Studies on the immunoepidemiology of bancroftian filariasis in East Africa

PhD Thesis

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DEDICATION

To my family – wife Akinyi and children Rehema and Imani – and to our families the Jaokos and the Simbiris

Table of Contents

Acknowledgment iv
Abstract vi
Glossary ix

Chapter 1: General overview 1

Introduction 2
Justification for the study 4
Objectives 5

Chapter 2: Background and literature review 7

Introduction 8
Biology and life cycle 8
Clinical presentation 10
Diagnosis of infection and disease 14
Patterns of infection and disease in the endemic populations 16
Patterns of infection and disease in individuals from non-endemic areas 17
Socio-economic and psychological consequences of bancroftian filariasis 17
Treatment of bancroftian filariasis 18
Prevention and control 19
Immunoepidemiology 20

Chapter 3: Infection, disease and filaria-specific antibody response patterns in two communities with high and low endemicity of *Wuchereria bancrofti* infection 25

Introduction 26
Materials and methods 26
Results 33
Discussion 47
Conclusions 54

Chapter 4: Filaria-specific antibody responses in two communities with high and low endemicity of *Wuchereria bancrofti* infection. I. Antibody profiles in relation to infection and clinical status 55

Introduction 56
Materials and methods 56
Results 57
Discussion 72
Conclusions 78

Chapter 5: Filaria-specific antibody responses in two communities with high and low endemicity of *Wuchereria bancrofti* infection. II. Antibody profiles in relation to infection and age 79

Introduction 80
Materials and methods 80
Results 81
Discussion 97
Conclusions 100

Chapter 6: Filaria-specific antibody responses in two communities with high and low endemicity of *Wuchereria bancrofti* infection. III. Antibody profiles in relation to infection and gender 103

Introduction 104
Materials and methods 104
Results 105
Discussion 125
Conclusions 128

Chapter 7: *Wuchereria bancrofti* in a community with seasonal transmission: stability of microfilaraemia, antigenaemia and filaria-specific antibody levels 131

Introduction 132
Materials and methods 132
Results 133
Discussion 140

Chapter 8: Conclusions and recommendations 143

Conclusions 144
Recommendations 145

References 147

Appendices 165

Appendix 1: An overview of published studies on filaria-specific antibody levels in relation to disease status in lymphatic filariasis endemic areas 166

Appendix 2: An overview of published studies on filaria-specific antibody levels in relation to infection status in lymphatic filariasis endemic areas 169

Appendix 3: An overview of published studies on antibody (filaria-specific and total IgG) levels in relation to age in lymphatic filariasis endemic areas....... 172

Appendix 4: References for Appendices 1-3 173

Appendix 5: Questionnaire used to obtain demographic information and medical history from participants in the study 175

Appendix 6: ELISA kit for detecting and quantifying *Wuchereria bancrofti* antigen in serum samples 176

Appendix 7: Buffers and substrate solution used in ELISA for the detection of filaria-specific antibodies 178

Appendix 8: A simple and quick method for enhanced detection of specific IgE in serum from lymphatic filariasis patients 179

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Abstract

This study assessed the effect of transmission intensity on the patterns of infection, disease and specific antibody response in bancroftian filariasis, by comparing observed patterns of infection, disease and specific IgG1, IgG2, IgG3, IgG4 and IgE profiles in two communities with high and low *Wuchereria bancrofti* endemicity. The communities were Masaika in Tanga Region, Tanzania, which was highly endemic for bancroftian filariasis, and Kingwede in Kwale District of Kenya, which had low endemicity. Detailed analyses of specific antibody responses were carried out in relation to infection and clinical status, age and gender. An additional smaller part of the study investigated if seasonal variation in transmission intensity influenced the stability of infection and specific antibody responses.

The larger part of the study was cross-sectional and included all consenting individuals aged 12 months and over. From each individual, demographic information and medical history was obtained, followed by clinical examination and blood sampling. Finger-prick samples were examined for microfilarie (mf) by counting chamber method, and venous samples were analysed for circulating filarial antigen (CFA) by the Trop Bio commercial kit for detecting *W. bancrofti* circulating antigen in serum, and for filaria-specific antibodies (IgG1, IgG2, IgG3, IgG4 and IgE) using ELISA technique. Mean intensities of mf, CFA and filaria-specific antibodies were all calculated as geometric means.

Overall, mf and CFA prevalence and mean intensities were significantly higher in Masaika than in Kingwede. In Masaika but not in Kingwede, mf and CFA mean intensities were significantly higher in males than in females. This was mainly due to gender differences in the 15-39 year age group. In both communities, infection prevalence was higher, although not significantly, in children of infected parents than in children of non-infected parents.

Chronic filarial disease manifestations (hydrocele and elephantiasis) among adults were more prevalent and presented earlier in Masaika than in Kingwede. The proportion of individuals reporting having experienced acute adenolymphangitis attacks during the one-year period preceding the survey was also significantly higher in Masaika than in Kingwede, and was higher in adults than in children, although this difference was statistically significant only in Masaika.

Overall, prevalence and mean intensities of IgG1, IgG2, IgG4 and IgE were significantly higher in Masaika than in Kingwede. The opposite pattern was seen for IgG3.

Antibody profiles were analysed in relation to clinical and infection status of the individuals in Masaika, but not in Kingwede where individuals with chronic disease were too few for such analysis. The profiles were similar in asymptomatic and chronic disease individuals. There was a highly significant association between antibody profiles of all the measured antibodies and infection status. IgG1 and IgG2 were more associated with mf status than with CFA status and IgG3 and IgG4 were more associated with CFA status than with mf status, while IgE was associated with

both mf and CFA status. These associations were not significantly influenced by clinical status.

Due to few chronic filarial disease cases in Kingwede, inter-community antibody profile comparison was restricted to asymptomatic individuals. In Masaika, IgG1 prevalence and intensity were significantly higher among mf negative individuals than among mf positive individuals. The opposite pattern was seen in Kingwede where both IgG1 parameters were highest among the mf and CFA positive and lowest among the mf and CFA negative. In Masaika, IgG3 profiles were associated with both mf and CFA, while in Kingwede they were more associated with mf than CFA. Furthermore, although in Masaika IgG2 and IgE were significantly associated with mf status, in Kingwede their profiles were uniform in all infection groups. Only IgG4 profiles were similar in the two communities, being highest among CFA positive individuals and lowest among CFA negative individuals.

Age-specific antibody intensity patterns for IgG1, IgG4 and IgE were similar in both communities. IgG1 and IgE decreased with age while IgG4 increased with age. IgG2 and IgG3 profiles differed between the communities. IgG2 intensity decreased with age in Masaika, but increased with age in Kingwede. IgG3 intensity remained uniformly low with age in Masaika but increased with age in Kingwede.

Despite clear gender differences in mf and CFA intensities in Masaika in the female reproductive age group, there were no clear gender differences in antibody intensities in this age group. IgG3 intensities were in general significantly higher among mf or CFA positive females than among their male counterparts. The opposite was seen for IgG4 intensities.

Overall, mean IgG4/IgE ratio was significantly higher in Masaika than in Kingwede. In Masaika, the ratios were higher among the chronic diseased than the asymptomatic individuals in each infection group. These findings contrast what is expected if this ratio indicates infection resistance level and if IgE mediates chronic filarial disease pathogenesis, as has been suggested.

These results suggest that transmission intensity influences levels and patterns of infection, disease and specific antibodies, and the association between infection intensity and gender, and that antibody responses are more associated with infection status than disease status. The study further suggests that the measured specific antibodies are not the basis for the observed gender differences in infection intensities in the female reproductive age group.

The last part of the study was longitudinal. A selected population of 37 CFA positive males aged 20 to 40 years participated. Blood samples from each individual were examined for mf, CFA and specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies at the beginning of the study, and at 6 and 12 months later. The time points corresponded to high, low and high transmission seasons, respectively. Transmission intensity during the study year was assessed entomologically by catching, dissecting and examining mosquito vectors for infective larvae.

W. bancrofti transmission was found to be seasonal, with highest intensities during the rainy season and lowest during the dry season in concert with mosquito vectors abundance. Despite the marked seasonal variation in transmission potential, no statistically significant variation was observed in the mf, CFA, measured filaria-specific antibody levels or IgG4/IgE ratios, suggesting that seasonal transmission may not result in seasonal fluctuations in the levels of infection, measured immune responses or resistance to infection.

Glossary

ADL	Acute adenolymphangitis
ADLA	Acute dermatolymphangioadenitis
AFL	Acute filarial lymphangitis
CFA	Circulating filarial antigen
DEC	Diethylcabarmazine
ELISA	Enzyme linked immunosorbent assay
GLM	Generalised linear modeling
GMI	Geometric mean intensity
HRP	Horseradish-peroxidase
IFN	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
Mf	Microfilaria/e
mM	Milimoles
OD	Optical density
OPD	Ortho-phenylene-diamine
PBS	Phosphate buffered saline
Prevalence of positivity	% with OD values above mean +2 SD of the control
S.D	Standard deviation
S.E	Standard error
Th	T helper cells
TPE	Tropical pulmonary eosinophilia
µl	Microlitre
WHO	World Health Organization

Chapter 1

General overview

Introduction

Bancroftian filariasis, resulting from infection with the mosquito borne filarial nematode *Wuchereria bancrofti*, is one of the major causes of morbidity in many tropical developing countries (WHO 1992), often debilitating and disfiguring (WHO 1994). It is estimated that at least 115 million people are infected worldwide, with more than one third of these living in sub-Saharan Africa (Michael & Bundy 1997; WHO 1994). The disease is a major public health problem along the Indian Ocean coast of eastern Africa, where it occurs with high endemicity (Wijers 1977a; McMahon *et al.* 1981; Estambale *et al.* 1994a; Meyrowitsch *et al.* 1995a; Simonsen *et al.* 1995a).

The natural history of development of infection and disease in bancroftian filariasis still remains unclear (WHO 1992; Michael *et al.* 1994). Thus, no simple direct relationship appears to exist between manifestation of disease and occurrence of infection (Bundy *et al.* 1991; Michael *et al.* 1994; Addiss *et al.* 1995; Simonsen *et al.* 1995a; Dreyer *et al.* 1996a). Microfilaraemia, until recently the most reliable marker of current infection, appears to have no simple correlation with the presence of overt disease. In endemic areas, several major categories of individuals, based on their microfilaraemia status and clinical presentation, can be recognised, namely those with disease manifestations with microfilaraemia, those with disease but without microfilaraemia, those with microfilaraemia but without disease, and finally those without microfilaraemia and without disease, the so-called 'endemic normals'. The proportions of people in these different categories vary from one endemic area to another (Day 1991; Meyrowitsch *et al.* 1995a; Simonsen *et al.* 1995a; Dzodzomenyo *et al.* 1999).

Previous studies have indicated that individuals with different infection status and different categories of clinical manifestations of bancroftian filariasis differ in the type and strength of the specific immune responses. Thus individuals with chronic disease have generally been seen to have higher specific IgG1, IgG2 and IgG3 levels but lower specific IgG4 levels (Ottesen *et al.* 1982; Hussain *et al.* 1987; Ottesen 1992; Kurniawan *et al.* 1993; Yazdanbakhsh *et al.* 1994b) and higher T-cell proliferative reactivity to parasite antigen (Ottesen 1984; King *et al.* 1993) than asymptomatic microfilariae positive individuals. Besides, uninfected individuals with chronic disease show a significantly elevated Th1-like response with high interferon gamma (IFN-γ) and interleukin 2 (IL-2) levels, but a low Th2-like response with low IL-4 and IL-5 levels (Nutman *et al.* 1987a; King *et al.* 1993; Almeida *et al.* 1996, 1998). The opposite is seen in asymptomatic but infected individuals. Despite this information on immune responses in bancroftian filariasis, two key questions remain unanswered. Firstly, do these immune responses play a role in regulating infection and in particular the development of acquired immunity? Secondly, do these responses play a role in the development of chronic disease pathology?

There is relatively little direct evidence that protective immunity exists in human bancroftian filariasis. Indirect evidence for protective immunity, often cited in literature, is based on convex prevalence/age curves which suggest that individuals

develop at least partial immunity to new infections after years of exposure to the parasite (Anderson & May 1991; Grenfell & Michael 1992; Michael & Bundy 1998; Weil *et al.* 1999). Further evidence comes from the observation that some individuals from endemic areas apparently develop immunological resistance to infective larvae (Freedman *et al.* 1989; Day *et al.* 1991a) and microfilariae (Simonsen 1983, 1985; Simonsen & Meyrowitsch 1998).

Several reasons underlie the uncertainty of the role of the observed immune responses in acquired immunity and in chronic disease pathogenesis. Firstly, investigating immune responses in individuals by clinical categories makes it difficult to establish cause and effect. Thus, it is not clear whether the immune responses seen lead to the clinical condition associated with them, or vice-versa.

Secondly, longitudinal studies, which are the ideal prerequisite for understanding infection, development of disease and acquired immunity to any pathogen, are difficult to implement in filariasis because of the longevity of infection. The alternative approach would be to examine re-infection rates and intensities in different ages after chemotherapy with a drug that kills adult worms. This has been done successfully for schistosomiasis and has provided strong evidence that humans acquire resistance to re-infection with increasing age and that this resistance is immune mediated (Hagan *et al.* 1991; Woolhouse *et al.* 1991; Dunne *et al.* 1992; Roberts *et al.* 1993). However, similar studies in bancroftian filariasis are hindered by the fact that diethylcarbamazine (DEC), the classical drug for treatment, does not kill all the adult worms (Richards *et al.* 1991; Weil *et al.* 1991; Kazura *et al.* 1993; Dreyer *et al.* 1994), and therefore re-infection data would be difficult to interpret. The alternative drug, ivermectin, which has recently become available, apparently has no adulticidal effect (Dreyer *et al.* 1996b; Eberhard *et al.* 1997).

Thirdly, a sensitive marker of infection is a prerequisite in studies on the interrelationship between infection, disease and host responses, but it is still not possible accurately to quantify the adult worm burden in bancroftian filariasis with currently available methods. Studies of *W. bancrofti* infection dynamics have generally been based on microfilariae (mf) counts in blood, which at best is a crude indirect measure of underlying adult parasite burden (Grenfell *et al.* 1990). Diagnosis based on mf detection is furthermore hampered by the existence of a large proportion of mf negative but infected individuals (Chanteau *et al.* 1994a; Dreyer *et al.* 1996a; Rocha *et al.* 1996; Weil *et al.* 1996). Hopefully, this impediment can be overcome by using newly developed diagnostic techniques based on circulating filarial antigen detection. Several of these techniques with extremely high sensitivity and with qualities for different purposes and settings (Turner *et al.* 1993; Chanteau *et al.* 1994b; Weil *et al.* 1996; Lalitha *et al.* 1998; Simonsen & Dunyo 1999; Pani *et al.* 2000) have recently become commercially available.

Fourthly, the observed immune responses have previously often been interpreted based on a static concept of infection and disease development (Ottesen 1989, 1992) which conflicts with more recent evidence suggesting that these processes as well as the associated immune responses may be dynamic entities (Bundy *et al.* 1991a; Maizels & Lawrence 1991; Grenfell & Michael 1992; Michael & Bundy 1998;

Michael 1999). In the static concept, the differing host responses and the different parasitological and clinical expressions observed reflect host predisposition and are a fixed characteristic of the individual. Thus, the population in an endemic area consists of clusters of individuals with differing responses to infection, which may result in the clearance of mf, resistance to further infection, immunopathological damage or some combination of these effects. On the other hand, the dynamic concept which is derived from mathematical analyses of epidemiological data, suggests that the processes involved in infection and progression to lymphatic pathology as well as anti-filarial immune responses are dynamic entities. According to this, endemic individuals pass through a sequence of events from a microfilaraemic asymptomatic state to subsequent disappearance of the microfilaraemia and development of pathology. The progression from microfilaraemia to amicrofilaraemia and to obstructive disease corresponds to low, medium and high levels of immune responsiveness (Bundy *et al.* 1991a). This dynamic hypothesis of development of infection and disease in lymphatic filariasis therefore implies that in addition to clinical categories, a community approach investigating age-related changes in immune responses within communities is required in order to determine the role of immunity in moderating infection and disease in filariasis.

Finally, the uncertainty concerning the role of observed immune responses in acquired immunity partly relates to the fact that studies looking at these responses have not taken into account exposure intensity. Recent analyses have highlighted the significance of exposure or transmission intensity in generating anti-filarial immunity in endemic communities (Michael & Bundy 1998). Therefore, studies aiming at detecting mechanisms behind such immunity must take this factor into account. Besides, it is not known whether seasonal variation in transmission intensity of *W. bancrofti* may also confound the interpretation of immune response data from different endemic communities.

The present study aimed at applying a new community-based approach to investigating the role of humoral immune responses in filarial infection, disease and acquired immunity, taking into account the above factors. This was done by investigating and comparing, in lieu of carrying out longitudinal studies, cross-sectional age and clinical patterns of specific antibody responses in two communities with differing transmission intensity. In addition, both mf and circulating filarial antigen (CFA) assay were used to assess infection status. The study further investigated if there was seasonal variation in intensities of these filaria-specific antibody responses.

Justification for the study

If, as suggested, a link between filarial immunity and exposure to infection exists, then control measures by inducing changes in exposure intensity will have the potential to alter both the strength and type of immunity in an endemic community. Thus, if these control measures reduce experience of infection but fall short of eradicating transmission, they will potentially reduce the level of such immunity in the population, and in turn lead to a rise in infection burden above the levels existing prior

to the beginning of control (Michael & Bundy 1998). Furthermore, if a balance between protective and pathological immune responses within the host exists, and if this balance is dependent on exposure to infective larvae, then reduction in community exposure by such control measures may produce a bias towards an increase in immunopathology.

To elucidate the role of transmission intensity on the patterns of infection, disease and protective immunity, these patterns need to be compared in individuals living in areas with different transmission intensities. Although a fair amount of immune response data from bancroftian filariasis areas with different endemicity exists, they often do not contain information on the levels of infection and disease, nor of transmission. Even where such information is available, the data are often still not comparable since different diagnostic methods have been used, and different age groups and disease spectrum covered. Comparative studies on infection, disease and humoral immune responses, carried out on the whole age range of individuals in endemic communities with different infection endemicity, would thus provide invaluable new information on the immuno-epidemiology of bancroftian filariasis. It is against this background that this study was carried out. The present study simultaneously examined profiles of microfilaraemia, circulating filarial antigenaemia, disease and specific antibody responses in a whole range of individuals in two communities with high and low bancroftian filariasis endemicity, using similar methods. The infection, disease and antibody response patterns were compared between the communities, and a detailed analysis was performed of antibody responses in these communities in relation to clinical and infection status, age and gender.

Besides infection endemicity, seasonal variations in transmission intensity of *W. bancrofti* may confound the interpretation of antibody response data. In some areas, infection transmission has been observed to be seasonal, with highest transmission occurring during and shortly after the rainy season (McMahon *et al.* 1981). Since antibody responses are directed at various stages of the parasite including the infective larvae, it may be that the intensity of these responses also varies with transmission seasons. If that is the case, the season during which studies are conducted must be taken into account, to allow for assessment of the impact of transmission intensity. In addition, therefore, the present study investigated the effect of *W. bancrofti* seasonal transmission intensity on infection and filaria-specific antibody response intensities.

Objectives

General objective

The general objective was to compare the patterns of infection, disease and specific antibody responses in two communities with high and low bancroftian filariasis endemicity, respectively, and to investigate the influence of seasonal variation in transmission intensity on the levels of infection and antibody responses in bancroftian filariasis.

Specific objectives

* To compare the profiles of microfilaraemia, circulating *W. bancrofti* antigenaemia and clinical manifestations in two populations with a high and a low bancroftian filariasis endemicity;
* To compare the profiles of filaria-specific antibodies (IgG1, IgG2, IgG3, IgG4 and IgE) in relation to clinical status and infection in two populations with a high and a low bancroftian filariasis endemicity;
* To compare the profiles of filaria-specific antibodies (IgG1, IgG2, IgG3, IgG4 and IgE), in relation to age in two populations with a high and a low bancroftian filariasis endemicity;
* To compare the profiles of filaria-specific antibodies (IgG1, IgG2, IgG3, IgG4 and IgE) in relation to gender in two populations with a high and a low bancroftian filariasis endemicity;
* To compare the levels of microfilaraemia, circulating filarial antigenaemia and filaria-specific antibodies (IgG1, IgG2, IgG3, IgG4 and IgE) at different infection transmission seasons among infected individuals living in a bancroftian filariasis endemic community.

Chapter 2

Background and literature review

Introduction

More than 1.1 billion people, approximately 20% of the world's population, live in lymphatic filariasis endemic areas and an estimated 120 million of these are infected (Michael *et al.* 1996; Michael & Bundy 1997). Bancroftian filariasis caused by *Wuchereria bancrofti* accounts for the majority, about 115 million, of the infections. The remainder result from *Brugia malayi* and *B. timori*. Bancroftian filariasis is endemic in large parts of Africa, northern South America, south and east Asia, Southeast Asia and the Western Pacific. An estimated 40 million of those with bancroftian filariasis have overt physical disabilities (WHO 1994).

Biology and life cycle

The adult *W. bancrofti* worms reside in the lymphatics of the human host. Females measure about 80-100 by 0.25 mm while males are smaller and measure about 40 by 0.1 mm. After mating, the adult females produce sheathed microfilariae (mf) measuring about 260 by 8 μm (McMahon & Simonsen 1996). The parasites are transmitted by various species of *Culex, Anopheles, Aedes* and *Mansonia* mosquitoes, which serve as intermediate hosts. Female mosquitoes ingest mf during a blood meal. The mf ex-sheath in the stomach to become first-stage larvae (L1), which then penetrate the stomach wall and migrate to the thorax muscles. Here they develop further by moulting twice, first to sausage-shape second-stage larvae (L2) and then to infective third-stage larvae (L3), which migrate to the mosquito's mouth parts. Development from mf to L3 in mosquitoes takes a minimum of 10-12 days and is dependent on ambient temperature and relative humidity. When the infective mosquito bites man, L3 larvae enter the skin through the puncture site made by the proboscis of the vector when taking blood, and migrate to the lymphatics. Here, they develop to adult worms by moulting twice, first to L4 then to young adults which later mature. When the mature adults mate, the fertilised females produce mf, which move from the lymphatics into the bloodstream. The life cycle of *W. bancrofti* is illustrated in Figure 2.1.

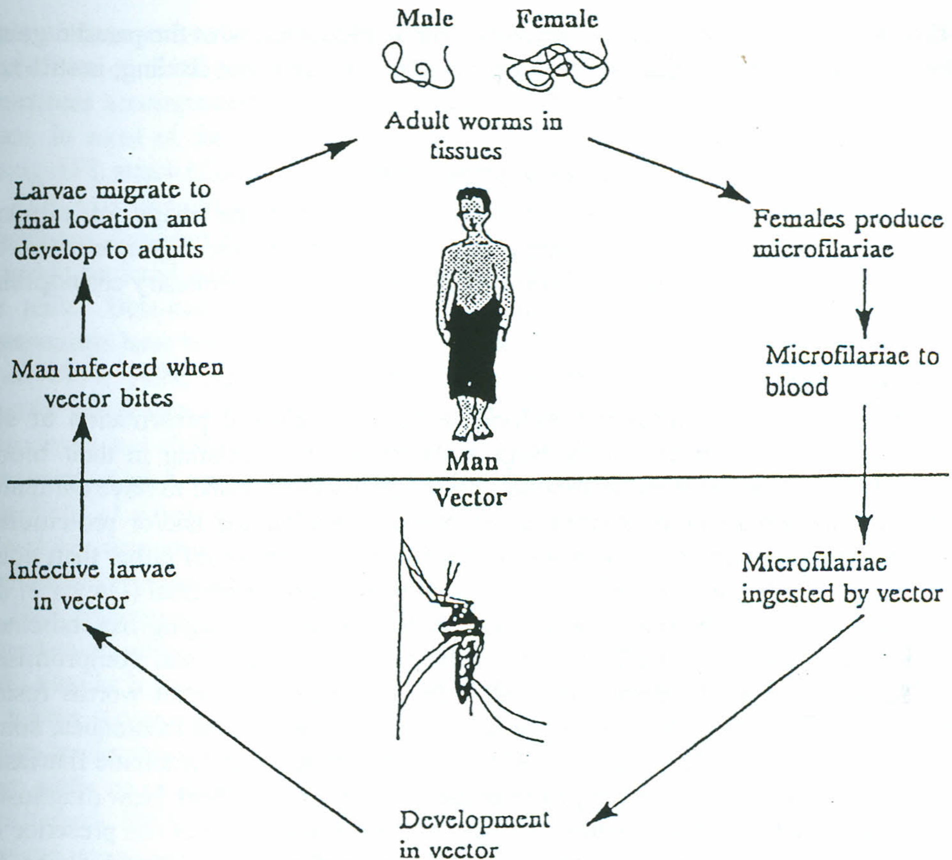


Figure 2.1. Life cycle of *Wuchereria bancrofti* (source: McMahon & Simonsen 1996).

The interval from infection to appearance of mf in peripheral blood, that is the pre-patent period, is approximately nine months. Adult worms may live and produce mf for more than 20 years, but on average their life span is shorter (McMahon & Simonsen 1996). The life span of the mf is about one year (WHO 1987). In most areas, *W. bancrofti* mf are nocturnally periodic with peak concentration in the blood being found around midnight and none or very few at midday. However, diurnally sub-periodic and nocturnally sub-periodic forms also exist. Here, mf are present continuously in peripheral blood, but their concentrations are higher than average during the day and the night, respectively. Periodicity is probably influenced by physiological signals from the host, such as oxygen tension in the blood and body temperature (Scott 2000). It coincides well with the biting time of the vector species in endemic areas. Reversing sleep schedule of an infected individual is known to lead to reversal of mf nocturnal periodicity to a diurnal pattern. Hence nocturnally periodic *W. bancrofti* in individuals who convert to working at night and sleeping during the day, become diurnally periodic. In addition, periodicity may also cease, as has been observed in patients hospitalised for long periods with resulting variable sleep and activity rhythm. It is obviously advantageous for mf to be in high numbers in blood at

the time when the vector species is actively feeding. However, what the parasite gains by being absent in blood during the time when the vector is not feeding, is still not clear.

Clinical presentation

Bancroftian filariasis is a spectral disease with a wide range of clinical presentations, which can be grouped into four major types: asymptomatic or subclinical disease; acute disease; chronic disease and tropical pulmonary eosinophilia (TPE).

Asymptomatic filariasis

Asymptomatic microfilaraemic individuals have no clinical presentation at all, despite some of them having very large numbers of mf circulating in their blood (Ottesen 1992). Some of these individuals have recently been found to have clinically silent renal abnormalities presenting as microscopic haematuria and/or proteinuria. These abnormalities appear to be associated with the presence of mf rather than adult worms since mf clearance from blood results in their complete reversal (Dreyer *et al.* 1992). Besides, ultrasonography and/or lymphoscintiographic imaging has indicated that some or perhaps even all of these individuals have dilated and compromised lymphatic function in the scrotal or limb lymphatics where the adult worms reside (Freedman *et al.* 1994, 1995; Norões *et al.* 1996). Using these new techniques, some mf negative individuals harbouring adult worms but with no overt lymphatic filariasis, have also been shown to have lymphangiectasia (Dreyer *et al.* 1996a). New diagnostic methods detecting circulating antigens thought to be an indication of the presence of adult worms have also shown some asymptomatic mf negative individuals to be positive for antigen (Chanteau *et al.* 1994a).

Acute disease

Acute lymphatic filariasis is also known as acute adenolymphangitis (ADL). It consists of intermittent episodes of lymphangitis, adenolymphangitis, funiculitis or epididymo-orchitis and is often accompanied by fever. Typically, these episodes occur once or twice a year, but they may be more frequent (Ramaiah *et al.* 1996). ADL is now thought to consist of two distinct entities, namely acute filarial lymphangitis (AFL) and acute dermatolymphangioadenitis (ADLA), with different aetiologies (Dreyer *et al.* 1999; Dreyer & Piessens 2000).

AFL is thought to result from the death of adult filarial worms, either spontaneously or as a result of treatment with a macrofilaricidal drug. The initial inflammatory response manifests as adenitis or lymphangitis, depending on location of the worm. Lymphangitis is retrograde, progressing distally, and is frequently accompanied by fever, headache and malaise, although these are relatively minor. Lymphoedema of limbs rarely develops and when it does, it spontaneously reverses in most patients. Because of the high prevalence of living adult *W. bancrofti* worms in the lymphatics of the spermatic cord, AFL triggered by spontaneous or drug induced

death of these worms is particularly common in the scrotal area (Dreyer *et al.* 1995b; Norões *et al.* 1996). This may present as orchitis, epididymitis or funiculitis, sometimes accompanied by acute hydroceles, which resolve spontaneously in most cases. In most of the cases, no bacteria are detected from the inflammatory sites during AFL attack (Connor 1932; Wartman 1944).

ADLA is thought to result from secondary bacterial infection of dysfunctional lymphatics damaged by the adult worms. The lower limbs are particularly prone to recurrent bacterial infections entering through trauma sites, inter-digital spaces and at the nails. Beta-haemolytic *Streptococci* and other organisms that are usually commensals have been isolated from blood or tissues during these attacks (Olszewski *et al.* 1997, 1999; Suma *et al.* 1997). The lymphangitis in ADLA develops in a reticular rather than a linear pattern. Local and systemic symptoms, including oedema, pain, fever and chills and accompanying lymphoedema are frequently more severe. Recurrent ADLA is a major risk factor for the development of elephantiasis, regardless of whether the initial cause of lymphatic dysfunction was filarial or not. Therefore, appropriate treatment and prevention of ADLA are key components of elephantiasis prevention.

The frequency of ADL attacks has been observed to be associated with transmission intensity. A study conducted in Ghana noted a decrease in the number of ADL attacks by over 50% during the dry season when transmission intensity should be lowest (Gyapong *et al.* 1996a). Furthermore, episodes of ADL attacks have been shown to decrease when individuals with filariasis move from an endemic area to an area with no transmission, even without treatment (Wartman 1947; Rajan & Gundlapalli 1997). Thus, it is probable that ADL attacks are associated with host immune responses to incoming L3 larvae, as has been proposed by Maizels & Lawrence (1991). On the other hand, the observed higher frequency of ADL attacks during the rainy season may be due to increased farming activities, compared to those during the dry season, probably resulting in higher incidences of farm injuries and increased bacterial infections.

Chronic disease

Chronic lymphatic pathology results from damage to the lymphatics, and may lead to gross abnormalities in severely affected individuals. Damaged lymphatics result in compromised lymphatic function, commonly presenting as limb lymphoedema [initially transient and reversible, but later permanent (elephantiasis)], hydrocele (in males) or chyluria (Ottesen 1992). The prevalence and severity of chronic clinical manifestations tend to increase with age. Most often, chronic lymphatic pathology with obstructive disease is preceded by repeated ADL attacks, but it also occurs in some individuals without prior recognisable attacks (WHO 1992).

Lymphoedema progressing to elephantiasis (see Plate 2.1) most commonly affects the lower limbs, but may also affect the upper limbs, scrotum, penis, vulva and breast (McMahon & Simonsen 1996). Some studies have shown it to be more common in women than in men, while others have indicated vice versa (Pani *et al.* 1991; Lammie *et al.* 1993; Gyapong *et al.* 1994; WHO 1994). It usually starts on one side, but often

becomes bilateral with time (McMahon & Simonsen 1996). Probably bacterial and fungal super-infections play a role in its aetiology (Addiss *et al.* 1994). The early stage of lymphoedema consists of pitting oedema, referred to as grade 1 lymphoedema. In the legs, swelling first appears around the ankles. Initially, swelling of the limbs returns to normal after attacks. However, after several attacks, the oedema becomes non-pitting, the skin becomes thickened and losses its elasticity. This is grade 2 lymphoedema. Further attacks leads to overt elephantiasis. This is characterised by dermatosclerosis and papillomatous lesions. It is referred to as grade 3 lymphoedema (WHO 1992).

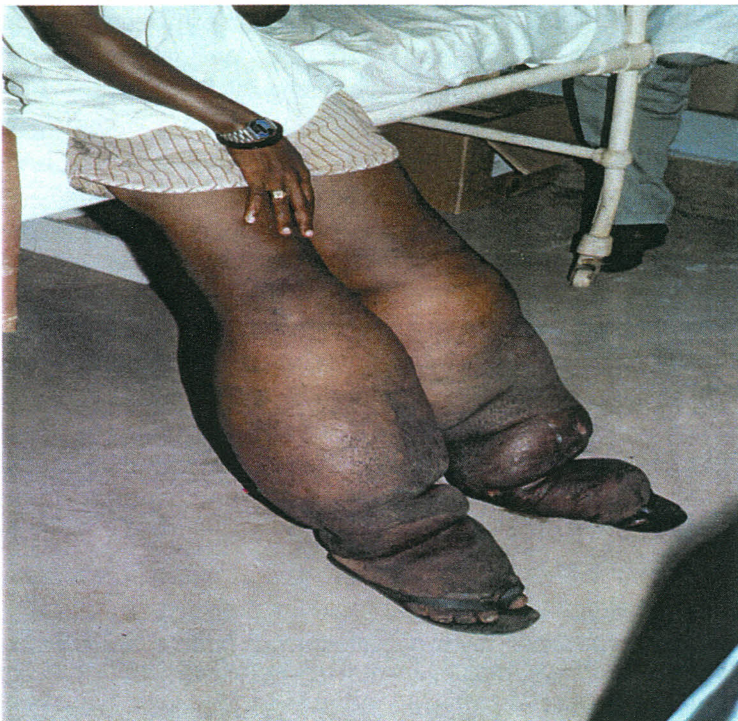


Plate 2.1. Bilateral elephantiasis of the lower limbs resulting from *W. bancrofti* infection.

Hydrocele (depicted in Plate 2.2) is the most common chronic clinical manifestation of bancroftian filariasis (Wijers 1977b; Wijers & Kinyanjui 1977; Estambale *et al.* 1994a; Gyapong *et al.* 1994). The scrotal enlargement results from fluid accumulation in the sac surrounding the testicles. Although the pathogenesis of hydrocele in bancroftian filariasis is still not clear (Ottesen 1992), it is related to the apparent preference of adult *W. bancrofti* for the scrotal area (Dreyer *et al.* 1994, 1995a; Norões *et al.* 1996). Hydrocele is often preceded by recurrent attacks of epididymo-orchitis or funiculitis. The attacks lead to blockage of the lymphatics that drain the retroperitoneal and subdiaphragmatic areas. In the beginning, the scrotal swelling disappears after each attack, but over time, it persists, leading to progressive enlargement of the scrotum with straw-coloured hydrocele fluid. This fluid may have a milky appearance if lymph from a ruptured lymphatic vessel pours into the hydrocele to form a chylocele. Long standing hydrocele or chylocele may be accompanied by scrotal elephantiasis.

Chyluria is the presence of chyle in urine. It results from a rupture of dilated lymphatics into the urinary tract. This commonly takes place in the renal pelvis and the bladder (McMahon & Simonsen 1996). Chylous urine appears milky, especially after a heavy fatty meal. Prolonged chyluria results in hypoproteinaemia, anaemia and weight loss. The prevalence of chyluria in most endemic areas is very low.

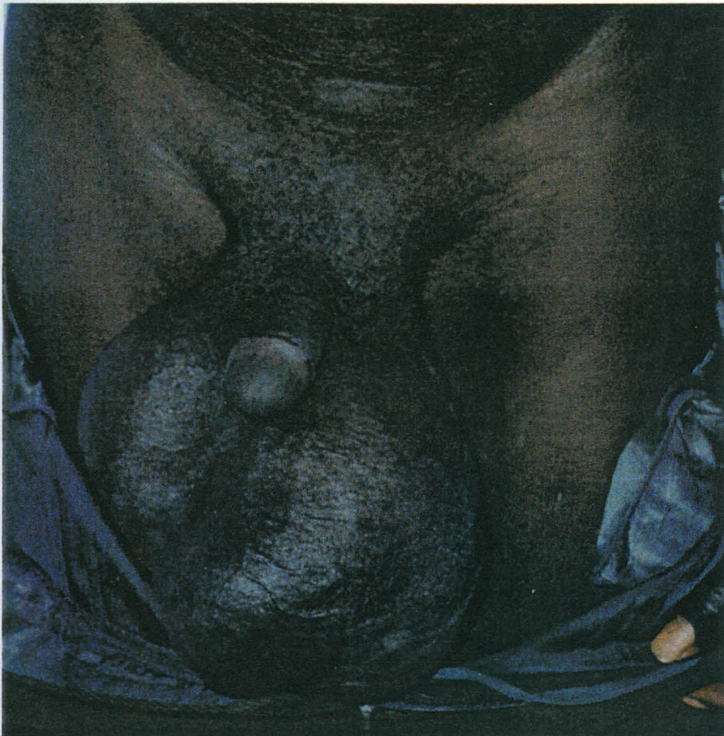


Plate 2.2. Bilateral hydrocele resulting from *W. bancrofti* infection.

Tropical pulmonary eosinophilia (TPE)

Tropical pulmonary eosinophilia (TPE) is a clinical manifestation that probably results from a hypersensitivity reaction to the microfilarial stages of *W. bancrofti* (Ottesen 1990). It affects males twice as often as females, and is rarely seen in children (WHO 1992). The clinical picture consists of paroxysmal nocturnal cough, wheezing, breathlessness, low-grade fever and scanty sputum production occasionally with haemoptysis, while some patients develop extra-pulmonary manifestations including splenomegaly, lymphadenopathy and hepatomegaly. Generally, these patients have very high IgE and eosinophil levels in peripheral blood (usually more than 3000 eosinophils/mm^3) and their chest X-rays show diffuse pulmonary lesions (Ottesen 1992). Although mf are not found in circulation, they have been demonstrated dead or dying in lung, liver and lymph node biopsies (Webb *et al.* 1960). Clinical response in TPE is usually dramatic following anti-filarial treatment. Untreated, pulmonary inflammatory damage progresses to a clinical picture of chronic interstitial fibrosis and finally to respiratory failure. Recent studies conducted in Tanzania show that TPE is probably rare in Africa (Magnussen *et al.* 1995), as elsewhere.

Diagnosis of infection and disease

Diagnosis of bancroftian filariasis is not always straightforward, and may require a series of investigations including documentation of clinical manifestations, search for the mf in blood, serological assays for parasite antigen or parasite-specific antibodies, and/or even the use of an imaging technique like ultrasonography or lymphoscintigraphy to visualise the adult worms or their effects on the lymphatics.

Clinical diagnosis

Bancroftian filariasis may present clinically as acute disease manifesting as fever with adenitis, lymphangitis, funiculitis or epididymo-orchitis, or as chronic disease manifesting as hydrocele or elephantiasis, mainly of the legs, but also of the arms, the breast or rarely of the vulva. Although these manifestations are highly suggestive of bancroftian filariasis in an endemic area on their own, they are not diagnostic since they are not unique to this disease.

Parasitological diagnosis of microfilaraemia

Definitive diagnosis of *W. bancrofti* relies on mf detection in peripheral blood. Parasitological methods commonly used for mf detection are the stained thick blood film technique, the membrane (nuclepore) filtration technique and the counting chamber technique (McMahon *et al.* 1979; WHO 1992; Wamae 1994; McMahon & Simonsen 1996). These techniques differ in their sensitivity, cost and appropriateness for field use. Detection of microfilaraemia as a diagnostic criterion, however, is hampered by the existence of a large proportion of amicrofilaraemic but infected persons (Chanteau *et al.* 1994a). Besides, the technique is often inconvenient to carry out in geographical areas where mf exhibit nocturnal periodicity, since the specimen must be obtained at the time when peak concentration of mf is expected, *i.e.* between

21.00 and 03.00 hrs in most areas (McMahon & Simonsen 1996). Mf of nocturnally periodic *W. bancrofti* can be provoked to appear in peripheral blood during the day for examination by administration of a low dose of diethylcarbamazine (DEC) to patients. DEC provocative day test has been found to be as sensitive as examination of night blood but mf intensities tend to be lower in the former than in the latter (McMahon *et al.* 1979). The DEC day provocative test has a significant filaricidal effect (Simonsen *et al.* 1997a) and therefore should not be used in longitudinal studies on microfilaraemia. Furthermore, since the dose of DEC used for provocation is sufficient to elicit severe reactions in patients with *Onchocerca volvulus* or *Loa loa*, the test cannot be used in areas where these infections concomitantly occur with *W. bancrofti*. Characteristic features of *W. bancrofti* mf such as size, the presence of a sheath and the absence of nuclei at the tip of the tail, distinguish them from mf of other human filarial species in stained blood smears.

Serological diagnosis

Serological tests measuring the presence of antibodies or filarial antigens are alternatives to parasitological techniques. Antibody detection as a diagnostic method is hampered by non-specificity resulting from cross-reactivity with other helminth infections. Besides, the method is limited by its inability to distinguish between exposure, current actual infection or past infection (Ottesen 1984). For these reasons, circulating filarial antigen (CFA) detection in blood is more accurate than antibody detection. Several techniques have now been developed for this purpose (Turner *et al.* 1993; Chanteau *et al.* 1994b; Weil *et al.* 1996; Lalitha *et al.* 1998; Simonsen & Dunyo 1999; Pani *et al.* 2000). Test kits for two of these techniques are currently available commercially. The first is based on the monoclonal antibody AD12 (Weil & Liftis 1987), while the second utilises the monoclonal antibody Og4C3 (More & Copeman 1990; Chanteau *et al.* 1994b; Lammie *et al.* 1994). Both techniques are thought to detect the same circulating adult worm antigens. The AD12 based antigen detection test is a card test (available in two versions for serum and blood, respectively) and simply indicates whether one is positive or negative without giving an indication as to the intensity of infection. It is quite ideal as a rapid diagnostic method in field studies especially where large numbers of individuals are to be tested and when only a positive or negative result is required. The Og4C3 based antigen detection test is an ELISA useful for both serum and filter paper blood specimen. Its main advantage over the AD12 based test is its ability to quantify indirectly the intensity of infection measured as antigen units.

Ultrasonography

Ultrasonography has recently been adopted for use as a diagnostic tool in lymphatic filariasis. It permits direct localisation of the adult worms especially in the scrotal area of males. It has also been used to locate the worms in the female breast (Dreyer *et al.* 1996c). The adult worms have characteristic movement patterns described as 'filarial dance sign', and cluster together at defined sites in 'nests' which remain remarkably stable. In addition, the lymphatic vessels are seen as abnormally

dilated several centimetres on either side of the worms (Amaral *et al.* 1994; Norões *et al.* 1996).

Lymphoscintigraphy

Lymphoscintigraphy using radio-labelled albumin or dextran to visualise peripheral lymphatic system is considered simple and safe to use (Ottesen 1994). Using the technique, abnormalities of limb lymphatic have been demonstrated even in asymptomatic microfilaraemic individuals with no evidence of oedema (Witte *et al.* 1993; Freedman *et al.* 1994).

Patterns of infection and disease in the endemic populations

In endemic communities, the prevalence of microfilaraemia generally increases gradually with age, with first cases usually seen at the age of 4-5 years, peaks in early adulthood then remains stable or declines in later years (Das *et al.* 1990; Grenfell & Michael 1992; Chanteau *et al.*1995; Simonsen *et al.* 1995a; Kazura *et al.* 1997). A similar pattern is seen for the overall age-specific mf intensity for whole communities (Brabin 1990). However, in general no clear relationship between mf intensity and age among the mf positive individuals has been found (Estambale *et al.* 1994a; Meyrowitsch *et al.* 1995a; Simonsen *et al.* 1996).

CFA is often detected at an earlier age than mf in the endemic areas. Its age-specific prevalence and mean intensity pattern is quite similar to that of mf, peaking in early adulthood and then remaining stable or declining in older age groups, but prevalences are much higher (Simonsen *et al.* 1996; Wamae *et al.* 1998).

In many communities, mf prevalence and mean intensities have been found to be higher in males than in females (Brabin 1990; Das *et al.* 1990; Pani *et al.* 1991; Chanteau *et al.* 1995). Several reasons have been advocated for the gender differences, including differences in exposure, hormonal and/or pregnancy related effects and differences in sampling time between men and women (Brabin 1990; Estambale *et al.* 1994a; Simonsen *et al.* 1997b).

Chronic lymphatic filarial disease is rare in children. The youngest hydrocele cases are often seen in males aged 15-20 years, and its prevalence increases with age (McMahon *et al.* 1981; Pani *et al.* 1991; Simonsen *et al.* 1995a). Leg elephantiasis, which is less common than hydrocele, generally starts to appear later in life and its prevalence increases with age (Wijers 1977b; McMahon *et al.* 1981; Pani *et al.* 1991; Meyrowitsch *et al.* 1995a; Simonsen *et al.* 1995a). Sex differences in the prevalence of elephantiasis vary from one area to another. Some studies indicate that males have higher elephantiasis prevalence than females (Simonsen *et al.* 1995a; Kazura *et al.* 1997) whereas others indicate the opposite (Kazura *et al.* 1984; Dunyo *et al.* 1996; Dzodzomenyo *et al.* 1999). The preponderance of males to females with chronic disease is due to occurrence of hydrocele in males, the most common clinical manifestations of bancroftian filariasis (Pani *et al.* 1991; WHO 1992; 1994).

Individuals with elephantiasis have often been thought to be mf negative, whereas males with hydrocele generally have proportionally higher prevalence of mf

(Estambale *et al.* 1994a; Gyapong *et al.* 1994; Meyrowitsch *et al.* 1995a). However, it has recently been found that individuals with chronic disease in general have equal chances of being mf positive or negative (Michael *et al.* 1994).

Patterns of infection and disease in individuals from non-endemic areas

Clinical presentation of lymphatic filariasis in expatriates or immigrants from non-endemic areas is characterised by the rapid appearance of signs and symptoms, not commonly seen in endemic populations. This may consist of fever, lymphangitis, lymphadenitis, epidydymitis, funiculitis, hepatomegaly or splenomegaly. These individuals rarely become microfilaraemic unless exposure is prolonged and continuous (WHO 1992). Such was the case for American, French and North African soldiers posted in filarial-endemic areas of Southeast Asia and The Pacific (King 1944; Wartman 1947; Galliard 1957), in immigrant populations from non-endemic to endemic areas in Indonesia (Partono 1987), and in humans experimentally infected with filarial parasites, as reviewed by Nutman (1991). Besides, expatriates or immigrants who remain in these endemic areas for many years tend to develop elephantiasis more often and much faster than individuals who were born and have lived their entire lives in the endemic areas (Partono *et al.* 1978; Partono 1987; WHO 1992).

Socio-economic and psychological consequences of bancroftian filariasis

Lymphatic filariasis is recognised as the second leading cause of permanent disability in the world (WHO 1995). Although it is not fatal, the chronic symptoms impose significant social and economic burdens not only to the affected individuals, but also to the society and the health care system, since treatment for elephantiasis, hydrocele and ADL attacks can be quite costly. The economic loss from bancroftian filariasis attributable to treatment cost, loss of man-hours due to repeated ADL attacks and to functional impairment from limb elephantiasis, have recently been computed (Gyapong *et al.* 1996b; Ramu *et al.* 1996; Ramaiah *et al.* 1997, 1998, 1999, 2000). The computed loss is quite considerable although it varies from one place to the other.

In addition to the economic impact of lymphatic filariasis, it has recently been documented that chronic physical disability resulting from bancroftian filariasis may have serious negative social and psychological consequences. Individuals with hydrocele or elephantiasis are often shunned and become isolated within their communities. Mocking and social stigmatisation are common towards those affected. Furthermore, hydrocele or other genital abnormalities in men of all ages can lead to the seldom mentioned sexual/social dysfunction, and lymphoedema of the limbs, the breast or the genitals may lead to social ostracism of young women (Dreyer *et al.* 1997; Ahorlu *et al.* 1999). Because individuals with these chronic disease manifestations are often unable to work or marry, they become dependent on care and financial support leading to further isolation, insecurity, shame and psychological stress.

Treatment of bancroftian filariasis

Depending on the parasitological and clinical status of an individual, the mainstay of treatment in bancroftian filariasis is chemotherapy, symptomatic treatment consisting of physiotherapy, exercises and personal hygiene, and surgery where necessary.

Chemotherapy

DEC has been used for decades as the drug of first choice in treating lymphatic filariasis (WHO 1992). It is effective against the *W. bancrofti* mf and also has some activity against the adult worms (WHO 1992, 1994; McMahon & Simonsen 1996). Administered in the WHO recommended standard dose of 6 mg/kg for 12 days, mf levels are decreased by 80-90% within days and remain low for more than six months (WHO 1992; Simonsen *et al.* 1995b; Meyrowitsch *et al.* 1996). Recently, it has been demonstrated that the efficacy of a single dose of 6 mg/kg or less is essentially equivalent to a full 12 days of treatment with the drug (Dreyer *et al.* 1994) and that low spaced dosages may be even more effective (Meyrowitsch & Simonsen 1998). The side effects of DEC consist of fever, headaches, muscle/joint pains and painful nodules, which are usually mild and are believed to be associated with the death of mf and some adult worms (Sabry *et al.*1991; Maizels & Denham 1992; Norões *et al.* 1997; Dreyer *et al.* 1998). DEC may however induce severe reactions in patients with onchocerciasis and loiasis (WHO 1992; 1994). For this reason, DEC is contraindicated in areas where these infections occur, which form a large part of the African continent.

An alternative drug that is currently being used for bancroftian filariasis in onchorcerciasis endemic areas of Africa is ivermectin (Richards *et al.* 1991; Chodakewitz 1995; Dreyer *et al.* 1995a). It is only microfilaricidal and has no effect on adult worms, and therefore treatment has to be repeated regularly until the natural death of the adult worms. It has recently been suggested that a combination of DEC or ivermectin with albendazole will enhance the effect of these drugs in bancroftian filariasis (Addiss *et al.* 1997; Ottesen *et al.* 1997; Beach *et al.* 1999) although the benefit of combining ivermectin with albendazole has also been questioned (Dunyo *et al.* 2000a,b).

Symptomatic treatment

Personal hygiene and prevention and/or treatment of secondary microbial infections are now an integral part of disease management in lymphatic filariasis (Ottesen *et al.* 1997; Dreyer *et al.* 1998). This arises from recent studies, which indicate that microbial super-infections play an important role in triggering acute and chronic filarial disease (Shenoy *et al.* 1995; Dreyer *et al.* 1999). Simple hygienic measures taking care of the feet in combination with the use of local antibiotic/anti-fungal cream application, where required, is effective in reducing the number of ADL attacks (Shenoy *et al.* 1995) and therefore progression of elephantiasis.

Surgery

Surgery is the definitive management of hydrocele. Aspiration of the hydrocele fluid with or without injecting sclerosing agents such as antazoline, ethanolamine, phenol or tetracycline, may be an alternative in areas where resources are scarce (Musa *et al.* 1995; Dreyer *et al.* 1998). However, this has a much higher recurrence rate. Surgical treatment of elephantiasis whereby the redundant tissue is excised from severely affected limbs has also been tried. However, the results have not been satisfactory. Elephantiasis patients can derive more benefit from lymphovenous surgery, but the procedure is still not available in most endemic areas (McMahon & Simonsen 1996).

Prevention and control

Recent advances in treatment and control options have resulted in new optimism that lymphatic filariasis can be eliminated as a public health problem, and this is now targeted to be achieved by the year 2020 (WHO 1997, 1999). Currently, WHO lymphatic filariasis prevention and control strategies aim at both reducing transmission and at reducing and preventing morbidity.

Reduction or elimination of transmission can be achieved by mass drug administration to entire populations at risk. This results in a reduction in the number of parasites in the blood to levels below which the mosquito vectors can no longer transmit infection. Annual single-dose combination of two drugs given for a period of four to six years is now recommended for this. Ivermectin with albendazole is the recommended combination for African countries that also have onchorcerciasis. This applies to all lymphatic filariasis endemic countries on the continent except Egypt and Kenya. DEC with albendazole is the recommended alternative for Egypt and Kenya, and lymphatic filariasis endemic areas outside Africa. DEC-medicated salt substituted for normal salt has been shown to be equally effective if used exclusively (Olszewski *et al.* 1993; Gelband 1994; Meyrowitsch *et al.* 1996; Meyrowitsch & Simonsen 1998). As an alternative, DEC-medicated salt is recommended for use for a period of one to two years.

Vector control can play an important supplementary role in control of lymphatic filariasis. It perhaps can not be relied upon on its own. Among the most promising control methods are biocides, especially *Bacillus sphaericus* (self-reproducing, toxin-producing bacterium) for *C. quinquefasciatus* mosquitoes, polystyrene beads to limit the breeding of vectors especially in enclosed urban breeding sites such as pit latrines and cesspools, insecticide-impregnated bed nets and curtains and indoor spraying of residual insecticides.

Morbidity resulting from elephantiasis can be reduced by palliative treatment. Intensive, but simple and effective local hygiene methods such as washing affected limbs using soap and water, and applying antibiotic and anti-fungal agents that prevent bacterial superinfection, is able to halt or even reverse lymphoedema and elephantiasis sequelae (Ottesen *et al.* 1997). This can be coupled with limb positioning, bandaging and exercises in order to enhance the symptomatic relief obtained.

Immunoepidemiology

Despite extensive studies on immune responses in bancroftian filariasis, the role of immunity in controlling infection and in disease development remains unclear. In the following, these studies will be reviewed as a background for the analyses carried out in the present thesis. In particular, an overview of studies on antibody responses in relation to infection and disease status will be listed in tabular form to ease comparison of results from former studies with those of the present study.

Immune responses in relation to chronic disease status

Clinical lymphatic filariasis among individuals living in endemic areas has often been viewed as a spectral disease with two poles. One pole has mf positive but asymptomatic individuals while the other has individuals with chronic disease characterised mainly by elephantiasis and often assumed to be mf negative. It is generally believed that differences in immune responses between the groups underlie their different infection and disease status. Thus, immunological studies have traditionally contrasted the immune responses seen between these two groups against each other (Ottesen 1989; Kurniawan *et al.* 1993).

Based on these studies, asymptomatic microfilaraemic individuals have generally been shown to have little or no *in vitro* lymphocyte proliferative responsiveness to filarial antigen, whereas those with chronic disease often respond remarkably well to such antigens (Ottesen *et al.* 1977; Piessens *et al.* 1980; Ottesen 1984; Nutman *et al.* 1987b; King & Nutman 1991; Maizels & Lawrence 1991; Lammie *et al.* 1993; Yazdanbakhsh *et al.* 1993; Maizels *et al.* 1995). This hypo-responsiveness, however, appears to be exclusively in response to filarial antigens and is not broad spectrum (Ottesen *et al.* 1977; Piessens *et al.* 1980; Ottesen 1992).

The hypo-responsive state in asymptomatic mf positive individuals has also been demonstrated with respect to specific filarial antibody levels in serum. Thus, these individuals have generally been found to have lower specific IgG1, IgG2, IgG3 and IgE levels compared to individuals with chronic disease (Ottesen *et al.* 1982; Hussain *et al.*1987; Ottesen 1992; Kurniawan *et al.* 1993; Yazdanbakhsh *et al.* 1993; Estambale *et al.* 1994b). However, asymptomatic mf positive individuals can be shown to be extremely hyper-responsive when parameters that down-regulate rather than promote inflammatory responses are evaluated. In this regard, they have generally been shown to produce extremely high amounts of specific IgG4 antibody (Ottesen *et al.* 1982; Hussain *et al.* 1987; Ottesen 1992; Kurniawan *et al.* 1993; Yazdanbakhsh *et al.* 1993; Estambale *et al.* 1994b).). In contrast, those with chronic disease have been observed to be relatively hyper-responsive and to produce large amounts of specific IgG1, IgG2, and IgG3, but minimal amounts of IgG4 (Ottesen *et al.* 1982; Hussain *et al.* 1987; Kurianwan *et al.* 1993; Yazdanbakhsh *et al.* 1993; Estambale *et al.* 1994b). Despite these general trends, specific antibody responses in relation to disease status in lymphatic filariasis vary considerably. This can be seen in the overview of published studies in Appendix 1. The studies quoted are only those in

which the levels of the specific antibodies are indicated or can be estimated from the figures shown.

The two above-mentioned groups of individuals also contrast in their cytokine production, in response to specific filarial antigen. It is thought that cytokine production is controlled by two subsets of $CD4^{+}$ (T-helper) lymphocytes. Interferon gamma (IFN-γ), a pro-inflammatory mediator of the immune system, is produced by the T-helper lymphocyte subset designated Th-1, while anti-inflammatory mediators, interleukin-4 (IL-4) and 5 (IL-5) are produced by the subset of T-helper lymphocytes designated Th-2. Several studies have indicated that upon antigenic stimulation the asymptomatic microfilaraemic individuals produce significantly higher IL-4 and lower IFN-γ, resulting in a higher IL-4: IFN-γ ratio than the individuals with chronic disease (Nutman *et al.* 1987a; Almeida *et al.* 1995; 1998). Other studies have shown, however, that although asymptomatic microfilaraemic individuals produce lower levels of IFN-γ upon filarial antigen stimulation, the two groups do not differ significantly in production of IL-4 (Maizels *et al.* 1995; King *et al.* 1993; Ravichandran *et al.* 1997). Thus, it appears that the relatively higher Th-2/Th-1 responses seen among asymptomatic microfilaraemic individuals are probably due to diminished Th-1 type responses rather than to heightened Th-2 type response. Some studies have shown that the relative hypo-responsiveness associated with lowered Th-1 type responses is partly due to inhibitory cytokines such as IL-10. In these studies *in vitro* neutralization of IL-10 partially reversed the lowered Th-1 type responses to parasite antigens (King *et al.* 1993; Mahanty & Nutman 1995). It seems therefore that pro-inflammatory elements of the immune system can be held in check by molecules secreted by other arms of the same system. Thus, asymptomatic microfilaraemic individuals might secrete these anti-inflammatory molecules resulting in lower Th-1 and hence higher Th-2/Th-1 type responses. In contrast, the individuals with chronic inflammatory pathology are those who respond to parasite antigen by producing the pro-inflammatory mediators of the immune system, *i.e.* Th1-type responses.

The above immunological explanation accounts well for the polar clinical presentations of mf negative asymptomatic individuals and mf positive individuals with chronic disease, but not for co-existence of lymphatic pathology and microfilaraemia, often seen in endemic areas (Michael *et al.* 1994). If filaria-induced immune response leads to inflammatory pathology without microfilaraemia, and if the down-regulation of such immune responses leads to asymptomatic microfilaraemia in the absence of inflammatory pathology, then pathology and microfilaraemia should not co-exist (Ottesen 1992). Besides, the Th1-type and Th2-type dichotomy does not strictly hold true. Microfilaraemic individuals with brugian filariasis have recently been found to have lower IL-5, a Th2-type cytokine (Sartono *et al.* 1997). In addition, specific IgE production in lymphatic filariasis does not fit well into the above-discussed Th-1/Th-2 dichotomy. Like specific IgG4, specific IgE production is stimulated by IL-4, a Th2-type cytokine (Lundgren *et al.* 1989; Ishizaka *et al.* 1990; Gascan *et al.* 1991). However, several studies have shown that unlike specific IgG4, specific IgE levels are often found higher among individuals with chronic disease than

among asymptomatic mf positive individuals (Hussain *et al.* 1981; Kurniawan *et al.* 1993; Yazdanbakhsh *et al.* 1993; Estambale *et al.* 1995). It is possible that the mf produce a substance that selectively inhibits IgE production but leaves IgG4 unregulated, as suggested by Kurniawan *et al.* (1993).

Immune responses in relation to infection status

The concept that chronic disease is uniformly associated with mf negative status has recently been challenged by a meta-analysis of data from several endemic areas showing that in fact these individuals are equally likely to be mf positive or negative (Michael *et al.* 1994). Thus, the polar classification of individuals with lymphatic filariasis into the infected asymptomatic and the uninfected chronic diseased is unsupported by field evidence. This classification lumps together individuals with chronic disease who have active filarial infection and those with chronic disease who have cleared their infection. Since immune responses are influenced by infection status, it is imperative that immunological studies must categorise endemic individuals by both clinical and infection status more accurately. Recently developed CFA assays, which presumably detect the presence of the adult *W. bancrofti* worms and hence are more sensitive determinants of infection (More & Copeman 1990; Chanteau *et al.* 1994; Addiss *et al.* 1995), have helped define an even more precise classification of individuals with chronic disease. These assays together with the knowledge that essentially all the infected have underlying pathology has led to new proposals for grouping endemic individuals in immunological studies. One of these proposes that individuals in bancroftian filariasis endemic areas be grouped into asymptomatic CFA positive, clinical chronic symptomatic CFA positive and clinical chronic symptomatic CFA negative (Freedman 1998). Alternatively, these individuals can first be grouped by their clinical status and then further categorised by their infection status using both their mf and CFA status (Nicolas *et al.* 1999).

Studies in which endemic individuals are classified with respect to both clinical and infection status have shown that specific antibody responses are more related to the presence of infection than to disease status. Most studies have indicated that specific IgG1, IgG2 and IgE are associated with mf status. Generally, mean intensities of IgG1, IgG2 and IgE are significantly higher among mf negative individuals than among mf positive individuals irrespective of their CFA status (Lammie *et al.* 1993; Addiss *et al.* 1995; Dimock *et al.* 1996; Simonsen *et al.* 1996; Nicolas *et al.* 1999). On the other hand, mean intensities of specific IgG3 and IgG4 are often more associated with CFA status than mf status. Thus, generally speaking, IgG3 levels are often higher among CFA negative than among CFA positive individuals, irrespective of their mf status (Nicolas *et al.* 1999). In contrast, mean specific IgG4 intensities are often higher among the CFA positive than among the CFA negative (Kwan-lim *et al.* 1990; Lammie *et al.* 1998; Wamae *et al.* 1998; Nicolas *et al.* 1999). Despite these general trends, specific antibody responses in relation to infection status in lymphatic filariasis vary considerably in different studies. This is demonstrated in an overview of published studies in Appendix 2. The studies included in this table are only those in which the specific antibody levels are indicated or can be estimated from given

figures. Similarly, cytokine responses have often been found to correlate to infection status. In a study by Almeida *et al.* (1995), antigen-specific IL-4 and IL-5 were not significantly different between infection groups, whereas IFN-γ was detected only in CFA negative but not in CFA positive individuals whether or not they had clinical filariasis. Dimock *et al.* (1996) similarly found Th-1-like antifilarial immune responses (both IL-2 and IFN-γ) significantly higher among CFA negative than CFA positive individuals regardless of clinical status, whereas IL-4 levels were not significantly different between different infection groups.

Cellular proliferative responses have also been found to be associated with infection status. Thus a study by Addiss *et al.* (1995) found that CFA negative bancroftian filariasis patients with hydrocele had a significantly greater antigen-specific lymphocyte proliferation response compared with matched CFA positive individuals.

Immune responses in relation to age and to gender

Relatively few studies have looked at the relationship between specific immune responses and age in bancroftian filariasis in detail. The prevalence of IgG4 with age has been the most consistently observed age-related immune response parameter. Generally, IgG4 prevalence increases sharply with age in early childhood, in consort with infection prevalence, followed by a plateau in the older age groups (Chanteau *et al.* 1995; Simonsen *et al.* 1996). The study by Simonsen *et al.* (1996) also observed that although the prevalence of specific IgG1 similarly increased rapidly with age, unlike IgG4, this was followed by a decline in the older age groups. Lammie *et al.* (1998) found the prevalence of IgG1, IgG2, IgG3 and IgG4 positively associated with age among the young children. Most studies looking at intensity of immune responses with age have generally compared the levels of these responses between the younger and the older individuals. From these results, it appears that the levels of most specific antibodies are often higher (although rarely significant) among the young than the old (Ottesen *et al.* 1977; 1982; Hitch *et al.* 1989; 1991; Estambale *et al.* 1994b; Simonsen *et al.* 1996). Thus, mean specific IgG1 and IgE have been found to be higher among the younger than the older individuals in all clinical categories (Estambale *et al.* 1994b). It has been suggested that the low levels of specific antibodies in the older individuals may indicate downward regulation of reactivity with increasing duration of exposure to the parasite (Ottesen *et al.* 1982; Ottesen 1984), as has been described in experimental animals (Osborne & Devaney 1999). In contrast, some studies indicate intensities of specific IgG4 and IgE antibodies higher among older than among younger individuals (Appendix 3). Despite the gender differences often seen with respect to infection and disease in bancroftian filariasis, only a few studies have looked at specific immune response in relation to gender, as a possible basis for this disparity in infection and disease patterns. A study by Hitch *et al.* (1989) found no correlation between the responses with either age or gender. Chanteau *et al.* (1995) however found the specific IgG4 prevalence to be significantly higher in males than in females, although the study did not compare its intensity in relation to gender.

Protective immunity

Relatively little is known about protective immunity in human filariasis. Indirect evidence for the existence of at least partial resistance is derived from age-infection curves which indicate that mf and CFA prevalence of infection rise with age reaching either a plateau or a decrease in the older age groups (Estambale *et al.* 1994a; Chanteau *et al.* 1995; Simonsen *et al.* 1996; Wamae *et al.* 1998). More specifically, convex age-specific mf and CFA prevalence patterns are thought to indicate that humans develop at least partial immunity to new infection after years of exposure to the parasite (Grenfell & Michael 1992; Michael & Bundy 1998; Weil *et al.* 1999). Further evidence comes from the observation that some individuals from endemic areas apparently develop immunological resistance to infective larvae (Freedman *et al.* 1989; Day *et al.* 1991a) and microfilariae (Simonsen 1983; 1985; Simonsen & Meyrowitsch 1998).

The occurrence of complete resistance to lymphatic dwelling filariae is however difficult to establish and is probably rare. A criterion for determining whether or not an individual or group of individuals is resistant to lymphatic filarial infection has been proposed (Kazura 2000). This includes life-long residence in endemic areas by the individuals, lack of mf in blood at the time of peak periodicity, absence of CFA (in cases of bancroftian filariasis) and epidemiological and/or entomological evidence for continued exposure to infective larvae. Several studies in endemic areas have identified individuals who are probably resistant to lymphatic filarial infection (Lammie *et al.* 1994; Chanteau *et al.* 1995; Almeida *et al.* 1996; Dimock *et al.* 1996; Steel *et al.* 1996; Weil *et al.* 1996; Kazura *et al.* 1997).

An alternative approach to determining resistance in lymphatic filariasis has been to compare the rate of change of infection levels among groups of individuals living in an endemic area, assuming a constant intensity of transmission. Using this approach, CFA levels have been found to increase significantly in children while remaining stable in adults, thus suggesting that children are more susceptible to new infections than adults (Day *et al.* 1991b). This has been supported by studies in Egypt and India showing higher increases in mf and antigen levels in children than in adults from the same household (Vanamail *et al.* 1989; Farid *et al.* 1997; Weil *et al.* 1999). Although children seem to experience significantly more mosquito bites than adults in the same house, the difference is too small to account for the observed difference in age-specific mf incidence (Farid *et al.* 1997).

Techniques using mathematical modelling of data from endemic areas indicate a higher level of peak infection intensity and a 'left peak shift' in the age at which this peak intensity is achieved (Michael & Bundy 1998). Thus, it has been suggested that the observed immunity in lymphatic filariasis is dependent on intensity of transmission (Michael & Bundy 1998).

Chapter 3

Infection, disease and filaria-specific antibody response patterns in two communities with low and high endemicity of *Wuchereria bancrofti* infection

Introduction

A cross-sectional study examining the patterns of infection, disease and filaria-specific antibody responses in a community with a high and a community with a low bancroftian filariasis endemicity was carried out along the East African coast. The study specifically compared prevalence patterns of acute and chronic disease and prevalence and mean intensity patterns of microfilaraemia, circulating filarial antigenaemia and filaria-specific antibodies, between the two communities.

Materials and methods

Study area and communities

Two communities, Masaika in Tanga Region of Tanzania and Kingwede in Kwale District of Kenya, each with approximately 1,000 inhabitants, were selected for the study. Both communities are located in the same coastal East African *W. bancrofti* transmission focus, but preliminary surveys had shown that the level of endemicity was markedly higher in the Masaika than in the Kingwede area. The distance between the study areas is approximately 80 km. The areas have two rainy seasons each year, the long rains in March-June and the short rains in October-November. Temperatures average 26°C throughout the year, while relative humidity ranges between 70 and 90% throughout the year. Both communities have a fairly stable population with few migrations.

Masaika is located in a hilly fertile area about 25 km inland from the Indian Ocean coast. The population consists mainly of subsistence farmers cultivating maize, cassava, rice and vegetables, and keeping chickens, ducks and goats as domestic animals. A few cash crops such as oranges, coconut and cashew nuts are also produced. Most houses in the village are made of mud walls and roofed with dried coconut leaves (*makuti*). The main source of domestic water in this community is shallow dugouts in the lower parts of the village and its periphery. One government owned and several privately owned dispensaries serve as the main health facilities in the community. Pangani District Hospital and a Mission Hospital in Muheza each about 20 km from the village, are the major referral health facilities. Both hospitals are fee charging and despite the low cost of treatment, this is unaffordable to most people due to the pertaining low socio-economic status in the community. The main ailments seen in the community are respiratory tract infections, diarrhoeal diseases, skin conditions such as scabies, and parasitic infections such as malaria and urinary schistosomiasis. Ethnically, the population is quite mixed, with more than 20 different tribes represented. The three largest tribes are the Makonde, Bondei and Zigua, who constitute about 54% of the population. The whole village population was included in the present survey.

Kingwede is located about 3 km from the Indian Ocean, in a flat lowland area. Similarly, the population consists of subsistence farmers cultivating maize, cassava, rice and green vegetables, and keeping chickens, ducks, goats and cattle. The few cash crops grown here consist of cashew nuts and coconuts. A large number of adult males are employed in jobs outside the village, mainly in Mombasa, a large city about 50 km

away. Some of the adult males are fishermen in the nearby ocean. Most houses are built from coral stones and/or mud walls and roofed with iron sheets or *makuti*, as depicted in Plate 3.1. A stream passing through the village and a number of deep wells with hand pumps, supply the village with domestic water. The nearest health facility in Kingwede is the government owned Msambweni District Hospital, situated about 10 km from the village centre. The district hospital offers both out-patient and in-patient services at a subsidised government rate, which however is still unaffordable to most of the people due to their low socio-economic status. The common ailments in the community include upper respiratory tract infections, skin conditions such as scabies, and parasitic diseases such as malaria and urinary schistosomiasis. The major ethnic group is the Digo constituting over 80% of the population. The village has more than 2,000 inhabitants, and therefore only half of it (the western part with the village centre) was included in the present survey.

Plate 3.1. Study area showing a house made of mud walls and roofed with dried coconut leaves (*makuti*).

Registration of inhabitants and field procedures

Before fieldwork commenced, permission to conduct the study was obtained from the health and administrative authorities, and meetings were held in the villages to explain the purpose of the study to the residents. Sketch maps of the villages were prepared, houses were numbered, and a house-to-house census was carried out in order to register the name, age and sex of the inhabitants. A repeat census was performed about two months later to verify information from the first census. During both census rounds inquiries were made about parent/child relationships for individuals less than 20 years. In addition, a group of elders was requested to independently provide information about parent/child relationships. Relationships were accepted and used in the study only when information from the census agreed with information from the group of elders. The cross-sectional survey during which individuals were examined clinically and during which blood samples were collected took place in July 1998 in Masaika and in August 1998 in Kingwede. For each registered individual, verbal consent to participate in the study was obtained (from parents or guardians in the case of children ≤15 years old), after which a standard questionnaire (Appendix 5) was administered to obtain demographic information and medical history relevant to lymphatic filariasis. The individuals were then examined clinically, and finger-prick (Plate 3.2) and venous blood samples taken in the evening for mf examination, and for examination for CFA and filaria-specific antibodies, respectively. Ethical clearance to carry out the study was obtained from the relevant national medical ethical committees of Kenya and Tanzania, and the Central Scientific Ethical Committee of Denmark.

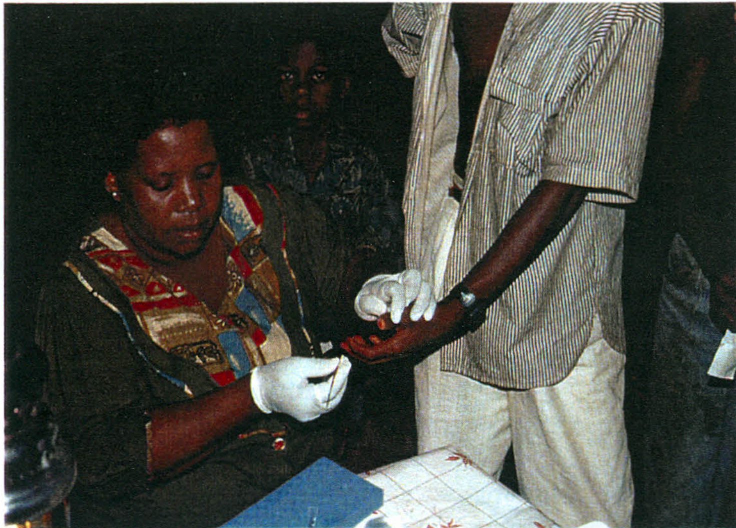


Plate 3.2 Finger-prick blood sampling for detection of *W. bancrofti* microfilaraemia.

Interviews and clinical examination

A house was chosen in each village for use as a private examination room. The standard questionnaire was administered and physical examination carried out on all individuals aged 12 months and over. During examination, individuals were asked in Ki-swahili, the local language, whether they had experienced an acute adenolymphangitis (ADL) attack in the past one-year period. *Mtoki*, the Ki-swahili local term used to describe ADL attacks, was adopted for use in the study. Those who gave a history of having had ADL attacks were asked to describe them, and cases were accepted as being true when the description fitted a condition of localised swelling accompanied by pain/tenderness and warmth, involving the limbs, scrotum or female breast, with or without fever. The number of attacks and their average duration (days) were also recorded.

Physical examination was carried out with emphasis on acute and chronic signs of lymphatic filariasis. In males, examination included the genitals, legs, arms, and lymph glands in the groin and axillae. Examination in females was confined to the legs and arms. Breast and genital examinations in females were included only if there was a history indicative of their involvement. Signs and symptoms of acute disease such as adenitis, adenolymphangitis, orchitis, epididymo-orchitis and funiculitis, and of chronic disease such as hydrocele and leg lymphoedema/elephantiasis were looked for and recorded. Hydrocele and leg lymphoedema/elephantiasis were graded as previously described by Meyrowitsch *et al.* (1995). Briefly, in cases of male genital manifestations, funiculitis (swelling of the spermatic cord) was recorded as hydrocele stage I, and true hydroceles, where the scrotum was enlarged to more than 6 cm, were graded as stage II (6.0-8.0 cm in length), III (8.1-11.0 cm), IV (11.1-15.0) and V (≥15.1 cm). For males with bilateral hydrocele, staging was done using the largest side. During the examinations, males were also asked if they had had hydrocelectomy. Leg lymphoedema/elephantiasis was graded as stage I (loss of contour, pitting lymphoedema), II (thickened skin with loss of elasticity, non-pitting oedema) or III (evident elephantiasis with skin folds and papules). Grading of hydrocele and elephantiasis has been omitted in this presentation. Instead, hydrocele ≥ stage II (true hydrocele) and elephantiasis ≥ stage I are presented as hydrocele and elephantiasis, respectively.

Microfilaria detection

Due to nocturnal periodicity of *W. bancrofti*, finger-prick blood samples were collected at night between 21.00 and 24.00 hours, and the time of collection was noted for each specimen. The counting chamber technique as described by McMahon *et al.* (1979) was used to enumerate *W. bancrofti* microfilariae in blood. Briefly, 100 μl of blood taken from a finger prick was collected in a heparinised capillary tube and washed into a plastic vial containing 0.9 ml of 3% acetic acid, before being transported from the field to the laboratory. At the laboratory, each specimen was transferred to a clean counting chamber and examined under a

compound microscope. The mf were counted and their concentration expressed as mf/ml of blood.

Serum preparation

Immediately after finger-prick blood sampling, 5 ml of venous blood was collected from each individual in plain vacutainer tubes. Serum was separated by centrifugation after overnight clotting in a refrigerator, and sodium azide was added to a concentration of 15 nm, as a preservative. Serum was initially frozen at -20°C in the field, and later stored at -80°C in the main laboratory until use. Before further handling and testing of sera, lipid coated vira were eliminated by incubating sera for six hours at room temperature with 3 µl/ml tri-N-butyl phosphate (SIGMA T-4908) and 10 µl/ml Tween 80 (SIGMA P-1754) as described by Poulsen and Sorensen (1993).

Circulating filarial antigen detection

Serum samples were tested for CFA by using the TropBio® enzyme linked immunosorbent assay (ELISA) kit for detection and quantification of *W. bancrofti* CFA in serum (JCU Tropical Biotechnology Pty Ltd., Townsville, Australia; catalogue No. 03-010-01), using rabbit anti-*Onchocerca* monoclonal antibodies (Og4C3) specific in detecting *W. bancrofti* CFA (More & Copeman 1990; Chanteau *et al.* 1994b; Lammie *et al.* 1994). The tests were performed according to instructions from the manufacturer (Appendix 6) and as described by Simonsen & Dunyo (1999). Briefly, 100 µl of each serum sample was mixed with 300 µl of sample diluent in an Eppendorf tube. The mixtures were pre-treated by boiling at 100°C for 5 minutes, and then centrifuged at 2000g for 15 minutes to obtain a supernatant fluid containing heat stable antigen; 50 µl of the supernatant was added to each well of an Og4C3 pre-coated ELISA plate. The plates were placed in a humid container and incubated overnight at room temperature, washed three times with wash buffer, then inverted and gently tapped to remove residual droplets. 50 µl of diluted rabbit anti-*Onchocerca* antibody was then added in each well and incubated for one hour. The plates were washed three times as previously, and 50 µl of diluted conjugate added to the wells and incubated for a further one hour. The plates were again washed as before and 100 µl of ABTS peroxidase substrate [2,2'-azino-di (3-ethyl-benzthiazoline-6-sulfonate) in glycine/citric acid buffer] was added as a chromogen. The plates were incubated for a final one hour, then read using an ELISA reader (Bio-Rad, USA) at a wavelength of 414 nm. Seven standards with known antigen content were included in each plate in duplicates, and the results used to prepare a standard curve relating optical density (OD) value and antigen content. All specimens were tested in duplicate and the mean measurements used to determine the response in antigen units (from the standard curve) as well as the antigen titre group (in relation to the seven standards) to which the specimen belonged. Specimens with ≥32 antigen units (≥ titre group 3)

were considered positive for CFA. Measurements that were ≥32,000 antigen units (≥ titre group 8) were assigned a fixed value of 32,000 units.

Antigen preparation

Antigen was prepared from *B. pahangi* adult worms (*W. bancrofti* adult worms are not easily obtainable in the laboratory), which had been maintained in jirds (*Meriones unguiculatus*). The worms were washed in phosphate buffered saline (PBS, pH 7.4)-poison containing 17.4 mg protease inhibitor (phenyl-methylsulphonylfluoride), 50 mg enzyme inactivator (L-1-Tosylamide-phenylchloromethyl ketone) and 2.5 mg papain and trypsin inhibitor (N-a-p-Tosyl-L-Lysinechloromethylketonehydrochloride) in 100 ml of PBS. The worms were then sonicated on an ice bath at maximum amplitude for five minutes, consisting of 15 seconds sonication bursts and 30 seconds rest intervals. The homogenate was incubated overnight at 4°C, centrifuged at 11,000 rpm for 20 minutes at the same temperature, and the supernatant filtered through a 0.45 µm filter (Minisart RC 15, Sartorius). Protein concentration as measured by Bio-Rad protein assay (BIO-Rad, USA) was 2.5 mg/ml. The obtained antigen was stored at -80°C until used.

Antibody detection

Sera were examined for filaria-specific antibodies (IgG1, IgG2, IgG3, IgG4 and IgE) by ELISA. Buffers used in ELISA were prepared according to Voller and Savigny (1981), as indicated in Appendix 7. Optimal dilutions of antigen, sera and conjugates were determined by serial dilutions. ELISA plates (Immuno-plates, Maxisorp 442404; Nunc A/S, Denmark) were coated by overnight incubation at 4°C with 100 µl of the *B. pahangi* antigen/well (antigen diluted in coating buffer to a protein concentration of 1 µg/ml for IgG1, IgG2, IgG3 and IgG4, and 2 µg/ml for IgE). All the next steps were carried out at room temperature. Following three 3-minute washes with washing buffer, 200 µl of 0.5% bovine serum albumin in washing buffer was added to each well as a blocking agent and incubated for one hour. The plates were then washed as above, and incubated with 100 µl of test serum diluted in washing buffer ($1^1/_2$ hours incubation with 1:1500, 1:500, 1:250 and 1:1000 serum dilutions for IgG1, IgG2, IgG3 and IgG4, respectively, and overnight incubation with 1:20 protein A absorbed serum dilution for IgE). The plates were washed as previously, and thereafter incubated with 100 µl horseradish-peroxidase (HRP) conjugated antisera diluted in washing buffer [1 hour incubation with 1:1500, 1:500, 1:500 and 1:2000 dilution of HRP conjugated monoclonal mouse-anti-human IgG1, IgG2, IgG3 and IgG4 (CLB, Netherlands) respectively, and 2 hour incubation with 1:1000 dilution of polyclonal rabbit-anti-human IgE (Dakopatts A/S, Denmark)]. The plates were then washed again as before. Finally, 100 µl of ortho-phenylene-diamine (OPD) substrate solution prepared from OPD tablets (Dakopatts A/S, Denmark) according to the manufacturer's instructions was added to each well. The reaction was stopped after reasonable development of colour (maximum of 20 minutes) by adding 50 µl

of 2.5 M H_2SO_4 per well. Optical density (OD) value was measured using an ELISA reader (Bio-Rad, USA) at 492 nm. Serum samples were tested in triplicate and the mean OD value calculated. A positive control serum was included on all plates, and the OD value of this serum was used to adjust for minor plate-to-plate variations. Serum was defined as being antibody positive when the OD value exceeded the mean + 2 standard deviations of OD values calculated from 30 sera obtained from people living in areas known to be free of lymphatic filariasis. These comprised 13 Kenyans infected with hookworm, 10 Kenyans with non-filarial lymphoedema, and seven Danes with no history of helminth infection.

Protein A absorption of sera

Filaria-specific IgE antibody responses were measured after protein A absorption of sera to remove IgG, in particular IgG4, blocking antibodies. In view of the large number of samples that were to be processed, it was anticipated that the conventional methods used for removing blocking IgG antibodies would be too laborious for the present study. Therefore, an easier and faster method for absorbing IgG4 from sera, based on protein A agarose beads solution in Eppendorf tubes, was developed for this purpose. Since this method had never been used previously, it was first tested for its usefulness prior to adoption for processing the test sera in the study. A detailed description of the materials and methods used, the results obtained and a discussion of these results appear in Appendix 8.

Data analysis

Data were analysed on a computer with SPSS version 9.0 software. Mf intensities were adjusted for sampling time by multiplying the counts with a time-specific factor, as described by Simonsen *et al.* (1997b). Geometric mean intensities (GMIs) of microfilaraemia, antigenaemia and filaria-specific antibody levels were calculated as antilog $[(\Sigma \log x+1)/n]-1$, with x being the number of mf/ml, number of CFA units and ELISA optical density (OD) values, respectively, and n being the number of individuals included. Upper and lower standard error values were calculated as antilog [(mean of log-transformed values) ± (standard error on log-transformed values)]. IgG4/IgE ratios were first calculated for each individual serum, and the IgG4/IgE ratio GMIs were thereafter calculated as described above. Prevalences were compared by χ^2 -tests, and GMIs were compared by *t*-tests or one-way analyses of variance (as appropriate) on log transformed values. The relationship between infection status (mf or CFA) of parents and that of their children aged 1-19 years was analysed by logistic regression with age group of children (1-4, 5-9, 10-14 and 15-19 years) as confounding variable. The odds of being mf or CFA positive for each chronic disease category were calculated from 2 x 2 table using a standard formula [odds ratio = $(a \times d)/(b \times c)$], where a, *b*, *c*, and *d* were the numbers of individuals infected and with disease, not infected but with disease, infected but with no disease, and neither infected nor with disease, respectively. A p-value <0.05 was considered to be statistically significant for all tests.

Results

Characteristics of the examined populations

Masaika had 950 inhabitants aged one year and above, and 47% of these were less than 20 years' old. The part of Kingwede selected for the study had 1,013 inhabitants aged one year and above, and 57% of these were less than 20 years' old (Table 3.1). The male to female ratio was significantly lower in Kingwede than in Masaika (χ^2-test, $p<0.001$) mainly because many young adult males (especially in the 20-29 year age group) had left Kingwede in search of employment elsewhere. Masaika had 285 inhabited houses (average of 3.3 individuals per house; range 1-14), and the selected part of Kingwede had 180 inhabited houses (average of 5.6 individuals per house; range 1-22).

Microfilaraemia

Eight hundred and forty-eight (89.2%) and 825 (81.4%) individuals in Masaika and Kingwede, respectively, had their blood examined for mf. The overall prevalence of microfilaraemia was significantly higher in Masaika (24.9%) than in Kingwede (2.7%, Table 3.1). The age-stratified mf prevalences in the two communities are shown in Tables 3.2 and 3.3, and in Figure 3.1. In both communities, microfilaraemia was rare among young children. The youngest mf positive individuals in Masaika and Kingwede were three and 14 years' old, respectively. The prevalence of mf increased with age in both communities. Among adults 20 years or more, mf prevalences were 35.2% in Masaika and 5.9% in Kingwede. In both communities, the mf prevalence was higher among males than among females. This was particularly so for those aged 20 years and more (Masaika: 45.2% vs. 25.5%, χ^2-test, $p<0.001$; Kingwede: 10.1% vs. 3.4%, χ^2-test, $p=0.015$).

Mf intensities among mf positive individuals ranged from 10-19160 mf/ml in Masaika and from 10-2520 mf/ml in Kingwede. The overall mf GMI was significantly higher in Masaika than in Kingwede, both when calculated for all examined individuals (3.6 vs. 0.1 mf/ml, respectively) and for mf positive individuals (458 vs. 174 mf/ml, respectively) as appears in Table 3.1. In Masaika, the first of these indices increased significantly by age to reach a maximum in the 50-59 year age group (Fig. 3.2a), whereas there was no significant difference between age groups in mf GMIs for mf positive individuals (Fig. 3.2b). Too few mf positive individuals were available in Kingwede for analysis of the effect of age on mf GMIs in this community.

Antigenaemia

Circulating filarial antigenaemia was determined in 837 (88.1%) and 770 (76.0%) of the inhabitants aged one year and above in Masaika and Kingwede, respectively. The overall CFA prevalence in Masaika (52.2%) was significantly higher than in Kingwede (16.5%, Table 3.1). The youngest CFA positive individuals in Masaika and Kingwede were two years and one year, respectively.

The prevalence of CFA positivity generally increased from the younger to the older age groups (Tables 3.2 and 3.3, and Fig. 3.1). The CFA prevalence in adults (≥20 years) was slightly, but not statistically significantly higher among males than females in Masaika (68.8% vs. 62.1%), whereas it was significantly higher among males than females in Kingwede (38.8% vs. 20.9%, χ^2-test, p=0.02). By far the majority of those who were mf positive were also CFA positive (97.6% for Masaika, 95.5% for Kingwede), but many were CFA positive without being mf positive. Thus, the CFA prevalence was 2.1 and 6.1 times higher than the mf prevalence in Masaika and Kingwede, respectively. This difference was more pronounced among young individuals (2.8 vs 17.3 times in 1-19 year age group) than among adults (1.9 vs. 4.3 times in the 20+ year age group). The proportion of mf negative individuals among those who were positive for CFA was significantly higher in Kingwede (83.3%) than in Masaika (52.8%), and it decreased with age in both communities (Fig. 3.3).

Table 3.1. Characteristics of the study populations in Masaika and Kingwede communities. Mf = microfilaria, CFA = circulating filarial antigen, GMI = geometric mean intensity.

	Masaika	Kingwede	
No. inhabitants ≥ 1 year	950	1013	
Male:Female ratio	1.09	0.81	χ^2-test, p<0.001
Mf prevalence (%)	24.9	2.7	χ^2-test, p<0.001
Mf GMI (mf/ml) for mf positives	458	174	t-test, p=0.016
Mf GMI (mf/ml) for all examined	3.6	0.1	t-test, p<0.001
CFA prevalence (%)	52.2	16.5	χ^2-test, p<0.001
CFA GMI (units) for CFA positives	6523	673	t-test, p<0.001
CFA GMI (units) for all examined	246	16	t-test, p<0.001
ADL reported history prevalence (%)	12.2	7.1	χ^2-test, p=0.001
Hydrocele prevalence in males ≥20 years (%)	25.3	5.3	χ^2-test, p<0.001
Elephantiasis prevalence in individuals ≥20 years (%)	4.0	0.9	χ^2-test, p=0.007

The overall CFA GMI was significantly higher in Masaika than in Kingwede (Table 3.1) both when calculated for all examined individuals (246 vs. 16 units) and for CFA positive individuals only (6523 vs. 673 units, Table 3.1). In Masaika, both of these indices increased significantly by age, but whereas the first reached a maximum in the 50-59 year age group (Figure 3.4a), the second continued to increase (Figure 3.4b). In Kingwede, CFA intensity both among all examined individuals and among positive individuals only, continued to increase gradually with age without reaching a clear peak (Figs. 3.4a and 3.4b).

Table 3.2. Population size and *W. bancrofti* microfilaraemia and antigenaemia in relation to age group in the high endemicity community (Masaika)

Age group (years)	Total population	Microfilaraemia				Antigenaemia			
		No. examined (% coverage)	No. mf positive (%)	GMI (mf/ml)* among all	GMI (mf/ml)* among positives	No. examined (% coverage)	No. CFA positive (%)	GMI (units) among all	GMI (units) among positives
1-4	99	84 (84.8)	1 (1.2)	-	-	73 (73.7)	6 (8.2)	9	1651
5-9	111	103 (92.8)	8 (7.8)	0.7	923	102 (91.9)	30 (29.4)	48	4715
10-14	139	129 (92.8)	23 (17.8)	1.9	356	129 (92.8)	60 (46.5)	105	2553
15-19	95	80 (84.2)	20 (25.0)	4.0	649	80 (84.2)	45 (56.3)	397	8691
20-29	162	142 (87.7)	47 (33.1)	6.2	388	143 (88.3)	86 (60.1)	477	8055
30-39	138	122 (88.4)	34 (27.9)	4.8	556	123 (89.1)	77 (62.6)	544	7356
40-49	83	77 (92.8)	29 (37.7)	6.8	235	76 (91.6)	54 (71.1)	788	5399
59-59	45	41 (91.1)	19 (46.3)	21.0	790	41 (91.1)	32 (78.0)	2235	10757
60+	78	70 (89.7)	30 (42.9)	14.1	564	70 (89.7)	47 (67.1)	1041	11885
Total	950	848 (89.3)	211(24.9)	3.6	458	837 (88.1)	437 (52.2)	246	6523

* Geometric means only given if > 3 mf positive individuals in group

Table 3.3. Population size and *W. bancrofti* microfilaraemia and antiagenaemia in relation to age group in the low endemicity community (Kingwede)

Age group (years)	Total population	Microfilaraemia				Antigenaemia			
		No. examined (% coverage)	No. mf positive (%)	GMI (mf/ml)* among all	GMI (mf/ml)* among positives	No. examined (% coverage)	No. CFA positive (%)	CFA (units) among all	CFA (units) among positives
1-4	163	141 (86.4)	0 (0.0)	-	-	108 (66.3)	4 (3.7)	8	403
5-9	154	139 (90.3)	0 (0.0)	-	-	129 (83.8)	9 (7.0)	9	218
10-14	157	145 (92.3)	1 (0.7)	-	-	139 (88.5)	19 (13.7)	13	595
15-19	102	78 (76.5)	2 (2.6)	-	-	77 (75.5)	15 (19.5)	18	706
20-29	179	125 (69.8)	6 (4.8)	0.3	137	122 (68.2)	29 (23.8)	24	800
30-39	104	86 (82.7)	8 (9.3)	0.6	172	86 (82.7)	21 (24.4)	24	873
40-49	58	37 (63.8)	1 (2.7)	-	-	35 (60.3)	8 (22.9)	20	477
50-59	52	41 (78.8)	1 (2.4)	-	-	40 (76.9)	8 (20.0)	18	750
60+	44	33 (75.0)	3 (9.1)	-	-	34 (77.3)	14 (41.2)	56	978
Total	1013	825 (81.4)	22 (2.7)	0.1	174	770 (76.0)	127 (16.5)	16	673

* Geometric means only given if > 3 mf positive individuals in group

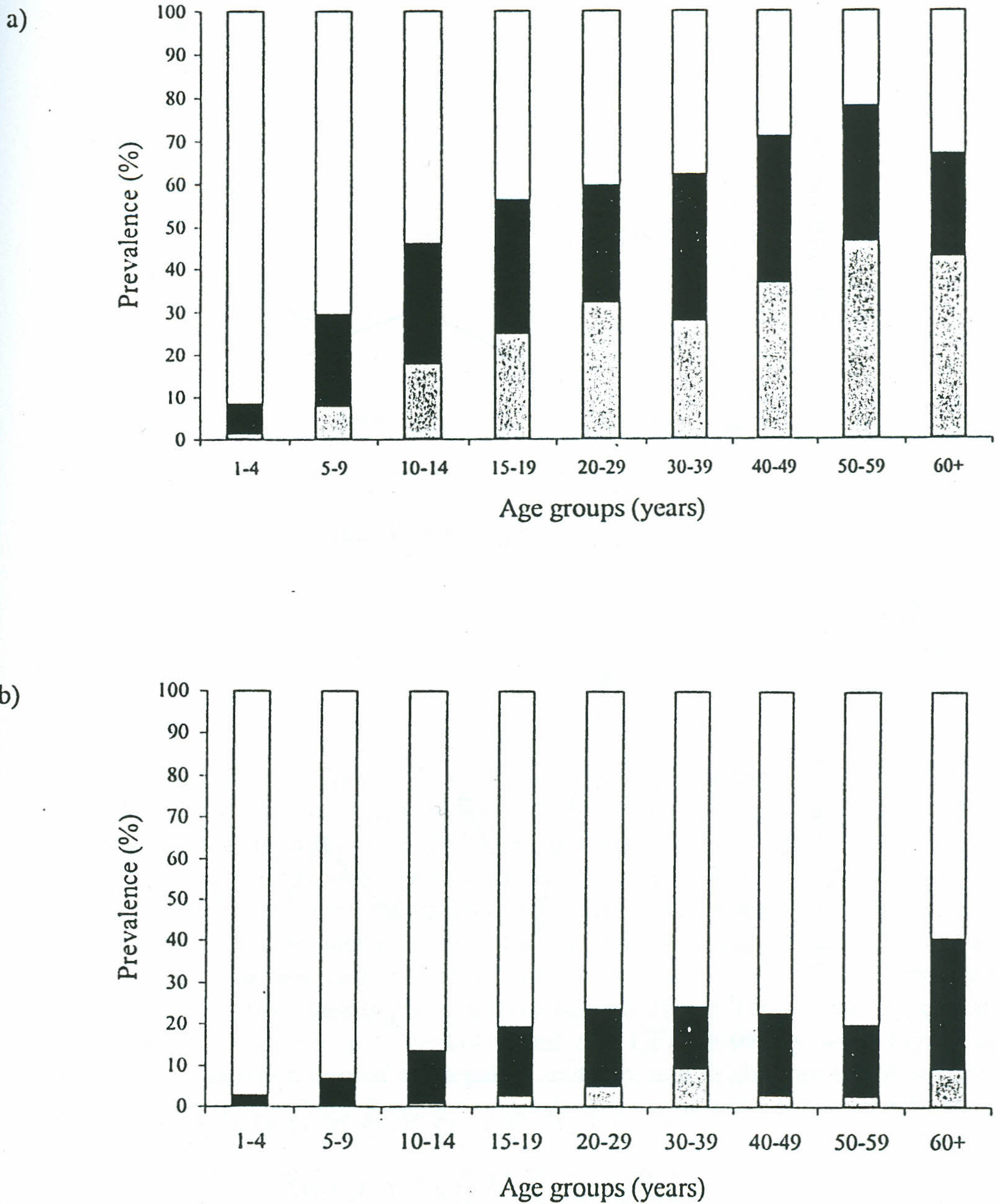
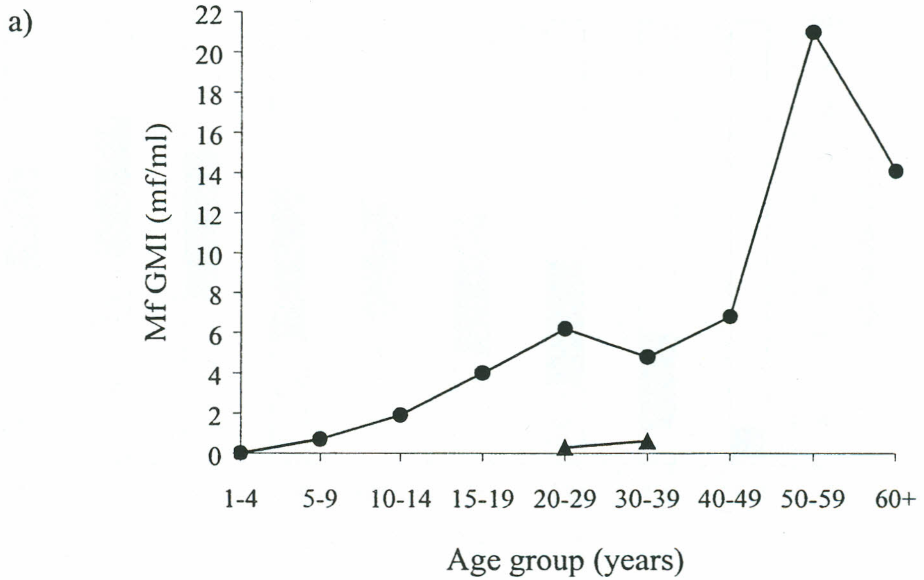


Figure 3.1. Age-specific prevalence of individuals being microfilariae and CFA positive (black part of bar), microfilariae negative but CFA positive (grey part of bar) and microfilariae and CFA negative (white part of bar) in Masaika (a) and Kingwede (b).

a)



b)

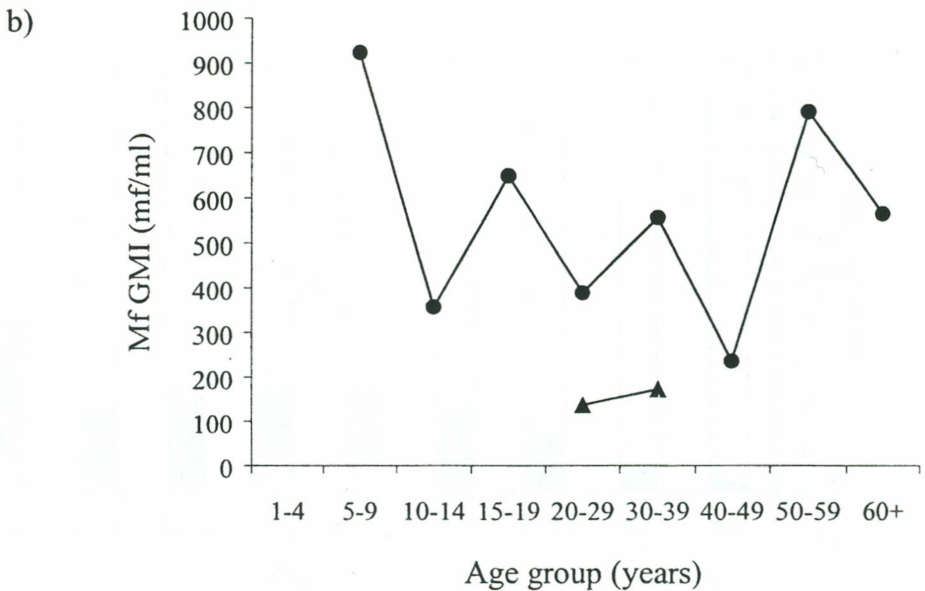


Figure 3.2. Age-specific geometric mean intensity of microfilaraemia among a) all examined individuals and b) microfilaria positive individuals only (indicated only where 3 or more in the group) in Masaika (•) and Kingwede (▲).

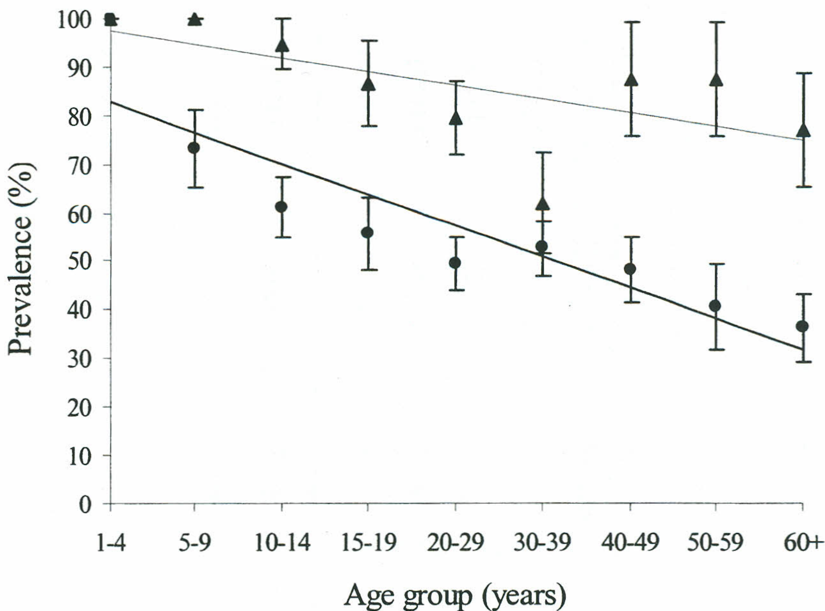


Figure 3.3. Age-specific prevalence of mf negative individuals among those being CFA positive in Maisaka (•) and Kingwede (▲), based on individuals examined for both indices. Trend lies are indicated.

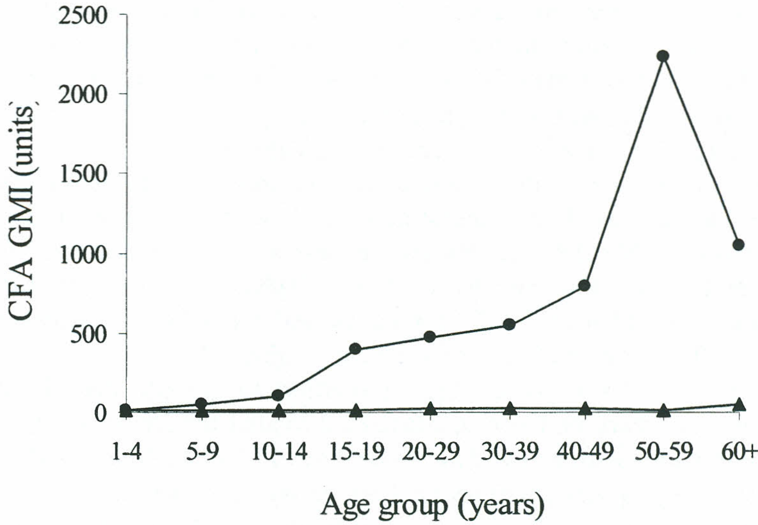
Relationships between mf and CFA status in children and their parents

Among mf and CFA examined individuals aged <20 years in Masaika, 286 (74.7%) had their mothers identified and examined for both mf and CFA, while 183 (47.8%) had their fathers identified and examined for both parameters. Among mf and CFA examined individuals aged <20 years in Kingwede, 312 (68.9%) had their mothers identified and examined for mf and CFA, while 149 (32.9%) had their fathers identified and examined for these parameters. The relationship between mf and CFA status of these individuals and that of their parents appears in Table 3.4. Generally, the prevalences of mf and CFA positivity were higher in children of mf positive than of mf negative mothers, and in children of mf positive than of mf negative fathers. Similarly, prevalences of mf and CFA positivity were higher in children of CFA positive than of CFA negative mothers and in children of CFA positive than of CFA negative fathers. However, none of the observed differences was statistically significant when analysed by logistic regression ($p>0.05$ for all tests).

Table 3.4. Relationship between mf and CFA status of children aged less than 20 years in relation to that of their parents (Masaika and Kingwede)

	Masaika			Kingwede		
	No. of children examined	No. of children mf +ve (%)	No. of children CFA +ve (%)	No. of children examined	No. of children mf +ve (%)	No. of children CFA +ve (%)
Mf +ve mothers	70	11 (15.7)	26 (37.1)	11	1 (9.1)	3 (27.3)
Mf -ve mothers	216	24 (11.0)	74 (34.3)	301	0 (0.0)	21 (7.0)
Mf +ve fathers	95	15 (15.8)	45 (47.4)	16	1 (6.3)	3 (18.8)
Mf -ve fathers	88	5 (5.7)	23 (26.1)	133	1 (0.8)	15 (11.3)
CFA +ve mothers	189	27 (14.3)	73 (38.6)	51	1 (2.0)	3 (5.9)
CFA -ve mothers	97	8 (8.2)	27 (27.8)	261	0 (0.0)	21 (8.0)
CFA +ve fathers	138	17 (12.3)	54 (39.1)	55	1 (1.8)	8 (14.5)
CFA -ve fathers	45	3 (6.7)	14 (31.1)	94	1 (1.1)	10 (10.6)

a)



b)

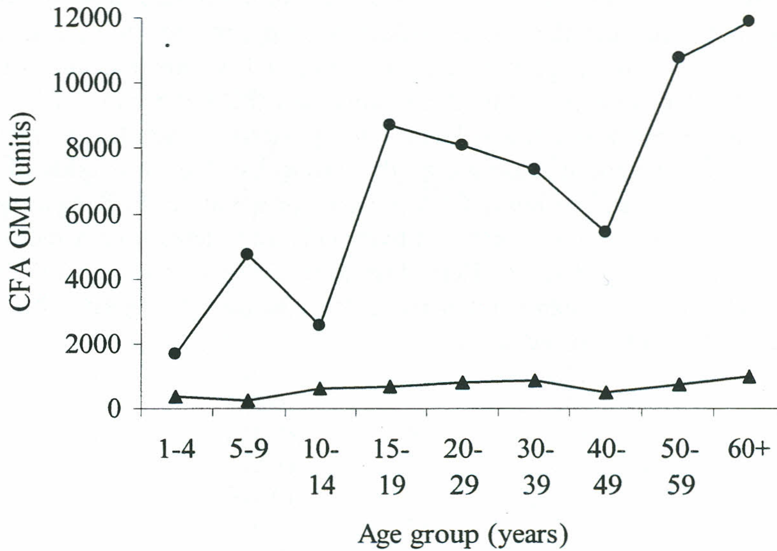


Figure 3.4. Age-specific geometric mean intensity of antigenaemia among a) all examined individuals and b) CFA positive individuals only in Masaika (•) and in Kingwede (▲).

Chronic filarial disease

The age-specific prevalence patterns of chronic filarial disease manifestations in the two communities appear in Tables 3.5 and 3.6. Hydrocele was the most common chronic manifestation in both communities. The youngest males with hydrocele in Masaika and Kingwede were 20 and 31 years old, respectively. Among adult males (≥20 years) the prevalence of hydrocele was significantly higher in Masaika (25.3%) than in Kingwede (5.3%, Table 3.1).

In both communities, elephantiasis was a less common clinical manifestation, and was confined to the legs. The youngest individuals with leg elephantiasis were 11 and 30 years old, in Masaika and Kingwede, respectively. Among individuals aged 20 years and above, the prevalence of leg elephantiasis was significantly higher in Masaika (overall 4.0%, 2.8% for males and 5.1% for females) than in Kingwede (overall 0.9%; one male and two females, Table 3.1).

Analysis of mf and CFA prevalences in relation to chronic manifestations among those aged 20 years or more in Masaika revealed that these were of the same magnitude in males with and without hydrocele (43.6% vs. 46.5% and 61.8% vs. 71.1%, respectively, χ^2-test, $p>0.05$ for both tests). In contrast, mf and CFA prevalences were considerably lower among individuals with elephantiasis than among those without (22.2% vs. 35.8% for mf and 37.9% vs. 66.7% for CFA), and this difference was statistically significant for CFA but not for mf (χ^2-test, $p=0.016$ and $p>0.05$, respectively). Too few cases of hydrocele and elephantiasis were present in Kingwede for statistical analysis of infection data for these.

The odds of infection between the various groups in Masaika and Kingwede are shown in Figure 3.5. In Masaika, the individuals with hydrocele had the same odds of being mf positive or negative, and of being CFA positive or negative. Individuals with elephantiasis in this community had the same odds of being mf positive or negative, but less odds of being CFA positive. When the groups were combined to form a chronic disease group, this group had the same odds of being mf positive or negative and of being CFA positive or negative. In Kingwede, this analysis was only possible with combined hydrocele and elephantiasis individuals into a group with chronic disease. Here, this group of individuals with chronic disease had higher odds of being mf positive than being mf negative, but equal odds of being CFA positive or negative.

Table 3.5 Clinical manifestations related to *W. bancrofti* infection in the high endemicity community (Masaika).

Age group (yrs)	Leg elephantiasis		Hydrocele*		History of ADL	
	No. Examined	No. positive (%)	No. Examined	No. positive (%)	No. interviewed	No. positive responses (%)
1-4	87	0 (0.0)	40	0 (0.0)	86	0 (0.0)
5-9	104	0 (0.0)	68	0 (0.0)	104	10 (10.0)
10-14	127	1(0.8)	72	0 (0.0)	127	13 (10.2)
15-19	80	0 (0.0)	38	0 (0.0)	79	12 (5.2)
20-29	141	1 (0.7)	67	10 (14.9)	140	19 (13.6)
30-39	124	7 (5.6)	59	10 (16.9)	123	26 (21.1)
40-49	75	4 (5.3)	33	8 (24.2)	75	11 (14.7)
59-59	41	1 (2.4)	22	8 (36.4)	41	5 (12.2)
60+	71	5 (7.0)	36	19 (52.8)	71	7 (9.9)
Total	850	19 (2.2)	435	55 (12.6)	847	103 (12.2)

* ≥ stage II, males only

Table 3.6 Clinical manifestations related to *W. bancrofti* infection in the low endemicity community (Kingwede).

Age group (yrs)	Leg elephantiasis		Hydrocele*		History of ADL	
	No. Examined	No. positive (%)	No. Examined	No. positive (%)	No. interviewed	No. positive responses (%)
1-4	150	0 (0.0)	74	0 (0.0)	150	2 (1.3)
5-9	144	0 (0.0)	67	0 (0.0)	144	4 (2.8)
10-14	145	0 (0.0)	70	0 (0.0)	145	18 (12.4)
15-19	81	0 (0.0)	33	0 (0.0)	81	11 (13.6)
20-29	137	0 (0.0)	46	0 (0.0)	137	14 (10.2)
30-39	91	2 (2.2)	37	1 (2.7)	91	5 (5.5)
40-49	38	0 (0.0)	17	2 (11.8)	38	2 (5.3)
59-59	43	0 (0.0)	15	1 (6.6)	43	5 (11.6)
60+	34	1 (2.9)	17	3 (17.6)	34	0 (0.0)
Total	863	3 (0.3)	376	7 (1.9)	863	61 (7.1)

*≥ stage II, males only

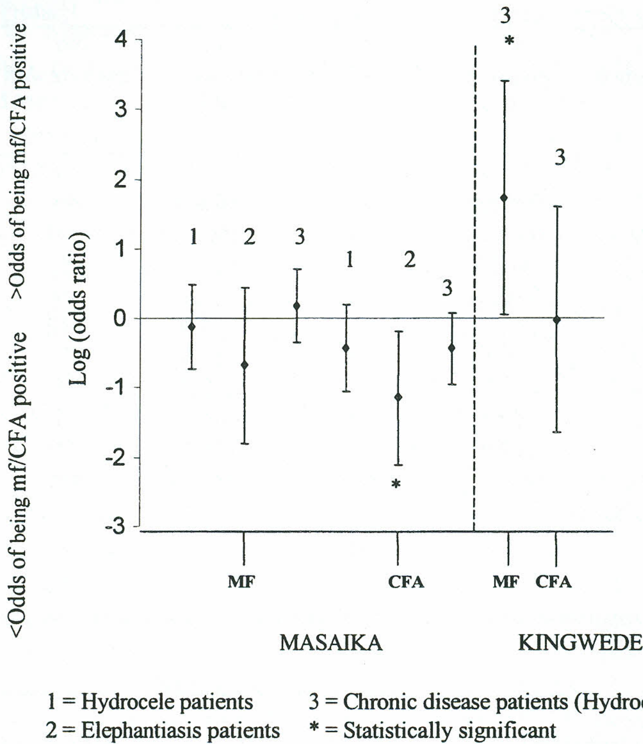


Figure 3.5. The odds ratio of being microfilaria or circulating filarial antigen positive among individuals with or without chronic obstructive disease in Masaika and Kingwede.

History of acute adenolymphangitis attacks

Significantly more individuals reported having experienced one or more ADL attack during the one-year period preceding the survey in Masaika (12.2%) than in Kingwede (7.1%, Table 3.1). In both communities, the prevalence of reported ADL attack was higher among individuals aged ≥20 years than in younger individuals (Table 3.4 and 3.5), but this difference was only significant for Masaika (15.1% vs. 8,.8%, χ^2-test, p=0.005), and not for Kingwede (7.6% vs. 6.4%, χ^2-test, p>0.05). In Masaika, the prevalence of reported ADL attacks was higher among males than among females (14.5% vs. 9.7%, χ^2-test, p=0.02), whereas gender differences in Kingwede were negligible (6.4% vs. 7.6%). Among those reporting attacks in Masaika, the mean numbers were 2.0 (2.1 for males and 1.9 for females) and the

range 1-5. Among those reporting ADL attacks in Kingwede, the mean number was 2.7 and the range 1-12.

In neither of the communities was there a statistically significant difference in prevalence of reported history of ADL attacks between mf positive and mf negative individuals (12.7% vs. 16.5% in Masaika, 15.8% vs. 7.0% in Kingwede) or between CFA positive and CFA negative individuals (13.4% vs. 17.8% in Masaika, 7.5% vs. 6.8% in Kingwede, χ^2-test, $p>0.05$ for all tests).

In Masaika, the prevalence of individuals reporting ADL was much higher among individuals with elephantiasis than in those without (72.2% vs. 12.7%, χ^2-test, $p<0.001$) whereas no difference was seen in relation to hydrocele status (16.4% vs. 18.8%). Too few individuals in Kingwede had hydrocele or elephantiasis for statistical analysis of their ADL status.

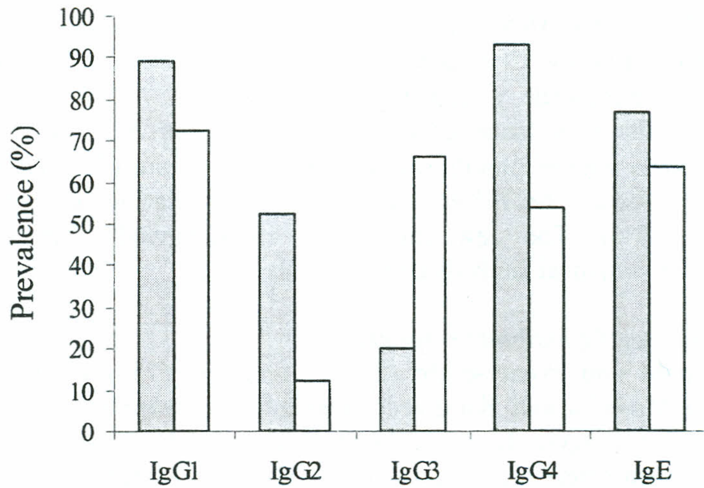
Overall filaria-specific antibody responses

Eight hundred and twenty-eight (87.2%) and 766 (75.6%) of the registered individuals in Masaika and Kingwede, respectively, were examined for all the filaria-specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies. For IgG1, IgG2, IgG4 and IgE, both parameters were significantly higher in Masaika than in Kingwede (χ^2 test and *t*-test, $p<0.001$ for all tests). Surprisingly, the opposite pattern was found for IgG3, for which both prevalence and mean intensity were significantly higher in Kingwede than in Masaika (χ^2 test and *t*-test, $p<0.001$ for both tests).

The overall prevalences of positivity and mean intensities of these antibodies in the two communities appear in Figure 3.6.

The overall mean IgG4/IgE ratios in the two communities are shown in Figure 3.7. The ratio for Masaika (2.35) was significantly higher than for Kingwede (0.48, *t*-test, $p<0.001$).

a)



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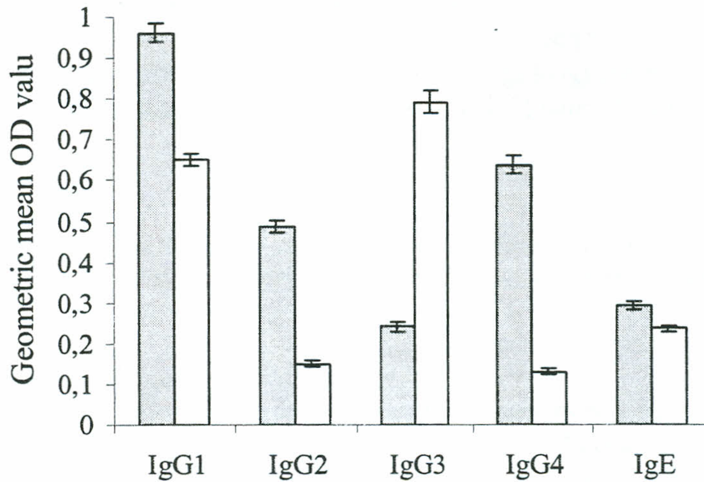


Figure 3.6. Overall prevalence (a) and geometric mean intensity (b) of filaria-specific IgG1, IgG2, IgG3, IgG4 and IgE in Masaika (grey bars) and Kingwede (white bars). Vertical lines indicate standard error bars.

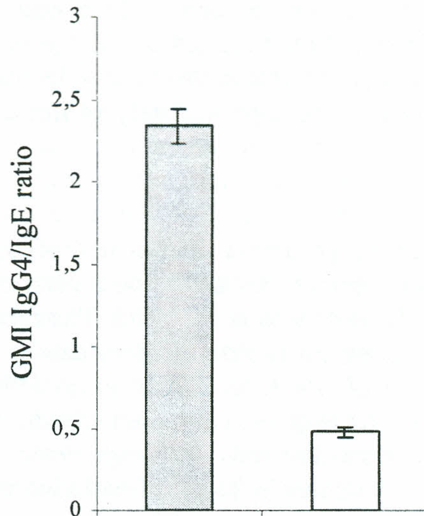


Figure 3.7. Overall mean filaria-specific IgG4/IgE ratio (± S.E) in Masaika (grey bar) and Kingwede (white bar).

Discussion

The patterns of infection, disease and filaria-specific antibody responses were analysed in two communities with high and low bancroftian filariasis endemicity. This was performed by examining and comparing profiles of microfilaraemia, antigenaemia, acute and chronic filarial disease, and specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies in the two communities. Overall community mf prevalences were used as the criteria for selecting high and low endemicity communities, being expected indicators of community transmission intensity.

Transmission intensity

None of the methods currently being used in measuring transmission intensity in human bancroftian filariasis are ideal. The most accurate index for this would be the rate of acquisition of sexually mature adult worms (Southgate 1992). However, since this cannot be measured by the currently available techniques, alternative albeit less satisfactory indices have been adopted. Commonly used indicators are microfilarial prevalence and mean intensity data (Southgate 1992). More accurate, but also more cumbersome to obtain, are entomological indices for which female mosquito vector populations are examined for infection over a period of time. The most common among these involves measuring the proportion of man-biting mosquitoes with developing and/or infective third-stage larvae (infection rate), the

proportion of these mosquitoes with infective third-stage larvae (infectivity rate) and the average number of infective larvae per vector mosquito (WHO 1984; Chanteau *et al.* 1995). In the present study, these entomological indices were subsequently measured in both communities, for a one-year period following the cross-sectional survey, as part of another PhD study. Using these data, the overall infectivity rate was estimated to be approximately 14 times higher in Masaika than in Kingwede.

Infection

A higher prevalence of antigenaemia than of microfilaraemia was observed in all age groups and in both communities as has been seen by others (Lammie *et al.* 1994; Chanteau *et al.* 1995; Simonsen *et al.* 1996). The fact that CFA was detected in all mf positive and in some mf negative individuals is also in agreement with previous studies (Turner *et al.* 1993; Simonsen *et al.* 1996; Wamae *et al.* 1998; Simonsen & Dunyo 1999), and implies that more individuals were actually infected than those in whom mf were detected. Although detection of mf confirms the existence of one or more living, mated, fecund female worms, it lacks sensitivity as regards detection of infection owing to the long pre-patent period in lymphatic filariasis, single-sex infections and the possible suppression of microfilaraemia by immune mechanisms (WHO 1984). Thus, a positive CFA test, which may be an indicator of the presence of adult worms (Chanteau *et al.* 1994a; Simonsen & Dunyo 1999), or perhaps even infection in general, is a better marker of infection than mf positivity. The higher discrepancy between CFA and mf prevalence in Kingwede than in Masaika in all age groups suggests that CFA detection was more useful in identifying infections missed by mf detection in the low than in the high transmission intensity community. This was probably related to the different levels of mf intensities in the two communities. Mf counts were much lower in the low than the high transmission intensity community and could have easily been missed by microscopy. Furthermore, individuals in the low transmission intensity community probably have low adult worm loads and therefore less chance of male and female worms meeting to produce mf.

Microfilaraemia started at a much younger age and mf and CFA prevalences in all age groups were much higher in the high transmission than in the low transmission intensity community. The low mf and CFA prevalence in young individuals and the gradual increase until old age seen in both communities, is quite typical for lymphatic filariasis (Wijers & Kiilu 1977; McMahon *et al.* 1981; Meyrowitsch *et al.* 1995a; Dunyo *et al.* 1996; Simonsen *et al.* 1996; Wamae *et al.* 1998), although in Masaika there is some evidence for a decrease in the rate of infection among the oldest individuals. The decrease in rate of acquisition of new infections among adults may be attributed to either a decrease in exposure or to acquired immunity (Anderson & May 1985; Woolhouse *et al.* 1991; Michael & Bundy 1998). There is no evidence in the present study to suggest that adults were less exposed to infection than children were. By contrast, mathematical models of helminth immunity predict that communities with higher transmission intensities

will have more convex age-prevalence curves and earlier age of peak prevalence than communities with low transmission intensities (Michael & Bundy 1998; Michael 2000). This suggests that acquirement of immunity may explain the decline in infection rate observed among the oldest age-class in Masaika, although it is clear that the overall impact of immunity on infection in this community if operational is very slight. Indeed, the lack of a clear convexity in the age-infection prevalence pattern in Masaika compared to Kingwede, despite vastly different transmission intensities in the two communities, could suggest that transmission in both communities was not sufficient to induce significant immunity in the older age groups.

The age-specific prevalence of mf negative individuals among those being CFA positive was higher in the low than in the high transmission intensity community, and decreased with age in both communities. The higher proportion of mf negative but CFA positive individuals in the low than in the high transmission intensity community may have been due to a higher prevalence of low-density microfilaraemia in the former than in the latter. Individuals in the low transmission intensity community probably had fewer adult worms than individuals in the high transmission intensity community. Thus, the worms in individuals in the low transmission intensity community had a lower mating probability and lower mf production, resulting in the individuals in that community having a higher prevalence of low-density microfilaraemia. A similar argument is advance for the observed decrease with increasing age in the proportion of mf negative but CFA positive individuals in both communities. Assuming that individuals in these communities continue to acquire infection with age, then the adult worm burden can be expected similarly to increase with age, increasing their mating probability and intensity of mf produced. Thus, the prevalence of low-density microfilaraemia would be expected to decrease with age, and subsequently the proportion of individuals mf negative but CFA positive would be expected to be less with increasing age.

The mf GMI among all examined individuals in the high transmission community increased with age as has been observed previously (Knight *et al.* 1979; Kazura *et al.* 1984; 1997). The numbers of individuals in the low transmission intensity community were too few to allow for a similar analysis. The increase in mf intensity with age can be attributed to new infections in the population arising from the continued transmission, thereby reducing the number of amicrofilaraemic individuals and therefore increasing the average mf intensity.

In contrast to increasing mean mf intensities with age among all examined individuals, mf intensities among positive individuals remained fairly stable with increasing age. Uniform mf intensities with increasing age among positive individuals have previously been observed in other endemic populations (Meyrowitsch *et al.* 1995a; Simonsen *et al.* 1995a; Dunyo *et al.* 1996). This may have been due to some regulating mechanism, probably immune mediated, within the host. The effect of transmission intensity on mf age-intensity pattern could not

be determined in the present study since the low transmission intensity community had very few mf positive individuals in each age group.

Mean CFA intensities in the two communities increased gradually with age, both among all examined individuals and among positive individuals only. Since CFA is probably derived from adult worms, it is suggested that the older individuals harboured more adult worms than the younger individuals. Thus, it would appear that the host's mf regulating mechanism suggested earlier did not have a similar effect in controlling the adult worm burden.

Mean CFA intensities were higher in the high transmission than in the low transmission intensity community in all the age groups. In the community with high transmission, CFA mean intensity reached a peak in the 50-59 year age group among all examined individuals, but there was no clear peak among the positive individuals alone. In the community with low transmission, CFA intensity continued to increase gradually among all the examined individuals and among positive individuals alone, without reaching a clear peak. Thus, the age at which peak CFA intensity was reached occurred much earlier in the high than in the low transmission intensity community. A left shift in the age of peak infection intensity in a high rather than a low transmission intensity community suggests the existence of a transmission driven acquired immunity (Woolhouse *et al.* 1991; Fulford *et al.* 1992; Woolhouse 1994; Michael & Bundy 1998). As earlier mentioned, the levels of transmission intensity were probably too low to induce immunity in older age groups, especially in the low transmission intensity community, to thereby allow for a clearer left shift in age of peak infection intensity.

Parental child relationships in infection

Recent studies have indicated that maternal but not paternal microfilaraemia correlates significantly with increased probability of microfilaraemia in children (Lammie *et al.* 1991; Hightower *et al.* 1993; Steel *et al.* 1994; Meyrowitsch *et al.* 1995b). Based on these observations, it was suggested that intra-uterine exposure to filarial antigens increases the risk of infection in the offspring, probably by diminishing their antifilaria immune responsiveness. On the other hand, other studies have indicated that children of both mf positive fathers and mothers are more likely to be mf positive than children of mf negative parents (Das *et al.* 1997; Weil *et al.* 1999). From these studies, it appears that parental and not only maternal infection is the more important risk factor for infection in the offspring.

As in previous studies, the present study had no information on the infection status of mothers during gestation of the children examined. However, since mf status in most individuals living in areas of uninterrupted infection transmission remains consistent for many years (Meyrowitsch *et al.* 1995b), it was assumed that the mf status of most of the women at the time of the study was similar to that during their pregnancies.

The relationship between circulating filarial antigenaemia in parents and their offspring has not been analysed previously. Circulating filarial antigen levels in the adults have previously been shown to remain fairly stable over time (Day *et al.*

1991b). It was therefore assumed equally that the CFA status of most of the women examined at the time of the study was similar to that during their pregnancies.

The study found that the children of infected parents generally had higher mf and CFA prevalences than the children of non-infected parents, and that this was not related to the mothers only. Although these differences were not statistically significant, they weigh more towards the notion that household exposure may be a more important risk factor in determining infection in children than maternal infection. Transmission intensity did not appear to influence this relationship between infection status of parents and that of their offspring, as the relationships were similar in the high and the low transmission intensity communities.

Chronic disease

In lymphatic filariasis, chronic disease is thought to result from mechanical damage to the lymphatics by worms or their products, local immunological responses to parasite antigen, and secondary opportunistic bacterial and/or fungal super-infection of the damaged lymphatic vessels (Freedman 1998; Almeida & Freedman 1999; Dreyer & Piessens 2000). The relative contribution of each of these components is not well known. In the present study, both hydrocele and elephantiasis appeared at an earlier age, and their prevalences were higher in the high than in the low transmission intensity community. This is in agreement with the suggestion that transmission intensity correlates with development of chronic disease in bancroftian filariasis (Kazura *et al.* 1997). However, in the study of Kazura *et al.* (1997), transmission intensity only correlated with leg elephantiasis and not with hydrocele. Gyapong *et al.* (1998) on the other hand found community microfilaraemia rates to be strongly correlated with hydrocele. Similarly, other studies have shown a strong correlation between a community's microfilaraemia prevalence and both elephantiasis and hydrocele (Dunyo *et al.* 1996). Higher prevalences of chronic disease in communities with higher transmission intensity may be due to several factors. Firstly, since these individuals have higher worm burdens, they have a higher probability of their lymphatics being damaged mechanically by the worms. Secondly, as these individuals are exposed to more frequent infective bites, they are more likely to produce more intense immune responses to the deposited L3 larvae as well as other larval and adult stages, hence resulting in more aggressive immunopathology to the lymphatics (Maizels & Lawrence 1991). Finally, lymphatic damage resulting from these two factors may make patients more vulnerable to secondary bacterial/fungal infections, thereby propagating chronic pathology.

The study investigated the association between chronic disease and infection status. It has generally been assumed that individuals with chronic lymphatic filarial disease are mostly mf negative. However, meta-analysis of data from several endemic areas has recently shown that individuals with and without chronic pathology are equally likely to be mf positive (Michael *et al.* 1994). Mathematical models assuming that mf positive individuals become amicrofilaraemic with time but reacquire microfilaraemia at a rate equivalent to that of initial infection, suggest

that the proportion of individuals with chronic obstructive filarial disease and mf in a community depends on the local incidence of infection (Bundy *et al.* 1991a). Thus, in areas of low transmission intensity, patients with chronic lymphatic disease are typically mf negative (Vanamail *et al.* 1989), whereas in areas of high transmission intensity, individuals with and without chronic disease have the same mf rates (Partono 1987). It has been argued that in high transmission intensity areas, re-infection rates are high resulting in more individuals with chronic disease becoming re-infected, hence being mf positive, whereas in low transmission intensity areas re-infection rates are low hence most of these individuals remain mf negative (Michael *et al.* 1994). This was not the case in the present study. Whereas the finding in the high transmission intensity community agreed with this argument, that in the low transmission intensity did not. In the high transmission intensity community individuals with chronic disease were equally likely to be mf positive or negative, while in the low transmission intensity community they were more likely to be mf positive. However, these results should be interpreted with some caution. Since in the low transmission intensity community very few individuals had hydrocele or elephantiasis, inter-community comparison was only possible with the two groups combined to form a chronic disease group. This may be misleading. The different patterns of microfilaraemia generally observed in these two conditions (those with hydrocele being more likely to be mf positive while those with elephantiasis being more likely to be mf negative) probably indicate that the two have different aetiologies (Meyrowitsch *et al.* 1995a). Thus, ideally, individuals with these conditions should not be simply grouped together as 'chronic disease'. In the present study, the chronic disease group in the low transmission intensity community consisted mainly of mf positive males with hydrocele. It was therefore not surprising that this group had high odds of being mf positive.

Acute disease

The present study relied on a history of acute adenolymphangitis (ADL) attacks, to measure acute clinical manifestations of bancroftian filariasis. This method may have limited reliability since one-year memory recall is bound to be quite inaccurate. Furthermore, it is possible that despite the stringent criteria used for defining an ADL attack, some of the so-called attacks may have been due to other febrile illnesses. As a result, these findings should be treated with caution. Higher proportions of individuals with ADL attacks were reported in the high than the low transmission intensity community. Furthermore, in the high but not in the low transmission intensity community, the proportion of individuals reporting ADL attacks was higher in the older than in the younger age groups and higher in males than in females. Other workers in Papua New Guinea have previously found higher ADL prevalence in males than in females (Alexander *et al.* 1999). From this, it seems possible that transmission intensity may influence the proportion of individuals reporting ADL attacks, and its relationship with age and with gender. No association was found between proportion of individuals reporting ADL attacks

and mf or CFA status. An association between ADL and microfilaraemia has been observed previously (Weller *et al.* 1982).

The proportion of individuals reporting ADL attacks in the high transmission intensity community was significantly higher in those with elephantiasis than in those without, as reported elsewhere (Pani *et al.* 1995; Shenoy *et al.* 1995; Ramaiah *et al.* 1996). It may be that the relationship between the two conditions is complementary. Thus, individuals with elephantiasis are predisposed to more ADL attacks, while more frequent ADL attacks lead to progression of the elephantiasis. It could not be examined whether the association between ADL and elephantiasis was affected by transmission intensity in the present study, since individuals with elephantiasis in the low transmission intensity community were very few.

Antibody responses

The intensity of exposure to transmission may be a key factor in determining anti-filaria immune responses (Bailey *et al.* 1995). Specific antibody prevalences and mean intensities differed significantly between Masaika and Kingwede. The overall prevalences and mean intensities of IgG1, IgG2, IgG3, IgG4 and IgE were significantly higher in Masaika than in Kingwede, whereas the opposite was seen for IgG3, implying that the production and/or regulation of these antibodies were influenced by transmission intensity. Thus, transmission intensity must be taken into account when examining for differences in specific antibody response patterns in different population groups.

IgG4 has often been claimed to be a marker of infection in lymphatic filariasis (Lal & Ottesen 1988; Kwan-Lim *et al* 1990; Kurniawan *et al.* 1993; Atmadja *et al.* 1995), and both prevalence and intensity of this isotype were higher in Masaika than Kingwede. However, in both communities its prevalence of positivity was much higher than that of both mf and CFA, as was also observed by Simonsen *et al.* (1996) and by Weil *et al.* (1999). Being IgG4 positive therefore does not necessarily denote active infection in individuals, and may be related to repeated exposure to infective larvae, whether this leads to a patent infection or not. Indeed, IgG4 has been suggested as a marker of intensity of larvae exposure (Bailey *et al.* 1995).

The higher IgG3 prevalence and intensity in the low transmission intensity community is surprising. This antibody isotype has previously largely been associated with pathology (Ottesen 1992; Yazdanbakhsh *et al.* 1993). It is probable that specific IgG3 production may be regulated by high infection intensity above a certain threshold, which had not been reached in the low transmission intensity community. However, it is also probable that a balance between this IgG isotype and other IgG isotypes exists. Thus, in the presence of high levels of IgG1, IgG2 and IgG4, the level of IgG3 is low, whereas in the presence of low levels of these other isotypes, the IgG3 level becomes high.

Studies in schistosomiasis indicate that IgG4 levels peak early in life and then slowly decline relative to increasing levels of IgE over time. The resulting decrease in IgG4/IgE ratio has been associated with age-related acquired immunity to

invasive schistosomiasis (Hagan *et al.* 1991). A similar theory in lymphatic filariasis has been suggested, whereby resistance and permissiveness to infection are associated with low and high IgG4/IgE ratio, respectively (Kurniawan *et al.* 1993). If this is the case, it would appear therefore that the community in Kingwede was more resistant to infection than Masaika, which is unlikely considering the observed age-intensity patterns of infection in the two communities.

The patterns of specific antibodies in relation to infection, chronic disease, age and gender will be analysed in subsequent chapters.

Conclusions

In conclusion, the present study suggested that transmission intensity influences the prevalence and intensity of microfilaraemia and antigenaemia, the age of onset of microfilaraemia and of chronic disease, the prevalence of acute and chronic filarial disease, the relationship between infection and gender, the relationship between acute filarial disease with age and with gender, and the overall filaria-specific antibody responses. However, transmission intensity did not appear to determine the type of chronic filarial disease that is more prevalent in a community or to influence the association between parental infection and that of their offspring. The effect of transmission intensity on development of resistance is suggested, but did not come out clearly probably because the levels of transmission in the communities were not great enough to cause a visible impact.

Chapter 4

Filaria-specific antibody responses in two communities with high and low endemicity of *Wuchereria bancrofti* infection. I. Antibody profiles in relation to infection and clinical status

Introduction

Filaria-specific antibody response patterns in relation to chronic disease presentation in lymphatic filariasis is still unclear. Previous studies looking at this relationship have often assumed that individuals with chronic disease have cleared their infection (since they are often mf negative), possibly by some immunological mechanisms. However, more sensitive diagnostic techniques indicate that some of these individuals still harbour active infection (More & Copeman 1990; Addiss *et al.* 1995; Dreyer *et al.* 1996). A meta-analysis of data from published epidemiological surveys has furthermore indicated that the probability of having microfilaraemia is not significantly different between individuals with and without chronic disease (Michael *et al.* 1994). Since the presence of active infection has profound effects on the immune response (Yazdanbakhsh *et al.* 1993), it is imperative that studies looking at antibody responses in lymphatic filariasis classify individuals according to clinical status as well as infection status rather than clinical status alone. In this chapter, antibody profiles in two communities with high and low bancroftian filariasis endemicity were analysed in relation to both the clinical and infection status of the individuals. Thus, individuals in each community were first classified clinically as being either asymptomatic or having chronic disease (hydrocele and/or elephantiasis). Individuals in each of these groups were then further categorised by their infection status using both microfilaraemia and antigenaemia. Therefore, asymptomatic individuals or individuals with chronic disease were either negative for both mf and CFA, negative for mf but positive for CFA positive, or positive for both mf and CFA. Specific IgG1, IgG2, IgG3, IgG4 and IgE antibody responses among asymptomatic individuals and individuals with chronic disease were then compared in relation to their infection status. These responses were further compared between the communities by clinical groups, also in relation to infection status.

Materials and methods

Study populations and field and laboratory procedures

This study was conducted with the sera obtained from individuals living in the two communities, Masaika and Kingwede. Only individuals who were clinically examined and tested for mf, CFA and all filaria-specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies were included for analyses. The study area and study populations, the procedures for clinical examinations, mf detection, serum preparation, measurements of CFA and of the above mentioned filaria-specific antibodies have previously been described in Chapter 3.

Data analysis

Data were analysed essentially as previously described (Chapter 3), with mean mf, CFA and specific antibody intensities calculated as geometric means. Further analyses were carried out using S-PLUS® computer software as described (S-PLUS 2000, 1999). Antibody prevalences and intensities between the asymptomatics and

individuals with chronic disease were analysed employing generalised linear model (GLM) using binomial errors and Gaussian errors for intensity data, with prevalence or intensity (log transformed OD values) as response and clinical status and infection status as factors. Chi-square tests and one-way analyses of variance (ANOVA) were used to compare each antibody's prevalences and GMIs in relation to infection status, respectively. Differences in antibody GMIs between communities were analysed by factor GLMs with antibody OD values as responses, and infection status and community as factors. The Turkey method was used to compare pair-wise differences among groups since group sizes were unequal. A p-value <0.05 was considered statistically significant for all the tests. The 95% confidence limits for differences in antibody prevalences were calculated as $\pm \{\sqrt{[p(1-p)/n]}\} \times 1.96$, with p being the proportion of individuals positive for the respective antibody in the respective infection group and n being the total of individuals in that group.

Results

Characteristics of the study populations based on their infection status

A total of 817 out of 950 (86%) individuals in Masaika and 763 out of 1013 (75.3%) individuals in Kingwede qualified for analysis in this part of the study. Based on their mf and CFA status, individuals were divided into three groups, namely mf and CFA negative, mf negative but CFA positive, and finally mf and CFA positive (Tables 4.1 and 4.2).

Table 4.1. The characteristics of the study population in Masaika grouped according to their infection status.

	Infection status of examined individuals		
	Mf -ve & CFA –ve (Group 1)	Mf –ve & CFA +ve (Group 2)	Mf +ve & CFA +ve (Group 3)
No. examined	388	226	203
Males/Females	196/192	98/128	129/74
Mean Age (years)	20.1	28.4	34.8
Mf GMI (Mf/ml)*	-	-	491
CFA GMI (units)*	6.8	2332	20253

*GMIs based on all examined individuals

Table 4.2. The characteristics of the study population in Kingwede grouped according to their infection status.

	Infection status of examined individuals		
	Mf -ve & CFA –ve (Group 1)	Mf –ve & CFA +ve (Group 2)	Mf +ve & CFA +ve (Group 3)
No. examined	638	104	21
Males/Females	265/373	50/54	14/7
Mean Age (years)	19.0	28.2	34.7
Mf GMI (Mf/ml)*	-	-	154
CFA GMI (units)*	7.2	368	13830

*GMIs based on all examined individuals

In both Masaika and Kingwede, individuals who were positive for both mf and CFA had a significantly higher male to female ratio than those in the other two groups (χ^2-test; $p<0.001$ and $p=0.040$, respectively). The mean age of the individuals in the groups was lowest in the mf and CFA negative, intermediate in the mf negative but CFA positive, and highest in the mf and CFA positive, in both communities. The differences in mean age between mf and CFA positive groups and the mf and CFA negative groups were statistically significant in both communities (F=47.3, df=2,815, $p<0.001$ for Masaika and F=22.2, df=2,760, $p<0.001$ for Kingwede). In both communities, GMI of CFA was significantly higher among mf and CFA positive individuals than among the mf negative but CFA positive individuals (20253 vs. 2332 units in Masaika and 13830 vs. 868 units in Kingwede, *t*-test, $p<0.001$ for each test).

Characteristics of the study populations based on their clinical status

Since clinical manifestations of lymphatic filariasis were confined to individuals aged ≥20 years, only individuals in that age group were included for this analysis. Based on the findings from clinical examinations, individuals were categorised into three groups as follows: asymptomatic individuals, males with hydrocele and individuals with elephantiasis (Tables 4.3 and 4.4). One male in Masaika who had both hydrocele and elephantiasis was assigned to the elephantiasis group. All elephantiasis in both communities was confined to the legs.

Table 4.3. The characteristics of the study population in Masaika aged 20 years and above grouped according to clinical presentation and infection status.

Clinical category	No. examined	Mean age in years (range)	MF -ve & CFA – ve (Group 1)	Mf -ve & CFA +ve (Group 2)	MF +ve & CFA + ve (Group 3)
Asymptomatic	368	38.4 (20-80)	120	122	126
Hydrocele	54	48.7 (23-76)	20	10	24
Elephantiasis	18	45.8 (20-84)	11	3	4
Total	440	39.9 (20-84)	151	135	154

Table 4.4. The characteristics of the population in Kingwede aged 20 years and above grouped according to clinical presentation and infection status.

Clinical category	No. examined	Mean age in years (range)	Mf - ve & CFA –ve (Group 1)	Mf -ve & CFA +ve (Group 2)	Mf +ve & CFA +ve (Group 3)
Asymptomatic	304	36.6 (20-76)	227	61	16
Hydrocele	5	60.2 (44-78)	3	0	2
Elephantiasis	3	43.0 (30-67)	3	0	0
Total	312	37.1 (20-78)	233	61	18

The age characteristics, infection and specific antibody status of males with hydrocele and individuals with elephantiasis in Masaika appear in Table 4.5. Since the individuals in these two groups were very similar (not significantly different) with respect to age, infection status and specific antibodies, they were combined to form a single group of individuals with chronic disease. This combined group was used in all further analysis.

Table 4.5. The characteristics, infection status and filarial-specific antibody responses of individuals with chronic disease manifestations in Masaika.

Clinical manifestation	Hydrocele	Elephantiasis	Statistics
No. of individuals positive (Males/Females)	54 (-)	18 (6/12)	
Mean age	48.7	45.8	*t*-test, $p>0.05$
No. mf positive (%)	24 (44.4)	4 (22.2)	χ^2-test, $p>0.05$
No. CFA positive (%)	34 (63.0)	7 (38.9)	χ^2-test, $p>0.05$
Mf GMI (Mf/ml)*	440	82	*t*-test, $p>0.05$
CFA GMI(units)*	13492	7495	*t*-test, p>0.05
No. positive for antibody (%)			
IgG1	51 (94.4)	17 (94.4)	χ^2-test, $p>0.05$
IgG2	32 (59.3)	10 (55.6)	χ^2-test, $p>0.05$
IgG3	14 (25.9)	4 (22.2)	χ^2-test, $p>0.05$
IgG4	54 (100)	18 (100)	χ^2-test, $p>0.05$
IgE	38 (70.4)	13 (72.2)	χ^2-test, $p>0.05$
Mean antibody intensity (OD values)			
IgG1	0.829	0.898	*t*-test, $p>0.05$
IgG2	0.406	0.431	*t*-test, $p>0.05$
IgG3	0.134	0.144	*t*-test, $p>0.05$
IgG4	0.989	0.805	*t*-test, $p>0.05$
IgE	0.202	0.200	*t*-test, $p>0.05$

*GMIs based on all examined individuals

Specific IgG1, IgG2, IgG3, IgG4 and IgE antibody profiles were first compared between asymptomatic individuals and individuals with chronic disease within Masaika, in relation to their infection status. This comparison was not possible for Kingwede due to the low number of individuals with chronic disease. Inter-community comparison of antibody profiles was made exclusively among asymptomatic individuals in relation to their infection status. Similarly, due to the few cases of individuals with chronic disease in Kingwede, the inter-community comparison of antibody profiles was restricted to the asymptomatic individuals. Finally, IgG4/IgE ratios were compared between the asymptomatic individuals and individuals with chronic disease in Masaika, and inter-community-wise among the asymptomatic individuals. These comparisons were similarly made in relation to infection status. In the following sections, the results of all these various comparisons are presented.

Antibody profiles among asymptomatic and symptomatic individuals in Masaika

The prevalence of positivity and mean intensity patterns of specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies in relation to infection status among asymptomatic and symptomatic individuals in Masaika are shown in Figure 4.1.

Overall, the prevalence and mean intensity patterns of each specific antibody followed a similar trend. When antibody prevalence was high, so was its mean intensity, and vice-versa. The patterns for these antibody parameters in relation to infection status were very similar between asymptomatic individuals and individuals with chronic disease. The prevalences and mean intensities of specific IgG1, IgG2, IgG3 and IgE antibodies were in general highest among individuals negative for both mf and CFA and lowest in individuals positive for both mf and CFA. The opposite pattern was seen for specific IgG4 antibody. Here, both prevalence and mean intensity were highest among individuals positive for both mf and CFA and lowest among individuals negative for both mf and CFA.

The levels of observed prevalences and mean intensities of each specific antibody were then compared between asymptomatic individuals and individuals with chronic disease, with respect to their infection status, for statistical significance. Tables 4.6 and 4.7 show analyses by generalised linear model fits using binomial errors for antibody prevalence data, and Gaussian errors for mean intensity data between asymptomatic individuals and individuals with chronic disease. The association between the prevalences of all the specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies and infection status were highly significant (χ^2; p=0.007, p<0.001, p<0.001, p=0.002 and p<0.001, respectively). Similarly the association between the mean intensities of all these specific antibodies and infection status was highly significant (F=58.6, df=1,436, p<0.001 for IgG1; F=43.4, df=1,436, p<0.001 for IgG2; F=44.3, df=1,436, p<0.001 for IgG3; F=45.9, df=1,436, p<0.001 for IgG4; and F=18.1, df=1,436, p<0.001 for IgE). The prevalences and mean intensities of IgG2 and IgG4 were also associated with clinical status but less significantly (Tables 4.6 and 4.7). The parameters for these antibodies were higher in individuals with chronic disease than among asymptomatic individuals. IgG1 intensity but not prevalence was marginally associated with clinical status, being higher among those with chronic disease than among the asymptomatic individuals. Importantly, clinical status did not significantly influence the association between the prevalence of the measured specific antibodies and infection status, nor between their intensities and infection status (Tables 4.6 and 4.7).

a)

b)

c)

d)

e)

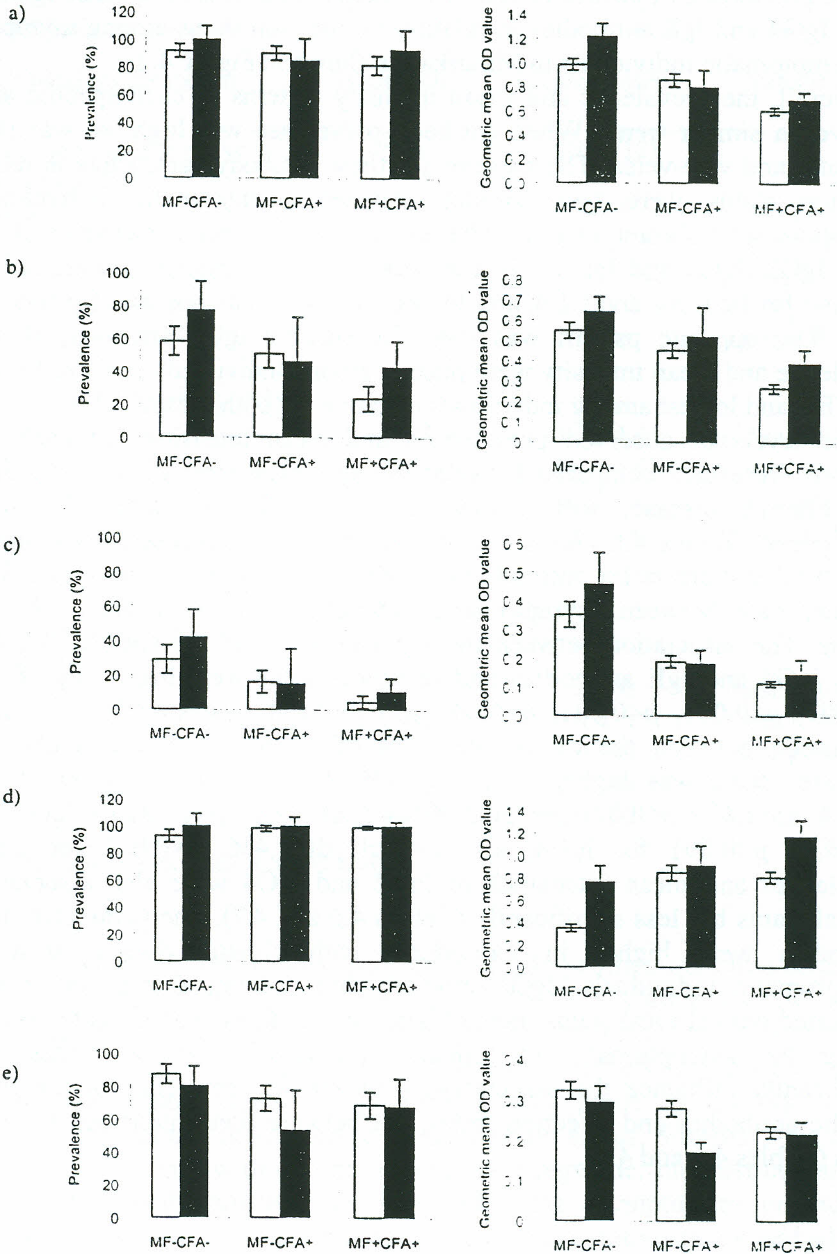


Figure 4.1. The prevalence of positivity (± 95% C.I) and the mean intensities (± S.E) of specific IgG1 (a), IgG2 (b), IgG3 (c), IgG4 (d) amd OgE (e) in relation to infection status among asymptomatic individuals (white bars) and among individuals with chronic disease (black bars) in Masaika.

Table 4.6. Analysis table for generalised linear model (GLM) fits to antibody prevalence data

	Residual Deviation	Degrees of freedom	Probability (χ^2)
IgG1			
Model Null	14.827	1, 5	
Clinical status	11.676	1, 4	0.076
Infection status	4.475	1, 3	0.007
Clinical status x Infection status	4.293	1, 2	0.669
IgG2			
Model Null	47.917	1, 5	
Clinical status	42.963	1, 4	0.026
Infection status	4.826	1, 3	0.000
Clinical status x Infection status	4.824	1, 2	0.967
IgG3			
Model Null	39.849	1, 5	
Clinical status	37.144	1, 4	0.100
Infection status	1.212	1, 3	0.000
Clinical status x Infection status	1.208	1, 2	0.949
IgG4			
Model Null	14.107	1, 5	
Clinical status	9.755	1, 4	0.040
Infection status	0.233	1, 3	0.002
Clinical status x Infection status	0.233	1, 2	0.988
IgE			
Model Null	18.064	1, 5	
Clinical status	17.101	1, 4	0.327
Infection status	4.568	1, 3	0.000
Clinical status x Infection status	4.186	1, 2	0.537

GLM fit to the prevalence data using binomial errors with antibody prevalence as response and clinical status and infection status as factors. Clinical status = asymptomatic or chronic disease (hydrocele or/and elephantiasis). Infection status = Mf-CFA-, Mf- CFA+ or Mf+CFA+. A *p*-value <0.05 in respect to clinical status and to infection status indicates significant association between antibody prevalence and clinical status or between antibody prevalence and infection status, as the case may be. A *p*-value <0.05 for clinical status x infection status indicates a significant influence of clinical status on the association between the prevalence of measured antibody and infection status.

Table 4.7. Analysis table for generalised linear model (GLM) fits to antibody intensity data.

	Mean squares	F value	Probability (F)
IgG1			
Clinical status	1.547	4.400	0.037
Infection status	20.620	58.638	0.000
Clinical status x Infection status	0.072	0.204	0.652
IgG2			
Clinical status	7.161	7.456	0.007
Infection status	41.658	43.372	0.000
Clinical status x Infection status	0.318	0.331	0.565
IgG3			
Clinical status	1.474	1.008	0.316
Infection status	64.859	44.348	0.000
Clinical status x Infection status	0.966	0.660	0.417
IgG4			
Clinical status	1.532	12.196	0.001
Infection status	5.773	45.947	0.000
Clinical status x Infection status	0.085	0.673	0.413
IgE			
Clinical status	0.418	0.746	0.388
Infection status	10.170	18.135	0.000
Clinical status x Infection status	0.067	0.120	0.729

GLM fit to the log transformed OD values using Gaussian errors with antibody intensity (log transformed OD values) as response and infection status and clinical status as factors; clinical status = asymptomatic or chronic disease (hydrocele or/and elephantiasis); infection status = Mf-CFA-, Mf- CFA+ or Mf+CFA+. A *p*-value <0.05 in respect to clinical status and for infection status indicates significant association between antibody intensity and clinical status or between antibody intensity and infection status, as the case may be. A *p*-value <0.05 for clinical status x infection status indicates a significant influence of clinical status on the association between the intensity of the measured antibody and infection status. Degrees of freedom for each test = 1, 436.

Further statistical analyses were carried out for each specific antibody in relation to the three infection groups, namely mf and CFA negative, mf negative but CFA positive, and mf and CFA positive. This was done with the two clinical groups combined since clinical status had been found not significantly to influence antibody responses in relation to infection status. The prevalences of specific IgG1, IgG2 and IgE were significantly higher among mf negative individuals (whether CFA negative or positive) than among mf positive individuals (χ^2-test, p=0.013, p<0.001 and p=0.018, respectively). Similarly, mean intensities of IgG1 and IgG2 were significantly higher among individuals negative for mf (irrespective of CFA status) than among those positive for mf (F=32.9, df=2, 436, p<0.001 for IgG1 and F=22.9, df=2,436, p<0.001 for IgG2). Significant differences in specific IgE mean intensities were only seen in relation to both mf and CFA, being higher among those negative for both mf and CFA than among those positive for both mf and CFA (F=8.3, df=2,436, p<0.001). The prevalences and mean intensities of specific IgG3 and IgG4 were associated more to CFA status than to mf status. Thus, the prevalence and mean intensity of specific IgG3 were significantly higher among the CFA negative individuals (whether mf negative or positive) than among the CFA positive individuals (χ^2-test, p<0.001 for prevalence, F=25.5, df=2,436, p<0.001 for intensity). In contrast to IgG3, both prevalence and mean intensity of specific IgG4 were significantly higher among the CFA negative than among the CFA positive individuals, irrespective of mf status (χ^2-test, p<0.003 for prevalence, F=28.3, df=2,436, p<0.001 for intensity)

Antibody profiles among asymptomatic individuals in Masaika and Kingwede

The effect of infection transmission intensity on specific IgG1, IgG2, IgG3, IgG4 and IgE antibody profiles was studied by comparing the patterns of these antibodies in Masaika and Kingwede. For reasons mentioned earlier, this comparison was restricted to asymptomatic individuals. The prevalence and mean intensity patterns of these specific antibodies in these asymptomatic individuals in Masaika and Kingwede, in relation to their infection status are shown in Figure 4.2.

The association between the observed GMIs of specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies and infection status in both communities are shown in Figures 4.3-4.7. Here, pair-wise analysis of antibody GMIs between individuals negative for both mf and CFA, those negative for mf but positive for CFA and those positive for both mf and CFA using ANOVA are presented. Differences in antibody GMIs between individuals negative for both mf and CFA and those negative for mf but positive for CFA, *i.e.* the first two groups, indicate the effect of CFA status alone on antibody levels. Differences in antibody GMIs between individuals negative for both mf and CFA and those positive for both mf and CFA, *i.e.* the first and the third groups, indicate the additional effect of mf status on the antibody levels. Finally, differences in antibody GMIs between individuals negative for mf but positive for CFA and those positive for both mf and CFA, *i.e.* the second and third groups, indicate the effect of mf status alone on antibody intensity.

The most remarkable difference in antibody pattern was seen for specific IgG1 antibodies. In Masaika, the prevalence and mean intensity of these antibodies were significantly higher among individuals negative for mf than among individuals positive for mf (χ^2-test, p=0.011 for prevalence, F=22.6, df=2,363, p<0.001 for intensity). The opposite pattern was seen in Kingwede. Thus, in this community both the prevalence and mean intensity of specific IgG1 were highest among individuals positive for mf and CFA, and lowest among those who were negative for mf and CFA, although not significantly (Figures 4.2 and 4.3).

The profiles of specific IgG3 also differed between Masaika and Kingwede. In Masaika, the prevalence and mean intensity of specific IgG3 was associated with the presence of both mf and CFA. Thus in this community, these parameters were highest among the individuals negative for both mf and CFA, intermediate among the individuals negative for mf but positive for CFA, and lowest among those positive for both mf and CFA (χ^2-test, p<0.001 for prevalence, F=16.3, df=2,363, p<0.001 for intensity) (Figures 4.2 and 4.5). However, in Kingwede specific IgG3 was more related to the presence of mf than to that of CFA. In this community, prevalence and mean intensity of specific IgG3 was higher among the mf negative individuals (CFA status not withstanding) than among the mf positive individuals (χ^2-test, p=0.020 and F=3.4, df=2,301, p=0.034, for prevalence and intensity, respectively) as seen in Figures 4.2 and 4.5.

Masaika and Kingwede also had different IgG2 and IgE prevalence and mean intensity patterns. In Masaika, prevalences of specific IgG2 and IgE were significantly higher among the individuals negative for mf than among those positive for mf (χ^2-test, p<0.001 and p= 0.017, for IgG2 and IgE, respectively). Similarly GMI of specific IgG2 and IgE were significantly higher among the mf negative individuals than among the mf positive individuals (F=17.7, df=2.363, p<0.001 for IgG2 and F=8.0, df=2,363, p=0.001 for IgE) (Figures 4.2, 4.4 and 4.7). In Kingwede, prevalences and mean intensities of IgG2 and IgE were uniform in all the three infection groups, with no statistical difference in their levels as seen in Figures 4.2, 4.4 and 4.7.

Only IgG4 prevalence and mean intensity patterns were similar in the two communities. These parameters were highest among the individuals positive for CFA and lowest among the individuals negative for CFA (F=32.9, df=2,363, p<0.001 for Masaika and F=29.5, df=2,301, p<0.001 for Kingwede). In both communities, mean IgG4 intensities differed significantly between individuals positive for CFA (irrespective of mf status) and those negative for CFA. In both communities, however, there were no significant differences between IgG4 mean intensities between individuals positive for CFA but negative for mf, and those positive for both CFA and mf (Figures 4.2 and 4.6).

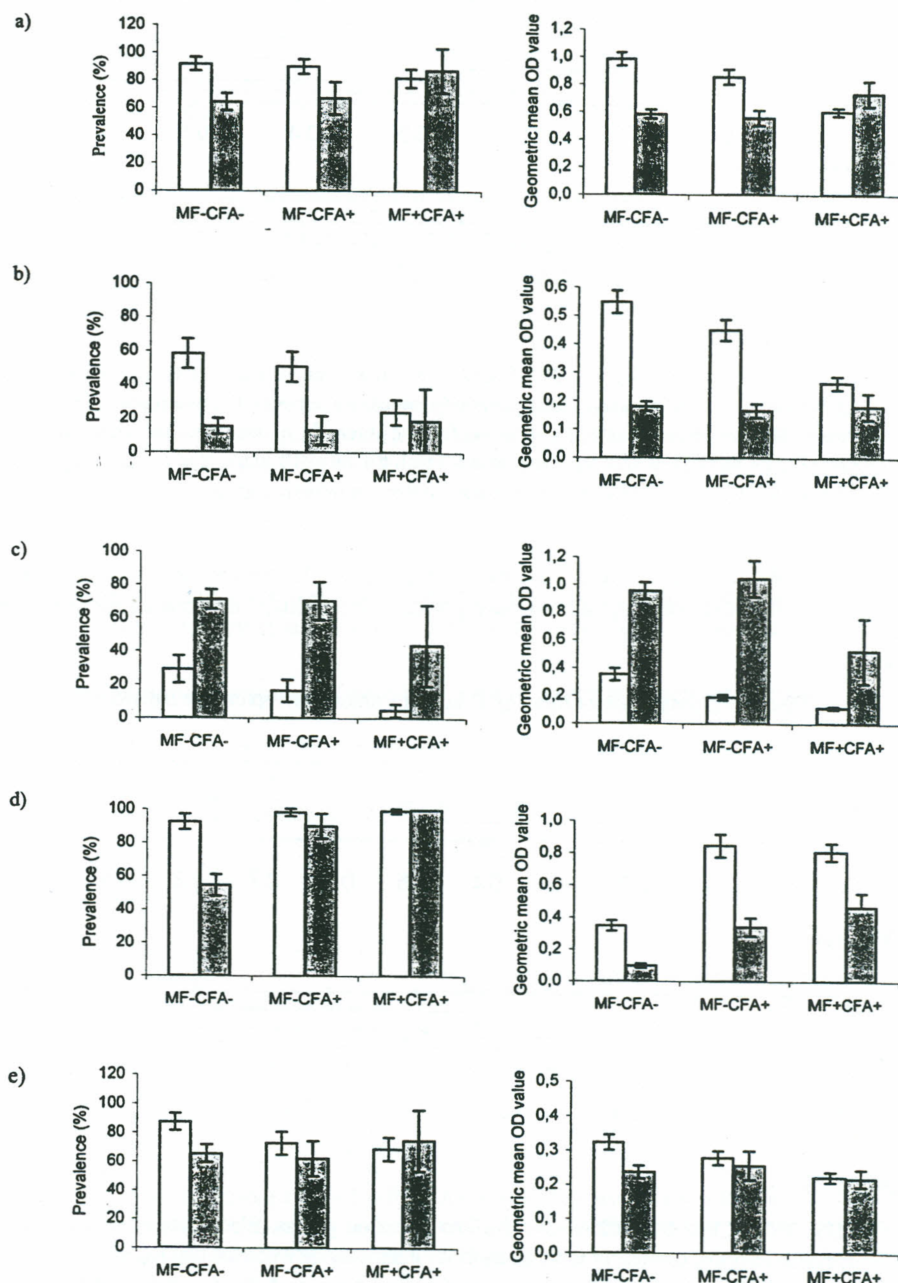
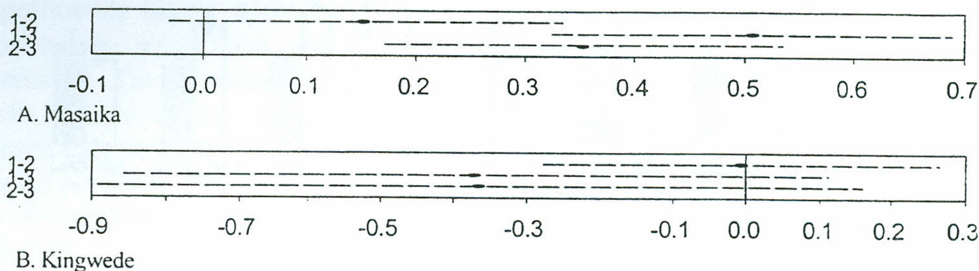


Figure 4.2. The prevalence of positivity (± 95% C.I) and mean intensities (± S.E) of specific IgG1 (a), IgG2 (b), IgG3 (c), IgG4 (d) and IgE (e) in relation to infection status among asymptomatic individuals in Masaika (white bars) and Kingwede (grey bars).

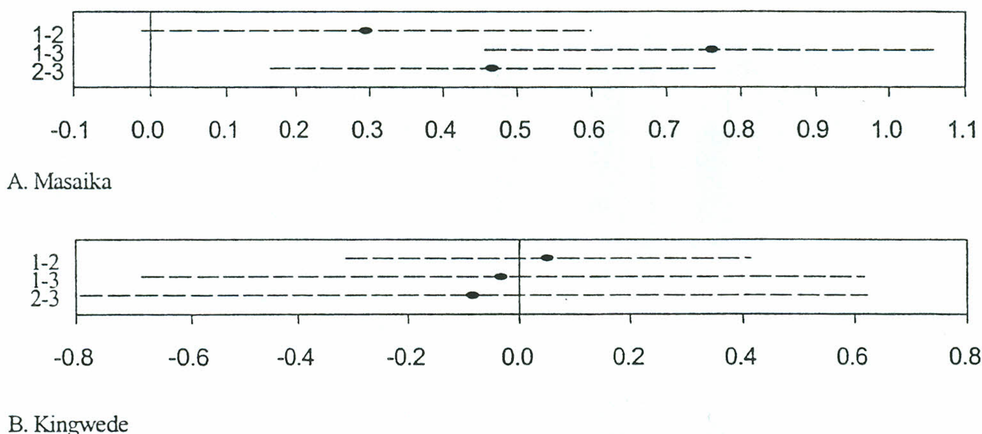
Intergroup comparison of mean IgG1 intensity among asymptomatic individuals



KEY: 1=Mf-CFA- 2=Mf-CFA+ 3=Mf+CFA+ infection groups. Black dots indicate estimated standard errors of differences in mean antibody intensities between the two respective infection groups. Broken lines indicate the 95% confidence intervals of the specified standard errors, using Tukey method. Where the lines do not reach the zero (0) mark, this indicates significant differences in mean antibody intensities between the two infection groups.

Figure 4.3. Standard error of the difference (± 95% C.I) in mean IgG1 intensities in relation to infection status among asymptomatic individuals in Masaika and Kingwede.

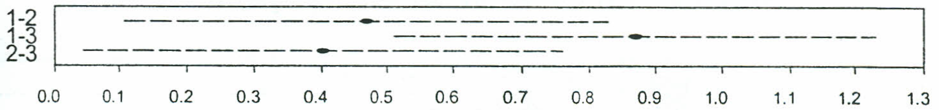
Intergroup comparison of mean IgG2 intensity among asymptomatic individuals



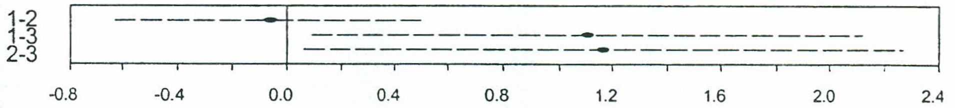
KEY: 1=Mf-CFA- 2=Mf-CFA+ 3=Mf+CFA+ infection groups. Black dots indicate estimated standard errors of differences in mean antibody intensities between the two respective infection groups. Broken lines indicate the 95% confidence intervals of the specified standard errors, using Tukey method. Where the lines do not reach the zero (0) mark, this indicates significant differences in mean antibody intensities between the two infection groups.

Figure 4.4. Standard errors of the difference (± 95% C.I) in mean IgG2 intensities in relation to infection status among asymptomatic individuals in Masaika and Kingwede.

Intergroup comparison of mean IgG3 intensity among asymptomatic individuals



A. Masaika

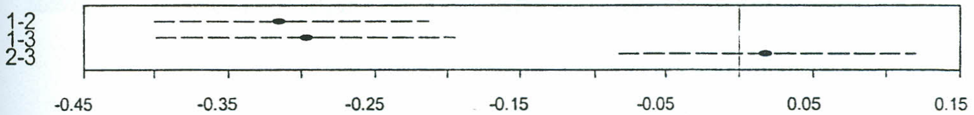


B. Kingwede

KEY: 1=Mf-CFA- 2=Mf-CFA+ 3=Mf+CFA+ infection groups. Black dots indicate estimated standard errors of differences in mean antibody intensities between the two respective infection groups. Broken lines indicate the 95% confidence intervals of the specified standard errors, using Tukey method. Where the lines do not reach the zero (0) mark, this indicates significant differences in mean antibody intensities between the two infection groups.

Figure 4.5. Standard errors of the difference (± 95% C.I) in the mean IgG3 intensities in relation to infection status among asymptomatic individuals in Masaika and Kingwede.

Intergroup comparison of mean IgG4 intensity among asymptomatic individuals



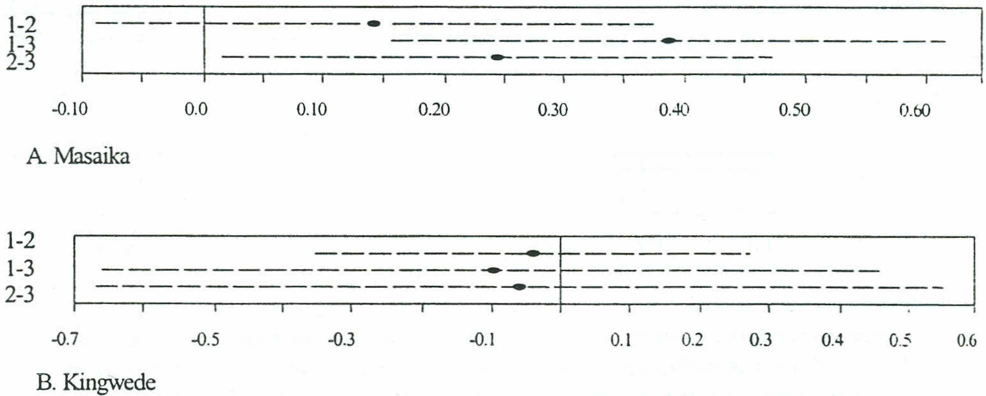
A. Masaika

B. Kingwede

KEY: 1=Mf-CFA- 2=Mf-CFA+ 3=Mf+CFA+ infection groups. Black dots indicate estimated standard errors of differences in mean antibody intensities between the two respective infection groups. Broken lines indicate the 95% confidence intervals of the specified standard errors, using Tukey method. Where the lines do not reach the zero (0) mark, this indicates significant differences in mean antibody intensities between the two infection groups.

Figure 4.6. Standard errors of the difference (± 95% C.I) in the mean IgG4 intensities in relation to infection status among asymptomatic individuals in Masaika and Kingwede.

Intergroup comparison of mean IgE intensity among asymptomatic individuals



KEY: 1=Mf-CFA- 2=Mf-CFA+ 3=Mf+CFA+ infection groups. Black dots indicate estimated standard errors of differences in mean antibody intensities between the two respective infection groups. Broken lines indicate the 95% confidence intervals of the specified standard errors, using Tukey method. Where the lines do not reach the zero (0) mark, this indicates significant differences in mean antibody intensities between the two infection groups.

Figure 4.7. Standard errors of the difference (± 95% C.I) in the mean IgE intensities in relation to infection status among asymptomatic individuals in Masaika and Kingwede.

IG4/IgE ratios in relation to clinical status and to infection endemicity

The mean ratios of IgG4 intensity to IgE intensity among asymptomatic individuals and among individuals with chronic disease in Masaika, in relation to their infection status appear in Figure 4.8.

The mean IgG4/IgE ratio was lowest among individuals negative for both mf and CFA and highest among those who were positive for both mf and CFA, both in the asymptomatic individuals and in the individuals with chronic disease. These ratios were however higher among individuals with chronic disease than among asymptomatic individuals in each respective infection status group, although only significantly for individuals negative for both mf and CFA (*t*-test, $p<0.001$).

The mean IgG4/IgE ratios among the asymptomatic individuals in Masaika and Kingwede, in relation to their infection status are shown in Figure 4.9. In both communities, the ratio was lowest among the individuals negative for both mf and CFA, and highest among the individuals positive for both mf and CFA. This ratio was significantly higher in Masaika than in Kingwede among the individuals negative for both mf and CFA (*t*-test, $p<0.001$), among the individuals negative for mf but positive for CFA (*t*-test, $p=0.002$) and among the individuals positive for both mf and CFA (*t*-test, $p=0.001$).

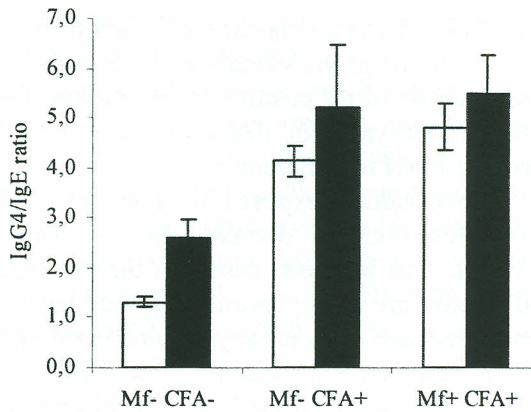


Figure 4.8. The mean specific IgG4/IgE ratios (± S.E) among asymptomatic individuals (white bars) and individuals with chronic disease (black bars) in Masaika, in relation to infection status.

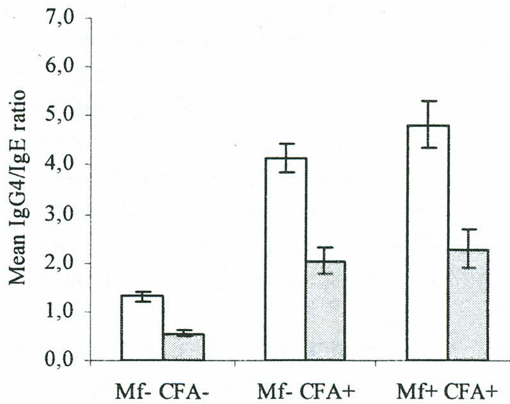


Figure 4.9. The mean specific IgG4/IgE ratios (± S.E) among the asymptomatic individuals in Masaika (white bars) and in Kingwede (grey bars), in relation to infection status.

Discussion

Immunological studies in human lymphatic filariasis have most often contrasted immune responses seen between individuals at each end of its spectral clinical manifestations. These consist of mf positive asymptomatic individuals at one end and individuals with chronic lymphatic disease at the other end. The studies have then often compared the observed immune responses in these two groups against the responses seen in individuals exposed to transmission but not infected or showing clinical manifestations of disease, the so-called 'endemic normals' (Kurniawan *et al.* 1993). This has been based on the assumption that individuals with chronic filarial disease are mostly uninfected, with increased specific cellular and humoral immune responses, whereas asymptomatic mf positive individuals are immunologically hypo-responsive (Piessens *et al.* 1980; King *et al.* 1992; Yazdanbakhsh *et al.* 1993). Studies based on this assumption have suggested a correlation between antibody responses and chronic disease manifestations. Thus, asymptomatic individuals have been observed to have higher mean intensities of specific IgG4 and lower mean intensities of specific IgG1, IgG2, IgG3 and IgE antibodies than individuals with chronic disease (Hussain *et al.* 1981; Ottesen *et al.* 1982; Hussain *et al.* 1987; Hitch *et al.* 1991; Maizels *et al.* 1995).

A recent meta-analysis of a large number of epidemiological studies has however shown that individuals with chronic filarial disease are equally likely to be mf positive or mf negative (Michael *et al.* 1994). Indeed, recently introduced more sensitive diagnostic methods that appear to detect *W. bancrofti* adult worm circulating filarial antigens (CFA) have confirmed this by showing that many individuals with chronic pathology are in fact actively infected (More & Copeman 1990; Kwan-lim *et al.* 1990; Chanteau *et* al. 1994a; Addiss *et al.* 1995). Thus, the often used classification erroneously puts individuals with chronic pathology who still have active filarial infection together with those who have cleared their infection (Freedman 1998). Besides, the use of antigen detection tests and ultrasonography have recently confirmed a long held suspicion that some of the 'endemic normal' individuals are actually actively infected (Chanteau *et al.* 1994a; Dreyer *et al.* 1996; Rocha *et al.* 1996).

Since active infection may profoundly affect immune responses (Yazdanbakhsh *et al.* 1993), it is imperative for immunological studies that individuals should be classified accurately both in terms of clinical and infection status (Almeida *et al.* 1996; Freedman 1998). With respect to chronic disease pathology, individuals in *W. bancrofti* endemic areas can clinically be categorised into asymptomatic and chronic disease groups. Based on both mf and CFA detection, the two clinical groups can be further divided into three infection groups. Firstly, there are those who are positive for both mf and CFA and therefore considered to harbour adult worms. Secondly, there are those who are mf negative but CFA positive. This group is considered to be harbouring adult worms, despite being mf negative, probably due to the mf being too low for detection by microscopy, or having single sex infections or some immune mechanisms destroying the mf produced. Finally, a

large proportion of individuals is negative for both mf and CFA, and considered to have neither adult worms nor mf. This last group is probably composed of two different categories of individuals. One may consist of those who have not been sufficiently exposed to acquire infection, despite living in these endemic areas. The other probably consists of those who are not infected, despite having been sufficiently exposed to *W. bancrofti* transmission. The latter category of individuals, especially if they are adults, is probably able to prevent the development of L3 larvae to adult worms and may be considered to be 'resistant' to development of *W. bancrofti* infection. Mf negative asymptomatic individuals, often designated 'putatively immune', must therefore be divided into two groups, those with and those without CFA, which can be considered to have different immune responses to filarial parasites. The present study used both clinical and infection status to classify individuals.

Infection status

In both communities, the mean age was lowest among the individuals negative for both mf and CFA, intermediate among those negative for mf but positive for CFA and highest among those positive for mf and CFA. Furthermore, in both communities, the mean age among individuals positive for both mf and CFA was significantly higher than that for individuals negative for both mf and CFA. Thus, the younger individuals were not infected, the older ones had adult worms but no mf, probably because the worms were still too few and therefore had low mating probability, and the oldest ones had more adult worms with high mating probability and therefore were mf positive. The significantly higher mean CFA intensity among individuals positive for both mf and CFA than among those positive for CFA but negative for mf supports the suggestion that the former had higher adult worm loads than the latter. In both communities, significantly more males than females were positive for both mf and CFA. Since males were furthermore harbouring the heaviest infection loads, the finding may suggest that females have a mechanism, behavioral, biological or otherwise, that protects them from acquiring heavy worm burdens.

Chronic disease status

In both communities, males with hydrocele outnumbered the individuals with elephantiasis. The number of individuals with chronic disease was large enough to allow for statistical analysis only in the community with high endemicity. Hence further analysis of the individuals with chronic disease was restricted to the high endemicity community. There was no age difference between males with hydrocele and individuals with elephantiasis in this community. Both males with hydrocele and individuals with leg elephantiasis consisted of a blend of mf and CFA negatives, mf negative but CFA positives, and mf and CFA positives. Thus, individuals with chronic disease pathology were heterogeneous, as has been observed previously (Faris *et al.* 1993; Lammie *et al.* 1993; Chanteau *et al.* 1994a; Addiss *et al.* 1995; Freedman 1998). Although males with hydrocele had higher mf

and CFA prevalences and mean intensities than individuals with elephantiasis, the differences were not statistically significant. Some studies have shown males with hydrocele and individuals with elephantiasis to differ remarkably in their mf status. These studies have shown males with hydrocele more likely to be actively infected than individuals with elephantiasis (Lammie *et al.* 1993; Addiss *et al.* 1995; Simonsen *et al.* 1995a). This has led to the theory that the two disease conditions may have different etiologies, with elephantiasis being more immune-mediated and therefore resulting in mf clearance, while hydrocele results more from mechanical damage to the lymphatics by the adult worms (Ottesen 1992). The similarity in antibody response patterns between males with hydrocele and individuals with elephantiasis is in agreement with a previous study that found these responses of limited value for distinguishing the two conditions (Lammie *et al.* 1993). However, in another study, marked differences in specific antibody responses between individuals with hydrocele and individuals with lymphangitis or elephantiasis were observed (Nicolas *et al.* 1999). It should be noted, though, that in the later study the infection status of these two groups was also statistically different. Most of the individuals with hydrocele were parasitised and exhibited high CFA levels, whereas most individuals with lymphangitis or elephantiasis were unparasitised or poorly parasitised and had much lower CFA levels.

Specific antibody responses in relation to infection and disease status

The patterns of specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies in relation to infection status were very similar among asymptomatic individuals and those with chronic filarial disease. In addition, the prevalences and mean intensities of all the specific antibodies were more significantly associated with infection than with disease status. Furthermore, clinical status did not significantly influence the association between the profiles of these specific antibodies and infection status. This finding is in contrast with that of other studies which have suggested that individuals with chronic disease produce higher amounts of specific IgG1, IgG2, IgG3 and IgE, and lower amounts of IgG4 than asymptomatic individuals (Ottesen *et al.* 1982; Ottesen *et al.* 1985; Hussain *et al.* 1987; Ottesen 1992; Kurniawan *et al.* 1993; Yazdanbakhsh *et al.* 1993). However, the present findings are in agreement with those of other workers who have found antibody responses to be more attributable to the presence or absence of microfilaraemia and/or of antigenaemia rather than to disease (Estambale *et al.* 1994b; Simonsen *et al.* 1996; Wamae *et al.* 1998; Nicolas *et al.* 1999). The findings are also in agreement with studies on lymphocyte cytokine responses which have been shown to be associated with the presence or absence of active infection and not with clinical status (Almeida *et al.* 1996).

The highest prevalences and mean intensities of specific IgG1, IgG2, IgG3 and IgE were among the mf and CFA negative (unparasitised) individuals, while the lowest were among CFA positive individuals (those harbouring adult worms). Mean specific IgG1 and IgG2 antibody levels were specifically associated with mf and not CFA. The intensities were higher among the mf negative than among the

mf positive, irrespective of the presence of CFA. Previous studies in other endemic areas have found low levels of specific IgG1 and/or IgG2 responses associated with the presence of mf (Lammie *et al.* 1993; Addiss *et al.* 1995; Dimock *et al.* 1996; Simonsen *et al.* 1996; Wamae *et al.* 1998; Nicolas *et al.* 1999). This suggests that specific IgG1 and IgG2 may be suppressing mf intensity, or that the presence of mf may be suppressing IgG1 and IgG2 production. On the other hand, Wamae *et al.* (1998) found levels of specific IgG2 also associated with CFA status. In that study, IgG2 levels were significantly lower among CFA positive than among CFA negative individuals. However, that study did not examine the mixed effect of mf and CFA status on antibody responses. It is therefore possible that most of the CFA positive individuals were also mf positive, and were the ones contributing most to the lower levels of IgG2. Thus, it may be that the observed lower IgG2 levels among the CFA positive may have been more related to these individuals' mf status rather than their CFA status.

The present study on the other hand found mean specific IgG3 negatively associated with the presence of CFA, that is the presence of adult worms, rather than mf. Lower mean IgG3 intensities were found among individuals positive for CFA, irrespective of their mf status, than among those negative for CFA. Other workers have similarly observed a negative association between IgG3 levels and presence of adult worms (Nicolas *et al.* 1999). Hence, it would appear that specific IgG3 production suppressed the burden of adult worm infection or that the presence of the adult worms suppressed its production.

Previous studies have indicated higher IgE intensities among individuals with chronic disease than among asymptomatic individuals (Hussain *et al.* 1981, 1985; Kurniawan *et al.* 1993; Yazdanbakhsh *et al.* 1993). It has even been suggested that specific IgE may be involved in the development of chronic lymphatic pathology (Kurniawan *et al.* 1993). In the present study, no differences were observed in the patterns or levels of specific IgE between asymptomatic individuals and those with chronic disease. Here, IgE prevalences and intensities were associated with the presence or absence of infection. These parameters were only significantly lower among individuals negative for both mf and CFA compared to the individuals positive for both mf and CFA. This finding suggested that IgE levels were associated with both mf and CFA status, in agreement with the findings by other workers (Nicolas *et al.* 1999).

In contrast to all the other specific antibodies, prevalence and mean intensity of specific IgG4 in the present study were significantly higher among the infected than among the uninfected individuals. Its mean intensity was higher among individuals positive for CFA, irrespective of their mf status, than among those negative for CFA. Thus, IgG4 production by the host appeared to be induced by the presence of adult worms, or the presence of IgG4 enhanced the survival of the adult worms. This may be the case if the presence of IgG4 blocks effector immune responses geared at eliminating the adult worms. The association between specific IgG4 and the presence of adult worms has been described previously (Dimock *et al.* 1996; Simonsen *et al.* 1996; Lammie *et al.* 1998; Wamae *et al.* 1998; Nicolas *et*

al. 1999). However, other studies have found IgG4 levels also associated with the presence of mf, being higher among mf positive than among mf negative individuals (Simonsen *et al*. 1996; Wamae *et al*. 1998). The higher levels of IgG4 among the mf positive individuals in these studies most probably is due to the individuals being CFA positive, since antibody responses in these studies were examined in relation to mf and to CFA but independently of the presence of each other.

Specific antibody responses in relation to infection transmission intensity

The present study examined the influence of transmission intensity on the patterns of specific antibody responses. This was accomplished by comparing the patterns of these responses in the two communities with high and low transmission intensity, respectively. Due to reasons mentioned earlier, analysis was restricted to asymptomatic individuals. Except for specific IgG4, other antibody response patterns between the two communities were quite different. Whereas in the high transmission intensity community, high mean intensities of specific IgG1, IgG2 and IgE were associated with the absence of infection, that was not the case in the low transmission intensity community. Here, the opposite pattern was seen for specific IgG1. Thus, high specific IgG1 mean intensity was associated with the presence of infection and low intensity with the absence of infection. Furthermore, in the low transmission intensity community, specific IgG2 and IgE mean intensities were not related to the presence or absence of infection.

Higher IgG1 intensity among infected individuals than among uninfected individuals has previously been reported in young children. A study in Haiti found IgG1 prevalence significantly higher in the CFA positive than in the CFA negative children (Lammie *et al*. 1998). Similarly, in Tanzania mean IgG1 intensity was found to be significantly higher in the CFA positive than in the CFA negative among the youngest but not in the older children (Simonsen *et al*. 1996). Assuming that young children are probably the ones who have experienced the lowest transmission intensities, it would appear that the pattern of IgG1 production changes with increasing transmission intensity. Thus, it is probable that when transmission is low, as is the case in the low transmission intensity community, IgG1 is positively associated with infection, but as transmission intensity becomes high as is the case in the high transmission intensity community, this association switches, becoming negative. This suggestion is in concert with the hypothesis that the hypo-responsiveness seen among infected individuals is dependent on the intensity of transmission (Yazdanbakhsh 1999). Individuals who migrate to lymphatic filariasis endemic areas from non-endemic areas have been observed to have markedly high Th-1 responses, which become down regulated with increasing length of stay in the endemic region. Thus, early infections are characterised by high Th-1 responses, but upon chronicity these responses are down modulated. It is possible that the infection intensity among the infected individuals in the low transmission intensity community was not large enough to induce this immunological suppression, thus leading to the individuals there still having high

Th-1 and high IgG1. This however does not explain why a similar pattern was not seen for the other antibody isotypes that are associated with Th-1 responses. The reasons for the observed different patterns between the two communities therefore remain unclear. It is also not clear whether the observed influence of transmission intensity would be true for individuals with chronic disease since this analysis did not include them. However, the findings suggest that infection transmission intensity is an important factor to be considered when comparing immune response patterns between communities.

IgG4/IgE ratios in relation to disease status

IgG4 is thought to act as a blocking antibody to IgE mediated hypersensitivity reactions in lymphatic filariasis (Ottesen *et al.* 1981; Ottesen 1989), and the IgG4/IgE ratio has been found to vary with the state of the disease in lymphatic filariasis. A study by Kurniawan *et al.* (1993) observed the IgG4/IgE ratio to be 10 times higher among mf positive asymptomatic individuals than among individuals with chronic filarial disease. Thus, it was suggested that IgE may be mediating pathogenesis of chronic disease, but that sufficient IgG4 levels may be protective. However, that study also found the IgG4/IgE ratio to be lowest among asymptomatic mf negative individuals thereby suggesting that high IgE with little IgG4 was not in itself sufficient to initiate pathology.

In the present study the IgG4/IgE ratio was higher among individuals with chronic disease than among asymptomatic individuals, although this difference was only significant among those negative for both mf and CFA. The results therefore did not suggest involvement of specific IgE in the development of chronic lymphatic pathology, in contrast to the observations by Kurniawan *et al.* (1993). It should however be noted that whereas in the present study chronic disease included both hydrocele and elephantiasis, in the study by Kurniawan *et al.* (1993), the group of individuals termed as having chronic disease was solely composed of elephantiasis patients. It has previously been hypothesized that pathogenesis of these two chronic disease manifestations are different (Ottesen 1992). In addition, it is probable that the low ratio observed among symptomatic individuals in the study by Kurniawan *et al.* (1993) may have been due to some individuals in the group actually being infected. That study, however, did not examine these individuals for infection and assumed on the basis of previous studies in the region that they were mf negative. Even if these individuals had been tested for mf and found negative, their infection status would still be uncertain since the more sensitive antigen detection technique has not yet been developed for brugian filariasis.

IgG4/IgE ratios in relation to infection transmission intensity

Studies in schistosomiasis indicate that low IgG4/IgE ratio is associated with acquired immunity (Hagan *et al.* 1991). A study of brugian filariasis patients suggested that IgG4/IgE ratio can serve as an indicator of permissiveness or

resistance to infection (Kurniawan *et al.* 1993). Thus, a low IgG4/IgE ratio would indicate resistance, while a high ratio, permissiveness.

In the present study, the lowest IgG4/IgE ratios in both communities were found among asymptomatic individuals negative for both mf and CFA. To the best of our knowledge, these individuals have neither adult worms nor mf. If this is the case, despite them being subjected to *W. bancrofti* L3 transmission through their continued stay in these endemic areas, this finding is in conformity with the resistance/permissiveness theory. The fact that individuals positive for both mf and CFA in both communities had the highest IgG4/IgE ratios is also in conformity with this theory.

However, the IgG4/IgE resistance/permissiveness theory in the present study breaks down when the ratio is compared between the two communities. If resistance is exposure-driven as has been suggested (Michael & Bundy 1998), and low IgG4/IgE ratio indicates the presence of resistance, then the ratio should be lower in the high than in the low transmission intensity community among the asymptomatic individuals negative for both mf and CFA. However, this was not the case in the present study.

Conclusions

In conclusion, the present study found that antibody responses are more associated with infection status than with disease status. The profiles of specific IgG1, IgG2 and IgE antibodies in the high endemicity community were more associated with mf than with CFA status. Their levels were higher among the mf negative than among the mf positive individuals. Specific IgG3 and IgG4 antibodies were on the other hand associated more with CFA than with mf status in the high endemicity community. Thus, IgG3 intensities were higher among the CFA negative than the CFA positive individuals, whereas IgG4 intensities were higher among the CFA positive than the CFA negative individuals. The different profiles for IgG1, IgG2 and IgE in relation to infection between the high and low endemicity communities may suggest that these responses are driven by transmission intensity. The pattern of the IgG4/IgE ratios in the present study did not indicate a clear role for these antibodies, or the ratio between them, for the level of resistance or permissiveness to infection in bancroftian filariasis.

Chapter 5

Filaria-specific antibody responses in two communities with high and low endemicity of *Wuchereria bancrofti* infection. II. Antibody profiles in relation to infection and age

Introduction

The role of acquired immunity in regulating human lymphatic filariasis remains unresolved (Ottesen 1992; Maizels *et al.* 1995). Indirect evidence from epidemiological data is suggestive of the operation of such immunity (Michael & Bundy 1998). A number of studies have looked at humoral immune responses to lymphatic filariasis among endemic populations, with the objective of understanding the role of acquired immunity in shaping the epidemiology of this infection. From these studies, *W. bancrofti* infection has been observed to induce strong antibody responses (Ottesen *et al.* 1982; Estambale *et al.* 1994b; Simonsen *et al.* 1996; Wamae *et al.* 1998; Nicolas *et al.* 1999), although protective responses have not yet been identified. These studies have generally looked only at part of the age groups of the endemic populations. Where broader age groups have been examined, specific antibody responses in relation to age have generally been compared only between two age groups, a younger and an older. Detailed studies on specific antibody responses in relation to age are thus greatly lacking. Immunoepidemiological models predict that if an age-specific protective immunity exists, a negative correlation between the protective response and infection intensity would be expected in the older age groups depending on the form and nature of acquired immunity (Anderson & May 1991; Woolhouse 1992). It has also recently been suggested that protective immunity in lymphatic filariasis may be dependent on transmission intensity (Michael & Bundy 1998). Thus, the age-intensity patterns of protective immune responses can be expected to be different between communities with different transmission intensities. In this chapter, the age-specific profiles of filaria-specific antibody responses and their relationship to infection intensity in individuals living in two communities with different bancroftian filariasis transmission intensity, and covering the whole age range ,are presented and analysed.

Materials and methods

Study population, field and laboratory procedures

The study areas and populations, and findings from clinical and parasitological examinations in the two communities, have previously been presented and analysed (Chapter 3). Only individuals who had been examined for microfilaraemia, antigenaemia and all filaria-specific antibodies (IgG1, IgG2, IgG3, IgG4 and IgE) were included for analysis in this chapter. Procedures for clinical examinations, mf detection, serum preparation and measurements of CFA and filaria-specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies have also been described in Chapter 3.

Data analysis

Data were analysed essentially as previously described (Chapter 3) with mean mf, CFA and specific antibody intensities calculated as geometric means. Briefly, the age-intensity patterns in each community were analysed using Gaussian generalized additive models (GAMs), with age as predictor and either mf, CFA or antibody intensity as responses. Age-antibody intensities between the two communities were

compared by fitting generalized linear models to lot transformed OD values using age and community as factors. In addition, partial correlation analysis was used to examine the relationship between antibody intensity and parasite burden (either mf or CFA intensity) for the whole population and within age groups. The variables controlled for in the partial correlation were host age (within age groups) and the other four antibody isotype responses. Due to the large number of tests carried out in partial correlation analysis, the level of significance was taken at p-value <0.01 as indicated in the tables, to reduce the likelihood of Type 1 errors. Tests for heterogeneity of correlation coefficients were carried out according to Sokal and Rolf (1981). Here, a p-value <0.05 was considered statistically significant.

Results

Characteristics of the examined population

Masaika had a total registered population of 950 individuals aged one year and above and 817 (86.0%) of these were examined for mf, CFA and filaria-specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies (Table 5.1). In Kingwede, a total of 1,013 individuals aged one year and above were registered and 762 (75.6%) of these were examined for mf, CFA and for the above mentioned filaria-specific antibodies (Table 5.1).

Age-specific antibody profiles in Masaika and Kingwede

The age-specific prevalence of specific IgG1, IgG2, IgG3, IgG4 and IgE antibodies is indicated in Figure 5.1. The mean intensities of these antibodies among the mf positive individuals alone and the CFA positive individuals alone are shown in Figure 5.2. Generally, both of the parameters followed similar patterns for each antibody type. The prevalence and mean intensities of IgG1, IgG2, IgG4 and IgE were higher in Masaika than in Kingwede for each age group, while the opposite pattern was seen for IgG3. In Masaika, age-specific prevalence and mean intensities of specific IgG1, IgG2 and IgE increased sharply with age in the early age groups and then declined gradually with age. By contrast, both prevalence and mean intensities of IgG3 were generally low and remained at the same level with increasing age. The age-specific prevalence and mean intensities of specific IgG4 in this community increased with age to reach a plateau in the older age groups.

In Kingwede, the age-specific prevalence and mean intensities of specific IgG1, IgG4 and IgE followed a similar pattern to that seen in Masaika. The parameters for IgG1 and IgE increased sharply in the early ages followed by a gradual decline in the older ages, while those for IgG4 generally increased with age to reach a plateau in the older ages. The age-specific prevalence and mean intensity patterns for specific IgG2 and IgG3 in this community were quite different from that seen in Masaika. In Kingwede, these parameters for IgG2 increased with age in the young to reach a plateau in the old ages. The prevalence of positivity and mean intensity of IgG3 in this community increased with age without reaching a plateau.

Table 5.1. Age-stratified characteristics of the populations in Masaika and Kingwede examined for filaria -specific antibodies (IgG1, IgG2, IgG3, IgG4 and IgE).

	Antibody examination				Antibody examination		
Age group (years)	No. examined for antibodies (% coverage)	No. Mf positive (%)	No. CFA positive (%)	No. of inhabitants	No. examined for antibodies (% coverage)	No. Mf positive (%)	No. CFA positive (%)
1-4	71(71.7)	1 (1.4)	6 (8.5)	163	105 (64.4)	0 (0)	3 (2.8)
5-9	100 (90.1)	8 (8.0)	30 (30.0)	154	129 (83.8)	0 (0)	9 (7.0)
10-14	126 (90.6)	23 (18.3)	59 (46.8)	157	138 (87.9)	1 (0.7)	19 (13.7)
15-19	78 (82.1)	20 (25.6)	45 (57.7)	102	77 (75.5)	2 (2.6)	15 (19.5)
20-29	138 (85.2)	46 (33.3)	83 (60.1)	179	121 (67.6)	6 (5.0)	29 (23.8)
30-39	120 (87.0)	34 (28.3)	74 (61.7)	104	86 (82.7)	8 (9.3)	21 (24.4)
40-49	74 (89.2)	27 (36.5)	53 (71.6)	58	35 (60.3)	1 (2.9)	8 (22.9)
50-59	41 (91.1)	19 (46.3)	32 (78.0)	52	40 (76.9)	1 (2.5)	8 (20.0)
60+	69 (88.5)	30 (43.5)	47 (68.1)	44	31 (70.5)	3 (9.4)	13 (40.6)
Total	817 (86.0)	208 (25.5)	429 (52.5)	1013	762 (75.2)	22 (2.9)	125 (16.4)

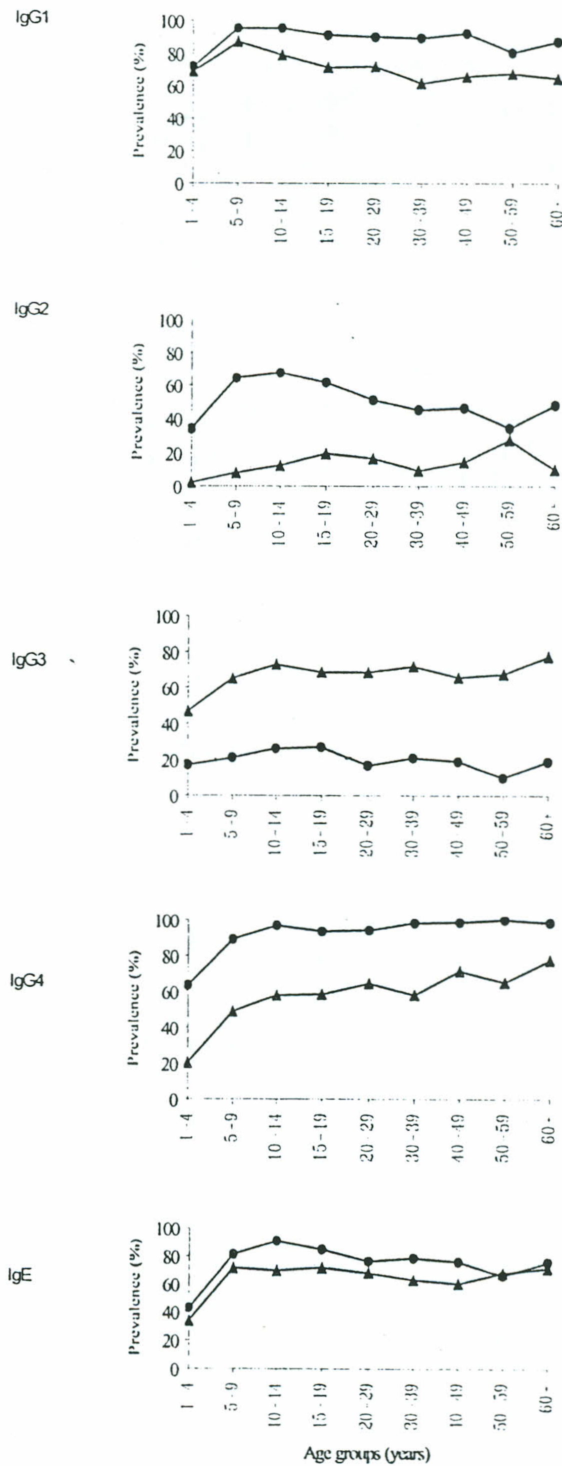


Figure 5.1. Age-specific IgG1, IgG2, IgG3, IgG4 and IgE prevalence of positivity in Masaika (●) and Kingwede (▲).

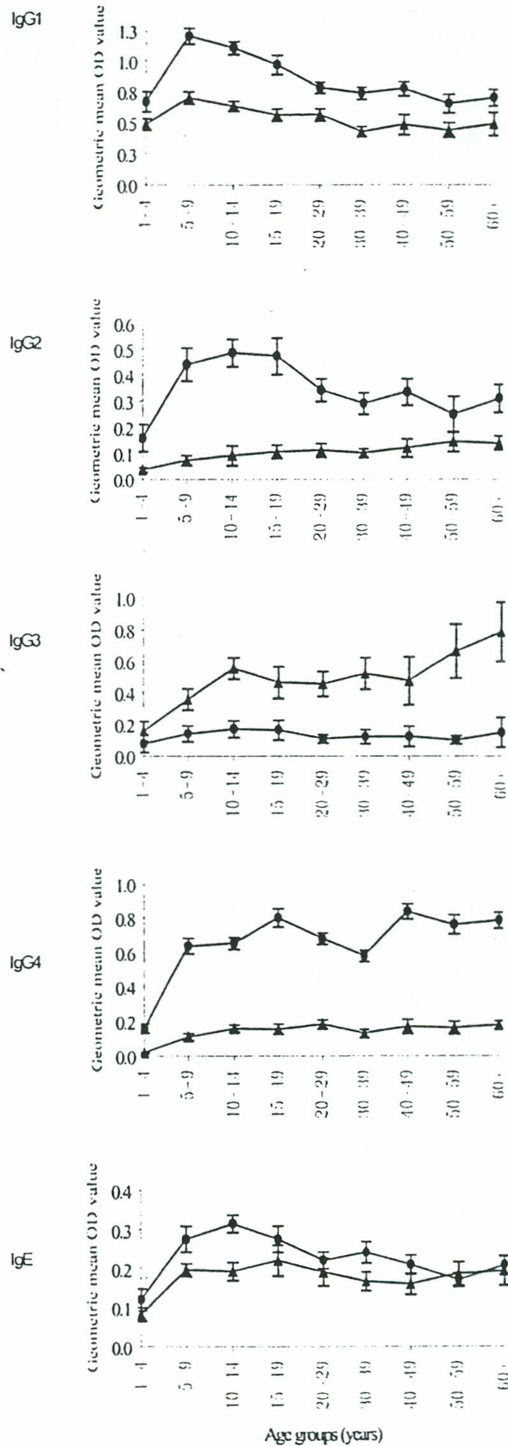


Figure 5.2. Age-specific mean IgG1, IgG2, IgG3, IgG4 and IgE intensities (OD values ± S.E.) in Masaika (●) and Kingwede (▲). Calculated among all examined individuals.

GLM fits to the antibody intensity data are presented in Table 5.2. The overall mean intensities of IgG1, IgG2, IgG4 and IgE were significantly higher in Masaika than in Kingwede, whereas IgG3 mean intensity was significantly higher in Kingwede than in Masaika ($F=154.0$, $df=1,1598$, $p<0.001$ for IgG1; $F=450.2$, $df=1,1598$, $p<0.001$ for IgG2; $F=411.0$, $df=1,1598$, $p<0.001$ for IgG3; $F=525.9$, $df=1,1598$, $p<0.001$ for IgG4 and $F=25.6$, $df=1,1598$, $p<0.001$ for IgE). Mean intensities of IgG1, IgG3 and IgG4 varied significantly with age ($F=52.4$, $df=1,1597$, $p<0.001$ for IgG1; $F=22.7$, $df=1,1597$, $p<0.001$ for IgG3 and $F=31.23$, $df=1,1597$, $p<0.001$ for IgG4), while those of IgG2 and IgE did not (Table 5.2). Mean IgG1 intensities decreased with age, while in contrast, mean IgG3 and IgG4 intensities increased with age. The age-intensity patterns for IgG1, IgG2, IgG3 and IgE differed significantly between the two communities ($F=4.45$, $df=1,1596$, $p=0.035$ for IgG1; $F=22.3$, $df=1,1596$, $p<0.001$ for IgG2; $F=29.3$, $df=1,1596$, $p<0.001$ for IgG3 and $F=7.5$, $df=1,1596$, $p=0.006$ for IgE), whereas IgG4 age-intensity patterns did not differ significantly between the communities ($F=3.45$, $df=1,1596$, $p>0.05$).

Age-relationships between specific antibody and infection intensity profiles in Masaika and Kingwede

The observed age-specific antibody intensity patterns were examined for interrelationships with age-specific infection intensity patterns. These were carried out by fitting age-intensity generalized additive models (GAMs) for each antibody type and comparing the patterns with that for age-intensity patterns for mf and for CFA. The estimated age-functions for mf, CFA and specific antibodies are shown in Figures 5.3-5.9, and indicate significant non-linearity with age for each response.

In Masaika, the GAM fits indicated a non-linear increase in both mf and CFA intensities with age. Mf intensity increased steeply in the younger ages then appeared to reach a plateau in the older ages (Fig. 5.3). The CFA age-intensity pattern in this community was similar to that of mf, but intensity dropped slightly in the oldest age group. In Kingwede, GAM fits indicated non-linear increases in mf and CFA intensities with age with no clear plateau or a drop in intensities of infection in the older ages.

IgG1 mean intensity in both communities showed an initial increase in the young ages followed by a decline in the older ages (Fig. 5.5). Thus, IgG1 and infection age-intensity patterns were in concert in the younger ages, but this reversed in the older ages. The decline in IgG1 intensity in the older ages appeared stronger in Masaika than in Kingwede, contributing to the marginal statistical differences in age-intensity patterns between the communities (Fig. 5.5 and Table 5.2). IgG2 age-intensity patterns were quite different between the two communities (Fig. 5.6). In Masaika IgG2 intensity increased with age in the younger ages in concert with infection intensity, but declined in the older ages as infection continued to rise to a plateau for mf and possibly a slight drop in the oldest age group for CFA.

In contrast, IgG2 intensity in Kingwede increased with age to a stable plateau in the older ages without declining. IgG3 intensity patterns also differed between the communities. In Masaika IgG3 intensity did not change with age, whereas in Kingwede, it increased non-linearly with age in concert with infection intensity (Fig. 5.7). IgG4 age-intensity patterns were quite similar in both communities. The intensities increased with age in the young ages to plateaus in the older ages in both villages (Fig. 5.8). Thus, IgG4 age-intensity appeared to closely mimic age-intensity infection trends in the respective communities (Figs. 5.1 and 5.2). IgE intensity patterns in the two communities were also somewhat similar. Its intensity increased in the young ages followed by a decline in the older ages. The decline however appeared steeper in Masaika than in Kingwede, probably resulting in the statistical difference in the age-intensity patterns between the communities (Fig. 5.9 and Table 5.2).

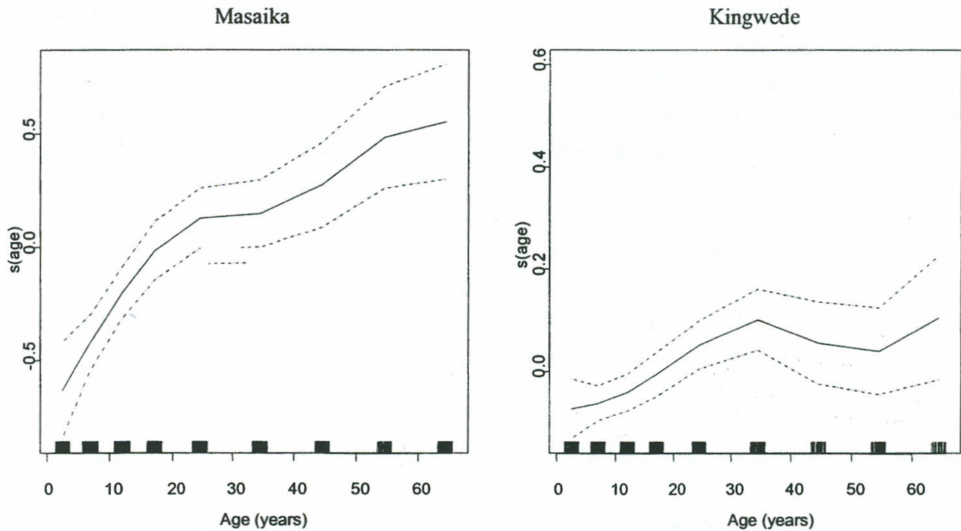


Figure 5.3
The fitted function for age for the microfilaria (mf) intensity data from Masaika and Kingwede. In the model, s(age) is an arbitrary smoothing function replacing mf intensity. The ticks at the bottom of the graphs indicates the density of data in each age class. Solid line represents the mean of mf intensity while dashed lines denote the respective point-wise standard errors of the estimated curve.

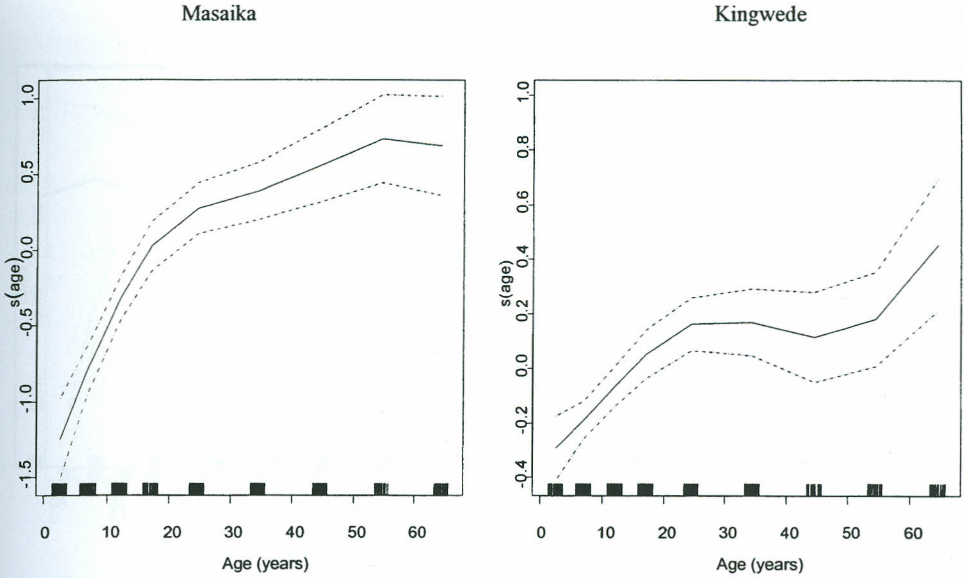


Figure 5.4
The fitted function for age for the circulating filarial antigen (CFA) intensity data from Masaika and Kingwede. In the model, s(age) is an arbitrary smoothing function replacing CFA intensity. The ticks at the bottom of the graphs indicates the density of data in each age class. Solid line represents the mean of CFA intensity while dashed lines denote the respective point-wise standard errors of the estimated curve.

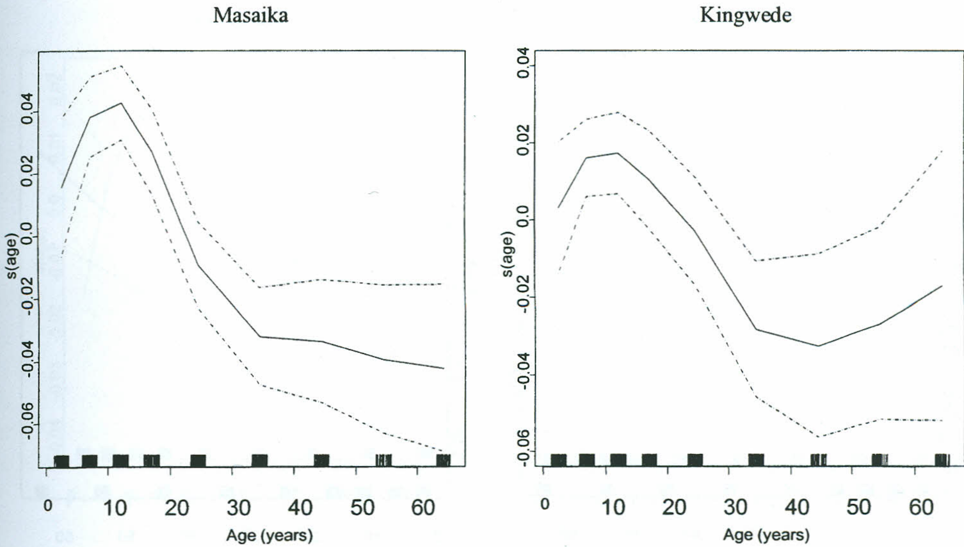


Figure 5.5
The fitted function for age for the IgG1intensity data from Masaika and Kingwede. In the model, s(age) is an arbitrary smoothing function replacing IgG1 intensity. The ticks at the bottom of the graphs indicates the density of data in each age class. Solid line represents the mean of the response while dashed lines denote the respective point-wise standard errors of the estimated curve.

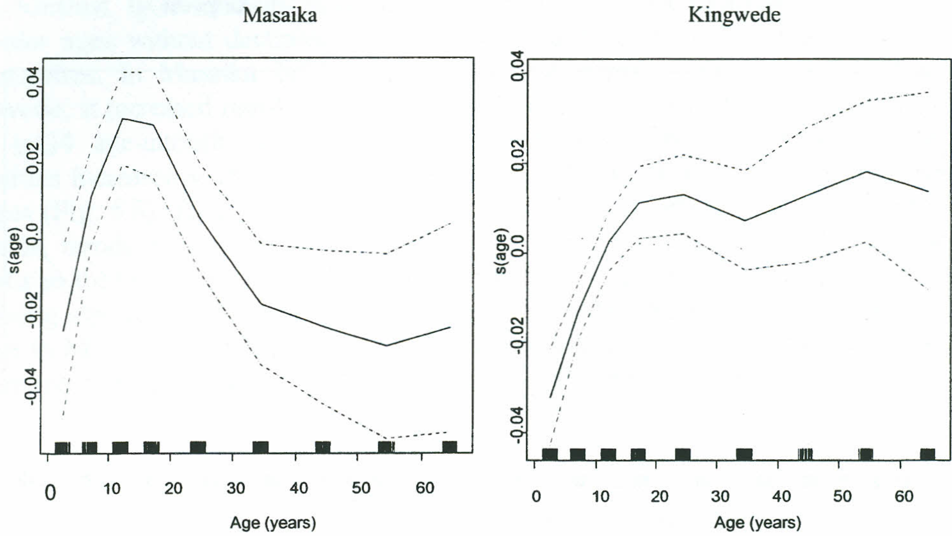


Figure 5.6
The fitted function for age for the IgG2 intensity data from Masaika and Kingwede. In the model, s(age) is an arbitrary smoothing function replacing IgG2 intensity. The ticks at the bottom of the graphs indicates the density of data in each age class. Solid line represents the mean of the response while dashed lines denote the respective point-wise standard errors of the estimated curve.

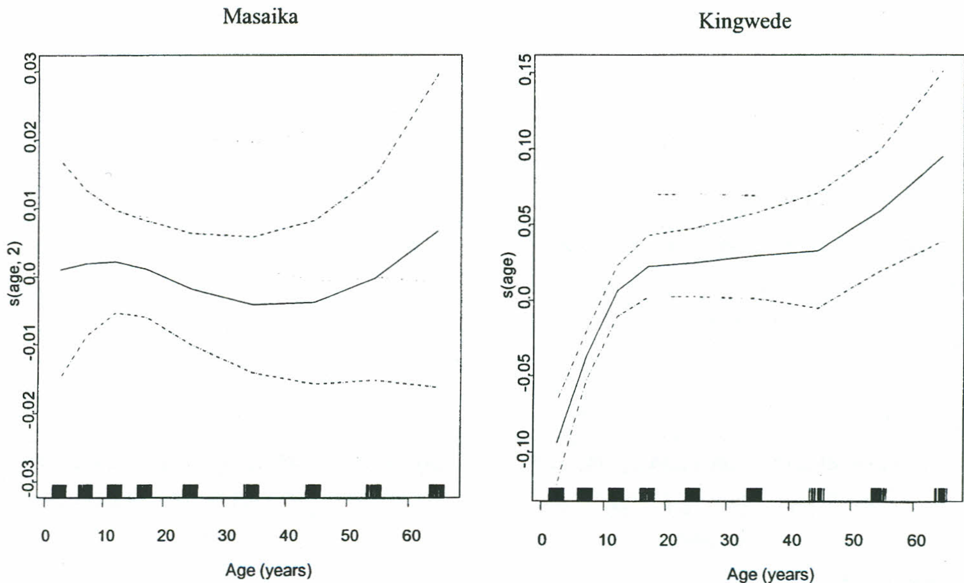


Figure 5.7
The fitted function for age for the IgG3 intensity data from Masaika and Kingwede. In the model, s(age) is an arbitrary smoothing function replacing IgG3 intensity. The ticks at the bottom of the graphs indicates the density of data in each age class. Solid line represents the mean of the response while dashed lines denote the respective point-wise standard errors of the estimated curve.

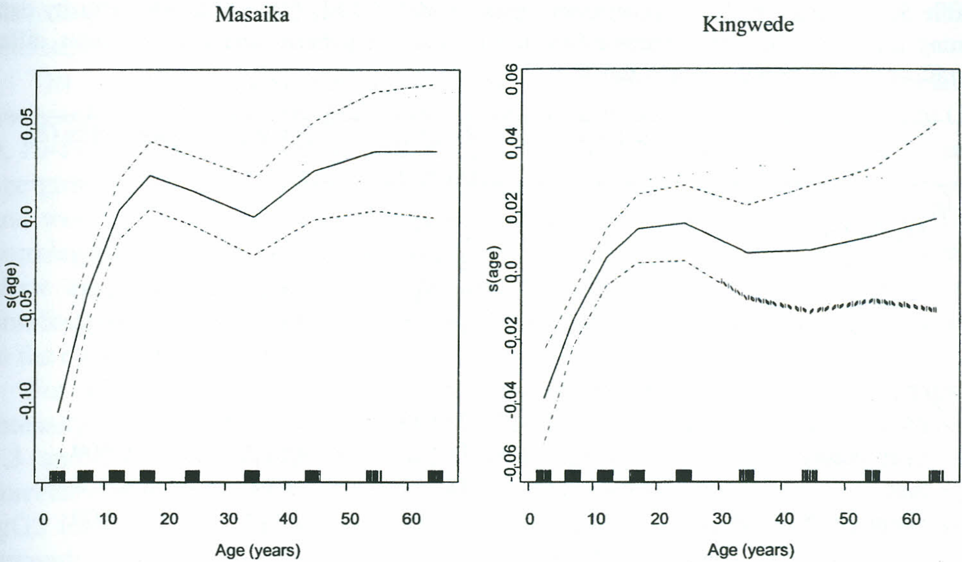


Figure 5.8
The fitted function for age for the IgG4 intensity data from Masaika and Kingwede. In the model, s(age) is an arbitrary smoothing function replacing IgG4 intensity. The ticks at the bottom of the graphs indicates the density of data in each age class. Solid line represents the mean of the response while dashed lines denote the respective point-wise standard errors of the estimated curve.

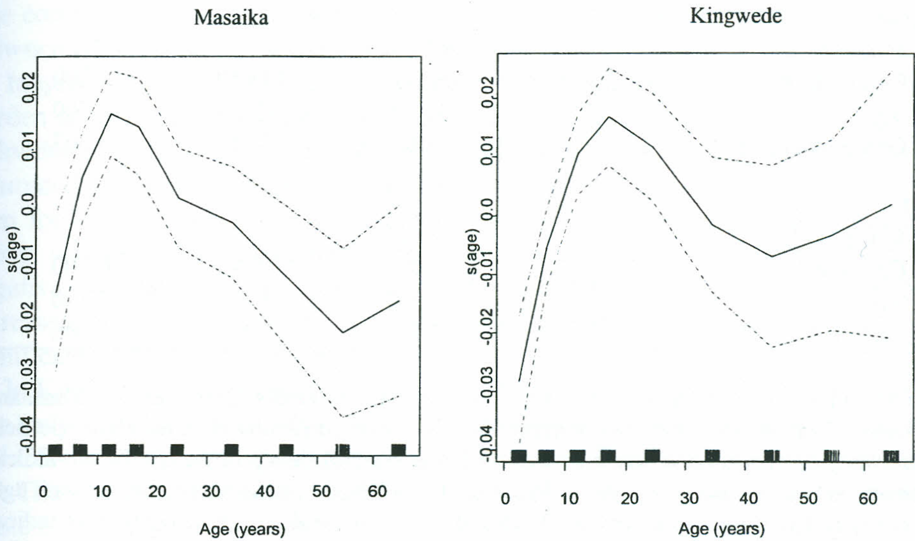


Figure 5.9
The fitted function for age for the IgE intensity data from Masaika and Kingwede. In the model, s(age) is an arbitrary smoothing function replacing IgE intensity. The ticks at the bottom of the graphs indicates the density of data in each age class. Solid line represents the mean of the response while dashed lines denote the respective point-wise standard errors of the estimated curve.

Table 5.2 Analysis table for generalised linear model (GLM) fits to antibody intensity data testing the levels between communities, the overall age-patterns and if age-patterns differ significantly between the communities

	Coefficient	Residual Deviation	F Value	Probability (F)
IgG1				
Model Null		26.211		
Community	–	23.977	154.01	0.000
Age	–	23.217	52.41	0.000
Community x Age		23.152	4.45	0.035
IgG2				
Model Null		23.037		
Community	–	18.024	450.23	0.000
Age	–	18.017	0.62	0.432
Community x Age		17.769	22.28	0.000
IgG3				
Model Null		50.141		
Community	+	40.133	410.96	0.000
Age	+	39.580	22.70	0.000
Community x Age		38.867	29.26	0.000
IgG4				
Model Null		42.752		
Community	–	32.327	525.89	0.000
Age	+	31.707	31.23	0.000
Community x Age		31.639	3.45	0.063
IgE				
Model Null		9.616		
Community	–	9.465	25.60	0.000
Age	–	9.454	1.72	0.190
Community x Age		9.410	7.51	0.006

GLM fit to the log transformed OD values using Gaussian errors. Community = Masaika or Kingwede. For antibody intensity comparisons between communities, a negative coefficient indicates higher intensities in Masaika than in Kingwede, while a positive coefficient indicates higher intensities in Kingwede than in Masaika. For antibody intensity comparison with age, a negative coefficient indicates decreased intensity with age and a positive coefficient indicates increased intensity with age. A p-value <0.05 in respect to community indicates significant differences in antibody intensity between the communities, whereas a p-value <0.05 for age indicates significant differences in antibody intensities with age. A p-value <0.05 in respect to community x age indicates significant differences in age-intensity patterns between the two communities. Degrees of freedom: Community = (1, 1598); Age = (1, 1597); and Community x Age = (1, 1596).

Statistical analysis of the correlation between antibody intensity and mf or CFA intensity

The age-patterns and associations observed in Figures 5.3-5.9 were formally analysed using Pearsons product moment partial correlation tests. Four age groups (1-9, 10-19, 20-39 and 40+ years) were chosen to give adequate sample sizes within each age group to allow for statistical tests. Partial correlations between antibody intensity and worm burden with age were first analysed among all examined individuals. The correlations were then carried out among infected individuals (mf positive individuals alone and then CFA positive individuals alone) to examine if the proportion of uninfected individuals contributed disproportionally to the overall increase or decrease in the coefficients within the age groups.

For all the examined individuals in Masaika, the correlation between antibody intensity and worm burden (either mf or CFA) within age groups is shown in Tables 5.3 and 5.4. IgG1, IgG2, IgG3 and IgE intensities were in general negatively correlated with worm burden in all age groups. The correlation coefficients between IgG2 intensities and mf intensities were significantly heterogeneous with age. The strength of the negative correlation for IgG1 and IgE intensity with worm burden decreased with age. This was more prominent in relation to CFA intensity than to mf intensity for both antibodies (Tables 5.3 and 5.4). In contrast, the strength of the negative correlation between IgG2 intensity and worm burden increased with age. The negative correlation between IgG3 intensity and worm burden did not show a clear pattern between age groups. IgG4 intensities positively correlated with worm burden. The correlation between IgG4 intensities and CFA intensities were stronger than that between IgG4 intensities and mf intensities.

In general, the trend seen for the correlation between antibody intensity and worm burden among infected individuals (mf positive individuals alone and CFA positive individuals alone) in Masaika (Tables 5.5 and 5.6) was similar to that seen among all examined individuals. The most clear correlation pattern among these individuals was seen for IgG2 intensity and worm burden. Here, IgG2 intensity in the youngest age group was positively correlated with worm burden. However, this switched to a negative correlation in the older ages. The strength of IgG2 correlation with mf increased with age, and the correlation coefficients between age groups were significantly heterogeneous ($p<0.001$).

In Kingwede, due to the low number of mf positive individuals, antibody intensity was only correlated with CFA intensity. The correlation between antibody intensity and CFA intensity within age groups for all examined individuals is shown in Table 5.7. This correlation among the infected (CFA positive individuals alone) is shown in Table 5.8. Similar to the pattern seen in Masaika, intensities of IgG1, IgG2, IgG3 and IgE antibodies in this community were in general negatively correlated with worm burden, whereas that of IgG4 was positively correlated with worm burden. Similarly, the strength of the negative correlation in general decreased for IgG1 and IgE, as was the case in Masaika. Correlation coefficients of IgG1, IgG4 and IgE intensities with CFA intensities were significantly heterogeneous between age groups among all examined individuals, but not among infected individuals alone (Tables 5.7 and 5.8).

In contrast to Masaika, the correlation coefficient of IgG2 with worm burden in Kingwede was not clear. Although IgG2 intensity and CFA intensity were in general negatively correlated, the strength of the correlation decreased with age unlike in Masaika. However, the correlation coefficients between age groups were significantly heterogeneous as was the case in Masaika (Tables 5.7 and 5.8).

IgG4/IgE ratios in relation to age

The age-specific mean IgG4/IgE ratios for all examined individuals in Masaika and Kingwede are shown in Figure 5.10. The ratios were higher in Masaika than in Kingwede for all age groups. In each community, this ratio was lowest in the youngest age group and was slightly increasing by age.

The age-specific mean IgG4/IgE ratios for CFA positive individuals alone are shown in Figure 5.11. Similarly, for these individuals, IgG4/IgE ratios were higher in Masaika than in Kingwede for all the age groups. With the exception of the youngest age group where the ratios were quite low in both communities, IgG4/IgE ratios were quite similar in all the other age groups. The age-specific mean IgG4/IgE ratios for mf positive individuals alone could not be compared between the two communities since Kingwede had very few mf positive individuals. These ratios among the mf positive individuals in Masaika were generally similar for all age groups.

Table 5.3. Partial correlation coefficients between filaria -specific antibody intensity and microfilaraemia intensity within age groups among all the examined individuals in Masaika

Age group	N	Percentage positive	IgG1	IgG2	IgG3	IgG4	IgE
1-9	172	5.2	-0.0976	-0.0283	-0.0964	0.1989**	-0.1059
10-19	208	20.7	-0.3643**	-0.0179	-0.0366	0.1384	-0.0836
20-39	261	30.3	-0.2478**	-0.2348**	-0.0669	0.2629**	-0.0586
40+	187	41.2	-0.2529**	-0.2130**	0.0094	0.3375**	-0.0221
χ^2 ᶲ			7.485	8.773*	1.111	4.866	0.710

**$p<0.001$; *$p<0.05$

ᶲ Test for homogeneity of correlation coefficients.

Table 5.4. Partial correlation coefficients between filaria -specific antibody intensity and circulating filarial antigenaemia intensity within age groups among all the examined individuals in Masaika

Age group	N	Percentage positive	IgG1	IgG2	IgG3	IgG4	IgE
1-9	172	20.9	0.0109	-0.1375	-0.1304	0.4807**	-0.1624
10-19	208	50.0	-0.2375**	-0.1162	-0.0727	0.5252**	-0.2857**
20-39	261	60.9	-0.3039**	-0.1711**	-0.1302	0.4976**	-0.1554
40+	187	71.1	-0.1906**	-0.2075**	-0.1051	0.4812**	-0.1026
χ^2 ᶲ			11.251*	0.984	0.466	0.451	3.946

**$p<0.001$; *$p<0.05$

ᶲ Test for homogeneity of correlation coefficients.

Table 5.5. Partial correlation coefficients between filaria -specific antibody intensity and microfilaraemia intensity within age groups among the mf positive individuals alone in Masaika

Age group	N	GMI mf intensity (mf/ml)	IgG1	IgG2	IgG3	IgG4	IgE
1-9	9	632	-0.9169	0.8554	-0.7762	-0.6424	-0.3804
10-19	43	470	-0.1821	-0.0642	0.0705	0.1399	0.0419
20-39	79	444	-0.1597	-0.1876	0.0348	0.1060	0.0614
40+	77	458	-0.0607	-0.2440	0.0452	0.2116	0.1608
$\chi^{2\,\phi}$			12.648**	13.305**	6.841	5.361	2.932

**p<0.001; *p<0.05

$^{\phi}$Test for homogeneity of correlation coefficients.

Table 5.6. Partial correlation coefficients between filaria -specific antibody intensity and circulating filarial antigenaemia intensity within age groups among the CFA positive individuals alone in Masaika

Age group	N	GMI CFA intensity	IgG1	IgG2	IgG3	IgG4	IgE
1-9	36	3959	-0.2486	0.0212	-0.2737	0.1435	0.0018
10-19	104	4293	-0.1938	0.0443	-0.0669	0.0103	-0.2644**
20-39	159	7469	-0.1627	-0.1119	-0.0610	0.2624**	-0.0705
40+	133	8422	0.0049	-0.0783	-0.0505	0.2918*	-0.2585*
$\chi^{2\,\phi}$			3.507	1.765	1.513	5.774	4.689

**p<0.001; *p<0.05

$^{\phi}$Test for homogeneity of correlation coefficients.

Table 5.7. Partial correlation coefficients between filaria -specific antibody intensity and circulating filarial antigenaemia intensity within age groups among all the examined individuals in Kingwede

Age group	N	CFA positive (%)	IgG1	IgG2	IgG3	IgG4	IgE
1-9	235	5.1	-0.0489	0.1293	-0.0163	0.1996*	0.0201
10-19	216	15.7	0.1457	-0.1532	0.0543	0.4656*	-0.2365*
20-39	208	24.0	-0.1055	0.0319	-0.1630	0.5075*	-0.0563
40+	107	27.1	-0.0898	-0.0825	0.0761	0.3923*	-0.0676
χ^2 ϕ			8.164 *	9.868*	6.400	16.488**	7.980*

**$p<0.001$; *$p<0.05$

ϕ Test for homogeneity of correlation coefficients.

Table 5.8. Partial correlation coefficients between filaria -specific antibody intensity and circulating filarial antigenaemia intensity within age groups among the CFA positive individuals in Kingwede

Age group	N	GMI CFA intensity	IgG1	IgG2	IgG3	IgG4	IgE
1-9	12	269	-0.2385	0.4733	0.0656	-0.1366	0.0783
10-19	34	642	-0.0942	-0.4023	0.2828	0.4961*	-0.5732*
20-39	50	830	0.0374	0.1649	-0.2336	0.3415	-0.2725
40+	29	737	0.1824	-0.1450	0.0069	0.3849	-0.1375
χ^2 ϕ			1.738	9.678*	5.319	3.291	5.771

**$p<0.001$; *$p<0.05$

ϕ Test for homogeneity of correlation coefficients.

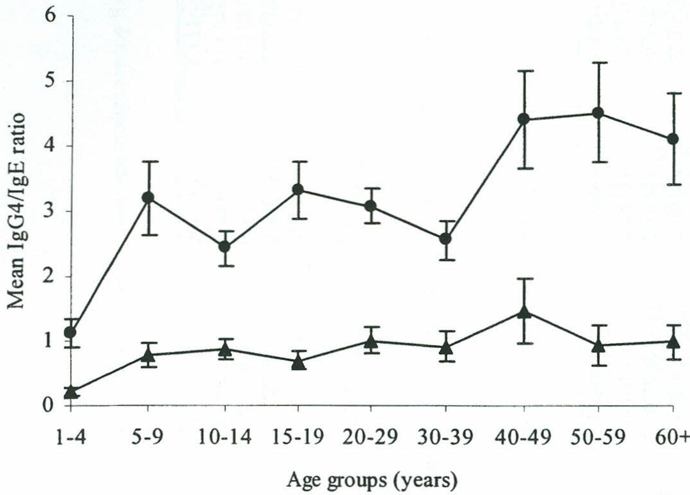


Figure 5.10. The age specific mean IgG4/IgE ratio (± S.E.) among all examined individuals in Masaika (●) and Kingwede (▲).

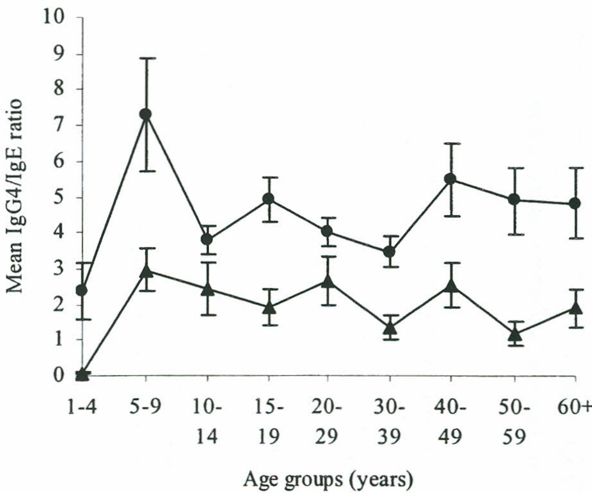


Figure 5.11. The age-specific mean IgG4/IgE ratio (± S.E) among circulating filarial antigen positive individuals in Masaika (●) and Kingwede (▲).

Discussion

The role of acquired immunity in regulating infection in human bancroftian filariasis is largely undetermined. Various studies have observed that some individuals from endemic areas apparently develop immunological resistance to infective larvae (Freedman *et al.* 1989; Day *et al.* 1991a) and to microfilariae (Simonsen 1983; 1985; Simonsen & Meyrowitsch 1998a). Other studies have observed that children have a higher incidence of new infections than adults (Day *et al.* 1991b; Weil *et al.* 1999). However, most evidence suggestive of the operation of acquired immunity in bancroftian filariasis is indirect and based on analysis of epidemiological data (Grenfell & Michael 1992; Michael & Bundy 1998). Thus, convex age-intensity curves suggest the presence of either acquired immunity or of age-dependent exposure to infection, or both. The relative contribution of exposure related factors and immunological processes as determinant of the observed patterns is difficult to elucidate. Part of the difficulty stems from the fact that infection level and immune responses are interdependent and both are related to age and exposure.

Several studies have shown that strong antibody responses to infection are induced in bancroftian filariasis (Ottesen *et al.* 1982; Estambale *et al.* 1994b; Addiss *et al.* 1995; Simonsen *et al.* 1996; Nicolas *et al.* 1999). However, none of these responses have yet been identified to be protective. Theoretically, immune responses would be expected to be positively correlated with worm burden. However, the correlation between immune response and parasite burden may change with host age if these responses are protective. Thus, the correlation coefficient can be expected to be positive in young children with the immune responses reflecting exposure, but as the hosts age, the correlation coefficient between a protective immune response and parasite burden may become negative as the immune response starts to regulate the worm burden (Bundy *et al.* 1991b; Woolhouse 1992, 1993).

Studies investigating protective immunity by correlating antibody responses with worm burden within age groups have been carried out for a number of helminth infections (Bundy *et al.* 1991b, Needham *et al.* 1992; Quinnell *et al.* 1995; Mutapi *et al.* 1997). However, no similar study has so far been documented for lymphatic filariasis. The present study used partial correlation analysis to examine the statistical correlation between filarial specific antibody responses and worm burden with increasing age (Quinnell *et al.* 1995). Certain factors need to be borne in mind when interpreting results from this approach. Firstly, the division of the population into age classes will result in wide confidence limits and any emerging patterns may be obscured by noise (Quinnell *et al.* 1995). Secondly, the calculation of many correlation coefficients means that the level of significance must be treated with caution or corrected for multiple comparisons. In this study, significance level was set at $p<0.01$. Finally, while theory predicts age-related changes in correlation coefficients, it does not require that the correlation itself be significantly different from zero. It is the changes in correlation with age that are meaningful (Quinnell *et al.* 1995).

Age-specific infection intensity patterns and specific antibody profiles

In the high transmission intensity community, infection intensity increased with age in the young ages but then either reached a plateau as seen for mf intensity or dropped as seen for CFA, in the oldest ages. This age infection intensity curve suggested the existence of some regulation in worm burden with age, probably from protective immunity, reduced exposure to infection by age, or both (Anderson & May 1991; Grenfell & Michael 1992; Michael & Bundy 1998). In the low transmission intensity community, infection intensity increased with age without reaching a plateau or dropping in the older ages. Thus, in this community, the age infection intensity curves did not suggest the existence of an age-dependent worm burden regulating mechanism (Michael & Bundy 1998). Mathematical models comparing age-intensity curves between areas with different transmission intensities in the presence of some protective immunity (particularly against larval establishment) predict higher peak infection intensities and the peaks occurring at a younger age in the high compared to the low transmission areas, the so called 'peak shift' phenomenon (Anderson & May 1991; Woolhouse 1992). This pattern was not clearly seen in the present study. It is possible that transmission intensities in the communities were not high enough to reach a threshold required to switch on protective immune responses.

The age-specific antibody prevalence and mean intensities in the present study generally followed similar patterns, and further analysis was carried out only with regard to antibody intensities. Mean intensities of specific IgG1 and IgE decreased with age in both communities whereas that of IgG2 decreased with age in the high but not in the low transmission intensity community. Most previous studies have generally shown higher specific antibody intensities in the younger than in the older age groups, although the differences have rarely been significant (Ottesen *et al.* 1977, 1882; Hitch *et al.* 1989, 1991; Estambale *et al.* 1994b; Simonsen *et al.* 1996). It has been suggested that the low antibody levels in the older age groups may indicate downward regulation of reactivity with increasing duration of exposure to the parasite (Ottesen *et al.* 1982; Ottesen 1984). Perhaps the different age-specific patterns for IgG2 in the two communities may also be explained on this basis. Thus, the increase in IgG2 intensity with age in the low transmission intensity community in contrast to the decrease in IgG2 intensity with age in the high transmission intensity community may suggest that exposure to the parasite was not enough to begin down-regulating the antibody levels in the former. However, this does not explain the similar IgG1 and IgE age-intensity patterns observed in the two communities. If this was the case, then IgG1 and IgE intensities would also have been expected to increase with age in the low transmission intensity community in contrast to the decreasing intensity with age in the high transmission intensity. A possible explanation why this was not the case may be that these IgG1 and IgE antibody isotypes probably require a lower threshold of exposure to antigen for them to start being down-regulated, and this was achieved in the low transmission intensity community.

Age-intensity patterns for IgG3 also differed in the two communities. Whereas in the high transmission intensity community its levels did not change with age, in the low transmission intensity community its levels were not only significantly higher but

also increased with age. The reason for the difference in the two communities is not clear, but suggests some down-regulatory role for worm burden.

In contrast to specific IgG1, IgG2 and IgE, the mean intensities of IgG4 in both communities increased with age. Higher IgG4 levels in the older individuals than in the younger individuals have previously been described (Hitch *et al.* 1989; Mahanty *et al.* 1994). This finding is not surprising since IgG4 intensity is generally positively associated with presence of infection (Estambale *et al.* 1994b; Dimock *et al.* 1996; Simonsen *et al.* 1996; Nicolas *et al.* 1999) and with intensity of infection (Wamae *et al.* 1998), and both of these were higher in the older than in the younger individuals in the present study.

Overall and age-specific antibody intensity correlation with worm burden

In the present study specific IgG1 and IgG2 intensity was more strongly negatively correlated with mf intensity than with CFA intensity, probably suggesting that these antibody isotypes may be suppressing mf production by the adult worms, or that the mf may be suppressing the production of the antibodies. If the antibodies suppress mf production, they probably do so through an anti-fecundity mechanism on the adult worm. On the other hand, specific IgG3 and IgE intensity was negatively correlated with CFA intensity and not with mf intensity. Thus, these antibodies may be having a suppressing effect on the intensities of the adult worms. In contrast to all the other antibody types, specific IgG4 intensity was positively correlated with CFA intensity.

The relationship between antibody intensity and worm burden across age groups was not obvious, making their interpretation rather difficult. This may have risen from the crude nature of the parasite extracts used, which may have given polyspecific responses against a multiplicity of antigens shared between various stages of the parasite. The clearest pattern was seen for IgG2 intensity in the high transmission intensity community being positively correlated with infection in the young age groups but switching to a negative correlation with infection in the older ages. Furthermore, the strength of the association increased with increasing age. Positive correlation between antibody levels and worm burdens in younger age groups are expected, simply because both will be correlated with exposure to the parasite. There are however two possible interpretations for the switch to a negative correlation between antibody and infection intensities in the older ages. Either the high antibody levels may be protective hence reducing worm burdens (Bundy *et al.* 1991b), or the very high worm burdens may be immunosuppressive and be reducing antibody levels as has been observed for intestinal nematodes (Pritchard *et al.* 1994). The age-intensity patterns indicated that the negative correlation between IgG2 and infection intensity in the older age groups was due to increasing infection intensities, both mf and CFA, as opposed to the decreasing IgG2 intensity with age. Thus, it is more likely that high worm loads were suppressing IgG2 intensities.

IgG1 and IgE intensities were negatively correlated with worm load and the strength of this correlation decreased with age. Since there was a decrease in the proportion of uninfected individuals with increasing age, these individuals may have been a major contributor to the strength of the correlation between the antibody

intensities and worm burden. This may also explain why the drop in the association was more prominent in relation to CFA than to mf, since age-specific decrease in proportion of non-infected individuals was higher for the former than the latter.

The present study also examined if the observed age-specific antibody and infection intensity correlation patterns differed with transmission intensity. This was accomplished by comparing the age-specific antibody intensity and worm burden correlation profiles between the high and low endemicity communities. The hypothesis was that if these specific antibodies had a role in protective immunity and that if this immunity was driven by transmission intensity, then these profiles would differ between the communities. In both communities IgG1, IgG2, IgG3 and IgE intensities were in general negatively correlated with worm burden in all age groups. However, the initial positive correlation between IgG2 intensity and worm burden followed by a switch to a negative correlation observed in the high transmission intensity community was not seen in the low transmission intensity community. Thus, as earlier suggested, perhaps exposure in the low transmission intensity community to the parasite antigen was not enough to begin down-regulating the IgG2 antibody levels.

IgG4/IgE ratios

It has been suggested that the IgG4/IgE ratios in lymphatic filariasis may be a measure of the strength of immunological resistance to infection (Kurniawan *et al.* 1993). Thus the ratio is expected to be low in the presence of resistance. The findings of low mean IgG4/IgE ratios in the low transmission intensity community compared to the high transmission intensity community are not in agreement with this theory. If IgG4/IgE ratios are indicators of resistance as suggested, the finding would suggest that resistance to infection in bancroftian filariasis is not affected by age. Alternatively, the finding may suggest that IgG4/IgE ratios may not be good indicators of resistance in this infection.

Conclusions

The present study found clear correlation between the overall specific antibody intensity and worm burden. IgG1 and IgG2 intensities were negatively correlated with mf intensity more than CFA intensity, whereas IgG3 and IgE were negatively correlated with CFA intensity more than mf intensity. In contrast, IgG4 intensity was positively correlated with CFA intensity more than mf intensity. The study however did not find clear age-related specific antibody and worm intensity correlation patterns. In the high transmission intensity community, IgG2 intensity in the youngest age group was positively correlated with worm burden as would be expected, since both worm burden and antibody responses are partly dependent on exposure to infection. However, this switched to a negative correlation in the older age groups, consistent either with a protective response or down-regulation of antibody intensity by increasing worm burden. The age-intensity patterns suggested the latter to be more likely. The age-intensity pattern observed for IgG2 in the low transmission intensity

community suggested lack of down-regulation of the antibody intensity by the worm burden, probably as a result of the infection intensity being below the threshold required for such a switch. The lack of conclusive evidence for the existence of either protective response or down-regulation of immune responses in this study underscores the difficulties in interpreting this kind of immunoepidemiological data, and emphasizes that the underlying immunological processes are complex and may not generate easily understood patterns. It is suggested that the use of more specific antigens for detecting antibodies may probably give clearer patterns. It is also possible that no clear evidence was seen for protective immunity in this study because transmission intensities in the two communities were not high enough to induce strong protective immune responses. A similar study in communities with much higher transmission intensities is therefore suggested.