The epidemiology and control of lymphatic filariasis and intestinal helminths in the lower Shire valley- Chikwawa District, southern Malawi.

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by

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Abstract

Work presented in this thesis has three related componets: First, a nation wide mapping survey for the distribution of lymphatic filariasis (LF), a mosquito borne disease caused by a nematode parasite *Wuchereria bancrofti.* Second, an intervention trial to evaluate the impact of a single round of mass drug administration (MDA) of albendazole and ivermectin in combination for the control of LF on intestinal helminths and associated morbidity. Third, to determine the potential role of anti-tuberculosis chemotherapy in the treatment of LF.

This is the first comprehensive description of the geographical distribution of LF throughout Malawi. The study, in the remaining 23 districts, involved 35 villages. Data from earlier studies in Karonga and in southern districts of Chikwawa and Nsanje were incorporated into a national distribution map. Village LF antigenaemia prevalence [based on immunochromatographic (ICT) card tests] ranged from 0% to 35.9%. In general, villages from the western side of the country and far removed from the lake shore tended to register lower prevalence. In all districts, except Chitipa in the north, there was at least one individual positive on ICT.

An opportunity to describe the distribution of intestinal helminths, malaria and their relationship to anaemia in a rural population of southern Malawi- Chikwawa District arose in connection with the baseline and follow-up surveys for the intervention trial designed to assess the impact of a single MDA for the control of LF. In addition, adult study participants were given a full body clinical exam for chronic clinical manifestations of LF. At the baseline survey, 1108 individuals from all the 18 study villages had a complete data set. Of these, 466 (42.1%) were anaemic (haemoglobin (Hb)< 11.0g/dl) and 71 (6.4%) had moderate to severe anaemia (MSA) (Hb<8g/dl). Malaria parasitaemia was found in 226 (20.4%) individuals. Based on a single, thick Kato-Katz stool smear, the prevalence for hookworm, *Schistosoma mansoni* and *Ascaris lumbricoides* was 18.9%, 5.4% and 1.7%, respectively. At this survey period, 21.3% of surveyed adult males had a hydrocele and 2.9% of adult female participants had lymphoedema. In addition, *W. bancrofti* microfilaraemia was found in 30.1% of individuals sampled at night. At the follow-up survey, a year after the MDA, statistically significant reductions in anaemia (intervention villages: 36.3% vs control villages 45.4%), hookworm infection (intervention villages: 19.1% vs control villages 26.5%) and density of *W. bancrofti* infection based on microfilaraemia between control and intervention villages were found. Further analysis of follow-up survey data has established that polyparasitism is common in southern Malawi with 50% of parasitic infections occurring as multi-species infections.

In conclusion, the data obtained in these studies suggest that, in Malawi, *W. bancrofti* infections are more widespread than previously thought and that the lake shore and the lower Shire Valley are priority areas for control activities. These studies have also revealed a significant burden of clinical disease associated with *W. bancrofti* infection, hookworm, malaria and schistosomiasis. Secondly, the strategy of mass drug administration for the control of LF is associated with a sustained reduction in intestinal helminths (hookworm in particular) and anaemia in Chikwawa District-southern Malawi. It is, therefore, appropriate to promote this as a deworming strategy that is associated with significant 'ancillary' benefits.

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Merck and GSK who provided drugs that were used in our mass treatment campaigns.

The World Health Organisation who provided my tuition fees through a TDR training grant.

My Mum and Dad who started me off on a narrow path that is now bearing fruit.

DEDICATION

This thesis is dedicated to

My wife Atusaye Maureen Ngwira and our 3 month old son Chigomezgo Elias Mdoni Mzomera Ngwira

For being there through thick and thin!!!

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Chapter 1

1.1 Introduction and aims

Lymphatic filariasis (LF), a chronic disease characterised by clinical manifestations such as lymphoedema, elephantiasis and/or hydrocele, is caused by the vector-borne nematodes, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* (Michael et al., 1996). *W. bancrofti* infection is more prevalent accounting for 90% of the estimated 120 million prevalent cases globally (Ravindran, 2003). It is distributed throughout the tropics and subtropics except in the Middle East where it seems to be restricted to Egypt and Yemen. In contrast, brugian filariasis is only endemic in South and Southeast Asia, including Southern China whereas *B. timori* is found only in Timor Letse (formerly East Timor) and adjacent islands of Indonesia (Flores and Alor) (Anon, 2001; Fischer et al., 2004; Melrose, 2002).

The World Health Organisation (WHO) ranks LF as one of the leading causes of permanent and long-term disability worldwide (World Health Organisation, 2005). It inflicts debilitating genital disease in more than 26 million men, lymphoedema or elephantiasis in an estimated 16 million individuals and lymphatic dysfunction in virtually all those infected (World Health Organisation, 1994). In 1993, the International Task Force for Disease Eradication considered LF as one of the six potentially eradicable diseases (Centres for Disease Control and Prevention, 1993). This was followed, in 1997, by the passing of resolution 50.29 by the World Health Assembly that called for member states of the WHO to support the elimination of LF as a 'public-health' problem (Molyneux and Taylor, 2001). In

consequence, the WHO started a Global Alliance to Eliminate Lymphatic Filariasis (GPELF) in 2000, a worldwide coalition of many organisations, each with a different mandate but all having the common goal of tackling the wide-ranging and complex process of science and practice that will result in the global elimination of LF as a 'public-health' problem (Anon, 2001).

The programme recommends that all those who live in at-risk communities be treated on mass, orally, once a year, with an appropriate two-drug combination (Molyneux and Taylor, 2001; Molyneux and Zagaria, 2002; Ottesen, 2000). Secondly, it aims to promote measures to alleviate and prevent both the suffering and disability caused by the disease. For mass treatment three antiparasitic drugs are recommended in this global effort: albendazole, ivermectin and diethylcarbamazine (DEC). In countries where onchocerciasis and LF co-exist, ivermectin and albendazole are the drugs of choice as DEC cannot be safely used due to its associated ocular pathology attributed to death of microfilaria in the eyes of individuals infected with *Onchocerca volvulus* (Greene et al., 1983). For the rest of the world, albendazole and DEC are to be used. Two-drug combinations have been shown to provide a more long-lasting suppression of microfilaraemia than using one drug alone (Ismail et al., 2001). This then ensures that mosquitoes are deprived of the opportunity to continue transmission. Despite this effect, it is appreciated that these drugs have a limited profile against the adult worms although some evidence exists that suggests they may adversely affect their survival and the female worm's ability to reproduce (Dreyer et al.,

1995c; El Setouhy et al., 2004; Fox et al., 2005). For the mass drug distribution (MDA) to be effective in the long term, a high percentage (≈ 70%) of the at-risk community need to be treated every year for up to six consecutive years (Molyneux and Zagaria, 2002). This period corresponds to the estimated reproductive life-span of the adult female worm. The principal strategy adopted to alleviate the suffering caused by the acute and/or the chronic consequences of LF focuses on reducing the possibility of secondary bacterial and fungal infections of limbs or genitals where the lymphatic function has already been compromised by filarial infection (Dreyer et al., 2000). Current thinking suggests that secondary infections are the primary determinant of the worsening of lymphoedema and elephantiasis (Addiss and Dreyer, 1999). The proposed package to prevent secondary bacterial and/or fungal colonization of the affected limbs includes regular washing with soap and water, daily exercising of the limbs, wearing of comfortable protective footware and where indicated use of antibiotic and antifungal cream (Dreyer et al., 2000; Dreyer et al., 1999; Vaqas and Ryan, 2003).

An essential first step in implementing programmes to eliminate LF is to determine the geographical distribution of the infection based on antigenaemia levels. Depending on the health delivery system, an endemic country identifies an administrative unit responsible for MDA. Such a unit is called an 'implementation unit'. A mapping survey is carried out in all such units to determine infection status. In implementation units where the infection level

exceeds 1%, everyone that meets the treatment criteria, regardless of their infection status is treated annually with an appropriate two-drug combination (World Health Organisation, 2005).

There are, however, outstanding issues that need addressing such as: 1) the most cost- effective and efficient way to distribute the drugs, 2) the most appropriate way to assess treatment coverage and monitor any impact on transmission, 3) the number of MDA rounds necessary to interrupt transmission and the role of any vector control, 4) appropriate indicators of interruption of transmission, 5) ways to maintain compliance with treatment particularly in areas where chronic manifestations are uncommon, 6) how to measure the effect of the LF elimination programme on local health systems, 7) how to measure the programme's impact on other diseases amenable to similar interventions.

From 2001 a series of LF related studies were initiated in Malawi. Malawi is a landlocked country situated in southeast Africa. It lies south of the equator (90 to 170 S) and in the eastern zone (330 to 360 E Lon). It shares international borders with the Republic of Zambia to the west and northwest; the United Republic of Tanzania to the north and northeast; and the people's Republic of Mozambique to the southwest, south and east. The country is 901 kilometres long and ranges in width from 80 to 161 kilometres. It has a total area of 118,484 square kilometres of which 94,276 square kilometres is land area.

It has numerous mountain ranges and hills whose peaks range from 1700 to 3000 metres above sea level. It forms part of the Great Rift Valley. The most prominent topographic feature is the freshwater lake called 'Lake Malawi' which

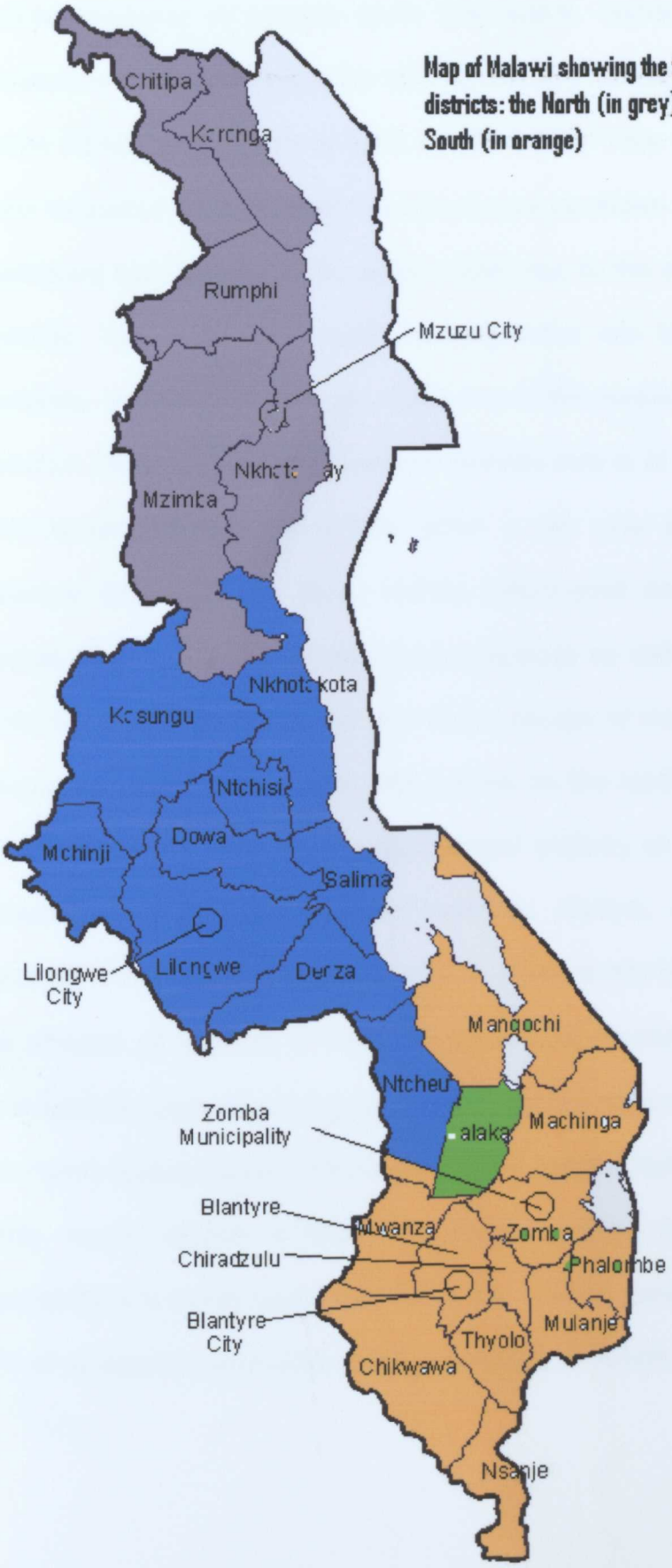
covers a third of the country's surface area. The Shire River drains Lake Malawi from its southern tip traversing the Lower Shire Valley on its course into the Zambezi River that it joins inside Mozambique.

There are two main seasons (dry and wet). The dry season extends from May to October while as the wet season spans November to April. Annual rainfall varies across Malawi with the highest being over the highlands. In the recent past, possibly as a result of the "El Nino" effect, rains have been erratic with prolonged droughts (Kovats et al., 2003). However, the Lower Shire Valley and some lakeshore districts have experienced repeated flooding. The highest temperatures are experienced in the Lower Shire Valley where it can approach 50 ^{0}C at the height of summer (October) with humidity level of over 85%.

Malawi has an estimated population of 11.2 million (UNAIDS, 2003). The country's population is mainly rural (86%, 1998 National census) whose main economic activity is farming. It is estimated that 80% of the population live below the poverty line. There are three main tribal groupings, the Yao in the southern region, the Chewa in the central region and the Tumbuka in the northern region. The main cash crop has been and remains tobacco. The government has recently embarked on an initiative to encourage irrigation based farming and crop diversification.

Malawi is administratively divided into three regions; the north, the centre and the south. These are further divided into 28 districts (see Figure 1). Six districts are in

the Northern Region, 9 in the Central Region and 13 in the Southern Region. Administratively, the districts are subdivided into Traditional Authorities (TAs), presided over by chiefs. Traditional Authorities are composed of villages, which are the smallest administrative units and these are headed by village headmen.



Map of Malawi showing the three regions and their districts: the North (in grey), Central (in blue) and South (in orange)

Sectoral analyses of poverty show that social, human capital and income indicators in Malawi are very poor with the country ranking 161 out of 174 on the Human Development Index in 2000 (UNDP 2001). Thus it is not surprising that health indicators have generally remained poor as shown in Table 1. Notably life expectancy has declined to 36 years mainly due to the advent of the HIV/AIDS epidemic. The infant and child mortality rates are high even by regional standards, at 104 and 189 per 1,000 live births respectively(Malawi National Statistical Office, 2000). The maternal mortality rate is at 1,120 per 100,000 live births which increased from 620 within a ten year period(Malawi National Statistical Office, 2000). These indices reflect poor access and coverage of general, maternal and child health care services as well as the general socio-economic conditions in Malawi. The major causes of mortality and morbidity in Malawi are mostly preventable, with malaria as the leading cause of outpatient visits (30% of the outpatient visits) (Malawi Ministry of Health, 2005). This is followed by diarrhoeal diseases, including cholera, and acute respiratory infections. HIV/AIDS constitutes a serious threat to the country as a whole as it has affected all aspects of Malawi's social and economic fabric. The Malawi National Aids Commission estimates that there are up to a million people infected with HIV in Malawi (Malawi Ministry of Health and the National Aids Commission, 2003). Nearly 80,000 of these are children under 15 years of age. More importantly it is worth noting that HIV/AIDS related conditions account for over 40% of all inpatient admissions (Malawi Ministry of Health, 2005).

The Ministry of Health's overall aim is to contribute to the achievement of sustainable poverty reduction through the enhancement of health development (Malawi Ministry of Health, 2003). In this regard, the ministry supports the Government's efforts in poverty reduction as outlined in the Malawi Poverty Reduction Strategy Paper which identified low human capital development as one of the root causes of poverty in Malawi (Malawi Government, 1998). To that effect, as from 1999, the ministry has embarked on the delivery of the Malawi Essential Health Package (EHP) as one of the components of the Malawi Poverty Reduction Strategy (Malawi Government, 1998). The EHP consists of a cluster of cost-effective interventions delivered together in order to reduce the total cost of the interventions by reducing the cost to patients obtaining the service as well as the cost of providing the service (Malawi Ministry of Health & Population, 2002). The EHP brings together interventions that can be delivered at the same level of the health delivery system, using similar technological sophistication and through the same facility and using multi-skilled health workers. The decision to select an intervention for inclusion in the EHP is based on the cost-effectiveness of each of the interventions as well as their ability to be controlled at less than US$100 per Disability-Adjusted Life Year (DALY) gained (Malawi Ministry of Health & Population, 2002). Each intervention is thus considered on the basis of what it costs to achieve one additional year of healthy life. The Malawi EPH has eleven interventions as listed below:

- Prevention and treatment of vaccine preventable diseases.

- Malaria prevention and treatment- promotion of impregnated bed nets, intermittent prophylactic treatment and case management.
- Reproductive Health Interventions- including Safe Motherhood Initiatives, Essential Obstetric Care and Prevention of mother to child transmission of HIV.
- Prevention, control and treatment of Tuberculosis and related complications.
- Prevention, control and treatment of Schistosomiasis and related complications.
- Management of acute respiratory infections and related complications.
- Prevention, treatment and care for Acute Diarrhoeal Diseases (including cholera).
- Prevention and management of Human immunodeficiency Virus (HIV) /Acquired Immunodeficiency syndrome (AIDS), Sexually Transmitted Infections (STI) and related complications (including Voluntary Testing and Counselling (VCT) and the provision of antiretroviral therapy (ARVT).
- Management of eye, ear and skin infections and related complications.
- Treatment for common injuries- including emergency care for accidents and trauma and their complications.

Support systems required:

- Essential laboratory services
- Drug procurement, distribution and management.

- Information, Education and Communication- Behaviour Change Interventions.
- Pre- and in-service training
- Planning, budgeting and management systems.
- Monitoring and Evaluation- including enhancing integrated disease surveillance activities
- Patient management system.

In addition, in its 4th National Health Plan (1999-2004), the ministry expressed its intention to adopt and develop the Sector Wide Approach (SWAp) to health development (Malawi Ministry of Health & Population, 1999). Because some programmes (malaria, HIV/AIDS and Tuberculosis) will remain vertical, a hybrid SWAp is being implemented allowing for project funding arrangements to continue. These changes are occurring following the decentralisation policy of health services in Malawi. District Health Management Teams (DHMTs) have assumed the responsibility of financial management, planning and expenditure on health services in their respective districts. The DHMTs will be assessed on the quantity and quality of services they deliver based on the EHP which will act as a convenient modality to compare performance.

Malawi has an extensive and comprehensive health delivery system infrastructure consisting of maternity units, dispensaries, health centres, district and central hospitals linked through a well-defined referral system. The health

delivery system emphasises primary health care. In each district there are several health centres at different geographical sites with one referral unit at the district headquarters. There is a primary health care worker [health surveillance assistant (HSA)] responsible for each village. These individuals, amongst other things, are responsible for all Expanded Programme for Immunisation (EPI) activities in their catchment area. In all government facilities health care is provided free of charge. In addition there are facilities that are managed by religious missions, not-for-profit non-governmental organisations (NGOs) and the private sector which charge for their services. A recent Malawi Health Facility Survey found that of the 617 health facilities, 392 (63.5%) are managed directly by the Ministry of Health (Malawi Ministry of Health and Japanese International Corporation Agency (JICA), 2002).

Despite this infrastructure access to health services remains low with only 54% of the rural population having access to formal health services within a 5- kilometre radius (Malawi Ministry of Health and Japanese International Corporation Agency (JICA), 2002). The coverage and quality of health services has been adversely affected by severe shortages of staff at facility level. It is estimated that vacancies on established posts are up to 50% at some institutions. This is in part as a direct consequence of the HIV/AIDS pandemic that has led to increased mortality even amongst health workers. In addition poor working conditions have led to increased skilled labour in the health sector migrating out of Malawi (Hongoro and McPake, 2004). The major challenge facing the health sector is how to ensure that it attracts and retains health workers.

The LF studies in Malawi had two components. First, a mapping component that included a rapid assessment survey at national level and a detailed survey in the northern LF focus (Karonga District). Second, an intervention trial conducted in the southern focus (Lower shire Valley- Chikwawa District).

1.1.1 Objectives for the mapping component

The national survey had three main objectives:

a) To measure the prevalence of *W.bancrofti* antigenaemia (based on ICT) in randomly selected villages which would determine district level endemicity status.

b) To develop a map of the spatial distribution of LF infection in Malawi based on village prevalence data.

c) To provide baseline data that would inform decision on instituting a "National LF Elimination Programme".

The Karonga survey had the following objectives:

a) To measure the current *W. bancrofti* infection rates and disease attributable to this parasite in Karonga District.

b) To determine the extent of *W. bancrofti* transmission area in Karonga District

1.1.2 Objectives for the intervention trial

The objectives for the intervention trial were as follows:

a) To provide a detailed descriptive epidemiological account of the prevalence, intensity and multiple helminth infections and associated disease in Chikwawa District.

b) To evaluate the impact of one round of mass distribution of albendazole and ivermectin in combination on *W bancrofti* transmission.

c) To measure the "ancillary benefits" derived from mass drug administration (MDA) for the control of LF.

1.1.3 Thesis structure

Chapter 2 is a review of the relevant LF literature. **Chapter 3** describes the mapping and the results of the surveys. The outline and methods of the intervention trial are presented in **chapter 4**. Baseline results for the intervention trial are summarised in **chapter 5**. **Chapter 6** describes findings of surveys conducted one year post-intervention. **Chapter 7** discusses polyparasitism in the lower Shire Valley. **Chapter 8** outlines a study to measure the effect of rifampicin on LF antigenaemia. **Chapter 9** includes a discussion of the research findings, conclusions and suggested recommendations.

Indicator	1999- 2003
Total population	11.3 million
Infant mortality rate per 1,000 live births	104
Under five mortality rate per 1,000 live births	189
Maternal mortality rate per 100,000 live births	1,120
Crude birth rate per 1,000 population	46
Life expectancy at birth	36.3 years
Antenatal coverage (%)	91.4
Attendance at birth by a trained person (%)	55
% low birth weight babies	13.1
Children under five years chronically malnourished (%)	49
Children 12-23 months fully immunized (%)	70
Immunisation BCG (%)	89.2
Immunisation measles (%)	64.2
Population per physician	101,000
Public Health Expenditure (PPP/US$) Private/ Public	11

Source: (Malawi Ministry of Health, 2003)

Table 1.1: Selected Health Indicators for Malawi

Chapter 2

2.1 Literature review

Since the only species of human lymph dwelling filariae found in Africa including Malawi is *W. bancrofti,* this review is focussed accordingly. Where appropriate differences with *Brugia* species found in Asia are highlighted. The potential 'ancillary' benefits from controlling filariasis related to the impact of mass drug distribution on intestinal helminths are discussed in the last section of this chapter.

2.1.1 History of lymphatic filariasis

Records of disorders resembling those of lymphatic filariasis (LF) exist from the beginning of recorded human history. An Indian physician Sushruta in his medical textbook completed in about 70 AD refers to a condition called *slipada* (*sli* elephant; *pada* leg) (Rajan, 2000). Westerners came into contact with filariasis following the advent of colonialism during the 18th and 19th Centuries. A French physician Jean-Nicolus Dermaquay, based in Somme district in Cuba, is credited with one of the earliest Western descriptions of filariasis (Pan American Health Organisation, 2003). He is believed to have tapped a milky white fluid from a scrotum of a patient originally from Havana, Cuba, which he later examined microscopically and his subsequent sketches leave no doubt that he saw microfilaria in the fluid. These observations went unnoticed until Otto Wucherer in 1868 described a worm species from urine of patients with tropical haematuria in Brazil which he distinguished from *Bilharzia (Schistosoma)*

hematobium (Routh and Bhowmik, 1993). Thomas Lewis, in 1870, further described microfilariae in both urine and blood from Indian patients (Routh and Bhowmik, 1993). However, the most prominent figure in the history of LF is Sir Patrick Manson whose work in China led to the incrimination of mosquito as a vector. Manson also described the periodicity and sequestration of microfilariae in the deep vasculature especially of the lungs during the period when they are not in the peripheral blood (Manson, 1899) . However, the full life cycle was not determined until 1899 by Joseph Bancroft based in Australia (Bancrofti, 1877). The distinction between bancroftian and brugian filariasis was not made until 1958. This followed an observation by Brug that microfilariae from patients in North Sumatra had features which distinguished them from those that caused bancroftian filariasis (Buckley, 1960).

2.1.2 General biology and life cycle of human filarial parasites

The natural primary host for *W. bancrofti, B. malayi* and *B. timori* is *Homo sapiens. Brugia* species occasionally infect animals such as cats and leaf monkey, *Presbytis* though this zoonotic reservoir does not appear to be an important source of human infection (Kazura, 2002). Mongolian jirds can be infected in the laboratory and are used as experimental animals for studies in filariasis. The adult worms reside in the lymphatic vessels with preferential localisation in those of the extremities and male genetalia (Cross et al., 1979). The adult female *W. bancrofti* worm measures up to 100mm long while the male extends to 40mm. Those of *Brugia* species are only half of these dimensions

(Simonsen, 2003). Insemination occurs when there is coupling of the male and female adult worms. The resultant zygotes develop *in utero* over a period of three weeks (Scott, 2000). This ovoviviparous reproduction results in the release of fully formed sheathed first stage larvae called L1 or microfilaria. The sheath, a remnant of the egg shell, is a morphological feature that distinguishes L1 stages of *W. bancrofti* and *Brugia* from other human filariae such as *Onchocerca volvulus* and *Loa loa* (Scott, 2000). The microfilariae leave the lymphatic channels and pass into the blood stream presumably via the thoracic duct where they are available to be ingested by the vector during a blood meal. There is marked daily fluctuation in the density of microfilariae in the blood which runs in consort with the biting behaviour of the principal vectors in an area (Simonsen, 2003). Two forms of peridiocity are recognised; nocturnal where the density peaks around midnight and diurnal where the peak is during the day. Subperiodic forms of these exist where microfilariae are continuously present in the blood but concentrations are higher than average during the night or day respectively.

Microfilariae are ingested by the mosquito as part of a blood meal. Within a short period, they penetrate the midgut wall and begin their migration through the haemolymph to the flight muscles. In the flight muscle bundle the microfilariae undergo a series of what are believed to be chemically programmed developmental changes (Scott, 2000). First, they develop into a shorter "sausage" form which then undergoes two moults generating L2 and subsequently L3 forms. The L3 form then migrates from the thoracic muscles to

the head where they position themselves within the feeding structure from where they escape onto the skin of their human host during a blood meal. The L3s enter the human host presumably through the puncture site made by the mosquito during the blood meal. Though not entirely understood, they are believed to secrete proteases that allow penetration through the connective tissue into the host's afferent lymphatic channels. They then undergo a further moult generating L4 forms which develop into adult worms within six to twelve months. The complete life cycle is shown in Figure 2.1.

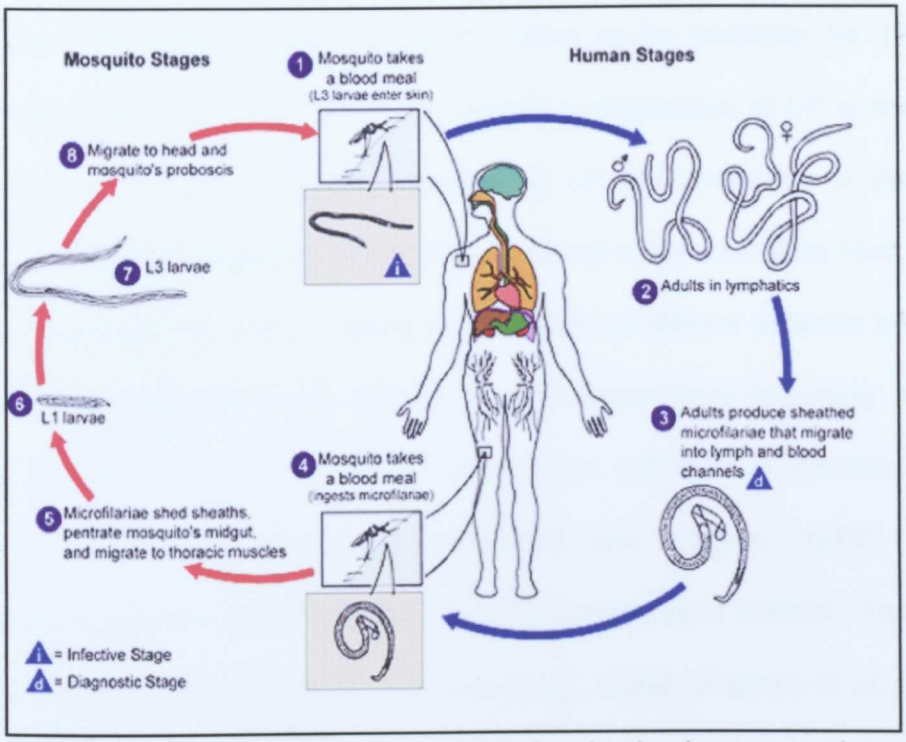


Figure 2.1: The life cycle of filarial nematodes in the human and mosquito hosts. *W. bancrofti*, *B. malayi*, and *B. timori* have a similar life-cycle. The female worm produces offspring, known as microfilariae, which leave the lymphatic system to enter the blood where they may be taken up by mosquitoes during a blood-meal. The microfilariae undergo about 14 days of development in the mosquito to become infective, third-stage larvae which migrate to the mosquito's mouthparts. These larvae may be transmitted to humans at the time the mosquito takes its next blood-meal. Once transmitted to humans, the larvae take approximately 6-12 months to mature into adult worms. The adult female has the capacity to produce several million microfilariae in its approximate 4-6 year reproductive lifespan (Source: www.dpd.cdc.gov/dpdx/HTML/Filariasis.htm).

2.1.3 The geographical distribution of LF

The worldwide distribution and countries known to be endemic for LF are shown in Figure 2.2 and Table 1.1. The regional distribution of LF is not fully described. Of note, rapid mapping surveys for LF are underway in Zambia, Zimbabwe and Mozambique (WHO/AFRO, personal communication from Dr L. Mubila). It appears that the LF focus in the north of Malawi extends into the Republic of Tanzania and that in the south into Mozambique (Hawking, 1977). Although sporadic case reports of LF have been reported in Zambia (Hira, 1976; Hira, 1977) and Zimbabwe (Goldsmid and Rogers, 1976), these countries seem not to have foci of intense transmission as in Malawi , Tanzania and Mozambique (Hawking, 1977; Ngwira et al., 2002; Nielsen et al., 2002; Pinhao, 1963).

2.1.4 LF distribution and its vectors in Malawi

Malawi has two previously known LF foci: one in the southern part (Shire valley) and the other in the northern region along the Songwe river which forms its border with Tanzania (Hawking, 1977; Oram, 1958). However there had been no detailed community based surveys for LF in Malawi apart from one in the northern focus which was conducted in 1960. This survey, based on microscopic examination (for microfilarae) of thick bloodsmears which were made from samples collected at night, showed a high prevalence of microfilaraemia amongst adults (40%) and suggested that human infection with *W. bancrofti* was confined to communities in close proximity to the Songwe River(Oram, 1960).

More recently, surveys in these two foci have reported high antigenaemia prevalence based on immunochromatographic (ICT) card tests that approached 80% in some of the sampled villages(Ngwira et al., 2002; Nielsen et al., 2002). There was also remarkably high prevalence of LF associated disease in both areas (4% lymphoedema and up to 18% hydrocele). In addition, the survey in Karonga established that *W. bancrofti* infection is more wide spread than previously known, whereas in the lower Shire valley a surprisingly high antigenaemia prevalence (55%) was found amongst children (aged 1-9 years) than has been reported anywhere else.

There has only been one entomological study aimed at identifying vectors for LF in Malawi. This study carried out in the southern foci determined the principal vectors to be *Anopheles gambiae s. str., A. arabiensis* and *A. funestus*. At the time of the survey there were relatively few *culex* than would be expected at other times of the year. Thus the role of this species as a vector of LF in southern Malawi remains to be determined.

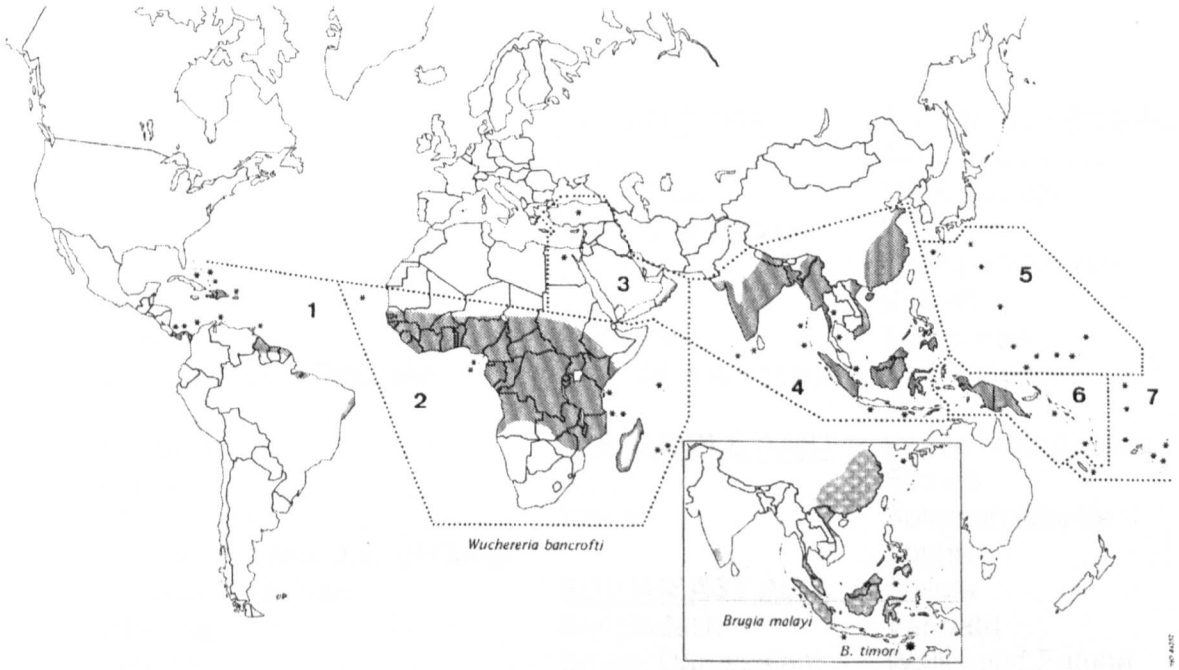


Figure 2.2: Map of the world showing the areas where bancroftian filariasis is endemic and, in the inset, where brugian filariasis is endemic. Large areas of endemicity are shaded but small foci are indicated with asterisks (*). Brugian filariasis is caused by *B. malayi* in all endemic countries, plus *B. timori* in Nusa Tenggara/ Timor Letse and the Indonesian Islands of (Alor, Flores and Timor islands). The zones 1-7 correspond to types of filariae and specific species of vectors; differences in mosquito species transmitting the parasites and the strain or species of parasite causing the disease account for epidemiological characteristics that are unique to each zone. Reprinted with permission from *Annals of Tropical Medicine and Parasitology* 96 (Suppl. 2), S3–S13 (2002).

AFRICA	THE AMERICAS	THE WESTERN PACIFIC
Angola	Brazil	American Samoa
Benin	Costa Rica	Cook Islands
Burkina Faso	Dominican Republic	Fiji
Burundi	Guyana	French Polynesia
Cameroon	Haiti	Kiribati
Cape Verde	Suriname	Micronesia
Central African Republic	Trinidad and Tobago	New Caledonia
Chad		Niue
Comoros	THE MIDDLE EAST	Papua New Guinea
Congo	Egypt	Samoa
Cote d'Ivoire	Yemen	Solomon Islands
Democratic Republic of Congo		Tonga
Equatorial Guinea	SOUTHEAST ASIA	Tuvala
Ethiopia	Bangladesh	Vanuatu
Gabon	Brunei Darussalam	Wallis and Futuna
Gambia	Cambodia	
Ghana	China	
Guinea	India	
Guinea- Bissau	Indonesia	
Kenya	Laos	
Liberia	Malaysia	
Madagascar	Maldives	
Malawi	Myanmar	
Mali	Nepal	
Mauritius	Phillipines	
Mozambique	Republic of Korean	
Niger	Sri Lanka	
Nigeria	Thailand	
Reunion	Vietman	
Rwanda		
Sao Tome and Principe		
Senegal		
Seychelles		
Sierra Leone		
Sudan		
Togo		
Uganda		
United Republic of Tanzania		
Zambia		
Zimbabwe		

Table 2.1: Countries with endemic filariasis adopted from (Anon, 2001)

2.1.5 Infection and disease

Accurate determination of an individual's infection and disease status for LF has been extraordinarily difficult. However, recent technological advances in immunological diagnosis and imaging have revolutionised our understanding of the pathophysiology for LF. Previously, the primary means of diagnosing active infection relied on the detection of bloodborne microfilariae, the progeny of fecund female worms. The parasitological techniques employed include:

2.1.5.1 Thick blood films

This technique uses a thick smear made from a finger prick blood sample. The smear is air-dried, stained with Giemsa (1: 10) for 30 minutes and examined under a microscope (McMahon et al., 1979a; Wamae, 1994). The staining allows the distinction of *W. bancrofti* microfilaria based on size, presence of the sheath and lack of nuclei in the tip of the tail from other human filarial species. If a measured volume of blood is used intensity of infection (per ml) can be determined. It is relatively cheap and requires minimal training for its use (Moulia-Pelat et al., 1992). However its major limitations are; 1) low sensitivity owing to small amounts of blood used and loss of microfilariae during staining procedures (Simonsen, 2003). 2) The technique is often inconvenient, as a blood sample needs to be obtained at night (in areas with nocturnal periodicity) to coincide with peak concentration of microfilaria, i.e. between 2100 hrs and 0200 hrs. This test can be modified by the administration of a low dose of diethylcarbamazine (DEC)

prior to drawing the sample (called DEC provocative day test). The DEC provokes microfilaria to leave the deep vasculature into the peripheral circulation and thus allows use of the test during the day (McMahon et al., 1979b). The contraindication of DEC in areas endemic for onchocerciasis limits the use of this test.

2.1.5.2 Knott's concentration technique

This test has been modified since its invention. One ml of anticoagulated venous blood is lysed in 10ml of 1-2% formalin or citrate-saponin solution depending on whether viable microfilaria are required (Melrose et al., 2000). The preparation is then centrifuged at 500 g for a minute. The resultant sediment is then examined under the microscope. The viscosity of the sediment may hamper microscopic examination of the sediment. Triton X-100 can be added to enhance visibility as it dissolves the proteingenous elements of the deposit (Melrose et al., 2000).

2.1.5.3 Counting chamber

The counting chamber technique is fast, quantitative and cheap (McMahon et al., 1979a). A 100 µl aliquot of blood is added to 0.9ml of 3% acetic-acid and examined in a counting chamber for microfilariae under low power of a compound microscope. This technique is ideal for routine hospital diagnosis as well as field surveys in areas where only one species of filaria is known to exist.

2.1.5.4 Membrane (Nucleopore) filtration technique

This technique involves filtration of a known volume of venous blood through a polycarbonate Nucleopore membrane (3 µm pore size) (Eberhard, 1991). This is followed by a large volume of pre-filtered water (35 ml) that lyses the red blood cells. The filter is then removed and placed on a glass slide for staining and subsequent examination. The staining allows species identification. In addition, this technique is highly sensitive and excellent for determining density of infection but due to the high cost of filters and the need for venous blood makes it impractical for field surveys (Desowitz et al., 1973).

2.1.5.5 The Quantitative Blood Count (QBC) technique

In this system heparin, EDTA and acridine orange are incorporated into a microhaematocrit tube (Freedman and Berry, 1992). Following centrifugation of the blood sample, the buffy coat interface is examined by fluorescence microscopy, where the acridine orange stained DNA of the nuclei within the microfilariae can be readily identified, and morphologic characteristics can be examined, allowing for differentiation of species (Bawden et al., 1994). Though this test is faster, its sensitivity is not superior to the thick blood film technique at low microfilarial densities.

2.1.5.6 Serological diagnosis

These tests described above do not allow for determination of whether microfilariae negative status is due to true absence of adult worms, infection with single sex or non-fecund worms or asymptomatic infection with microfilarial densities below limits of detection (More and Copeman, 1990). A further limitation is lack of the ability to accurately classify individuals according to their relative adult worm burden, as the precise correlation between microfilarial density and adult worm burden is unclear.

2.1.5.7 Circulating filarial antigen

The demonstration that parasite antigen is present in the circulation of infected individuals led to the development of two commercially available assays capable of detecting parasite antigen in whole blood or serum. The first to become available was the one that uses the monoclonal antibody Og4C3 marketed as TropBio-test and manufactured by TropBio Pty Ltd, Australia (More and Copeman, 1990). This is a semiquantitative sandwich enzyme-linked immunosorbent assay (ELISA) that recognises a major protein moiety of a phosphorylcholine-containing circulating antigen of adult *W. bancrofti* worms. This assay has been shown to have no cross-reactivity to *Brugia* species or other common gastrointestinal helminths (Simonsen and Dunyo, 1999). In high intensity microfilaraemia its sensitivity approaches 100%, but falls to less than 75% in those with low level microfilaraemia (Chanteau et al., 1994; Rocha et al., 1996). This test can also be used on filter paper blood samples but its sensitivity

has been reported to be as low as 50.3% when used on such preparations (Gyapong et al., 1998a). It has, however, a high specificity of between 99-100% (Chanteau et al., 1994; More and Copeman, 1990)

The second assay uses the monoclonal antibody AD12. This has been marketed as a rapid-format immunochromatographic card test (ICT), originally manufactured by ICT diagnostics (Balgowlah, New South Wales, Australia) and recently taken over by Binax, USA. The ICT utilises capillary as well as venous blood and thus is simple enough and can be used in the field at any time of day by people with minimum training (Weil et al., 1997). Results are available within ten minutes and the test has now become a method of choice in community surveys for rapid assessment of endemicity. Studies evaluating the performance of the ICT against thick films and filtration techniques have reported a sensitivity of 100%, specificity 96.3%, positive predictive value 70.7% and a negative predictive value of 100% (Phantana et al., 1999; Simonsen and Dunyo, 1999).

2.1.5.8 Molecular diagnosis

An alternative to the identification of the parasite, or parasite antigen is the identification of parasite DNA within the infected individual. The identification of non-coding sequences from *B. malayi* and *W. bancrofti* has enabled the development of DNA-based techniques for these parasites (Siridewa et al., 1994; Zhong et al., 1996). By incorporation of a 3' biotin on one PCR primer, and using an internal fluorescein-labelled probe, these PCR-based diagnostic methods

have subsequently been refined to allow for ELISA-based detection of PCR products (Nutman et al., 1994). In evaluation studies the method has been shown to be of equivalent or greater sensitivity compared to parasitological methods, detecting patent infection in almost all infected individuals (McCarthy et al., 1996). In addition the technique is able to detect cryptic infections. This technique can also be used on saliva and pathological specimens to detect parasite DNA (Abbasi et al., 1996).

2.1.5.9 Serodiagnosis using parasite extract

Although methods to detect humoral immune responses to filarial infection have been available for at least 60 years, the development of serodiagnostic assays of sufficient sensitivity and specificity has proven problematic (Ambroise-Thomas, 1974; Fairley, 1937). The major difficulty has been their poor specificity. There is extensive cross-reactivity in the sera of individuals infected with closely related helminths, and even some protozoal parasites (Maizels et al., 1985). Further, it is difficult to distinguish previous infection, or exposure to the parasite (aborted infection), from current active infection. Notably most residents of endemic regions are antibody positive (Ottesen et al., 1982). However, these assays may have a role in making a diagnosis as a negative result effectively excludes past or present infection. The anti-filarial IgG4 antibody, which is produced in abundance during active infection, has been found to be the most useful (Chanteau et al., 1995; Terhell et al., 1996). It has also been shown to be a good index of intensity and duration of infection in endemic populations (Mahanty et

al., 1994). In addition it correlates well with microfilariae counts (Maizels et al., 1995). Despite the limitations cited above the newly introduced dipstick incorporating IgG4 antibody shows promise as an epidemiological tool in *Brugia*-endemic areas (Rahmah et al., 1998; Rahmah et al., 2001). Another area that is showing diagnostic potential is the use of filarial-specific enzymes (such as filarial acetylcholinesterase) either as antigens for antibody assays or by the detection of the enzyme itself (Misra et al., 1993; Sharma et al., 1998).

2.1.5.10 Imaging techniques

The use of high-frequency ultrasound in conjunction with Doppler techniques have revolutionised our understanding of the location of adult worms in humans. These techniques have made it possible to visualise adult worms in the female breast, scrotum and spermatic cord (Amaral et al., 1994; Dreyer et al., 1996b; Noroes et al., 1996a). Live adult worms have a distinctive pattern of movement within the lymphatic channels (called "filarial dance sign") (Amaral et al., 1994). Adult worms seem to remain in a constant location referred to as a "nest" in the lymphatic channels (Dreyer et al., 1994). The loss of the "filarial dance sign" has been used to monitor the efficacy of anti-filarial chemotherapy (Dreyer et al., 1996a; Dreyer et al., 1995a; Dreyer et al., 1995c; Taylor et al., 2005).

2.1.6 The clinical manifestations of lymphatic filariasis

2.1.6.1 Historical perspectives

Previously, classification of disease manifestations in lymphatic filariasis has been based on two models, clinical and immunological (Ottesen, 1992). In the clinical model individuals were divided into asymptomatic, acute and chronic. Later an immunological model based on criteria used in other infectious diseases such as leprosy was adopted (Ottesen, 1992). In this model extreme poles were identified as being "asymptomatic microfilaraemic carriers" (believed to be relatively hyporesponsive to filarial antigens) and those with tropical pulmonary eosinophilia (TPE) (felt to be hyper-responsive to filarial antigens). There have been difficulties in adopting either model of clinical classification for several reasons. In endemic areas, it is not uncommon to see patients who have overlap syndromes. Traditionally it has been accepted that three groups of people will be found in a filarial-endemic area (Partono, 1987): 1) those who are exposed but with no evidence of disease- so called "endemic normals"; 2) those with "asymptomatic microfilaraemia"; 3) those with chronic disease such as lymphoedema, hydrocele and elephantiasis. People in all these groups can also suffer episodes of acute filarial disease. In some areas, notably in Asia, another manifestation of filariasis is reported- 'Tropical Pulmonary Eosinophilia' (TPE) (Ottesen, 1992).

In filariasis, 'endemic normal', refers to people who despite constant exposure to filariasis and circulating anti-filarial antibodies, are amicrofilaraemic and have no evidence of disease(Ottesen, 1989). Whether these people are truly immune to filariasis has recently been challenged. A number of studies have shown that many of the so called 'endemic normals' have a cryptic infection because they have circulating filarial antigen which is identical to that found in people with microfilaraemia and ultrasound examination has revealed that many men who are classified as such have adult worms in their scrotum (Weil et al., 1996) (Dreyer et al., 1996c) (Noroes et al., 1996a) (Faris et al., 1998).

'Asymptomatic microfilaraemia' is the most common manifestation of filariasis in endemic populations and has been reported even in children less than a year old (Witt and Ottesen, 2001). The prevalence of microfilaraemia increases with age and usually reaches a plateau between 20- 30 years of age; during the childbearing years the prevalence tends to be higher among males than females (Brabin, 1990). It is often regarded as a non-disease state as the infected individuals are unaware that their blood contains large numbers of microfilarae. This situation may persist for years without progression to overt clinical disease (Ottesen, 1992; Rajan, 2005). Recent studies, however, have shown that asymptomatic microfilarae is not a benign phase as considerable occult lymphatic, tissue and organ damage may be occurring (Freedman et al., 1994; Freedman et al., 1995b). Ultrasound has shown that approximately half of the asymptomatic microfilariae positive men have a adult worm nests in their scrotal

lymphatic channels: 'the filarial dance sign' (Amaral et al., 1994). Lymphoscintigraphy has also revealed profound changes in that the 'asymptomatic microfilaraemias' tend to have markedly dilated lymphatics with collateral channelling and increased lymph flow. In these individuals for several centimetres on either side of the worms the lymphatics are abnormally dilated but there is no evidence of an inflammatory response (Noroes et al., 1996b; Ottesen, 1993; Simonsen, 2003). In addition it has been recognised that as many as 40% of the asymptomatic microfilaraemias have haematuria and/ or proteinuria that suggests low-grade renal damage (Dreyer et al., 1992). These renal abnormalities seem to be associated with microfilaria as clearing them results in complete recovery of the renal function (Dreyer et al., 1992).

The acute clinical manifestations of filariasis are characterised by recurrent attacks of fever associated with inflammation of the lymph nodes (adenitis) and/or lymph vessels (lymphangitis) termed adenolymphangitis (ADL). Two distinct types of acute attacks are described in residents of endemic communities (Dreyer et al., 1999). Filarial acute attacks so called 'acute filarial lymphangitis' (AFL) and ADL secondary to bacterial and fungal infections. AFL are believed to be triggered by the death of adult filarial worms in the lymphatic channels (Dreyer et al., 1999). AFL are characterised by localised pain and redness centred on the location of the dead adult worm. In the lower or upper limbs the most common presentation is that of a cord-like structure associated with retrograde lymphangitis. In the scrotal area or breast it may present as a painful nodule

(Dreyer et al., 1999). Funiculo-epididymoorchitis is the usual feature of the disease involving the male genitalia. In this situation the scrotum is painful with testicular tenderness (Dreyer et al., 1999). These are usually subclinical with the affected individuals generally being afebrile with mild constitutional symptoms (if any) and having neither exofoliative dermatis nor oedema (Dreyer et al., 1999). Thus these are rarely reported and consequently comprise a small percentage (≈ 3%) of reported acute attacks (Dreyer et al., 1999). Treatment with diethylcarbamazine (DEC) may exacerbate AFL due to its macrofilaricidal action (Dreyer et al., 1999).

In contrast to the relatively mild AFLs, acute attacks secondary to bacterial or fungal infection are overt and more severe. They are now called acute dermatolymphangioadenitis (ADLA) as the cascade of events in the acute process seem to start in the skin and then spread along the lymphatics to the lymph nodes (Olszewski et al., 1997; Olszewski et al., 1999). The existence of adult worms in the lymphatic vessels induces dilatation and compromises flow of lymph. This process results in the accumulation and leakage of protein-rich interstitial fluid in the dependant areas such as the foot, leg and scrotal area (Olszewski et al., 1992). Skin lesions caused by fungal infections or injury provide portals of entry for bacteria that find a rich media in the fluid under the skin (Swoboda-Kopec et al., 2001). The clinical picture can include fevers, chills, myalgia, prostration and other manifestation of systemic bacteraemia. Oedematous inflammatory plaques clearly demarcated from normal skin are

usually seen. Occasionally vesicles, ulcers and hyperpigmentation may be present. These episodes can last up to a week with the oedema of the affected part taking longer to subside and often not regressing completely. This is commonly associated with the pathognomonic exfoliative dermatitis (skin peeling) (Dunyo et al., 1998; Olszewski et al., 1993). ADLA episodes recur at irregular intervals with the resulting oedema taking longer to resolve. With time the oedema never regresses, resulting in chronic lymphedema of the affected part of the body. Repeated episodes of ADLA have been shown to be important in the progression of the disease. A direct relationship between the frequency of ADLAs and the grade of lymphoedema has been observed (Pani et al., 1995). Individuals with severe oedema are more prone to recurrent episodes of candidiasis as their toes are closely apposed and the moisture in the interdigital spaces supports infection (Suma et al., 2002). Treatment with DEC neither affects the duration nor the frequency of ADLA episodes, since these episodes result from bacterial infections and past lymphatic vessel damage, and similarly disease progression is unaffected by treatment (Kerketta et al., 2005). Of note, local hygiene, prevention and treatment of entry lesions reduce the frequency of ADLAs and halt or reverse the progression of chronic lymphoedema (Dreyer et al., 1999).

2.1.6.2 Chronic manifestations of lymphatic filariasis

The chronic manifestations of filariasis include hydrocele, lymphoedema/ elephantiasis (Partono, 1987). They can affect the lower and/or upper limbs,

scrotum, vulva and breast. They rarely develop before the age of 15 years, and a small proportion of the infected individuals manifest these signs (Simonsen, 2003). This contrasts with the knowledge that new immigrants to endemic areas tend to develop chronic manifestations more often and sooner (sometimes within 1-2 years) than do the indigenous populations of endemic areas (Partono, 1987).

Hydrocele is the commonest chronic manifestation in areas endemic for bancroftian filariasis (Partono, 1987; Wamae et al., 1998). Its prevalence has been shown to be a relatively accurate index for the prevalence of *W. bancrofti* microfilaremia in endemic populations (Gyapong et al., 1998b). The tunica vaginalis, the membrane that surrounds testes, produces lubricating fluid that allows free movement of the testes within the scrotum. This fluid is normally drained by the lymphatic channels of the spermatic cord thus preventing its accumulation within the scrotum. In men who are infected with *W. bancrofti* , worm-induced damage of the spermatic cord lymphatics leads to build up of fluid due to inefficient drainage. When the adult worm in the intrascrotal lymphatics dies, these lymphatic channels may be blocked due to the body's inflammatory response to the disintegrating worm leading to rapid accumulation of fluid and consequently a hydrocele (Gyapong et al., 1998b). In the early stages the blockage may be temporary and thus drainage is re-established and they may temporary regress. However, a chronic hydrocele develops when the damage to the channels has become more extensive and permanent (Gyapong et al., 1998b).

Lymphedema/elephantiasis of the leg is the next common manifestation. Most surveys have reported a female preponderance (Wamae et al., 1998). Lymphedema/ elephantiasis of the scrotum and other parts of the genitalia also occur, although less frequently. With repeated ADLs, lymphedema progresses to elephantiasis. Generally in the initial stages, skin elasticity and thickness are normal with pitting oedema that reverses on elevating the leg. With time non-pitting oedema and loss of elasticity ensue. As the lymphatic dysfunction worsens, small pouches and vesicles that leak protein rich lymph fluid develop (Olszewski and Jamal, 1994). This promotes fungal colonisation and wart formation leading to a condition called 'mossy foot' (Olszewski et al., 1993).

Other conditions such as chyluria and chylocele occur less commonly (Kumaraswami, 2000). Chyluria occurs when the lymphatic channels in the pelvis of the kidney rupture and a communication with the urinary system is established. Typically the patient complains of passing 'milky urine'. Chylocele describes a condition where the intrascrotal lymphatic channels rupture releasing lymph into the scrotum and this may adversely affect the testes leading to infertility (Kumaraswami, 2000). In general the pathogenesis of chronic manifestations is poorly understood. For instance the reasons why relatively few individuals in endemic communities develop these signs are unknown. In addition, the geographical variation in prevalence of these manifestations has yet to be explained. For example up to 90% of men in coastal East Africa develop a

hydrocele by the age of 70, but elephantiasis is observed relatively rarely (Wamae et al., 1998). In contrast, hydrocele is less prevalent among Indian men- in Pondicherry, India, where 45% of men develop hydrocele by age 60- but lymphoedema is much more common (Pani et al., 1991). Admittedly these differencies may be spurious as they may result from differencies in case definitions and sampling methodology (e.g, the above mentioned East African studies did not include women).

In Malawi and Chikwawa District, in particular, up to 20% of men aged over 15 years have a hydrocele while as lymphoedema/elephantiasis is found in less than 3% of the adult population (Nielsen et al., 2002).

2.1.7 Treatment and control of W. bancrofti

In both bancroftian and brugian filariasis the mainstay of treatment for the past 50 years has been diethylcarbamazine citrate (DEC) (Ottesen, 1985). In the past a course of treatment lasted 12 to 14 days at 6mg/kg daily dosage. The microfilaricidal effect of DEC has been widely documented (Addiss and Dreyer, 1999). Microfilariae are usually rapidly cleared from the peripheral blood, though at high intensity of infection clearance may be delayed (Kimura et al., 1985). The 12 day course of DEC treatment has recently been challenged. Recent data suggest that single-dose treatment with 6 mg/kg of DEC has comparable macrofilaricidal and long-term microfilaricidal efficacy (Dreyer et al., 1995c). The 12-day course provides more rapid short-term microfilaricidal suppression but

when other factors are considered such as cost, convenience and patient compliance it seems reasonable to recommend a single-dose regimen (Ottesen, 2000).

The degree to which DEC kills adult worms has until recently been debatable because direct methods of monitoring adult worms have not been available. Evidence for adult worm death following DEC treatment have included prolonged suppression of microfilaraemia, development of local nodules and identification of degenerating worms in biopsies of these nodules (Addiss and Dreyer, 1999; Figueredo-Silva et al., 1996). Recently, investigators have used ultrasound to monitor the effect of DEC on adult worms *in vivo* (Dreyer et al., 1998). In the largest study to date, 31 men examined before treatment had 53 individual adult worm nests detected by ultrasound (Noroes et al., 1997). After treatment with DEC the filarial dance sign became undetectable in 22 (42%) of these nests. Thirty-nine percent of the men had cessation of the dance sign in all their previously detectable adult-worm nests. These observations have also been demonstrated in children from Haiti (Fox et al., 2005). These ultrasonographic findings, which confirm the variable macrofilariacidal effect of DEC, confirm the clinical observations that up to 45% of infected men develop scrotal nodules after a single 6mg/kg dose of DEC (Dreyer et al., 1995b). Side effects of DEC may be systemic (eg fever, headache, myalgia, malaise and haematuria) related to the death of microfilaria or localised (eg nodules, pain, adenitis and retrograde lymphangitis) suggesting an inflammatory reaction due to the death of the adult

worm at a particular anatomical site (Addiss and Dreyer, 1999). DEC is contraindicated in individuals co-infected with *O. volvulus* as it may lead to a worsening of ocular manifestation associated with onchocerciasis (Molyneux et al., 2003).

Several investigators have reported a decrease in the incidence of filarial adenolymphangitis following treatment with DEC (Simonsen et al., 1995). This would be consistent with current knowledge that true acute adenolymphangistis is caused by the death of the adult worm in the lymphatic channels. Thus, by killing a number of adult worms, DEC may reduce the number of subsequent acute attacks. The evidence that DEC reverses chronic manifestations of LF is contradictory. Using ultrasound, investigators in Brazil reported no change in lymphatic function in persons with lymphoedema 1 year after treatment with DEC (Freedman et al., 1995a). However, several observations of resolution of early stage hydrocele and lymphoedema have been reported following community-wide mass treatment with DEC (Dunyo and Simonsen, 2002; Meyrowitsch et al., 1996). Lack of control groups in these studies makes conclusive inferences difficult. Of note, there is agreement, though not universal, that DEC has no effect on longstanding lymphoedema or hydrocele. This is not surprising as the majority of these cases usually do not have active infections.

The second agent that is recommended for the treatment of *W. bancrofti* is a macrolide used in veterinary medicine called ivermectin. Ivermectin is a drug of

choice for the treatment and control of onchocerciasis (Ottesen and Campbell, 1994). It has also been shown to be a potent microfilaricidal agent against lymphatic filariasis. In a single 200- 400µg/kg dose it suppresses *W. bancrofti* microfilariae for periods of 6- 24 months (Kazura et al., 1993; Richards et al., 1991). A macrofilaricidal effect of ivermectin has been suggested but this has not been borne out by recent ultrasonographic investigations even at total doses of 4800 µg/kg over a period of 6 months (Dreyer et al., 1998; Dreyer et al., 1995c; Ismail et al., 1996). The systemic side effects for ivermectin are similar to DEC which include fever, headache, myalgia and malaise but there are no local side effects associated with death of the adult worms seen in DEC treatment (Cartel et al., 1991). However, serious adverse events have been reported where ivermectin is used for onchocerciasis control in *Loa loa* endemic areas (Twum-Danso, 2003a; Twum-Danso, 2003b). *L. loa* is a filarial worm that is transmitted by a tabanid fly of the genus *Chrysops* (Boussinesq and Gardon, 1997). Individuals with high intensity *Loa* microfilaraemia have an increased risk of developing *Loa* encephalopathy following ivermectin treatment (Boussinesq and Gardon, 1997; Twum-Danso, 2003a). *Loa* is endemic in west and central Africa and manifests as an eye worm or Calabar swelling (transient subcutaneous oedema) when adult worms migrate through the eye and cutaneous tissues.

The third agent licensed for the control of LF is albendazole. A broad spectrum benzimidazole carbamate with efficacy against a wide range of human and animal helminth parasites (Horton, 2000). The mechanism of action of

albendazole is still unclear. However, there is evidence that it disrupts cytoplasmic microtubule formation by inhibiting beta tubulin polymerase thus interfering with metabolism (Lacey et al., 1987). This contrasts with the mode of action of non-benzimidazole anthelminthics which act on the parasite neuromuscular pathways and paralyse them. Jayakody et al, (1993) conducted the first formal study on the effectiveness of albendazole in *W. bancrofti* infection. High dosages (400 mg twice daily) were used for three weeks on 15 microfilaraemic men and the results were compared with those of 12 other microfilaraemic men of comparable age and weight who had received DEC (6 mg/kg/day) for three weeks. Although clearance of microfilaria was more rapid with DEC but there was no significant difference between the groups at 3, 6 and 18 months post treatment. However, this dramatic microfilaricidal effect was associated with pain and inflammation of the scrotal sac in these men presumably induced by dying adult worms. Though these reactions are also seen following DEC treatment they discouraged further study of high dose albendazole treatment in LF.

The next phase in the control of LF saw the introduction of two-drug combinations. DEC or ivermectin in combination with albendazole given as a single dose. Single doses of albendazole (600 mg) given in combination with either ivermectin (400 µg/kg) or DEC (6 mg/kg), proved to have both long-term effectiveness and safety in decreasing microfilaraemia in *W. bancrofti* infections (Ismail et al., 1998). These findings were corroborated at the lower drug dosages

(albendazole 400 mg and ivermectin 200 µg/kg), commonly used in the treatment of intestinal helminths and onchocerciasis, respectively (Addiss et al., 1997; Ismail et al., 2001). Furthermore the addition of albendazole did not result in an increase in frequency of adverse reactions compared with DEC treatment alone (Ismail et al., 2001). In addition to the significant microfilaricidal activity induced by the two drug combinations, circulating filarial antigen levels, presumably reflecting the presence of viable adult worms, were seen to fall progressively. Of note the combination with DEC had consistently lower antigen levels than the one with ivermectin probably reflecting the superior macrofilaricidal effect of DEC (Ottesen et al., 1999).

These combinations are being evaluated in mass treatment programmes for the control of LF as explained above and some of the effectiveness trials have now published their results. In Africa, because of the co-endemicity of onchocerciasis with LF in several communities, DEC is not recommended for use. The combination of choice is therefore ivermectin and albendazole annually for up to six years. The effectiveness of albendazole in treating individuals with LF has recently been reviewed by the International Filariasis Review Group and published in the Cochrane Library (Addiss et al., 2005). Two trials met the inclusion criteria for the meta-analysis; one from Ghana and the other from Haiti (Beach et al., 1999; Dunyo et al., 2000). There was no difference when albendazole was compared to placebo both in Ghana and Haiti in reductions on either microfilaraemia or antigenaemia. A meta-analysis of these trials suggested

a statistically significant reduction in the prevalence of microfilaria when the analysis was restricted to microfilaria positive individuals at baseline, in favour of ivermectin. Beach et al (1999) reported that the combination of albendazole and ivermectin was better at reducing microfilaria loads than ivermectin alone after four months of follow-up, but in the Ghana trial the two regimen were not different at 12 months of follow-up. These inconsistent findings are probably due differences in time-points of evaluation, differences in evaluation techniques and also differences in dosages. These trials did not evaluate the effect of albendazole in mass drug administration campaigns and thus should be extrapolated with caution. Recently, a less stringent review also found limited evidence to support the use of albendazole in combination regimen in LF control programmes (Tisch et al., 2005). Despite this assertion the authors recognise the potential peripheral benefits of albendazole use such as broad spectrum anthelminthic action against co-occuring intestinal helminth infections, thus potentially enhancing the health benefits of filariasis control.

Of particular concern for any public health programme that relies on broad, community-wide drug treatment strategies is the potential for development of drug resistance. Resistance to benzimidazole (e.g. albendazole) anthelmintics and to ivermectin have arisen relatively easily in nematode parasites of animals when repeated mass treatment has been used to control nematode populations with little *refugia* from drug selection (Wolstenholme et al., 2004). To date resistance to albendazole or ivermectin has not been unequivocally

demonstrated in nematodes of humans (Schwab et al., 2005). This is because in contrast to animals, it is difficult to conduct controlled *in vivo* or *in vitro* studies of drug efficacy against filarial parasites in humans. However, there are reports of reduced parasitological and clinical responses to benzimidazole and ivermectin in nematode parastite of humans which could be indicative of drug resistance development and of genetic changes in *W. bancrofti* and *O. volvulus* (Awadzi et al., 2004; McCrathy, 2005; Schwab et al., 2005). In view of this, efforts are currently underway to develop genetic tools to monitor for albendazole and ivermectin selection in human filariae.

Despite the above mentioned promise accorded by the two drug combinations used in mass drug administration campaigns, there is broad recognition of the need for a macrofilaricide to enable programme closure. Recently the focus on the potential of antibiotics as adult worm sterilants has become a subject of intense investigations (Molyneux and Taylor, 2001; Taylor et al., 2005). This has come about due the realisation that *Wolbachia* endosymbionts are potential targets for a number of antibiotic compounds. In addition, *Wolbachia* have been shown to contribute to disease pathology and adverse reactions following treatment (Cross et al., 2001; Taylor et al., 2001). Thus, use of antibiotics to eliminate these endosymbionts offers a novel approach to the treatment of filarial nematodes (Hoerauf et al., 2000). Doxycycline, 100mg/day for 6 weeks, permanently sterilises *Onchocerca* adult female worms. Doxycycline seems to be effective against *Wolbachia* from *W. bancrofti.* Hoerauf et al, (2003a) using

quantitative PCR, were unable to detect any *Wolbachia ftsz* genes within four months of starting doxycycline therapy in 26 out of 29 cases and more importantly there was no microfilaraemia a year after starting therapy. This would be consistent with complete block of embryogenesis observed in onchocerciasis. Recently data published from a randomised double blind controlled trial from Tanzania has shown that doxycycline, 200 mg per day for 8 weeks, almost completely cleared microfilaraemia within 8- 14 months and that 22% versus 88% (in placebo group) still had adult worms detected by ultrasound at 14 months (Taylor et al., 2005).

2.1.8 Successful elimination programmes

Though none of the programmes using the newly available, optimal tools for lymphatic filariasis control/elimination has yet been in existence long enough to record complete success, even with the older less effective tools and strategies then available, elimination of lymphatic filariasis has already been achieved in certain areas. Active programmes based on DEC administration or vector control eliminated bancroftian filariasis in Japan in recent years, and similar programmes resulted in the elimination of LF from large parts of China, Malaysia, Korea and some islands of the Pacific (Sasa, 1976). Passively (probably as a result of improved population socio-economic status and sanitation) LF has disappeared from endemic areas of southeastern United States, northeastern Australia and possibly in a small foci in Benin (Myung et al., 1998).

2.1.9 Ancillary benefits

The soil- transmitted nematodes *Ascaris lumbricoides, Trichuris trichiura* and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) are among the commonest chronic infections of humans. Current estimates suggest that at least a quarter of the world's population, most of them living in developing countries, is infected with one or more species of these worms (Chan, 1997). Helminth infections have been associated with undernutrition, iron deficiency anaemia, growth stunting and impairment of learning (de Silva, 2003). These infections can interfere with appetite, decrease food absorption and cause intestinal blood loss (Stephenson et al., 2000b). The deficits reported in most studies can be divided into three broad areas: physical growth, iron status and cognitive function. Ascariasis, trichuriasis and hookworm have all been implicated in impaired physical growth in school children as measured by gain in weight, height, mid-upper arm circumference and skin-fold thickness.

A number of studies, conducted in South East Asia, Sub Saharan Africa, Latin America and the Caribbean, have examined the impact of anthelminthic treatment on physical growth. They recruited children between the age of 2 and 6 years. Individual parasite prevalences varied greatly but were generally not less than 50% in all study areas. The intervention study that has demonstrated highest impact on growth was carried out in Kenya, among school children who had very high prevalence of hookworm, trichuriasis and ascariasis (Stephenson et al., 1993a; Stephenson et al., 1989). At follow-up, 6 months after treatment

with a single dose of albendazole 400 mg (n= 78) or placebo (n= 72), the treated group had gained significantly more weight than the control group (1.3 kg) and also height (0.6 cm). The improvements were attributed more to the decrease in hookworm egg counts than the two other parasites. Two subsequent studies in the same population provided further evidence of improved growth after albendazole treatment of children with multiple helminth infection (Stephenson et al., 1993a; Stephenson et al., 1993b).

A much larger intervention trial with a 2 year follow-up period was carried in Myanmar, involving 1206 children between the ages of 2 and 12 years, in 21 villages with a high prevalence of *A. lumbricoides* (Thein et al., 1991b). Children in the intervention villages were given levamisole every 3 months, while children in the non-intervention villages were not given any anthelminthic treatment until the final survey. Significant increments in height were observed from the sixth month onwards, but weight gain became significant only at 24 months. By the end of the follow-up period, the height and weight gains per child were 0.65 cm and 0.93 kg respectively, in the treated group as compared with the control group.

The effect of removing *Ascaris* on the growth of schoolchildren was also studied in a highland Indian town in Guatemala (Watkins and Pollitt, 1996). Children between the ages of 7 and 10 years were randomised to receive either albendazole 400 mg (n= 116) or placebo (n=112) at baseline and 3 months. At the 6 month follow-up, the treatment group showed a small gain in weight (0.18

kg) compared to the placebo group, but no improvement in height or mid-arm circumference. A study carried out in Bangladesh examined the effect of 2-monthly mebendazole therapy on the growth of predominately *Ascaris* infected preschool children but failed to find any improvement in the treated group (n= 688) compared with the placebo group (n= 714) at 18 months (Rousham and Mascie-Taylor, 1994). The effect of treating asymptomatic mild-to-moderate trichuriasis was investigated in 407 Jamaican children aged 6- 12 years (Simeon et al., 1995a). The children were randomised to receive either albendazole (400 mg daily for 2 days) or placebo, at baseline, 3 and 6 months. In addition to *Trichuris*, 46% of the children also had *Ascaris*. At the six month follow-up, only children with lower *Trichuris* infection intensities at baseline had increased significantly in Body Mass Index (BMI) after treatment. Overall, treatment did not result in improvement of growth as measured by BMI and height for age.

The largest reported intervention trial to date has been carried as part of the school based de-worming programme in Zanzibar, where children have very high prevalence rates of hookworm, *T. trichiura* and *A. lumbricoides (Stoltzfus et al., 1997a)*. Children in four primary schools were given thrice-yearly mebendazole 500 mg (n= 1019), and children from another set of four schools received twice yearly mebendazole 500 mg (n= 990), and finally children in four other schools did not receive an anthelminthic (n= 1054). At the end of 12 month follow-up, children below the age of 10 years had gained more weight (0.27 kg) in the twice yearly group and significantly more height (0.3 cm) in the thrice yearly group

compared with the controls. The authors conclude that the deworming programme improved growth of the school children, especially those who were young and less stunted. Overall improvements were small but among non-stunted children, the dewormed children gained about 20% more weight than the control group.

The findings are evidently variable and may be influenced by several factors which include the age and sex mix of the study population, baseline nutritional status, the relative prevalence and intensity of the three types of nematodes, the choice of drugs and dosage used and the time period between intervention and final assessment. The majority of the studies have been carried out through primary schools but only a few have included pre-school and children who may have entered pubertal growth spurt. The Zanzibari study found that treatment improved growth of children under 10 years, whereas there were no improvements among the older children (Stoltzfus et al., 1997a). The same study found that among the older children, girls grew less than boys in response to deworming (Stoltzfus et al., 1997a). Only three studies included preschool children and two of these did not show significant improvements in growth after anthelminthic treatment. However, the Bangladesh study may have been confounded by a significant difference in the prevalence of giardiasis in the treatment and placebo groups at 18 month follow-up (Watkins and Pollitt, 1996). In the third study, however, significant improvements were observed in gains in both weight and height and the increments in the 2- 5 year age group were

similar to those in the 5-10 year group (Thein et al., 1991b). There was no correlation between the degree of malnutrition at the onset of the study and the incremental gains in height and weight after treatment in the Myanmar study (Thein et al., 1991b), but Zanzibari children who were less stunted appeared to benefit more in gains in both height and weight than those with severe stunting at the time of intervention (Stoltzfus et al., 1997a). Watkins and Pollit, (1996) suggest that a possible reason for the lower growth rates seen among their Guatemalan children when compared with children of similar age in the Myanmar study (Thein et al., 1991b) could be the higher prevalence of stunting (low height for age) among the Guatemalan children.

In communities where ascariasis is the predominant problem, it appears that improvements in physical growth are minimal after chemotherapy and repeated treatment over an extended period is required to show significant benefits. In Myanmar, although improvements in height were seen from 6 months onwards, a significant difference in weight gain was seen only at 24 months, after regular treatment with levamisole every 3 months (Thein et al., 1991b). In Guatemala, despite high intensities of ascariasis, only a small increment in weight gain was seen at 6 months after treatment with albendazole, with no significant improvement in height or mid-arm circumference (Watkins and Pollitt, 1996). The growth response to mebendazole therapy did not differ between Zanzibari children with and without *A. lumbricoides* infections at baseline (Stoltzfus et al., 1997a). It is possible, however, that the high prevalence rates of other helminth

infections and malaria in these children could have obscured any effects on growth. Several studies have demonstrated a relationship between the initial worm burden, and growth after treatment. Burmese children with higher *Ascaris* worm burdens were found to gain less in height and weight than children with lower worm burdens (Thein et al., 1991b). Similarly, among Jamaican children with trichuriasis, those with low infection intensity showed a significant improvement in body mass index after treatment whereas those with high intensities did not (Simeon et al., 1995a). The intensity of the three geohelminth infections at baseline were not found to be related to either weight or height gain in the Zanzibari study (Stoltzfus et al., 1997a).

Where ascariasis is the major helminth infection, the choice of anthelminthic is of little consequence since all the commonly used anthelminthics give good egg reduction rates (De Silva et al., 1997). However, it is of more importance in dealing with hookworm infections and trichuriasis. Although pyrantel, levamisole, mebendazole and albendazole are all effective against hookworm, in a single dose formulation, albendazole probably achieves the best reductions in worm burden (De Silva et al., 1997). For trichuriasis too, where the choice of drug is more limited, single dose albendazole is probably more effective than single dose mebendazole. This may be why the largest growth responses to anthelminthics have been reported in the Kenyan studies, where albendazole was used. Stoltzfus et al (1997a) suggest that the poorer control of hookworm and trichuriasis by mebendazole could be one of the reasons why their Zanzibar

study showed smaller growth responses than the Kenyan studies, despite similar epidemiology. While the most commonly used dosage for albendazole is a single dose of 400 mg, higher dosages such as 600, 800 and 1200 mg have also been tried, especially where trichuriasis is a particular problem. However, these higher doses appear to have little added benefit.

A factor that may explain the differences in observed gains in height and weight between studies could be the duration of the follow-up period. It is possible that anthelminthic therapy causes only a temporary growth spurt that is not sustained. Stoltzfus et al (1997a) postulate that schoolchildren may have multiple 'gates', growth inhibitors or missing growth factors that limit their rate of growth. Geohelminths may be one such growth inhibitor but when this burden is alleviated, the child grows at a normal rate only until bumping into the next 'gate'. Thus the growth effect seen over a long period (a year or more) may seem relatively small compared with results from shorter trials. It is suggested that deworming causes an immediate increase in weight gain that is not sustained beyond 6 months, whereas height gains occur gradually throughout the follow-up period. It has also been shown that appetite improves after treatment of *Ascaris* infection (Hadju et al., 1996) and this too would mediate improvements in growth rates after anthelminthic treatment (O'Lorcain and Holland, 2000).

Adult hookworms attach themselves to the mucosa of the upper small intestine, ingesting tissue and blood and changing their feeding site every 4- 6 hours

(Layrisse and Roche, 1964). Blood is primarily lost when it passes through the hookworm's intestinal tract and is subsequently expelled during feeding, but secondary loss also occurs from bleeding of the damaged mucosa. Thus, as with any other disease which causes chronic blood loss, hookworm infections invariably lead to iron-deficiency anaemia. The amount of blood loss is strongly correlated with the worm load and the faecal egg count (Albonico et al., 1998). The correlation is basically linear, with no evidence for a threshold in the relationship between hookworm infection intensity and intestinal blood loss (Stoltzfus et al., 1996). However, since the development of iron deficiency anaemia from hookworm depends on iron intake and iron stores in addition to the intensity and duration of infection, a threshold effect is sometimes observed in the development of anaemia. The threshold varies depending on the iron intake and stores in the affected community or population subgroup (Stoltzfus et al., 1996). In populations with poor iron status and iron stores to buffer the losses caused by hookworms, decline in haemoglobin levels may be apparent even at the lowest levels of hookworm infection intensity (Stoltzfus et al., 1997c). Several controlled trials have demonstrated a positive impact of anthelminthic treatment on the iron status of school children. Because deworming acts primarily by decreasing iron loss, in populations with iron-poor diets, the effect would be to halt or slow the decline in iron status associated with hookworm infection, rather than actually increasing iron stores and haemoglobin levels. Among Kenyan schoolboys with mixed infections of hookworm, *T. trichiura* and *A. lumbricoides*, haemoglobin concentrations dropped significantly by 6 g/l in the placebo group

(n= 26) at the 4 month follow-up, whereas they showed a non-significant decrease of 2 g/l in the group treated with a single dose of albendazole 600 mg (n= 27). The difference of 4 g/l between the two groups was statistically significant (Stephenson et al., 1993a). Similar results were found among Tanzanian schoolchildren infected with hookworm or *Trichuris* in addition to *S. haematobium*. At the 4 month follow-up, haemoglobin concentrations had dropped by 3.5 g/l in the placebo group (n= 123), whereas in the group treated with a single dose of albendazole 400 mg along with praziquantel (n= 127), haemoglobin levels fell significantly less, by only 1.1 g/l (Beasley et al., 1999). Among the Zanzibari schoolchildren who had very high baseline levels of hookworm and trichuriasis as well as malaria and a poor iron intake, the deworming programme had no impact on haemoglobin levels (Stoltzfus et al., 1998). Thrice yearly deworming with mebendazole 500 mg did however improve iron status as measured by protoporphyrin (a measure of iron-deficient erythropoiesis) and serum ferritin (a measure of iron stores) and markedly reduced the incidence of moderate to severe anaemia in children with heavier hookworm infections at baseline (Stoltzfus et al., 1998). Although the problem of iron deficiency is particularly prevalent and severe in pre-school children, and theoretical calculations suggest that hookworms could contribute to iron deficiency in preschoolers (Stoltzfus et al., 1997c), there are no reports on the effects of anthelminthic therapy on iron status in this age group in communities with endemic hookworm.

T. trichiura also causes blood loss but to a much lesser degree than hookworms (Stephenson et al., 2000a). Rarely it may take the form of gross haemorrhage (as a part of dysentery or of rectal prolapse), which would lead to a severe, sometimes life-threatening anaemia. The question of whether whipworms actually suck and ingest blood remains controversial. More commonly the blood loss is believed to be part of an exudation from the superficial lamina propria and damaged epithelium and to be proportional to the parasite burden (Bundy and Cooper, 1989). It has also been suggested that the anaemia seen in trichuriasis may be primarily due to a chronic reduction in food absorption and therefore iron intake, due to anorexia resulting from production of tumour necrosis factor (TNF) alpha (Stephenson et al., 2000a).

Among 658 Panamanian schoolchildren (6- 12 years of age), haemoglobin levels were found to be significantly lower in children with heavier *T. trichiura* infections (>5000 epg faeces, n= 14) (Robertson et al., 1992). The incidence of anaemia was significantly more in children with mixed *T. trichiura* and hookworm infections than in those with single infections. However, at follow-up, 12 months later, there was no significant improvement in haemoglobin levels in children who had lost hookworm or *Trichuris* infections during the preceding year, or in those who had a significant reduction in the intensity of infection. Ramdath et al, (1995) also found that Jamaican children with heavy infection intensities of over 10 000 epg faeces (n= 21) had significantly lower

haemoglobin levels, whereas those with lighter infections (n= 400), had no evidence of iron deficiency or anaemia. Mahendra Raj (1999), however, failed to find evidence of significant occult gastrointestinal bleeding in Malaysian children with heavy *Trichuris* infections and no dysenteric syndrome. Forrester et al (1998), also failed to find a significant association between intensity of infection and haemoglobin levels in their Mexican study population. On the other hand, significant increases in haemoglobin concentration were seen after 11 months when South African school children with moderate levels of trichuriasis and no hookworm, were given 4-monthly albendazole along with a daily cup of iron-fortified soup for 6 months (Kruger et al., 1996). However, the results are somewhat difficult to interpret, since trichuriasis levels fell in the placebo group as well as the albendazole groups.

Unlike ascariasis and trichuriasis, hookworm infection intensities often continue to rise in adulthood (Bradley et al., 1993). Thus, where hookworm infection is endemic, it is usually common in pregnant and lactating women, a group that is particularly vulnerable to anaemia. A study carried out among pregnant women living in the plains of Nepal found 74.2% of them to be infected with hookworms; 72.6% were anaemic, and hookworm infection intensity was the strongest predictor of iron status (Christian et al., 2004; Dreyfuss et al., 2000). Seventy-five percent of pregnant women attending an antenatal clinic in coastal Kenya were infected with hookworm and 76% of this study population were anaemic (Geissler et al., 1998). Hookworm egg output was a significant predictor of haemoglobin

status among the multigravidae but not primigravidae in this Kenyan antenatal population. Olsen et al (1998), found 70% of adult women in Western Kenya to be infected with hookworm; even light hookworm infections contributed significantly to low haemoglobin levels in this adult population. It has been estimated that at any given time, about 44 million pregnant women around the world are infected with hookworm (Bundy et al., 1995). In sub-Saharan Africa alone, about 7.5 million women are reckoned to be both infected and pregnant and probably a million of these are likely to harbour 40 or more hookworms and so be at risk of clinical disease, i.e. developing iron-deficiency anaemia (Crompton, 2000). Concern about safety of anthelminthic drugs for the foetus has limited efforts to control hookworm infection as a cause of iron deficiency anaemia in pregnant women. In Sri Lanka however, deworming in addition to iron-folate supplementation has been a routine part of public health antenatal care for many years (de Silva et al., 1999). A cross sectional study carried out against this background found that mebendazole therapy is safe during pregnancy as it is not associated with a significant increase in major congenital defects (de Silva et al., 1999). The effect of deworming in addition to iron-folate supplementation during pregnancy has been evaluated in three published studies. The first involved 195 pregnant tea plantation workers in Sri Lanka where hookworm is known to be prevalent (Atukorala et al., 1994). Women received intervention which was delivered by the public health service; the investigators did not control the intervention in any way. Thus it happened that some women received iron-folate tablets and some did not, but all the women

who received deworming (mebendazole 200 mg daily for 3 days) also received iron-folate tablets. Receiving the combination of mebendazole with iron-folate supplementation (n= 51) was more effective in improving the women's iron status during pregnancy than receiving iron-folate supplementation alone (n= 64), and resulted in a marked increase in haemoglobin and improved iron status as indicated by protoporphyrin and serum ferritin levels. A subsequent study conducted in Sierra Leone also measured the impact of a single dose albendazole with or without daily iron-folate supplements, on haemoglobin and serum ferritin concentrations during pregnancy (Torlesse and Hodges, 2000). At baseline (during the first trimester of pregnancy), the prevalences of hookworm and trichuriasis were 66.5% and 71.9%, respectively; 58.7% of the 125 women were anaemic and 21.2% had low serum ferritin levels indicative of depleted iron stores. The haemoglobin and serum ferritin concentrations of the women who received albendazole along with iron-folate supplements did not change significantly from baseline to the third trimester. However, among women who received either anthelminthic or iron-folate supplements alone, or who received neither, these values decreased significantly. After controlling for baseline haemoglobin concentration and season, the authors found that the mean benefit of anthelminthic therapy relative to the control was 6.6 g/l of Hb; the corresponding value for iron-folate supplements was 13.7 g/l. The effects of anthelminthic treatment and iron-folate supplements were additive. The third study conducted in Nepal assessed the impact of albendazole given in the second trimester and found a 59 g gain in birthweight of infants born to mothers

who had received two doses and similarly infant mortality fell by 41%. These gains were in addition to significant reductions in rates of moderate to severe anaemia amongst the treated group (Christian et al., 2004).

Thus, it appears that even in populations where iron intake is poor, if hookworm infections are prevalent, deworming alone can improve the iron status of the population, particularly by reducing the incidence of moderate to severe anaemia. The effect of deworming on the iron status of populations with trichuriasis and little or no hookworm is less clear. In any case, deworming alone seems not to be sufficient as an anaemia control strategy: Thus, increased iron must be provided through supplementation, fortification or an improved diet.

Many studies have reported an association between soil-transmitted nematode infections and impaired cognitive function and school performance, but reports of controlled intervention trials examining this relationship are few. Five of the eight reported studies have concentrated on trichuriasis in Jamaican children. Extremely severe infections of *T. trichiura* in the form of Trichuris Dysentery Syndrome (TDS) have an undoubted impact on cognitive function (Callender et al., 1992). Follow-up after 4 years showed that although these children showed catch-up growth in height, their intelligence quotients, school achievement and cognitive function remained significantly lower than those of controls (Callender et al., 1998). As many more children have mild to moderate trichuriasis, rather than heavy infections such as seen in TDS, it is important to determine the level at which detrimental effects occur. This may, however, vary from area to area

depending on other variables that also influence the detrimental effects measured. A clinical trial carried out in Jamaican children with moderate to heavy infections of trichuriasis found that children who received albendazole 400 mg daily for 3 days, improved more than the group receiving a placebo, in three of eight tests of cognitive function at follow up, 9 weeks later (Nokes et al., 1992). However the pattern of cognitive improvement was not consistent across specific functions and was therefore difficult to explain (Baddeley, 1992). A similar randomised clinical trial (using albendazole 400 mg on each of 2 days) was carried out in a larger sample of 289 Jamaican children with mild to moderate infections of *Trichuris* (Simeon et al., 1995b). At follow-up, 12 weeks later, the investigators failed to find a consistent improvement in cognitive function following anthelminthic treatment. However, children with evidence of undernutrition improved much more with treatment than the better nourished children in verbal fluency. In the same study population, followed up over a longer period of time, spelling and school attendance improved after treatment in the more heavily infected and shorter children, respectively (Simeon et al., 1995a). A third study carried out among Jamaican children with mild or moderate trichuriasis also failed to find significant improvements in cognitive function after albendazole therapy (Gardner et al., 1996). These findings suggest that *T. trichiura* infections of low to moderate intensity in children of adequate nutritional status are unlikely to have a substantial detrimental effect on cognitive function. Schoolchildren in Guatemala (infected with both *Ascaris* and *Trichuris*) also failed to show improvement in several tests of cognitive function and school

performance 6 months after treatment with repeated doses of albendazole (Watkins et al., 1996); and Malaysian school children with moderately high levels of ascariasis and trichuriasis did not show any improvement in school attendance 4 months after treatment with albendazole (Mahendra Raj et al., 1997). One study carried out among 6- 8 year olds in Jakarta, Indonesia, found that children with ascariasis improved in two of six tests of cognitive function 5 months after treatment with single dose mebendazole when compared with controls. However, children subjected to health education in addition to mebendazole treatment failed to show a similar improvement, and subsequent analyses showed that the treatment groups were not similarly matched in some important characteristics (Hadidjaja et al., 1998). Iron deficiency anaemia has been linked to lowered performance on development tests and school attainment but more research is needed to establish or reject the role of hookworms in this sphere of childhood development (Crompton, 2000).

In general assessment of changes in cognitive performance is difficult due to lack of appropriate tools and those available are standardised for children in developed countries and may not necessarily be applicable across cultures. In addition, malnutrition, anaemia and learning impairment are linked to poverty. This association is of vital importance in assessing effectiveness of helminth intervention programmes. In a recent Cochrane review, Dickson et al, (Dickson et al., 2000a; 2000b), did not find evidence for improvement in cognitive

performance in children treated with chemotherapeutic agents but did find a small effect on growth.

Public health programmes designed to decrease the prevalence of intestinal helminths have focused on two areas: 1) decreasing transmission through improved sanitation and handling of human waste. 2) Reducing human infections through drug treatment. The potential advantage of drug treatment is a rapid reduction in infections. The problem with this approach is that these effects may be temporary as the population is rapidly re-infected. In Japan, following the Second World War, an intensive drug treatment programme was implemented at the same time that sanitation conditions were being improved (Yokogawa, 1985). Although this strategy proved successful in controlling helminth infections, the programme was both time and resource intensive and spanned several decades.

As discussed above, albendazole is as effective and safe a drug for treating intestinal helminth infections (including hookworms) as any available, and it has effects against other parasities such as *Ascaris* and *Trichuris* as well. Therefore, its inclusion in two-drug treatment regimens for the control or elimination of LF might result in a public health impact far greater than the elimination of LF alone, especially because the filariasis elimination strategy calls for the community-wide treatment in endemic areas of all those 'at risk' of LF infection. A significant departure from the approach advocated by the Soil Transmitted Helminth Control Initiative of targeted bi-annual treatment in high prevalence communities (Hall et

al., 1997; World Health Organisation, 2004). Furthermore, when albendazole is used in combination with ivermectin, the public health benefit might be even broader as has recently been demonstrated in Haiti (Beach et al., 1999; De Rochars et al., 2004). Not only is ivermectin itself effective against many intestinal nematodes, strongloides and even ectoparasites, such as Tunga, scabies and lice (Heukelbach et al., 2004), it seems to synergise with albendazole such that the activity of the two drugs in combination against *Trichuris* is far greater than that of either drug alone (Beach et al., 1999; Lawrence et al., 2005). Extrapolation from earlier studies would not only predict improvements in anaemia levels and anthropometric (e.g. height and weight) measurements but also cognitive development amongst children and increased productivity (i.e. fewer work days lost) amongst adults.

Because many of these 'ancillary benefits' of albendazole and ivermectin can be directly perceived by individuals receiving treatment for filariasis, the compliance in these communities is likely to be appreciably enhanced. This would result in higher coverage rate which is predictably the most important factor in having a successful mass control/elimination programme.

Chapter 3

3.1 Materials and Methods

3.1.1 Background to the LF mapping methodology

In 1998, the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) initiated research to develop a method for the Rapid Geographical Assessment of Bancroftian Filariasis (RAGFIL) that could be suitable for large-scale application by control programmes. A workshop was held first to review spatial patterns of filariasis in sites for which detailed survey-information was available (World Health Organisation, 1998). On the basis of this review it was postulated that filariasis foci tend to be large with a diameter of at least 50 km. A rapid mapping method was proposed which used a spatial sampling grid with 50 km between sampled villages and spatial analysis techniques to estimate the distribution of filariasis. Grid sampling was proposed instead of random sampling as it would ensure better spatial coverage of the target area. A multi-country study was subsequently undertaken in Ghana, India, Myanmar and Tanzania to test the RAGFIL method in the field (Gyapong and Remme, 2001).

The first step in the RAGFIL method is to exclude areas where filariasis is unlikely to be found because of demographic and ecological features that are incompatible with the parasite, vector, and disease (e.g., uninhabited areas, deserts, national parks, high mountain ranges). For the remaining areas, a spatial

sample of villages is taken using a grid with 50 km between grid nodes in a north-south and east-west direction. Using Geographical Information System (GIS), the grid is overlayed on a map of the study area and the nearest village to each grid node is selected as a sample village. In the selected sample villages rapid assessment surveys (RAS) of the prevalence of *W. bancrofti* antigenaemia are done.

3.1.2 Sampling frame for Malawi

Malawi is administratively divided into northern, central and southern regions. These are further divided into 28 districts. Recent LF prevalence data were available for three districts; Karonga District in the northern region, Chikwawa and Nsanje Districts in the southern region. Thus the national survey did not cover these districts. In the remaining districts we aimed to sample a random selection of villages for antigen testing. A database of villages by district was made available via the WHO's HealthMapper software. A programme incorporated in the software was used to provide a random sample of villages to be surveyed shown in Figure 3.1. The selected villages had a 50km buffer zone as recommended by the WHO's RAGFIL method(Gyapong and Remme, 2001) (Gyapong et al., 2002). Two additional villages were chosen in the field in Mzimba District- northern Malawi from inhabited areas where the database did not contain any villages. The testing protocol adopted followed recommendations of the RAGFIL method that are based on Lot Quality Sampling (LQAS) scheme(Anon, 1999). Briefly, if at least 10 (20%) of the first 50 randomly selected

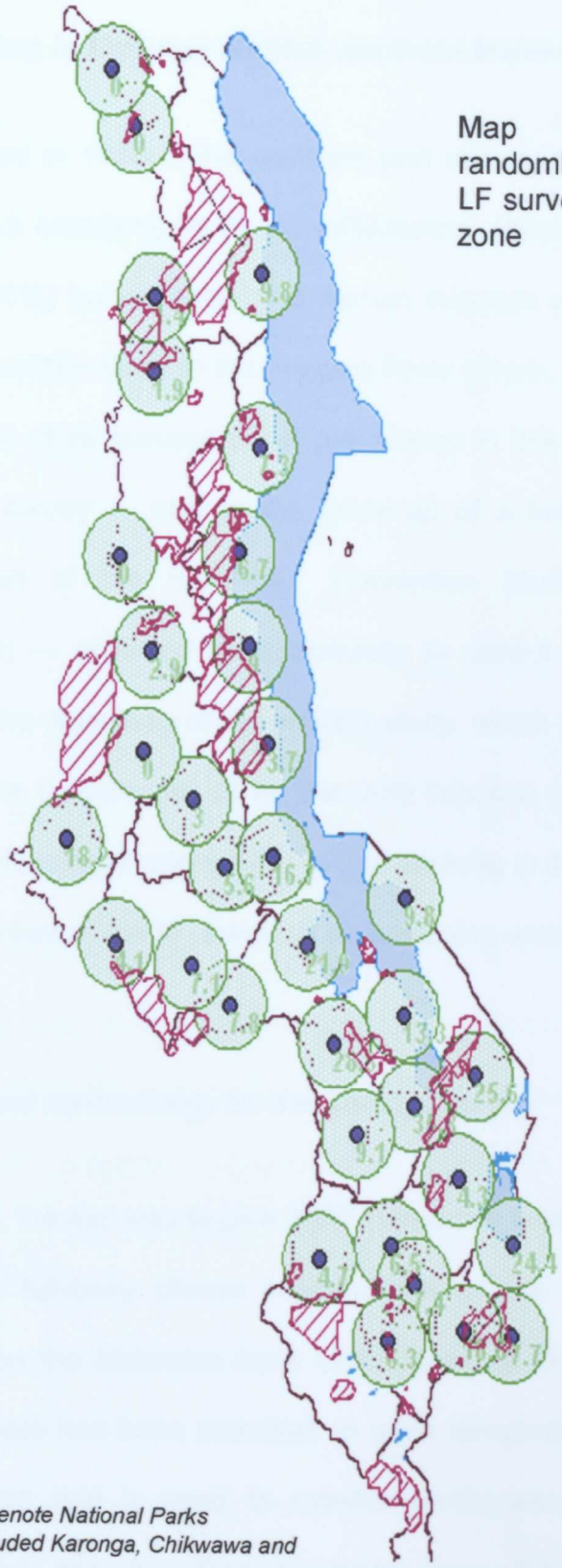
individuals (aged >15 years and resident in the village for >5 years) tested were positive testing could be stopped; otherwise up to 100 individuals were to be tested per sampling point (Anon, 1999). However since most villages are sparsely populated thus those adjacent to the selected one were also invited to participate in order to achieve the required sample size. Hence, random selection of subjects was not feasible in most villages. Before testing could be carried out a meeting with village members was held and the objectives of the survey were explained. Each consenting individual, who had been resident in the selected village for more than five years and was aged 15 years and above, provided demographic data (age and sex) and a finger prick blood sample. The whole blood obtained was immediately applied onto the ICT (Binax Inc., Portland, ME) card and read within ten minutes according to the manufacturer's instructions. If two lines appeared in the viewing window that particular individual was positive for *W. bancrofti* antigen (Weil et al., 1997). Individuals found positive were treated on the spot with albendazole (400 mg) and ivermectin (200µg/kg body weight). All sampled villages had geo-coordinates determined by a portable Geographical Positioning System (GPS- Garmin eTrex®) machine.

3.1.3 Ethics

The survey received ethical clearance from the Malawi Ministry of Health Sciences Research Committee (HSRC). Individual consent was obtained from each participant or (if they were aged <16) from one of their parents or a guardian.

3.1.4 Data management

Data were entered into the computer using EPINFO 2000 (CDC, Atlanta) software. The data were subsequently exported into STATA version 8 (Stata Corporation, College Station, TX) for statistical analysis. Village geographical coordinate data were used to produce a map showing the spatial distribution of LF infection using the WHO's HealthMapper software. Results for the survey are presented and discussed in **Chapter 4**.



Map of Malawi showing randomly selected villages for LF survey with their 50km buffer zone

- *Hatched areas denote National Parks*
- *This survey excluded Karonga, Chikwawa and Nsanje Districts*

Figure 3.1

3.1.5 LF mapping in Karonga district- northern Malawi

A survey conducted in 1960 in the northern part of Karonga district, based on examination of thick bloodsmears for microfilaraemia, showed a high prevalence amongst adults (40%) but indicated that human infection with *W. bancrofti* was restricted to communities close to the Songwe River (Oram, 1960). Unfortunately, there were then no other surveys of LF prevalence in this area for the next 40 years. A leprosy survey — part of the follow-up of a large trial of a leprosy vaccine, itself part of the Karonga Prevention Study (1996; Fine and Ponnighaus, 1988) — provided an opportunity to collect fresh data on LF in Karonga district. The objectives of the present study, which exploited this were 2-fold: to measure the prevalences of *W. bancrofti* infection (using ICT cards) and of the clinical manifestations attributable to this parasite in the Songwe area; and to determine the extent of the *W. bancrofti* transmission area in Karonga district.

3.1.5.1 Subjects and methodology for the Karonga survey

As part of the KPS, the aim was to give 3000 individuals living in the flood plain of the Songwe River full-body clinical examinations to see if they had leprosy. Adjacent villages on the Malawian bank of the Songwe River were sampled in turn until 3000 people had been recruited. In each sampled village, the list of all heads of household that is used to monitor participation on local self-help projects was obtained from the village headman. Every third household on each list was then visited and each member of these households present and

consenting was given a full-body clinical examination (for leprosy and the various manifestations of LF).

Lymphoedema (leg, arm and breast) and hydrocele were graded according to the recommendations of the World Health Organization's Expert Committee on filariasis (WHO, 1992). In four representative villages (chosen to cover all sections of the Songwe area), the prevalence of *W. bancrofti* antigenaemia was measured using an appropriate, rapid, commercial ICT: the AMRAD-ICT card test for filariasis (AMRAD Corporation, Richmond, Victoria, Australia) (Weil et al., 1997). A small sample of venous blood (<1 ml) was drawn from each consenting individual who lived in a selected household and was aged ≥ 20 years. Each sample was then applied to an ICT card and the result (positive/negative) read within ten minutes according to the manufacturer's instructions. A second reader at the field camp checked the result registered in the field on the same day. If the two results for one card were discordant, perhaps because of faint lines, the two card readers discussed the card until a consensus was reached. All ICT-positive individuals (except pregnant women and epileptics) were treated with albendazole (400 mg) and ivermectin (200 µg/kg body weight).

To determine the geographical extent of the *W. bancrofti* transmission area, ICT tests were also performed on adults aged ≥ 20 years in eight villages (selected so that they were 10–20 km apart) south of the Songwe area, using the same method to select households as used further north. The testing protocol adopted for this part of the survey was as described above.

3.1.5.2 Parasitological Examination

In order to confirm microscopically the existence of *W. bancrofti* in the study area, thick smears were prepared of finger prick samples of blood collected at night (21.00–02.00 hours) from 100 consenting subjects who were ICT-positive (and whom it was logistically possible to visit at night). These smears were fixed then stained with Giemsa's stain (1:10) for 30 minutes and then examined under the microscope at X40 magnification.

3.1.5.3 Data Management

All data were double-entered and verified at the KPS headquarters in Karonga, using Foxpro (version 2.6A; Microsoft), and subsequently analysed using Stata 6 software (Stata Corporation, College Station, TX).

3.1.6 Methodology for the cluster randomized trial in Chikwawa District-southern Malawi

The study was conducted in Chikwawa district in the Lower Shire Valley-southern Malawi. This is a rural area whose population is mainly engaged in subsistence farming. The main crops cultivated include maize, sorghum, sugar cane and cotton. This area lies between 100 and 300m above sea level. The rainy season extends from December to March. Temperatures can rise to as high as 50^0C in the months preceding the rainy season. Malaria is known to be holoendemic (Verhoeff, 2000).

3.1.6.1 Sample size calculation *and selection of villages*

Since the intervention was going to be introduced at village level, a cluster randomized study was planned. In determining the sample size the following factors were taken into consideration:

- As there was only going to be one round of treatment, the biggest impact was going to be on intensity of infection as measured by the mean egg output per gram of stool for intestinal helminthes and mean microfilarial load for *W. bancrofti*.
- With regard to anaemia a 30% reduction in prevelance was anticipated. This was used in determining the number of clusters required.

- The following formula, as recommended by Hayes et al, (2000), was used in calculating the number of clusters :

$$C = 1 + (z_{\alpha/2} + z_{\beta})^2 [\pi_0 (1 - \pi_0)/n + \pi_1 (1 - \pi_1)/n + k^2 (\pi_0^2 + \pi_1^2)] / (\pi_0 - \pi_1)^2$$

Where:

$c=$	*number of clusters*
$\pi_0=$	*prevalence in the control villages*
$\pi_1=$	*prevalence in the intervention villages*
$n=$	*Number of people sampled per cluster*
$k=$	*coefficient of variation of true proportions between clusters*
$(z_{\alpha/2} + z_{\beta})^2=$	*multiplying factor for power (7.9 for 80% and 10.5 for 90%)*

A baseline anaemia prevalence of 40% (+/- 10%) was expected based on previous studies in the area. For this calculation *k* was set at 0.13. Computing the above formula gave a total number of 9 clusters in each group for 80% power to detect a 30% difference in prevalence. This sample size was judged adequate to detect significant differences in intensity of infection for hookworm.

3.1.6.2 Selection of villages

Eighteen villages were selected for this study. In three of these, a survey mapping the distribution of LF had recently been completed (Nielsen et al.,

2002). Four of these villages were taking part in malaria related entomological studies aimed at mapping the genetic diversity of *Plasmodium falciparum* as well as a parallel LF vector incrimination study (Merelo-Lobo et al., 2003).

3.1.6.3 Baseline measurements

A baseline survey was undertaken in the 18 villages from December 2002 to May 2003. In each village a meeting to explain the aims and objectives of the survey in the local language was initially held with the respective chiefs and all village members. A total population census was then undertaken. Each village was given a two-digit code. The village code and a three-digit number determined a household number. Each household member was given a unique seven-digit identification number derived from the village code and household number and a consecutive two-digit number of household members in ascending order from the oldest member of the household. Ten percent of the households were subsequently randomly selected for baseline survey using random number tables.

3.1.6.4 Recruitment of participants

In the selected households all members aged one year and above were invited to participate. Consenting individuals had their demographic details completed and were then given a full body exam (except genitals for females) for chronic manifestations of LF. In addition they had anthropometric measurements taken

and were then asked to provide a single fresh stool and urine sample. All individuals (aged >1 year) were requested to provide a finger prick blood sample.

3.1.6.5 Anthropometric measurements

The height and weight for the participants were measured with them wearing light clothing and no shoes, according to procedures described by Jellife, (1966). Height was recorded to the nearest 0.1cm using a fixed base statiometer with a sliding headpiece parallel to the base (CMS Weighing Equipment, UK). The same statiometer was used throughout the study. Weight was measured to the nearest 0.1kg on a Soehnle electronic balance (CMS Weighing Equipment, Uk). The height-for-age (HAZ) and weight-for-age (WAZ) were expressed as Z-scores using EpiInfo 2000 software and the Centers for Disease Control (2000) reference values. Because EpiInfo will not calculate WAZ and HAZ for children older than 18 and those less than 3 years, this analysis was restricted to those aged 3- 18 years. Similarly, body mass index (BMI), which is the weight in kilograms divided by height in square meters was also calculated. Stunting and wasting were defined as HAZ and WAZ <- 2 s.d. from the reference median, respectively.

3.1.6.6 Chronic manifestation of LF

All participants were examined for clinical manifestations of LF. A hydrocele was present if there was collection of fluid in the tunica vaginalis with associated scrotal swelling of at least 6 cm (the longest axis) (see Figure 3.3).

Lymphoedema was diagnosed when there was limb (arm and/or leg) swelling of lymphatic origin (see Figure 3.5). This was graded according to the WHO's recommendation (World Health Organisation, 1992) as follows: Grade1: pitting oedema that is spontaneously reversible on elevation; Grade 2: non- pitting oedema that is non reversible on elevation; Grade 3: gross swelling with dermartosclerosis and papillomatous lesions. An 'acute attack' was diagnosed if there were constitutional symptoms such as fever associated with sudden painful increase in the swelling of the affected limb with exfoliative dermatitis (see Figure 3.4).

Figure 3.2: Showing Lake Lisuli (an ox-bow lake off the Shire River) and the Shire River in Chikwawa District

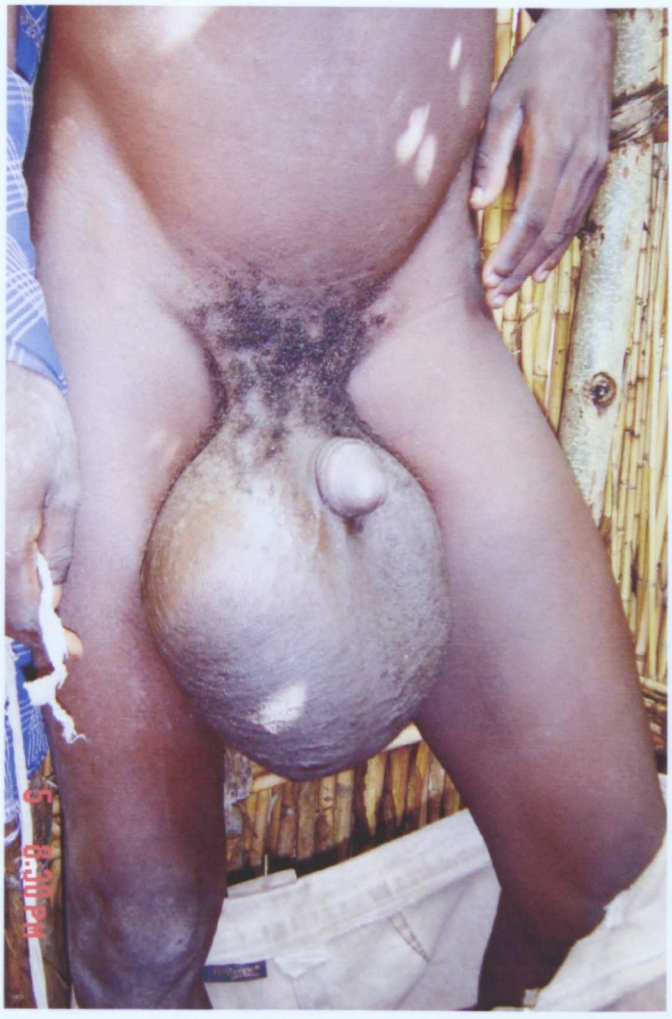


Figure 3.3: Showing a 17 year old boy with an advanced hydrocele in Chikwawa District- Traditional Authority Maseya

Figure 3.4: Showing a 50 year old lady with lymphoedema of the left arm and a resolving episode of 'acute attack' displaying the pathognomonic exfoliative dermatitis.

Figure 3.5: Showing a lady in Chikwawa District- Traditional Authority Maseya, with elepahntiasis of the left leg displaying typical skin folds associated with this stage of the disease.

3.1.7 Laboratory procedures

3.1.7.1 Stool samples

Fresh stool samples were transported in a cool box to the laboratory at Montfort Hospital and processed within four hours of collection. A single Kato- Katz thick smear was prepared from each sample and immediately examined under a light microscope for hookworm eggs (within 15- 20 minutes) and subsequently for other enteric parasites by an experienced technician. Standardised and quality controlled procedures were followed. Briefly 41.7 mg of sieved stool was placed on a microscope slide through a punched plastic template (Katz et al., 1972). Ova for each parasite observed were counted and expressed as eggs/gram (epg) of stool. Five percent of the slides were randomly selected for re-examination for quality control purposes.

3.1.7.2 Urine samples

Urine samples were processed on the day of collection. A measured volume (maximum 10 ml) was centrifuged at 300 rpm for five minutes. An experienced technician then examined the sediment under a light microscope. The number of eggs seen was counted and the intensity of infection per 10 ml of urine accordingly determined. All those infected were treated with praziquantel at 40 mg/kg.

3.1.7.3 Haemoglobin measurement

Haemoglobin (Hb) was determined on the spot from a finger prick blood sample by a portable HaemoCue (HaemoCue AB, Angelhom, Sweden). Anaemia was present in those individuals who had a Hb <11g/dl. A Hb of <8g/dl was classified as moderate to severe anaemia (MSA).

3.1.7.4 Malaria parasitaemia

A thick blood film was prepared in the field from a finger prick blood sample, air-dried and transported to the laboratory where it was stained with Giemsa then examined under a light microscope at high magnification by an experienced laboratory technician. *Plasmodium falciparum* infection density was determined by counting the number of asexual parasites per 200 leukocytes (WBC) and assuming as standard a white cell count of 8000/μL of blood (Trape, 1985). Slides were considered negative if no parasites were found after examining 100 high-powered fields. Ten percent of the slides, randomly selected, were re-read at the Welcome Trust Malaria Research Laboratory for quality control.

3.1.7.5 Immunochromatographic (ICT) card test

A hundred micro-liters of whole blood obtained from a finger prick from each individual was applied on to a sample pad on an ICT card (Binax). The pad is impregnated with dried colloidal gold-labelled polyclonal antifilarial antibodies that bind to adult worm filarial antigen in the blood sample. Once the card is closed and a gentle squeeze applied, the antibody-antigen complexes rise via capillary

action on the test strip. The nitro-cellulose test strip has a monoclonal antibody (AD12.1) traps the antibody-antigen complexes, as well as, unbound antigen. If an individual is positive for filarial antigen the complexes form a pink line next to a control line which is present on all valid cards. The results were read within ten minutes according to the manufacturer's instructions. Results (positive or negative as previously discussed) were immediately recorded on a results form for each individual.

3.1.7.6 Night blood sampling

In a selected village night blood survey were carried out between 21 hours and 1 am to coincide with peak blood microfilarial concentration. A hundred microlitre of blood was added to nine hundred microliters of 3% acetic acid. The acetic acid haemolysed the red blood cells and also acted as a fixative/preservative for the microfilaria. The samples were later examined in a one ml Sedgewick graduated counting chamber under the microscope. Microfilaria identified were counted and a density of infection (per ml) of blood was determined by multiplying the number of microfilaria seen by ten.

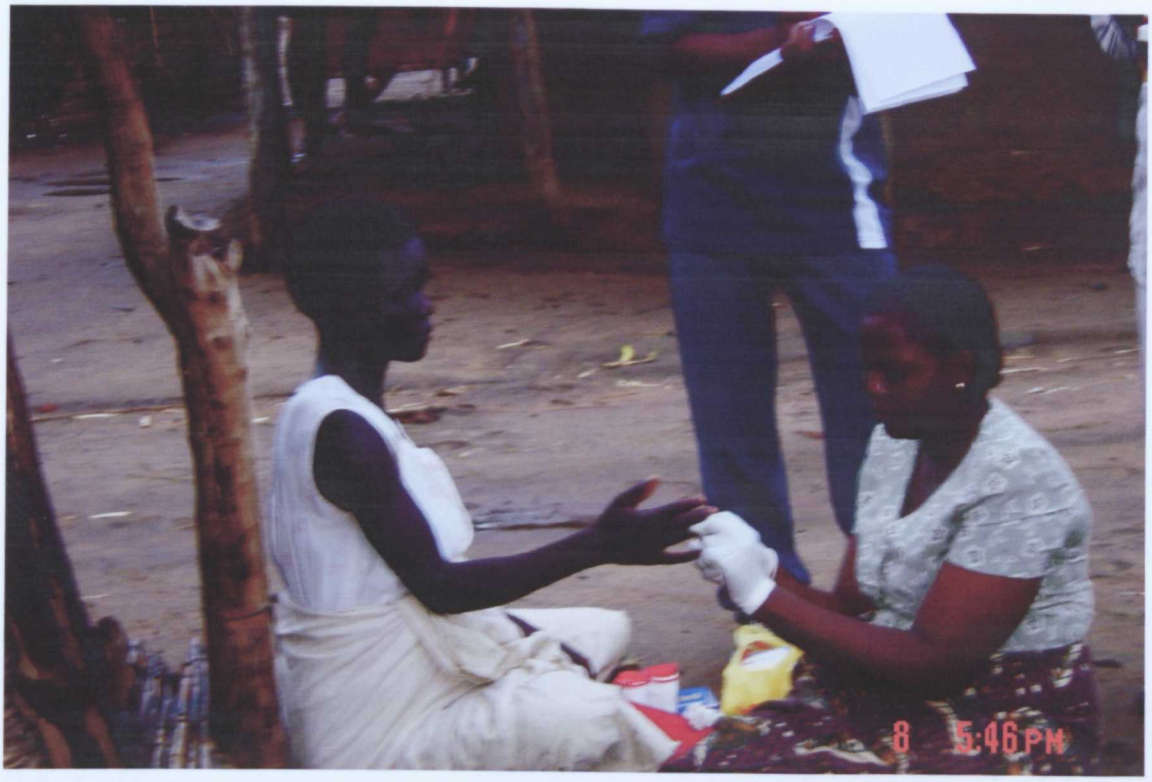


Figure 3.6: Finger prick blood sample collection for Hb, malaria parasite and ICT testing in Chipula Village- Traditional Authority Kasisi.

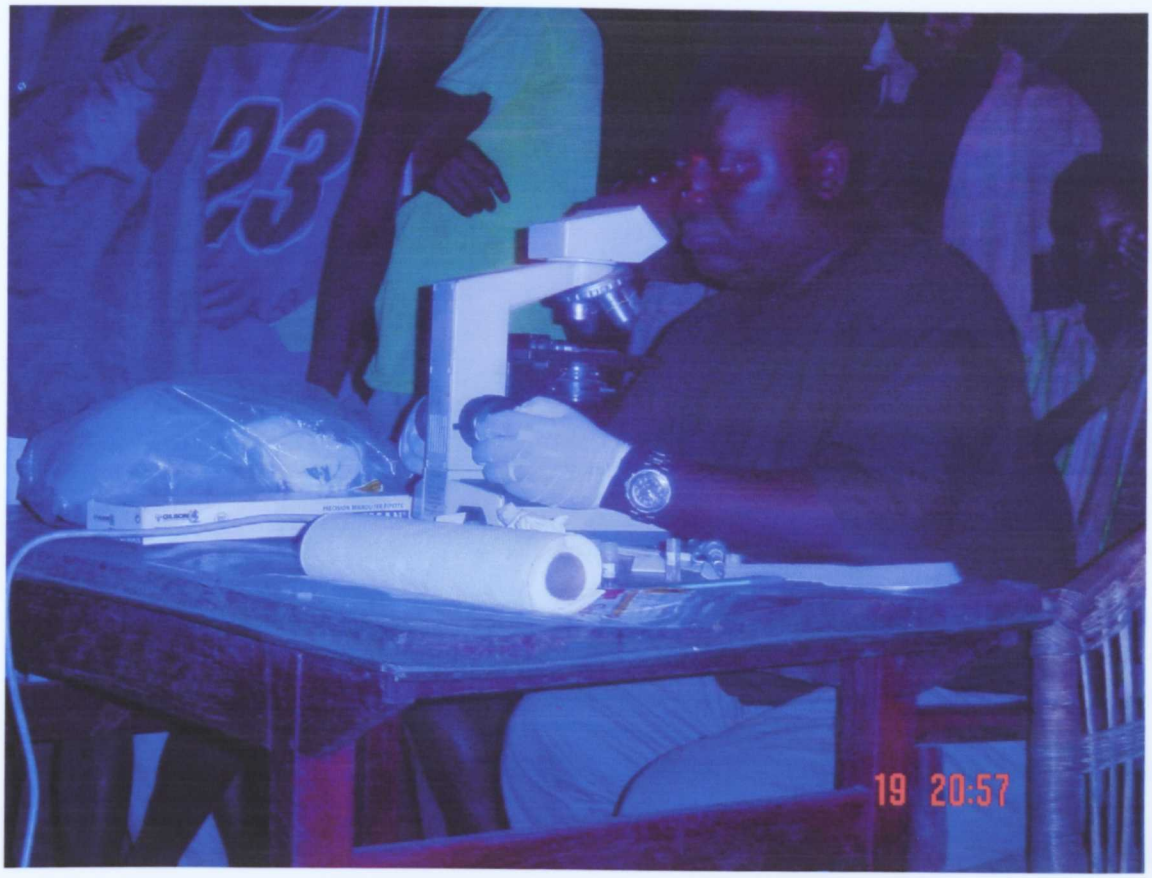


Figure 3.7: Dr Bagrey Ngwira examining night blood samples for microfilaria using a Sedgewick counting chamber in Mbande Village- Traditional Authority Maseya.

3.1.8 Data management and analysis

Data were double entered and validated using Epinfo 2002 (CDC, Atlanta) and exported into Stata version 8 (Stata Corporation, College Station, TX) for statistical analyses. Where appropriate differences in proportion of those anaemic between groups of categorical variables (sex, hookworm infection, *Schistosoma. mansoni*, *Ascaris*, malaria parasitaemia and history of fever) were assessed using the Pearson's chi-square test. Differences in means for haemoglobin between groups were examined using the unpaired Student t test. Logistic regression models were developed in a step-wise manner to estimate the effect on being anaemic of the following explanatory variables: age (categorical), sex, hookworm, *S. mansoni*, *Ascaris*, malaria parasitaemia, history of fever and village. Likelihood-ratio tests were used to examine the effect of each independent variable having adjusted for the other covariates. Variables with a $p<0.05$ were included in the final model. Where significant interaction was observed stratum-specific odds ratios are reported.

3.1.9 Ethics

Ethical clearance for this study was provided by the Malawi College of Medicine Research Ethics Committee (COMREC) and the Ethics Committee of the Liverpool School of Tropical Medicine.

3.1.10 Treatment allocation

Villages were randomly selected for treatment by selecting numbered pieces of paper from a plastic bag. The list of villages and their treatment allocation is presented in Table 3.1. The geographical location of intervention and control village is shown in Figure 3.9. The intervention villages underwent the first round of mass treatment with albendazole and ivermectin in May 2003. The dosing schedule that was followed based on height (determined by a drug pole- see Figure 3.8) is outlined in Table 3.2. Pregnant, lactating (1^{st} 3 weeks), epileptic and underfive individuals were excluded from treatment. Also excluded were individuals with a serious intercurrent illness.

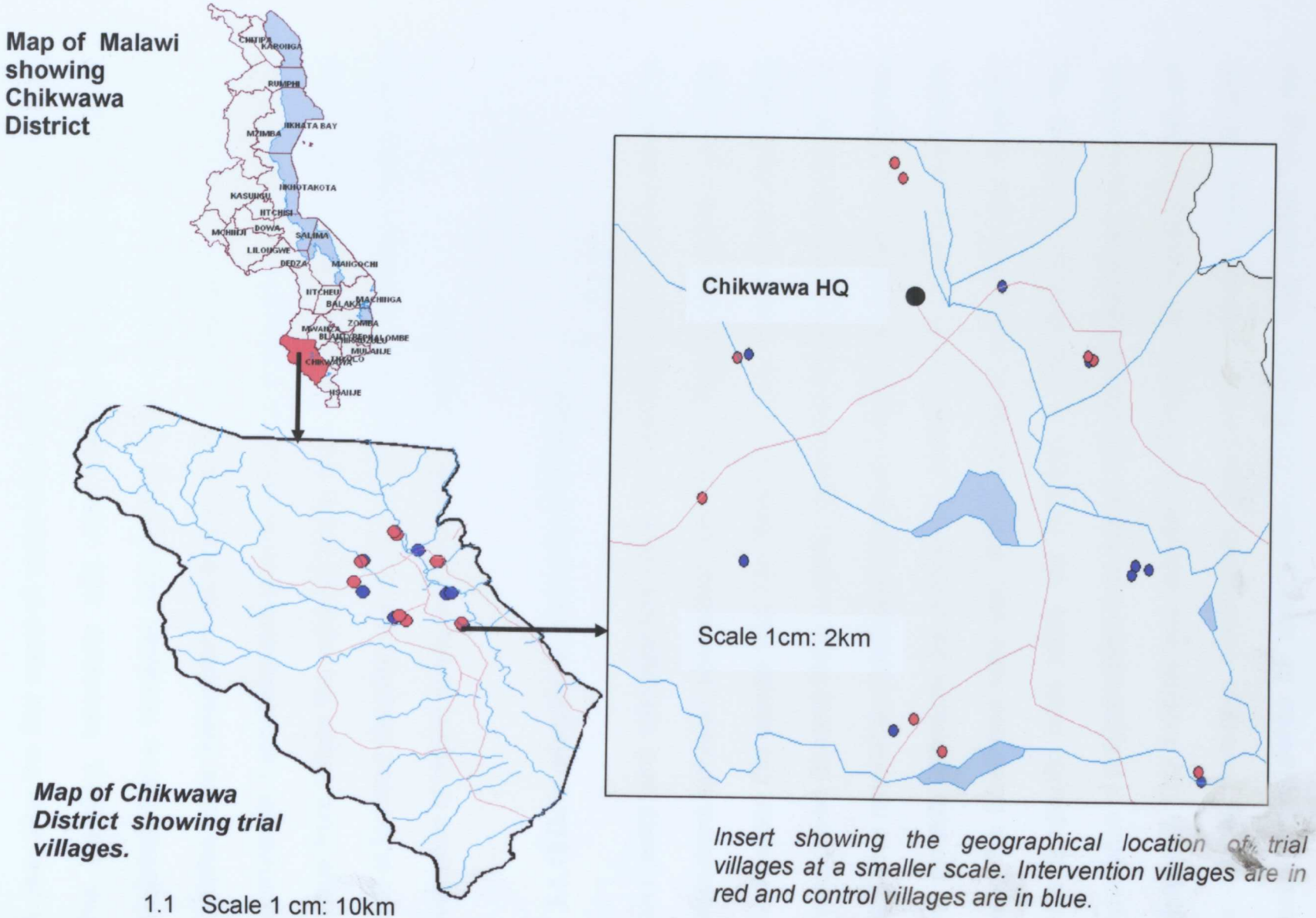
Figure 3.8: Showing a drug distributor determining the required dosage using a drug pole

Village Code	Village Name	Traditional Authority	Population	Treatment
01	Belo	Mulilima	992	Control
02	Kela	Mulilima	241	Intervention
03	Nkhwitcho	Mulilima	893	Control
04	Mvula	Mulilima	203	Intervention
05	Chipula	Kasisi	224	Intervention
06	Kadzumba	Maseya	678	Control
07	Nenenji	Maseya	586	Control
08	Sekela	Kasisi	214	Intervention
09	Nkata	Maseya	846	Control
10	Misili 1	Maseya	192	Control
11	Misili 2	Maseya	297	Intervention
12	Mbande	Maseya	978	Intervention
13	Andireya	Maseya	865	Intervention
14	Ng'ombe	Maseya	663	Control
15	Nyamphota	Katunga	774	Control
16	Chikalumpha	Katunga	563	Intervention
17	Pende1	Kasisi	344	Control
18	Pende 2	Kasisi	449	Intervention

Table 3.1: A list of villages with their population and treatment allocation.

Height (cm)	Ivermectin tablets	Albendazole tablets
< 90	0	0
90- 119	1	1
120- 140	2	1
141- 158	3	1
> 158	4	1

Table 3.2: Dosing schedule based on height for albendazole and ivermectin used in the MDA



Map of Chikwawa District showing trial villages.

Insert showing the geographical location of trial villages at a smaller scale. Intervention villages are in red and control villages are in blue.

Figure 3.9

3.1.11 Evaluation of the impact of mass treatment

A year after the first round of mass treatment a repeat survey was carried. The sampling, laboratory and data management procedures were as described above. However, following the death of the main technician, stool samples were transported on a daily basis to a laboratory in Blantyre for examination. Laboratory staff were not aware of the treatment allocation of the village from where the samples came from. Urine and malaria slides were read at Montfort Hospital by a newly recruited senior technician. Results of the evaluation of impact survey are presented in **Chapter 6**.

3.1.12 Data analysis for evaluation of impact

Data entry and management were as described above. Comparisons in proportions between intervention and control villages were carried out using the Pearson Chi square test. For continuous data, means were tested using the unpaired Student t-test. Logistic regression models were developed in a step-wise manner to investigate significant explanatory variables for anaemia and parasite infections (eg hookworm). Models were tested for significance using the Likelihood ratio test. Where significant interaction was observed stratum specific odds ratios are reported. A sub-analysis to explore the prevalence of polyparasitism was carried out and the findings are presented in **Chapter 7**. This analysis was restricted to individuals with a complete data set for all the parasites investigated. Comparison between expected and observed prevalence for each parasite combination was carried using the Pearson chi square test. Logistic regression models for multivariate analysis

were set up for co-infections of interest where individuals with such infections were defined as cases. A p- value of 0.05 was considered significant.

Chapter 4

4.1 Results of the mapping surveys

As pointed in Chapter 1, Malawi has two previously known LF foci: one in the southern part (Shire valley) and the other in the northern region along the Songwe river which forms its border with Tanzania (Hawking, 1977; Oram, 1958). However there had been no detailed community based surveys for LF in Malawi apart from one in the northern focus which was conducted in 1960. This survey, based on microscopic examination (for microfilaria) of thick blood smears which were made from samples collected at night, showed a high prevalence of microfilaraemia amongst adults (40%) and suggested that human infection with *W. bancrofti* was confined to communities in close proximity to the Songwe River (Oram, 1960).

More recently, surveys in these two foci have reported high antigenaemia prevalence based on immunochromatographic (ICT) card tests that approached 80% in some of the sampled villages (Ngwira et al., 2002; Nielsen et al., 2002). There was also remarkably high prevalence of LF associated disease in both areas (4% lymphoedema and up to 18% hydrocele). In addition, the survey in Karonga established that *W. bancrofti* infection is more wide spread than previously known, whereas in the lower Shire valley a surprisingly high antigenaemia prevalence (55%) was found amongst children (aged 1-9 years) than has been reported anywhere else.

Towards the end of 2003, a nation-wide mapping exercise using ICT cards was completed. The main objective was to obtain baseline data on the geographical distribution of LF in the remaining districts in Malawi as a

prerequisite to initiating national LF elimination activities. This chapter presents findings from the 2003 survey and in addition it incorporates data from recent surveys in the two known foci that have already appeared in the scientific literature to produce, for the first time, a complete map of the distribution of LF infection (based on adult worm antigenaemia) in Malawi. The implications of this distribution for LF control programme planning and eventual implementation are discussed in **Chapter 9**.

4.1.1 National LF mapping survey results

A total of 35 data points were sampled. Of these, three were chosen in the field in inhabited areas where there were no villages on the Healthmapper database. A total of 2913 individuals were examined. The age and sex distribution of the survey participants is shown in Figure 4.1. There was a female excess (64%) amongst the study participants (more marked in the 20-24 age bracket). Overall, there were 269 (9.2%) individuals positive for circulating filarial antigen (CFA) based on ICT results. Significantly more males than females tested positive (11.0% vs 8.2% p=0.01). Figure 4.2 shows the proportion of those positive for CFA by age and sex. Amongst the males, those positive, tended to be older (student-*t* test p=0.08). This relationship was not observed in their female counterparts.

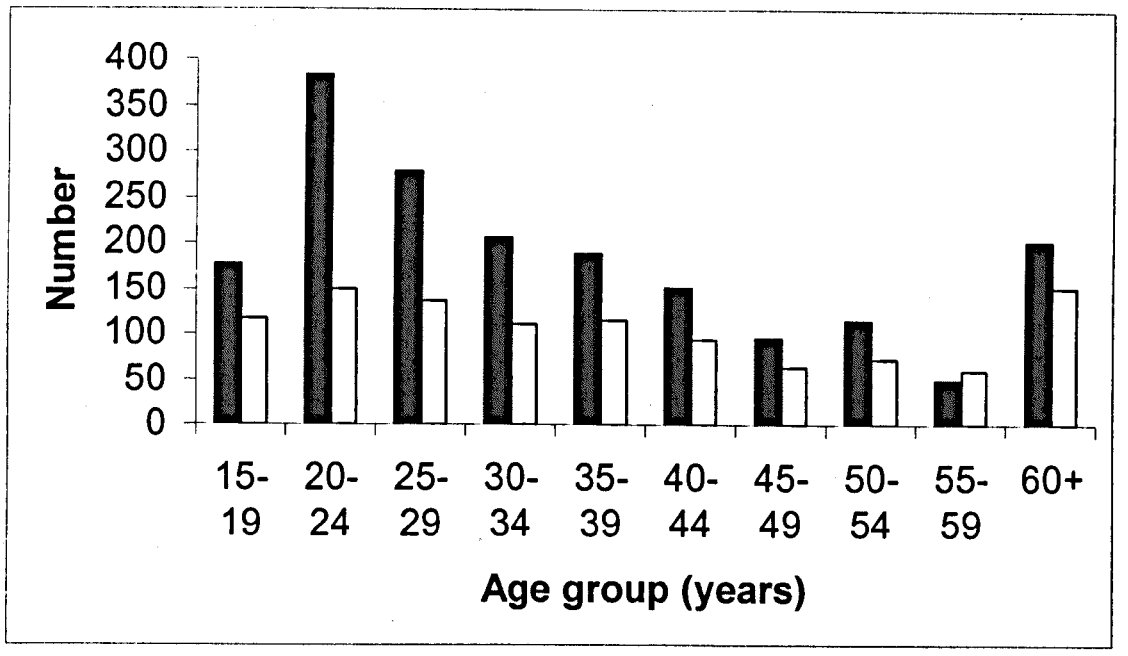


Figure 4.1: The age and sex [female (■) and male (□)] distribution of national survey participants.

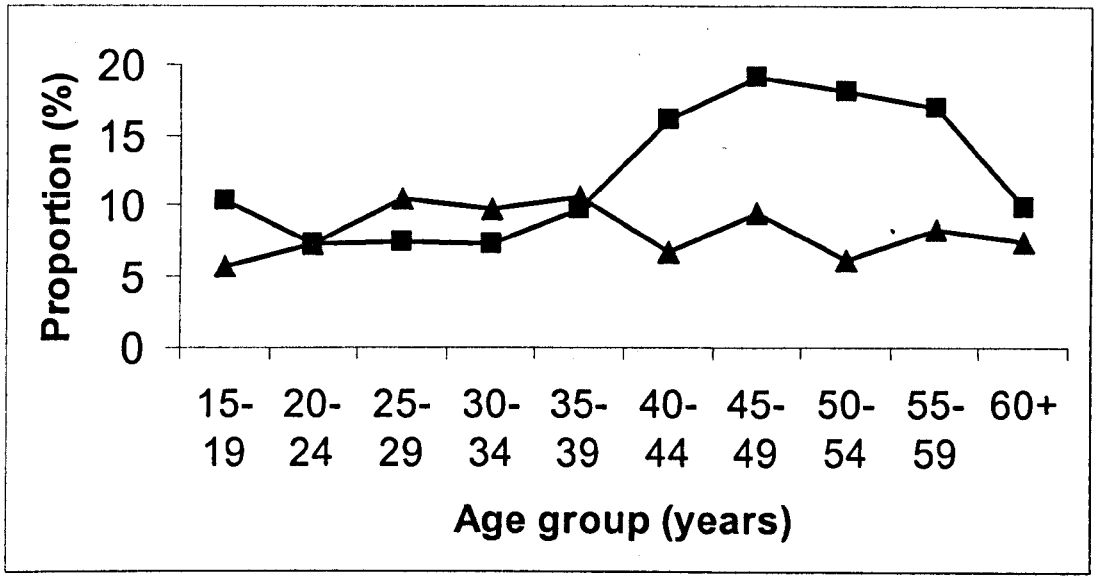


Figure 4.2: The proportion of males (■) and females (▲) positive for CFA by age from the national survey.

Survey prevalence data by district and village are summarised in Table 4.1. This ranged from 0% to 35.9%. The spatial distribution of the sampled villages with their prevalence category are shown in Figure 4.3. In general, villages in the western side of the country registered a CFA prevalence of less than 10%. This is with the exception of Mzenga Village in Mchinji District along the Malawi-Zambia border where a prevalence of 18.2% was found. Prevalence of over 20% was observed from villages in Salima and Mangochi Districts along the southern shore of Lake Malawi. Also in Ntcheu district (Bwanje Valley), Balaka district near Lake Malombe and finally in Phalombe district along the shores of Lake Chilwa. The highest prevalence (35.9%) was recorded at Kalembo village in Balaka district in southern Malawi.

District	Village	Number tested	Number positive	Prevalence	Latitude	Longitude
Balaka	Kalembo	53	19	35.8	14.84500	35.16900
Blantyre	Masanjala Lilangwe	77	5	6.5	15.54490	35.02184
Chiradzulu	Mbalame	81	6	7.4	15.70000	35.10000
Chitipa	Chisenga	85	0	0	9.97500	33.38977
Chitipa	Siyombwe	77	0	0	9.68441	33.24764
Dedza	Kamenyagwaza	64	5	7.8	14.40750	34.98750
Dowa	Chimangamsasa	72	4	5.6	13.70964	33.99795
Kasungu	Kadyaka	65	0	0	13.07633	33.48360
Kasungu	Kaluluma	105	3	2.9	12.58077	33.51870
Lilongwe	Mwenda 1 T/A Chadza	84	6	7.1	14.14074	33.78825
Machinga	Phuteya	70	3	4.3	15.19000	35.09887
Mangochi	Chilawe	92	9	9.8	13.80000	35.10300
Mangochi	Chiponde	90	12	13.3	14.38300	35.10000
Mangochi	Mtuwa	82	21	25.6	14.68400	35.55100
Mchinji	Chalaswa	98	4	4.1	14.11689	33.32919
Mchinji	Mzenga	99	18	18.2	13.60427	32.73460
Mulanje	Gawani	78	6	7.7	15.98100	35.78300
Mulanje	Mbewa	69	13	18.8	15.99970	35.48611
Mwanza	Chapita A	64	3	4.7	15.63022	34.59139
Mzimba	Milingo-Jere	101	0	0	12.20374	33.33340
Mzimba	Kambombo	102	2	1.9	11.17551	33.52649
Nkhata-Bay	Kalumpha	104	7	6.7	12.08733	34.05695
Nkhata-Bay	Mizimu	103	8	7.8	11.55820	34.18150
Nkhotakota	Mowe	122	11	9	12.55496	34.13366
Nkhotakota	Tandwe	81	3	3.7	13.02981	34.26246
Ntcheu	Gwaza	92	26	28.3	14.52800	34.68000
Ntcheu	Nkonde-1	66	6	9.1	14.98570	34.82825
Ntchisi	Kalulu	99	3	3	13.33129	33.74804
Phalombe	Maguda	78	19	24.4	15.51774	35.78996
Rumphi	Bongololo	72	1	1.4	10.81276	33.52233
Rumphi	Mhango	82	8	9.8	10.81000	33.52379
Salima	Chipoka-Nkwizi	73	16	21.9	14.03676	34.50614
Salima	Kasonda	78	13	16.7	13.59828	34.29268
Thyolo	Nkaombe	95	6	6.3	15.99271	35.04998
Zomba	Kapenda	57	2	3.5	15.35885	35.40305

Table 4.1: ICT prevalence data by village and district from the 2003 national survey.

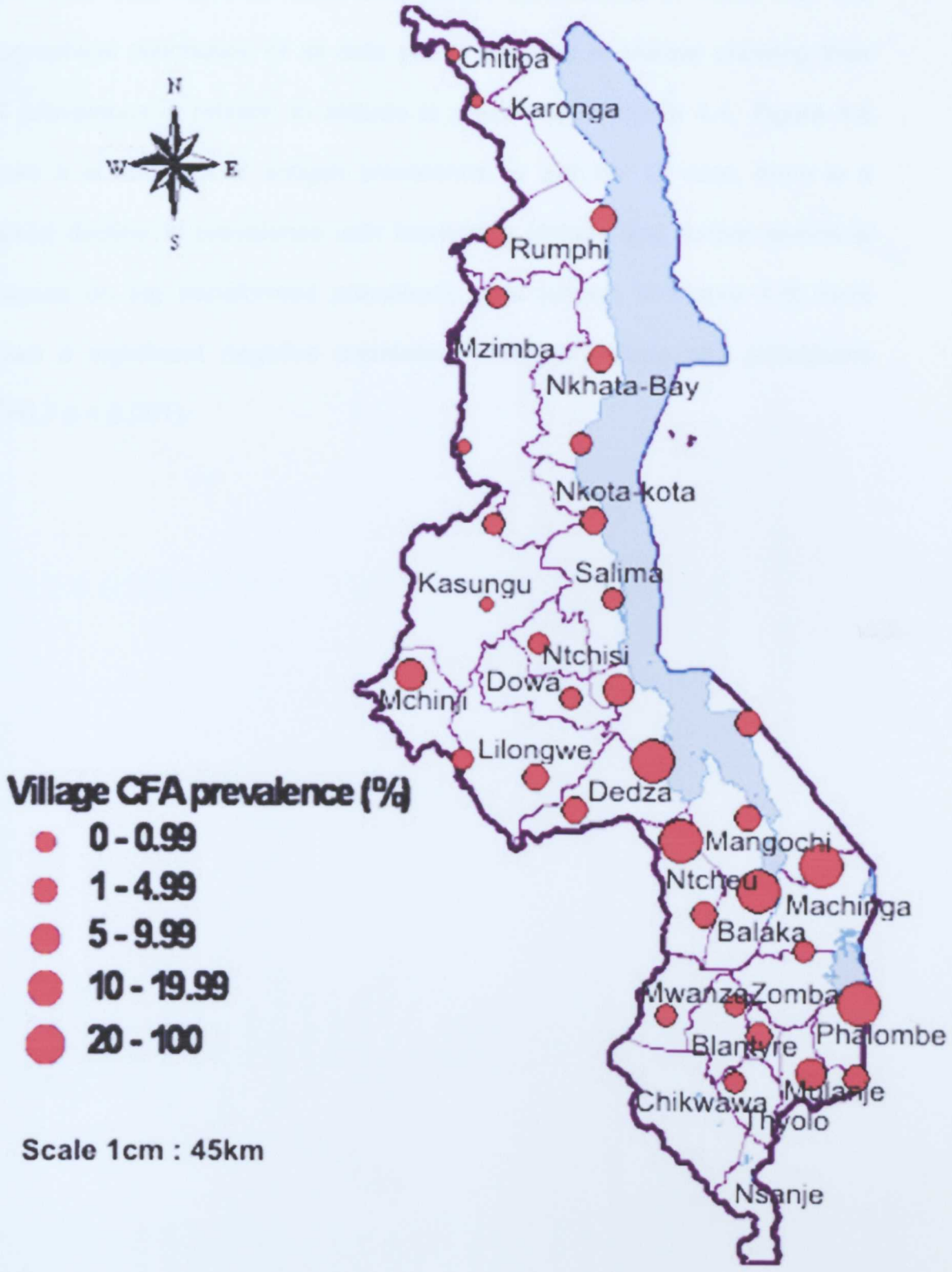


Figure 4.3: CFA prevalence data by village and district from the 2003 national mapping survey.

Prevalence data from the 2000 surveys are summarised in Table 4.2. The geographical distribution of all data points sampled in Malawi showing their ICT prevalence in relation to altitude is presented in Figure 4.4. Figure 4.5 shows a scatter plot of antigen prevalence by altitude. Of note, there is a marked decline in prevalence with increasing altitude and further statistical analyses on log transformed prevalence data [shown in Figure 4.6] have shown a significant negative correlation between altitude and prevalence (R^2=0.7 $p < 0.001$).

District	Village	Number tested	Number positive	Prevalence	Latitude	Longitude
Karonga	Mwenitete	42	20	47.6	9.71257	33.92973
Karonga	Mwakyusa	91	44	48.7	9.69795	33.89313
Karonga	Mwenepela	102	59	57.8	9.67193	33.82520
Karonga	Kashata	50	22	44	9.73315	33.88652
Karonga	Mwamsaku	50	22	44	9.80920	33.86483
Karonga	Mwambetania	50	29	58	9.86747	33.86892
Karonga	Kafikisila	51	23	45.1	9.91213	33.93105
Karonga	Mwenitete-mpata	50	24	48	9.94957	33.82237
Karonga	Ngosi	50	15	30	10.01228	33.94907
Karonga	Mwakabanga	50	15	30	10.14422	34.01782
Karonga	Kanyuka	51	14	27.5	10.30768	34.12692
Karonga	Bonje	50	28	56	10.49027	34.17098
Nsanje	Chazuka	148	60	40.5	16.84261	35.25259
Nsanje	Nchacha18	148	86	58.1	16.63617	35.17126
Nsanje	Gamba	84	56	66.7	16.58110	35.14076
Chikwawa	Nchingula	128	76	59.4	15.99828	34.48297
Chikwawa	Zilipaine	129	96	74.4	16.07998	34.88262
Chikwawa	Mbande	108	76	70.4	16.16167	34.79332
Chikwawa	Pende	116	79	68.1	16.04362	34.72428
Chikwawa	Belo	196	155	79.1	16.02093	34.8162
Chikwawa	Mfunde	87	29	33.3	16.19929	35.01652
Chikwawa	Kasokeza	60	34	56.7	16.11213	34.92532
Chikwawa	Khumbulani	59	9	15.3	15.99232	34.87910
Chikwawa	Muyaya	78	21	26.9	16.04667	34.90783

Table 4.2: Prevalence data from 2000 surveys in Karonga, Nsanje and Chikwawa Districts.

Source for Chikwawa and Nsanje Districts: (Nielsen et al., 2002)

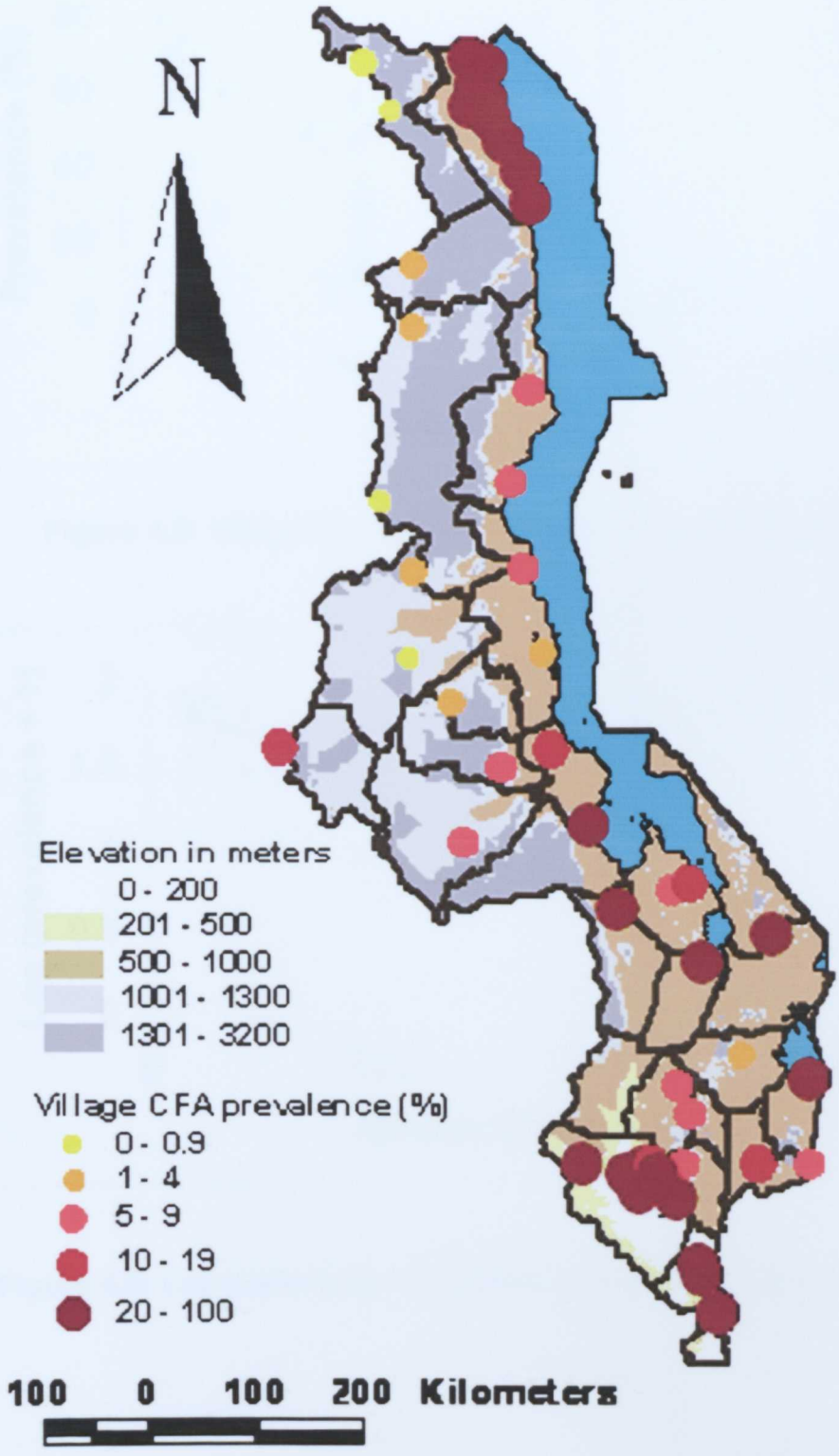


Figure 4.4: Map of Malawi showing CFA prevalence from all sampled villages in relation to altitude (metres).

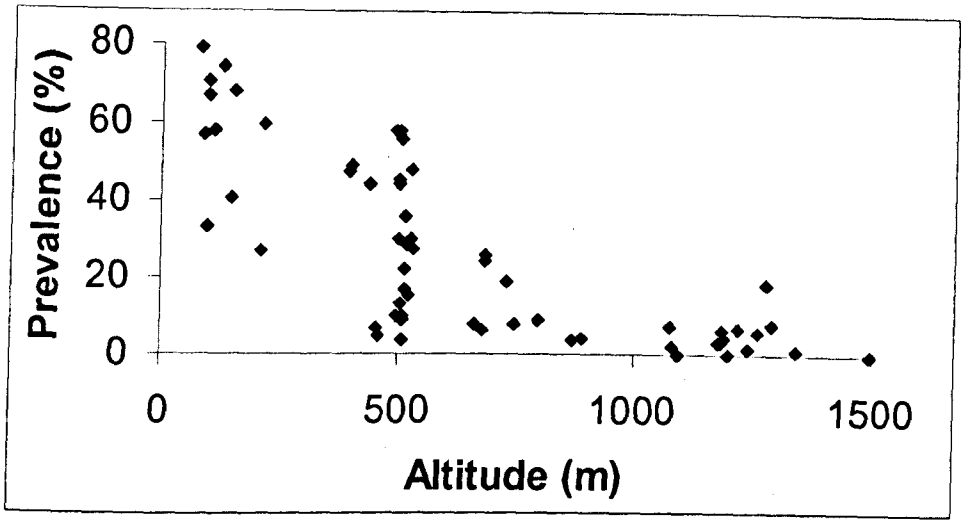


Figure 4.5: Village CFA prevalence plotted by altitude (M)

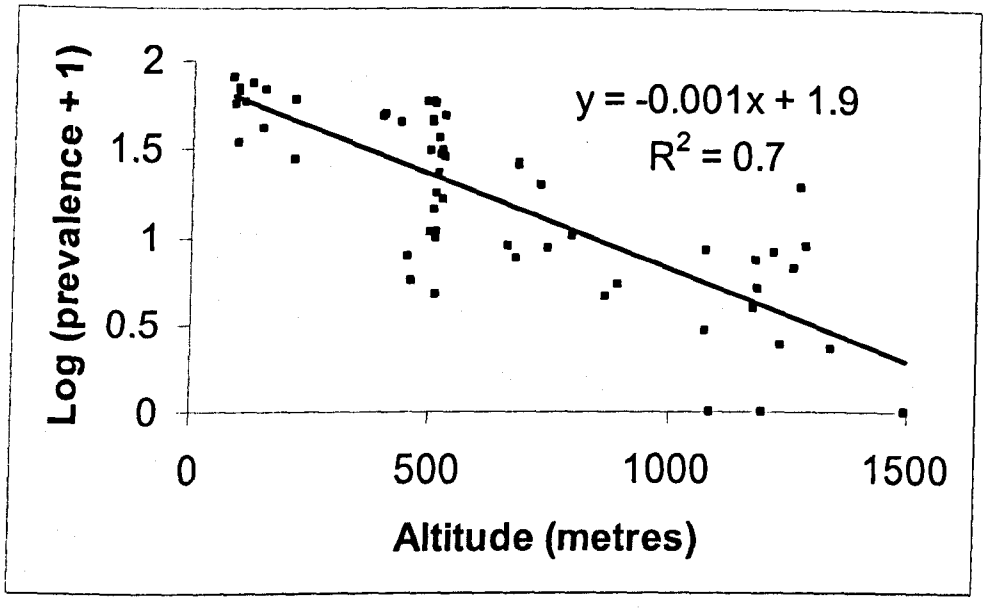


Figure 4.6: Log (prevalence + 1) plotted against altitude (metres).

4.1.1.1 Summary of the national mapping survey results

In summary, Village antigenaemia prevalence from the national survey [based on immunochromatographic (ICT) card tests] ranged from 0% to 35.9%. In general, villages from the western side of the country and far removed from the lake shore tended to register lower prevalence. This is with the exception of Mzenga village in Mchinji district along the Malawi-Zambia border where a prevalence of 18.2% was found. In contrast villages from lake shore districts [Salima, Mangochi, Balaka and Ntcheu (Bwanje valley)] and Phalombe had prevalence of over 20%.

Incorporating data from the 2000 surveys in Karonga, Chikwawa and Nsanje districts shows a marked decline of prevalence with increasing altitude. Further analysis revealed a strong negative correlation (R^2=0.7 $p < 0.001$) between altitude and prevalence. Of note, these data suggest that the lake shore, Phalombe plain and the lower Shire valley will be priority areas for the Malawi LF elimination programme. Implications of these findings as regards implementing a national LF elimination programme in Malawi are discussed in **Chapter 9**.

4.1.2 Detailed presentation of results from the Karonga survey

A separate detailed LF mapping survey was carried out in Karonga District as outlined in Chapter 3. Briefly, the main objectives for this survey included to determine the extent of the transmission area for *W. bancrofti* and also the prevalence of disease attributable to this parasite within the district. ICT results for the Karonga survey by village are included in Table 4.2. Overall, there were 687 ICT tests performed of which 315 (46%) were positive. The antigen prevalence is above 25% in all the sampled villages. Of note, villages in the Songwe area and those north of Karonga Township (within 35 km of the Songwe area) registered similar and higher prevalence (above 40%) than those in the south with the exception of Bonje (in the Hara area) as shown in Figure 4.7. The *W. bancrofti* antigen prevalence is constant (but lower in the south-excluding Bonje) over the age range tested in each geographical area shown in Figure 4.8. Of note, 50% of individuals tested in the Songwe area were *W. bancrofti* antigen positive by the age of 20. Overall, males had a higher prevalence compared to females in age groups beyond 20-29 (OR 1.45 p=0.016 95% CI 1.07-1.97).

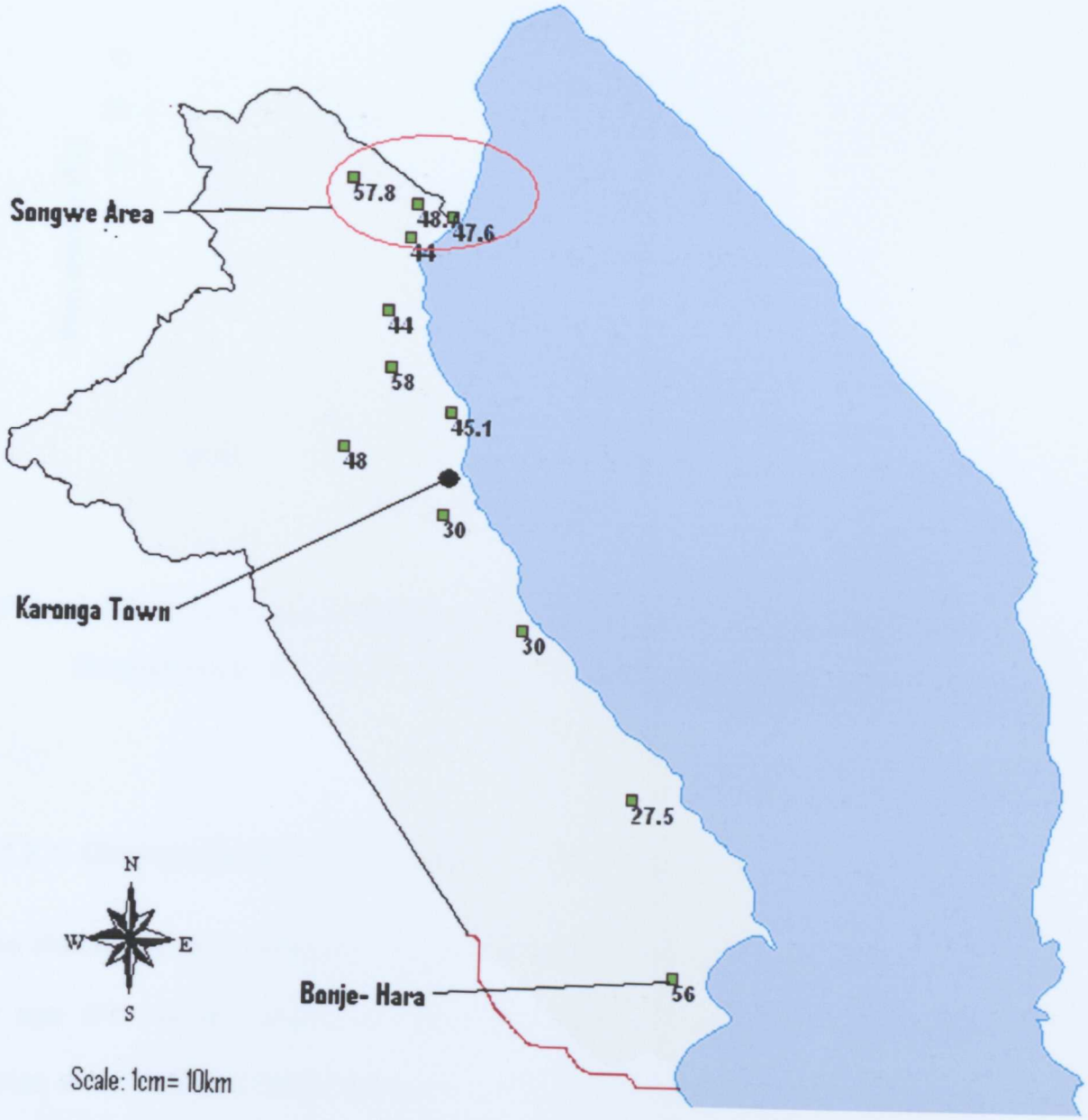


Figure 4.7: Map of Karonga District showing geographical positions of sampled villages and their ICT prevalence.

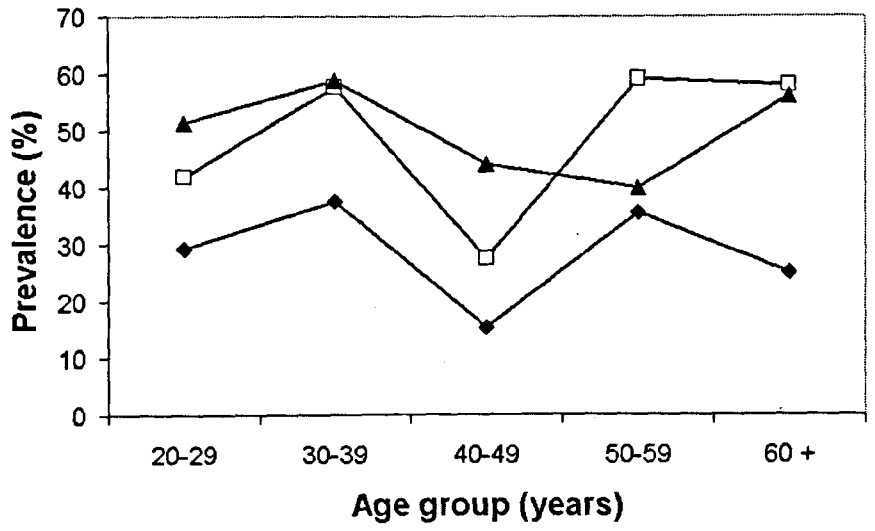


Figure 4.8: *W. bancrofti* antigen prevalence by age and geographical area: Songwe area (▲), north (□), south (excluding Bonje village) (♦).

4.1.2.1 Chronic clinical manifestation in Karonga District

The distribution and proportion of individuals with clinical manifestation of LF by age and sex are shown in Figure 4.9. Overall 80/685 (11.7%) of adult males examined had hydrocele and 7 (1.0%) had lymphoedema (6 in the leg alone, 1 in both arm and leg). Of 729 adult females examined, 29 (3.7%) had lymphoedema or elephantiasis (28 in the leg alone and 1 in both arm and leg). Hydrocele is the commonest manifestation of LF in this population. The prevalence of both hydrocele and lymphoedema increases with age.

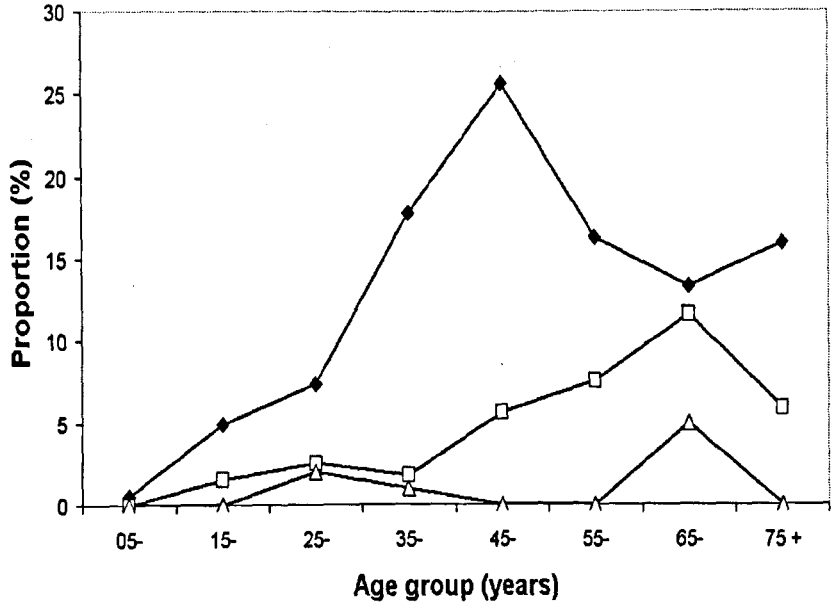


Figure 4.9: Age and sex distribution of disease attributable to *W. bancrofti* infection in Karonga District, northern Malawi: hydrocele (♦), female lymphoedema (□), male lymphoedema (Δ).

4.1.2.2 Thick blood films from the Karonga survey

A total of 107 ICT positive individuals were visited at night for finger prick blood sampling. Of the 107 thick films made, 33 (30.8%) were positive for microfilaria. All were identified as *W. bancrofti.* The microfilarial positivity did not vary with age (χ^2 for trend p=0.5).

4.1.2.3 Summary of the Karonga results

In summary, the Karonga survey results suggest that *W. bancrofti* infection is more widespread than previously reported (Oram, 1960). However, there exists a cline in antigenaemia prevalence from the northern parts of the district to the south. In addition, an LF focus seems to have been established

in the Hara area as a result of movement of people from the northern part of the district. These findings are discussed in detail in **Chapter 9**.

Chapter 5

5.1 Baseline survey of anaemia, geo-helminths, malaria, lymphatic filariasis and anthropometric indices in Chikwawa District- southern Malawi

Intestinal helminths and malaria infections are widespread in sub-Saharan Africa. Hookworms are mostly implicated as a cause of iron deficiency anaemia (Roche and Layrisse, 1966; Stoltzfus et al., 1996) due to enteric blood loss, which is dependent on worm burden and species (Roche and Layrisse, 1966). Malaria infections, with their associated destruction of red blood cells, may lead to haemolytic anaemia (Abdalla et al., 1980; Das et al., 1999). The respective contribution of these parasitic infections including *Schistosoma spp, S. haematobium* in particular, to the aetiology of anaemia in a particular area will depend on the local epidemiology and the frequency of co-infections (Guyatt et al., 2001; Stephenson et al., 1985). Enteric infection with some helminths (eg *Ascaris lumbricoides*) has also been associated with increased frequency of malaria fevers (Le Hesran et al., 2004; Spiegel et al., 2003).

In Chikwawa District- southern Malawi there have been no detailed total population based epidemiological surveys of intestinal helminths a part from a small early nutritional survey (Burgess et al., 1973). However extensive studies on anaemia and malaria amongst pregnant women and infants have been carried out over the past ten years (Brabin et al., 2004; Verhoeff et al., 1999).

In recent years there has been renewed interest in the control of intestinal helminths, especially amongst school children, through bi-annual distribution of anthelmintic treatment (Anon, 1998; World Health Organisation, 2004). An opportunity to reduce morbidity associated with intestinal helminths has emerged as a result of the implementation of the Global Programme to Eliminate Lymphatic Filariasis. The essential strategy of the GPELF in sub-Saharan Afirca includes mass drug distribution of two broad-spectrum antiparasitic agents, albendazole and ivermectin, in combination in communities endemic for lymphatic filariasis (Cline et al., 2000).

The aim of the present study was to describe the distribution and relationship of helminth infections, malaria and anaemia in communities taking part in the mass distribution of albendazole and ivermectin for the control of lymphatic filariasis in southern Malawi.

5.1.1 Results of the baseline survey

A total of 1242 individuals were recruited into the study from 313 households as shown in Table 5.1. Of these, 1108 (90.5%) have a complete set of data including stool results. Age and sex distribution of the study participants is shown in Figure 5.1. There were a total of 607 (54.9%) female study participants. Fifty-five percent of the participants were less than 20 years old.

Characteristic	Male	Female	Pooled
Study population			
Total recruited (%)	567	675	1242
With complete data (%)	501	607	1108
Age (years)			
Mean (range)	21.9 (1-87.5)	24.7 (1- 87.9)	23.4 (1-87.9)
Median	14.6	18.7	16.3
Anaemia (n= 1228)			
Mean haemoglobin in g/dl (range)	11.7 (4.4- 20)	11.0 (3.2- 17)	11.4 (3.2- 20)
Anaemic (%)	185 (33.0)	254 (38.0)	439 (35.8)
Moderate to severe anaemia (%)	35 (6.3)	49 (7.3)	84 (6.8)
Enteric parasites (n= 1113)			
Hookworm positive (%)	88 (17.5)	114 (18.7)	202 (18.2)
S. Mansoni positive (%)	33 (6.6)	28 (4.6)	61 (5.5)
A. lumbricoides positive (%)	12 (2.4)	7 (1.2)	19 (1.7)
Malaria (n= 1242)			
MPS Positive (%)	120 (21.5)	133(19.9)	253 (21.5)
Episode of Fever within past 1 month (%)	250 (44.7)	324 (48.4)	574 (46.7)
Bed net use (n= 1242)			
Slept under nets the previous night (%)	37 (6.5)	40 (5.9)	77 (6.2)

Table 5.1: Characteristics of study participants at the baseline survey

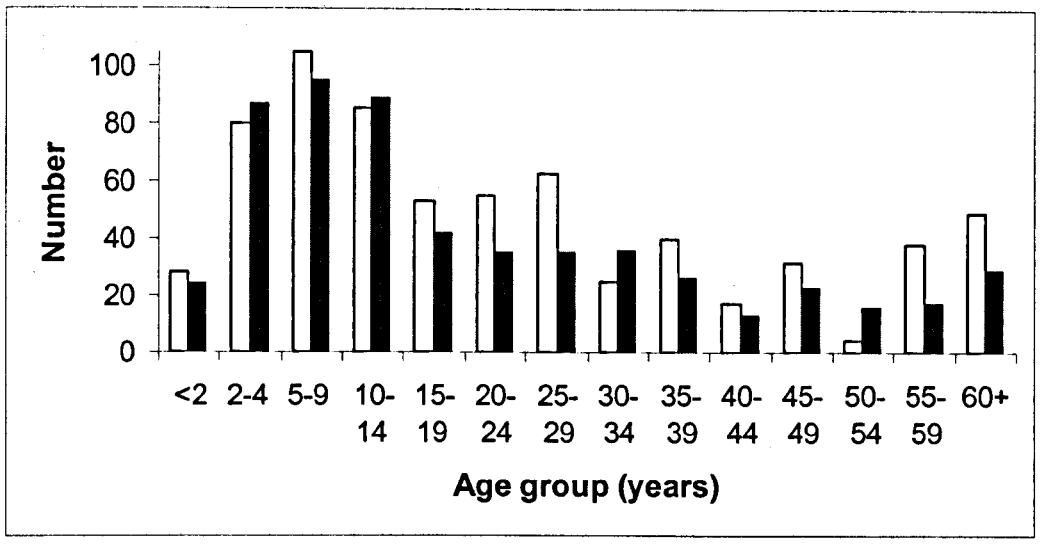


Figure 5.1: Age and sex distribution of male (■) and female (□) participants recruited for the baseline survey.

5.1.1.1 Anaemia prevalence at the baseline survey

The age-specific prevalence of anaemia by sex is shown in Figure 5.2. Of the 1228 individuals with haemoglobin level results 523 (42.6%) were anaemic (Hb <11g/dl). Amongst the anaemic individuals 84 (6.8%) had moderate to severe anaemia (Hb <8g/dl). Anaemia was most prevalent (95%) in the youngest age group (< 2 years old). The anaemia prevalence decreased to either below, or above 20%, for females and males respectively beyond 15 years of age. This is reflected in the corresponding increases in the mean haemoglobin by age as shown in Figure 5.3. Mean Hb increased with age for both sexes to early adolescence. Females aged ≥15 years had significantly lower mean Hb (12.9g/dl vs 11.3g/dl t= -7.56 p<0.001) than males. This difference was not seen in younger subjects (10.9g/dl vs 10.7g/dl t= 1.35 p=0.18). Among individuals with moderate to severe anaemia Hb levels were negatively correlated with age (Spearman's rho= - 0.24 p= 0.04).

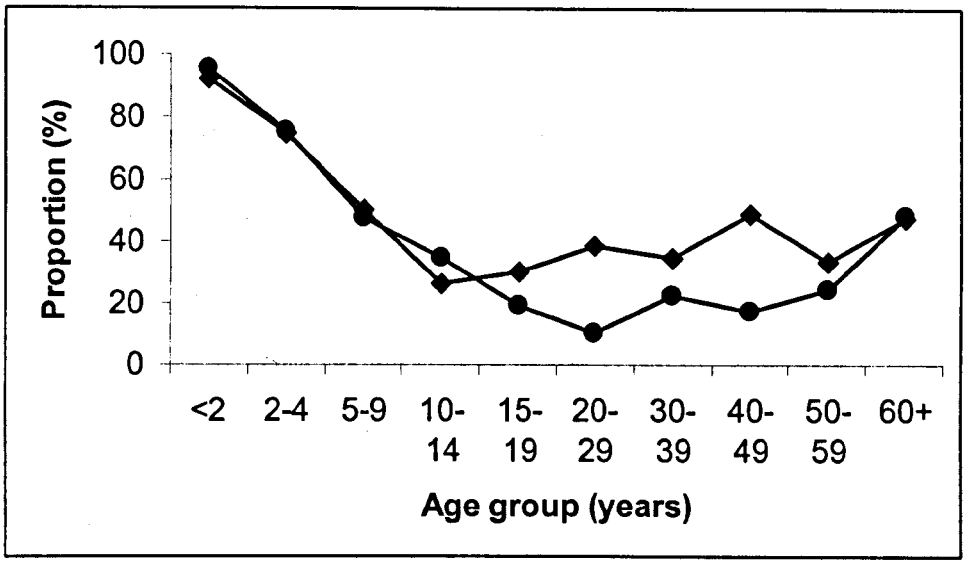


Figure 5.2: Age specific proportions of individuals with anaemia by age and sex [Male (●) Female (♦)]

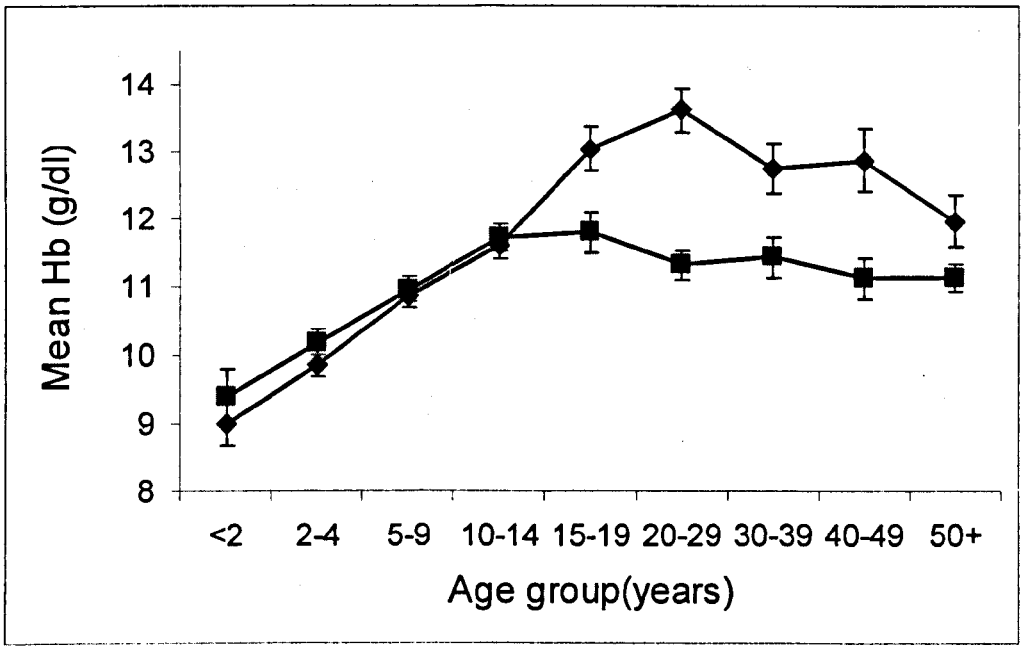


Figure 5.3: Mean Hb by age and sex [males (♦) females (■)] with standard errors.

5.1.1.2 Enteric parasites at the baseline survey

A total of 1113 individuals had stool microscopy. Hookworm ova were found in 202 (18.1%) samples. Prevalence increased with age from 10% in those under 2 years of age to 30% amongst those over 40 years (χ^2 for trend p= <0.001) as shown in Figure 5.4. All of the hookworm infections were of light intensity (range 24- 888 *eggs per gram (epg)* of stool) based on the WHO recommended classification of hookworm infection intensity. However the distribution of hookworm infection intensity in the sampled population is shown in Figure 5.5. This is typically skewed to the right with the majority of the study participants being negative. *Schistosoma mansoni* ova were observed in 61 (5.5%) participants. Prevalence remained under 10% across all age groups. There were 19 cases of *Ascaris lumbricoides*, 2 of *Enterobius vermicularis* and 1 of *Strongyloides stercoralis*. Hookworm co-infection with *S. mansoni* and *A. lumbricoides* occurred in 8 (0.6%) and 3 (0.3%) individuals respectively.

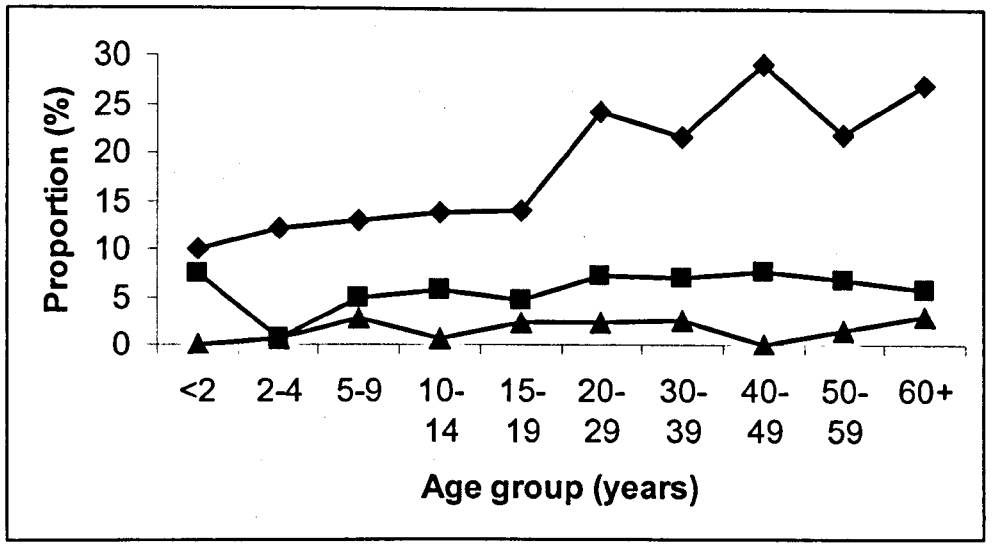


Figure 5.4: The age specific prevalence of hookworm (♦), *S. mansoni* (■) and *A. lumbricoides* (▲) from the baseline survey.

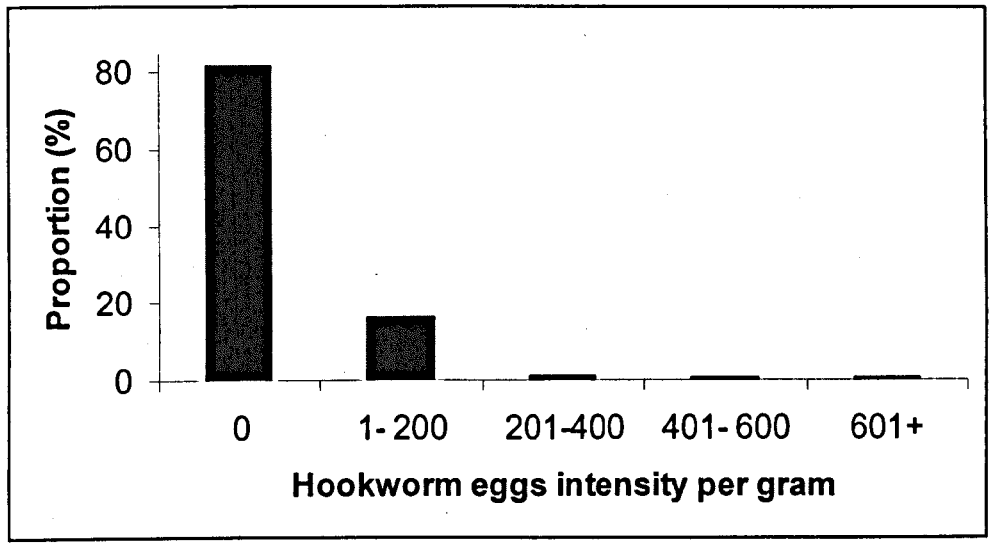


Figure 5.5: Distribution of hookworm eggs per gram (epg) of stool.

5.1.1.3 Malaria parasitaemia prevalence at baseline

Thick smear slides were examined for 1229 individuals. *Plasmodium falciparum* infection was detected in 253 (20.6%) individuals. Prevalence decreased with age (χ^2 for linear trend $p< 0.001$). It was highest (31.6%) amongst under five children (see Figure 5.6). The majority of the positive

cases [192 (74.4%)] had low parasite density (≤50 parasites per 200 WBC). The overall distribution of intensity of malaria parasitaemia is shown in Figure 5.7. History of fever for which antimalaria treatment was received in the previous 1 month was reported by 574 (46.7%) individuals of whom 250 were males.

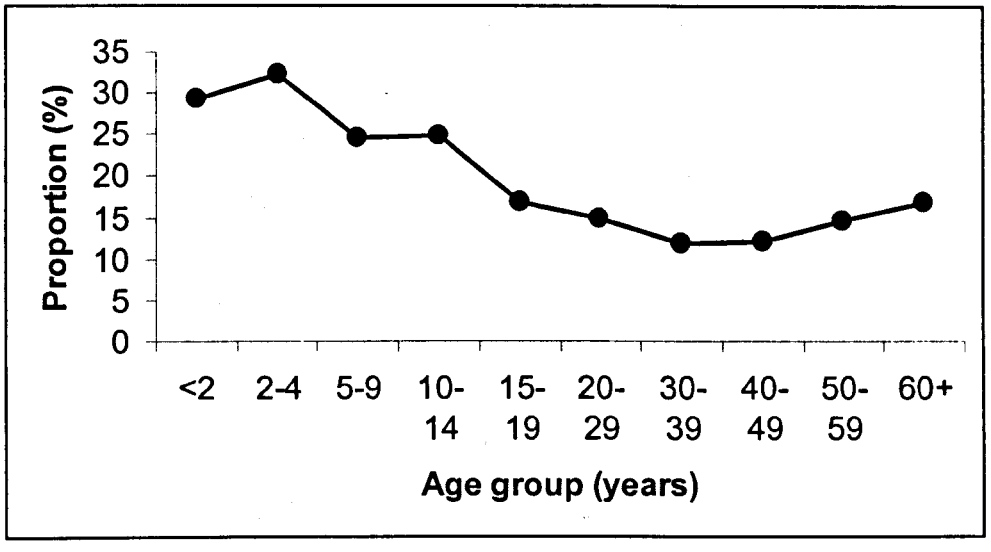


Figure 5.6: The age specific prevalence of malaria parasitaemia from the baseline survey.

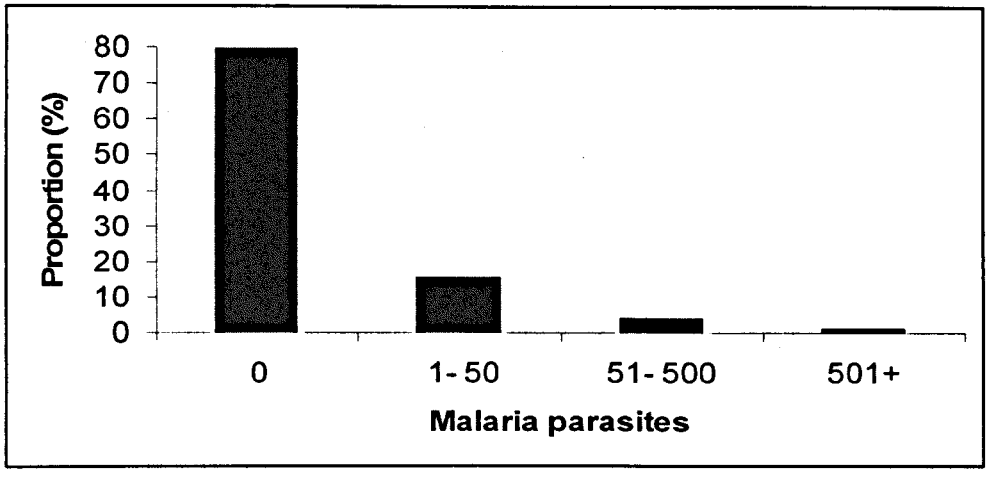


Figure 5.7: Distribution of individuals by intensity of malaria parasitaemia category (malaria parasites per µL of blood).

5.1.1.4 Baseline anthropometric measurements

This part of the analysis is restricted to those aged between 3 and 18 years for which the EpiInfo's Nutstat programme used to perform this analysis is designed to work. A total of 563 children met the age criteria and had weight and height measurements. Of these, 283 (50.3%) were female. Relative to the softwares reference population (CDC- 2000), this group of children were 41.9% (236/563) underweight and 44.7% (252/563) stunted. Amongst the boys 46.1% (129/280) were underweight and 51.4% (144/280) were stunted. The corresponding proportions for the girls were 37.8% and 38.2% respectively. The difference in proportion between boys and girls was statistically significant for the underweight (X^2 p= 0.05) and for those with stunting (X^2 p= 0.002). These differences are apparent when age specific proportions for weight-for-age and height-for-age are plotted by age and sex as show in Figure 5.8 and 5.9. There is an apparent increase with age in age-specific proportions for the underweight amongst the boys while for the girls

the level seems to remain constant. As for stunting the age-specific proportions for the boys are similar across the age range which contrasts with the pattern observed for the girls which shows a decrease with age.

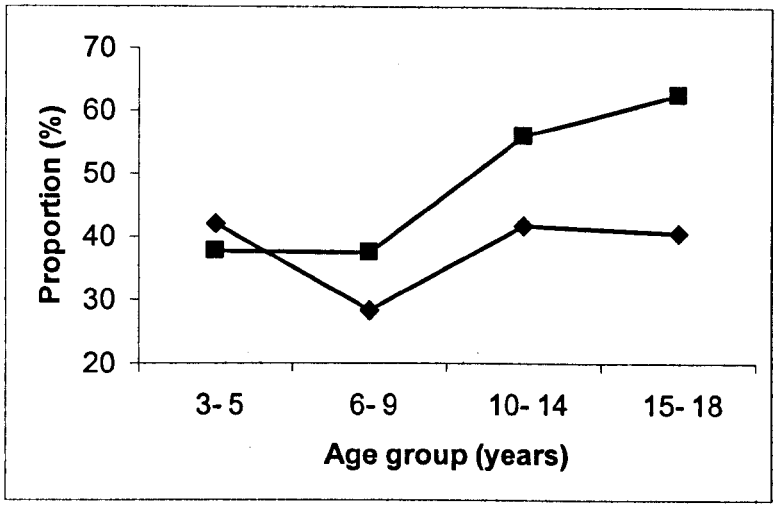


Figure 5.8: Showing the proportion of those underweight amongst the sampled boys (■) and girls (♦) in Chikwawa District.

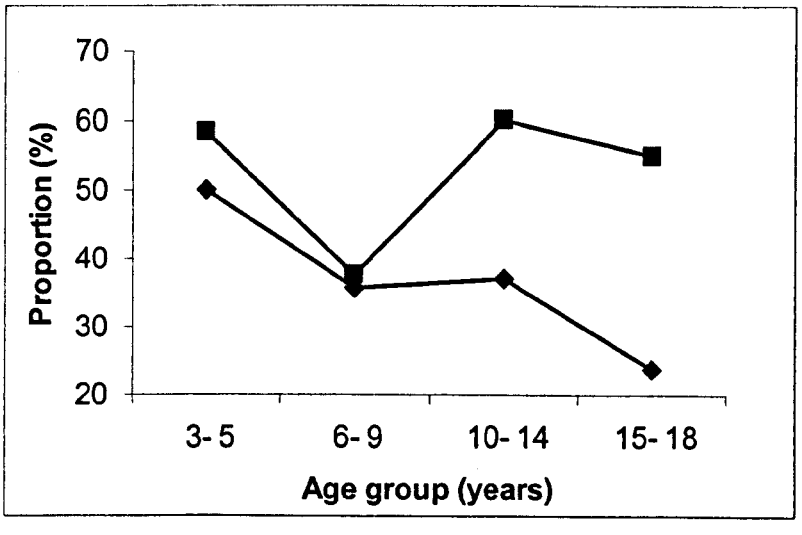


Figure 5.9: Showing the proportion of stunting amongst the sampled boys (■) and girls (♦) in Chikwawa District.

Figures 5.10 and 5.11 show the mean values of z-scores for weight-for-age and height-for-age by age and sex. There appears to be a progressive decrease in z-scores for both weight-for-age and height-for age for boys over the age range. In contrast the z-scores for the girls do not show such a pattern but have a marked upturn most noticeable amongst the 15-18 age group.

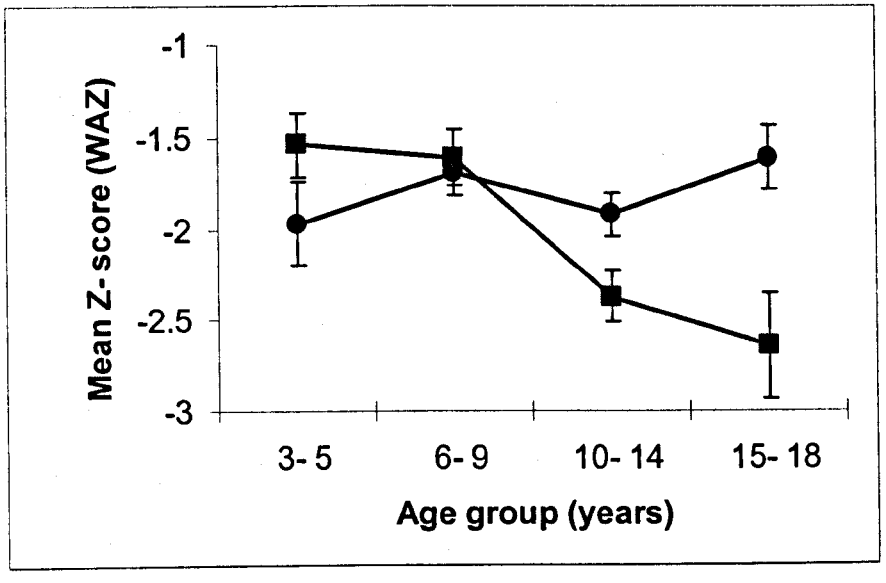


Figure 5.10: Showing age specific mean z-score for weight-for-age for boys (■) and girls (●)

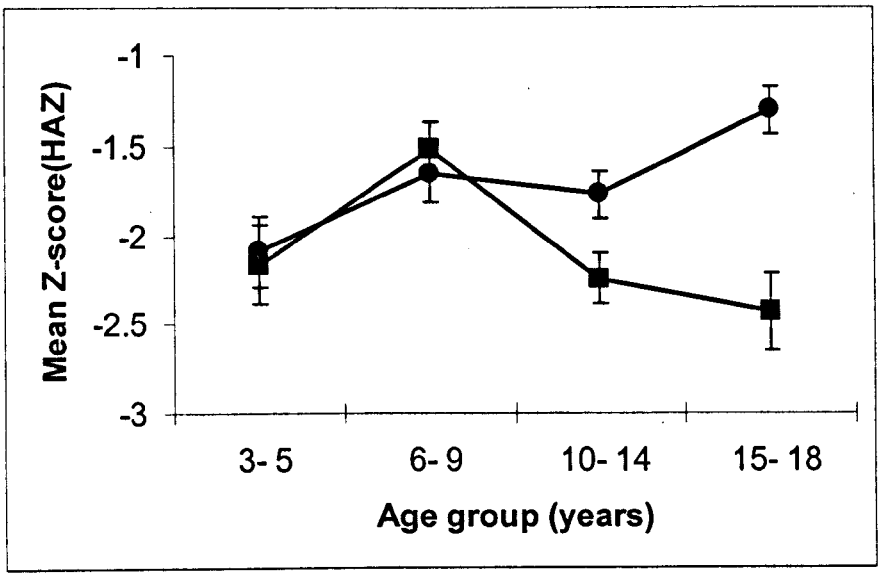


Figure 5.11: Showing age specific mean z-score for height-for-age for boys (■) and girls (●)

5.1.1.5 Chronic manifestations of LF at the baseline survey

All adults aged 15 and above were given a full body clinical examination for chronic manifestations of LF. The age-specific prevalence of these sequelae of LF in this population is presented in Figure 5.12. The prevalence for both lymphoedema and hydrocele increases with age. Of these manifestations hydrocele is the commonest. Amongst the 376 adult female participants 11(2.9%) had lymphoedema of the leg with 3, at the elephantiasis stage. Of the 272 adult males 58 (21.3%) had a chronic manifestations with 55 carrying a hydrocele of at least 6cm in diameter (range 6-24cm), 2 had lymphoedema of the leg alone and 1 had both lymphoedema and hydrocele.

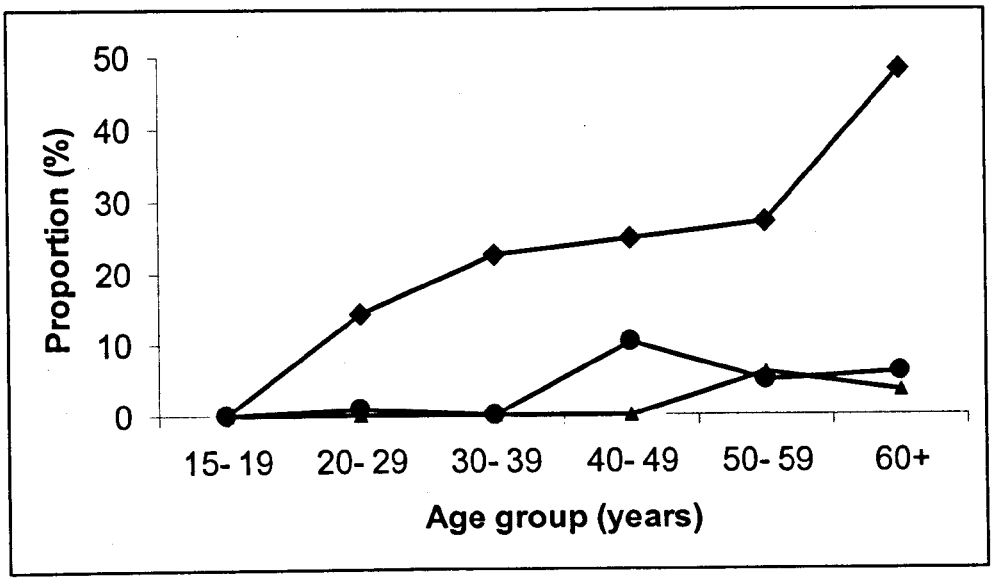


Figure 5.12: The age-specific prevalence for chronic manifestations of LF from the baseline survey, hydrocele (♦), lymphoedema-females (●) and lymphoedema- males (▲)

5.1.1.6 Microfilaraemia at the baseline survey

Microfilaraemia was assessed in willing volunteers in a total of 5 villages between 2100 hours and 0200 hrs. A total of 209 individuals participated. Of these, 158 (75.6%) were males. The overall mean age was 23.5 years while that for the male and female participants were 23.7 and 22.8 years, respectively. The age difference between sexes was not significant (Student ttest t= -0.043 p= 0.67). Up to 63 (30.1%) individuals were carrying *W. bancrofti* microfilaria. Amongst the male participants microfilaraemia prevalence was 29.1% (46/158) and that for the female was 33.3% (17/51). The difference in microfilaraemia by sex was not significant (X^2 chi= 0.33 p= 0.57). The proportion of positive cases and microfilaraemia intensity by age is shown in Figures 5.13 a and b, respectively. In general the proportion of

individuals who were microfilaria positive and microfilaraemia intensity increased by age. The overall geometric mean intensity of microfilaraemia amongst positive cases was 354 mf/ml of blood.

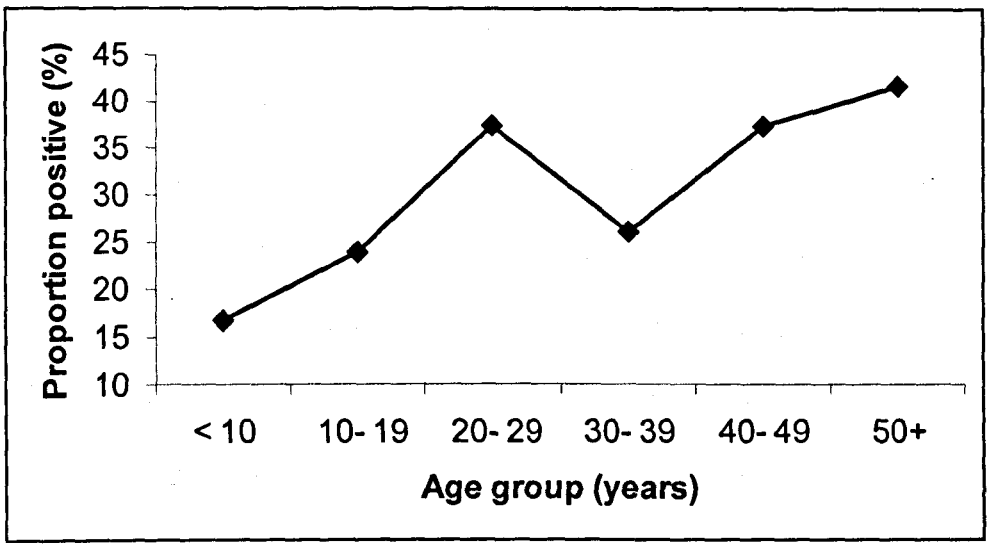


Figure 5.13a: Proportion of individuals microfilaria positive by age. There was one positive case amongst those aged less than 10 years.

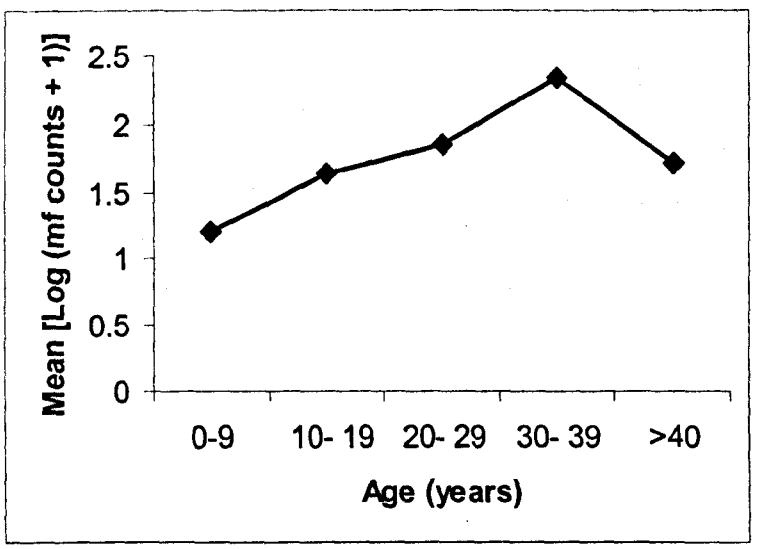


Figure 5.13b: Microfilaraemia density (mf/ml of blood) by age on the log scale.

5.1.1.7 Socio-economic status of recruited households at the baseline survey

Table 5.2 presents the socio-economic characteristics of the households selected for survey. A total of 313 households were sampled. Notably, only a third of the households had at least 1 member on regular income (including small scale business) while 45% and 42% reported owning a radio or a bicycle respectively. Cattle and goats were owned by a third of the households. It is noteworthy that up to 96.5% of the households had access to safe drinking water however only 29.1% had a pit latrine. The distribution of socio-economic scores for the recruited households is shown in Figure 5.14. The distribution is highly skewed with 207 (66.1%) of the households falling in or below band 3 on the socio-economic scale and only 36 (11.5%) being above the 90th (band 6) percentile.

Characteristic	Number (%)
Total number of households recruited	313 (100)
At least 1 member on regular income	97 (31.0)
Radio in the household	141 (45.1)
Bicycle in the household	132 (42.2)
Ownership of animals (cattle and goats)	95 (30.4)
House with cement floor	23 (7.4)
House with corrugated iron roof	57 (18.2)
Borehole as main source of drinking water	302 (96.5)
Pit latrine	91 (29.1)

Table 5.2: Socio-economic characteristics of households randomly selected for baseline survey.

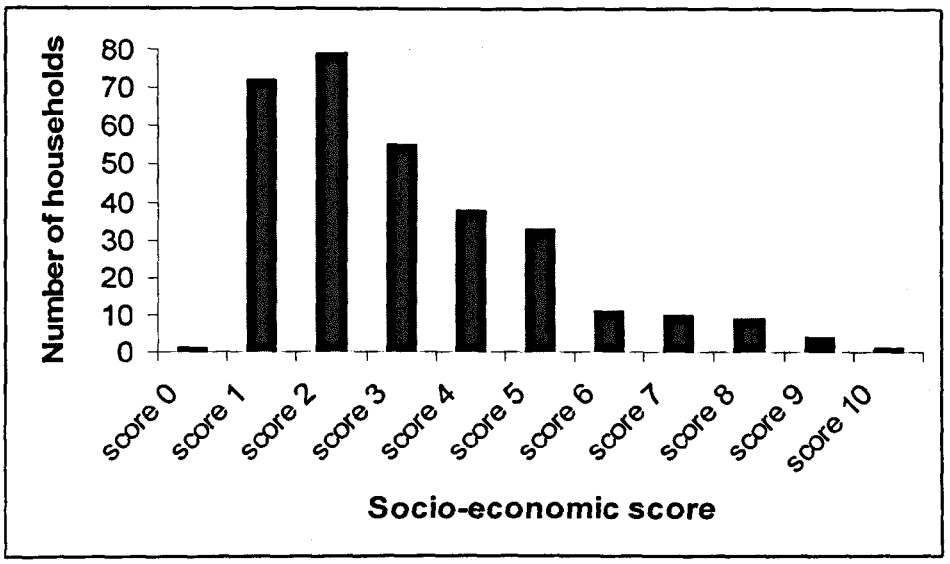


Figure 5.4: Distribution of socio-economic scores of households from the baseline survey.

5.1.1.8 Association between various potential risk factors and haemoglobin level

An investigation into the relationship between various factors and anaemia was carried out. The distribution of the various potential risk factors for anaemia by Hb level is presented in Table 5.3.

Factor	Moderate to severe anaemia (Hb < 8g/dl)	Anaemic	Normocaemic	χ^2–p
Median age in years	19.8	9.5	21.4	-
Sex				
Male (%)	*33(6.9)*	*166 (33.1)*	*302 (60.3)*	*NS*
Female (%)	*38 (6.3)*	*229 (37.7)*	*340 (56.0)*	
Hookworm				
Positive (%)	*26 (12.9)*	*62 (30.9)*	*113 (56.2)*	*<0.001*
Negative (%)	*45 (5.0)*	*333 (36.7)*	*529 (58.3)*	
S. mansoni				
Positive (%)	*4 (6.7)*	*20 (33.3)*	*36 (60.0)*	NS
Negative (%)	*67 (6.4)*	*375 (35.8)*	*606 (57.8)*	
Ascaris				
Positive (%)	*1 (5.3)*	*8 (42.1)*	*10 (52.6)*	NS
Negative (%)	*70 (6.4)*	*387 (35.5)*	*632 (58.0)*	
Malaria parasitaemia				
Positive (%)	*18 (8.0)*	*94 (41.6)*	*114 (50.4)*	0.03
Negative (%)	*53 (6.0)*	*301 (34.1)*	*528 (59.9)*	
History of fever				
≤1 month (%)	*41 (8.1)*	*191 (37.6)*	*276 (54.3)*	*0.03*
> 1 month (%)	*30 (5.0)*	*204 (34.0)*	*366 (61.0)*	

Table 5.3: Distribution of potential risk factors for anaemia

Among the factors explored in this crude univariate analysis age, hookworm infection, malaria parasitaemia and history of fever seem to be associated with haemoglobin level. Increasing age, as already observed above, has a negative effect on anaemia. This is confirmed by the difference in median age between those with and without mild anaemia (9.5 vs 21.4 median test Pearson X^2 =68.86 p<0.001). In contrast median age for those with severe anaemia was not significantly different from the non-anaemic group (19.8 vs

21.4 median test Pearson X^2 =0.05 p= 0.83). Though sex does not seem to be associated with anaemia in this crude analysis it has been observed above that there is an association which is dependent on age. Amongst the enteric parasites only hookworm has a significant association with anaemia. This is largely due to the fact that a larger proportion of those with moderate to severe anaemia (MSA) as compared to the mild and non-anaemic groups were carrying hookworms. In addition there appears to be a trend of decreasing mean haemoglobin by hookworm egg intensity shown in Figure 5.5. Malaria parasitaemia also seems to be significantly associated with anaemia in this population. There seems to be a trend of decreasing mean haemoglobin level with increasing density of malaria parasitaemia (shown in Figure 5.6) as was seen with hookworm. Similarly those who reported to have experienced an episode of fever within the previous month seem to be more likely to have anaemia than if they did not. The fever episode is presumed to be due to malaria.

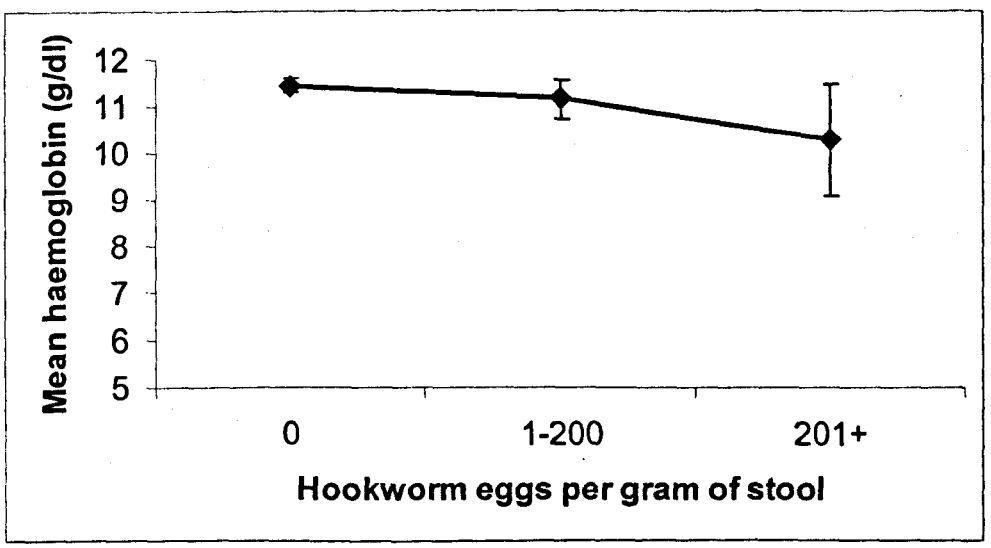


Figure 5.5: Mean haemoglobin by hookworm egg intensity per gram of stool.

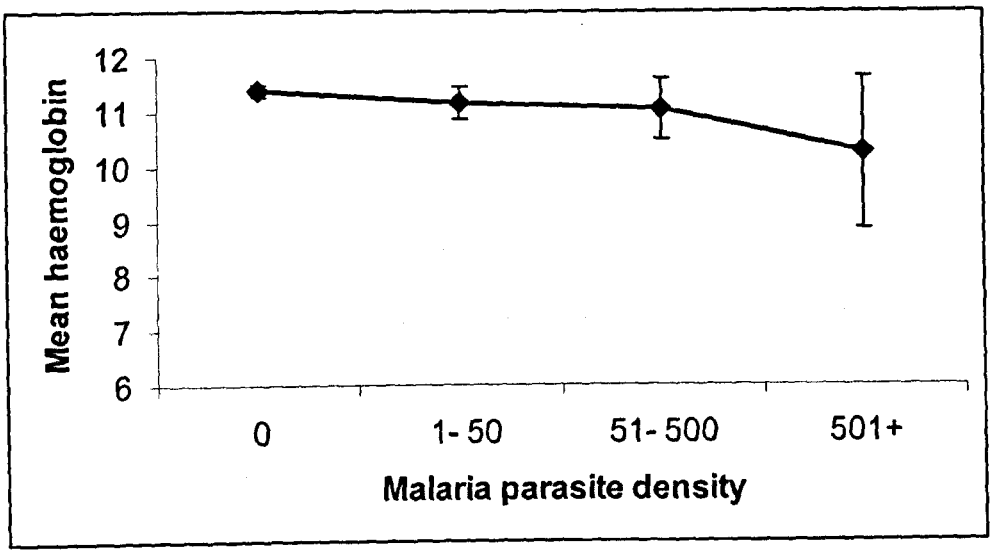


Figure 5.6: Mean haemoglobin by malaria parasite density

Grouped by quartile levels, household socio-economic score does not seem to predict anaemia levels. However, when individual socio-economic factors were analysed, it was found that coming from a house with corrugated iron roof and cement floor had a negative association with anaemia in that such individuals were less likely to have anaemia. Of note all houses with cement floor had iron roofs. Because there were a few houses with iron roofs without cement floor thus in further analyses iron roof only was used as a covariate. None of the anthropometric indices seemed to be associated with anaemia.

5.1.1.9 Multivariate analysis

The relationship of the potential risk factors with anaemia was further investigated in a multivariate analysis using logistic regression. Since anaemia prevalence did not change monotonically across the age range, age was

categorised for multivariate analysis so that each stratum had a reasonable number of individuals for model stability. A significant interaction between age and sex was observed. Allowing for this interaction the crude and adjusted odds ratios (AOR) for having anaemia for the various risk factors are summarised in Table 5.4. The OR for each age stratum remained significant even after controlling for the other covariates. A protective effect of over 80% in those above five years of age was suggested. However, because of the significant interaction between age and sex a stratified analysis was carried out. The sex effect on anaemia was determined in those younger and older than 15. In those less than 15 years of age there was no association between sex and anaemia (AOR 0.93, 95% CI 0.62- 1.37). Of note, in this age group being older than 5 years was associated with 86% (AOR= 0.16, 95% CI 0.10-0.26) and for those coming from a household with corrugated iron roof 60% (AOR= 0.40, 95% CI 0.24- 0.67) protection against having anaemia respectively. In those older than 15, being female was associated with a doubling of risk of having anaemia (AOR 2.15, 95% CI 1.44- 3.19). None of the other factors assessed, including age, showed any association with anaemia in these older individuals. Similarly, hookworm, *S. mansoni*, *Ascaris*, malaria parasitaemia, history of fever and socio-economic score did not show any association with anaemia in the adjusted analyses. In contrast coming from a household with corrugated iron roof was significantly associated with a reduction in risk of having anaemia in both the univariate and the adjusted analysis (AOR= 0.56, 95% CI 0.41- 0.77)

A further analysis restricted to those with moderate to severe anaemia (MSA) was carried out. In this analysis age, hookworm and having an iron roofed household were significantly associated with having MSA as shown in Table 5.5. A protective effect against having MSA of between 83% and 94% by age was observed across the age strata. Conversely hookworm was associated with a more than fourfold (AOR = 4.27, 95% CI 2.15- 8.47) increased risk of having MSA in this population. Coming from an iron roofed house conferred a 79% (AOR= 0.21, 95% CI 0.08- 0.55) reduction in the risk of developing MSA.

The impact of hookworm on MSA was further investigated by calculating the attributable proportion (AP) using the following formula:

$$AP = AP(e).p1$$

(note: AP(e)= prevalence ratio- 1/prevalence ratio)

where p1 is the proportion of cases that are exposed and AP(e) is the attributable proportion for the exposed population.

The unweighted AP of MSA for hookworm was found to be 22.6%.

Factor	Crude OR	95% CI	Adjusted OR	95% CI
Age				
<5	*ref*		*ref*	
5- 14	*0.18*	*0.11- 0.27*	*0.15*	*0.09- 0.25*
15- 24	*0.09*	*0.05- 0.14*	*0.05*	*0.02- 0.11*
25- 34	*0.11*	*0.07- 0.20*	*0.05*	*0.02- 0.13*
34- 54	*0.11*	*0.07- 0.19*	*0.05*	*0.01- 0.12*
55+	*0.14*	*0.08- 0.24*	*0.02*	*0.00- 0.14*
Sex amongst those aged <15 years				
Male	*ref*		*ref*	
Female	*0.91*	*0.63- 1.30*	*0.93*	*0.62- 1.37*
Sex amongst those aged ≥15 years				
Male	ref		ref	
Female	2.17	1.47- 3.20	2.15	1.44- 3.19
Hookworm				
Negative	*ref*		*ref*	
Positive	*0.98*	*0.71- 1.36*	*1.19*	*0.84- 1.70*
S. mansoni				
Negative	*ref*		*ref*	
Positive	*0.91*	*0.53- 1.56*	*1.14*	*0.63- 2.05*

Factor	Crude OR	95% CI	Adjusted OR	95% CI
Ascaris				
Negative	*ref*		*ref*	
Positive	*1.34*	*0.53- 3.41*	*2.64*	*0.97- 7.23*
Malaria parasites				
Negative	*ref*		*ref*	
Positive	*1.53*	*1.12- 2.08*	*1.29*	*0.92- 1.82*
History of fever episodes				
≤1 month	*ref*		*ref*	
1-3 month	*0.94*	*0.65- 1.36*	*0.99*	*0.66- 1.47*
>3 months	*0.74*	*0.56- 0.97*	*0.92*	*0.68- 1.24*
Quartile of socio-economic score				
1st quartile	*ref*		*ref*	
2nd quartile	*1.29*	*0.90- 1.8*	*1.29*	*0.88- 1.90*
3rd quartile	*1.05*	*0.72- 1.54*	*1.15*	*0.77-1.74*
4th quartile	*0.95*	*0.70- 1.29*	*1.00*	*0.71- 1.39*
Household with corrugated Iron roof				
No	*ref*		*ref*	
yes	*0.58*	*0.43- 0.78*	*0.56*	*0.41- 0.77*

Table 5.4: Crude and adjusted odds ratio for anaemia (Hb <11g/dl) for the various risk factors.

Factor	AOR	95% CI
Age		
<5	ref	
5- 14	0.13	0.05- 0.32
15- 24	0.06	0.02- 0.20
25- 34	0.17	0.07- 0.47
34- 54	0.14	0.05- 0.37
55+	0.15	0.05- 0.46
Hookworm		
Negative	ref	
Positive	4.27	2.15- 8.47
Household with iron roof		
No	ref	
Yes	0.21	0.08- 0.55

Table 5.5: Adjusted odds ratio (AOR) for moderate to severe anaemia compared with non-anaemic

5.1.2 Summary to the baseline findings

In summary, anaemia is a common public health problem in this community. It seems to be influenced by age, sex, geohelminths (hookworm- in particular), malaria and socio-economic status. In addition, over 40% of boys and girls in this community are either stunted and/or underweight. These results suggest a need for innovative approaches to the control of anaemia in this population. A detailed discussion of these findings and alternative control measures is presented in **Chapter 9**.

Chapter 6

6.1 The impact of a single mass drug administration to eliminate lymphatic filariasis in the lower Shire Valley- Chikwawa District- southern Malawi

A second survey to evaluate the impact of a single round of mass drug administration in Chikwawa District- southern Malawi was carried out between 13- 15 months post MDA. A total of 1642 individuals were recruited to the study. Of these, 831 were recruited from control villages. In the control villages 466 (56.1%) participants were female. Of the 811 participants recruited from the intervention villages 436 (54.6%) were female. There was no difference in the composition of the two groups by sex (X^2 chi= 0.5 p=0.47). Similarly there was no difference in the mean age between participants from the control and intervention villages (mean age 20.5 vs 21.9 years, Student ttest t= -1.61 p= 0.11). The age and sex distribution of the study participants is shown in Figure 6.1. The general pattern in the distribution of age-specific proportions is similar between the two groups except among the 30-39 age group where there are more females from the control villages compared to the intervention and vice versa in the 40- 49 age group. A similar pattern is seen in males but in reverse with more males from the intervention villages amongst the 30-39 age group. The characteristics of the recruited population are outline in Table 6.1.

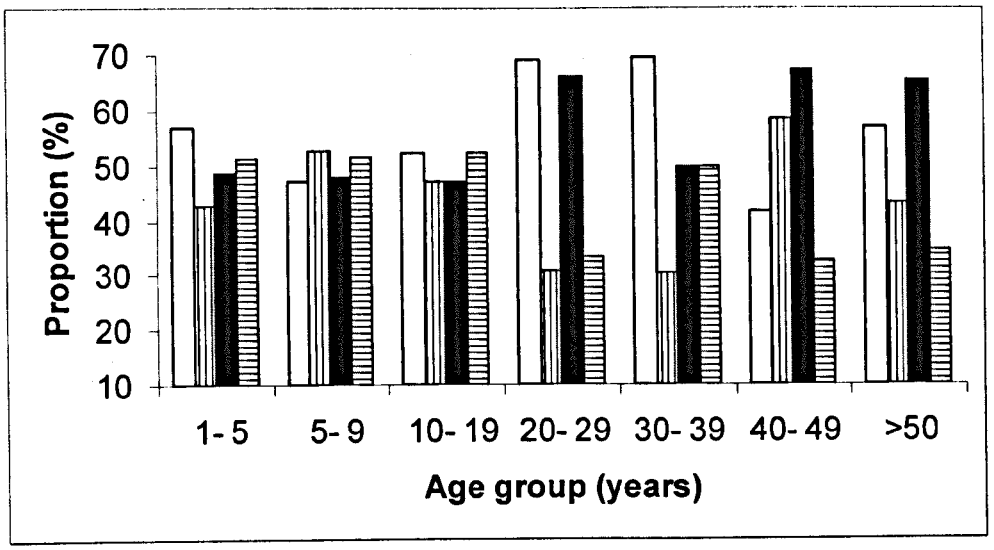


Figure 6.1: Age and sex distribution of participants recruited into the post-MDA survey; female-control (□), male-control (▥), female-intervention (■) and male-intervention (▤)

Table 6.1: Characteristics of the recruited population

Characteristic	Control villages		Intervention villages		Pooled
	Male	Female	Male	Female	
Study population					
Total recruited (%)	365 (44.0)	465 (56.0)	368 (45.4)	443 (54.6)	1642 (100)
Age (years)					
Mean (range)	20.0 (1- 73)	20.9 (1-79)	19.6 (1- 98)	24.0 (1- 81)	21.3 (1- 98)
Median	12.3	15.3	12.4	20.4	14.4
Anaemia (n= 1358)					
Mean haemoglobin in g/dl (range)	11.6 (4.4- 22.2)	10.8 (2.2- 15.4)	12.0 (6.7- 17.2)	11.2 (3.8- 15.7)	11.3 (2.2- 22.2)
Anaemic (%)	122 (38.4)	210 (50.9)	82 (29.9)	145 (41.1)	559 (41.2)
Moderate to severe anaemia (%)	12 (3.8)	27 (6.5)	7 (2.6)	13 (3.7)	59 (4.3)
Enteric parasites (n= 1415)					
Hookworm positive (%)	84 (25.7)	109 (27.1)	53 (17.9)	78 (20.1)	324 (22.9)
S. Mansoni positive (%)	16 (4.9)	28 (7.0)	8 (2.7)	19 (4.9)	71 (5.0)
A. lumbricoides positive (%)	10 (3.1)	6 (1.5)	3 (1.0)	2 (0.5)	21 (1.5)
Malaria (n= 1516)					
MPS Positive (%)	39 (11.5)	43 (9.7)	41 (12.5)	48 (11.9)	171 (11.3)
Episode of Fever within past 1 month (%)	116 (31.8)	161 (34.6)	104 (28.3)	135 (30.5)	516 (31.4)
S. haematobium					
Number positive (%)	41 (13.4)	66 (17.7)	58 (24.5)	68 (23.7)	233 (19.4)
LF antigen (ICT)					
Number positive (%)	123 (38.8)	138 (34.4)	93 (35.6)	97 (30.2)	451 (34.7)
Bed net use (n= 1641)					
Slept under a net the previous night (%)	87 (23.8)	93 (20.0)	66 (17.9)	77 (17.4)	323 (19.7)

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6.1.1 MDA coverage

Assessment of coverage of deworming treatment was done at baseline and follow-up surveys. At the baseline survey participants were asked if they had received deworming treatment from the routine health services or other community based programmes within 12 months prior to the survey. It was established that there had been no mass treatment programmes in this area in the preceding years. However, at the baseline survey up to 7.6% (95/1248) individuals had reported having received deworming treatment within the previous year. There was no difference in the number of people having been treated between the control [8.8% (61/690)] and intervention villages [6.1% (34/558)] (X^2 chi= 3.3 p= 0.07). At the follow-up survey in the intervention villages deworming had increased to 46.5% (308/662) of the target population and in the control villages it went up to 12.9% (83/644). In the control villages most of those treated were individuals who had been screened in the previous year and were found to be harbouring intestinal helminths. Thus on ethical grounds they had to receive appropriate treatment. The coverage found at the follow-up survey in the intervention villages was markedly different from what was determined at a coverage survey carried out within one week after the mass treatment. During this survey an average of 78.5% coverage of the target population was found.

6.1.2 Impact of a single MDA on anaemia in Chikwawa District

Haemoglobin results are available for 1358 individuals (82.7% of the recruited population). Of these, 559 (41.2%) had anaemia (Hb <11g/dl). Overall, the anaemic individuals were significantly younger than the non-anaemic (mean

age in years 16.7 vs 24.6 Student ttest t = 8.21 p= <0.001). Amongst the anaemic cases 355 (63.4%) were female. In addition the proportion of anaemic females [46.3% (355/766)] was significantly higher (X^2 chi= 19.8 p <0.001) than that for their male counterparts [34.5% (204/592)].

6.1.2.1 Anaemia prevalence by treatment area

Results for haemoglobin are available for 731 and 627 individuals from control and intervention villages respectively. There was no difference by sex in the composition of the two groups. Up to 413 (56.5%) individuals from the control and 353 (56.0%) from the intervention villages were female (X^2 chi= 0.03 p= 0.86). Similarly, there was no difference in age distribution between the two groups [control mean age in years= 20.6 (95% CI 19.4- 21.9) vs intervention mean age in years= 22.2 (95% CI 20.7- 23.6) student ttest t= -1.6, p= 0.06]. Of the participants recruited from control villages, 332 (45.4%) were anaemic and the corresponding number in the intervention villages was 226 (36.3%). This difference in proportion of individuals with anaemia was statistically significant (X^2 chi= 11.6 p= 0.001). Figure 6.2 shows the proportion of individuals by respective haemoglobin level. Of note, due to small numbers, the difference in proportion seen amongst the moderate to severe anaemia category was not statistically significant.

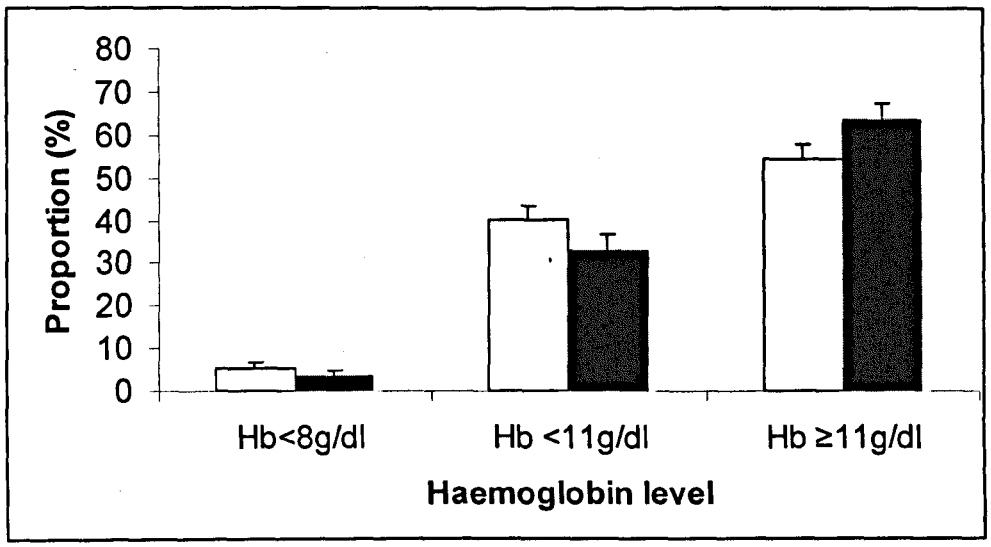


Figure 6.2: Proportion of individuals by haemoglobin level from the control (□) and intervention (■) villages at 12- 14 months post MDA (error bars are 95% CI).

However, it is worth mentioning that the proportion of individuals in respective haemoglobin categories (outlined above) at baseline between the two groups was similar (shown in Figure 6.3) and the overall proportion of anaemic persons was not different (control: 42.21% vs intervention: 43.22% X^2 chi= 0.13 p= 0.72).

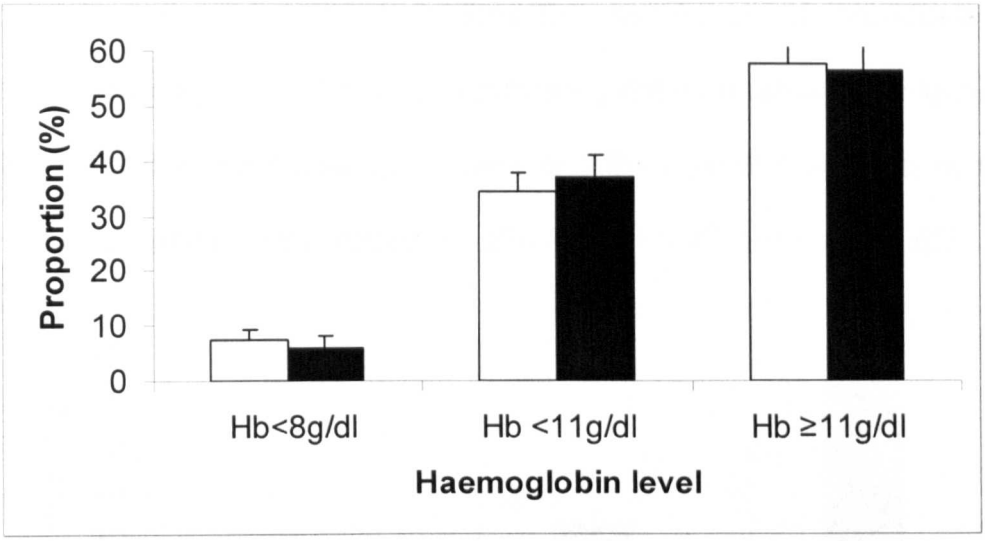


Figure 6.3: Proportion of individuals by haemoglobin level at baseline from the control (□) and intervention (■) villages

Also noteworthy is the fact that there was no difference in proportion by haemoglobin level between baseline and follow-up surveys in the control villages (as shown in Figure 6.4) as well as the overall proportion of anaemic individuals (42.21% vs 45.4% X^2 chi= 1.47 p= 0.22).

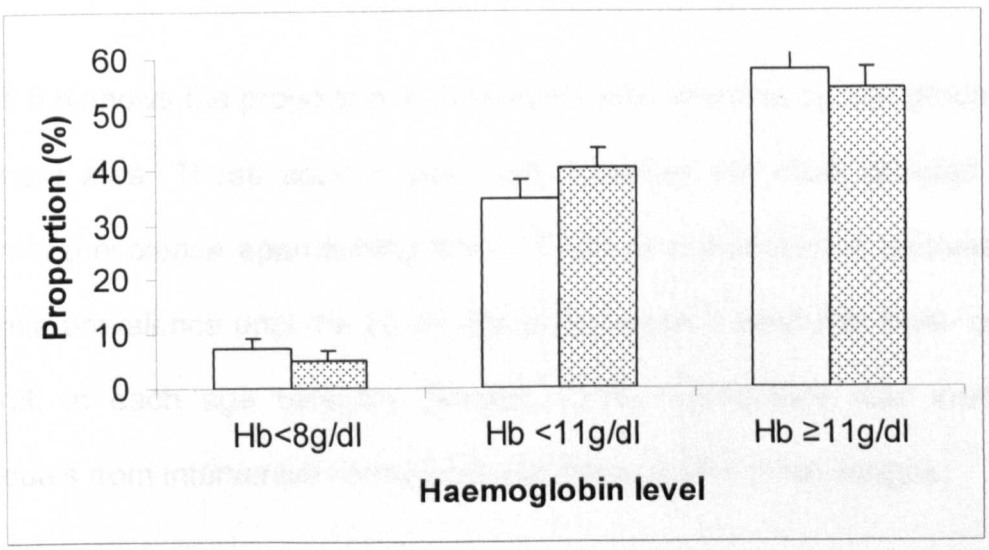


Figure 6.4: Comparison of proportion of individuals with respective haemoglobin level from control villages at baseline (□) and after 14 months (▨).

In contrast, in the intervention villages the distribution of individuals with respective haemoglobin levels was significantly different (shown in Figure 6.5) between baseline and follow-up surveys and the overall proportion of those anaemic was significantly reduced (43.2% vs 36.4% X^2 chi= 5.6 p=0.02).

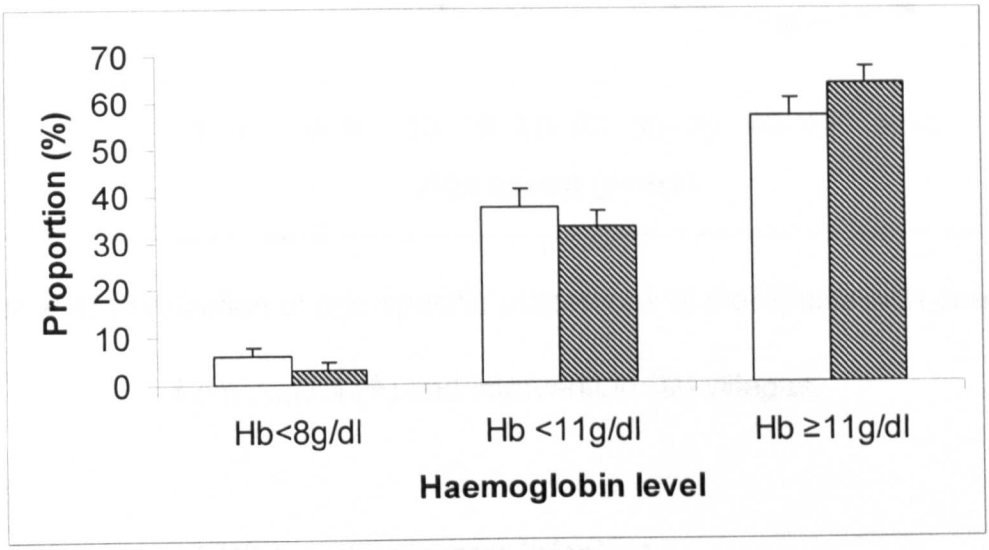


Figure 6.5: Proportion of individuals with respective haemoglobin levels from intervention villages at baseline (□) and after 14 months- post MDA (▨)

Figure 6.6 shows the proportion of individuals with anaemia by age group and treatment area. Those aged 5 years and younger are most affected with anaemia prevalence approaching 80%. There is a progressive decrease in anaemia prevalence until the 20-29 age group when it seems to level- off. In general, in each age category (except 10-19), prevalence was lower in individuals from intervention compared with those from control villages.

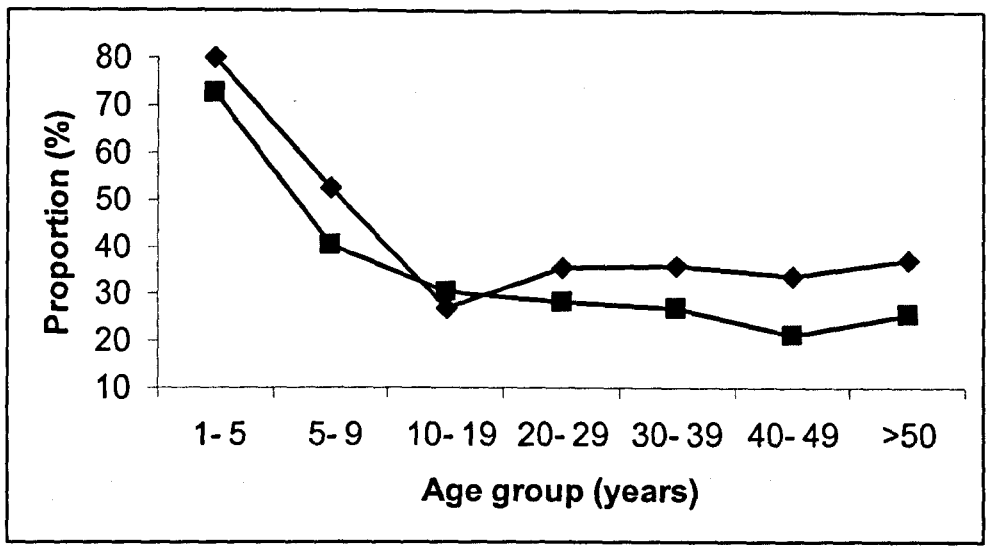


Figure 6.6: Distribution of age-specific proportions of individuals with anaemia from control (♦) and intervention (■) villages.

6.1.3 Impact of MDA on hookworm infection

Hookworm infection status was evaluated in 1409 (86.2% of the recruited study population) individuals. Of these, 729 were recruited from control villages. Of these participants from the control villages 402 (55.1%) were female. The corresponding figures from the intervention villages were 680 recruited and 388 (56.3%) females. There was no difference in the sex mix of the two groups (X^2 chi= 0.2 p= 0.7). Similarly, there was no difference in age between the two groups [mean age in years; control 21.0 (95% CI 19.8- 22.3 vs intervention 22.5 (95% CI 21.1- 23.9) Student ttest t= -1.5 p= 0.1].

Overall, a total of 323 (22.9%) individuals had hookworm ova detected in their stool. This was a significant (X^2 chi= 8.6 p= 0.003) increase from the overall prevalence of 18.2% (202 out of 1113 individuals) found at baseline. The respective prevalence of hookworm by treatment area was 26.5% (192/729) in the control villages and 19.1% (130/680) in the intervention villages. The

hookworm prevalence for the control villages was significantly (X^2 chi= 10.8 p= 0.001) higher than that for the intervention villages. This contrasts with the situation found during baseline survey. At this survey period the prevalence in the control villages was 17.3 % (106/613) which was not significantly different (X^2 chi= 0.58 p= 0.45) from 19.1% (96/504) found in the intervention villages. The hookworm prevalence between the two surveys was significantly higher (X^2 chi= 16.2 p= <0.001) at follow-up in the control villages whereas there was no difference in the intervention villages (X^2 chi= 0.001 p= 0.98) as shown in Figure 6.7.

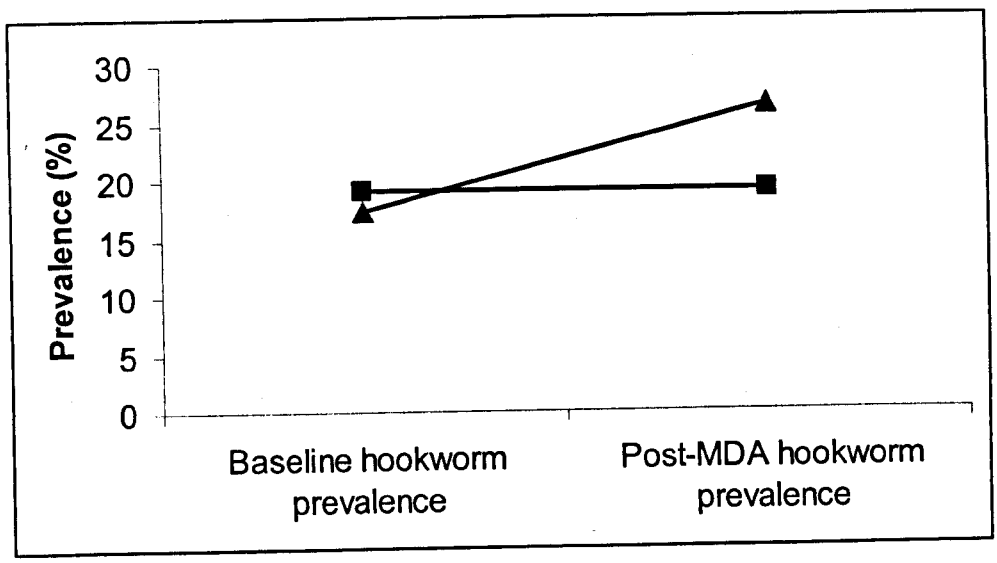


Figure 6.7: Showing hookworm prevalence at baseline and post MDA in control (▲) and intervention (■) villages.

Age-specific proportions of individuals infected with hookworm by treatment area are presented in Figure 6.8. In both sets of villages prevalence increases with age. In those aged less than 20 years, prevalence is similar. However, in the over 20's, the prevalence in the control is consistently higher than in the intervention villages across the age range. This contrasts with the observation made during baseline survey when prevalence was remarkably similar across the age range as shown in Figure 6.9.

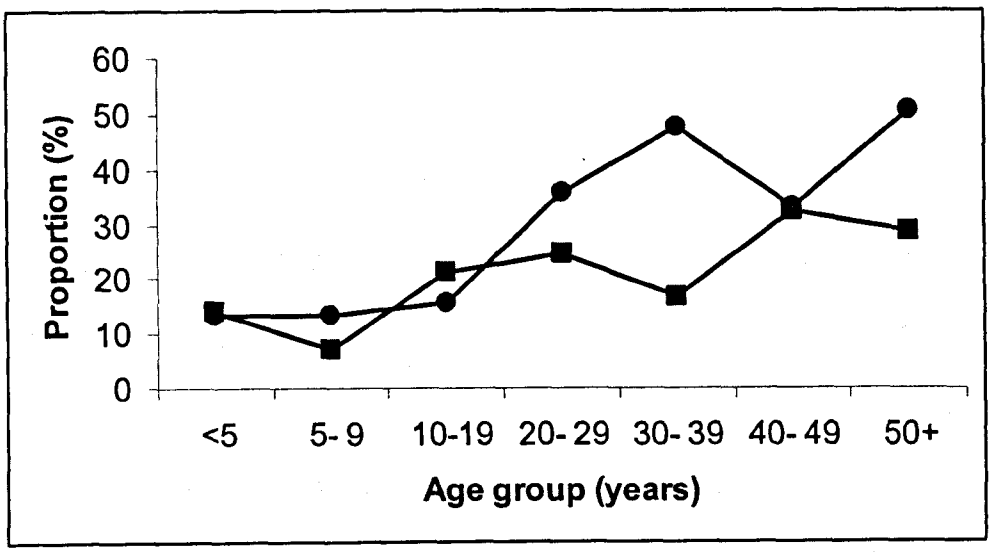


Figure 6.8: Age- specific proportion of individuals with hookworm infection at follow-up from the control (●) and intervention (■) villages.

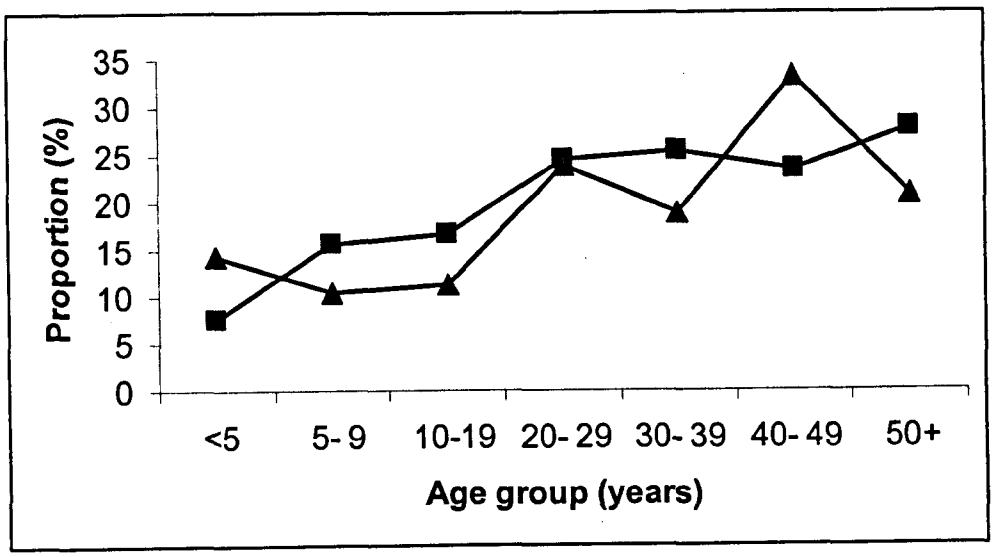


Figure 6.9: Proportion of individuals with hookworm infection at baseline from the control (▲) and intervention (■) villages.

Distribution of hookworm intensity of infection, based on eggs per gram (epg) of stool according to WHO recommended categories, for all study participants with stool results is presented in Figure 6.10. There is over-dispersion of egg counts with the majority of cases having zero counts and few in the high intensity category. Though the general pattern in the distribution of egg counts is similar between control and intervention villages (shown in Figure 6.11) the proportion with light intensity is lower in the intervention villages and correspondingly higher amongst the negative. The pattern in the heavily infected group is not clear due to small numbers. This observation is confirmed by a significantly lower median epg for intervention as compared to control villages [intervention median 96 epg and control median 144 epg (median test; X^2 chi= 4.23 = 0.04)].

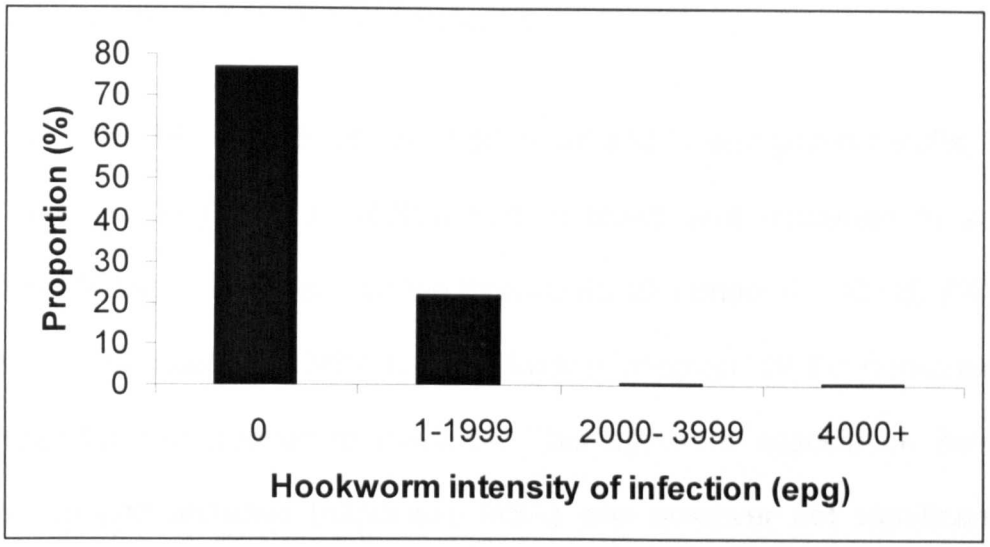


Figure 6.10: Proportion of individuals in respective hookworm intensity of infection (epg) categories from control and intervention villages combined.

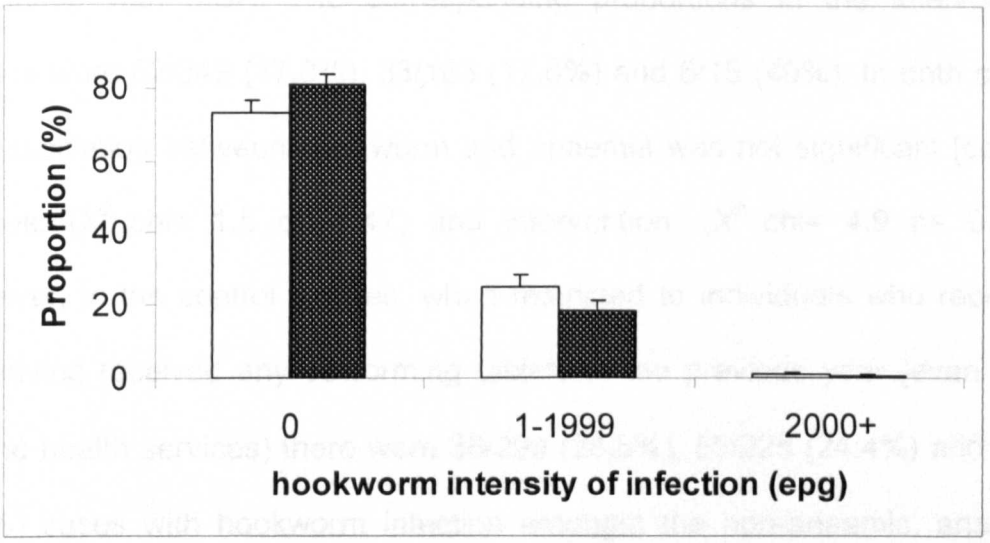


Figure 6.11: Proportion of individuals in respective hookworm intensity of infection (epg) categories from control (□) and intervention (■) villages.

6.1.3.1 Hookworm infection and anaemia

Amongst the 1192 individuals with both stool and haemoglobin results, there were 444 (37.3%) and 51 (4.3%) had anaemia and moderate to severe anaemia (MSA) respectively. Of the individuals with anaemia, 92 (20.7%) and 16 (31.4%) of those with MSA had hookworm infection. Of the non-anaemic, 157 (22.5%) had hookworm infection. This apparent association between hookworm and anaemia (especially MSA) was however not significant (X^2 chi= 3.1 p= 0.21). The pattern was similar when stratified by treatment area. In the control villages, there were 95/348 (27.3%), 59/256 (23.1%) and 10/36 (27.8%) cases with hookworm infection amongst the non-anaemic, anaemic and those with MSA. The corresponding proportions in the intervention villages were 62/349 (17.8%), 33/188 (17.6%) and 6/15 (40%). In both cases the association between hookworm and anaemia was not significant [control villages: (X^2 chi= 1.5 p= 0.47) and intervention: (X^2 chi= 4.9 p= 0.09)]. However, in the control villages, when restricted to individuals who reported not having received any deworming tablets in the previous year (even from routine health services) there were 86/299 (28.8%), 55/225 (24.4%) and 2/26 (7.7%) cases with hookworm infection amongst the non-anaemic, anaemic and those with MSA respectively. This was significant (Fisher's exact test, p= 0.04). This suggests an association between hookworm and anaemia. In contrast when restricted to those who reported having taken MDA tablets in the intervention villages there were 26/299 (15.5%), 11/66 (16.7%) and 0/2 (0%) cases with hookworm infection amongst the non-anaemic, anaemic and those with MSA respectively. This was not statistically significant (Fisher's exact test, p= 0.9). In addition, it is worth noting that in this population there

appears to be a decreasing trend of mean haemoglobin with increasing hookworm intensity of infection as shown in Figure 6.12.

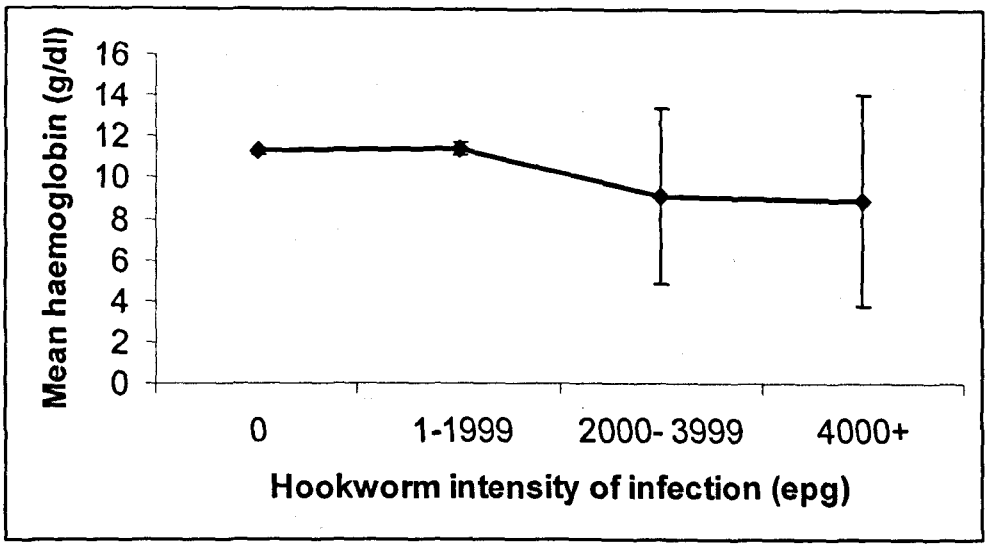


Figure 6.12: Mean haemoglobin by hookworm intensity of infection category (error bars are 95% Confidence Intervals)

6.1.4 Impact of MDA on other enteric parasites

Of 1410 samples examined 71 (5.0%) were positive for *S. mansoni*. Of the positive cases 47 were female. There was no difference in prevalence between males and females (X^2 chi= 3.3 p= <0.07). The age-specific prevalence of *S. mansoni* is presented in Figure 6.13. Prevalence tends to increase with increasing age until the 20- 29 age group when it starts to fall. Of note all the *S. mansoni* cases were found in 4 of the 18 villages (see Figure 6.13b). The 4 villages are within a 5 kilometre radius of each other. *A. lumbricoides* occurred in 21 (1.5%) of the 1410 stool samples. There were 8 and 13 positive cases among the females and males, respectively. Prevalence remained under 5% across the age range as shown in Figure

6.13. The prevalence of *S. mansoni* and *A. lumbricoides* was not different between baseline and follow-up. In addition there were 9 cases of Taenia and 3 of *Enterobius vermicularis.*

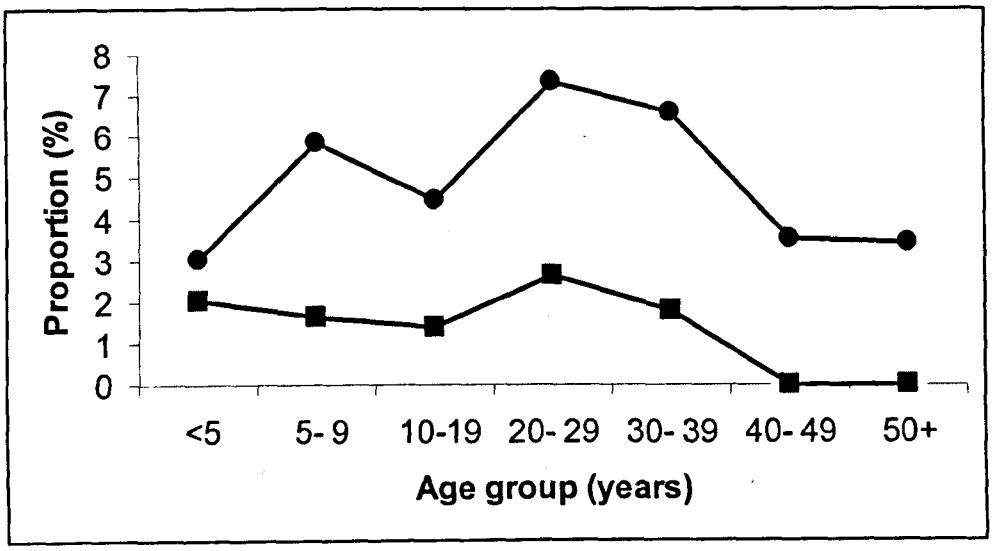


Figure 6.13: Age-specific prevalence for *S. mansoni* (●) and *A. lumbricoides* (■) from the follow-up survey irrespective of treatment area.

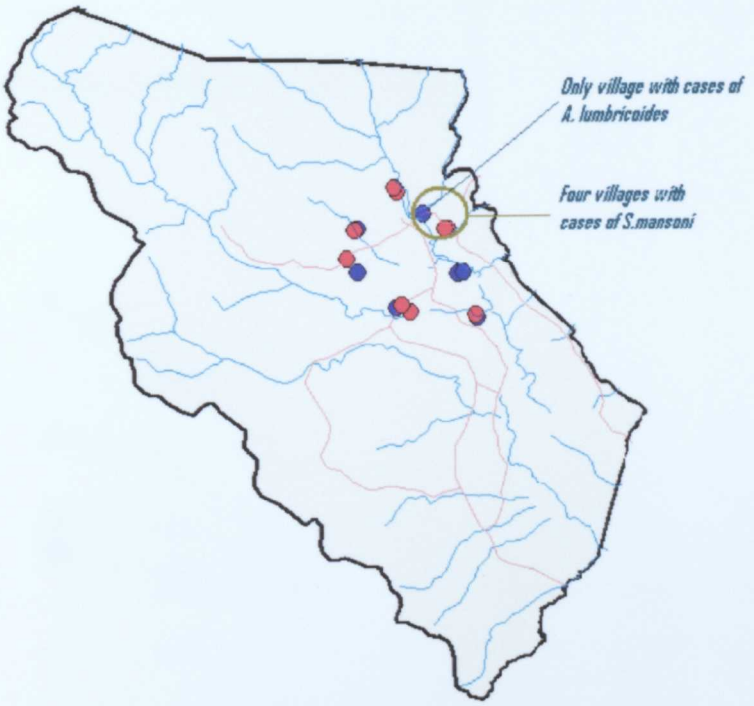


Figure 6.13b: Map of Chikwawa District showing the four villages that had cases of *S.mansoni* and the only village that had *A. lumbricoides* infected individuals

6.1.5 Malaria parasitaemia at follow-up

Thick blood smears were examined for 1362 individuals. Of these, 139 (10.2%) were positive for *P. falciparum*. Amongst the positive cases 77(55.4%) were female. The female excess amongst the positive cases was not significant (X^2 chi= 0.081 p= 0.78). However, the positive cases were significantly younger than those who were negative for *P. falciparum* (mean age in years 21.6 vs 14.5 Student ttest t= 4.5 p <0.001). This observation can be appreciated in Figure 6.14 which shows the age- specific proportions of individuals positive for malaria parasitaemia (pooled as well as broken down by density). Consistent with observations made at baseline both prevalence as well as density of infection is highest in the youngest age group. Of note

none of those aged over 20 years was found to have a malaria parasitaemia density of 500 or more parasites per µL of blood.

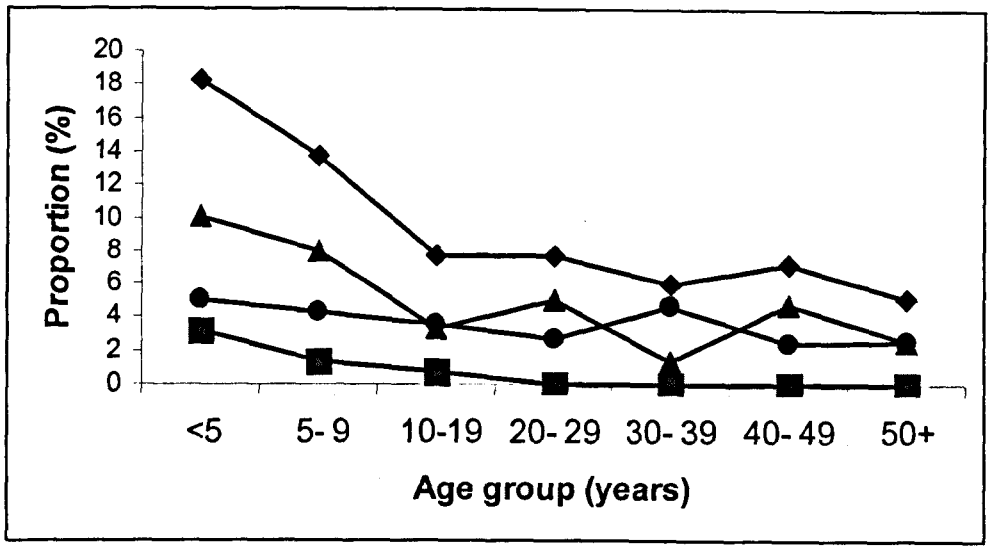


Figure 6.14: Showing age-specific proportion of individuals with malaria parasitaemia: overall (♦), those with 1-50 parasites/ µL (●), those with 51-500 parasites/ µL (▲) and those with >500 parasites/ µL (■)

6.1.5.1 Malaria parasitaemia and anaemia at follow-up

Overall those positive for *P. falciparum* were more anaemic than their negative counterparts with up to half a gram difference in their respective means (mean hb 10.8 vs 11.3 Student ttest t= 2.6 p <0.008). Though mean haemoglobin tended to decrease with increasing density of parasitaemia with the highest category having the smallest mean. However, the numbers in this category are small and hence the estimate has a wide confidence interval. Similarly, this pattern did not vary between control and intervention villages as shown in Figure 6.15.

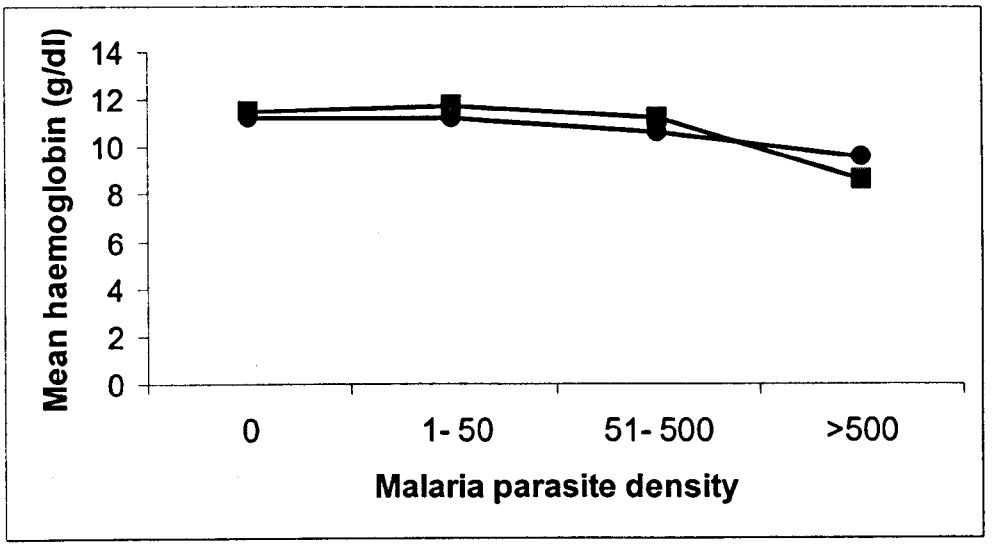


Figure 6.15: Mean haemoglobin (g/dl) by malaria parasite density (per µL of blood) and treatment area [control (●) and intervention (■) villages]

6.1.6 S. haematobium at follow-up survey

An overall prevalence of 19.2% for *S. haematobium* was found from a total of 1273 urine samples. Up to 9 (0.7%) cases had high intensity (50 or more eggs per 10 ml of urine) and 235 (18.5%) were of light to moderate intensity of infection. There was no difference in prevalence by sex [male 19.2% vs female 20.2% (X^2 chi= 0.88 p= 0.6)] and the sex and age distribution of the infected cases was accordingly similar as shown in Figure 6.16. In general those infected were younger [median age in years 22.9 vs 19.4 (median test X^2 chi= 5.8 p= 0.02)]. *S. haematobium* infection prevalence rose rapidly from the under-fives to a peak of approximately 25% in those aged between 5 and 10 years (see Figure 6.16). This then started to progressively decrease to less than 15% amongst those over 40 years of age. The egg-count distribution was typically over-dispersed with 95.1% of the infected cases falling in the 1-9

eggs per 10 ml of urine category shown in Figure 6.17. Children (> 5 years of age) and adolescents harboured most of the high intensity infections.

Overall, there was no difference in proportion of anaemic individuals by *S. haematobium* infection status [positive; 40% vs negative; 39.5% X^2 chi= 0.02 p= 0.9) and the mean haemoglobin did not vary by intensity of infection category as shown in Figure 6.18. Note there were only 7 cases in the 50+ category and none of them was anaemic.

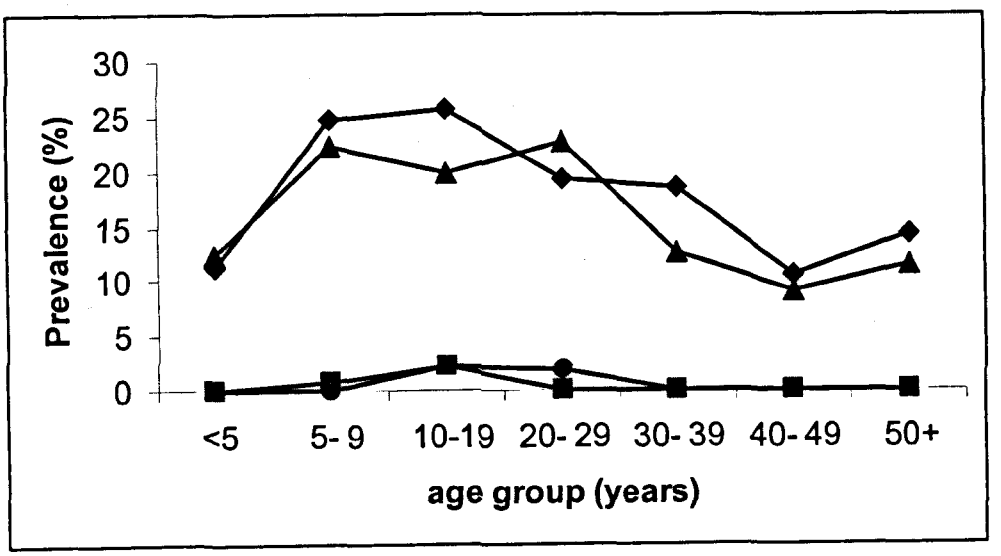


Figure 6.16: Sex and age- specific proportions of individuals with *S. haematobium* infection; mild to moderate-males (▲), high intensity (≥ 50 eggs/ centilitre of urine)- males (●), mild to moderate-females (♦), high intensity (≥ 50 eggs/ centilitre of urine)-females (■).

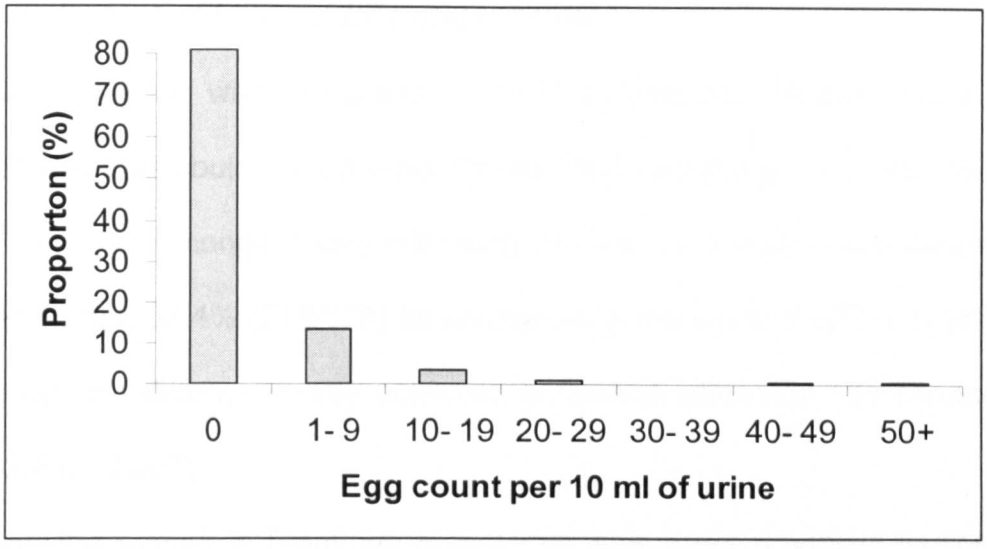


Figure 6.17: Distribution of *S. haematobium* egg-counts per 10 ml of urine from the follow-up survey.

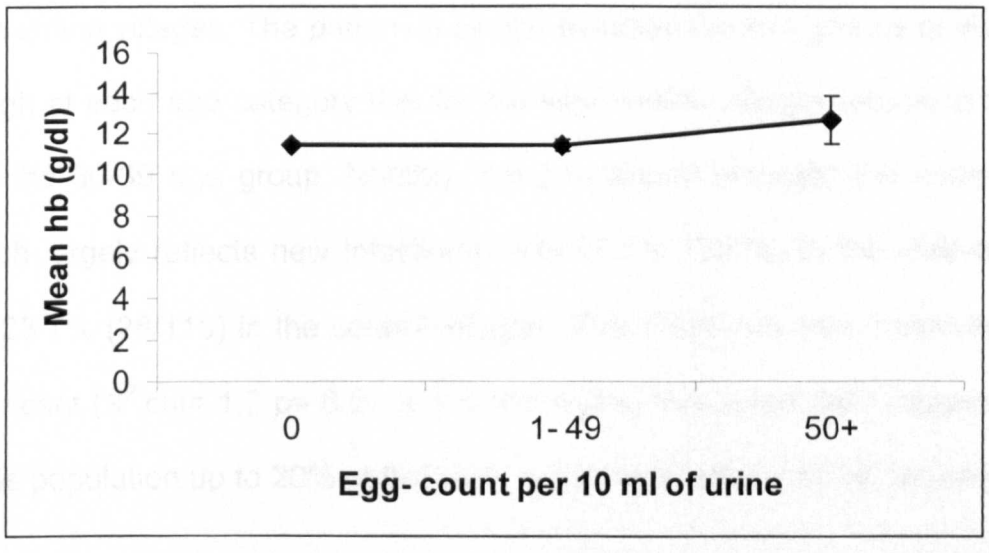


Figure 6.18: Mean haemoglobin by egg-count per 10 ml of urine category. Note there were only 7 cases in the 50+ category and none of them was anaemic.

6.1.7 Impact of MDA on LF antigenaemia

LF antigenaemia was evaluated in 1321 individuals. Twenty cards were invalid and thus could not be read. Of the 1301 remaining cards 451 (34.7%) were positive. Amongst those with data on sex, LF antigen prevalence was higher in men 37.4% (216/578) as compared to the women 32.5% (234/723). However this difference only achieved borderline statistical significance (X^2 chi= 3.4 p= 0.067).

Though the overall ICT antigen prevalence was lower amongst participants from intervention villages- 32.7% (190/582) as compared to the control villages- 35% (261/719), this was not statistically significant (X^2 chi= 1.9 p= 0.2). Generally, ICT antigen prevalence increased monotonically with age. Figure 6.19 shows age-specific LF antigen prevalence for control and intervention villages. The pattern is similar between the two groups of villages though at each age category that for the intervention villages remained lower until the 30-39 age group. Notably, the prevalence amongst the underfives (which largely reflects new infections) was 16.0% (12/75) in the intervention and 23.7% (28/118) in the control villages. This difference was, however, not significant (X^2 chi= 1.7 p= 0.2). It is worth noting that these data suggest that in this population up to 20% of the underfives are infected with *W. bancrofti.*

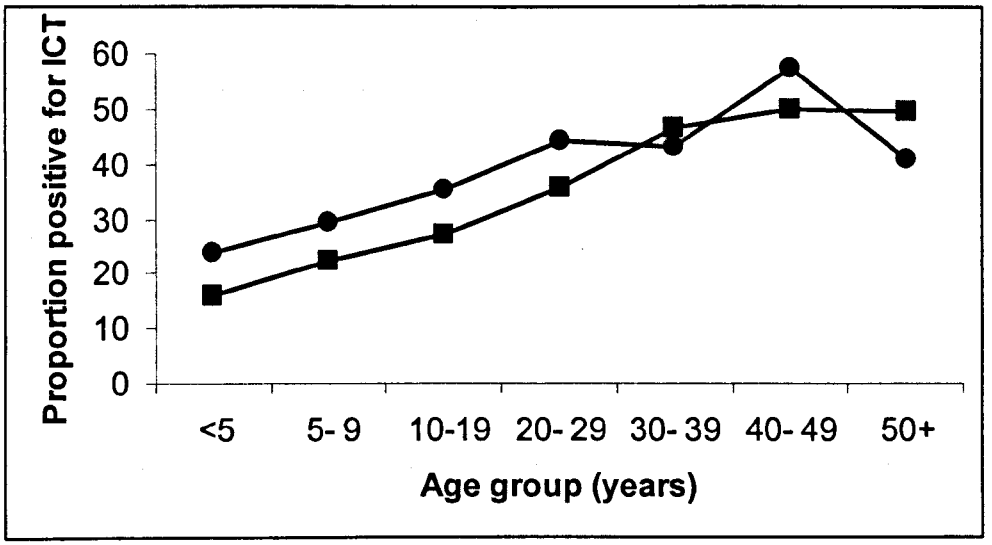


Figure 6.19: Age-specific proportion of individuals positive for LF antigen by ICT for control (●) and intervention (■) villages

6.1.8 Impact of MDA on microfilaraemia

Post- MDA microfilaraemia assessment surveys were carried out in selected villages from both control and intervention areas at night from 21 hours to 2 am. A total of 364 individuals were assessed. Of these, 186 were recruited from control villages. Amongst the 188 participants from intervention villages, 58 (30.9%) were female. In the control villages there were 60 (32.3%) females. There was no difference by sex between the two groups (X^2 chi= 0.42 p= 0.80). Similarly, there was no difference in age distribution between the two groups (mean age in years in control villages: 19.8 vs intervention 21.2, Student ttest t= -1.00 p= 0.32). Overall there were 87 (23.3%) participants positive for microfilaria. There were 52 (28.0%) cases positive in the control villages while as in the intervention villages 35 (18.6%) individuals were found to be carrying microfilaria. The difference in microfilaria prevalence at the follow-up phase, between the two village groups, was

statistically significant (X^2 chi= 4.57 p= 0.03). Of note, however, the difference between baseline and follow-up microfilaria prevalence for the control [31.9% vs 28.0% (X^2 chi= 0.6 p= 0.44)] and intervention [26.5% vs 18.6% (X^2 chi= 1.9 p= 0.2)] villages was not significant.

An assessment of microfilarial density at follow-up found a significant difference between the two groups of villages. The microfilaria geometric mean for the control villages was 545 mf/ml (range 10- 4810) of blood and that for the intervention villages was 346 mf/ml (10- 4190). The microfilaria count was significantly lower in the intervention villages than the control villages [Wilcoxon ranksum (Mann-Whitney) test z= 2.5, p= 0.01]. In addition there was a 30% reduction in microfilarial density in the intervention villages at follow-up compared with the baseline (492 mf/ml) shown in Figure 6.20. In contrast there was a 5.1 % increase in microfilarial density in the control villages at follow- up (control villages; baseline geometric mean 518 mf/ml).

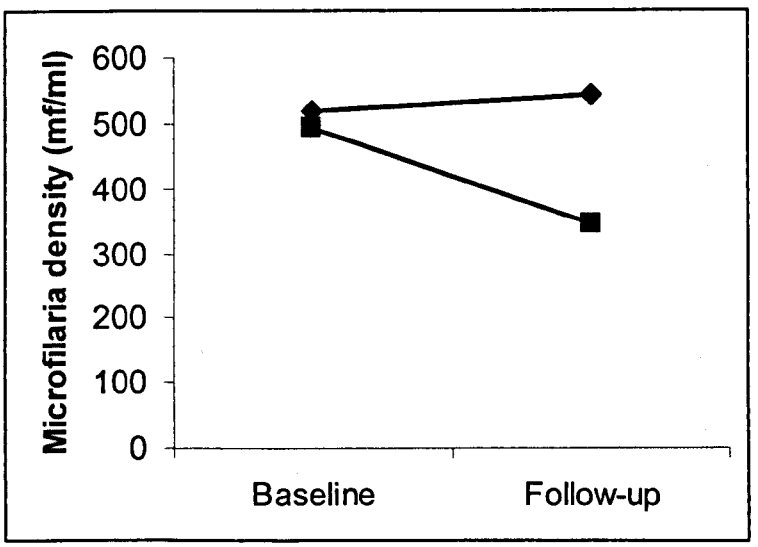


Figure 6.20: Microfilaria density (mf/ml of blood) at baseline and follow-up surveys in the control (♦) and intervention (■) villages.

6.1.9 Impact of MDA on anthropometric indices

Anthropometric indices were determined in children aged between 3 and 18 years in accordance with the EpiInfo 2000 Nutristat software restrictions. There were 808 children with 409 (50.6%) girls. Of these 422 were recruited from control villages. There was no difference in the composition of the groups by sex (X^2 chi= 0.81 p= 0.37). The mean body weight for children from the control villages was 24.3 kg and that for the intervention villages was 24.6 kg. The difference was not statistically significant (Student ttest t= -0.32 p= 0.75). Similarly mean body mass index (BMI) was identical between the two groups (16.2 vs16.2 Student ttest t= 0.18 p= 0.85). In addition, the proportion underweight [control; 28.9 % vs intervention; 29.0% (X^2 chi= 0.001 p= 0.97)] and stunted [control; 37.4 % vs intervention; 36.3% (X^2 chi= 0.12 p= 0.7)] were not different between the control and intervention villages. The age-specific mean z-scores for weight-for-age (WAZ) and height-for-age (HAZ) for the control and intervention villages are shown in Figures 6.21 and 6.22. Though it appears as if children from the control villages had better anthropometric indices, this was not statistically significant for both underweight (overall mean z-score WAZ: control -1.32 vs intervention -1.48 Student ttest t= 1.64, p= 0.1) and stunting (overall mean z- score HAZ: control, -1.48 vs intervention, -1.65 Student ttest t= 1.35, p= 0.18).

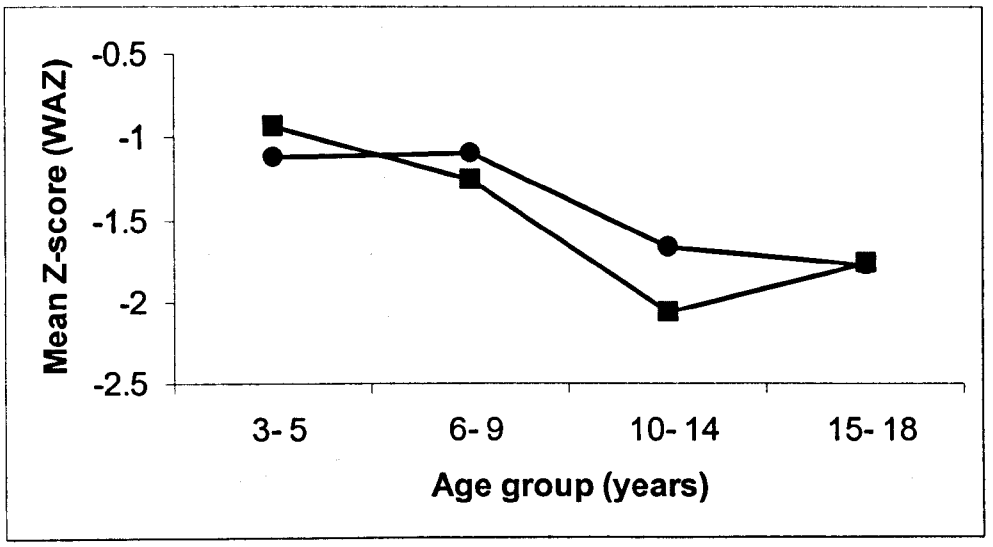


Figure 6.21: Overall mean Z- score for weight for age (WAZ) at follow-up survey from the control (●) and intervention (■) villages

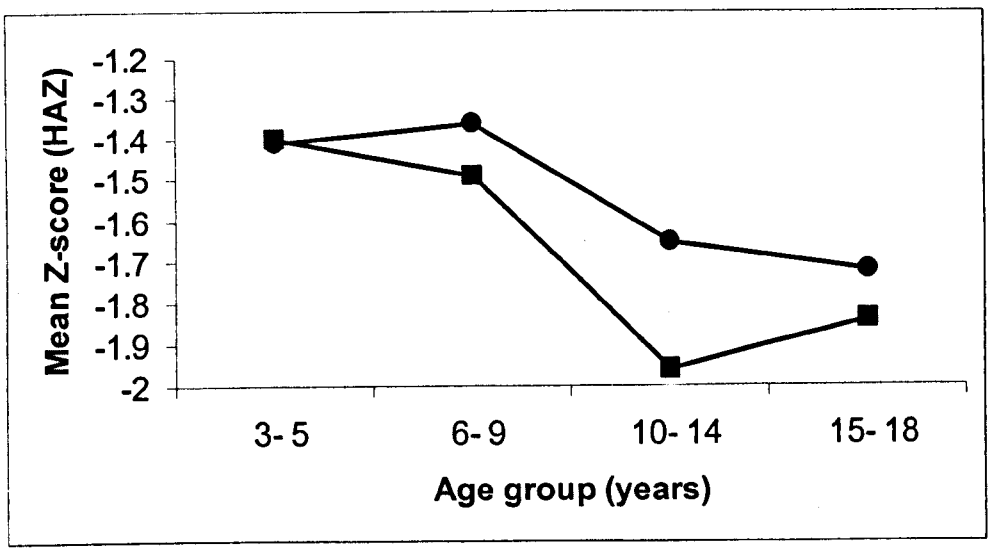


Figure 6.22: Overall mean Z- score for height for age (HAZ) at follow-up survey from the control (●) and intervention (■) villages

Of note, consistent with our findings at the baseline survey, overall age-specific anthropometric indices for girls were largely better and showed some catch-up growth in adolescent years for both weight-for-age (WAZ) and height-for-age (HAZ) while as those for boys got progressively worse and their respective means reached the underweight and stunted cut-off points by age ten (see Figures 6.23 and 6.24). Consequently, the overall proportion for those underweight [girls; 24.9% vs boys; 33.1% (X^2 chi= 6.5 p= 0.01)] and stunted [girls; 32.5% vs boys; 41.4% (X^2 chi= 6.8 p= 0.009)] was significantly lower for the girls as compared to the boys. A similar pattern was observed in the intervention villages for underweight [girls; 23.8% vs boys; 34.0% (X^2 chi= 4.87 p= 0.03)] as well as stunting [girls; 30.7% vs boys; 41.6% (X^2 chi= 4.99 p= 0.03)]. However, in the control villages though the sex-specific patterns were similar to those of the overall and intervention villages for both the underweight [girls; 32.5% vs boys; 41.4% (X^2 chi= 6.8 p= 0.009)] and the stunted [girls; 32.5% vs boys; 41.4% (X^2 chi= 6.8 p= 0.009)] the difference in proportions was not statistically significant for the two indices.

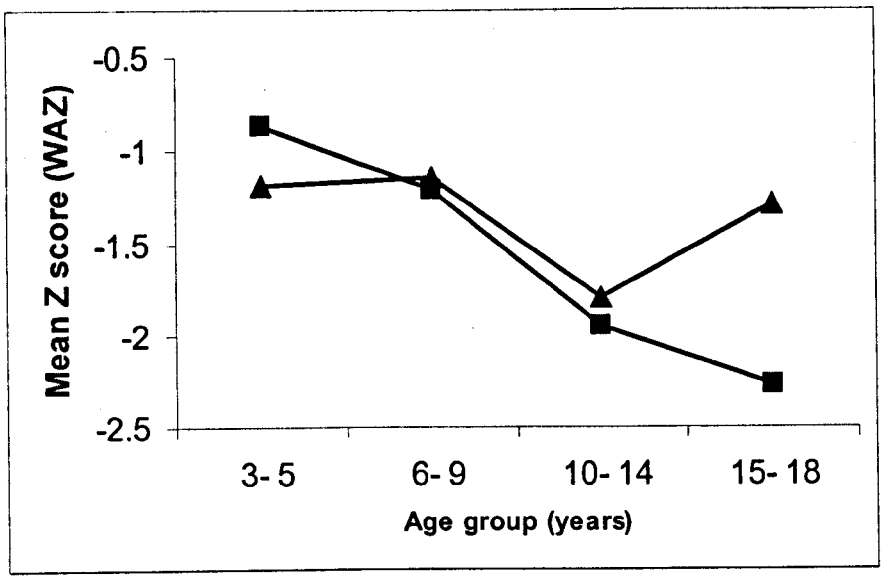


Figure 6.23: Overall mean z-score for weight-for-age (WAZ) for girls (▲) and boys (■) at follow-up survey (-2 s.d is cut-off for underweight).

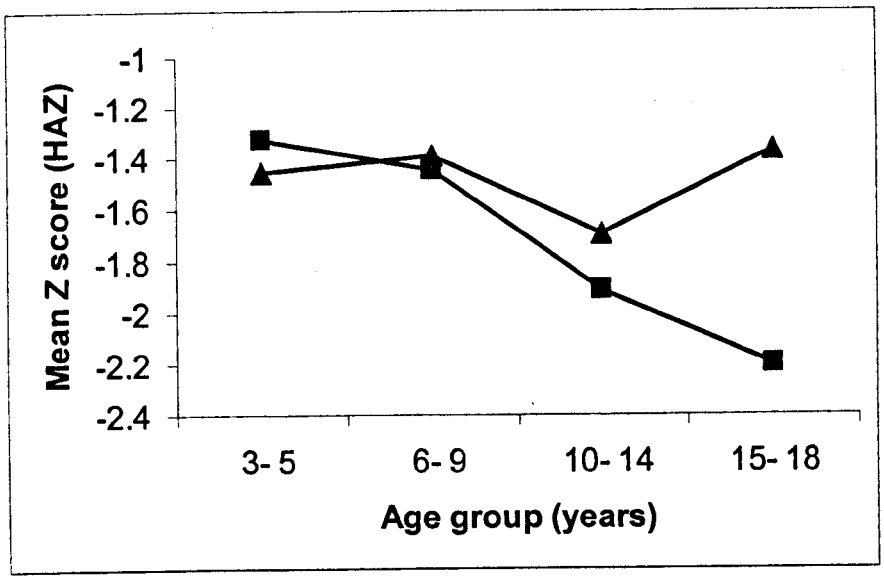


Figure 6.24: Overall mean z-score for height-for-age (HAZ) for girls (▲) and boys (■) at follow-up survey (-2 is cut-off point for stunting).

Table 6.2a presents a summary of the impact of a single MDA on the main indicators in chikwawa District as discussed above.

Prevalence	Control villages	Intervention villages	P- value
Anaemia	45.4	36.3	**0.001**
Hookworm	26.5	19.1	**0.001**
LF antigenaemia	35	32.7	0.20
Microfilaraemia	28.0	18.6	**0.03**
S.mansoni	6.0	4.0	0.48
S.haematobium	15.7	13.8	0.18
Malaria parasitaemia	10.5	12.2	0.56
Anthropometric indices			
Underweight	28.9	29.0	0.97
Stunted	37.4	36.3	0.70

Table 6.2a: Impact of a single MDA on the prevalence of selected indicators

6.1.10 Socio-economic scores of follow-up households

The socio-economic characteristics and overall economic scores of households recruited during the follow-up survey are presented in Table 6.2b and Figure 6.25 respectively. The general characteristics are similar to what was found at the baseline survey. In addition there was no difference in household material ownership between the control and intervention villages shown in Table 6.2b. Similarly, the socio-economic scores are also highly skewed as shown in Figure 6.25. There was no difference in socio-economic scores between control and intervention villages.

Characteristic	Total Number (%)	Control (%)	Intervention (%)
Total number of households recruited	361 (100)	177 (100)	184 (100)
At least 1 member on regular income	72 (19.9)	35 (19.8)	37 (20.1)
Radio in the household	200 (55.6)	91 (51.4)	109 (59.6)
Bicycle in the household	153 (42.7)	70 (39.6)	83 (45.9)
Ownership of animals (cattle and goats)	120 (33.2)	59 (33.3)	61 (33.2)
House with cement floor	34 (9.2)	16 (9.0)	18 (9.8)
House with corrugated iron roof	57 (15.8)	28 (15.8)	29 (15.8)
Borehole as main source of drinking water	346 (99.8)	176 (99.5)	184 (100)
Pit latrine	111 (28.4)	51 (28.8)	50 (27.2)

Table 6.2b: Showing household ownership of various items for intervention and control villages.

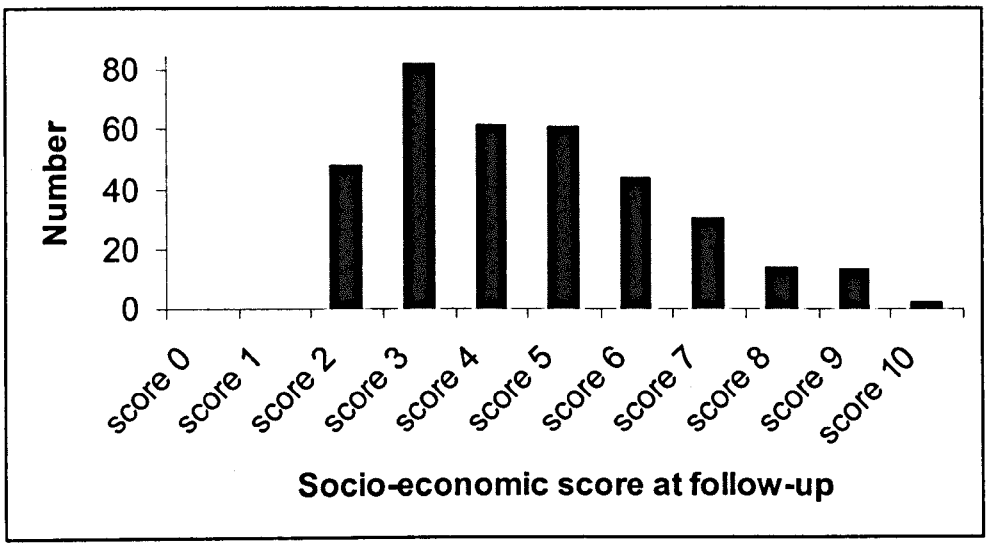


Figure 6.25: The distribution of socio-economic scores of the households sampled at follow-up.

6.1.11 Multivariate analysis for factors affecting haemoglobin level at follow-up

Logistic regression analysis was carried to determine which of the potential risk factors influenced haemoglobin level after pre and post-adjustment for the covariates at follow-up. The crude and adjusted odds ratios are reported in Table 6.3. In the univariate analysis age, sex (for those aged >15 years), hookworm (infection intensity), malaria (infection intensity), history of fever, treatment area and deworming showed significant associations with anaemia (Hb<11 g/dl). Increasing age (categories shown in Table 6.3) was associated with between 88% to 72% reduction in the likelihood of having anaemia (AOR ranged from 0.12- 0.28). This is consistent with what was observed at the baseline survey. Similarly, the relationship between sex and anaemia mirrored that found at baseline. Because of a significant interaction between age and sex, stratum specific odds ratios are reported. Notably in those over 15 years

of age being male was associated with 74% reduction in the risk of having anaemia (AOR= 0.26, 95% CI 0.17- 0.41). In those younger than 15 years, sex did not seem to influence the presence of anaemia. The association between hookworm as a binary (positive vs negative) variable and anaemia was not significant. However, when looked at by intensity of infection, those in the moderate to high intensity category of infection showed a markedly increased risk for having anaemia (Crude OR 4.9 and AOR 12.55). Of note, because of small numbers in this category, the confidence intervals are wide. Malaria parasitaemia showed a similar pattern to hookworm in that those with high intensity infection seemed more likely to have anaemia; however, this relationship was not observed after adjustment (AOR 1.21 95%CI 0.73- 2.02). Similarly the suggested association observed in those with a history of fever more than 3 months previously did not hold after adjustment. In the crude analysis being resident in the intervention villages was associated with a 32% reduction in the likelihood of having anaemia (OR 0.68, 95% CI 0.55- 0.85). However this was not significant after adjusting for other covariates including deworming (that included those in the control villages who had received deworming treatment). In contrast, the protective effect of 'deworming' remained significant even after adjustment (AOR 0.62, 95%CI 0.46- 0.82). *S. mansoni, A. lumbricoides, S. haematobium* and household roofing material was not associated with anaemia at follow-up.

When restricted to those with MSA (hb<8 g/dl) sex, hookworm infection and malaria parasitaemia had a significant association in the univariate analysis. When adjusted for the other independent variables, being male was associated with a 62% protection from having MSA ((AOR 0.38 95%CI 0.20-

0.74). As for hookworm those with heavy infection had over a 20 fold increase in the risk of having MSA (AOR 22.87 95%CI 2.42- 215.6). Though the corresponding confidence intervals are extremely wide this was statistically significant. The crude OR (2.54 95%CI 1.08- 5.97) for a high malaria parasitaemia (>50 parasites/μL of blood) infection suggested a more than two fold increase in the likelihood of having MSA; however, this did remain after adjustment (AOR 2.36 95%CI 0.85- 6.43).

6.1.11.1 Factors influencing hookworm infection at follow-up

The potential factors influencing hookworm infection at follow-up and their adjusted OR are presented Table 6.4. Of these only 'deworming' and age showed a significant association with the risk of having hookworm infection. There was a 40% reduction in the risk of having hookworm ova in stool amongst individuals who had received deworming tablets in the previous year (AOR 0.60 95%CI 0.42- 0.86). Age (notably those over 20) was associated with an increased risk in having hookworm infection. However, this association was only significant for the over 40 (AOR 1.67 95%CI 1.07- 2.62).

Factor	Crude OR	95% CI	Adjusted OR	95% CI
Age				
<5	ref		ref	
5- 9	0.26	0.18- 0.40	0.28	0.18- 0.44
10- 19	0.12	0.08- 0.14	0.12	0.08- 0.19
20- 29	0.14	0.09- 0.22	0.12	0.07- 0.21
30- 39	0.14	0.09- 0.22	0.14	0.08- 0.24
40+	0.1	0.09- 0.20	0.12	0.07- 0.20
Sex effect amongst those aged <15 years				
Female	ref		ref	
male	0.89	0.66- 1.20	0.87	0.63- 1.22
Sex effect amongst those aged ≥15 years				
Female	ref		ref	
male	0.25	0.16- 0.37	0.26	0.17- 0.41
Hookworm intensity				
Negative	ref		ref	
1- 1999 epg	0.91	0.69- 1.21	1.29	0.94- 1.76
>2000 epg	4.9	1.01- 23.72	12.55	1.82- 86.20
S. mansoni				
Negative	ref		ref	
Positive	1.58	0.98- 2.56	1.39	0.83- 2.33

Factor	Crude OR	95% CI	Adjusted OR	95% CI
Ascaris				
Negative	*ref*		*ref*	
Positive	*0.53*	*0.19- 1.52*	*0.44*	*0.15- 1.33*
Malaria parasite density				
Negative	*ref*		*ref*	
1- 50 parasites/µL of blood	*0.65*	*0.37- 1.15*	*0.57*	*0.30- 1.06*
> 50 parasites/µL of blood	*1.60*	*1.02- 2.49*	*1.21*	*0.73- 2.02*
History of fever episodes				
≤1 month	*ref*		*ref*	
1-3 month	*0.94*	*0.65- 1.36*	*0.97*	*0.69- 1.36*
>3 months	*0.74*	*0.56- 0.97*	*0.82*	*0.61- 1.10*
S. haematobium				
Negative	*ref*		*ref*	
Positive	*1.02*	*0.75- 1.38*	*0.93*	*0.65- 1.31*
Household with corrugated Iron roof				
No	*ref*		*ref*	
yes	*0.70*	*0.49- 1.03*	*0.68*	*0.55- 0.90*
Treatment area				
Control villages	*ref*		*ref*	
Intervention villages	*0.68*	*0.55- 0.85*	*0.78*	*0.60- 1.01*

Factor	Crude OR	95% CI	Adjusted OR	95% CI
Deworming				
No	*ref*		*ref*	
Yes	*0.58*	*0.45- 0.75*	*0.62*	*0.46- 0.82*

Table 6.3: Crude and adjusted OR's for the potential risk factors for anaemia at the follow-up survey.

Factor	Adjusted odds ratio (AOR)	95% CI
Treatment area		
Control villages	*ref*	
Intervention villages	*1.14*	*0.86- 1.52*
Deworming		
No	*ref*	
Yes	*0.60*	*0.42- 0.86*
Sex		
Female	*ref*	
Male	*1.19*	*0.9- 1.57*
Age		
<5	*ref*	
5-9	*0.54*	*0.34- 0.88*
10- 19	*0.94*	*0.60- 1.49*
20- 29	*1.23*	*0.75- 2.02*
30- 39	*1.34*	*0.80- 2.25*
40+	*1.67*	*1.07- 2.62*
House with iron roof		
No	*ref*	
Yes	*1.02*	*0.71- 1.46*

Table 6.4: Adjusted OR's for potential risk factors for hookworm infection at follow-up.

6.1.12 Summary of follow-up survey results

These data suggest that a single MDA had a significant impact on intestinal helminths (hookworm in particular), LF and anaemia. It is plausible to postulate that the significant reductions in hookworm infections and intensity led to improvements in anaemia levels in the intervention villages. The impact on microfilaria levels was expected. However, it seems MDA did not have an appreciable impact on anthropometric indices in this population. A detailed discussion of these results is presented in **Chapter 9**.

Chapter 7

7.1 *Polyparasitism in the lower Shire valley- southern Malawi: a case for integrated disease control.*

In tropical environments, malaria, lymphatic filariasis, schistosomiasis and geohelminths are widespread parasitic infections posing an enormous toll on the socio-economic development of the infected individuals as well as that of the general population. It is currently estimated that 200 million people are infected with schistosomiasis, 85% of whom are concentrated in sub-Saharan Africa including Malawi (Chitsulo et al., 2000; Engels et al., 2002). It has recently been argued that the estimated 1.25 million disability adjusted life years (DALYs) lost in sub-Saharan Africa as a consequence of schistosomiasis infection (King et al., 2005; Murray and Lopez, 1996) might be a gross underestimation (Bergquist, 2002). Of the schistosomes, *S. mansoni* is the most widespread form, and its public health importance is increasing due to ecological changes and population movements (Chitsulo et al., 2000). In Malawi, however, *S. haematobium* is the predominant form (Bowie et al., 2004). Infections with hookworms (*Necator americanus* and *Ancylostoma duodenale*) affect an estimated 1200 million people worldwide (World Health Organisation, 1996), leading to approximately 0.3 million DALYs lost in sub-Saharan Africa (Hotez et al., 2005; Molyneux et al., 2005; Murray and Lopez, 1996).

Schistosomiasis and hookworm infections tend to be highly aggregated in a relatively small, heavily infected, proportion of the population. The majority harbour few or no worms. It has been suggested that predisposition to a heavy infection is a result of the interplay between social, behavioural,

ecological and immunological factors (Butterworth et al., 1985; Schad and Anderson, 1985). The large overlap in the geographic distribution of malaria, lymphatic filariasis, *S. haematobium, S. mansoni* and hookworm implies that co-infections might occur, and they have been reported frequently (Lwambo et al., 1999; Raso et al., 2004). However, little is known with regard to the association between the different parasites and the underlying mechanisms, and the results of previous observations are inconclusive. In a study undertaken in 30 schools of Tanzania, there was a significant negative correlation between the prevalence of *S. mansoni* and hookworm infections in school age children (Booth et al., 1998). In Co^te d'Ivoire, a study conducted in over 5000 children, found a positive association between the two parasites at the individual level (Utzinger et al., 1998). Similarly, significant positive associations had been reported among rural community members of Co^ te d'Ivoire (Keiser et al., 2002). In Mali, the prevalence of concomitant infections with schistosomes (*S. mansoni* and *S. haematobium*) and hookworms was significantly less than expected, however, there were important geographical differences (De Clercq et al., 1995). In addition, a re-analysis of several cross-sectional studies carried out in sub-Saharan Africa supported independent distributions of schistosomes and hookworm (Brooker et al., 1999). However, great care is needed in the interpretation of these findings, since parasitological diagnoses were usually based on a single stool specimen thus this may have resulted in the investigators missing an important proportion of the infections (de Vlas and Gryseels, 1992).

7.1.1 Polyparasitism in the lower Shire valley

Data presented in Chapter 6 were further analysed to investigate the epidemiology of multiple species parasite infections in the lower Shire valley. The parasites investigated in this analysis included hookworm, *S. haematobium, S. mansoni*, malaria, *A. lumbricoides*, lymphatic filariasis, *Taenia species* and *Enterobius vermicularis*. There were 947 individuals with a complete data set for this analysis. Of these 527 (55.7%) were female. The age and sex distribution of these individuals is show in Figure 7.1. There is a female excess in all the age groups except the 5- 9 year olds and this is more marked amongst the 20-29, 30-39 and the over 50. This is probably a result of males being absent working on the sugar cane estate where they obtain temporary employment.

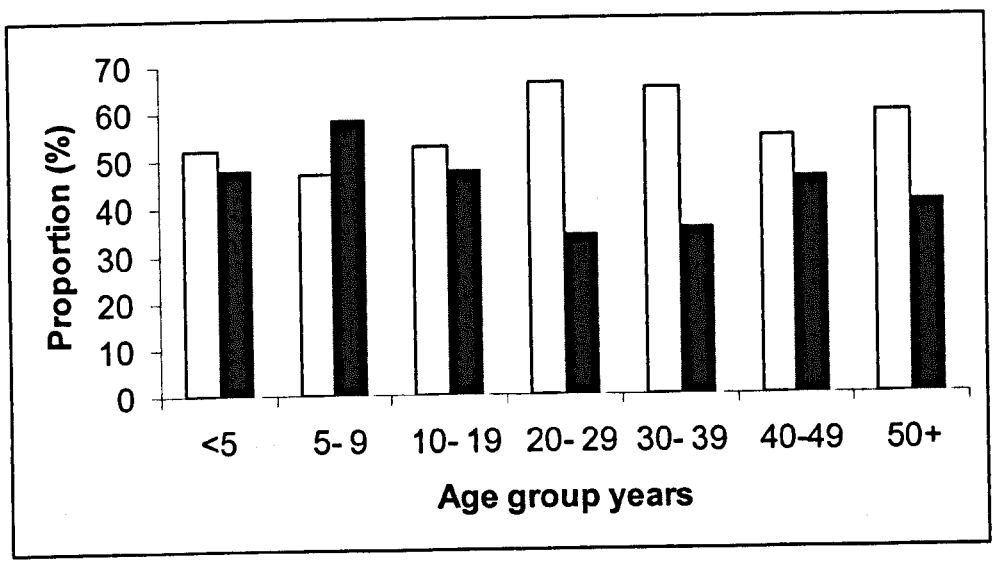


Figure 7.1: The age and sex distribution of female (□) and male (■) study participants with a complete data set for the polyparasitism analysis.

Table 7.1 presents the prevalence for the various parasites amongst the 947 individuals. The commonest infection was *W. bancrofti* with a prevalence of 36.2%, followed by hookworm 22%, *S. haematobium* 20.2%, malaria parasitaemia 9.5% and *S. mansoni* 6.8%. The other parasites were not common.

Parasite species	Number (%)
Total	947
Enteric parasites	
Hookworm	208 (22.0)
S. Mansoni	64 (6.8)
A. Lumbricoides	17 (1.8)
Taenia	6 (0.6)
E. vermicularis	3 (0.3)
S. haematobium	191 (20.2)
Malaria parasitaemia	90 (9.5)
Lymphatic filariasis (ICT)	343 (36.2)

Table 7.1: Showing the prevalence of the various parasites examined in the analysis for polyparasitism.

The prevalence by the number of species is shown in Table 7.2. Of note only 35% of these study participants were negative for all parasites. Thus 65% had at least one parasite species infection diagnosed. There were 374 (39.5%) individuals with a mono-species infection. Up to 185 (19.5%) individuals had a double- species infection while as 50 (5.3%) had triple-species. Four- species infection was found in 7 (0.7%) participants. It is worth noting that of the

infected individuals, 242/616 (39.3%) had a multi-parasite species infection. There was no difference by sex (X^2 chi= 1.62 p= 0.8) in polyparasite infections as shown in Figure 7.2.

Number of species	Number (%)
Total	947
Negative	331 (35.0)
Single- species	374 (39.5)
2- species	185 (19.5)
3- species	50 (5.3)
4- species	7 (0.7)

Table 7.2: Prevalence by the number of species amongst the study participants included in the analysis for polyparasitism.

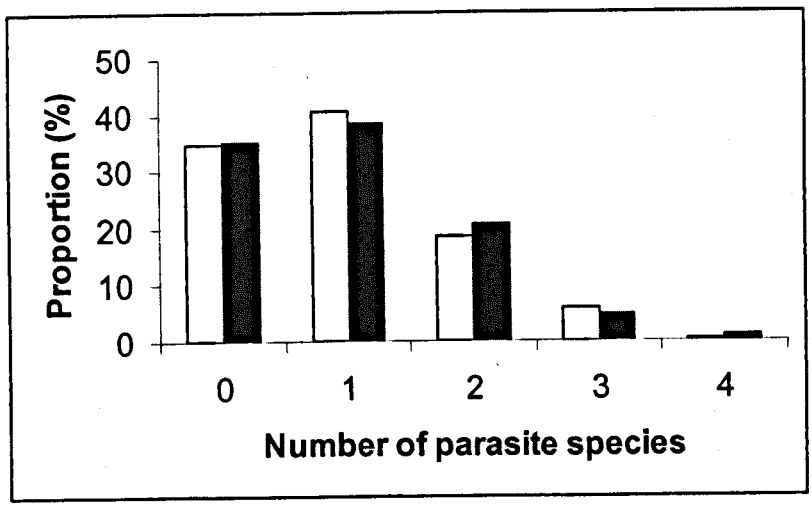


Figure 7.2: The distribution of parasite infections by the number of species for females (□) and males (■) included in the analysis for polyparasitism.

The parasite infection prevalence by the number of species and age is shown in Figure 7.3. There is progressive decrease in the proportion of individuals that are parasite free by age. The proportion of individuals with single-species infection did not fall below 35% across the age range. In contrast, there was a clear increasing trend of the proportion of individuals carrying two-species infections that approached 30% amongst the 30- 39 years age group. The proportion of individuals with three-species infection did not go above 10% across the age range while that for the four-species remained under 2%. The cases with four-species infections were all in the 5-9 age-group.

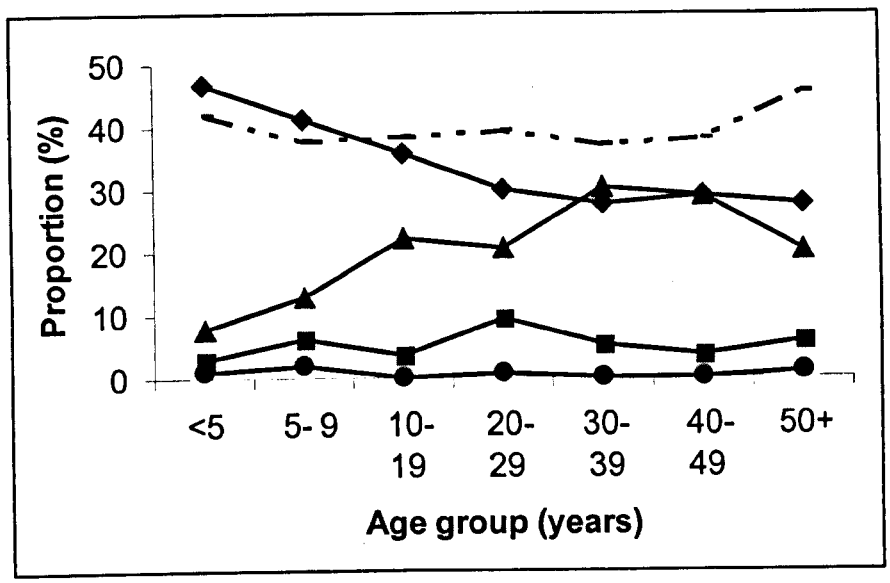


Figure 7.3: Parasite infection prevalence by the number of species and age. Negative (♦), single species (---), 2- species (▲), 3- species (■) and 4- species (●)

7.1.1.1 Species- specific co-infections

Dual- species infections were determined and the findings are presented in Table 7.3. Also included in this table are expected (based on the prevalence

for each parasite outlined in Table 7.1) prevalence for each parasite combination.

Parasite combination	Number n= 947	Observed prevalence (%)	Expected Prevalence (%)	X^2 p value
Hookworm + lymphatic filariasis	98	10.3	7.9	**<0.001**
Hookworm + *S. haematobium*	42	4.4	4.4	NS
Hookworm + *S. mansoni*	12	1.3	1.5	NS
Hookworm + malaria parasitaemia	19	2.0	2.1	NS
Hookworm + *A. lumbricoides*	6	0.6	0.4	NS
Hookworm + other	0	0	0.2	NS
S. haematobium + lymphatic filariasis	72	7.6	7.3	NS
S. haematobium + *S. mansoni*	25	2.6	1.4	**<0.001**
S. haematobium + malaria parasitaemia	23	2.4	1.9	NS
S. haematobium + *A. lumbricoides*	0	0	0.4	NS
S. haematobium + other	5	0.5	0.1	NS
S. mansoni + lymphatic filariasis	28	3.0	2.4	NS
S. mansoni + malaria parasitaemia	6	0.6	0.6	NS
S. mansoni + *A. lumbricoides*	3	0.3	0.02	NS
S. mansoni + other	1	0.1	0.05	NS
A. lumbricoides + lymphatic filariasis	5	0.5	0.07	NS
A. lumbricoides + malaria parasitaemia	1	0.1	0.17	NS
A. lumbricoides + other	0	0	0.1	NS
malaria parasitaemia + lymphatc filariasis	27	2.9	3.4	NS
Malaria parasitaemia + other	0	0	0.07	NS
lymphatic filariasis + other	4	0.4	0.3	NS

NS= Not significant;

'other', includes 7 cases of Taenia and 2 of *Enterobius vermicularis.*

Table 7.3: Showing the observed and expected prevalence for each parasite combination.

The association was significant for the combination of hookworm with lymphatic filariasis and for *S. haematobium* and *S. mansoni*. This suggests that, in this population co-infections of hookworm and lymphatic filariasis were observed more frequently than would be expected to occur by chance based on the prevalence for each parasite reported in Table 7.1. This observation also applies to concomitant infections with *S. haematobium* and *S. mansoni*. It is important to note that more than 50% of all infections with each of the commonest parasites investigated in this analysis occurred as a multi-species infection shown in Figure 7.4.

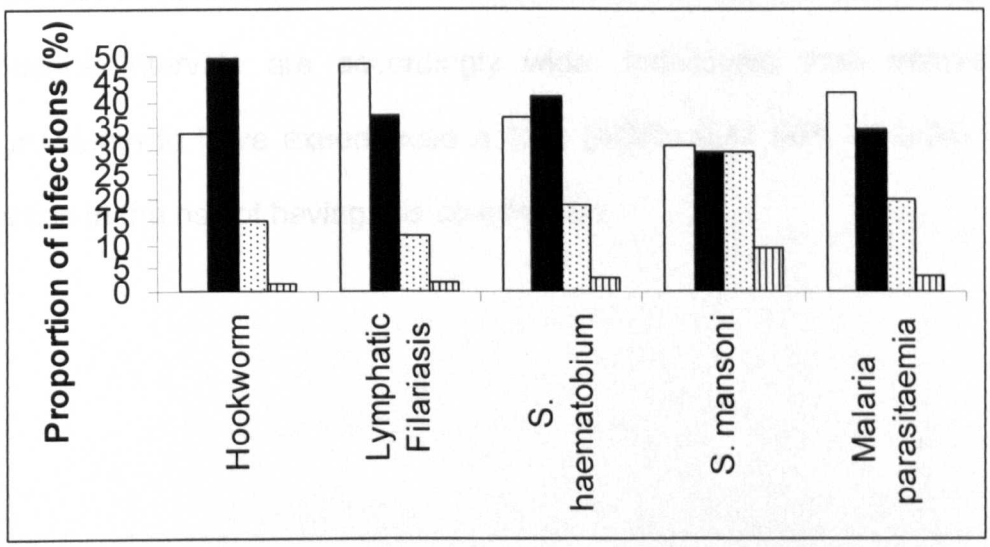


Figure 7.4: Proportion of parasite infection for the five commonest parasites as single- species (□), 2- species (■), 3- species (▨) and 4 species (▥)

7.1.1.2 Factors influencing hookworm and LF co-infections

A multivariate analysis using logistic regression was carried out to determine factors that influenced co-infections with hookworm and LF in this population. The adjusted odds ratios (AORS) are presented in Table 7.4. In this analysis individuals with hookworm and LF co-infection were classified as cases. Of the factors explored, age and treatment area had statistically significant association with the risk of having the co-infection. Increasing age was associated with up to six-fold increase with the risk of having hookworm and LF co-infection. Of note this was only significant for those beyond 30 years of age and in addition, because of small numbers, the corresponding 95% confidence intervals are accordingly wide. Individuals from intervention villages seem to have experienced a 53% [AOR= 0.47 95% CI 0.24- 0.91] reduction in the risk of having this co-infection.

Factor	AOR	95% CI
Age		
<5	*ref*	
5- 9	*0.89*	*(0.24- 0.91)*
10- 19	*1.60*	*(0.42- 6.04)*
20- 29	*2.06*	*(0.49- 8.64)*
30- 39	*3.93*	*(1.02- 15.15)*
40- 49	*6.27*	*(1.49- 26.48)*
50+	*6.71*	*(1.84- 24.48)*
Sex		
Female	*ref*	
Male	*1.40*	*(0.79- 2.49)*
Treatment area		
control	*ref*	
Intervention	*0.47*	*(0.24- 0.91)*
Household roof		
Thatch	*ref*	
Iron	*1.12*	*(0.56- 2.32)*

Table 7.4: Showing the adjusted odds ratio for the potential factors influencing co-infection with hookworm and LF.

7.1.1.3 Morbidity associated with polyparasitism

An evaluation of the impact of multi-species infection on anaemia and nutrition was carried out. For anaemia, there were 299, 348, 171, 44 and 7 individuals available for analysis for each of these categories of parasite infection; 0, single-species, 2-species, 3-species and 4- species respectively. As for assessment of nutrition, there were 213, 211, 83, 26 and 5 individuals with data for each respective multi-species infection category as outlined above. There is a decreasing trend for mean haemoglobin by the numbers of species detected especially for individuals with three or more species shown in Figure 7.5. However, the 4- species category included only 7 individuals,

and thus the estimate obtained is imprecise and the corresponding confidence intervals are expectedly wide.

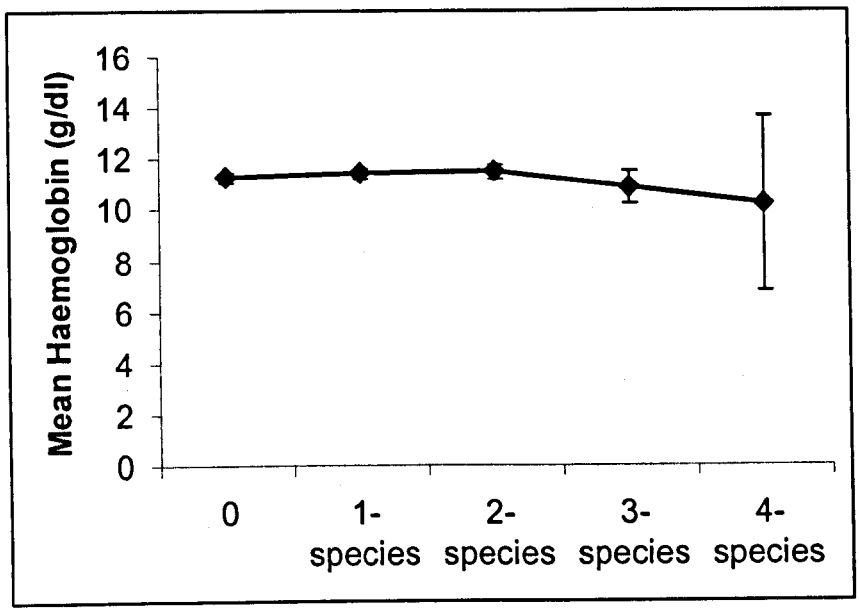


Figure 7.5: Mean haemoglobin by the number of parasite species detected (bars are 95% confidence intervals).

Similarly, the anthropometric indices became worse with increasing number of parasitic species an individual carried shown in Figure 7.6. In general, mean z-scores for weight-for-age were better than those for height-for-age across the range of species combinations. However they all markedly decreased for the 3 and 4-species infection categories. For this part of the analysis, only 5 individuals were in the 4-species category, and thus the estimates are imprecise.

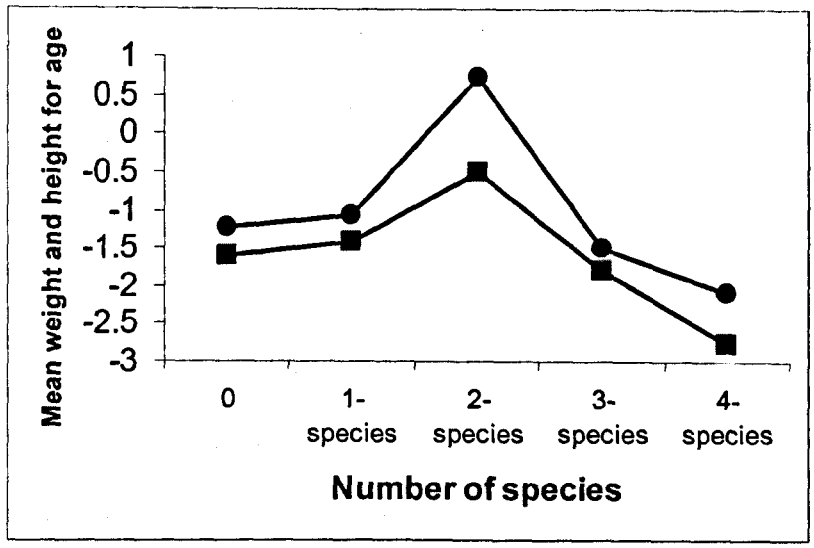


Figure 7.6: Mean z-scores for weight-for-age (●) and height-for-age (■) by the number of parasite species detected.

7.1.2 Summary of findings for the analysis for polyparasitism in Chikwawa District

This analysis has shown that polyparasitism is common in the population of the lower Shire Valley- Chikwawa District. Of note, 50% of infections for each parasite investigated occurred as a multiple infection. In addition, the data suggests that morbidity may be associated with the number of parasite species an individual is carrying. The implication of these results for the control of parasitic infections in the Chikwawa District and Malawi as a whole is discussed in **chapter 9**.

Chapter 8

8.1 The potential role of anti-tuberculosis chemotherapy in the treatment of lymphatic filariasis

Electron microscopic studies of several species of filarial nematodes have shown the presence of intercellular rickettsia-like organisms that are related to a group of arthropod endosymbionts known as *Wolbachia* (Bandi et al., 1998). They were first found in hypodermal tissues of lateral chords, uterine wall and in embryos of filarial nematodes, embedded as single or multiple organisms in host-derived vacuoles. They exist in different shapes (oval, round or rod- shaped) and are 0.6-1.5 µm in size. The body is covered with a double membrane enclosing the cytoplasm which is rich in ribosomes. These endobacteria were first identified in filarial worms in the late 70's, and it was speculated that antibiotics could be used to treat filarial infections in the discussion of those papers (Kozek, 1977). All life cycle stages of filarial worms are infected with these bacteria, but the intensity of the infections varies between life cycle stages (McGarry et al., 2004), and it appears they have their own developmental life cycle within the worms which is yet to be clearly defined. Several filarial nematodes have been shown to contain these bacteria (Taylor and Hoerauf, 1999) and, interestingly, only a few species (for example: *Acanthocheilonema viteae*, *Loa loa* and *Onchocerca flexuosa*) do not carry these bacteria (Buttner et al., 2003). The observation that *Wolbachia* can be used as a drug target against filarial nematodes holds great promise as a therapeutic option available for filariasis treatment, especially against adult worms.

8.1.1 Wolbachia as a target for therapy in animal models

A number of research groups (Rao and Well, 2002; Smith and Rajan, 2000) have shown that antibiotics active against Rickettsiaceae, particularly the tetracyclines, rifampicin and chloramphenicol, were effective in reducing the filarial larval molt (from L3 to L4) and their development *in vitro*. In contrast, effect of tetracycline analogues lacking antirickettsial properties also affected larval molting indicating that the drug might have other pharmacological effects on worms (Rajan, 2004). Antibiotics also seem to affect adult filarial worms *in vitro* by reducing their ability to produce microfilariae and their viability (Rao et al., 2002). Several reports have shown effects of antibiotics on filarial nematodes in experimental animal models (Hoerauf, 2000; Volkmann et al., 2003). More importantly members of tetracycline family (tetracycline, oxytetracycline, doxycycline, and minocycline) were found to be effective against adult worms. Modes of action of these antibiotics are generally on bacterial RNA polymerases, protein synthesis, and other processes, and these agents may affect similar pathways in both worms and their *Wolbachia*. In several nematode worm infections these antibiotics have multiple effects on worm growth and development; worm fertility (particularly female worm embryogenesis) and worm survival, with evidence suggesting that prolonged treatment can be detrimental to worms (Hoerauf et al., 1999). Moreover, when microfilaraemic animals were treated, their microfilarial numbers were considerably reduced in the circulation (Hoerauf et al., 1999). In contrast, in animals infected with *A. viteae* worms, which do not carry these bacteria, similar long-term treatment had no effect on worm biology and development (Hoerauf et al., 1999), suggesting that these bacteria play

an important role in the growth and reproduction of filarial worms that harbour them. The combination studies with rifampicin in animal models have been found promising to achieve acceptable short-term regimen plans with doxycycline (Volkmann et al., 2003). Interestingly, in addition to anti-*Wolbachia* properties (Volkmann et al., 2003), tetracyclines markedly affected the normal embryogenesis profiles by causing damage and degeneration of intrauterine embryos(Volkmann et al., 2003). Polymerase chain reaction (PCR) assay also confirmed the clearance of *Wolbachia* DNA after prolonged therapy (Volkmann et al., 2003).

8.1.2 Wolbachia as a target of therapy against pathogenic human filarial infections

The pharmacological effect of doxycycline has encouraged clinical investigators to test their hypothesis that elimination of *Wolbachia* is beneficial in reducing the human filarial infections. The first clinical trials were undertaken in people with *O. volvulus* infections. A 6 week course of daily doxycycline treatment (100 mg/day) depleted *Wolbachia* in adult *O. volvulus* worms, and caused extensive degeneration of embryos by 4 months post treatment (Hoerauf et al., 2003a). The worms became sterile after the loss of *Wolbachia*, and infected individuals also had significantly fewer or no microfiladermia (Hoerauf et al., 2003a). The combination therapy with doxycycline and ivermectin also remarkably reduced microfiladermia following reductions in *Wolbachia* in adult worms (Hoerauf et al., 2001). Similar effects were observed in *W. bancrofti*- infected patients after multiple doses of doxycycline (200 mg/day for 6 wk) (Hoerauf et al., 2003a). In this

study, patients were treated with doxycycline followed by a single dose of ivermectin. Doxycycline treatment alone reduced *Wolbachia* numbers (96%) after 4 months of treatment, followed by 99 per cent reductions in number of microfilariae by one year of treatment. It would be interesting to see whether *Wolbachia* can repopulate in these worms after cessation of antibiotic therapy. Additional studies are needed to effectively measure macrofilaricidal activity of these drugs in clinical studies. Despite this demonstrated efficacy, multi-dose antibiotic therapy and their use in mass treatment campaigns is considered impractical. Therefore, the efficacy of short-term antibiotic treatments along with antifilarial drug combinations such as diethylcarbamazine (DEC) and albendazole in various endemic countries remains to be evaluated. As there have been a limited number of studies using doxycycline treatment and in selective populations carrying onchocerciasis, loaisis, or lymphatic filariasis (Hoerauf et al., 2003a; Hoerauf et al., 2003b; Taylor et al., 2005), no clinical trial with this potent antibiotic has been reported in other endemic areas. Therefore, it is still premature to have a consensus regarding the effective universal dosage and duration of treatment for either microfilaria clearance or adult worm sterility. Moreover, the results of treatment may be affected by the immunological status of the host, age, host susceptibility and total worm burden. The filarial *Wolbachia* genome sequencing has recently been completed (Foster et al., 2005) and several new targets necessary for the bacteria are being identified. These might lead researchers to investigate a new class of anti-*Wolbachia* drugs that may benefit lymphatic filariasis chemotherapy research.

8.1.3 Rationale for the study in Chikwawa

As discussed above, one of the drugs that has been shown to have potency against *Wolbachia* is rifampicin. In Malawi, rifampicin is one of the anti tuberculosis (TB) chemotherapeutic agents. In those with smear positive TB and cases of relapse, rifampicin is given for 8 or 12 months respectively. This study aimed to investigate the effect of anti-tuberculosis treatment on the filarial nematode *W. bancrofti*. The hypothesis for this study is that newly diagnosed TB patients, who are coinfected with *W. bancrofti,* would show reductions in both microfilaraemia and adult filarial (*W. bancrofti)* worm specific antigen levels following anti-TB treatment.

8.1.3.1 Sample size determination

The sample size is based on the primary outcome, a reduction in microfilaraemia. In this area of Malawi, mean microfilaraemia is estimated to be 650 (+/- 180 SD)/ ml of blood. In order to detect a difference in microfilaraemia of 30% between TB cases and the community controls, difference d =195 at a significance level *P*= 0.05 and 90% power, factor F = 10.51, the required sample size was determined by solving the following equation:

$N > 2 \times F \times SD^2 / d^2 = 18$ individuals are required in each group,

Therefore total patients = 2 x 18 = 36

Approximately 50% of people do not complete their TB therapy (Volmink and Garner, 2001). In addition, a 10% reduction in compliance within the trial is expected, thus with a drop out rate of 60%, approximately 90 patients will need to be recruited to the trial. This sample size was judged to be adequate to detect significant differences in antigenaemia levels.

8.1.3.2 Recruitment procedures

All newly diagnosed adult TB patients starting chemotherapy at Chikwawa District Hospital were invited to participate. The exclusion criteria included those who were severely ill and those likely to leave the district before the end of the follow-up period. In order to map the natural behaviour of antigenaemia (such as stability over time within an individual) in this population, an area, age (+/- 5 years) and sex matched cohort of individuals (referred to as community controls) who had never received TB treatment was identified. After informed consent was obtained, 10 mls of venous blood was provided by each consenting individual (TB cases as well as community controls). The serum was then separated from the sample and stored in – 80 °C freezer before shipping to the UK.

8.1.3.3 Recruited individuals

Overall, there were 146 adult TB cases recruited into the study from April to July 2004. Of these, 80 (54.8%) were female. The mean age for female and male participants was 37.8 and 40.4 years respectively. Though the males

were slightly older, this was not statistically significant (Student ttest t= -1.18, p= 0.2). The age and sex distribution of this cohort is presented in Figure 8.1. It is apparent that the majority of the TB cases are young adults. Though not significant in this cohort, the epidemiology of TB in Malawi, is such that there tends to be a significant female excess amongst young adults. This mirrors the distribution of HIV infections whereby females are infected at a younger age compared to their male counterparts. In addition to the TB cases, 82 community controls have been recruited into the study. These have all provided a baseline serum sample.

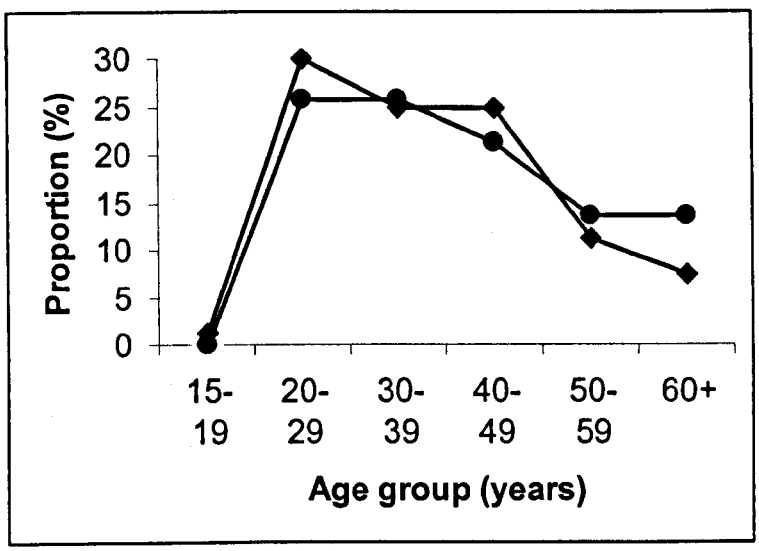
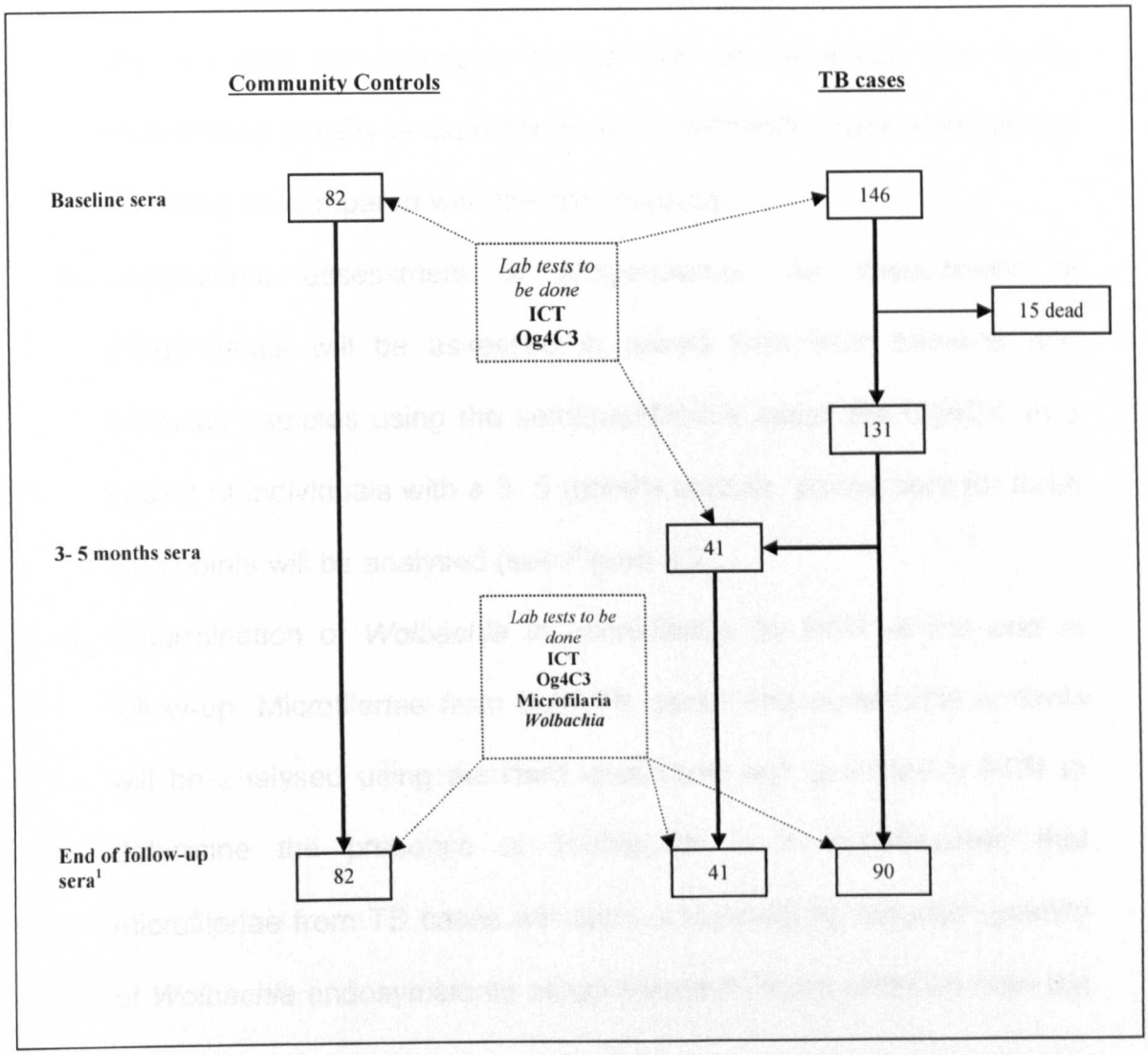


Figure 8.1: The age and sex distribution of TB cases recruited into the study [male (●) and female (♦)]

8.1.3.4 Follow-up and analysis plan

A scheme of the analysis plan is provided in Figure 8.2.



[1] *will be collected in 2006*

Figure 8.2: Schematic presentation of the analysis plan

These data will allow the following comparisons:

1. Antigenaemia levels at baseline. This will be evaluated for both the TB cases and community controls using ICT cards and the Og4C3 assay.

The antigen levels at baseline are anticipated to be similar between the two groups.

2. Microfilaraemia at the end of follow-up. Since it was logistically not possible to collect samples for microfilariae examination at baseline, this will only be assessed at the end of follow-up. The mean microfilariae density is expected to be significantly lower amongst the TB cases as compared with the control group.
3. Longitudinal assessment of antigenaemia. An assessment of antigenaemia will be assessed in paired sera from baseline and follow-up samples using the semiquantitative assay the Og4C3. In a subset of individuals with a 3- 5 months sample, paired sera for three time points will be analysed (see Figure 8.2).
4. Determination of *Wolbachia* in microfilariae by PCR at the end of follow-up. Microfilariae from both TB cases and community controls will be analysed using standard qualitative and quantitative PCR to determine the presence of *Wolbachia*. It is hypothesised that microfilariae from TB cases will carry a significantly reduced quantity of *Wolbachia* endosymbionts as compared to those obtained from the control group.

8.1.3.5 Unexpected development

Unfortunately, this study experienced prolonged delay in starting because of the following reasons:

1. Ethical approval was delayed because the Malawi committee felt it was unethical to hold treatment, especially for community controls, for the duration of the follow-up period. This was resolved when it was agreed that LF is a chronic infection and in indigenous populations it seems to take years for disease to develop. Thus, the fact that infected study participants would receive appropriate treatment at the end of follow-up was deemed ethical.
2. Within the first 3 weeks of starting this study, the district TB officer who was trained to obtain informed consent had to leave to go for further training. Thus, the study could not continue until a replacement had been found and trained.

This introduced a delay of over 12 months from the originally planned start date. The implication of this, being that, the cohort of patients that has since been recruited has not been followed up for long enough to allow detection of a significant decrease in antigenaemia levels. The current thinking is that the macrofilaricidal effect of chemotherapy targeted at *Wolbachia*, as detected by a decline of *W. bancrofti* antigenaemia, is only apparent beyond 14 months of follow-up (Taylor et al., 2005). Thus, though the baseline sera for both TB cases and community controls including the 41 '3- 5 months' sera from a subset of the TB cases are in Liverpool, it was deemed inappropriate to run the assays based on the scientific knowledge as outlined above. There are, therefore, no results to report for this component of the LF studies in Malawi.

Chapter 9

9.1 Discussion of the respective study results

9.1.1 Discussion of the national LF mapping survey results

The national survey, in the remaining unmapped districts in Malawi, has shown that infection with *W. bancrofti* as determined by antigenaemia prevalence, is more widespread than previously appreciated. The female excess observed amongst our survey population probably reflects the fact that males are often out in the field during the day thus not available for testing. The implication of this being that the prevalence found in some of the sampled villages is likely to be an under-estimate of the true prevalence. This is due to the fact that in most communities significantly more males tend to carry the infection as has been observed in this survey and in other surveys from Malawi and elsewhere in Africa (Ngwira et al., 2002; Wamae et al., 1998).

In all districts, except Chitipa in the north, there was at least one individual who was positive on ICT. The low prevalence found in villages from the western side of Malawi could be explained by the fact that these areas are dry, of relatively higher altitude and thus not ideal for extensive mosquito breeding. The 18.2% prevalence observed at Mzenga Village in Mchinji along the Zambia border is intriguing. This is particularly so as there have been no anecdotal reports of LF disease from either the Malawi or Zambia side of the border in this area. Of note is that this village is in close proximity to a perennial stream that sustains a reasonable amount of irrigated onion farming. Whether this setting is conducive for supporting extensive mosquito breeding and thus driving *W. bancrofti* infection as has been observed in Northern Malawi and Ghana will need further investigation (Dzodzomenyo et al., 1999; Hunter, 1992; Ngwira et al., 2002). Ideally this should be coupled with human night blood examination for microfilaria.

It is also interesting to note that some villages from districts (Rumphi, Nkhata-Bay and Nkhotakota) along the lake shore had prevalence of less than 10%.

A possible explanation could be due to the fact that these districts are mountainous and thus well drained consequently limiting potential mosquito breeding sites.

The relatively high prevalence found in Salima, Ntcheu (Bwanje Valley), Balaka, Mangochi and Phalombe was unexpected. However there have been isolated unpublished reports of cases with chronic manifestation of LF (hydrocele and elephantiasis) in these areas. It is worth noting that the ecological conditions in these districts are ideal for supporting large potential LF vector populations. Incorporating data from 2000 surveys clearly shows that the priority areas for LF control activities in Malawi will be the lakeshore districts, Phalombe plain and the Lower Shire Valley.

The decline in LF prevalence with increasing altitude has also been reported from other settings in Africa (Onapa et al., 2005). This is believed to be due to the influence of altitude on temperature which is known to be critical for survival of the vector and development of the parasite within the vector (Lardeux and Cheffort, 2001).

These findings have important implications for initiating the "Malawi LF Elimination Programme". First, following WHO's recommendation that all implementation units with a prevalence on ICT of over 1% be considered endemic and thus treated, the Malawi programme would involve 26 districts with a target population of over ten million. The population affected is far greater than ever envisaged. Secondly, both the northern (Karonga) and Southern foci (the Lower Shire Valley) share international borders which are largely porous. This calls for innovative approaches in carrying out control activities as they have to be synchronised with those in neighbouring countries. Thirdly, in some districts (Phalombe, Mulanje, Thyolo, Chikwawa and Mwanza) where LF is co-endemic with onchocercisasis the two programmes will need to be merged. Fourthly, the LF programme will need to establish links with other programmes that are delivering community based interventions such as the ministry of education's deworming and feeding

programme and the expanded bed net distribution under the malaria control programme.

9.1.2 Discussion of the Karonga LF mapping survey results

This survey has shown that, in Karonga, *W. bancrofti* infection is more widespread than was previously reported (Oram, 1960). In all the sampled villages antigen prevalence is over 25%. This has implications for the implementation of control activities. The cline in infection levels from north to south may be explained by prevailing climatic and ecological conditions. The north including the Songwe area is wetter and mainly rice growing. The relatively high prevalence found in Bonje-Hara in the south could be explained by the fact that it is within a rice irrigation scheme which was settled by tenants recruited from the northern part of the district. This association between irrigation schemes and LF has been shown in other parts of Africa (Dzodzomenyo et al., 1999; Hunter, 1992). It is possible that similar migration patterns have established LF foci in locations outside those historically known.

The observed *W. bancrofti* antigen prevalence patterns in the respective geographical areas in Karonga suggest differences in transmission dynamics. The intensity of infection is likely to be higher in the Songwe area and in those villages in the north than in the southern villages. The constant prevalence by age among adults has been noted in other populations (Lammie et al., 1994). This could reflect restriction to some susceptible portion of a population, but is likely to reflect a dynamic "equilibrium" as adults move in and out of detectable antigenaemia over time. The male excess in infection levels has been shown in other surveys (Wamae et al., 1998). In Karonga as well as elsewhere in Africa hydrocele is the most common clinical manifestation of LF (Wamae et al., 1998). Its application in rapid mapping of LF has been proposed (Gyapong et al., 1998b). However there is mounting anecdotal evidence from Karonga and other centres in Africa that despite high antigen prevalence some areas are spared chronic clinical manifestations of LF. This suggests there are important co-factors that predispose to the development of this disease. This has significant

ramifications for mass drug distribution coverage, as compliance is likely to be low in areas where there is no evident disease but high infection level.
It is interesting to note that only 30% of the thick films made from night blood samples were positive for microfilariae despite having been made from individuals who were ICT positive. A possible explanation could be that the ICT test detects adult worm antigen (Weil et al., 1997). Thus, individuals infected with single sex or non-fecund worms may be positive on ICT (Nuchprayoon et al., 2003) without a possibility of having circulating microfilariae.

9.1.3 Discussion of baseline survey results for the intervention trial

Anaemia was an extremely common problem affecting over 40% of individuals in this population. The present study confirmed earlier observations in this population by Prinsen-Geerligs *et al.*, (2003) that: a) anaemia has the highest prevalence amongst the youngest, with prevalence falling with increasing age. This pattern is similar to that for malaria parasitaemia. b) Females older than 15 years have significantly lower haemoglobin levels than males suggesting sex differences in risk factors leading to anaemia.

There is a complex interplay of factors related to the evolution of anaemia in southern Malawi. Age, sex, hookworm infection and history of malaria are of major importance. Young children (<2 years) were especially at risk, probably related to birth with low iron levels and limited reserves required for growth (Geerligs et al., 2003). The sex differences in anaemia risk could be attributed to differences in host exposure to various parasites although sex differences in host susceptibility and differences in growth requirements between males and females may play an important role. The observation that

young adult females are more anaemic than their male counterparts is possibly related to excess demands for iron following early and repeated pregnancies, as well as menstrual losses. It has previously been shown in this population that nearly all pregnant women (92%) tend to be anaemic at their first antenatal visit (Verhoeff, 2000).

The relationship between hookworm and anaemia in southern Malawi confirms findings from other studies in sub-Saharan Africa (Stoltzfus et al., 1997b). Similarly, the fact that increasing intensity of hookworm infection (see Figure 5.15) is associated with decrease in mean haemoglobin level has been previously established elsewhere in Africa (Stoltzfus et al., 1996). Hookworm infection rates were higher in those over 20 years (24.7% vs 12.9% χ^2 p= <0.001), which is consistent with the known epidemiology of hookworm (Behnke et al., 2000). It suggests lack of acquired immunity and possibly that increased exposure occurs with increasing age (Bradley et al., 1992). The finding that hookworm has an association with MSA has important implications for public health. Those individuals with MSA are likely to suffer serious morbidity. The AP suggests that hookworm may be responsible for up to 20% of all MSA cases in Chikwawa District. The number of hookworm-infected individuals with severe anaemia was relatively small (26 of 71). Thus this finding needs further investigation. It seems, however, that hookworm plays a major role in determining MSA risk in southern Malawi as in other parts of sub- Saharan Africa (Stoltzfus et al., 2000; Stoltzfus et al., 1997b). Thus the mass treatment with the two broad-spectrum anti-parasitic drugs (albendazole and ivermectin) for the control of LF is likely to impact on

anaemia levels in these communities. The prevalence for hookworm (18.9%) was lower than expected. A survey carried out in Karonga District- northern Malawi reported a hookworm prevalence of over 60% amongst school children (Randall et al., 2002). This could be due to the fact that we performed a single Kato-Katz smear, which is known to be less sensitive, although preferred for determining intensity of infection. However, the low prevalence in Chikwawa seems to be stable over time as a nutritional survey carried out 30 years ago reported a prevalence of 15% (Burgess et al., 1973). The fact that the majority of infections were of light intensity does not preclude an association with anaemia (Stoltzfus et al., 1997c). This is because the impact of such infections depends on the population's iron status and availability of iron stores to buffer increased iron losses (de Silva, 2003).

It is surprising that although the study period spanned the rainy season, a lower (especially in adults) than expected prevalence (20.4%) for malaria parasitaemia was found in these mainly asymptomatic individuals. The reasons for this are unclear. Laboratory error is unlikely because of the quality control procedures. Inoculation rates may have been lower during the study period. The relatively low prevalence and the fact that the majority of infections were of low parasite density could explain the lack of association with anaemia. However, consistent with the known epidemiology of malaria there appears to be a decrease in mean haemoglobin with increasing parasite density (Dreyfuss et al., 2000). The reduction in MSA risk associated with a fever episode (presumed to be due to malaria) occurring more than a

month previously is plausible. In these individuals low haemoglobin levels may have recovered. Though delayed recovery may occur as a consequence of poor nutritional status due to main diets lacking iron, folate and other essential elements (Brabin et al., 2004).

The poor diets and the multiple parasitic infections all impact on the growth patterns of children in this and other tropical areas. The observation that over 40% of the children were either underweight and/or stunted was similar to those reported from Tanzania (Lwambo et al., 2000). Also similar is the fact that girls seem to display catch-up growth in adolescence and the reasons for this are not fully understood (Lwambo et al., 2000). These could be genetic, hormonal and/or cultural.

This analysis has shown that the aetiology of anaemia is multifactorial in southern Malawi. Its control requires innovative and integrated approaches. These should be community based in order to reach a significant proportion of the population (Molyneux and Nantulya, 2004). Mass treatment strategies as used in the control of LF and onchocerciasis aim to treat over 70% of the population (excluding pregnant women, epileptics, seriously ill, under fives and those <90cm in height) yearly. This is likely to be of strategic importance as an anaemia control measure.

9.1.4 Assessment of impact of a single MDA in Chikwawa District-Southern Malawi

Ultimately, the success of the LF elmination program will be judged by its impact on microfilaria prevalence and intensity. Although the program is still in its infancy, it is clear that mass treatment leads to significant reductions in

filarial infection level and in transmission (Bockarie et al., 1999; Dunyo et al., 2000).

Demonstrating collateral public health benefits, such as reductions in the burden of filarial disease or intestinal helminth infections, is also important for building political and social (community) support for the LF elimination effort. Here, data are presented for the first evidence that MDA for lymphatic filariasis also leads to significant and sustained reductions in hookworm and anaemia prevalence at the population level after only a single round of MDA with albendazole and ivermectin in combination.

9.1.5 Impact of MDA on anaemia in the lower Shire valley- Chikwawa District –southern Malawi

The finding of a 20% (from 43.2% to 36.4%) reduction from the baseline measurement in anaemia in the intervention villages is remarkable. The fact that such a decrease was not seen in the control villages illustrates the impact of deworming with albendazole/ivermectin combination on anaemia in this population. The 9.1 percentage-point difference between intervention and control villages, which is statistically significant, in anaemia prevalence between control and intervention villages emphasises this impact. Though previous deworming studies have demonstrated improvements in haemoglobin levels, these have mainly concentrated on school children and have been undertaken in the context of clinical trials (Beasley et al., 1999; Stephenson et al., 1993a; Stephenson et al., 1989; Stoltzfus et al., 1998). This study is unique in that it is population based and the intervention was administered by Community Drug Distributors (CDDS) with minimal training

and external supervision. This was aimed at replicating national programme conditions.

9.1.6 Impact of MDA on prevalence and intensity of hookworm infection

Though the overall prevalence of hookworm had increased between baseline and follow-up surveys, this was largely due to increases in the control villages. This is supported by the statistically significant difference between the hookworm prevalence in the control and intervention villages. The increase in prevalence in the control villages could probably be explained by the season and change of laboratory technician. The follow-up survey was conducted in the months immediately after the rainy season. These are cooler and thus would promote the survival of hookworm larvae in the soil. This would be consistent with findings from Anambra State in Nigeria and also in Kwazulu Natal South Africa (Mabaso et al., 2003; Mabaso et al., 2004; Nwosu and Anya, 1980). Since during the rainy season people spend considerable amount of time barefoot working the fields, this would probably result in increased hookworm transmission. It is worth noting that sanitary facilities are limited (29% of households had a pit latrine) in these villages. In fact, defecation is done in communal shaded grounds in close proximity to the villages, which are probably ideal environments for the development of hookworm larvae (Schad and Anderson, 1985). This increase in prevalence could also be explained, at least in part, by the fact that a different technician read the slides at the follow-up survey. This was due to the sudden death of the technician who read slides at the baseline survey. However, the same

supervisor provided quality control at the two surveys which ensured that the same high standards in preparation of smears and microscopy were maintained. Of note, however, is the fact that the above factors and others unknown that may have influenced hookworm infection should have been equally distributed amongst villages. This was ensured by the random allocation of the intervention. Indeed this analysis has identified no significant differences in the distribution of potential confounding factors between the two groups of villages including age, sex and socioeconomic characteristics. This, therefore, suggests that the observed difference in prevalence was as a result of the MDA. This is confirmed in the multivariate analysis where an adjusted odds of 0.60 (95% CI 0.42- 0.86) was found. This would suggest a reduction of 40% in risk of having hookworm infection associated with having received the deworming tablets. In addition to the impact on prevalence a significant effect on hookworm intensity of infection was found. This would be consistent with findings from Haiti where an evaluation of the LF program's impact on intestinal helminths was also undertaken (De Rochars et al., 2004). The drug combination deployed in Haiti included DEC instead of ivermectin and the evaluation was carried out after two rounds of treatment. These observations would suggest significant public health benefits for this community. The reduction in hookworm intensity of infection as well as prevalence is likely to lead to reductions in associated morbidity. As was observed at baseline, hookworm infection per se, may be associated with MSA in Chikwawa District. Similarly, this observation was seen in control villages at follow-up. In addition data at baseline as well as follow-up suggested that moderate to high intensity hookworm infections may be

associated with a decrease in mean haemoglobin. This is in line with the known epidemiology of hookworm infection and its effect on haemoglobin level (Stoltzfus et al., 1996; Stoltzfus et al., 2000). It follows, therefore, that the improvements in anaemia observed in the intervention villages, are probably, as a result of the MDA's impact on hookworm infections. Of particular importance is that this effect extends to those with MSA. A group that is particularly prone to experiencing morbidity and mortality associated with anaemia. Seen in context, these results suggest that an LF elimination programme would have marked ancillary benefits in Chikwawa District and Malawi as a whole. The same will probably apply in other LF endemic settings with similar epidemiology for hookworm and anaemia.

9.1.7 Impact of MDA on other enteric parasites

This study has not demonstrated an impact on *S.mansoni* (prevalence at baeline=5.4% vs follow-up 5.0%). This is hardly surprising as the drugs used are not known to be potent against this parasite. However, the fact that both *S. mansoni* and *S. haematobium* are prevalent in this area, calls for efforts to integrate the two programs at least at district level. This is particularly so because both these parasites tend to be localised. However, Bowie et al (2004) established that *S. haematobium* is widely distributed in Malawi with pockets of high prevalence and intensity. It is clear that such pockets are likely to benefit from integrated control initiatives and thus should be identified (Hopkins et al., 2002). This could be easily achieved by deploying rapid assessment methods such as the 'red urine question' as discussed by Bowie et al (2004). Data from the national schistosomiasis survey, as well as

the current analysis, suggest *S. mansoni* is more restricted in its distribution. The main outstanding issue is lack of evidence supporting the safety of co-administration of albendazole, ivermectin and praziquantel. Thus the current strategy advocates at least a one week 'washout period' between the administration of the albendazole/ivermectin and praziquantel (Hopkins et al., 2002). In public health terms, this would be a challenge as it would mean running two MDAs instead of one. Thus, research is urgently needed to determine whether these drugs could safely be given simultaneously. An assessment of efficacy of these three drugs, when given concurrently, would be imperative.

The prevalence of *Ascaris* did not change between the two survey periods (baseline 1.7% vs follow-up 1.5%). In addition, the overall prevalence is low and thus would require a much larger sample size to demonstrate an impact. More importantly at this overall prevalence, *Ascaris* may not be a major 'public health' problem affecting the population of Chikwawa District.

9.1.8 Impact of MDA on anthropometric indices in the lower Shire-Chikwawa District

Assessment of anthropometric indices for children aged between 3 and 18 years (due to restrictions of the software) revealed that one round of treatment, assessed after a year post treatment, did not show a statistically significant difference. These findings are similar to those reported by Fox et al, (2005) but different from those of Beach et al (1999). Beach et al (1999) found significant gains in height (among the hookworm infected) and weight (among *Trichuris*- infected) in a placebo controlled trial at four months follow-

up using a DEC based combination. In contrast, Fox et al, (2005), working in the same area in Haiti did not find improvements in z-scores except for small though significant increase in weight in *Trichuris* infected children who had received albendazole alone or in combination with DEC. The reason advanced for this lack of impact is low baseline infection intensities. It is intuitive that, though STH are associated with nutritional deficiencies and consequently growth faltering, this is likely to depend on the local epidemiology of the parasites and nutritional values of the staple foods for the population in question. In Chikwawa District, the predominant STH species is hookworm, whose main effect is on iron status (Crompton, 2000). It is possible; therefore, that this may have contributed towards lack of a demonstrable impact on anthropometric indices in the current study. In addition, assessment was carried out a year after the MDA and more importantly following the rainy season (post lean months of December-February). Thus, the long follow-up and the fact that the follow-up survey was carried out during the harvest season may have diluted any nutritional gains that might have accrued.

9.1.9 Impact of MDA on LF antigenaemia and microfilarimae

It is appreciated that a single MDA is unlikely to produce a significant impact on antigenaemia prevalence. This is particularly so for albendazole/ivermectin combination in contrast to that which includes DEC. This is because of the limited impact of these drugs on adult worms. DEC based combinations seem to have superior efficacy against adult worms (El Setouhy et al., 2004; Ottesen et al., 1999). In addition, it is thought that in the

event that an adult worm dies it may take over 12 months for the associated antigen to decay and become undetectable by ICT (Taylor et al., 2005). Thus it is not surprising that in this evaluation MDA seems not to have had an effect on antigen prevalence. Though the numbers were small (thus could not achieve statistical significance), nonetheless, it was interesting to note that underfive children from the intervention villages had a lower antigen prevalence as compared to those from the control villages and indeed the antigen prevalence amongst the under 30's was consistently lower in the intervention villages for each age category (see Figure 6.19). The observation that the geometric mean for microfilaria counts was significantly lower in the intervention villages would be consistent with findings from other settings in Africa (Dunyo et al., 2000; Dunyo and Simonsen, 2002). More importantly there was a 30% reduction in geometric mean intensity in the intervention villages between the two surveys. This contrasts with a 5% increase in the control villages. It is important, however, to appreciate that there might have been a bigger difference had it been that assessment was carried out over a shorter time period as was the case in Ghana (Dunyo et al., 2000; Dunyo and Simonsen, 2002). However, this was meant to replicate programme conditions and it seems to suggest that the essential strategy of the Global Alliance for the Elimination of Filariasis may apply in Chikwawa District. Repeat rounds of MDA would probably result in further reductions of geometric mean intensity of microfilaria which would in turn lead to reductions in force of infection by denying mosquitoes the opportunity of picking up microfilaria during blood meals for onward transmission. Whether the 6 rounds of MDA advocated by the Global Alliance for the Elimination of

LF will be adequate is currently unclear. In Chikwawa District, a recent vector incrimination study has established that the principal vectors for LF are *Anopheles* mosquito species- *A. gambie* and *A. funestus* (Merelo-Lobo et al., 2003), this offers an opportunity to control LF through synergistic efforts with the current Roll Back Malaria initiatives aiming to distribute impregnated bed nets to vulnerable sections of the population. In Malawi, a social marketing model has been adopted, with targeted groups (pregnant women and underfive children) buying nets at $0.5. This analysis has shown that bed net use had increased from 6% at baseline to 19% at the follow-up survey. Whether such a targeted coverage of ITNs, would have an impact on LF transmission in Chikwawa District and indeed in all LF endemic communities with anopheline transmission, needs to be evaluated.

9.1.10 Other benefits associated with MDA

It seems that the de-worming effect of treatment of filariasis is perceived as a benefit by people in the community and serves as an important factor in promoting compliance. Knowledge, attitude and perception surveys and anecdotal reports suggest that this contributes to the acceptance of the program (De Rochars et al., 2004). In Chikwawa District, there were several reports of children passing worms following MDA and this was commended by the population. To date, these de-worming benefits of treatment have not been emphasized as part of social mobilization strategy, in part, because of concerns that once a year treatment might not produce a long-term reduction in intestinal helminth infection. The results of the present study suggest that

increased emphasis on de-worming would be appropriate and would benefit the LF program in Malawi and elsewhere.

Current efforts to control intestinal helminth infection through mass treatment are largely focused on chemotherapy targeted to specific risk groups, e.g., school children. Although it is clear that school-based de-worming programs can reduce the morbidity of helminth infections in children, the indirect benefits of this targeted treatment to younger children and adults who reside in the community but are untreated may be limited, depending on the proportion of infected persons who are treated (Olsen, 1998). As the proportion of treated persons increases, untreated persons begin to benefit through a reduction in transmission intensity and decreased infection (Thein et al., 1991a). In contrast, as already pointed out, the LF programme is likely to impart public health benefits to a much larger proportion of the population as it targets over 70% of those 'at risk' of *W. bancrofti* infection. In addition, it is clear that treatment targeted to school children, at least in Chikwawa District, would miss populations that would benefit from treatment. Hookworm infections in particular, are more prevalent in adults than young children. Since LF programs are based on mass treatment, most age groups (except underfives) are expected to benefit from MDA regimens that include albendazole. In conclusion, LF elimination programs provide important collateral benefits through the reduction of intestinal helminth burdens. Because DEC has only a limited effect on intestinal parasites, greater collateral health benefits should be expected where albendazole is combined with ivermectin. To increase compliance, as the LF program is launched in

Malawi and elsewhere, health educational messages should place greater emphasis on the de-worming benefits of such a program.

9.1.11 Polyparasitism in the lower Shire Valley- Chikwawa District

This is the first detailed analysis of polyparasitism in Chikwawa District and indeed Malawi as a whole. It has demonstrated that multiple species infections are common and that 50% of infections with parasite species investigated in this analysis occur in combination with at least one other. Nonetheless it is worth pointing out that the observed parasite combinations in this analysis are likely to underestimate the true prevalence of co-infections for three reasons. First, soil transmitted helminths (STH) and *S. mansoni* were diagnosed using a single a Kato Katz thick smear. This is likely to miss light intensity infections and especially those due to *S. mansoni* which has been shown to exhibit day-to-day fluctuations in egg output (Booth et al., 2003; Utzinger et al., 2001). Though particular attention was paid to examine all stool smears within 15 minutes of preparation to minimise the risk of hookworm eggs dissolving in glycerol and thus infections being missed, this has not been evaluated in this setting to determine whether this time frame is optimum. It is, therefore, possible that some hookworm infections may have been missed due to over clearing (Martin and Beaver, 1968). Second, both hookworm and malaria infections are known to have seasonal patterns as discussed above (Malawi Ministry of Health, 2005; Nwosu and Anya, 1980). Since, the follow-up survey spanned the cooler months of July and August, the observed hookworm co-infections may be higher than would have been found at the height of summer. As for malaria,

the co-infections might be lower than if the survey had included the peak malaria season (rainy-season of December to April). Third, the fact that only 947 individuals out of the whole sample had a complete set of data is of concern regarding possible selection bias. It is, however, reassuring that the composition of this group by sex and the overall age distribution is not different from the parent data set. In addition, the prevalence for the major parasites was not statistically different from the original data set. This suggests that selection bias is unlikely to be playing a major role.

The observation that hookworm and LF co-infections occur at a higher frequency than would be expected by chance (based on prevalence data) is interesting. Though this association could be explained, at least partially, by ecological and socio-economic factors prevailing in this community, it might also reflect selective susceptibility of a subset of the population. The underlying factors for such susceptibility may be immunological, genetic or nutritional. It has long been appreciated that geo-helminth infections modulate the immune system towards predominately Th2-biased responses. Additionally, several studies have shown that infection with nematode (including *W. bancrofti*) can lead to a suppressed immune response against tetanus toxoid, BCG and other vaccine antigens (Cooper et al., 2003a; Cooper et al., 2000; Cooper et al., 2003b; Malhotra et al., 1999; Sabin et al., 1996). Influence of helminth infections on nature and outcome of clinical disease has also been observed (Cooper et al., 2003b; Lyke et al., 2005). For example, in Thailand it was found that nematode infected individuals are more prone to malaria, but are protected from severe disease (Nacher et al., 2000). These findings were not reproduced in studies conducted under

different epidemiological conditions in Africa (Le Hesran et al., 2004; Shapiro et al., 2005). It is conceivable therefore that the first parasite that establishes an infection may modulate the immune response in such a way that it makes it easier for the next. As regards the hookworm/LF combination in Chikwawa District, one could postulate that *W. bancrofti* is likely to be the first parasite the majority of the population come in contact with and at a much younger age (see Chapter 6 Figure 6.19).

The more than expected prevalence of *S.mansoni* and *S. haematobium* co-infection observed here is probably a result of the ecological conditions in this area. Of note, as already pointed out, *S. mansoni* was detected in indivudals coming from four villages (see Figure 6.13b) which are within 5 km of each other and to a large water body (called Lake Lisuli- see Figure 3.2). This observation suggests that the local environment in these four villages supports the intermediate hosts for both parasites and more importantly *Biomphalaria spp* are restricted in their geographical distribution even at this scale.

In view of polyparasitism being so common in Chikwawa District- southern Malawi and most of Sub-Saharan Africa, several important implications need to be discussed. First, there is growing recognition in public health circles for integrated and combined control approaches, so that pro-poor health benefits can be maximized with limited resources. It has recently been proposed to combine the filariasis elimination programme with control efforts aimed at malaria, which is, among other reasons, justified on the basis of technical similarities of effective and current interventions (Molyneux et al., 2005; Molyneux and Nantulya, 2004). Further justification for combined control

approaches can be made from recent data in Senegal; severe malaria attacks were significantly lower among individuals free of helminth infections, thus treatment against soil-transmitted helminths might protect from malaria episodes (Spiegel et al., 2003). However, noteworthy is the fact that studies in Côte d'Ivoire and Uganda have not corroborated this observation, as no significant associations were found between either *Plasmodium* infection or clinical malaria and any of the soil-transmitted helminths investigated (Raso et al., 2004; Shapiro et al., 2005).

Second, it has previously been suggested that individuals with multi-species parasite infections are at an increased risk of morbidity [though not conclusively established in our data albeit such a pattern for anaemia (see Figure 7.5) and anthropometric indices (see Figure 7.6) emerged]. Thus there is urgent need for targeted research on the pathophysiology of multiple parasite infections and associated disease in the human host. Notwithstanding that interaction between different parasite species are complex and thus inherently difficult to elucidate.

Finally, it is important to appreciate that historically, polyparasitism used to be common in industrialised nations. But social and economic advances, environmental sanitation, use of preventive measures, improved hygiene and, finally, development and access to efficacious drugs led to a decrease in the frequency of parasitic infections and hence polyparasitism as a direct response to these economic and social changes (Saathoff et al., 2004; Yokogawa, 1985). This illustrates that without addressing the root ecological and behavioural causes, i.e. improved sanitation, coupled with sound hygiene, multiparasitism will remain common in the developing world.

9.2 Suggested recommendations

Plans are currently underway to set up the National LF Elimination Programme in Malawi. Data presented in this thesis will help inform decisisons on how best to proceed with initiation of LF elimination activities. It is, therefore, appropriate to make recommendations to be taken on board as the plans develop.

1. The national LF mapping survey has established that, in Malawi, LF is endemic in all districts except one. The implication being that the LF elimination programme will need to reach a population of over ten million. It is clear that some districts, though endemic by the WHO's classification, do not have LF as a major 'public health' problem. The national programme will, therefore, need to identify priority areas where to initially launch and concentrate elimination efforts before extending to non-priority districts.

2. In Malawi, as a result of erratic rains, a large section of the population experiences yearly famine. As a response, the government has embarked on promotion of irrigated farming as well crop diversification involving smallholder farmers especially in areas near large water bodies where water can easily be harnessed. Data from Karonga and Mchinji Districts suggest that LF transmission is influenced by agricultural practices. It would be adviseable for the LF programme and indeed others that target diseases whose equilibrium is likely to be affected by such practices (eg

malaria and schistosomiasis) to make plans to prevent establishment of such transmission foci.

3. Data from Chikwawa District suggests that MDA with albendazole and ivermectin leads to a sustained reduction in hookworm infection and anaemia prevalence in addition to its impact on LF transmission. It is, therefore, appropriate for the Malawi programme to promote the deworming aspect of this programme as an important and far reaching 'ancillary' public health benefit.

4. It is apparent from the Chikwawa data that polyparasitism is common in this population. Data from the scientific literatuture suggest that parasite infections may modulate the clinical course of each other. It is plausible, to infer, that control efforts targeted at one infection may impact on the epidemiology of the other. Thus, integration of control efforts (in particular for diseases with similar control startegies such as onchocerciasis and STH) would be recommended even at district level which would be in keeping with the Malawi decentralisation policy being advocated by the Sector Wide Approach (SWAp) intiatives in Malawi. This would maximise the use of already scarce resources.

5. Though vector control is not the main strategy being advocated by the Global Alliance for the Elimination of Filariasis, it would be recommended for the Malawi programme to invest in research to determine its potential

synergistic effect with MDA. This is especially so as the principal vectors for LF seem to be *Anopheles* mosquitoes and the fact that the government is promoting the use of impregnated nets with the aim of achieving the Abuja targets [60% of the vulnerable groups (pregnat women and children under 5) to be protected by ITNs by 2010]. Such research would determine whether vector control would lead to a reduction in the number of MDA rounds needed to achieve elimination of LF in this setting.

6. Findings from the TB study will provide valuable information in determining the potential role of antibiotic chemotherapy as an alternative treatment for LF. It is, therefore, highly recommended that this study is completed and its findings published.

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Appendices

Chikwawa Lymphatic filariasis study identifier form 15/03/06

(To be completed for each study participant)

Ident: |_____-__|

Name: _____

Sex: **M F** Date of birth: |____/_____| *(month/year)*

(<1900 <1910 <1920 <1930 <1940 <1950 <1960 <1970 <1980)
A B C D E F G H I

Name of mother: _____ |_____-__|

Village of birth: _____ |_____|

Current village: _____ |_____|

Current household: _____ |_____|

Position: **a** Head **b** Member **c** Visitor **d** Other ..... |__|

Relationship to the head of household_____|____|

Occupation _____

Highest class attended _____ *std* |___|

****

History

How often do you

a) Wash (clothes)/bath/swim in the Shire River and/or its tributary? 1 **Never** 2 **Rarely** 3 **Often**

b) fish (hook or nets) in the Shire River and/or its tributary? 1 **Never** 2 **Rarely** 3 **Often**

Do you grow crops such as rice along the Shire River and/or its tributary? **Y N**

Are you currently passing blood in your (a) stool? **Y N** (b) urine? **Y N**

Are you experiencing pain when passing urine? **Y N**

Do you ever have problems with abdominal pain? 1 **Never** 2 **Rarely** 3 **Often**

Have you ever had episodes of fever associated with localised lymphadenopathy and limb swelling?

Y N

If yes, how often in the last year? |_____|

When was the last episode? |_____| day(s)/week(s)/month(s)/year(s)

When was the last time you received treatment for malaria? |_____| day(s)/week(s)/month(s)/year(s)

Ask if ever received deworming tablets during the school mass treatment campaign **Y N**

Did you sleep under a mosquito net last night? **Y N**

If yes, check colour of the net (a) **white** (b) **other**

Examination

Weight|_____| Kg Height |_____| cm MUAC |____| inch BCG scar **Y N**

Are there signs of chronic clinical manifestations of lymphatic filariasis? **Y N**

If yes, then complete the following section:

Lymphoedema/Elephantiasis

Part affected	Leg	**L**	Y/N |____| cm	**R**	Y/N |____| cm
	Arm	**L**	Y/N |____| cm	**R**	Y/N |____| cm
	Breast	**L**	Y/N |____| cm	**R**	Y/N |____| cm

If leg and/or arm affected

(1) Is it pitting? **Y N**

(2) Does oedema reverse on elevation? **Y N**

(3) Is the skin thickened? **Y N**

(4) Are there warty (papillomatous) lesions? **Y N**

Hydrocele (testicle) **L** Y/N |____|cm **R** Y/N |____|cm **Both** Y/N |____|cm

****

Specimen taken **Y N**

****

Date |____|____|____| Staff code |____| checker |_____| *v1 April 16, 2002*

Chikwawa Lymphatic filariasis study-Household Questionnaire

(One form per household)

House number |_____|

Name of household head : _____

Sex: **M F** Date of birth: |____/_____| *(month/year)*

(<1900 <1910 <1920 <1930 <1940 <1950 <1960 <1970 <1980)

Birth estimate: **A B C D E F G H I**

Village: _____

Mother's name: _____

****

Income

Do you or any member of your household have a regular source of income? **Y N**

Do you or any member of your household have a bank and/or a post office savings book? **Y N**

Do you or any member of your household have (*ring if owned*) a) **paraffin lamp** b) **radio** c) **bicycle** d) **motorcycle** e) **video set** f) **an ox-cart** g) **cattle** h) **goats**

What is used for cooking in your household? a) **firewood** b) **charcoal** c) **electricity**

****

House

What is the main material of 1 Floor? a) **earth/dung** b) **cement**

2 Walls? a) **bamboo/wood** b) **Unburnt bricks** c) **Burnt bricks**

3 Roof? a) **thatch** b) **iron sheets** c) **Other**

What is the main source of drinking water for members of your household?

a) **unprotected well**
b) **protected well**
c) **surface water** (spring, stream, river, pond etc)
d) **piped/borehole**
e) **Other**

What kind of toilet facility does your household use?

a) **pit latrine**
b) **flush toilet**
c) **no facility**

Do you share this facility with other households? **Y N**

****

Date |____/____/____| Staff code |____| Checker |____|

Chikwawa Lymphatic filariasis study-SPECIMEN FORM *April 2002-04-16*

(One form per specimen)

Ident: |_____|

Name: _____

Sex: **M F** Date of birth: |____/_____| *(month/year)*

(<1900 <1910 <1920 <1930 <1940 <1950 <1960 <1970 <1980)

Birth estimate: **A B C D E F G H I**

Head of Household:_____ *Attach specimen no. here*

Village: _____

Mother's name: _____

****

Stool

Consistency: 1 **hard** 2 **soft** 3 **watery**

Blood stained: **Y N** lab no. |_____|

****

Urine

Blood stained: **Y N** lab no. |_____|

****

Blood *(ring type)*

Finger prick / Venepuncture: volume: _____

Tests required:

Malaria parasites	**Y N**	
Microfilarae	**Y N**	
ICT	**Y N**	
haemoglobin	**Y N**	
other	**Y N**	lab no. |_____|

Time collected (if for MF).....HRS

****

Date collected |____/____/____| Staff code |____|

Date received in lab |____/____/____| Lab staff code |____| Checker |____|

Publications

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

Dr B Ngwira
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Our Ref: 05/206

22 December 2005

Dear Dr Ngwira

The geographical distribution of lymphatic filariasis infection in Malawi

B. Ngwira, P. Tambala, M. Perez, C. Bowie, D. Molyneux

The above manuscript has now been accepted for publication in the Annals of Tropical Medicine and Parasitology. Should any queries arise during editing I will write to you again.

Best wishes

Yours sincerely

Keith Wallbanks

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

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The geographical distribution of lymphatic filariasis infection in Malawi

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Abstract

Results from a nation wide mapping exercise for lymphatic filariasis (LF) in Malawi are presented. A total of 35 villages were sampled from 24 districts excluding three districts (Karonga, Chikwawa and Nsanje) that had recently been mapped and Likoma island where access was not possible in the time frame of the survey.

Village antigenaemia prevalence [based on immunochromatographic (ICT) card tests] ranged from 0% to 35.9%. In general, villages from the western side of the country and far removed from the lake shore tended to register lower prevalence. This is with the exception of Mzenga village in Mchinji district along the Malawi-Zambia border where a prevalence of 18.2% was found. In contrast villages from lake shore districts [Salima, Mangochi, Balaka and Ntcheu (Bwanje valley)] and Phalombe had prevalence of over 20%.

Incorporating data from the 2000 surveys in Karonga, Chikwawa and Nsanje districts shows a marked decline of prevalence with increasing altitude. Further analysis revealed a strong negative correlation (R^2=0.7 $p < 0.001$) between altitude and prevalence. Of note, these data suggest that the lake shore, Phalombe plain and the lower Shire valley will be priority areas for the Malawi LF elimination programme. Implications of these findings as regards implementing a national LF elimination programme in Malawi are discussed.

Introduction

Lymphatic filariasis (LF), a disabling parasitic disease, has been identified as a major public health problem in most of the tropical and sub-tropical countries. It is currently estimated that up to 120 million people are infected with *Wuchereria bancrofti* in about 83 endemic countries (World Health Organisation, 2005). Of these, 40 million have evidence of chronic manifestations such as hydrocele and lymphoedema/ elephantiasis. In addition the affected individuals suffer repeated episodes of adenolymphangitis ('acute attacks') which result in marked loss in their economic productivity (Ravindran, 2003). Improved therapies and diagnostic methods have led to the realisation that it should be possible to interrupt transmission and eliminate LF by repeated, annual cycles of mass drug administration (MDA), with single dose combination regimens (Ottesen, 2000). Thus, in 1997 the World Health Assembly passed a resolution calling for strengthening of activities leading to the elimination of LF as a "public health problem" (Molyneux and Taylor, 2001). This resulted in the initiation of the now well-established Global Program to Eliminate Lymphatic Filariasis (GPELF) in 2000.

Malawi has two previously known LF foci: one in the southern part (Shire valley) and the other in the northern region along the Songwe river which forms its border with Tanzania (Hawking, 1977; Oram, 1958). However there had been no detailed community based surveys for LF in Malawi apart from one in the northern focus which was conducted in 1960. This survey, based on microscopic examination (for microfilarae) of thick bloodsmears which were made from samples collected at night, showed a high prevalence of microfilaraemia amongst adults (40%) and suggested that human infection with *W. bancrofti* was confined to communities in close proximity to the Songwe River(Oram, 1960).

More recently, surveys in these two foci have reported high antigenaemia prevalence based on immunochromatographic (ICT) card tests that approached 80% in some of the sampled villages(Ngwira et al., 2002; Nielsen et al., 2002). There was also remarkably high prevalence of LF associated disease in both areas (4% lymphoedema and up to 18% hydrocele). In addition, the survey in Karonga established that *W. bancrofti* infection is more wide spread than previously known, whereas in the lower Shire valley a surprisingly high antigenaemia prevalence (55%) was found amongst children (aged 1-9 years) than has been reported anywhere else.

Towards the end of 2003 we completed a nation-wide mapping exercise using ICT cards. The main objective was to obtain baseline data on the geographical distribution of LF in the remaining districts in Malawi as a prerequisite to initiating national LF elimination activities. This paper presents findings from the 2003 survey and in addition it incorporates data from recent surveys in the two known foci that have already appeared in the scientific literature to produce, for the first time, a complete map of the distribution of LF infection (based on adult worm antigenaemia) in Malawi. The implications of

this distribution for LF control programme planning and eventual implementation are discussed.

Materials and methods

Malawi is administratively divided into northern, central and southern regions. These are further divided into 27 districts. Two of these (Likoma and Mwanza Neno Districts) were demarcated after this survey had been planned and thus are considered with their parent districts of Nkhata-Bay and Mwanza, respectively. LF prevalence data were available for three districts; Karonga District in the northern region, Chikwawa and Nsanje Districts in the southern region. Thus the latest survey did not cover these districts. In the remaining districts we aimed to sample a random selection of villages for antigen testing. A database of villages by district was made available via the WHO's HealthMapper software. A programme incorporated in the software was used to provide a random sample of villages to be surveyed. The selected villages had a 50km buffer zone as recommended by the WHO's rapid assessment for the geographical distribution of lymphatic filariasis (RAGFIL) method(Gyapong and Remme, 2001) (Gyapong et al., 2002). Three additional villages were chosen in the field from inhabited areas from where the database did not contain any villages. The testing protocol adopted followed recommendations of the RAGFIL method that is based on Lot Quality Sampling (LQAS) scheme (Anon, 1999). Briefly, if at least 10 (20%) of the first 50 individuals (aged >15 years) tested were positive testing could be stopped; otherwise up to 100 individuals were to be tested per sampling point(Anon, 1999). However since most villages are sparsely populated thus those adjacent to the selected one were also invited to participate in order to achieve the required sample size. Hence random selection of subjects was not feasible in most villages. Before testing could be carried out a meeting with village members was held and the objectives of the survey were explained in the local language. Each consenting individual provided demographic data (age and sex) and a finger prick blood sample. The whole blood obtained was immediately applied onto the ICT (Binax Inc., Portland, ME) card and read within ten minutes according to the manufacturer's instructions. If two lines appeared in the viewing window that particular individual was positive for LF(Weil et al., 1997). Individuals found positive were treated on the spot with albendazole (400 mg) and ivermectin (200µg/kg body weight). All sampled villages had geo-coordinates determined by a portable Geographical Positioning System (GPS- Garmin eTrex®) machine.

Ethics

The survey received ethical clearance from the Malawi Ministry of Health Sciences Research Committee (HSRC). Individual consent was obtained from each participant or (if they were aged <16) from one of their parents or a guardian.

Data management

Data were entered into the computer using EPINFO 2000 (CDC, Atlanta) software. The data were subsequently exported into STATA version 7 (Stata

Corporation, College Station, TX) for descriptive statistical analyses. In order to investigate the relationship between prevalence and altitude, log transformation of the prevalence data was carried out using the formula $\log_{10}(x + 1)$. Village geographical coordinate data were used to produce a map showing the spatial distribution of LF infection using the WHO's HealthMapper software.

Results

A total of 35 data points were sampled. Of these three were chosen in the field in inhabited areas where there were no villages on the Healthmapper database. A total of 2913 individuals were examined. The age and sex distribution of the survey participants is shown in Figure 1. There was a female excess (64%) amongst the study participants (more marked in the 20-24 age bracket). Overall there were 269 (9.2%) individuals positive for circulating filarial antigen (CFA) based on ICT results. Significantly more males than females tested positive (11.0% vs 8.2% p=0.01). Figure 2 shows the proportion of those positive for CFA by age and sex. Amongst the males, those positive, tended to be older (student *t test* p=0.08). This relationship was not observed in their female counterparts.

Survey prevalence data by district and village are presented in Table 1. This ranged from 0% to 35.9%. The spatial distribution of the sampled villages with their prevalence category are shown in Figure 3. In general villages in the western side of the country registered a CFA prevalence of less than 10%. This is with the exception of Mzenga Village in Mchinji District along the Malawi-Zambia border where a prevalence of 18.2% was found. Prevalence of over 20% was observed from villages in Salima and Mangochi Districts along the southern shore of Lake Malawi. Also in Ntcheu district (Bwanje Valley), Balaka district near Lake Malombe and finally in Phalombe district along the shores of Lake Chilwa. The highest prevalence (35.9%) was recorded at Kalembo village in Balaka district in southern Malawi.

Prevalence data from the 2000 surveys are summarised in Table 2. The geographical distribution of all data points sampled (ICT) in Malawi showing prevalence in relation to altitude is presented in Figure 4. Figure 5 (a) shows a scatter plot of antigen prevalence by altitude. There is notable decline in prevalence with increasing altitude and further statistical analyses on log transformed prevalence data [Figure 5 (b)] have shown a significant negative correlation between altitude and prevalence ($R^2=0.7$ $p < 0.001$).

Discussion

The present survey, in the remaining unmapped districts in Malawi, has shown that infection with *W. bancrofti* as determined by antigenaemia prevalence is more widespread than previously appreciated. The female excess observed amongst our survey population probably reflects the fact that males are often out in the field during the day thus not available for testing. The implication of this being that the prevalence we found in some of our sampled villages is likely to be an under-estimate of the true prevalence. This

is due to the fact that in most communities significantly more males tend to carry the infection as has been observed in this survey and in other surveys from Malawi and elsewhere in Africa (Ngwira et al., 2002; Wamae et al., 1998).

In all districts, except Chitipa in the north, there was at least one individual who was positive on ICT. The low prevalence found in villages from the western side of Malawi could be explained by the fact that these areas are dry, of relatively higher altitude and thus not ideal for extensive mosquito breeding. The 18.2% prevalence observed at Mzenga Village in Mchinji along the Zambia border is intriguing. This is particularly so as there have been no anecdotal reports of LF disease from either the Malawi or Zambia side of the border in this area. Of note is that this village is in close proximity to a perennial stream that sustains a reasonable amount of irrigated onion farming. Whether this setting is conducive for supporting extensive mosquito breeding and thus driving *W. bancrofti* infection as has been observed in Northern Malawi and Ghana will need further investigation (Ngwira et al., 2002) (Dzodzomenyo et al., 1999) (Hunter, 1992). Ideally this should be coupled with human night blood examination for microfilariae.

It is also interesting to note that some villages from districts (Rumphi, Nkhata-Bay and Nkhotakota) along the lake shore had prevalence of less than 10%. A possible explanation could be due to the fact that these districts are mountainous and thus well drained consequently limiting potential mosquito breeding sites.

The relatively high prevalence found in Salima, Ntcheu (Bwanje Valley), Balaka, Mangochi and Phalombe was unexpected. However there have been isolated unpublished reports of cases with chronic manifestation of LF (hydrocele and elephantiasis) in these areas. It is worth noting that the ecological conditions in these districts are ideal for supporting large potential LF vector populations. Incorporating data from 2000 surveys clearly shows that the priority areas for LF control activities in Malawi will be the lakeshore districts, Phalombe plain and the Lower Shire Valley.

The decline in LF prevalence with increasing altitude has also been reported from other settings in Africa(Onapa et al., 2005). This is believed to be due to the influence of altitude on temperature which is known to be critical for survival of the vector and development of the parasite within the vector (Lardeux and Cheffort, 2001).

These findings have important implications for initiating the "Malawi LF Elimination Programme". First, following WHO's recommendation that all implementation units with a prevalence on ICT of over 1% be considered endemic and thus treated, the Malawi programme would involve 26 districts with a target population of over ten million. The population affected is far greater than ever envisaged. Secondly, both the northern (Karonga) and Southern foci (the Lower Shire Valley) share international borders which are largely porous. This calls for innovative approaches in carrying out control activities as they have to be synchronised with those in neighbouring

countries. Thirdly, in some districts (Phalombe, Mulanje, Thyolo, Chikwawa and Mwanza) where LF is co-endemic with onchocercisasis the two programmes will need to be merged. Fourthly, the LF programme will need to establish links with other programmes that are delivering community based interventions such as the ministry of education's deworming and feeding programme and the expanded bed net distribution under the malaria control programme.

Acknowledgement

This survey received financial support from the Gates Foundation through the WHO's AFRO office (Sticker No. AF/02/P227728) and the Lymphatic Filariasis Support Centre (supported by the UK's Department of International Development) at the Liverpool School of Tropical Medicine. Technical and administrative support was provided by the Malawi Ministry of Health through the National Onchocerciasis Task Force Office and the Malawi College of Medicine. BN received financial support from a WHO/TDR fellowship.

Figure 1

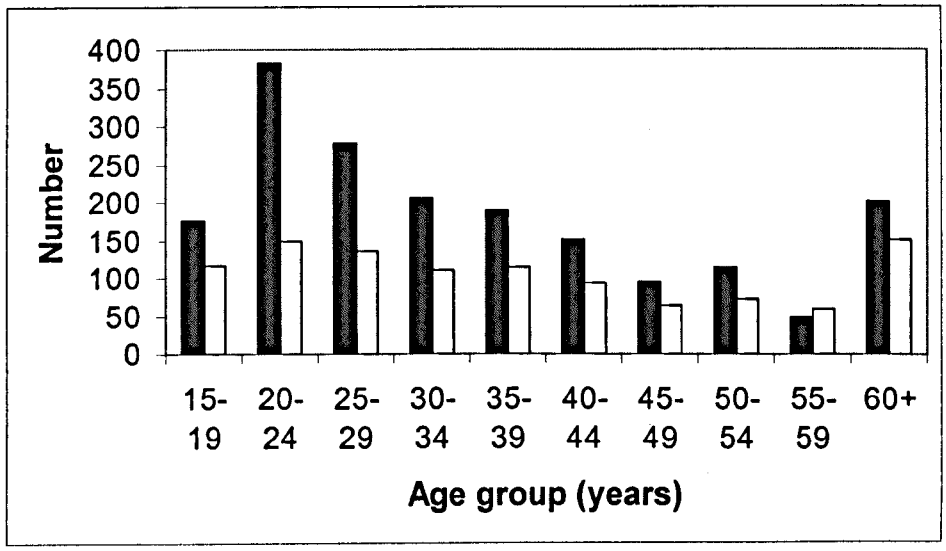

Number
400
350
300
250
200
150
100
50
0
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60+
Age group (years)

Figure1: The age and sex [female (■) and male (□)] distribution of survey participants.

Figure 2

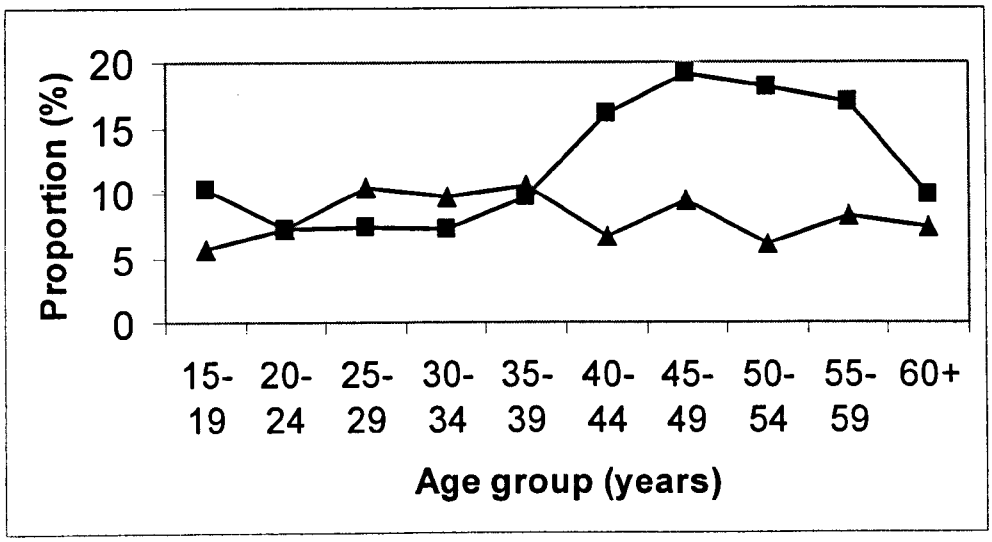
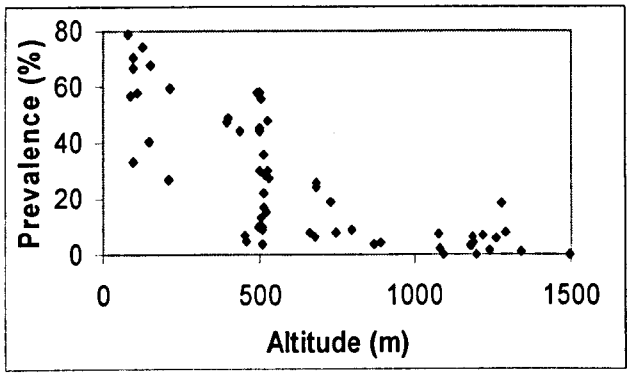
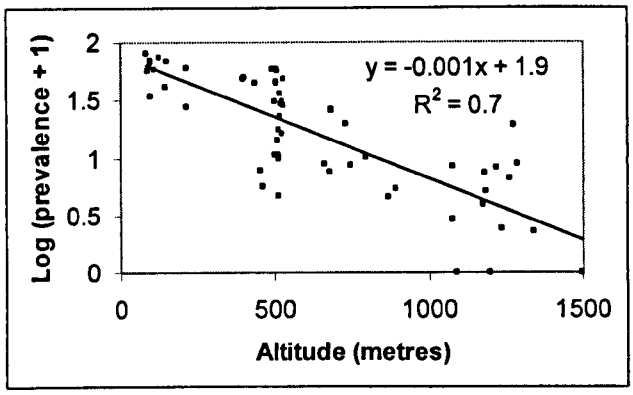

Proportion (%)
20
15
10
5
0
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60+
Age group (years)

Figure 2: The proportion of males (■) and females (▲) positive for CFA by age.

Figure 5


Prevalence (%)
80
60
40
20
0
0
500
1000
1500
Altitude (m)

(a)


Log (prevalence + 1)
2
1.5
1
0.5
0
y = -0.001x + 1.9
R2 = 0.7
0
500
1000
1500
Altitude (metres)

(b)

Figure 5: Prevalence plotted against altitude (metres) (a) and log transformed (prevalence + 1) plotted against altitude (metres) (b).

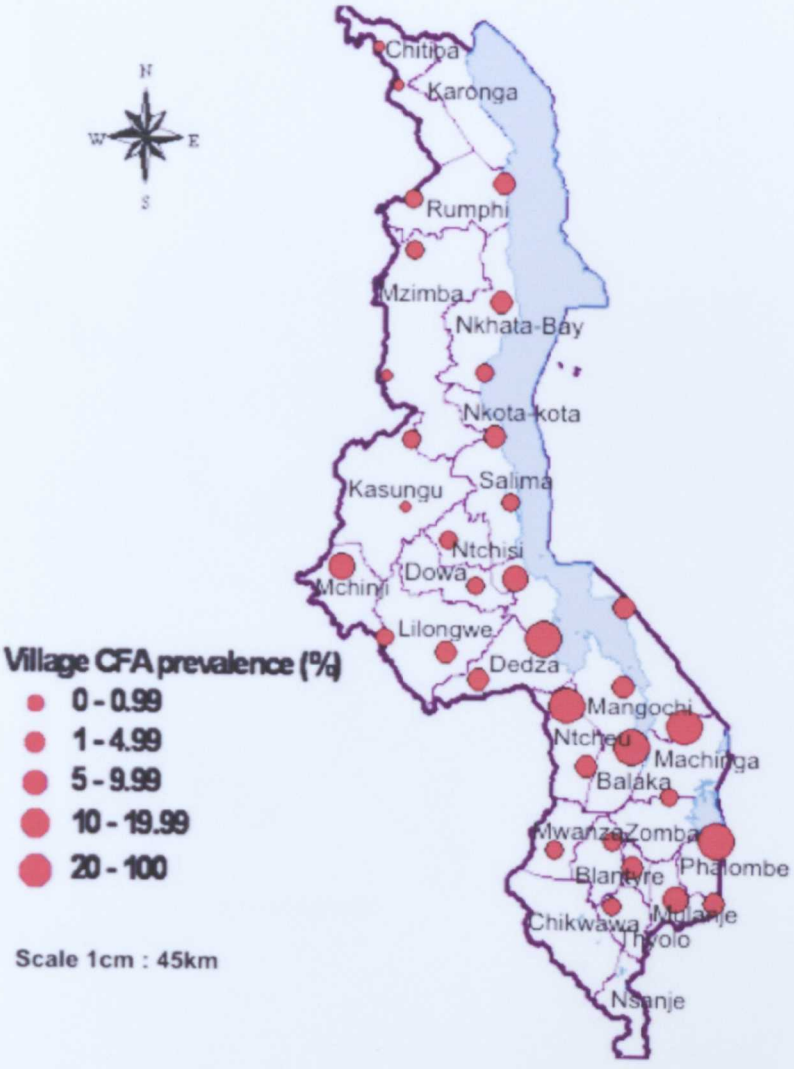

N
W
E
S
Chitipa
Karonga
Rumphi
Mzimba
Nkhata-Bay
Nkota-kota
Kasungu
Salima
Ntchisi
Dowa
Mchinji
Lilongwe
Dedza
Mangochi
Ntcheu
Machinga
Balaka
Mwanza
Zomba
Phalombe
Blantyre
Chikwawa
Mulanje
Nsanje
Village CFA prevalence (%)
0 - 0.99
1 - 4.99
5 - 9.99
10 - 19.99
20 - 100
Scale 1cm : 45km

Figure 3: Showing village CFA prevalence from the 2003 national mapping survey.

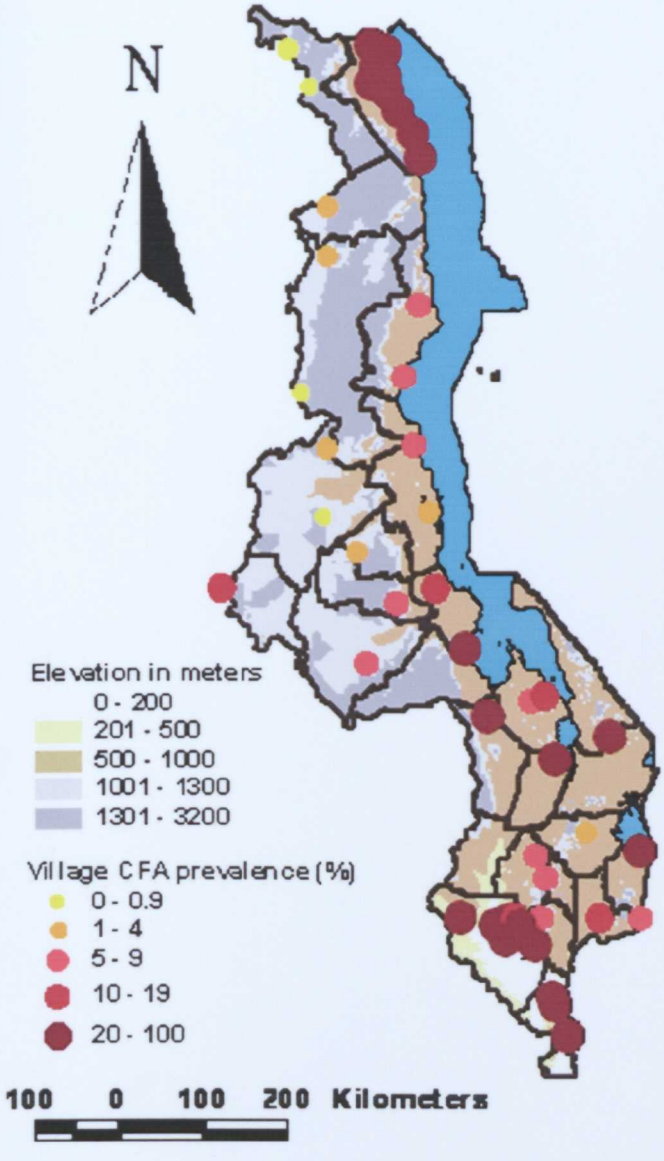

N
Elevation in meters
0 - 200
201 - 500
500 - 1000
1001 - 1300
1301 - 3200
Village CFA prevalence (%)
0 - 0.9
1 - 4
5 - 9
10 - 19
20 - 100
100 0 100 200 Kilometers

Figure 4: Map of Malawi showing prevalence of all sampled villages in relation to altitude (metres).

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Haematological profiles of the people of rural southern Malawi: an overview

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An integrative review of the results of two published and two unpublished studies of anaemia in children, adolescent females, pregnant women and adults living in southern Malawi is presented. Anaemia was universally present in all age-groups, with the higher prevalences in infants (100%) and adolescent primigravidae (93.8%). Nutritional deficits of iron and vitamin A were major contributory factors but chronic malarial haemolysis also significantly contributed to the anaemia. Among boys, anaemia was more common among those with glucose-6-phosphate-dehydrogenase (G6PD) deficiency than in those without this deficiency ($P < 0.002$). This enzymopathy, which occurred in 23.5% [95% confidence interval (CI) = 16.7%–30.1%] of the male and 30% (CI = 17.3%–42.7%) of the female infants examined, was also associated with neonatal jaundice.

The overall prevalences of the $-\alpha^{3.7}/\alpha\alpha$ and $-\alpha^{3.7}/-\alpha^{3.7}$ thalassaemia genotypes were estimated at 41.0% (CI = 28.3%–53.7%) and 8.7% (CI = 1.5%–15.9%), respectively. Haemoglobin AS was present in 18.1% (CI = 12.8%–23.4%) of the infants and haemoglobin SS in 2.5% (CI = 1.4%–3.6%). As the prevalence of infection with *Plasmodium falciparum* was significantly higher in infants with haemoglobin AS than in those with AA (21.4% *v.* 6.7%; $P < 0.001$), an increased risk of early-onset moderate parasitaemias in young infants probably stimulates the development of immunity, protecting older heterozygotes from severe malarial infection. Innovative community approaches are required to break the cycle of ill health that anaemia supports in those living in rural areas of southern Malawi. Interventions in adolescent girls could be of particular importance, as they could break the cycle in both pregnant women and their infants.

Iron-deficiency and malaria-attributable anaemia continue to be two of the most important public-health problems in developing countries. It is estimated that iron-deficiency anaemia affects as many as 2000 million people world-wide (World Health Organization, 2000). Malaria has an equally wide global distribution but approximately 90% of the estimated 300 million–500 million, new, clinical cases of malaria that develop each year occur in sub-Saharan Africa (Goodman *et al.*, 2000). In this region, 23 million pregnant women are exposed to malarial infection annually (Goodman *et al.*, 2000).

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Before the effectiveness of interventions for reducing iron-deficiency or malarial anaemia can be assessed, the haematological profile of the targeted population must be explored. In developing countries, there have been many studies on anaemia in particular age-groups but few profiles have been published on the haematological status of all age-groups, or of pregnant and non-pregnant women, within the same population or community. Four studies of anaemia in children, adults and pregnant women have been undertaken over the past few years amongst the people of the lower valley of the Shire river, in southern Malawi, where malaria is holo-endemic. The results of two of these investigations have already been published (Verhoeff, 2000; Prinsen-Geerligs *et al.*, 2003) but, until now, those of the other two studies have not appeared in the scientific literature. Although these studies were conducted semi-independently, each with its own primary purpose, they have provided the data used in the present, secondary and summary interpretation, with an analysis from infancy through to adulthood. The influence of genetic traits was also considered in the present study. During the period when the data were collected, anaemia control in the study area was based on case-management and vitamin-A supplementation in young children and post-partum women.

SUBJECTS AND METHODS

The haematological data summarized were all collected in one rural region of southern Malawi, approximately 300 m above sea level. Malaria transmission is holo-endemic in this area, with the main rains occurring from December–March. The small-scale cultivation of maize, sorghum, cotton and sugar-cane forms the primary source of food and income. There is a high level of illiteracy (71.8%) and poverty in the area. For convenience, the two unpublished investigations providing some of the data analysed were named UP1 and UP2. In all four studies, ethical approval was given by the Health Sciences Research Committee of the College of Medicine in Blantyre, and levels of malarial parasitaemia (trophozoiteses/μl blood) were determined for every subject. Thick blood-smears were prepared, stained, usually with Giemsa's stain, and examined by oil-immersion light microscopy. Trophozoites were counted against 50 leucocytes and each subject was assumed to have 8000 leucocytes/μl.

The Four Sources of Data

The oldest data analysed came from the study by Verhoeff (2000), which was undertaken, between March 1993 and June 1994, in the lower Shire valley. The main objective of this investigation was to determine the concentrations of haemoglobin and zinc protoporphyrin (ZP) in blood samples collected from pregnant adolescents and adults (at their first antenatal visit to the rural, district hospitals in Chikwawa and Montfort). The blood samples were collected by venepuncture after obtaining verbal informed consent. Haemoglobin (Hb) was measured using a cyanomethaemoglobin method and a haemoglobinometer (Biotron, Sydney). A ZP haematofluorometer (Model 206; AVIV Biomedical, Lakewood, NJ) and washed blood samples were used to measure ZP concentrations (Chimsuku, 1996). Subjects found to have >3.1 μg ZP/g Hb were considered to be suffering from iron deficiency. Serum concentrations of vitamin A were also measured, by HPLC (Catignani and Bieri, 1983), using blood samples obtained at delivery, when available, or otherwise those collected at recruitment.

The main objective of UP1, a study undertaken between July and August 1994, was to determine the prevalences of erythrocytic glucose-6-phosphate-dehydrogenase (G6PD) deficiency, malaria and anaemia in children aged 0–6 years. Most (203) of the 208 subjects were selected, by systematic sampling, from the children of non-consanguinous

marriages attending the 'under-fives' clinic at Blantyre; children with recent and severe haemolytic crises and high reticulocyte counts were excluded. The remaining five subjects were jaundiced infants; four neonates and a 6-month-old boy. Each blood sample was screened for G6PD deficiency using the semi-quantitative, Cresyl-Blue dye test (Motulsky and Campbell-Kraut, 1964), which mainly identifies subjects with almost complete enzyme deficiency (i.e. female homozygotes, male hemizygotes and an unknown proportion of female heterozygotes). Haemoglobin concentrations were again determined by the cyanomethaemoglobin method (Dacie and Lewis, 1984), subjects with <100 g Hb/litre being considered anaemic.

UP2 was undertaken, between March 1994 and September 1995, in the lower Shire valley, to determine the prevalences of the various α-thalassaemia polymorphisms and of sickle-cell anaemia, using cord-blood or infant-follow-up blood samples. Cord-blood samples were available from almost 700 infants born in Chikwawa District Hospital or Montfort Hospital. From these, 76 samples were selected, at random, for analysis, and 58 of these 76 were successfully characterized for α-thalassaemia genotype, by Southern-blot analysis (Old and Higgs, 1983). Screening for HbS genotypes was completed on 222 samples of infant blood, of which 95% came from subjects aged >26 weeks. During a 12-month follow-up period, the effect of α-thalassaemia and sickle-cell haemoglobin on haematological indices and malarial-parasite prevalence was also assessed in infants who attended regularly for follow-up. Haemoglobin variants were separated and identified by standard electrophoresis (Dacie and Lewis, 1984). Haemoglobin concentrations were again estimated by the cyanomethaemoglobin method. ZP levels and transferrin saturation were determined using commercial kits (Sigma) based on a colorimetric method (Labbé *et al.*, 1999).

Prinsen-Geerligs *et al.* (2003) collected their fingerprick blood samples in May 2000, with the objective of determining Hb concentrations and blood ZP levels (as measures of iron status) in rural Malawian villagers, of all ages except young infants, from the Shire valley. The two study villages, Meja and Tsamba, were selected because of their accessibility by road, the willingness of their populations to participate, and their large size. Up to six subjects — the father and mother in the nuclear family, adolescent girls and children aged 5 months–11 years — were selected from each household willing to participate. The youngest children in the household were selected in preference to older children and adolescent girls in preference to other children. Blood concentrations of Hb, measured on enrolment using a Hb photometer (HemoCue, Lake Forest, CA), and age-specific cut-off concentrations of Hb indicating anaemia (Stoltzfus and Dreyfuss, 1998) were used to calculate prevalences of anaemia. All subjects found to be anaemic were treated with a therapeutic course of iron, or referred to hospital if symptomatic. ZP was measured, for a random sample of participants, using the same methods and haematofluorometer as used by Verhoeff (2000). Again, ZP concentrations exceeding 3.1 μg/g Hb were considered indicative of iron deficiency (Labbé *et al.*, 1999).

RESULTS

Verhoeff (2000)

Nearly all the pregnant adolescents and adults checked at their first antenatal visits [mean (S.D.) gestation = 21.6 (6.2) weeks] were anaemic (Table 1). Adolescent primigravidae had the lowest mean Hb concentration and the highest prevalence of anaemia (93.8%) but were at an equivalent risk for iron deficiency as the non-adolescent primigravidae. Adolescent primigravidae had a high prevalence of *Plasmodium falciparum* parasitaemia (35.2%). The seasonal prevalence of iron deficiency, anaemia and malaria parasitaemia amongst pregnant adolescents is shown in the Figure. The peak prevalence of iron deficiency occurred in February 1994

TABLE 1. *Haematological parameters, at first antenatal visit, of the pregnant adolescents and adults studied by Verhoeff (2000)*

Parameter	Adolescents		Adults	
	Primigravidae	Multigraviade	Primigravidae	Multigravidae
No. of participants	528	166	355	2972
Mean age (years)	17.4	18.2*	21.7	27.7*
Mean (S.D.) gestational age (weeks)	20.6 (5.9)	20.7 (6.4)	20.2 (6.4)	22.0 (6.2)
Age range (years)	12–19	12–19	20–34	20–52
Mean (S.D.) haemoglobin (g/litre) and [no. of subjects investigated]	86.7 (16.5) [528]	92.5 (16.5)* [164]	89.4 (17) [355]	92.6 (17.5) [2972]
No. and (%) of participants found anaemic‡	495 (93.8)	144 (87.7)†	322 (90.7)	2614 (88.0)
Mean (S.D.) zinc protoporphyrin (μg/g haemoglobin)	3.7 (1.9)	3.4 (1.7)	4.0 (2.3)	3.6 (2.3)
No. and (%) of participants found iron-deficient/no. tested§	291/523 (55.6)	76/166 (45.8)	198/354 (55.9)	1327/2958 (44.9)*
No. and (%) of participants found smear-positive for malaria/no. tested	179/509 (35.2)	33/159 (20.3)	122/345 (35.4)	401/2862 (14.0)

*Value significantly different from that for the primigravidae of the same age-group ($P < 0.001$).
†Value significantly different from that for the primigravidae of the same age-group ($P < 0.01$).
‡Any participant with <110 g haemoglobin/litre was considered anaemic.
§Any participant with >3.1 μg zinc protoporphyrin/g haemoglobin was considered iron-deficient.

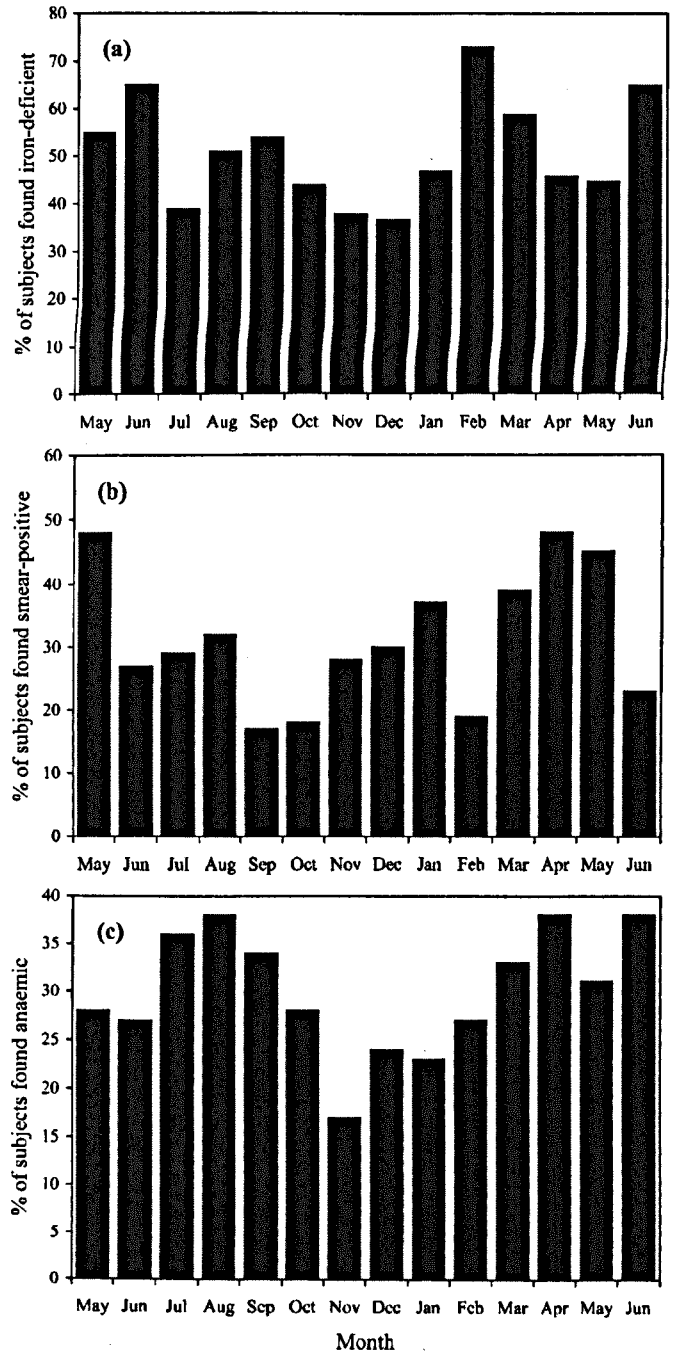

FIG. The seasonal prevalences of iron deficiency (a), malaria (b) and anaemia (c) amongst pregnant adolescents at first antenatal visit, as observed, by Verhoeff (2000), between May 1993 and June 1994. Any subject with < 80 g haemoglobin/litre was considered to be anaemic.

(73.2%) whereas that of anaemia (<80 g Hb/litre) occurred in June 1994 (38.2%) and malaria prevalence peaked in April 1994 (48.3%). Of the 179 women tested for vitamin-A deficiency, 65.3% were deficient (<0.70 μmol/litre) and 21.2% severely

deficient (<0.35 μmol/litre). The women checked for vitamin-A deficiency represented approximately 13.0% of the women delivering in the two district hospitals during the study period.

UP1

Of the 153 boys from the 'under-fives' clinic who were screened, 36 tested positive for G6PD deficiency, giving a frequency of the X-linked, recessive gene responsible for the deficiency of 0.235 [95% confidence interval (CI) = 0.167–0.301] in this group. Fifteen (30.0%; CI = 17.3%–42.7%) of the 50 girls from the same clinic who were checked were also found to have this deficiency. The frequency of the deficiency in the girls was higher than expected from the relevant gene frequency in the males; assuming a Hardy–Weinberg equilibrium, the gene frequency among the boys would indicate that 36% and only 5.5% of the females from the same population should be G6PD-normal heterozygotes and G6PD-deficient homozygotes, respectively. Boys with G6PD deficiency were much more likely to be anaemic than other boys ($P = 0.002$; Table 2). Amongst the five clinical cases of jaundice investigated, all three male neonates (but not the one female) were severely anaemic, deeply jaundiced and G6PD-deficient, and the older jaundiced boy was also deficient (Table 3).

UP2

Although 76 cord-blood samples were selected for the detection of α-thalassaemia genes ($-\alpha^{3.7}$) in UP2, sufficient DNA for the Southern-blot analysis was only obtained from 58 (76.3%). Of these 58 samples, 21 (36.2%) were heterozygous and 10 (17.2%) homozygous (all α+ homozygous). With correction for the population distribution of MCV, the prevalences of $-\alpha^{3.7}/\alpha\alpha$ and of $-\alpha^{3.7}/-\alpha^{3.7}$ were estimated to be 41.0% (CI = 28.3%–53.7%) and 8.7% (CI = 1.5%–15.9%), respectively. The estimated frequency of the $-\alpha^{3.7}$ gene, calculated using the Hardy–Weinberg equilibrium, was 0.29. Twenty-two infants were followed to study the effect of their α-thalassaemia genotypes on their haematological indices (Table 4). There were no significant differences at follow-up, between genotypes, in mean Hb concentrations. The mean transferrin saturations (at approximate ages of both 10 and 26 weeks) were, however, significantly lower in the normal infants than in those with α-thalassaemia deletions ($P < 0.02$; Table 4).

TABLE 2. *The association between glucose-6-phosphate-dehydrogenase (G6PD) deficiency and anaemia in the male infants investigated in UP1*

Category	G6PD-deficient	Normal	P*
No. of infants investigated	36	117	
No. and (%) found anaemic (i.e. with < 100 g haemoglobin/litre)	8 (22.2)	5 (4.5)	<0.002

*From a Fisher's exact, two-tailed test.

TABLE 3. *Anaemia and glucose-6-phosphate-dehydrogenase (G6PD) status of the jaundiced infants investigated in UP1*

Age	Sex	Haemoglobin (g/litre)	Diagnosis	Treatment
6 months	Male	62	Deep jaundice, G6PD-deficient	None (died 24 h after diagnosis)
2 days	Male	<70	Deep jaundice, G6PD-deficient	Exchange transfusion
1 day	Male	70	Deep jaundice, G6PD-deficient	Exchange transfusion
3 days	Male	62	Deep jaundice, G6PD-deficient	Exchange transfusion
6 days	Female	102	Deep jaundice, G6PD-normal	Phototherapy

TABLE 4. *α-Thalassaemia and haematological indices during the follow-up of the infants studied in UP2*

Parameter*	Genotype	Infant age (weeks): 5–14	24–31	>45
Mean (S.D.) haemoglobin (g/litre)	αα/αα	99 (35) [10]	87 (7) [10]	90 (11) [7]
and [no. of subjects investigated]	−α/αα or −α/−α	94 (19) [12]	91 (10) [12]	81 (22) [10]
Mean (S.D.) transferrin saturation (%)	αα/αα	26.7 (3.7) [10]	26.7 (3.4) [10]	26.8 (3.6) [10]
and [no. of subjects investigated]	−α/αα or −α/−α	30.4 (5.4)† [22]	31.8 (6.0)† [22]	30.3 (5.3) [22]

*Sample size was determined by subject compliance.
†Value significantly different from that for the αα/αα of the same age-group ($P < 0.02$).

Among the 12 infants with sequential ZP measurements, mean ZP levels at 10, 26 and 50 weeks of age were consistently higher in the babies with α-thalassaemia genotypes than in their normal counterparts, although the differences were not statistically significant.

Of the 222 samples used to determine the prevalence of HbS genotypes, five (2.5%; CI = 1.4%–3.6%) had HbSS, 37 (18.1%; CI = 12.8%–23.4%) had HbAS, and 18 could not be characterized. Application of the Hardy–Weinberg equilibrium gave a sickle-Hb-gene frequency of 0.11. Prior to 50 weeks of age, those with normal and sickle-cell genotypes had similar mean Hb and ZP concentrations and transferrin saturations ($P > 0.05$ for each). At 50 weeks of age, however, four infants with sickle-cell anaemia had a mean Hb concentration that was lower, by 8 g/litre, than that of their normal counterparts. At the same age, the 23 infants with HbAS then investigated also had significantly higher mean Hb levels than the 123 with HbAA (90 *v.* 85 g/litre; $P < 0.05$). Most of the children had >4.0 μg ZP/g Hb, indicating that they had chronic iron deficiency. A mean of 44 infants attended at each of the scheduled follow-up visits during their first 12 months. At these follow-up visits, infants with sickle-cell trait and those with sickle-cell anaemia were significantly more likely to have malaria parasitaemias than the HbAA children ($P < 0.001$ for each), with prevalences of 14.8% (48/325), 21.4% (12/56) and 6.7% (176/1735), respectively.

Prinsen-Gerlings *et al.* (2003)

Most (95.3%) of the 753 residents of Tsamba and Meja who were asked to participate in the study by Prinsen-Gerlings *et al.* (2003) gave their informed consent. The results for the 347 residents who, having satisfied the inclusion criteria, were enrolled in the study are summarized in Table 5. The prevalence of anaemia was generally high, varying from 100% in the infants to 32.4% in the adult males. Similarly, the prevalence of iron deficiency varied from 100% in the children aged <5 years to 80% in the adult males. The prevalence of malarial (*P. falciparum*) parasitaemia was highest in the children aged 2–5 years (72.7%), decreasing to 26.7% in the adult males and 20.3% in the adult females.

DISCUSSION

There is some heterogeneity among the four studies summarized, which reflects differences in the age-groups, sample sizes and study periods. The nutritional and malariometric studies were completed in May (Prinsen-Gerlings *et al.*, 2003) or between March and June (Verhoeff, 2000). The main rainy season in southern Malawi, which is associated with peak malaria transmission, occurs from December to March. The genetic studies (UP1 and UP2) were, however, unlikely to be influenced by study month(s). There were no droughts during any of the four studies although a severe

TABLE 5. *Haematological parameters of the various age-groups investigated by Prinsen-Geerligs* et al. *(2003)*

Parameter	Infants (aged 4 months–<2 years)	Children aged: 2–<5 years	Children aged: 5–9 years	Adolescent females (aged 10–19 years)	Adults: Males	Adults: Non-pregnant females
No. of participants	10	46	74	41	74	102
Mean age (years)	1.0	3.1	7.0	13.9	41.4	35.0
Age range	4–23 months	2–4 years	5–9 years	10–19 years	12–89 years	20–87 years
Mean (S.D.) haemoglobin (g/litre)	63.9 (24.6)	83.8 (12.9)	99.8 (18.1)	115.3 (12.3)	133.8 (20.7)	108.4 (20.5)
No. and (%) of participants found anaemic/no. tested*	10/10 (100)	43/46 (93.5)	61/74 (82.4)	30/41 (73.2)	24/74 (32.4)	72/102 (70.6)
Mean (S.D.) zinc protoporphyrin (μg/g haemoglobin)	10.8 (4.3)	9.3 (3.0)	7.3 (3.2)	5.0 (1.7)	4.2 (1.6)	5.9 (4.7)
No. and (%) of participants found iron-deficient/no. tested†	6/6 (100)	21/21 (100)	28/29 (96.6)	29/33 (87.9)	36/45 (80.0)	46/52 (88.5)
No. and (%) of participants found smear-positive for malaria/no. tested	5/8 (62.5)	24/33 (72.7)	37/62 (59.7)	12/29 (41.4)	12/45 (26.7)	15/74 (20.3)

*Children aged <5 years, children aged 5–9 years, adolescent and adult females, and adult males were considered anaemic if they had <110, <115, <120 or <130 g haemoglobin/litre, respectively.

†Any participant with >3.1 μg zinc protoporphyrin/g haemoglobin was considered iron-deficient.

drought had affected the study area for a few years prior to 1992. Among the adolescent pregnant girls investigated for a year by Verhoeff (2000), the peak prevalences of iron deficiency and malaria preceded that of severe anaemia (Fig.).

In the study by Prinsen-Gerlings *et al.* (2003), a clear age-specific trend was observed for the prevalence of anaemia, which fell sequentially from 100% in infants to 70.6% in non-pregnant, adult women and 32.4% amongst adult males. The prevalence of iron deficiency showed a similar downward trend with age but never fell below 80%. The reduction in anaemia prevalence with increasing age corresponds with a decreasing prevalence of malarial parasitaemia, from over 80% in young children to 20%–27% in adults. Despite this reduction in the prevalence of malarial infection, a high proportion of adults, especially of the women, remained anaemic. This indicates that the aetiology of anaemia amongst the people of southern Malawi is complex. In addition to iron deficiency and malaria, several other factors contribute. The seroprevalence of maternal HIV infection was high (24.0%–25.1%) among the pregnant women described by Verhoeff (2000) and the adolescent nulliparae screened as part of the same study (Brabin *et al.*, 1998). Given its high prevalence in the study area, HIV infection is likely to contribute to anaemia in all age-groups other than children of 5–9 years (most HIV-positive neonates will have died before the age of 5 years whereas the incidence of HIV infection only becomes high during adolescence). Even though HIV infection is relatively rare in those aged 5–9 years, Prinsen-Gerlings *et al.* (2003) still found 82.4% of children of this age-group to be anaemic and almost all to be iron-deficient (Table 5).

Hookworm infection, which could contribute to the observed iron deficiency, was not assessed in any of the four studies summarized here. Only one of 35 children admitted, with protein-energy malnutrition, to the Queen Elizabeth Central Hospital in Blantyre (the main referral hospital for the lower Shire valley) was found to have a hookworm infection (Mbewe, 1993). In a recent, unpublished, cross-sectional survey in the Shire valley, however, hookworm eggs were detected in Kato–Katz smears of faecal samples from 18.1% of the 1130 subjects (B. M. Ngwira; unpubl. obs.). *Schistosoma haematobium* infection is also probably common in the study area. Much of the iron that is consumed by the villagers in southern Malawi, who generally have poor diets, is probably not bio-available The main food source is maize, complemented with ground nuts and, seasonally, with Chinese cabbage and pumpkin leaves. Without irrigation, all crops are unpredictable.

Vitamin-A deficiency occurred in two-thirds of the pregnant women studied by Verhoeff (2000) and was severe in one in five such women. Of the 113 non-pregnant, adolescent girls from the Shire valley checked by Fazio-Tirrozzo *et al.* (1998), using the retinal dose–response test (Tanumihardjo *et al.*, 1990), 45 (40.2%) had vitamin-A deficiency. Although >88% of the pregnant women investigated by Verhoeff (2000) were found to be anaemic, only about 50% were iron-deficient. Maternal malarial infection and other nutrient deficiencies (e.g. of folate and vitamin B_{12}) may explain these findings, although ZP alone is not an ideal measurement of iron status in an area were malaria is common. No information is available on folate or vitamin-B_{12} deficiencies in the area where Verhoeff (2000) worked but both such deficiencies are likely to be common because of the lack of animal-derived foods in the diet and the limited intake of green vegetables. Van den Broek (1998) found that 21% of the pregnant women attending clinics in a different district of southern Malawi had combined folate and vitamin-B_{12} deficiency.

Anaemia in pregnant women is a major problem and, as in children, is multifactorial in aetiology. Among the pregnant villagers studied by Verhoeff (2000), the highest

prevalences of anaemia, iron deficiency and malaria were all found in the adolescent girls, with a mean age of 17.4 years (Table 1). Primigravidae, whether adolescent or adult, had higher prevalences of anaemia and malaria than the multigravidae. This parity difference is well described for women living in areas where malaria is holo-endemic, with women in their first pregnancies being particularly susceptible to *P. falciparum* infection (Brabin, 1983). The high frequency of anaemia in pregnant adolescent girls, and especially the increase in prevalence of anaemia from 73.2% in the non-pregnant adolescent girls to 87.78% in the pregnant (Table 1; Verhoeff, 2000), are major causes of concern. Iron deficiency in the pregnant villagers at their first antenatal visit was no more frequent in the adolescents than in the adults. It is likely that malaria was a main contributor to the anaemia observed during pregnancy. Of the non-pregnant adolescent girls investigated by Fazio-Tirrozo *et al.* (1998), 88.7% were anaemic and 4.4% severely anaemic (with <70 g Hb/litre). In Kenya, Leenstra *et al.* (2003) found that the control of malaria, by the use of insecticide-impregnated bednets, was effective in reducing the prevalence of anaemia in young adolescents. Although, by the standards of sub-Saharan Africa, the attendance at antenatal clinics by villagers from the lower Shire village might be considered reasonable, it clearly remains inadequate to provide effective anaemia control in pregnant women. From adolescence to grand multiparae, anaemia remains a chronic problem in the area. Integrated strategies, that involve communities more effectively and the village-based distribution of impregnated bednets, antimalarials and haematinics, will be required if the problem of anaemia during pregnancy is to be solved.

G6PD deficiency was found to be common during UP1, with an estimated frequency as high as 36% for female heterozygotes. Overall hemizygote prevalence for the A^- phenotype in African male populations has been reported to vary from 3% in East Africa to 22% amongst the Yoruba (Beutler, 1978). The frequency observed in UP1, in Malawian infants, is amongst the highest estimates for Africa. In a separate study of 101 pregnant women from the Shire valley, 24 (23.8%) were found to have G6PD deficiency (Howarth, 1996). The clinical importance of this polymorphism is clearly shown by its association with anaemia (22.2% of the deficient but only 4.5% of the enzyme-normal infants in UP1 had anaemia), and its presence in neonates with severe haemolytic anaemia requiring exchange transfusion. In Nigeria and Jamaica, jaundice was found more frequently in G6PD-deficient infants than in their normal counterparts (Capps *et al.*, 1963; Gibbs *et al.*, 1979; Dawodu *et al.*, 1984). Although G6PD deficiency provides some protection from *P. falciparum* infection (Ruwende and Hill, 1998), this was not apparent in the results of UP1, possibly because of the use of antimalarial drugs in the study area. G6PD-deficient erythrocytes are more susceptible to oxidative stress and have a shorter life-span than enzyme-normal erythrocytes (Bernini and Latte, 1964). In those with symptomatic malarial infections, the deficient erythrocytes may therefore be more severely damaged by oxidant antimalarial drugs such as sulfadoxine–pyrimethamine (the only readily available antimalarial drug used in the first-line treatment of malaria in the lower Shire valley). G6PD deficiency may also cause chronic subclinical haemolysis in the steady state (May *et al.*, 2000) and increased infection-induced haemolysis as a result of oxidative membrane damage (Beutler, 1978). Infection-related haemolysis in G6PD deficiency is usually mild but occasionally may be severe. The recovery of the Hb concentration to normal is also often delayed by the marrow suppression that ordinarily accompanies infection. Bacterial pneumonia is a common cause of infection-induced haemolysis in the G6PD-deficient (Tugwell, 1973), and acute respiratory infection is one of the commonest causes for health-centre attendance by young

children in Malawi. Between 5% and 10% of the children in the communities investigated in UP1 and UP2 and by Verhoeff (2000) and Prinsen-Gerlings *et al.* (2003) may have been HIV-positive, as the seroprevalence of maternal infection with HIV was about 25% at the time of these studies.

In UP2, the infants with α+ thalassaemia genotypes were found to have a lower mean Hb concentration and significantly higher transferrin saturations than those with normal genotypes. Although α-thalassaemia is another erythrocytic variant, characterized by elevated susceptibility to oxidative stress and protection against malaria, that could lessen the risk of malarial anaemia (Yuthavong and Wilairat, 1993), there is no evidence of this in the results of UP2. The sample size in UP2 was so small, however, that the differences seen in mean Hb levels between genotypes did not reach statistical significance and may have been influenced by confounding caused by developmental changes. Nurse (1979) suggested that, in α thalassaemia, there could be a reduced rate of α-globin chain production, while transferrin is fairly saturated. There could then be a reduction in haem synthesis, a slowdown in release of iron from transferrin, and an excess of haem precursors. This might explain why, in UP2, those with the α-thalassaemia genotypes had slightly higher ZP values than the other subjects.

The frequency of sickle-haemoglobin genotypes in infants was high in UP2, as would be expected in a highly malarious area of Africa. The prevalence of malarial parasitaemia in infants was significantly higher in those with the AS and SS genotypes than in those with the AA. This contrasts with the findings from a cohort study of infants (aged 2–16 months) in western Kenya, in which those with the sickle genotypes apparently had a reduced risk of high-density parasitaemia and of severe malarial anaemia (Aidoo *et al.*, 2002). In Ghana, however, Ringelhann *et al.* (1976) also reported relatively high prevalences of malarial parasitaemia in children (aged <5 years) with HbAS. If infants with HbAS are generally more likely to be found parasitaemic than their HbAA counterparts, the persistent moderate parasitaemias may stimulate the development of immunity, protecting older heterozygotes against severe *P. falciparum* infection. In UP2, mean Hb values in the children with the sickle-cell trait were, in general, not significantly different from those in the HbAA children. Only among the infants aged 12 months was the mean Hb concentration significantly higher in those with HbAS than in those with HbAA, indicating that the sickle-cell trait may offer some protection from malarial anaemia in the older infants.

This overview provides a profile of the burden of anaemia amongst a poor, essentially rural, Malawian community, and indicates that, from birth to adulthood, individuals are faced with substantial nutritional deficits and infection loads that almost preclude the achievement of a normal haematological status. Despite the knowledge of the causes and mechanisms of anaemia in tropical African communities, effective, integrated strategies for its prevention and treatment have rarely been deployed. Innovative, community-based approaches are required in order to break the cycle of ill health that anaemia holds on these communities.

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Identification of the vectors of lymphatic filariasis in the Lower Shire Valley, southern Malawi

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Abstract

An investigation of lymphatic filariasis vectors in Malawi is reported. *Anopheles funestus*, *A. arabiensis*, and *A. gambiae sensu stricto* had high rates of filarial infection (2.2–3.1%) and carried infective larvae. *Anopheles funestus* was the predominant species collected (77.6%) and was the primary vector during the study period of April to May 2002.

Keywords: lymphatic filariasis, *Wuchereria bancrofti*, transmission, *Anopheles funestus*, *Anopheles gambiae*, *Anopheles arabiensis*, *Culex quinquefasciatus*, Malawi

Introduction

Lymphatic filariasis (LF) is a leading cause of long-term and permanent disability worldwide (WHO, 1995). Many species of anopheline and culicine mosquitoes can transmit the causative agents, *Wuchereria bancrofti* and *Brugia* spp. In sub-Saharan Africa, the nocturnally periodic form of *W. bancrofti* is transmitted by night-biting *Culex* and *Anopheles* species, primarily *C. quinquefasciatus* and the malaria vectors *A. gambiae sensu lato* and *A. funestus s.l.* (Sasa, 1976; McMahon & Simonsen, 1996). Determining which of these potential vectors are responsible for LF transmission is essential prior to vector control, as well as for monitoring the efficacy of a drug-based, transmission-reducing intervention.

Nielsen *et al.* (2002) recently reported on a previously neglected highly endemic focus of filariasis transmission in southern Malawi, where microfilariae (mf) prevalence and circulating filarial antigen (CFA) levels reached 22.2% and 79.1%, respectively, in the population, with unexpectedly high CFA rates in children. *Anopheles gambiae sensu stricto*, *A. arabiensis*, *A. funestus*, and *C. quinquefasciatus*, all known vectors of *W. bancrofti* in Tanzania and Kenya, are present in the region (Donnelly & Townson, 2000; Spiers *et al.*, 2002) but their role as vectors in this area is unknown. With a mass drug administration (MDA) control programme for LF scheduled to begin in late 2002 within the Lower Shire Valley, this study set out to determine the role of local mosquito species as vectors of LF.

Materials and Methods

Mosquitoes were sampled from Belo village in Chikwawa district, southern Malawi (16°01′12″S, 34° 49′04″E), where the prevalence of *W. bancrofti* infection, detected by the immunochromatographic test (ICT) for adult CFA, was previously reported to be 79.1% in all age groups and 77.9% in individuals aged > 15 years (Nielsen *et al.*, 2002). Mosquitoes were collected by pyrethrum knock-down from 2–3 houses per day between 06:30 and 08:00, 3 times per week from 15 April to 20 May 2002. In total, 54 houses from across the study village were sampled. The purpose of the work was explained to each householder recruited to the study. Permission to enter the house was sought and the right to refuse or withdraw at any time was respected. Temperature and barometric data were recorded for each house using a handheld Global Positioning System receiver (Magellan, CA, USA). Female mosquitoes were identified morphologically using the taxonomic keys of Edwards (1941), Gillies & Coetzee (1987), and Service (1990). After provisional species identification all female mosquitoes were separated into head, thorax, and abdomen in a drop of saline and examined for the presence of *W. bancrofti*. Infection and infectivity status were recorded. Following dissection, all specimens identified as *A. gambiae s.l.* were stored over desiccant for later species identification by polymerase chain reaction (PCR). *Anopheles funestus s.l.* is also a species complex but as only *A. funestus s.str.* has been identified in the Lower Shire (Nora Besansky, personal communication) morphological identification of *A. funestus* specimens was sufficient. DNA was extracted following the Collins *et al.* (1987) protocol from mosquitoes identified as *A. gambiae s.l.* This DNA was used as a template for the *A. gambiae* species identification PCR of Scott *et al.* (1993). Since the extraction procedure yields both filaria and mosquito DNA, all *A. gambiae s.l.* found to be infected by dissection were subjected to a second PCR analysis to amplify *W. bancrofti* specific sequences following an adaptation of the protocols of Lenhart (2002) and Farid *et al.* (2001).

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Results

The number, species, infection and infectivity status of all mosquitoes collected and dissected are shown in the Table. The number of mosquitoes harvested each day ranged from 29 to 435 (mean ± SD, 198.6 ± 117.1). At the time of collection, daily mean temperature and atmospheric pressure ranged from 20.8 °C to 28.2 °C and 1017 mbar to 1027 mbar, respectively. Infection rates with all filarial stages and infective L_3 larvae were highest in *A. gambiae s.str.* (3.1% and 3.1%, respectively) followed by *A. arabiensis*, (3.0% and 2.3%, respectively) and *A. funestus* (2.2% and 1.6%, respectively). However, these differences were not statistically significant ($P > 0.05$) (*t* test for proportions, significance adjusted for multiple tests by Bonferonni procedure). A single *Mansonia* was found infected with 2 infective filarial larvae (see below). No filarial infections were found in any *Culex* species.

A total of 9 *A. arabiensis*, 2 *A. gambiae s.s.*, 1 *A. gambiae s.l.* and 1 *Mansonia* sp. were found to be infected with filarial parasites and were processed for *W. bancrofti* detection by PCR. All were confirmed as infected with *W. bancrofti* except 1 *A. gambiae s.str.* and the single *A. gambiae s.l.* and *Mansonia* specimens.

Discussion

This is the first study to investigate transmission of LF in southern Malawi. During the study period the predominant mosquito species was *A. funestus* (77.6%) followed by *A. arabiensis* and *A. gambiae s.str.* (10.8% and 2.3%, respectively). Another study in this area found that *A. gambiae* and *A. arabiensis* are more abundant at other times of the year (Spiers *et al.*, 2002). Thus, while infective females of all 3 anopheline species were found in the present study, their relative importance as vectors, in terms of their annual transmission potentials, remains to be determined.

Table. Checklist and filaria infection status of mosquito species collected by pyrethrum knock-down in Belo village, Malawi, April–May 2002

Species	Collected *n* (%)	Mean no./house	Infected[a] *n* (%)	Mean no. infected/house	Infective[b] *n* (%)	Mean no. infective/house
A. funestus	2159 (77.63)	40	48 (2.2)	0.89	35 (1.6)	0.65
A. gambiae s.str.	64 (2.30)	1.2	2 (3.1)[d]	0.04	2 (3.1)	0.04
A. arabiensis	299 (10.75)	5.5	9 (3.0)[e]	0.17	7 (2.3)	0.13
A. gambiae s.l.[c]	2 (0.07)	0	1 (50)	0.02	0	0
M. uniformis/africana	87 (3.13)	1.6	1 (1.1)	0.02	1 (1.1)	0.02
C. univittatus	155 (5.57)	2.9	0	0	0	0
C. neavei	11 (0.4)	0.2	0	0	0	0
C. quinquefasciatus	3 (0.11)	0.1	0	0	0	0
C. annulioris	1 (0.04)	0	0	0	0	0
Total	2781	51.5	61 (2.2)	1.13	44 (1.6)	0.84

[a]Infection defined as microfilariae, L_1, L_2 or L_3 stage filaria larvae in mosquito carcass.
[b]Infectivity defined as L_3 (infective) stage filaria larvae in mosquito head, thorax or mouthparts.
[c]These specimens were identified morphologically as belonging to the *Anopheles gambiae* complex but did not PCR-amplify for specific identification.
[d]One confirmed as *Wuchereria bancrofti* infection by PCR.
[e]All confirmed as *Wuchereria bancrofti* infection by PCR.

Since very few *Culex* were found (6% of the total catch), of which only 3 were *C. quinquefasciatus*, the role of this species as a vector in the area also remains to be determined. *Culex quinquefasciatus* is known as an important vector of filariasis elsewhere in East Africa (Maxwell *et al.*, 1990; Bogh *et al.*, 1998; Pedersen *et al.*, 1999). Whether the LF refractory phenotype is expressed in this region requires further investigation (Omar & Zielke, 1978).

Of the remaining species found, *Mansonia* transmits *W. bancrofti* in parts of Asia but has not been identified as a vector in Africa (Sasa, 1976; Service, 1990). Time constraints prevented specific identifications for all *Mansonia* although both *M. uniformis* and *M. africana* were found in the sample. In areas of Africa, both species are known to transmit the animal filariae *Dirofilaria immitis* and *D. repens* (Service, 1990), allowing speculation on the identity of the filarial parasites found in the single infected *Mansonia*. However, there is little doubt that the overwhelming majority of filarial infections found in the *Anopheles* mosquitoes are *W. bancrofti*. These *Anopheles* species are highly anthropophilic and are unlikely to transmit animal filaria. Furthermore, developing stages were found in the thoracic musculature of the insects whereas *Dirofilaria* develop in the Malpighian tubules.

Because anophelines show a facilitation pattern in infections with filaria (Southgate & Bryan, 1992), any reduction in the mf level of the human population following drug distribution is likely to lead to a decrease in transmission of *W. bancrofti* by these vectors. Moreover, these findings provide unequivocal evidence that insecticide-treated bednets, currently being promoted vigorously for malaria prevention throughout Malawi, will protect sleepers against another major mosquito-borne disease.

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Book Review

Manson's Tropical Diseases, 21st edition. Gordon C. Cook & Alimuddin Zumla (editors). London/Philadelphia: Saunders/Elsevier Science Ltd, 2003. xviii + 1848 pp. Price £122.00 hardback; £22.00 softback specifically for developing countries. ISBN 0-7020-2640-9 hardback; 0-7020-2790-X softback.

This 21st edition of *Manson's Tropical Diseases* is a remarkable volume in many ways. Following the long tradition originally set out by Patrick Manson in 1898 in his monograph entitled *Tropical Diseases: A Manual of the Diseases of Warm Climates*, this modern treatise maintains the tradition and has an updated feel about it; many of the 87 chapters have been entirely rewritten, indeed 11 are *de novo*, and balance both disease-specific and system-based approaches. The attractive hardcover, depicting a mosquito, provides an appropriate visual theme and throughout this volume there are many photographic images (more than 1000) carefully selected to illustrate typical clinical conditions; most of these are produced in black and white but over 50 have been duplicated in vivid colour (slightly more than in the 20th edition). In addition there are numerous well-selected illustrations, graphs, and clear clinical management diagrams, all very useful for teaching purposes as well as for general reference. With extensive cross-referencing throughout all chapters, there are very few instances of undue duplication, but perhaps what is most remarkable is the low cost of this volume. At £122 the book is very reasonably priced and well within the affordability of educational institutes, medical surgeries, and the individual pockets of clinicians and/or scientists who have chosen to pursue a career in tropical medicine. The softback version, specifically available for developing countries, is very generously priced. Indeed those with an interest in travel medicine, or general infectious disease, will not be disappointed with a copy of this acknowledged 'bible' of tropical medicine.

The 87 chapters are arranged within a framework of 12 sections that include: Underlying factors in tropical medicine, Symptoms and signs, System-oriented disease, Related specialities in the tropics, Environmental/genetic disorders, Viral infections, Rickettsial infections, Bacterial infections, Mycotic infections, Protozoan infections, Helminth infections, and Ectoparasites, with 5 subsequent appendices. With an assembled international authorship of 119, many of whom have spent their working lives within the tropics, much of their clinical and scientific acumen has been wisely passed on. Personally, I have had occasion to consult this volume on several areas outside my immediate interests and have not been disappointed in the guidance this book offers. The new chapters include treatises on: Primary Care, Epidemiology, Traditional medicine, Genetics, Economics, Ethics and tropical diseases: some global considerations, Tropical oral health, and Sources of information on tropical medicine. The latter contains a wealth of information which, after consultation, could carefully streamline further searching on both hardcopy and electronic resources. The associated web-based resources, e.g. e-TALC, were particularly informative and good initial points to work from.

At the start of each section there is a brief taster of what each subsequent chapter offers; it is outside the goal of this review to consider all 87 but I would wish to highlight a few. The first chapter on *History of tropical medicine, and 'medicine in the tropics'* should be considered essential reading for those wishing to understand how this discipline originated and evolved, giving rise to the London and Liverpool Schools; both of which still remain of international renown. The chapter on *Epidemiology of disease in the tropics* brought home the shocking burdens of HIV and related AIDS conditions, tuberculosis and malaria as well as those from diarrhoeal-related diseases. Indeed the latter disease spectrum forms the major source of ill health for those travelling abroad and was duly highlighted in a dedicated chapter entitled *Travel medicine.* The often expanded chapters on the former diseases were particularly informative. On considering the tradition of practical advice within *Manson's*, I was disappointed by *Ethics and tropical disease* as I thought the balance between theory and practice was misplayed. Ethical issues are indeed complicated and there are many disparities to correct but a pragmatic approach needs to be fostered otherwise, as even on simple issues, we could fall into a trap of procrastination.

In the light of Manson's keen knowledge and expertise in helminthology and protozoology, the 21st edition still provides much to keep the medical parasitologist happy and *Clinical laboratory diagnosis* describes many of the simpler diagnostic tests possible even within a resource impoverished setting. The results of research on emerging and worsening drug resistance and new treatments for several diseases were presented and where appropriate alternative potential therapeutic targets or novel drug regimes were explored. Amongst others, the chapters on *Opthalmology in the tropics and subtropics, Dermatological problems, Malaria, Tuberculosis, Animal toxins,* and *Sexually transmitted infections (excluding HIV)* were particularly thorough and very impressive.

To close, this latest edition of *Manson's Tropical Diseases* sets another very high standard within its long history and although rather heavy in weight, will soon be seen on the book shelves of many a grateful reader who seeks authoritative advice. The book is clearly an invaluable addition to the existing literature and owes greatly, without doubt, to the extensive experience and expertise of the present editors and to the distinguished panel of authors.

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Lymphatic filariasis in the Karonga district of northern Malawi: a prevalence survey

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In Malawi, two main foci of lymphatic filariasis (LF) are known to exist: one in the south, in the Shire valley, and the other in the north, along the Songwe River, on the border with Tanzania. There have been no formal surveys in the Songwe area since the 1960s but an opportunity arose in 2000–2001 to map LF in this area, in the context of a leprosy survey that formed part of the follow-up of a large leprosy and tuberculosis vaccine trial.

Overall 687 immunochromatographic (ICT) tests were carried out. *Wuchereria bancrofti* antigenaemia was found in >25% of adults in each of the 12 villages sampled (four in the Songwe area and eight in the rest of the Karonga district), with village prevalences varying from 28%–58%. Of the 685 adult male residents of the Songwe area who were each given full-body clinical examinations, 80 (11.7%) were identified as cases of hydrocele. Lymphoedema was found in seven (1.0%) of these adult males and in 29 (3.7%) of the 769 adult female residents of the Songwe area who were also examined. Microfilariae were detected in 33 (30.8%) of the 107 thick smears of night-blood samples that were made from individuals with positive ICT cards. The *W. bancrofti* infection focus in Karonga district is therefore wider than was previously known. This has important implications for the implementation and eventual impact of LF-control activities in this area.

Wuchereria bancrofti, the major cause of human lymphatic filariasis (LF), is estimated to infect over 100 million people worldwide (Michael and Bundy, 1997). The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000, following a resolution by the 50th World Health Assembly (in 1997) that endemic countries should develop programmes to eliminate filariasis as a public-health problem.

The strategy of the global LF-elimination initiative is based upon the mass distribution of a combined drug regimen in endemic communities, to interrupt the transmission of *W. bancrofti* (Molyneux and Taylor, 2001). Presently, there is inadequate information on the geographical distribution of LF in Africa on which to establish elimination programmes. Malawi has two known LF foci: one in the south (in the Shire valley) and the other in the north, in Karonga district, close to the Songwe River, which forms part of the border with Tanzania (Oram, 1958).

A survey conducted in 1960 in the northern part of Karonga district, based on the examination of thick bloodsmears for microfilariae, showed a high prevalence of microfilaraemia amongst adults (40%) but indicated that human infection with *W. bancrofti* was restricted to communities close to the Songwe River (Oram, 1960). Unfortunately, there were then no other surveys of LF prevalence in this area for the

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next 40 years. A leprosy survey — part of the follow-up of a large trial of a leprosy vaccine, itself part of the Karonga Prevention Study (KPS; Anon., 1995) — provided an opportunity to collect fresh data on LF in Karonga district. The objectives of the present study, which exploited this opportunity, were 2-fold: to measure the prevalences of *W. bancrofti* infection [using immunochromatographic (ICT) tests] and of the clinical manifestations attributable to this parasite in the Songwe area; and to determine the extent of the *W. bancrofti* transmission area in Karonga district.

SUBJECTS AND METHODS

As part of the KPS, the aim was to give 3000 individuals living in the flood plain of the Songwe River full-body clinical examinations to see if they had leprosy. Adjacent villages on the Malawian bank of the Songwe River were sampled in turn until 3000 people had been recruited. In each sampled village, the list of all heads of household that is routinely used to monitor participation on local self-help projects was obtained from the village headman. Every third household on each list was then visited between November 2000 and January 2001 and each member of these households who was present and consenting was given a full-body clinical examination (for leprosy and the various manifestations of LF) and a standard KPS form was completed for each subject.

Lymphoedema (leg, arm and breast) and hydrocele were graded according to the recommendations of the World Health Organization's Expert Committee on Filariasis (WHO, 1992). In four representative villages (chosen to cover all sections of the Songwe area), the prevalence of *W. bancrofti* antigenaemia was measured using an appropriate, rapid, commercial ICT: the AMRAD-ICT card test for filariasis (AMRAD Corporation, Richmond, Victoria, Australia; Weil, 1997). A small sample of venous blood (<1 ml) was drawn from each consenting individual who lived in a selected household and was aged ⩾20 years. Each sample was then applied to an ICT card and the result (positive/negative) read according to the manufacturer's instructions. The result registered in the field was checked by a second reader at the field camp on the same day. If the two results for one card were discordant, perhaps because of faint lines, the two card readers discussed the card until a consensus was reached. All ICT-positive individuals (except pregnant women and epileptics) were treated with albendazole (400 mg) and ivermectin (200 μg/kg body weight).

Determining the Extent of the LF Focus

To determine the geographical extent of the LF focus, ICT tests were also performed on adults aged ⩾20 years in eight villages (selected so that they were 10–20 km apart) south of the Songwe area, using the same method to select households as used further north. The protocol adopted for this part of the survey followed the recommendations of the TDR Task Force on the Rapid Assessment of the Geographical Distribution of Filariasis (RAGFIL; Anon., 1999). Briefly, if at least 10 (20%) of the first 50 individuals tested in a village were found positive then testing was stopped; otherwise 100 individuals were tested.

Parasitological Examination

In order to confirm microscopically the existence of *W. bancrofti* in the study area, thick smears were prepared of finger-prick samples of blood collected at night (21.00–02.00 hours) from 100 consenting subjects who were ICT-positive (and whom it was logistically possible to visit at night). These smears were fixed at the KPS laboratory in Karonga and transported to London (to the Parasitology Reference Laboratory at the London School of Hygiene and Tropical Medicine), where they were stained with

Giemsa's stain (1:10) for 30 min and then examined under the microscope at ×40 magnification.

Ethics

Ethical clearance for this study was granted by the Health Sciences Research Committee (HSRC) of the Malawi Ministry of Health and by the Ethics Committee of the London School of Hygiene and Tropical Medicine. Individual consent was obtained from each participant or (if they were aged <16) from one of their parents or a guardian.

Data Management

All data were double-entered and verified at the KPS headquarters in Karonga, using Foxpro (version 2.6A; Microsoft), and subsequently analysed using Stata 6 software (Stata Corporation, College Station, TX).

RESULTS

The ICT results are presented in the Table, by geographical area and village. The location of each sampled village is shown in Figure 1. Overall, 687 ICT tests were performed and 315 (46%) gave a positive result. The prevalence of antigenaemia was >25% in all of the sampled villages, with those north of Karonga *boma* (township) having similarly higher prevalences (>40%) than those south of the *boma* (with the exception of Bonje, in the Hara area), a χ^2 test for heterogeneity giving a P-value of 0.003. Thirty-four (11%) of the 315 positive cards had faint lines. The age-group of the subject appeared to have little effect on the prevalence of antigenaemia (Fig. 2) but, in each age-group except the youngest (20–29 years), male subjects were more likely to be antigenaemic than their female counterparts (odds ratio = 1.45; 95% confidence interval = 1.07–1.97; P = 0.016).

TABLE. *The results of testing blood samples with a rapid immunochromatographic test (ICT) for* Wuchereria bancrofti *antigen, split by geographical area and village*

Village	Latitude	Longitude	Total no. and (no. of males/no. of females): Tested with ICT	ICT-positive	Prevalence of antigenaemia and 95% confidence interval (%)
NORTH OF KARONGA BOMA					
Songwe area					
Mwenitete	9°42.754 S	33°55.784 E	42 (20/22)	20 (10/10)	47.6 (32.0–63.6)
Mwakyusa	9°41.877 S	33°53.588 E	91 (44/47)	44 (25/29)	48.7 (37.7–59.1)
Mwenepela	9°40.316 S	33°49.512 E	102 (52/50)	59 (29/30)	57.8 (47.7–67.6)
Kashata	9°43.989 S	33°53.191 E	50 (23/27)	22 (9/13)	44.0 (30.0–58.7)
Other area					
Mwamsaku	9°48.552 S	33°51.890 E	50 (25/25)	22 (10/12)	44.0 (30.0–58.7)
Mwambetania	9°52.048 S	33°52.135 E	50 (18/32)	29 (14/15)	58.0 (43.2–71.8)
Kafikisila	9°54.728 S	33°55.863 E	51 (23/28)	23 (13/10)	45.1 (31.1–59.7)
Mwenitete (Mpata)	9°56.974 S	33°49.342 E	50 (21/29)	24 (10/14)	48.0 (33.7–62.6)
SOUTH OF KARONGA BOMA					
Ngosi	10°00.737 S	33°56.944 E	50 (15/35)	15 (8/7)	30.0 (17.9–44.6)
Mwakabanga	10°08.653 S	34°01.069 E	50 (25/25)	15 (9/6)	30.0 (17.9–44.6)
Kanyuka	10°18.461 S	34°07.615 E	51 (19/32)	14 (7/7)	27.5 (16.2–42.5)
Bonje (irrigation scheme)	10°29.416 S	34°10.259 E	50 (22/28)	28 (12/16)	56.0 (41.3–70.0)

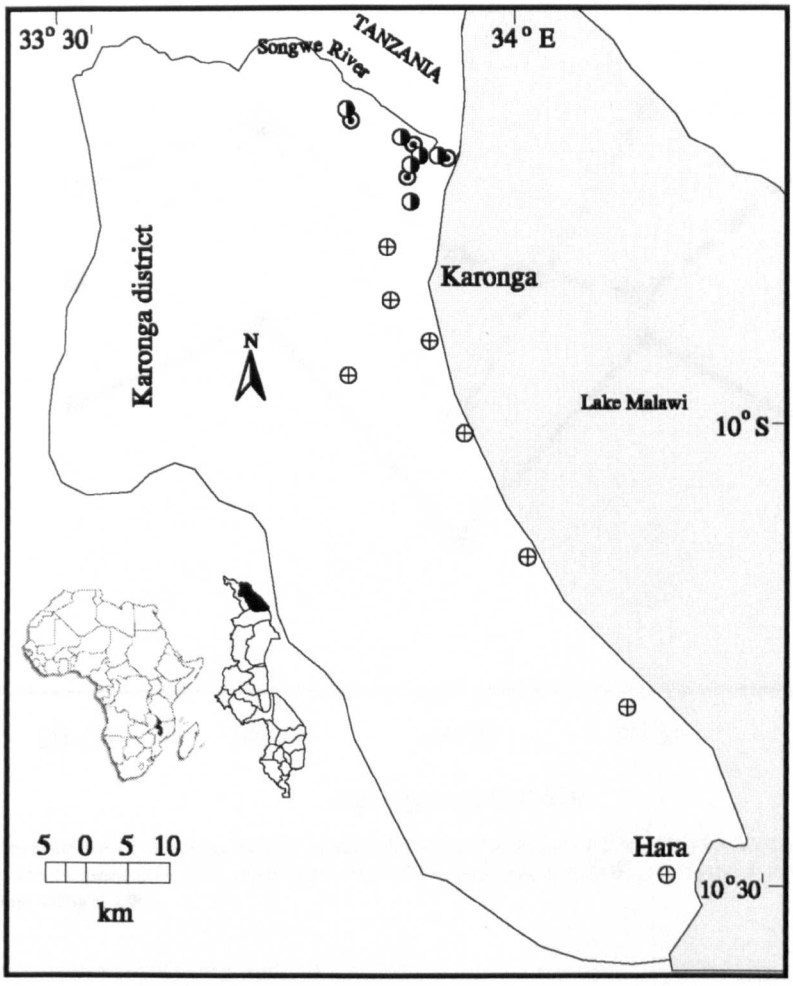

FIG. 1. Map of Karonga district, showing the villages where immunochromatographic tests (ICT) and clinical examinations (○), on ICT tests (⊕), or only clinical examinations (◑) were conducted.

Chronic Clinical Manifestations

The age–prevalence plots for each major clinical manifestation of LF are shown in Figure 3. Overall, 80 (11.7%) of the 685 adult males examined had hydrocele and seven (1.0%) had lymphoedema (six in the legs and one in both arms and legs). Of the 729 adult females also examined clinically, 29 (3.7%) had lymphoedema or elephantiasis (28 in the legs and one in both arms and legs). Hydrocele is therefore the commonest manifestation of LF in the study population as a whole. The prevalence of both hydrocele and lymphoedema increased with age (Fig. 3).

Thick Bloodsmears

Of the 107 thick smears made, 33 (30.8%) were positive for microfilariae. All the parasites were identified as *W. bancrofti.* Microfilarial positivity did not vary with age ($P \gg 0.5$).

DISCUSSION

The present results show that *W. bancrofti* infection is more widespread in Karonga district than was previously reported (Oram, 1960). In all of the villages investigated in

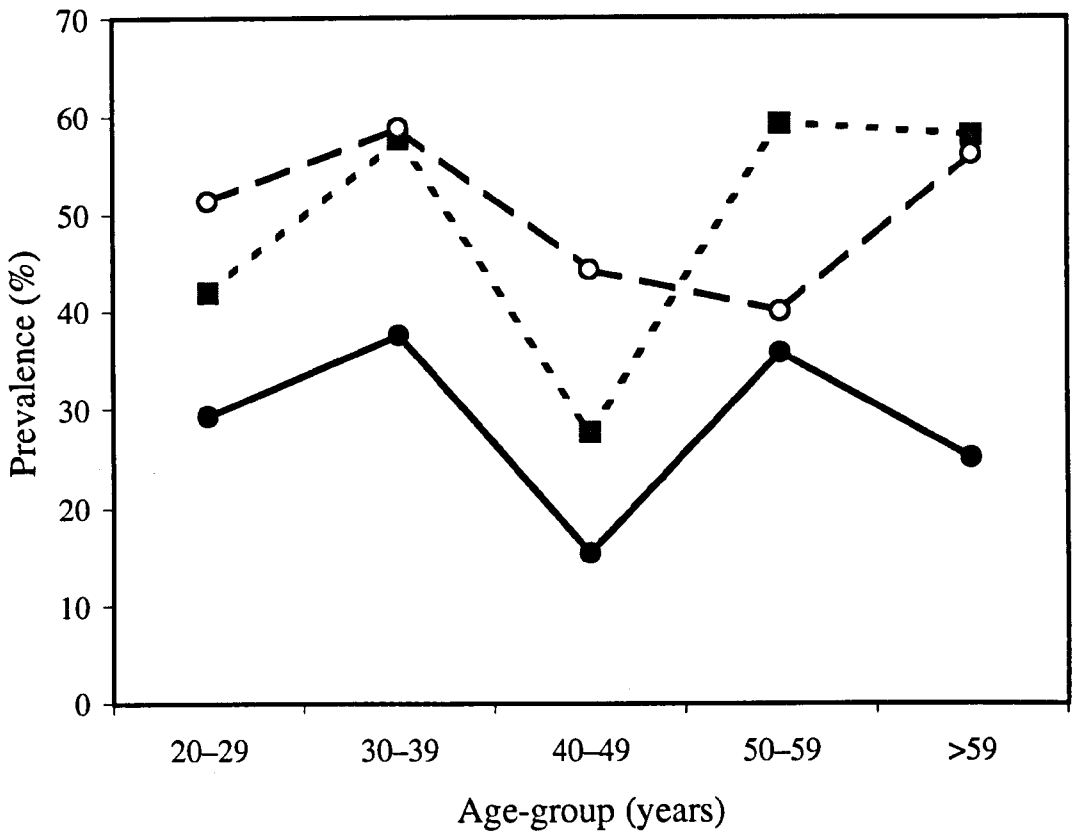

FIG. 2. The prevalence of *Wucheria bancrofti* antigenaemia according to the age of the subject and whether he or she lived in the Songwe area (○), elsewhere north of the Karonga *boma* (■), or south of the Karonga *boma* (excluding Bonje village) (●).

the present study, including those considerably south of the Songwe River, the prevalence of *W. bancrofti* antigenaemia exceeded 25%. This result has implications for the implementation of local LF-control activities. The cline in antigenaemia prevalence, from relatively high in the north to low in the south, may be explained by the prevailing climatic and ecological conditions. The north, including the Songwe area, is a relatively wet, rice-growing region and presumably supports more breeding of the mosquito vectors of *W. bancrofti* (and therefore more intense transmission of this parasite) than the drier south. The relatively high prevalence found in the Bonje (Hara) area in the south is interesting not only because Bonje lies within a rice irrigation scheme but also because the rice growers in this area are mainly tenants recruited from the northern part of the district. An association between irrigation schemes and LF has been detected in other parts of Africa (Dzodzomenyo *et al.*, 1999). It is possible that other migrations from endemic areas have established LF foci in other locations once thought free of this infection.

The fairly constant prevalence of antigenaemia by age among adults has been noted in other populations (Lammie *et al.*, 1994). This could reflect the parasite's restriction to a susceptible portion of a population, but is more likely to reflect a dynamic 'equilibrium' in which adults move in and out of detectable antigenaemia over time. The higher prevalence among males than females has

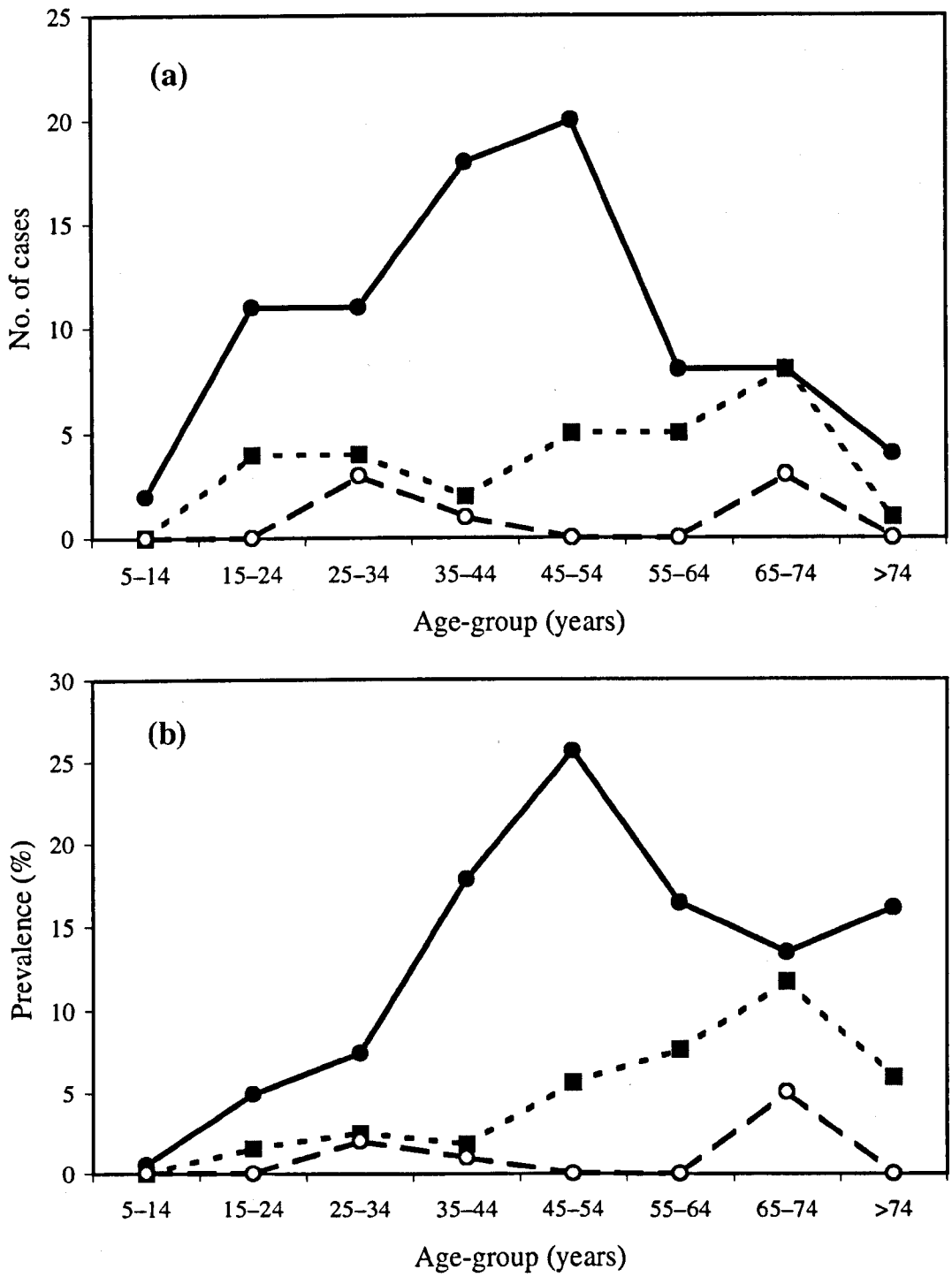

FIG. 3. Age distributions of the hydrocele (●), female lymphoedema (■) and male lymphoedema (○) attributable to *Wuchereria bancrofti* infection in Karonga district, northern Malawi. The plots show the actual numbers of cases detected (a) and the prevalences of hydrocele in the men, lymphodoema in the women, and lymphodoema in the men investigated (b).

also been observed before (Wamae *et al.*, 1998). In Karonga district, as elsewhere in Africa, hydrocele is the most common clinical manifestation of LF (Wamae *et al.*, 1998). Although the rapid mapping of LF could perhaps be based on the detection of hydrocele (Gyapong *et al.*, 1998), there is mounting anecdotal evidence, from Karonga and other areas of Africa, indicating that high prevalences of antigenaemia occur in some areas where the chronic clinical manifestations of LF are rare or even absent. There therefore appear to be important co-factors that predispose only some infected individuals to the development of chronic disease. This is likely to have an impact on geographical and treatment coverages in the mass drug distributions, as compliance will probably be low in areas where there is no evident disease despite a high prevalence of infection.

It is interesting to note that only 30% of the thick smears checked for microfilariae were found to contain them, even though all of these smears were of blood from individuals who had been found positive for *W. bancrofti* antigenaemia in the ICT. As the ICT test detects adult-worm antigen (Weil, 1997), individuals who carry only adult worms of one sex or non-fecund adult worms may be positive by ICT although they have no microfilariae. Data emerging from other centres in Africa are showing similar values for the proportion of ICT-positives who appear to be microfilaraemic (D. H. Molyneux, unpubl. obs.). However, there is clearly a need to evaluate rigorously the performance of the ICT test under various conditions, in particular in areas where multiple helminth infections (which may increase the potential for cross-reactivity) are likely. Hookworm, *Schistosoma haematobium* and *S. mansoni* are all common in Karonga district (Randall *et al.*, 2002). Further studies of the epidemiology of filariasis in this population are currently under discussion.

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