased to at least 80%. More than six rounds of mass treatment might be required to interrupt the transmission of *W. bancrofti* infection in areas where coverage is significantly lower than 80%.

The finding that *An. gambiae s.l.* and *An. funestus* are the vectors of *W. bancrofti* in the area along R. Sabaki has important implications on the potential for interruption of transmission in this important endemic focus. Anopheline mosquitoes exhibit a vector-parasite relationship known as facilitation where the vectors are

efficient above a critical threshold, but below which the well-developed pharyngeal armatures destroy the majority of microfilariae. These findings have led to the conclusion that interruption of filariasis is easier to achieve when the local vectors are anopheline and not culicine mosquitoes. The decline in levels of infection observed in the present study appears to support this hypothesis.

9.3 Epidemiological monitoring tools

The standard test for diagnosis of filarial infection has been detection of microfilaria in the peripheral blood. The advantages of using microfilaria detection include low cost, relative technical simplicity of the test and availability of materials in many endemic countries. Also, observation of the parasites under the microscope provides direct evidence for presence of infection. Disadvantages include the need to collect blood at night in many areas due to nocturnal periodicity of microfilariae in peripheral blood. Another disadvantage of microfilaria detection is that the sensitivity is lower when the density of microfilariae is low. The counting chamber method used in the present study uses 100 µl of fingerprick blood which is significantly higher than the 20 – 60 µl used in thick blood smears.

Previous studies have shown that circulating filarial antigen is a more sensitive measure of infection than microfilaraemia. A major advantage of circulating antigen-based assays is that antigen levels remain diurnally constant. Therefore, blood samples can be collected during the day which is more convenient than collecting blood for microfilaria detection at night. The major disadvantage of circulating filarial antigen tests is cost. Comparative evaluation of the two currently available antigen tests, the Og4C3 ELISA assay and the ICT test, has provided new information on the

performance of the two tests in the context of a filariasis elimination programme. Although both tests had similar sensitivities at pre-treatment (Section 4.4), the sensitivity of the ICT test was lower after two rounds of mass treatment (Section 6.4.1), presumably when the levels of circulating antigen decline. This finding indicates that the ICT test will be less reliable for identifying individuals with low-level infection after several rounds of mass treatment during filariasis elimination programmes. Such an underestimate of the prevalence of infection could inadvertently lead to stopping mass treatment before complete elimination is achieved.

The results of the present study suggest that the more sensitive Og4C3 ELISA assay may be more useful when making the critical decision to stop annual mass treatment. The assay could be used to determine impact of MDA using specimens collected at pre-treatment, at a mid-term time point and after the final round of mass treatment. Another advantage of the Og4C3 ELISA assay over the ICT test is that it gives quantitative measurements which are useful in assessing the impact of treatment on the density of adult *W. bancrofti* in a community.

As many countries move towards completion of the proposed 4 to 6 rounds of mass treatment under GPELF there is a need for development of sensitive and specific markers of exposure to filarial infection that could provide data to be used to confirm interruption of transmission. In the present study, the level of antifilarial IgG1 was similar in individuals with and without antigenaemia (Figure 4.8). Also, the pre-treatment level of antifilarial IgG1 response was higher in children than in adults (Figure 4.7). These findings strongly argue that antifilarial IgG1 antibody is more of a marker of exposure than for active infection. These are important findings for the GPELF because they indicate that antifilarial IgG1 could be used as a proxy for

monitoring the impact of mass treatment on transmission of *W. bancrofti* during elimination programmes. A major limitation of antibody-based methods is the requirement for venous blood samples for serum. Communities in many areas are opposed to repeated blood sampling, especially where venous blood is required, which can result in significant loss due to follow-up in longitudinal studies. An alternative is to collect fingerprick blood for serum because fingerprick blood sampling is generally more acceptable compared to venous blood collection.

As for circulating filarial antigen, the length of time required for antifilarial antibodies to clear after the implementation of mass treatment to eliminate lymphatic filariasis is currently unknown. The decline in the prevalence of antifilarial IgG1 by 45% after two rounds of mass treatment (Figure 6.12) compared to 13% for antifilarial IgG4 (Figure 6.13) was higher than expected. This finding provides further support for the hypothesis that antifilarial IgG1 is a marker of exposure to infection. Longitudinal monitoring of the follow-up populations in the current study would provide information on clearance of antifilarial antibodies and their suitably for measuring the impact of mass treatment when infection is low.

The decrease in the level of anti-Bm14 IgG4 (85%; Table 6.4) was very similar to the decline in levels of antigenaemia (90%; Figure 6.9) and intensity of anti-Bm14 IgG4 in antigen-positive individuals was more than 16-fold higher compared to antigen-negative persons (Figure 4.10). This is an important finding because unlike antifilarial IgG4 to crude parasite extract, anti-Bm14 IgG4 does not suffer from cross-reactivity to intestinal helminth infections. This means that the anti-Bm14 IgG4 ELISA assay could provide a suitable antibody-based assay for

monitoring active infection during filariasis elimination programmes. However, the Bm14 recombinant antigen is currently not readily available.

The apparent early and strong antifilarial IgG1 response in children compared to adults suggests that monitoring of children born after the implementation of the elimination programme could be used for surveillance of resurgence of transmission. Incident antifilarial IgG1 in such a carefully selected group would imply that there is presence of renewed transmission.

In the present study, the overall proportion of blood fed vector mosquitoes with parasite DNA (24.9%) was comparable to the overall prevalence of microfilaraemia (20.5%), although there was marked variability among the 4 communities. This finding suggests that xenomonitoring could be a valuable tool for rapid identification of communities to be included for mass treatment. Although the *Ssp*I PCR assay used is sensitive and specific for *W. bancrofti*, the disadvantage of this strategy is that many laboratories in endemic areas may not be able to perform the assay because it requires a significant amount of technical expertise and the equipment might not be available.

9.4 Impact of mass treatment on chronic filarial disease

Results on the assessment of the impact of mass chemotherapy on chronic disease due to *W. bancrofti* infection are presented in Chapter 7. The clinical benefits observed in the present study mainly as a result of resolution of hydrocele are very encouraging. Resolution of hydrocele was observed in 26.5% of males aged more than 14 years after the two rounds of mass chemotherapy (Section 7.1.1). The results indicate that mass treatment has direct benefits to patients with lymphatic disease.

This is an important observation which can be used by the GPELF in terms of programme advocacy. It is anticipated that additional rounds of treatment will result in further resolution of lymphatic pathology due to reduction of adult worm loads. Individuals that resolve lymphatic pathology after mass treatment could be identified and used as case examples during mobilization campaigns to improve community compliance to mass treatment.

There was no significant decline in lymphoedema and advanced stages of hydrocele in the present study. The principal objectives of the GPELF are to interrupt transmission of infection and alleviate the suffering of those already having chronic disease. It is therefore important to find ways of offering assistance to those individuals that already have hydrocele or lymphoedema. For hydrocele, the elimination programme could undertake to pay for the costs of surgery at the district hospital. Alternatively, programme managers can identify and invite philanthropic urologists to perform hydrocele surgeries. Implementation of improved hygiene regimen for those affected by lymphoedema should be considered to prevent advancement of the disease process.

Previous studies have implicitly suggested that inguinal adenopathy in lymphatic filariasis-endemic areas might be related to the presence of adult worms in the lymph nodes. The significant increase in the prevalence of inguinal adenopathy after mass chemotherapy is unclear (Figure 7.1). However, it could be related to increased death of adult worms due to the effect of the antifilarial treatments. We recommend that further long-term follow-up be conducted in the communities in the present study to enable us to get more knowledge on the evolution of the inguinal adenopathy.

In conclusion, the present study has provided important information on the dynamics of bancroftian filariasis during a period of annual mass treatment conducted under the GPELF. The observation that the levels of microfilaraemia, antigenaemia, antifilarial antibody responses and proportion of infected mosquitoes declined in parallel is an encouraging finding for the GPELF suggesting that elimination of filariasis may be feasible. This observation indicates that these parameters could be used to monitor impact of mass treatment during programmes to eliminate lymphatic filariasis. In addition, the present work provided knowledge on the dynamics of clinical manifestations during filariasis elimination programme.

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APPENDICES

APPENDIX A

INFORMED CONSENT EXPLANATION FORM FOR PERSONS BELOW 18 YEARS

Participation information: Your child/dependent is asked to participate in a medical research study on filariasis. It is important that you understand the following general principles that will apply to all participants in the study:

1. Participation is entirely voluntary.
2. You may withdraw your child from this study at any time without penalty or loss of benefits.

Purpose of study: Filariasis is a disease caused by parasites that are transmitted during mosquito bites. After transmission to human beings, the parasites invade and grow in the lymphatic vessels. If not diagnosed and treated promptly, filariasis can lead to severe disability due to the resulting lymphoedema and hydrocele. The recommended treatment is a combination of two drugs namely, DEC and albendazole given once a year to all individuals living in endemic areas. Currently, different methods are used to evaluate and monitor the effectiveness of mass treatment during filariasis control programmes. The current study aims to compare the effectiveness of different methods that have been proposed. The results of this study will provide the Ministry of Health with information that will become very useful during filariasis elimination programmes to be launched in different parts of the country.

Procedures to be followed: The study nurse or technician will prick your child's finger for a drop of blood to find out if it has filariasis parasites. He/she may be requested to provide 2 ml of blood (½ teaspoon) from the arm on 3 other occasions during the next 3 years. The blood will not be tested for HIV and unless you request, the results of the various tests will not be returned to you.

Risks: The risk from participation in this study is minimal. Blood drawing may cause a slight pain and possibly a bruise at the site where blood is drawn.

Benefits: Your child's blood will be checked for filariasis parasites. You and the rest of the community will be given mass treatment free of charge.

Assurance of confidentiality: Your child's name and other records about him/her will remain confidential and will not appear when we present this study or publish its results. You will receive copies of the consent explanation and agreement forms.

Contact: If you have any questions relating to this study, please contact Mr. Sammy M. Njenga, Kenya Medical Research Institute (KEMRI), P. O. Box 54840, Mbagathi Road, Nairobi; Telephone 02-722541.

I acknowledge that this consent form has been fully explained to me in a language that I understand and agree to allow my child/dependent to participate in the study.

Parent's or guardian's name: _____

Parent's or guardian's signature or thumb print: _____ Date: _____

Child's name: _____

Study No.: _____

Name of witness: _____

Signature of witness: _____

Investigator's signature: _____

I certify that this is an accurate and true translation.

Translator's Name: _____

Translator's signature:_____ Date: _____

Address: _____

_____

_____

_____

APPENDIX B

INFORMED CONSENT EXPLANATION FORM FOR PERSONS AGED 18 YEARS AND ABOVE

Participation information: You are asked to participate in a medical research study on filariasis. It is important that you understand the following general principles that will apply to all participants in the study:

1. Participation is entirely voluntary.
2. You may withdraw from this study at any time without penalty or loss of benefits.

Purpose of study: Filariasis is a disease caused by parasites that are transmitted during mosquito bites. After transmission to human beings, the parasites invade and grow in the lymphatic vessels. If not diagnosed and treated promptly, filariasis can lead to severe disability due to the resulting lymphoedema and hydrocele. The recommended treatment is a combination of two drugs namely, DEC and albendazole given once a year to all individuals living in endemic areas. Currently, different methods are used to evaluate and monitor the effectiveness of mass treatment during filariasis control programmes. The current study aims to compare the effectiveness of different methods that have been proposed. The results of this study will provide the Ministry of Health with information that will become very useful during filariasis elimination programmes to be launched in different parts of the country.

Procedures to be followed: The study nurse or technician will prick your finger for a drop of blood to find out if it has filariasis parasites. You may be requested to provide 2 ml of blood (about ½ teaspoon) from your arm on 3 other occasions during the next 3 years. The blood will not be tested for HIV and unless you request, the results of the various tests will not be returned to you.

Risks: The risk from participation in this study is minimal. Blood drawing may cause a slight pain and possibly a bruise at the site where blood is drawn.

Benefits: Your blood will be checked for filariasis parasites. You and the rest of the community will be given mass treatment free of charge.

Assurance of confidentiality: Your name and other records about you will remain confidential and will not appear when we present this study or publish its results. You will receive copies of the consent explanation and agreement forms.

Contact: If you have any questions relating to this study, please contact Mr. Sammy M. Njenga, Kenya Medical Research Institute (KEMRI), P. O. Box 54840, Mbagathi Road, Nairobi; Telephone 02-726125.

I acknowledge that this consent form has been fully explained to me in a language that I understand and agree to participate in the study.

Participant's name: _____

Participant's signature or thumb print: _____ Date: _____

Study No.: _____

Name of witness: _____

Signature of witness: _____

Investigator's signature: _____

I certify that this is an accurate and true translation.

Translator's Name: _____

Translator's signature:_____Date: _____

Address: _____

_____

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APPENDIX C

CLINICAL EXAMINATION FORM

DISTRICT: _____ SUB-LOCATION: _____

VILLAGE:_____

DATE: _____ H/H No. _____ M. NO. _____

NAME: _____ AGE: ____(yrs) SEX: M F (circle)

RESIDECY PERIOD: ____(yrs). PREVIOUS RESIDENCY: _____

CLINICAL EXAMINATIONS

SIGNS	STAGE													
	I		II		III		IV		V		VI		VII	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Groin glands														
Hydrocele														
Leg lymphoedema														
Scrotal lymphoedema														
Other signs: (breasts, arms etc.)														
Remarks														

LEGENDS TO CLINICAL STAGES

Inguinal glands:

Stage I: Approx. 3 cm, often visible under skin

Stage II: > 3 cm, always visible

Stage III: Hanging groin (skin folded under gland)

Hydrocele:

Stage I: Testis 6–8 cm in length (longitudinal axis)

Stage II: Testis 8-11 cm in length

Stage III: Testis 11 - 15 cm in length

Stage IV: Testis >15 cm in length

Scrotal lymphoedema:

Stage I: Skin hardened and thickened

Stage II: Fully developed scrotal elephantiasis

Leg lymphoedema:

Grade I: Swelling spontaneously reversible at night (patient to be asked).

Grade II: Skin is normal but swelling does not disappear at night.

Grade III: Skin has shallow folds.

Grade IV: Skin has irregular growths.

Grade V: Skin has deep folds.

Grade VI: Skin has moss-like growths.

Grade VII: Patient requires assistance with daily activities.

R = right; L = left

APPENDIX D

Antifilarial Immunoglobulin (Ig) G1 and IgG4 ELISA Buffers

1. To make 500 ml of bicarbonate coating buffer, 0.1 M $NaHCO_3$, pH 9.6

FW of $NaHCO_3$ = 84.01
Thus, 1 M $NaHCO_3$ = 84.01 g in 1000 ml distilled water (or 42 g in 500 ml)
Therefore, 4.2 g in 500 ml d.H_2O = 0.1 M $NaHCO_3$
Add 4.2 g into about 400 ml d.H_2O
Adjust pH to 9.6 (using conc HCl)
Add distilled H_2O to make volume 500 ml
Filter through 0.2 micron filter and store at +4 °C

2. To make 500 ml of 10% Diethanolamine (DEA) buffer, 3 mM $MgCl_2$, pH 9.8

Add 50 ml of DEA to about 400 ml of distilled H_2O (warm in water bath if crystallized).
Adjust pH to 9.8
Add 1.5 ml of 1 M $MgCl_2$
Add distilled H_2O to make volume 500 ml
Filter through 0.2 micron filter and store at +4 °C

3. To make 1 L of blocking solution, 0.01M PBS/0.3% Tween 20, pH 7.2

Ready to use Phosphate Buffered Saline (PBS) solution was provided by CDC.
Add 3 ml of Tween 20 to 1 L of 0.01 M PBS, pH 7.2
Filter through 0.2 micron filter and store at +4 °C

4. Sample diluent/washing solution, 0.01M PBS/0.05% Tween 20, pH 7.2

Add 500 µl of Tween 20 to 1 L of 0.01M PBS, pH 7.2