LYMPHATIC FILARIASIS CONTROL IN AN HIV ENDEMIC AREA IN NORTHERN MALAWI

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy

By

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August 2018

Declaration

I hereby declare that this PhD thesis is a presentation of my original research work. Material contained herein has not been previously published, accepted or presented for the award of any University degree. Wherever contributions of others are involved, every effort has been made to indicate this clearly, with due acknowledgement to the relevant sections made in the thesis.

Acknowledgements

I thank God Almighty for the gift of life and good health and giving me opportunities to further my education, and strength to complete this work.

I am most grateful to my supervisors, Dr Miriam Taegtmeyer and Professor Neil French who provided academic direction and supervision of this work and for their tremendous and untiring support throughout the whole period that this work was conducted. Thank you very much Miriam and Neil for your kind-hearted spirit, patience, endurance and guidance and for pushing me on during my lowest moments.

My sincere thanks and great appreciation to the Centre for Neglected Tropical Diseases (CNTD), Liverpool School of Tropical Medicine (LSTM), United Kingdom (UK) for providing funding for my PhD study through a grant from the Department for International Development (DFID). I thank all the CNTD staff for welcoming me to Liverpool and for their tremendous support during my numerous visits to Liverpool.

I thank the people of Chilumba and Songwe areas in Karonga district, northern Malawi for their participation in the studies included in this thesis. I also thank Karonga Prevention Study (KPS) management, field, laboratory, data office and administrative staff for their work and support for this PhD study.

Special thanks to Dr Louise Kelly-Hope, Dr Michelle Stanton, and Dr Hannah Betts of CNTD who helped with the ArcGIS software and the preparation of the maps, and Faye Moody of LSTM for her assistance in formatting the thesis.

DEDICATION

This thesis is dedicated to my dearest wife Alice

For her support and encouragement, and always being there for me through thick and thin.

And to my dearest sons, Dalitsolathu and Ungweruwithu for being my source of inspiration and bringing joy into my life.

Abstract

Lymphatic filariasis (LF) and human immunodeficiency virus (HIV) are major public health problems in Malawi. Coinfections are widespread and there is possibility of important interactions between these two pathogens with consequences for LF control and elimination. There is little available evidence on co-infection and interaction of LF and HIV infections and the study set out to investigate associations and interactions between these two pathogens and determine possible consequences for LF control and elimination through annual mass drug administration (MDA).

This thesis presents results from adult study participants in two rural sites in Karonga district, northern Malawi, a region endemic for both LF and HIV. Stored blood samples and data from two geographically separate studies were used. One was a clinical trial of anti-filarial agent dosing regimens in Songwe in the northern part of the district (the Songwe clinical trial), and the other a whole population annual HIV sero-survey with longitudinal follow up data from the southern part of the district (the Karonga Health and Demographic Surveillance System or KHDSS). The main objectives were:

1. To determine the prevalence of LF and HIV co-infections as quantified by the Og4C3 ELISA in a large cross-sectional study in Karonga district, rural northern Malawi.
2. To investigate whether higher and/or more frequent doses of albendazole and ivermectin were more effective in eliminating *Wuchereria bancrofti* microfilariae than the World Health Organisation (WHO) approved standard regimen.
3. To determine the relationship of circulating filarial antigen (CFA) and microfilaria count (MF) by HIV status.
4. To monitor the impact of Mass Drug Administration (MDA) on LF antigenaemia by HIV status by following a cohort of LF antigen positive individuals identified in objective one.
5. To assess the contribution of insecticide treated bed nets (ITNs) to changes in CFA.

These objectives were met through three filarial research studies using data from two distinct geographical locations: Study 1, cross-sectional assessment of the relationship of HIV and markers of LF infection; Study 2, clinical trial of anti-filarial dosing regimens and Study 3, longitudinal assessment of the impact of MDA on LF antigenaemia by HIV status.

In study 1 (cross-sectional assessment), 1,851 consecutive adult volunteers from the Songwe area of Karonga district were screened for HIV and LF infection. Overall CFA prevalence was 24.1% (447/1851) while CFA prevalence was 25.4% (43/169) in HIV-positive and 23.6% (351/1487) in HIV-negative participants (p=0.57). Geometric mean concentrations (GMC) of

CFA were 859 and 1660 antigen units per ml of blood (Ag/ml) respectively, geometric mean ratio (GMR) 0.85, 95% CI 0.49-1.50. In addition, a further 7,863 adults from the KHDSS part of the district had an overall CFA prevalence of 23.8% (1875/7863), a CFA prevalence of 20.9% (86/411) in HIV-positive and 24.0% (1789/7452) in HIV-negative participants (p=0.15) at baseline. GMC CFA was 630 and 839 Ag/ml respectively (GMR 0.75, 95% CI 0.60-0.94). In the HIV-positive group, antiretroviral therapy (ART) use was associated with a lower CFA prevalence, 12.7% (18/142) vs. 25.3% (67/265), (OR 0.43, 95% CI 0.24-0.76). Prevalence of CFA decreased with duration of ART use, 15.2% 0-1 year (n=59), 13.6% >1-2 years (n=44), 10.0% >2-3 years (n=30) and 0% >3-4 years treatment (n=9), $p<0.01$ $\chi 2$ for linear trend.

In study 2 (clinical trial), seventy individuals with confirmed circulating LF antigen had microfilarial counts >80 microfilariae/ml and were randomised as part of a controlled open label clinical trial. The clinical trial compared three modified treatment groups to standard dosage of ivermectin and albendazole in adults. Participants were followed up every six months for two years for repeat microfilarial counts and safety assessments. All treatment groups achieved a significant reduction of microfilariae levels by 12 and 24 months of follow up. Doubling the standard dose and giving it twice yearly showed a non-significant tendency towards faster and more complete clearance. There were no serious adverse reactions.

In study 3 (longitudinal assessment), the cohort was derived from study 1 and comprised of 1722 baseline CFA positive individuals who had a follow up blood sample and a random sample of 939 baseline CFA negative individuals. Of the 1722 baseline CFA positive individuals, 524 (30.4%) remained CFA-positive, a clearance rate of 325/1000 person years of follow up while all but two of the 939 CFA-negative individuals at baseline remained negative at follow up, an incidence of 1/1000 person years of follow up. Using logistic regression, two doses of annual MDA was independently associated with decreased CFA positivity while bed net ownership and HIV status were not associated with CFA positivity. In the HIV-positive subgroup, ART use did not show any association with CFA positivity at follow up.

This is the first investigation of this magnitude into HIV and LF co-infection in Malawi and it adds significantly to existing knowledge in the field. The cross-sectional study of two distinct LF-exposed populations confirms that there is no evidence that HIV infection has an impact on LF epidemiology that will interfere with LF control measures. A significant association of ART use with lower CFA prevalence merits further investigation to understand this apparent beneficial impact of ART. At follow up, MDA effectively reduced CFA prevalence and worm burden and the effectiveness of MDA treatment is unaffected by HIV co-infection and ART status.

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Appendix 7: Consent Form for HIV Testing in The Filariasis Study

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List of Acronyms

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
AWOL	Anti-Wolbachia Consortium
BLM	Banja La Mtsogolo
CDC	Centre for Disease Control
CDD	Community Drug Distributors
CFA	Circulating Filarial Antigenaemia
CHAM	Christian Health Association of Malawi
CI	Confidence Interval
CITC	Client-Initiated Testing and Counselling
CNTD	Centre for Neglected Tropical Diseases
DEC	Diethylcarbamazine
DFID	Department for International Development
DHMT	District Health Management Team
DHO	District Health Officer
DOTS	Directly Observed Treatment Strategy
DTO	District TB Officer
EH	Ethambutol and Isoniazid
EHP	Essential Health Package
ELISA	Enzyme-linked Immunosorbent Assay
EPTB	Extra Pulmonary TB
FDC	Fixed Dose Combinations
GIS	Geographical Information System
GMC	Geometric Mean Concentration
GMR	Geometric Mean Concentration
GNP	Gross National Product
GPELF	Global Programme to Eliminate Lymphatic Filariasis
HIV	Human Immunodeficiency Virus
HSA	Health Surveillance Assistant
HTC	HIV Testing and Counselling
ICT	Immunochromatographic Card Test
IRS	Indoor Residual Spraying

ITN	Insecticide Treated Net
KHDSS	Karonga Health and Demographic Surveillance System
KPS	Karonga Prevention Study
KPT	Karonga Prevention Trial
LEP	Lepra Evaluation Project
LEPRA	Leprosy Relief Association
LF	Lymphatic Filariasis
LSTM	Liverpool School of Tropical Medicine
MACRO	Malawi AIDS Counselling and Resource Organization
MDA	Mass Drug Administration
MF	Microfilaria
MOH	Ministry of Health
NGO	Non-Governmental Organisation
NOCP	National Onchocerciasis Control Programme
NTCP	National TB Control Programme
NTD	Neglected Tropical Disease
OR	Odds Ratio
PITC	Provider-Initiated Counselling and Testing
PMTCT	Prevention of Mother-to-Child Transmission
PTB	Pulmonary TB
RG	Reporting Group
SLA	Service Level Agreement
TA	Traditional Authority
TB	Tuberculosis
VCT	Voluntary Counselling and Testing
VMMC	Voluntary Medical Male Circumcision
WHO	World Health Organisation

Chapter 1 Introduction to the Thesis

This chapter provides a background to the study, a summary of the justification and rationale to the study, the aims and objectives as well as a summary of the study design and main methods used in the study. The layout of the thesis is desciribed in the last section of this chapter.

1.1 Background to the study

1.1.1 Lymphatic filariasis control

Lymphatic filariasis (LF), a helminth infection caused by the mosquito-borne filarial nematodes, *Wuchereria bancrofti, Brugia malayi* and *Brugia timori*, is a major public health problem in the tropics and according to the World Health Organisation (WHO) is the second leading cause of permanent and long-term disability globally after mental illness [1]. At the launch of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000, an estimated 1.3 billion people in 73 endemic countries were at risk of LF and 120 million people were infected, with 91% of cases caused by *W. bancrofti* and 40 million of them disfigured and incapacitated by chronic manifestations of the disease [2, 3]. Currently, 856 million people in 52 countries worldwide remain threatened by LF and require preventive chemotherapy [4].

The GPELF aims to eliminate LF through annual mass drug administration (MDA) using single-dose ivermectin 150 µg/kg and albendazole 400 mg annually for 4–6 years to affected populations living in areas in Africa that are also endemic for onchocerciasis [5, 6]. Rapid and sustained clearance is desirable for public health impact and the ideal annual dosing regimens remain unclear with documented ‘hot spots’ of new transmission remaining in areas that have received MDA and reports of individuals who remain microfilaria (mf) positive despite treatment [7].

1.1.2 Frequency and dosing of mass drug administration

Higher annual doses or more frequent dosing regimens might have the potential to achieve a successful and accelerated outcome of mass treatment programmes. This could be at a lower overall cost to national programmes by achieving critical programmatic milestones much earlier [8]. However, available data to guide and support such a large programmatic shift are limited and no data are available from HIV endemic areas. A randomised clinical trial in Egypt reported that multi-dose diethylcarbamazine (DEC) and albendazole treatment was more effective than single dose treatment [9]. The efficacy of ivermectin was shown to be higher when given twice yearly in French Polynesia [10]. Small studies in Haiti and Mali have shown

higher and biannual dosing to be more effective than the standard dose regimen in suppressing microfilariae [11, 12].

1.1.3 Helminths and HIV co-infection is common

Human Immunodeficiency Virus (HIV) infection is another major public health problem and leading cause of morbidity and mortality worldwide. Approximately 35 million people are infected with HIV worldwide and although Africa accounts for 14% of the population in the world, sub-Saharan Africa remains the region most heavily affected, accounting for 70% of all HIV infections worldwide [13]. Helminth and HIV co-infection are widespread in sub-Saharan Africa, including Malawi and there is substantial geographic and social overlap in their distribution leading to opportunities for HIV, parasite and drug interactions at both individual and population scales [14].

1.1.4 Mixed evidence of interactions between helminths and HIV

Several studies have examined the interaction of different helminths infections with HIV and have reported conflicting results. Some have found no association between helminth infections or treatment and HIV, while others have found evidence that treatment of helminth infections is associated with improvement in markers of HIV disease progression. A cohort study of HIV positive patients from Ethiopia reported that treatment of intestinal worms significantly decreased HIV plasma viral load in co-infected individuals [15]. This finding was supported by a systematic review of three randomized controlled trials that evaluated the effect of different interventions (praziquantel, albendazole, and DEC) on different helminth infections (schistosomiasis, soil-transmitted helminths, and *W. bancrofti*) respectively, which reported significant benefit of deworming on both plasma HIV-1 RNA and CD4 counts [16]. Similar findings were also reported in another prospective observational study from Ethiopia which found that intestinal helminths were associated with an increased HIV viral load which reduced significantly after treatment [17].

While the impact of helminth infection on HIV disease has received considerable attention, there is limited data on how co-infection with HIV might influence the dynamics of helminth infection and the studies that have investigated this relationship have been less clear [18]. A prospective study of HIV-1 and intestinal helminth co-infected adults in Zambia that assessed the impact of antihelminthic treatment of on plasma concentrations of HIV-1 RNA did not find an overall association between treatment of intestinal helminth infections and reduction in viral load in co-infected adults [19]. Similarly, Webb et al in their review article examined the epidemiological and immunological evidence for HIV and helminth interactions and found limited and inconsistent evidence of any important interactive effects [20].

1.1.5 LF and HIV co-infection

At the outset of this research there were few studies reported that had investigated LF and HIV co-infection. A cross-sectional study undertaken in Tanga region of Tanzania reported a positive association between HIV and *W. bancrofti* circulating filarial antigen (CFA) infection detected by the immunochromatographic rapid card test (ICT) [21]. A more detailed immunologic evaluation of a subpopulation of this cohort did not provide any evidence for an interaction between HIV and *W. bancrofti* infections, in terms of an association between *W. bancrofti* co-infection and HIV viral load, CD4% and CD4/CD8 ratio and, an association between HIV infection and CFA intensity or cytokine levels in response to filarial antigen stimulation [22]. Evaluation of the effect of diethylcarbamazine (DEC) treatment, a common drug of choice that kills both the microfilariae and some of the adult worms of *W. bancrofti* infection, found a significant decrease in HIV viral load in Tanzanian individuals co-infected with HIV and LF 12 weeks after treatment with DEC, indicating possible benefit of LF treatment in managing HIV progression [23]. In urban southern India, no quantitative difference in *W. bancrofti* CFA levels was found in a study of HIV-positive and HIV-negative patients [24]. Another study from the same cohort compared HIV replication and progression in HIV-positive patients with and without *W. bancrofti* infection and found no differences in HIV disease progression between the groups after treatment with DEC and albendazole in contrast to previously reported findings from a similar study from Tanzania [23].

The results from these two studies are divergent and mainly focused on the effects of helminth or filarial infection on the markers of HIV infection. Turning this around, the impacts of HIV infection on *W. bancrofti* infection are less clear. At the outset of the study, there were no available data on this interaction and thus a lack of clarity for LF control programmes on whether and how to change the current mass drug administration (MDA) approaches in HIV endemic areas. Further clinico-epidemiological investigations were needed in order to better understand how LF and HIV infections interact and to either provide reassurance that the current approach to control LF is acceptable in HIV endemic areas and if not, to identify targets for change.

1.2 Justification and rationale to the study

Understanding the inter-relationship between HIV and lymphatic filariasis is essential for ensuring effective intervention strategies. A better understanding of this relationship will ensure that HIV will not present roadblocks to LF control in populations where HIV prevalence is high and will assist in identifying areas for public health action. In addition, establishing the efficacy of anti-filarial drug doses may help drive the search for alternative treatment

approaches for LF. This study therefore aimed to investigate the interaction of HIV infection and lymphatic filariasis in northern Malawi, an area where HIV and LF prevalence rates are high and control programs are underway. It tested various doses and frequencies of albendazole and ivermectin for LF control. It also aimed to investigate the impact of HIV on the success of annual treatment with microfilaricidal drugs and the effect of other concurrent measures including insecticide treated nets and HIV treatment programmes. The data generated from this study will add to the body of scientific knowledge on these major public health problems and provide key data for LF control programs operating in regions of high HIV prevalence, data that at present are lacking.

1.3 Aim and objectives

The aim of the study was to assess the interaction between lymphatic filariasis and HIV that might affect the success of lymphatic filariasis elimination programmes in areas with a generalised HIV epidemic.

Objectives:

1. To determine the prevalence of LF and HIV co-infections as quantified by the Og4C3 ELISA in a large cross-sectional study in Karonga district, rural northern Malawi.
2. To investigate whether higher and/or more frequent doses of albendazole and ivermectin are more effective in eliminating *W. bancrofti* microfilariae than the WHO–approved standard regimen.
3. To determine the relationship of CFA and microfilaria count (MF) by HIV status.
4. To monitor the impact of Mass Drug Administration (MDA) on LF antigenaemia by HIV status by following a cohort of antigen positive individuals identified in objective one.
5. To assess the contribution of insecticide treated bed nets (ITNs) to changes in circulating filarial antigen (CFA).

1.4 Study summary and methods

This thesis presents results from adults in two rural sites in Karonga district, northern Malawi, a region endemic for both LF and HIV. Two geographically separate studies (see Figure 1 and Figure 4) were used: one was a clinical trial of anti-filarial agent dosing regimens in Songwe in the northern part of the district (the Songwe clinical trial), and the other a whole population annual HIV sero-survey with longitudinal follow up data from the southern part of the district (the Karonga Health and Demographic Surveillance System or KHDSS).

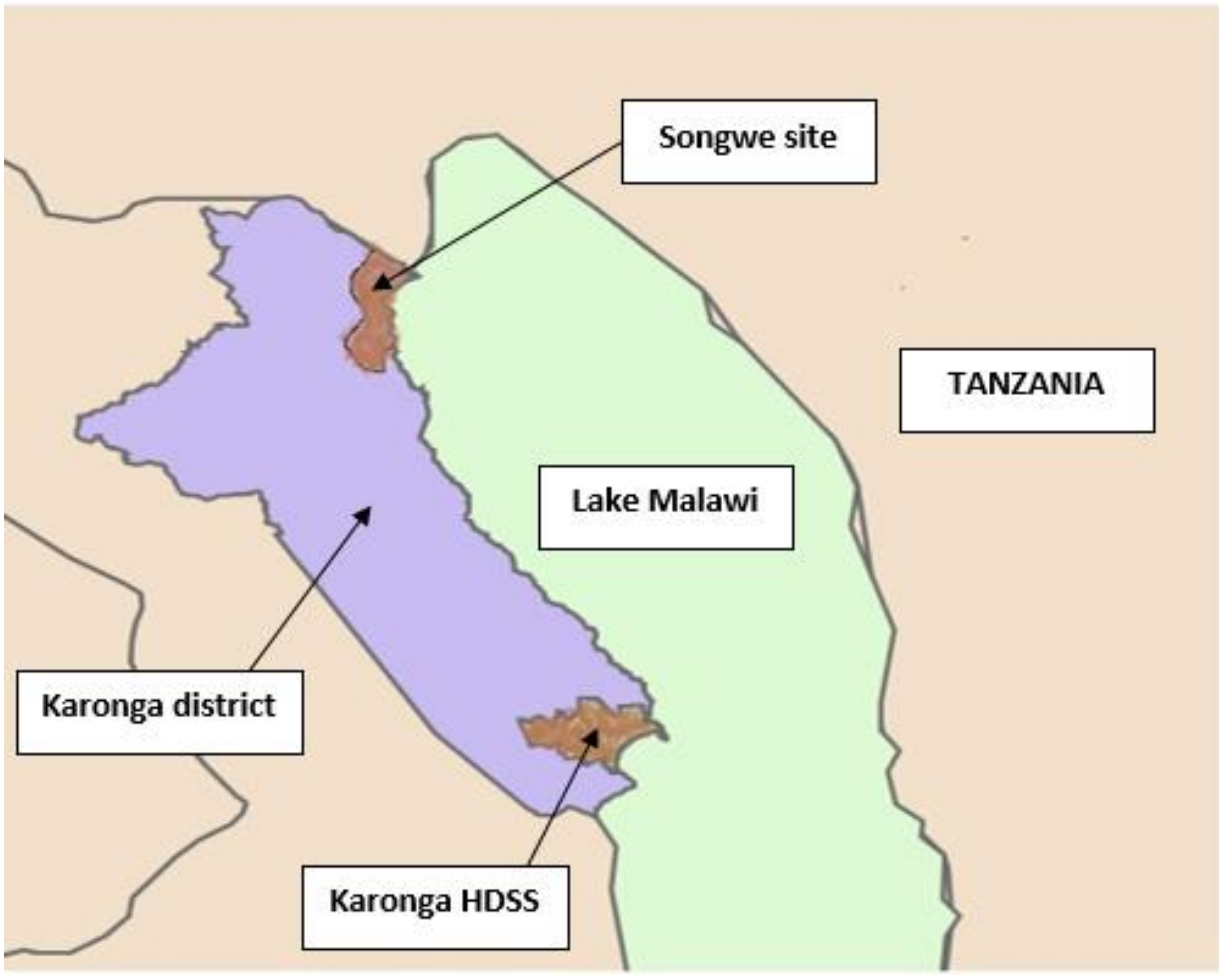


Figure 1: Map of Karonga District Showing Songwe and KHDSS Areas

Three filarial research studies were undertaken using stored blood samples and data from the two distinct geographical locations:

1. Study 1, cross-sectional assessment of the relationship of HIV and markers of LF infection.
2. Study 2, clinical trial of anti-filarial dosing regimens.
3. Study 3, longitudinal assessment of the impact of MDA on LF antigenaemia by HIV status.

1.5 Role in the research

The work included in this thesis is entirely my own. As the trial coordinator for the Songwe clinical trial, I oversaw all aspects of the day-to-day running and coordination of the trial, including its design, procurement of equipment, supervision of a team of five people, door-to-door data collection, night blood sample collection, laboratory work and analysis for the trial. I

randomised and selected stored blood samples from the KDHSS and the clinical trial for retesting and conducted the laboratory work with the assistance of a lab technician, following standard operating procedures (SOPs). The statistical and geospatial analysis are also my own work.

1.6 Thesis Layout

Chapter 1 provides a general introduction of the thesis including a summary of the justification and rationale to the study, the aims and objectives as well as a summary of the main methods and layout of the thesis.

Chapter 2 reviews general and local literature on LF and HIV coinfections and provides a comprehensive study and interpretation of this literature. It particularly provides a detailed account of lymphatic filariasis that includes historical perspective, burden of the disease, life cycle and vectors of the parasites, clinical manifestations, diagnostic methods, treatment, LF and HIV coinfection, and public health approaches to LF control and elimination.

Chapter 3 presents a detailed description of the methodology employed in this study. The chapter first describes the Karonga Prevention Study (KPS) in Karonga, northern Malawi. It then outlines the relevant Karonga Prevention Study studies within which this thesis is nested, including their linkage to each other. For the study to make sense, the timelines of the various interventions related to LF control in the district are summarised. The chapter then gives a detailed description of the methods for each of the filarial research studies that make up this thesis. For each the study design, characteristics and selection of the populations studied, description of the information collected from the participants, the variables used, and sample size considerations are described. Common methods across all the studies are presented separately and these include a description of laboratory methods, data handling and management, statistical analyses, mapping methods and analyses and ethical considerations.

Chapter 4 presents the study findings of each of the three filarial research studies that make up this thesis. It is divided into three sections corresponding to the three filarial research studies. The first section presents the cross-sectional analysis of the relationship between LF and HIV infections. The second section describes the Songwe clinical trial participants and the microfilarial clearance in the different arms while the third and last section presents results of samples taken before and after a period of mass drug administration including participant characteristics at baseline and follow up, CFA clearance rates and an analysis of the impact of bed net ownership and an HIV subgroup analysis.

Finally, chapter 5 examines and gives details of the main findings and discusses these in the light of the existing evidence from elsewhere, summarises the conclusions of this thesis and provides suggestions for future work. It also provides alternative explanations of the findings and summarises and acknowledges the main limitations of the study. Finally, it draws generalisations from the important findings and implications for public health, makes recommendations for LF control programmes and outlines areas for further research.

Chapter 2 Literature Review

This chapter presents a comprehensive study and interpretation of the existing literature on lymphatic filariasis and HIV coinfections. It provides a detailed and critical analysis of the available literature and identifies relevant previous research and methods on the subject area. It also identifies and demonstrates controversies and knowledge gaps in the existing literature on the subject area and justifies the rationale for the research question and study. It begins with a description of the methodology that was used to conduct the search for the existing literature on the subject area followed by a detailed account of the two main sections of the chapter.

The first section gives a detailed account of the existing general literature on lymphatic filariasis that includes historical perspective, burden of the disease, life cycle and vectors of the parasites, clinical manifestations, diagnostic methods, treatment, LF and HIV coinfection, and public health approaches to LF control and elimination.

The second section, the Malawian national context, gives a detailed account of the local context and available literature on lymphatic filariasis and LF and HIV coinfection.

2.1 Literature search methods

A comprehensive and systematic approach was used to conduct the search for the relevant existing literature on the research topic. The research question for the study – Is there an interaction between lymphatic filariasis and HIV that might affect the success of lymphatic filariasis elimination programmes in areas with a generalised HIV epidemic? – was used to derive key components of the subject area and the following key search terms and synonyms that were used for searching the literature: filariasis, filarial infection, lymphatic filariasis, elephantiasis, *Wuchereria bancrofti*, helminths, parasites, worms, nematodes, HIV, HIV-1, HIV-2, HIV/AIDS, Human Immunodeficiency Virus, AIDS, Acquired Immunodeficiency Syndrome, control, elimination, coinfection, interaction, relationship and association.

These key search terms were used in combination or isolation, to conduct the literature search using the searching option on the chosen databases or search engines. The ‘AND’ and ‘OR’ Boolean operators were used to combine the search terms together. The ‘OR’ Boolean operator was used to combine synonyms of the key components of the subject area while the ‘AND’ Boolean operator was used to combine the key components of the subject area. Other search tools that were used to help with the literature search were truncation and use of wildcards.

The literature search was performed on the following electronic healthcare databases: PubMed, Scopus and the Cochrane library. Other sources of information used were books, search engines, reference lists of key articles, authors and journals of key articles, and grey literature (conference papers and abstracts, theses, reports and unpublished research).

2.2 Lymphatic filariasis

2.2.1 Introduction

Lymphatic filariasis is a parasitic helminth infection caused by the mosquito-transmitted filarial nematodes *W. bancrofti, B. malayi and B timori* with most cases (91%) and all cases in Africa caused by *W. bancrofti* [2, 25]. The parasites are transmitted to humans via the bite of an infected mosquito vector and cause damage to the lymphatic system which leads to swellings in the legs, arms, breast and genitalia and significant disability and disfigurement of the affected individuals [26]. LF is a major public health problem worldwide affecting 120 million people in 72 endemic countries with an estimated at-risk population of 1.39 billion people [27]. It is one the leading causes of long-term disability and an estimated 40 million of the LF-infected people suffer from the chronic, debilitating and stigmatising clinical manifestations of LF, mostly lymphoedema or elephantiasis (15 million people) and scrotal hydrocoele (25 million people) [2]. LF is classified as one of the world's Neglected Tropical Diseases (NTDs), a diverse group of diseases that thrive mainly among the poorest and deprived populations of the world in Africa, Asia and Latin America [27].

The World Health Assembly (WHA) meeting in 1997 urged member states through the adoption of resolution WHA 50.29 which called for LF endemic countries to eliminate the disease as a public health problem [28]. In response to this resolution and the significant global LF burden, WHO launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in the year 2000 with the goal to eliminate LF by 2020 through interruption of transmission of the filarial parasite using MDA and management and prevention of disabilities associated with the disease [2].

2.2.2 Life cycle and vectors of LF parasites

Anopheles and *Culex* mosquito vectors transmit LF from person to person. *Anopheles gambiae s.l.* and *Anopheles funestus,* which are also the principal vectors of malaria, transmit the disease in rural areas of Africa while *Culex quinquefasciatus* is the main vector in urban and peri-urban areas [27, 29]. The human phase of the life cycle (Figure 2) begins when the mosquito takes a blood meal and deposits infective larvae of microfilariae onto the human skin. The infective larvae of microfilariae enter the human body, their definitive host, via the mosquito bite wound. They migrate to the lymphatic system mostly in the inguinal and genital

areas where they reside and develop into male and female adult worms that are 4-8 cm long. The adult worms have a lifespan of 5-8 years during which they mate and produce thousands of sheathed microfilariae. The worms live in mutual symbiosis with *Wolbachia endobacteria* which are essential for growth, development and maturation of the filarial parasites and also responsible for inflammatory disease pathogenesis [30].

The microfilariae resemble tiny worms and measure about 100-150µm in length. They periodically migrate into the bloodstream at night and peaks between 9 pm and 4 am, a phenomenon called nocturnal periodicity. This period coincides with the period of most active feeding by the mosquito vectors. The mosquito phase of the life cycle begins when the mosquito vector that is the intermediate host, ingests the microfilariae during a blood meal on an infected human. Inside the mosquito, the microfilariae migrate to the thoracic muscles where they develop into three larval stages in about 10-14 days. The third-stage larvae then migrate to the head and proboscis of the mosquito and may infect other humans when the mosquito takes a blood meal again.

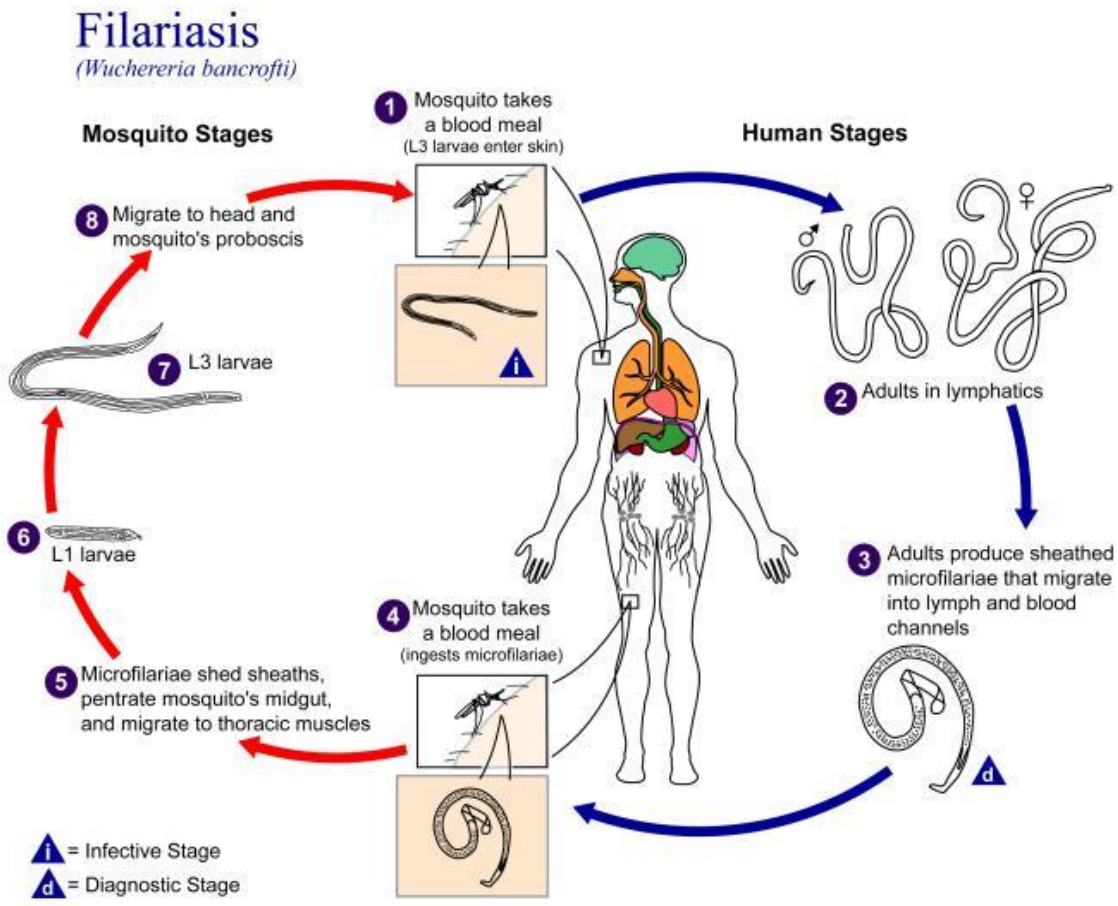


Figure 2: Lymphatic Filariasis Life Cycle

(Source: Public Health Image Library, CDC [http://phil.cdc.gov])

2.2.3 Clinical manifestations

LF infection is generally acquired in childhood but clinical signs and symptoms usually appear several years later in life. Most infected individuals remain asymptomatic and never develop clinical manifestations of LF, while a small proportion of infected individuals will develop clinical signs and symptoms. Males are more often infected than females and have the most severe forms of clinical manifestations of LF. Treatment options are discussed in more detail in section 2.2.5 below.

Asymptomatic infection

Asymptomatic individuals have adult worms and microfilaraemia but do not exhibit any clinical signs and symptoms of LF.

Acute LF manifestations

These include periodic attacks of adenolymphangitis (local inflammation of skin, lymph nodes and lymphatic vessels), fever and malaise. They are caused by the body's immune response to the parasite and secondary bacterial infection of the skin and lymph system, facilitated by partial loss of the body's immune response and damage of the lymphatic system (Figure 3).

Chronic LF manifestations

These include hydrocoele in males, lymphoedema, elephantiasis and chyuria (Figure 3). Hydrocoele is the fluid collection and swelling of the scrotum. Lymphoedema is the fluid collection and swelling mostly affecting the legs, arms, breasts and genitalia. Elephantiasis is the hardening and thickening of the skin which is caused by frequent secondary bacterial infections of the skin and lymph system. Chyuria is the presence of chyle or milky white lymphatic fluid in urine that makes urine appear white.

Tropical pulmonary eosinophilia

LF infection may also manifest a condition called tropical pulmonary eosinophilia syndrome. It is characterised by cough, shortness of breath and high levels of immunoglobulin E (IgE) and antifilarial antibodies.

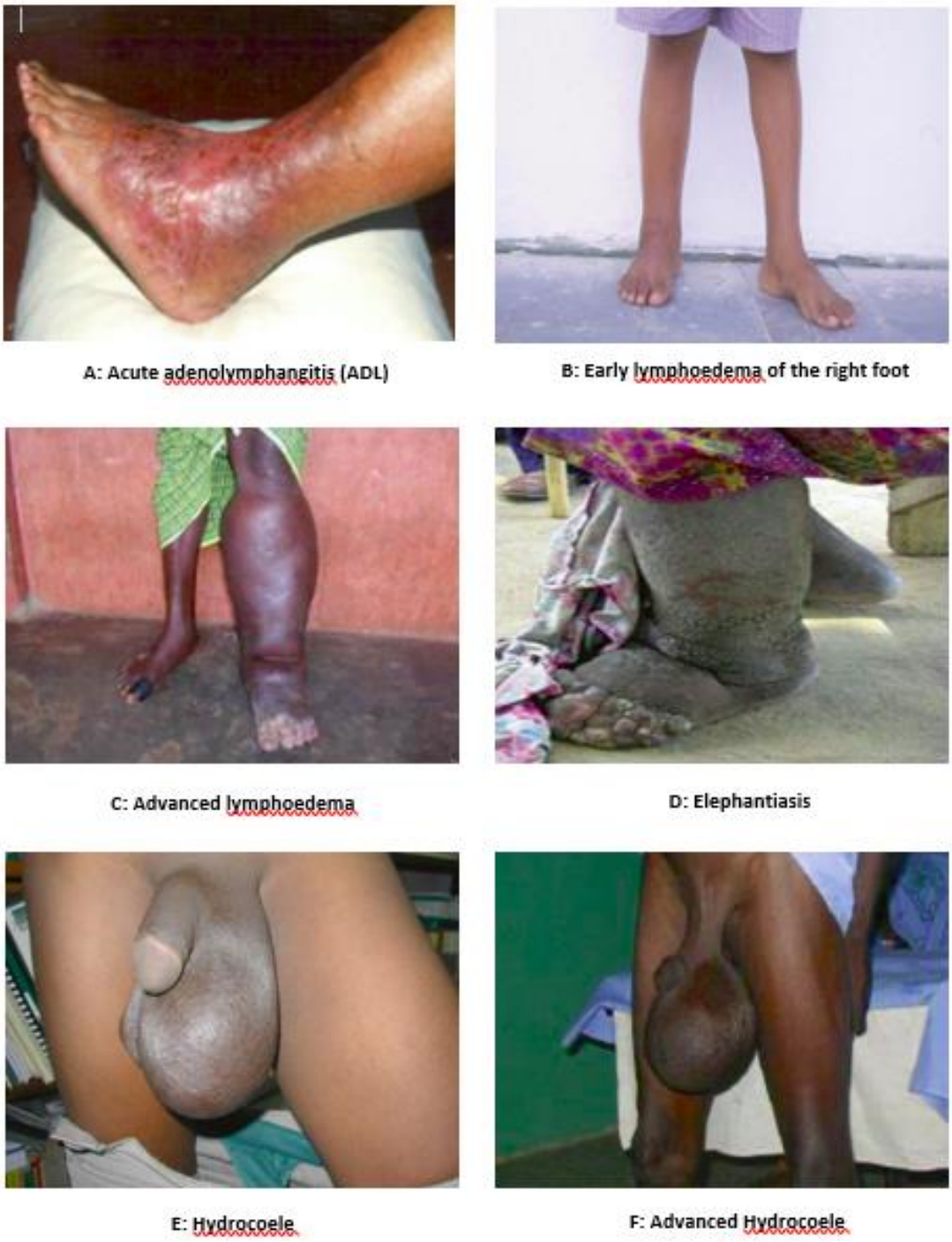


Figure 3: Clinical Manifestations of LF

(Source: Neglected Tropical Diseases – Sub-Saharan Africa)

2.2.4 Diagnostic methods

Clinical diagnosis

Clinical diagnosis of LF can be made in any patient from an endemic area with a history of unilateral or bilateral lymphoedema of the limbs or hydrocoele in males associated with

thickening of skin and repeated episodes of fever and pain in the affected organs in the absence of other obvious causes of oedema such as congestive cardiac failure or diseases of the lymphatic system [31].

Ultrasound scanning

Adult filarial worms residing in the lymphatics can be detected by ultrasound scanning of the appropriate area, usually scrotal lymphatics in males and breast lymphatics in females. The worms are visualised rapidly moving on the scan, a phenomenon referred to as the filarial dance sign (FDS) [32]. This diagnostic technique is expensive and not suitable for mass or routine diagnosis of LF [31].

Detection of microfilariae

Identification of microfilariae by microscopy on a blood smear from an infected individual is considered as the standard method to diagnose active LF infection [33]. The blood requires to be collected at night usually between 10pm and 2am because the microfilariae are only present in the blood circulation at night (nocturnal periodicity). There are four common methods for microfilarial detection:

Thick blood smear

A 20-60µL finger-prick blood sample volume is applied on a microscope glass slide, dried overnight, fixed and stained with Giemsa stain and later examined for microfilariae under a microscope. This method has low sensitivity due to small blood sample volume used and loss of microfilariae during preparation of the slide [34].

Knott's concentration method

A 1mL venous blood sample collected in an anticoagulant-containing tube is mixed with 10mL 2% formalin that acts as a preservative and lysing solution. The mixture is left to stand for 15 minutes and later centrifuged. The supernatant is discarded, and the remaining sediment is later examined for microfilariae under a microscope. A drop of methylene blue may be added to the sample to aid examination [34].

Counting chamber method

A 100µL finger-prick blood sample volume is transferred to a tube containing 0.3% acetic acid that acts as a preservative and lysing solution. The mixture is shaken gently and later transferred to a Sedgewick Rafter counting chamber and examined for microfilariae under a microscope [34].

Membrane filtration method

A venous blood sample volume of 1-5mL is collected in an anticoagulant-containing tube and mixed with a red blood cell lysing solution. The mixture is then filtered through a 5µM pore size Nucleopore filter held within a leak-proof reusable filter holder. The filter is later removed using forceps and placed on a glass slide and examined for microfilariae under a microscope [34].

Alternative methods to microscopy are assays for circulating filarial antigens and antibodies and ultrasound scanning for detecting adult filarial worms. There are two assays for detecting filarial antigens currently available, the Og4C3 enzyme-linked immunosorbent assay (ELISA) and the immunochromatographic card test (ICT). These tests have high sensitivity ranging from 96 to 100% and a specificity of 99% unlike microscopy for microfilariae detection which has a low and variable sensitivity [31].

Og4C3 ELISA

This test kit is W. bancrofti specific and manufactured by TropBio Pty Ltd. (Queensland, Australia). It yields quantitative results (see laboratory methods section) but its use is limited and not suitable for mass or routine diagnosis because it requires a well-equipped laboratory as well as skilled laboratory personnel [34].

ICT

This is a rapid card test manufactured by Binax (Scarborough, USA) and is also specific for *W. bancrofti.* It yields qualitative (positive/negative) results (see laboratory methods section) and is used extensively for mass and routine diagnosis and is ideal for field settings because it uses finger-prick blood, is easy to administer, has high sensitivity and yields results in a short time [34].

Anti-Bm14 IgG4 assay

The filariasis CELISA anti-Bm14 IgG4 assay (Cellabs Pty Ltd., Manly, Australia) is a filarial antibody IgG4-specific ELISA kit with plates that are coated with the recombinant Bm14 antigen [35]. This antigen reacts with sera from patients with bancroftian filariasis and is highly sensitive (over 90%) [35]. Its limitation is cross reactivity with sera from other helminths parasites [34].

2.2.5 Treatment of lymphatic filariasis

Treatment of LF is generally aimed at preventing, reversing or halting the progression of disease and to interrupt transmission of the parasite [36].

Non-drug treatment of clinical manifestations

This is targeted at alleviating the suffering and decreasing the disability in lymphatic filariasis patients [37]. It is achieved by providing training and support for improved hygiene including daily washing of the affected areas with soap and clean water, elevating and exercising of the swollen limbs to increase lymph flow, disinfecting wounds and treatment of secondary infections, and increases access to hydrocoelectomy [37].

The drugs and how they work

Diethylcarbamazine (DEC), ivermectin and albendazole are the three drugs currently in use for the treatment and control of LF [38]. These drugs are given orally either alone or in combinations.

a. Diethylcarbamazine

This drug is a synthetic derivative of piperazine that is mainly microfilaricidal but also possesses some macrofilaricidal effects [39]. It acts by inhibiting arachidonic acid metabolism in microfilariae and making them more susceptible to host immune attack [38]. It rapidly reduces microfilariae after administration and this effect is sustained several months afterwards before the filarial load begins to increase again [38]. DEC also reduces circulating filarial antigenaemia, but this effect is variable probably because of its limited macrofilaricidal effects. It has no effect on already established lymphatic damage or chronic manifestations but can halt the development of chronic manifestations and is therefore recommended for treatment of asymptomatic microfilariae or antigen positive LF patients [38].

Common side effects include fever, headache, malaise, muscle, and joint pains. DEC is contraindicated in individuals co-infected with onchocerciasis due to severe adverse reactions and symptom progression especially in onchocercal eye disease. It is also contraindicated in pregnancy.

b. Ivermectin

Ivermectin, a macrocyclic lactone, is a broad-spectrum anti-parasitic drug of the avermectin group of compounds that is mainly microfilaricidal and has no macrofilaricidal effects [38]. It acts by inhibiting nerve and muscle function of the parasite. The recommended dosage is 150-400µg/kg single dose and is contraindicated in pregnant and breastfeeding women and under-five children.

c. Albendazole

Albendazole is a benzimidazole derivative that is used for the treatment of a number of helminths infections [39]. It acts by interfering with microtubule assembly and blocking glucose

uptake in the parasite. It is usually given in combination with DEC or ivermectin because of its microfilaricidal effects and this combination results in a sustained longer lasting effect.

d. Antibiotics and LF

Certain antibiotics have been reported to be active against filarial nematodes due to their symbiotic relationship with *Wolbachia* bacteria species that lives within the filarial nematodes [40]. These antibiotics include doxycycline and rifampicin.

e. Doxycycline

Doxycycline is a member of the tetracycline group of antibiotics. It is used in the treatment of many types of bacterial and protozoal infections. Multiple studies have shown that treatment of bancroftian filariasis with a 4-, 6- or 8-week course of 200 mg per day dose of doxycycline is effective in killing filarial adult worms and reducing circulating antigenaemia and microfilaraemia [41-43]. In addition, doxycycline treatment also leads to long-term improvements in lymphatic pathological features and decreased severity of lymphoedema and hydrocoele [42].

f. Rifampicin

This is a bactericidal drug belonging to the rifamycin group of antibiotics. It is used to treat several bacterial infections including tuberculosis and leprosy. Rifampicin has been shown to have some macrofilaricidal effects in murine filariasis infected mice where a 14 day treatment with rifampicin alone or in combination with doxycycline led to the depletion of *Wolbachia* endobacteria and inhibition of worm development, embryogenesis and adult worm survival [44]. Rifampicin has also been shown to have partial macrofilaricidal activity in a pilot study that investigated treatment of human LF with doxycycline alone or in combination with rifampicin [45].

2.3 LF co-infections and co-endemicity

Multiple infections are constantly present in particular geographical areas or population groups. Co-endemic infections are especially widespread in sub-Saharan African countries, including Malawi, and may result in co-infection, which is the simultaneous infection of an individual host with multiple pathogens [46]. Co-infection is of particular clinical significance because pathogens interact with one another within the host in a positive or negative way. Positive co-infection interactions involve enhancement of disease transmission and progression while negative co-infection interactions involve suppression of virulence or colonisation of one pathogen by another [47]. LF is co-endemic in many regions in sub-Saharan African with other infections and this may lead to potential interactions in co-infected

individuals. This section will focus on two important and overlapping LF co-infections with HIV and malaria.

2.3.1 LF and HIV Co-infection

Lymphatic filariasis (LF) and HIV are both major public health problems worldwide and where they co-exist, have the potential to interact. These interactions may have important consequences for annual mass drug administration (MDA), the main strategy for LF elimination, particularly whether HIV, through its impact on the immune system interferes with the effectiveness of this approach to control and eliminate LF. It has been suggested that co-infection with HIV and filarial helminths may have bi-directional deleterious interactions by affecting susceptibility to HIV, impacting on HIV progression and potentially worsening clinical outcomes of filarial infection [48]. Findings from several studies suggest that helminth infection may adversely affect HIV-1 progression and that treating helminth infection in HIV-1 co-infected adults appears to impart beneficial effects on both HIV-1 viral load and CD4 counts [15, 17, 49]. In-vitro studies have shown helminth infections to increase susceptibility of peripheral blood mononuclear cells to HIV infection, and this susceptibility decreased once the individuals were treated for their filarial infection [50].

While the consequences of helminth infection on HIV disease have received considerable attention, there is limited data on how co-infection with HIV might influence the dynamics of filarial infection [51]. It has been hypothesized that immunodeficiency arising from HIV infection can alter the clinical course and dynamics of filarial infections but studies that have investigated this relationship have found conflicting results. In two small studies of co-infection with the filarial infection *Onchocerca volvulus*, those infected with HIV had more significant onchocercal skin disease and were less likely to have antibodies in response to onchocercal antigens , while in another study, Fischer and colleagues found no effect of HIV-1 infection on ivermectin efficacy [52].

A cross-sectional study of 907 adults undertaken in Tanga region of Tanzania reported a positive association between HIV and *W. bancrofti* circulating filarial antigen (CFA) infection detected by ICT rapid card test , although a further evaluation of a subgroup of these individuals in another cross-sectional study did not support any association between HIV and *W. bancrofti* infection in terms of an association between *W. bancrofti* co-infection and HIV viral load, CD4% and CD4/CD8 ratio and, an association between HIV infection and CFA intensity or cytokine levels in response to filarial antigen stimulation [22]. Evaluation of the effect of diethylcarbamazine (DEC) treatment, a common drug of choice that kills both the microfilaria and some of the adult worms of *W. bancrofti* infection, found a significant decrease

in HIV viral load in Tanzanian individuals co-infected with HIV and LF 12 weeks after treatment with DEC, indicating possible benefit of LF treatment in managing HIV progression [23]. Similarly, in urban southern India, no quantitative difference in *W. bancrofti* CFA levels by HIV status was found in a study of 432 HIV-positive and 99 HIV-negative patients [24]. A further study from the same cohort that compared HIV replication and progression in HIV-positive patients with and without *W. bancrofti* infection found no differences in HIV disease progression between the groups after treatment with DEC and albendazole in contrast to previously reported findings from a similar study from Tanzania [23].

2.4 Public health approaches to LF control

2.4.1 MDA

WHO launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000 with the aim of eliminating LF as a public health problem by the year 2020 [5, 53]. The main strategy recommended by WHO that is used is to interrupt LF transmission in endemic areas is mass drug administration (MDA), a therapy that uses combinations of two antihelminthic drugs that are administered to entire at-risk population in endemic areas [54]. The antihelminthic drugs are used to reduce microfilaraemia in infected persons to levels so low that that they can no longer sustain transmission by mosquito vectors to new hosts. The target population for MDA is all individuals aged 5 years and above living in LF endemic areas. Annual MDA is given for at least 5 years in a row, which is generally considered the reproductive life span of the adult filarial worms in humans. The number of rounds may vary and depends on the initial prevalence of infection, the initial intensity of transmission, the efficacy of medicines, the combinations of parasites and vectors, and the density of vectors [54]. MDA aims for coverage of at least 65% of the total at-risk population in endemic areas each year as this is considered as the minimum level of coverage adequate to achieve LF elimination [55, 56].

Drug selection, dose and frequency

MDA is delivered using a single annual dose of either ivermectin and albendazole in Africa, where onchocerciasis is also endemic because of the contraindications of the use of diethylcarbamazine (DEC) in patients with onchocerciasis, or DEC and albendazole in all other LF endemic regions of the world, where onchocerciasis is not endemic [54]. Co-endemicity with *Loa loa* in parts of Central and West Africa is a major challenge that places people with these infections at risk of serious adverse reactions if they receive ivermectin and the current MDA strategy cannot be initiated.

Community-based distributors

MDA is usually administered at community level. This is achieved using one of two drug delivery methods. The first method is delivery via regular health services and uses employed community health workers as drug distributors [57, 58]. The second method is delivery through a system of community-directed treatment in which local health workers train and sensitise communities to direct the intervention [57, 58]. The MDA drugs are delivered to the target population using door-to-door distribution or fixed posts delivery and uses directly observed treatment strategy (DOTS) to ensure compliance. Another strategy used to deliver MDA drugs to at-risk populations is through the use of table salt or cooking salt fortified with DEC. DEC-fortified salt has been used successfully in by China to eliminate LF and is a safe, low-cost and effective alternative to MDA of tablet drugs [59].

Success of MDA programmes

Since the launch of the GPELF in 2000, national control programmes in LF endemic countries have registered dramatic progress and success in implementing MDA [53]. Between 2000 and 2015, more than 820 million people received mass treatment in 64 LF endemic countries, considerably reducing transmission in many countries and the number of people requiring MDA by 25% (351 million) due to successful implementation of the MDA programmes [60]. Success of MDA programmes depends both on achievement of high compliance levels among individuals and high coverage of populations for 4-5 successive years. Individual compliance can be challenging since people have to accept to take the treatment when they have no symptoms. Constraints to coverage include fragile health care systems and infrastructures in resource-limited countries, difficult to access populations, limited enumeration of households, poor supervision of community-based distributors and inadequately funded national programmes [61].

2.4.2 Vector control

LF is a mosquito-borne parasitic disease and the mosquitoes that transmit the disease are night biting. There is an overlap between LF and malaria in many aspects. The two diseases occur concurrently in many tropical regions of the world, share common mosquito vectors and susceptibility to the same control interventions. Vector control is a major part of malaria control and it is likely that this strategy may also significantly impact on LF transmission and prevalence.

The main methods for vector control in malaria are insecticide treated bed nets (ITNs) and indoor residual spraying (IRS). An ITN is a bed net that has been treated with safe residual insecticide that provides a physical barrier against malaria-transmitting mosquitoes, and kills and repels mosquitoes [62]. WHO recommends that ITNs be distributed free of charge or

heavily subsidised to all people living in at risk communities and in order to achieve universal coverage, one ITN should be distributed for every two persons in a household [63]. ITNs are estimated to reduce child mortality by 17% and uncomplicated malaria cases by 50% [64].

IRS is the application of a residual insecticide to walls and other surfaces of a house. The insecticide kills malaria-infected mosquitoes and other insects that encounter these surfaces and prevents disease transmission. The proportion of at risk populations protected from malaria by IRS in Africa increased from 5% in 2005 to 11% in 2011 [65].

The integration of these vector control interventions with national LF elimination programmes has been widely advocated following evidence that ITN use and IRS for malaria significantly reduces filarial rates and because of problems with MDA in some areas [66]. Field studies and mathematical models have shown that vector control can have an important complementary role in the elimination effort resulting in lower coverage levels and fewer annual rounds would be needed to achieve elimination.

2.5 National context in Malawi

This section gives an introduction of the country of Malawi. It describes the country's geography, population, political, economic and health systems. It also gives details on lymphatic filariasis, HIV and the control strategies for these diseases currently underway in Malawi. Sources of information used were relevant published literature, unpublished reports and national policy documents available in Malawi. Personal communication records in form of personal interviews and conversations, letters, memos and emails were also used.

2.5.1 Population and geography

2.5.1.1 Geography and climate

Malawi is a small landlocked country located in south-eastern Africa bordering Tanzania to the north and northeast, Mozambique to the east, south and southwest and Zambia to the west and northwest (Figure 4).

Figure 4: Map of Malawi Showing Region and District Boundaries

(Source: Malawi Demographic and Health Survey 2015-16)

It lies south of the equator in the southern end of the Great East African Rift Valley between latitudes 09^0 25'S and 17^0 08'S and longitudes 32^0 40'E and 35^0 55'E. The country is 901 kilometres long and ranges in width from 80 to 161 kilometres covering a total area of 118,484 square kilometres of which 94,276 square kilometres is land. The rest of the area is covered with water and the major water body in the country is Lake Malawi, which is about 475 kilometres long and is the third largest lake in Africa and the eighth largest freshwater body in the world, and runs down Malawi's eastern boundary with Tanzania and Mozambique [67].

The countryside and catchment area of Lake Malawi is characterised by fertile plains, hills, and mountain ranges whose peaks range from 1700 to 3000 metres above sea level. There are also several river systems in the country and the largest is the Shire River which is the outlet of Lake Malawi and drains into Zambezi River in Mozambique [67].

The country has a tropical continental climate with two main distinct seasons: the rainy season from November to April and the dry season from May to October. The rainy season is hot and humid while the rest of the year is mainly dry and cool. There is variability in rainfall and temperature depending on altitude and proximity to the lake [67]. It experiences good rainfall during the rainy season with an annual mean of 1,037mm and the mean monthly temperatures range from 10-16°C in highland areas and 20-29°C along the lakeshore areas [68].

The topography of the land together with a good network of rivers and water bodies and the tropical climate contribute to extensive mosquito breeding and the epidemiology of mosquito-borne diseases in the country. In general, a low prevalence of mosquito vectors is found in the western side of the country, due to the fact that these areas are dry and of higher altitude and thus not ideal for extensive mosquito breeding, while higher prevalence is found along the lakeshore areas and areas bordering major rivers [69].

2.5.1.2 Population and demography

The population of Malawi was estimated at 16.7 million in 2014 with an average annual growth rate of 3.1% [70]. There are more females than males (51% vs. 49%), about 59% of the population are children under the age of 20 years, and about 17% are children under the age of 5 years [67]. Malawi is one of the poorest countries in the world and this is reflected in its health indices. The estimated total life expectancy at birth in Malawi is at 63 years, the infant mortality rate is 44 deaths per 1,000 live births and under-five mortality rate is 70 deaths per 1,000 live births. The total fertility rate is 5.7 per woman and maternal mortality ratio is 510 per 100,000 live births. About 47% of children under-five years of age are chronically malnourished and stunted. The adult literacy rate is 61% and most of the population (84%)

live in rural areas. There are over 20 tribes in Malawi, however, Chichewa and English are regarded as the two official national languages [67, 71].

2.5.1.3 Political organisation and socio-economic profile

Administratively, Malawi is divided into three regions, the northern, central, and southern regions, which are further divided into twenty-eight districts, six districts in the Northern Region, nine in the Central Region, and thirteen in the Southern Region. The districts are subdivided into approximately 250 traditional authorities (TAs), presided over by chiefs. Each TA is composed of villages, which are the smallest administrative units and are presided over by village headmen. The capital city of Malawi is Lilongwe located in the Central Region and is the largest city. Blantyre located in the Southern Region is the commercial centre and the second largest city while Mzuzu located in the Northern Region is the third largest city.

Malawi remains one of the poorest countries in the world with over 60% of Malawians living below the poverty line and is among the world's least developed countries [68]. The backbone of Malawi's economy is agriculture and accounts for 30% of the Gross Domestic Product (GDP) [67]. The main occupation of the people is subsistence farming, fishing and livestock rearing and agricultural produce is the main contributor to the Gross National Product (GNP).

Malawi maintains good diplomatic relations with all African countries including its three neighbouring countries of Tanzania to the north and northeast, Mozambique to the east, south, and southwest and Zambia to the west and northwest. The people of Malawi and its neighbouring countries have also maintained relationships across the borders and this interrelationship of communities in the region partly forms the historical basis for the existence of informal cross-border movements. Informal cross-border movement is facilitated by the largely porous borders between Malawi and its neighbouring countries and has the potential to undermine disease control activities in border regions.

2.5.2 The health system in Malawi

2.5.2.1 Ministry of Health structures

The Ministry of Health (MOH) is responsible for overall health care provision in the country. It is divided into three administrative levels namely ministry headquarters (central level), zonal (regional) offices and district health offices.

At the ministry headquarters, there is the Secretary for Health who heads the ministry and is assisted by directors of seven technical directorates that make up the ministry headquarters. The seven technical directorates are clinical services, nursing services, reproductive health services, preventive health services, planning and policy development, finance and administration and central monitoring, evaluation and research development.

Five zonal health offices provide technical support to District Health Management Teams (DHMTs) in planning, delivery and monitoring of health service delivery at the district level and facilitation of central hospitals' supervision to districts.

Health service provision is free of charge and is delivered through a three-level system at the community, district hospital and central hospital level and these levels are linked through a referral system. At the community level, health care provision is delivered by community-based cadres such as community health workers (known as health surveillance assistants in Malawi), community-based distributors and other health care volunteers. Their official roles include the provision of community-based preventive health services conducted through door-to-door visitations, village clinics, mobile clinics, and manned or unmanned health posts. In practice, however, coverage may be patchy and the performance of health surveillance assistants variable.

Primary health care is delivered through health centres and community hospitals built in the communities. These are staffed mostly by medical assistants and nurses/midwives who provide both curative and preventive Essential Health Package (EHP) services.

District hospitals provide secondary level healthcare. There is one hospital for each district in Malawi and they serve as referral facilities for health centres and rural hospitals and have an admission capacity of 200 to 300 beds. They are staffed by doctors, paramedics and nurses and they provide general services, PHC services and technical supervision to lower units. They also provide in-service training for health personnel and other support to community-based health programs in the provision of EHP.

Tertiary healthcare is provided by central hospitals where specialists are found, and they provide specialist referral services for their respective regions. There are currently 4 central hospitals namely, Queen Elizabeth Central in Blantyre, Kamuzu Central in Lilongwe, Mzuzu Central in Mzimba and Zomba Central in Zomba with admission capacities of 1250, 1200, 300 and 450 beds, respectively. Queen Elizabeth and Kamuzu Central Hospitals are also teaching hospitals because of their proximity to College of Medicine and Kamuzu College of Nursing. Central hospitals, however, also provide EHP services which should essentially be delivered by district health services [72].

The public health care system in Malawi has limited capacity and is chronically under resourced and staffed. Malawi has only 3 health workers per 10,000 population and falls way too short of the WHO recommended minimum of 23 health workers per 10,000 population [73]. The doctor to patient ratio is 2 per 100,000 population and the few doctors that are available are mostly concentrated in urban areas or are not working in the areas where they

are needed most, and many lack the necessary resources and equipment needed to perform effectively.

2.5.2.2 Other health care providers

The MOH is the largest provider of public health services in Malawi. Other health care providers are also available and complement the MOH in the provision of health care in Malawi. Private sector health care providers charge user fees for their services and consist of private for profit and private not for profit health care providers. Private for-profit health care providers have clinics and hospitals which are mostly located in towns and cities but are unaffordable to most Malawians.

Christian Health Association in Malawi (CHAM) is the largest partner of MOH in the private sector and it provides primary level and secondary level not for profit health services through its health centres, rural hospitals and mission hospitals which are mostly located in rural areas. CHAM also owns 11 health training institutions which train various cadres of health care workers. Although the user fees charged by the CHAM health facilities are at more affordable rates, they are a major barrier to accessing health care services for most poor Malawians hence MOH heavily subsidises CHAM facilities by financing drug and local staffing costs. A smaller, but significant other partner in service provision, especially to women, is the Banja La Mtsogolo (BLM) a Malawian non-governmental organisation. MOH has signed service level agreements (SLAs) with CHAM and BLM facilities to remove user fees for the delivery of maternal and neonatal health services and a few facilities have SLAs for all health services provided. SLAs involve the transfer of a fee from the District Health Office (DHO) to a CHAM facility in exchange for services offered [74].

Other health care providers in Malawi include non-governmental organisations, companies and firms, private pharmacies and grocery sales of drugs. Traditional health care providers in form of traditional healers and traditional birth attendants also play a significant role in health service provision in Malawi and many people use the traditional and modern health sectors simultaneously or consecutively [75].

2.5.2.3 Laboratories and their role in LF and HIV

Laboratories are an essential part of an effective health delivery service. They provide confirmatory diagnosis and improved management of disease, essential public health information and disease surveillance and constitute an important part of many disease control programmes [76].

HIV is mainly diagnosed through rapid diagnostic tests designed to be used in the field, but the laboratory is essential to provide quality assurance to ensure accurate results. The

laboratory also provides diagnosis of HIV in infants and newly infected individuals. Another key role of the laboratory is in the provision and monitoring of antiretroviral therapy through measurements of CD4 counts and HIV viral load [76].

Rapid diagnostic tests are also available for LF diagnosis. There are simple, finger-prick tests that detect infection within minutes and are designed to be used in the field. Again, the laboratory is essential to provide quality assurance for these tests to ensure accurate results. These tests are also used in mapping of LF endemic areas for inclusion in LF control programmes. Laboratory examination of blood for circulating microfilariae is another method of LF diagnosis. It is used for monitoring progress of LF treatment and MDA programmes. Other laboratory LF diagnostic methods used to monitor treatment and MDA programmes include demonstration of specific filarial antibodies and demonstration of parasite DNA in mosquito vectors (molecular xenomonitoring) or in human blood samples [77].

2.5.3 Lymphatic filariasis in Malawi

2.5.3.1 Epidemiology

Lymphatic filariasis, commonly known as elephantiasis, is a major public health problem in Malawi [78]. It is the second most common vector-borne parasitic disease after malaria and is the second commonest cause of long-term disability after mental illness [79]. Previously, Malawi was known to have two foci of lymphatic filariasis, one in the southern region (Shire valley) and the other in the northern region along the Songwe river bordering Tanzania [80]. Following a nationwide survey in 2003, it is now known that in Malawi LF infection is more widespread than previously appreciated. It is endemic (as defined by WHO guidelines as areas with an LF prevalence on ICT of 1% or higher) in all the districts in the country except Chitipa [81].

Both men and women suffer from lymphatic filariasis but the prevalence of infection and disease manifestations are significantly higher in males compared to females [82]. The nationwide mapping of LF in Malawi in 2003 also significantly found that males were more LF antigenaemic than their female counterparts (11.0% vs. 8.2%) [78].

Overall transmission of bancroftian filariasis varies seasonally as well as geographically. This variation reflects differences in transmission potential among individual mosquito vectors for LF as well as varying climatic and environmental conditions such as rainfall, temperature, solid drainage and presence of and/or proximity to permanent or temporal water bodies which favour extensive breeding of the mosquito vectors [83].

Nearly 90% of the population engages in subsistence farming. Smallholder farmers produce a variety of crops, including maize, beans, rice, cassava, tobacco, and groundnuts. Rice-

growing areas are relatively wet and support more breeding of the mosquito vectors of disease and more intense transmission of parasites [80].

2.5.3.2 Lymphatic filariasis control programme in Malawi

In 1997 the World Health Assembly (WHA) passed a resolution to eliminate lymphatic filariasis as a public health problem [28]. Following this resolution the Global Programme for Elimination of Lymphatic Filariasis (GPELF) with the overall goal of eliminating LF by the year 2020 was launched in the year 2000 and the strategy it adopted was to interrupt transmission of the parasite by its vectors through mass drug administration (MDA) once yearly for at least 4-6 years and to manage and prevent LF-related disabilities [25].

The stages of any national LF elimination programmes involve an initial assessment and mapping of the distribution of infection followed by MDA rounds and disability management and prevention [77]. Malawi completed mapping for LF in 2003 in a nationwide survey mentioned above [78]. LF is co-endemic in Malawi with onchocerciasis in 8 districts in the southern region namely Blantyre, Chikwawa, Chiradzulu, Mulanje, Mwanza, Neno, Phalombe and Thyolo [68]. This mapping was followed by the formation of the Malawi National LF Elimination Programme in 2008 and Malawi qualified for a free donation of ivermectin and albendazole from Merck and Co. and Glaxo Smith Kline respectively for its MDA programme. MDA began in 2008 in the 8 LF and onchocerciasis co-endemic districts in the southern region [2] where the national onchocerciasis control programme (NOCP) had been operating since 1997 and had well established structures [79].

Scaling up of MDA to all the 27 LF endemic districts in Malawi followed in 2009 and thus far two annual country-wide MDA rounds have been completed. Community drug distributors (CDDs) and health surveillance assistants (HSAs) (section 3.6.1) under the supervision of district LF coordinators deliver MDA in the communities. The CDDs and HSAs are placed at health centres and health posts and at other specific places within the communities where the drugs are distributed. Training is done by trainers of trainers who are themselves trained at central level. These train district trainers responsible for cascading training to health facility level staff and health surveillance assistants who in turn train the CDDs at community level.

The MDA programme and other LF control activities in border districts of Malawi need to take into account LF control activities underway in the neighbouring countries as there is extensive cross-border movement of people and unless efforts are made to integrate or synchronise LF control activities, there is great potential of undermining LF control activities in Malawi and its neighbouring countries [78].

The vectors for LF transmission in Malawi are not well known [84]. A study on the role of local mosquito species as LF vectors done in 2002 in southern Malawi showed that the potential vectors of LF in Malawi are *Anopheles funestus, Anopheles gambiae s.s* and *Anopheles arabiensis* [85]. These same vectors are also responsible for malaria transmission in the country [2]. Currently there are no vector control programmes specific for LF in Malawi but malaria vector control interventions such as bed net distribution and indoor residual spraying (IRS) (sections 2.5.6.2 and 2.5.6.3) are currently underway through the malaria control programme. These are likely going to play a significant role in LF elimination in the country.

The Malawi National LF Elimination Programme keeps track and monitors progress of the MDA programme in the country. MDA is monitored in the country by measuring the geographical coverage i.e. proportion of target areas covered by the programme and population coverage as well as proportion of the target population that received the drugs during each round of MDA. This is achieved by use of special registers and forms that record this information during each round of MDA. In the community, the reports are completed by the CDDs who submit their reports to district LF coordinators who in turn submit their reports to the national LF coordinator.

Supervisors also make random field visits to monitor coverage and the quality of data recording during each round of MDA. After each round of MDA, the supervisors audit the reported coverage and identify obvious signs of error [84].

The National LF Elimination Programme also states that it will conduct periodic assessment of the impact of MDA on the population through review meetings at various levels, independent validation of reported coverage through random sampling of household surveys and operational research [84].

2.5.3.3 Perceptions of lymphatic filariasis in Malawi

LF is a disfiguring and debilitating disease not well understood in many societies and often carries considerable stigma. It is sometimes believed that the condition is the result of witchcraft or as a penalty for sins committed and other events that can victimise the affected persons [77].

Misconceptions about the cause and/or transmission of the disease are also common in Malawi. A baseline survey of malaria, LF and other neglected tropical diseases in southern Malawi reported various misconceptions in over 75% of the respondents where sexual intercourse with a menstruating woman, bad weather and HIV/AIDS were among the common perceived causes of the disease [79].

Such kind of misconceptions and local perceptions of the cause and/or transmission of LF are key to control interventions of the disease since they are the basis for treatment seeking behaviour and community participation in the control strategies such as MDA. These disease control interventions are most effective if they take a multidisciplinary approach and incorporate these lay knowledge and perceptions [86].

2.5.4 HIV and HIV control in Malawi

The prevalence of HIV among adults aged 15 to 64 years in Malawi was estimated at 9.2% in 2016 and ranks among the ten countries with the highest rates in the world. An estimated one million people were infected with HIV in 2016 and 10% of them were children under the age of 15 years [87]. In the same year, there were an estimated 36,000 new HIV infections and 24,000 AIDS-related deaths in the country [88]. Co-infections with a range of opportunistic infections, including tuberculosis, are more common in individuals infected with HIV largely because HIV infection compromises the immune system and the individual becomes vulnerable to multiple infections [89]. HIV infection may alter the natural history of concurrent infections in an unfavourable way, impede diagnosis and response to treatment of concurrent infections while concurrent infections may facilitate HIV transmission as well as HIV disease progression [90].

The Government of Malawi has responded to the HIV/AIDS epidemic by initiating a number of policies and intervention strategies on prevention, treatment, care and support such as behaviour change intervention for HIV/AIDS and sexual reproductive health, voluntary medical male circumcision (VMMC), HIV testing and counselling (HTC), prevention of mother to child transmission (PMTCT) and antiretroviral therapy (ART) scaling up [87].

HTC services are provided through client-initiated testing and counselling (CITC), also known as voluntary counselling and testing (VCT), and provider-initiated counselling and testing (PITC). These HTC services have increased over the years in Malawi. VCT services started in 1985 using ELISA testing and HTC using whole blood rapid testing was initiated by Malawi AIDS Counselling and Resource Organization (MACRO) in 2001 and was adopted by MOH in 2003 [91]. By 2008, HTC was provided through a combination of PITC services focused on ANC, TB patients and STI patients, static, mobile and outreach sites, as well as home-based door-to-door testing and HTC national campaign events.

PMTCT services have also significantly expanded since July 2011 when Malawi became the first country to implement the Option B+ programme in which all pregnant women diagnosed with HIV are offered antiretroviral treatment for life irrespective of their CD4 count or WHO clinical stage [92].

ART was rolled out in Malawi in 2004 and by the end of 2007, 145,000 patients had been registered for therapy at 109 ART treatment facilities in the public sector and 45 in the private sector countrywide [93]. Malawi's ART programme has been successful managing to put 68% of HIV-infected Malawians on treatment by 2016 [88], with the dual impact of improved health of those that are already infected and of prevention of onward transmission through decreased onward transmission of HIV [94]. ART clinics also provide diagnosis, treatment and prevention service for common opportunistic infections.

These strategies and plans have assisted the country to achieve a slight decline and stabilisation in HIV prevalence from around 16.2% in 1999 to around 10.6% in 2010 and 9.2% in 2016. Malawi is also on track to achieve the UNAIDS 90-90-90 targets by 2020, which include 90% of people with HIV knowing their status, 90% of these accessing ARVS and 90% of those on treatment being virally suppressed [92].

ART is delivered by both the public and private sector at ART clinics situated in hospitals (central, district, mission, and rural), health centres or other stand-alone sites [93]. By December 2014, there were 706 static ART sites in Malawi owned by government, mission, NGOs and the private sector [95]. The minimum staff required per ART clinic is one clinician, one nurse and one clerk. Medical officers, clinical officers, medical assistants and nurses initiate and prescribe ARV drugs in the ART clinics and are required to have attended a pre-service ART training course recognised by the MOH [93].

The Malawi Ministry of Health has developed new guidelines for clinical management of HIV in children and adults following new WHO recommendations for ART and PMTCT in resource limited countries and have been implemented since May 2016 [96]. According to these new HIV treatment guidelines, all HIV positive children and adults are started on ART regardless of CD4 count or clinical stage (universal coverage).

There are five different standard ART first line regimens for use in Malawi. The regimens are numbered 0 – 6 for ease of reference as below. Fixed dose combinations (FDC) are shown with a slash sign (e.g. AZT **/** 3TC **/** NVP) while combinations made up of different tablets are shown with a plus sign (e.g. AZT/3TC **+** EFV). There are also five different second line ART regimens, numbered 7 – 11, in Malawi and these are used for patients who have confirmed treatment failure on a first line regimen. The appropriate second line regimen is determined by the 1st line regimen that the patient was taking when failing. A third line regimen is available as a last resort for patients failing on the second line in spite of good adherence and can only be initiated by a specialised ARV clinician upon authorisation of a review committee [96].

2.5.5 Co-endemicity and shared risk factors for HIV, malaria and LF in Malawi

In Malawi, there is overlap between LF and HIV in the whole country. While HIV prevalence is highest in the southern parts of the country it is still high in the adult population in the north of the country [97]. As already described in section 2.5.2.1 above LF is most common in low lying areas that are close to water bodies including the Songwe river area of Karonga in the north and lower Shire valley are of Chikwawa in the south [29]. Malaria is endemic across two thirds of Malawi and Karonga is one of the more highly endemic parts of the country for malaria. Both malaria and LF are more common in rural areas of the country with HIV being more common in the urban areas [97].

There are actual and theoretical shared risk factors for the three conditions in Malawi. Firstly, LF and malaria are other transmitted by night biting vectors so failure to sleep under a bed net, proximity to water bodies and poor housing conditions increase night biting risks. Poverty, social deprivation and educational levels have all been cited as increased risks for the three conditions which area associated with poorer knowledge of transmission and prevention of infectious diseases and poor access to prevention and treatment services in Malawi.

2.5.6 Coverage and uptake of LF, malaria and HIV control measures

This section will review coverage and uptake of control measures. It focuses on HIV, LF and malaria control efforts since HIV LF co-infection is the subject of your enquiry and since malaria control is likely to impact LF control through targeting night biting mosquito vectors and is therefore an important confounder in this thesis. The section focuses specifically on the coverage and update of control measures in Malawi, highlighting factors that have influenced coverage and uptake that are of particular relevance to co-infection.

2.5.6.1 Coverage and uptake of HIV services

Despite the successful ART programme and scale-up, the scope of the HIV/AIDS epidemic in Malawi has placed enormous challenges on the health care system due to its limited capacity with more than 50% of the hospital beds in Malawi being occupied by patients with HIV-related illnesses. This has increased demands and workload on the already poorly staffed and inadequately resourced health care system of Malawi [98]. At the time of the study, only just over half of the adults aged 15-49 knew their HIV status with men, young people, people in hard to reach areas and key populations being underserved [99]. Inequities in access to treatment and significant loss to follow up from ART services were also noted as major programmatic challenges. While supply side factors (service delivery) such as limited resources have limited the expansion of testing and treatment services, demand side factors

(at individual and community level) such as poor access to services, stigma, masculinity and cultural norms also play a role in creating these gaps in HIV control efforts.

2.5.6.2 Scale up of mass drug administration in Malawi

MDA to eliminate LF in Malawi started in eight districts in the southern region namely Blantyre, Chikwawa, Chiradzulu, Mulanje, Mwanza, Neno, Phalombe and Thyolo in 2008 and was scaled up in 2009 to all the 27 LF endemic districts in Malawi to reach 100% national geographic coverage for the target population of 13 million people at risk [100]. Since then until 2014, six consecutive rounds of MDA have been administered and treatment coverage remained over 80% for each MDA round. Currently Malawi is conducting transmission assessment surveys (TAS) that are required to determine if LF transmission has been interrupted. The first TAS was conducted in 2014 in 11 evaluation units (EUs) following WHO guidelines and the LF infection rate for each EU was below the critical cut-off level required to stop MDA. The NTD Regional Programme Review Group (RPRG) for the WHO African Region reviewed and approved the TAS results in 2015 indicating that MDA could be stopped in Malawi [100].

2.5.6.3 Scale-up of insecticide treated nets

Since 2005 Malawi has scaled up malaria control measures focusing on vector control, case management and protection of vulnerable groups including pregnant women and infants. Current vector control intervention strategies being implemented by the national malaria control programme include distribution and use of ITNs and indoor residual spraying (IRS).

The ITN policy involves usage and free distribution of ITNs. Free ITNs are distributed countrywide to all newborn children born in health facilities, children attending their first vaccination (if it was not received at birth) and pregnant women attending their first antenatal visit. National free distribution campaigns of ITNs targeting pregnant women and under-five children are also conducted every 2-3 years.

According to the report of the Malawi National Malaria Indicator survey conducted in March and April 2010, over 5 million nets have been distributed in Malawi in the two years preceding the survey. Nearly 60% of households had at least one ITN while 55.4% of children under the age of five years and 49.4% of pregnant women slept under an ITN the night before the survey [101].

2.5.6.4 Coverage of indoor residual spraying

IRS is the latest malaria vector control programme to be adopted by the national malaria control programme. It involves application of a long-acting insecticide on the walls of all houses in order to kill the mosquito vectors that land and rest on these surfaces. The insecticide of

choice in Malawi is ICON-CS and is one of the recommended insecticides by WHO. IRS was initiated in 2007 as a pilot in the lakeshore district of Nkhotakota in the Central Region and in December 2010 MOH expanded IRS to seven high malaria prevalence districts along the lakeshore and in the Shire valley, including Karonga district and covering a population of 2.7 million people. However, the programme was discontinued between 2011 and 2012 due to logistical problems including evidence of emerging insecticide resistance until 2013 when it was resumed [29].

2.5.5.6 Relevance of LF, malaria and HIV disease control programmes to coinfection

The three control programmes for LF, malaria and HIV are of relevance to co-infection prevention and clinical management. The overlap of LF and malaria control are of particular relevance as vector control can impact on both conditions simultaneously. Stanton et al in 2014 developed a multiple intervention score map for Malawi that quantified malaria and LF control activities and mapped them to areas of the country [29]. The district score maps indicated areas that have received high and low coverage of LF-impacting interventions. High coverage areas included the LF-onchocerciasis endemic areas in the southern region of the country and areas along the shores of Lake Malawi, where malaria vector control had been prioritized. This kind of combined mapping allowed the team to prioritize three districts with high baseline LF prevalence measures but low coverage of multiple interventions as these were most at risk of ongoing transmission or re-emergence. HIV control efforts are also relevant as these vertically funded programmes have brought additional resources, health systems strengthening and clinical expertise into previously underserved districts. All three control programmes are designed as vertical programmes at national level yet work through community health workers or community health distribution agents, creating platforms for integration at the lowest level of the health system in Malawi [39].

2.6 Summary and study rationale

This chapter has reviewed available literature on LF and HIV and other significant co-infections putting them into a Malawian context. Available evidence adds further justification to the need for a study on LF and HIV co-infection in Malawi, first outlined in section 1.2 of the introductory chapter. At the outset of this study there was no clear evidence of an association or relationship between HIV and LF (equipoise). The expectation was that HIV might disrupt LF control based on the assumption that an individual requires an intact immune system to clear LF infection when MDA is given. A poorer response to antihelminthics could therefore be expected in immunosuppressed HIV positive individuals. This expected impact would then have public health implications for the dose, duration and scheduling of MDA in areas of high

HIV prevalence. Before major changes to LF programmes evidence was needed that interrogated the association between LF and HIV, including the ability to clear LF over time.

Chapter 3 Methodology

This chapter presents the research methodology that was used to conduct the study. The chapter first describes the Karonga Prevention Study (KPS) in Karonga, northern Malawi. It then outlines the relevant Karonga Prevention Study studies within which this thesis is nested, including their linkage to each other. For the study to make sense, the timelines of the various interventions related to LF control in the district are summarised. The chapter then gives a detailed description of the methods for each of the filarial research studies that make up this thesis. For each the study design, characteristics and selection of the populations studied, description of the information collected from the participants, the variables used, and sample size considerations are described. Common methods across all the studies are presented separately and these include a description of laboratory methods, data handling and management, statistical analyses, mapping methods and analyses and ethical considerations.

3.1 Background to Karonga and the Karonga Prevention Study

3.1.1 Karonga Prevention Study

The research programme in Karonga district was started in 1979 with support from the British Leprosy Relief Association (LEPRA) to study the incidence and risk factors of leprosy in Karonga district [102, 103]. Following a pilot leprosy survey in 1979, a total population leprosy survey known as the Lepra Evaluation Project (LEP) was conducted from 1980 to 1984 as a large cohort of leprosy covering the entire district of Karonga with a population of 112,000 people. A second total population leprosy survey was conducted from 1986 to 1989 to measure leprosy incidence and to recruit for a large double-blind, randomised, controlled vaccine trial comparing the efficacy of a single BCG dose, repeat BCG dose and a WHO-sponsored combined BCG plus killed *M. leprae* vaccine in protecting against both leprosy and tuberculosis (TB) [104]. The vaccine trial became known as the Karonga Prevention Trial (KPT) while the combined epidemiological studies (LEP) and the vaccine trial (KPT) became known as the Karonga Prevention Study (KPS). The programme assumed responsibility for tuberculosis diagnosis and treatment in the district from 1984 onwards and in 1987 began studies of HIV. Annual trial follow ups for leprosy and TB and case control studies of HIV as a risk factor for leprosy and TB were conducted from 1990 to 1996.

The trial follow up results led to two five-year Wellcome Trust supported programmes. The first programme from 1996 to 2001 involved case control genetic studies on leprosy and TB, controlled trials comparing BCG efficacy in adolescents and young adults between Malawi and the UK and a retrospective cohort study of HIV-infected individuals [105, 106]. The second Wellcome Trust programme from 2001 to 2006 followed this and had three main themes:

immune responses to BCG vaccination, epidemiology of *M tuberculosis* infection and disease and the demographic impact of HIV. In this programme, a cohort of infants was recruited to examine responses to BCG, the TB case control study continued to examine the evolving relationship between TB and HIV in adults, the TB molecular epidemiology studies were extended and a demographic surveillance system of 33,000 individuals was initiated in the southern part of the district [103].

A third Wellcome Trust funded programme from 2006 to 2011 continued the TB epidemiology and immunology studies adding pneumococcal and filariasis research studies. HIV became the central focus of the programme with studies on HIV epidemiology and its interaction with other major infectious diseases and the mitigating effects of antiretroviral therapy.

Research work on filariasis began in the mid-90s with community prevalence surveys identifying Karonga district as one of the high prevalence regions of Malawi. Several KPS studies with available data and stored blood samples for nested observational studies on filariasis were available and three key on-going studies at KPS were suitable for filariasis research since they had robust datasets and available stored blood samples with consent from participants and ethical approval for retesting. These were the Karonga Health and Demographic Surveillance System (KHDSS) and the Filariasis Elimination Dosage Study (also called the Songwe Filariasis Clinical Trial due to its location). These are outlined in sections 3.1.2, 3.2 and 3.3 respectively.

3.1.2 The Karonga Health and Demographic Surveillance System and HIV sero-surveys

In 2002, the KHDSS was established to provide cross-sectional estimates of population size and structure, and to provide a sampling frame for epidemiological and immunological studies [103]. The KHDSS population was 35,730 in 2011 and covers an area of 135km^2 in the southern part of the district (Figure 1). The surveillance system collects data on vital events and migration for individuals and households, cause-specific mortality for all ages, vaccine coverage and socio-economic status data.

Between 2007 and 2011, the KHDSS was combined with annual household HIV sero-surveys (HSS). All households including approximately 16,000 individuals over the age of 15, underwent an annual census visit following which all consenting adult members aged 15 or over underwent a rapid HIV antibody test. Results were provided at their home. This process was repeated on an annual basis over a four-year period. Consenting adults provided a 5ml blood sample for rapid testing for HIV using the standardised national serial testing algorithm (where all reactive results were confirmed by a second rapid test), pre- and post-test

counselling protocol [107, 108]. The venous blood sample collected was also used for quality control and further laboratory analysis including HIV drug resistance testing Remaining blood samples after HIV testing were stored in the KPS laboratory for later testing for other diseases related to HIV or of importance in Karonga district.

As part of the demographic surveillance system, information on socio-economic status of individuals and households was collected using a structured questionnaire (See Appendix 1: Individual Socio-Economic Survey Form). The questionnaire enquired about education, occupation, vaccine, bed net ownership and MDA history.

The KHDSS is located in the south where the HSS was conducted and the Songwe Filariasis Clinical Trial took place in the north of the district (see Figure 1). Thus, these studies were geographically separate. Each study collected GPS coordinates and unique identifiers meaning that individuals who appear in more than one dataset can be linked. This is particularly relevant to the large overlapping KHDSS and HSS datasets where HIV status can be linked to behavioural and intervention variables such as MDA and bed net ownership. Not all individuals who participated in the KHDSS annual census also consented to participate in the HSS. This makes the HSS a subset of the KHDSS.

3.1.3 Timelines of relevant public health interventions and relationship to overall KPS timelines

Between 2004 and 2012 there have been a number of district wide interventions in an effort to control endemic diseases. These include vector borne diseases such as malaria, soil transmitted helminths (STH), TB and HIV control among others. Filariasis is a vector borne disease treated by the same regiments as soil-transmitted helminths and affected by antibiotics and treatment interventions related to malaria, STH and TB control. These are described in the text below and summarised in Figure 5. Details of HIV prevention and control and other illnesses are not covered in this chapter.

Since 2007, ***free insecticide treated bed nets*** (ITNs) are distributed at health facilities to children born in health facilities, children attending their first immunisation visit if not received at birth and pregnant women attending their first antenatal care visit through the national malaria control programme [109].

Indoor residual spraying (IRS) is one of the primary preventive strategies for malaria against the vector mosquitoes. It involves application of a long-acting insecticide on inside walls of houses and shelters to kill vector mosquitoes when they land and rest on the sprayed surfaces. Following a successful pilot of IRS in Nkhotakota district in 2007, the Malawi Ministry of Health

scaled up IRS in 2011 to seven highly endemic districts along the lakeshore including Karonga and in the Shire valley.

Six annual MDA rounds as part of the Malawi LF elimination programme have been undertaken in Karonga district. The first MDA, using a combination of albendazole and ivermectin was done between November and December 2009. The second MDA round was conducted between November and December 2010, the third MDA between December 2011 and January 2012, the fourth in November 2012, the fifth in November 2013 and the sixth in August 2014. Access to antihelminthic drugs in Malawi is through STH control activities which is part of the national school health and nutrition programme, launched in 2007. In this programme, albendazole is given annually to all school age children in all schools in Malawi.

The first round of HSS fell between September 2007 and September 2008, round two between October 2008 and September 2009, round three between October 2009 and October 2010 while the fourth round fell between November 2010 and December 2011. The Songwe clinical trial was conducted from January 2009 to March 2012.

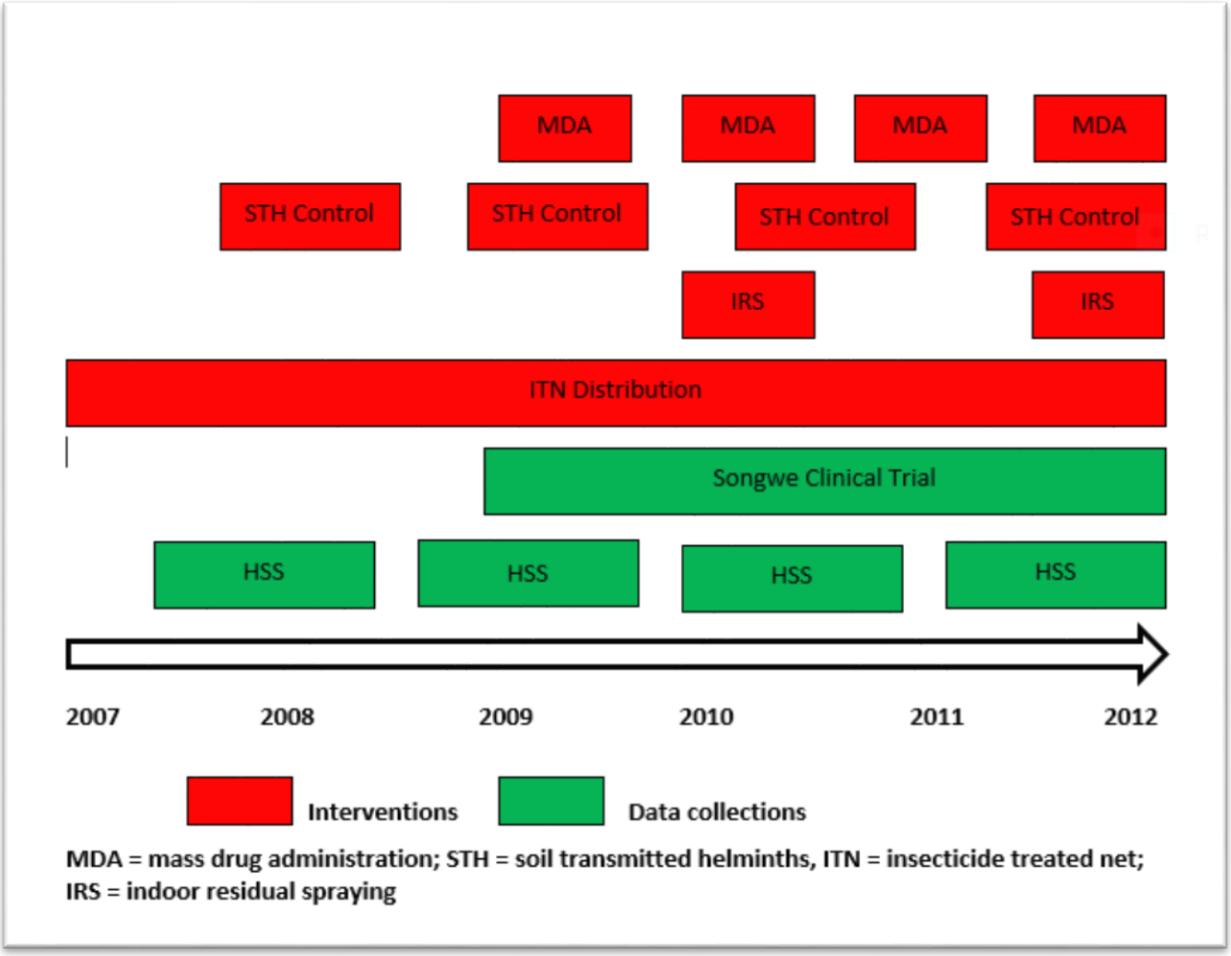


Figure 5: Timelines of Interventions and Data Collections

3.2 Filarial Research Study 1: Assessment of Relationship of HIV and Markers of Lymphatic Filariasis Infection

3.2.1 Study design and links to parent studies

This cross-sectional study used available stored serum and data from the two geographically separate studies. It aimed to investigate the relationship between HIV and circulating filarial antigenaemia (CFA) and microfilaraemia. The overall population of Karonga district during the study period was 272,789 [110] with the KHDSS population accounting for 33,500. HIV prevalence in the KHDSS in the 2007–2008 baseline HIV survey was measured at 7.4% but estimated to be 10.4% when adjusted for non-testing by those who already knew they were HIV-positive [99]. In the baseline survey, 54.8% of those aged 15 years or more reported previous HIV testing.

3.2.2 Data and samples from the Songwe Clinical Trial

The Songwe Filariasis Clinical Trial was conducted in Songwe area in the northern part of Karonga district. More details on this study may be found in section 3.3 as the full study also forms part of the filariasis research (Filarial Research Study 2) in this thesis. Study participants included in this part of the assessment of qualitative CFA and HIV were residents of the Songwe area who participated in the screening phase of the Songwe clinical trial. The study participants were screened for CFA by the ICT card test and HIV infection. HIV testing procedures were done as in the KHDSS HIV sero-survey (section 3.2.3). In addition, all individuals with baseline microfilarial counts who had stored plasma samples available for retesting were included in the assessment of microfilaraemia and quantitative CFA and HIV (see Figure 6).

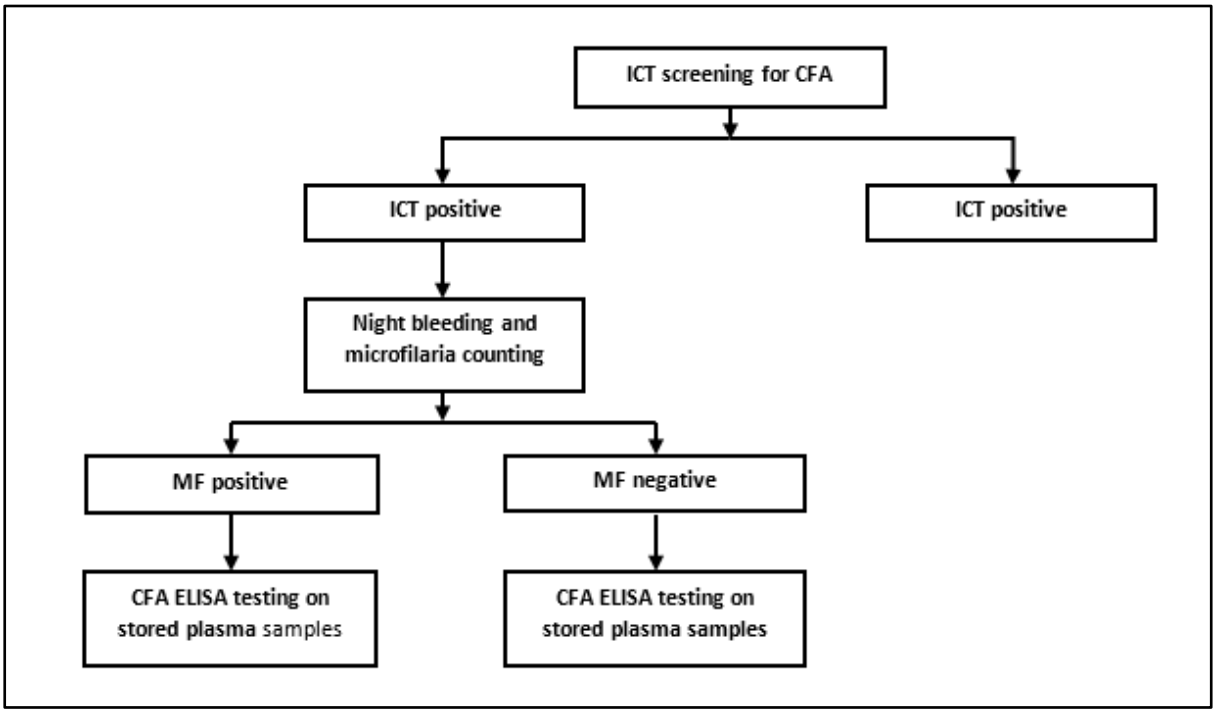


Figure 6: Testing Algorithm for Circulating Filarial Antigen and Microfilarial Counting

Qualitative CFA, HIV and baseline microfilarial counts from Songwe clinical trial participants were available from the KPS database and were linked with socio-demographic characteristics from a structured questionnaire by unique study identifiers. Stored plasma samples from night blood specimens used for microfilarial counting were available from the KPS laboratory archive and were reassessed for LF antigen detection and quantification using the Og4C3 ELISA (see section 3.6.4 below).

312 individuals (29 HIV-infected and 283 HIV-uninfected) had stored baseline night blood samples. A ratio of 29 HIV-infected and 87 HIV-uninfected individuals was expected to detect a 20% difference or greater in mean CFA titre at significance of 1% and power of 88% assuming a geometric mean titre of 2000 CFA units (SD 500) in the HIV-uninfected.

3.2.3 Data and samples from the KHDSS

Data and samples from a comprehensive population-based HIV sero-survey nested within the Karonga Health and Demographic Surveillance Site (KHDSS) were used to further explore the relationship between LF and HIV [13]. Since its establishment, the initial population of the KHDSS has been under continuous demographic surveillance. In addition, between September 2007 and October 2011 four annual HIV sero-surveys were conducted in all individuals aged 15 or more years using rapid point-of-care HIV tests on finger-prick whole blood samples [14]. Samples from the first surveillance round, which took place between September 2007 and October 2008, prior to MDA introduction, were included in this study.

Community sensitisation meetings to explain the aims and procedures of the study were held in each village and were followed by house-to-house visits by counsellors to recruit participants. The counsellors were trained and certified by Malawi Ministry of Health staff to perform HIV counselling and testing and referral using standard procedures [15]. At each home visit, the study was introduced and explained to all eligible participants and written informed consent was requested (See Appendix 2 – HIV Sero-survey Consent form). All consenting adults were offered HIV pre-test counselling and asked to provide a 5ml blood sample for immediate HIV testing at the home, quality control and storage for further laboratory analysis including for other diseases of importance in Karonga district. All participants who wanted to know their test results were post-test counselled and informed of their results at the home, with referral to local clinical services if found to be HIV positive. Plasma samples from consenting participants were stored in the project laboratory archive at -20°C and were assessed for LF antigen detection and quantification using the Og4C3 ELISA (see section 3.6.4 below). Viral load, CD4 counts and clinical staging were not measured as a part of the survey.

3.3 Filarial Research Study 2: Songwe Filariasis Clinical Trial

The filariasis elimination dosage study (FED) also called the Songwe Filariasis Clinical Trial was a clinical trial undertaken in the northern part of Karonga district in Songwe area (see Figure 1) along the Tanzania border, 110km north of the KHDSS site [111].

This was a randomised, controlled, four-arm, open-label, clinical trial. The study took place from January 2009 to March 2012 in all 16 villages in a pre-defined study area close to the Songwe River in the north of Karonga District of northern Malawi. This area was known to be highly endemic for *W. bancrofti* infection and no mass treatment intervention had been undertaken prior to commencement of this study. The trial was registered with Clinical trial.gov, number NCT01213576.

3.3.1 Study participants

Eligible participants were all adults aged between 18 and 55 years living within the study area who understood and signed the informed consent (Appendix 3 – Consent Form for Lymphatic Filariasis Study), were willing to undergo night time blood drawing every 6 months for 2 years, had haemoglobin levels ≥9 g/dL and microfilarial levels >80 mf/ml. Exclusion criteria were pregnancy, lactation and history of taking albendazole or ivermectin for any reason within the previous 6 months or a known allergy to either drug.

3.3.2 Study procedures

At the initiation of the study, field study personnel explained procedures at village sensitisation meetings. Recruitment was then done through house-to-house visits. Consenting, eligible individuals were initially screened for circulating filarial antigenaemia (CFA) using the Immunochromatographic (ICT) card test (Binax, Portland, ME) [112]. The ICT card test (Figure 7) was administered according to the manufacturer's instructions with measurements read at 10 minutes and if two lines were visible in the viewing window that individual was recorded positive for CFA on the ICT result form (Appendix 4 – ICT Result Sheet). Individuals whose samples produced faint lines were recorded as weakly positive and deemed as positive individuals for subsequent investigations. Participants were informed of their test results at the home and individuals who were CFA positive but declined to participate in the clinical trial or did not meet the eligibility criteria for the trial were offered standard dose antifilarial therapy with albendazole and ivermectin.

Individuals who were positive for CFA by the ICT card test and consented to provide a night blood sample were evaluated with a brief medical history and physical examination and visited at their households between 22:00 and 02:00 hours for collection of two venous 'night blood' samples. One EDTA sample was used for full blood count and the other, a sodium citrate sample, was used for microfilaria measurement by the Nucleopore membrane filtration technique [113] expressed as density of microfilariae (mf) per ml of blood.

3.3.3 Randomisation

Participants who fulfilled the eligibility criteria underwent further informed consent and were assigned to study treatment groups using a computer-generated randomisation list. An independent investigator pre-generated the list which had an allocation ratio of 1:1:1:1 and restricted block sizes of 4. Study field personnel enrolled and allocated participants to the treatment groups using allocation letters on the randomisation list consecutively for each individual recruited into the study. The study field personnel kept the allocation list in a field site locker and no blinding was employed since the trial design was open-label.

3.3.4 Interventions

Eligible participants were randomly allocated to the four treatment study arms (see Figure 15): the standard regimen of single dose albendazole (400 mg) and ivermectin (200 mg/kg) once-yearly (control group [SDA]), a twice-yearly standard regimen (SDT), a high-dose regimen of albendazole (800 mg) and ivermectin (400 mg/kg) given either once-yearly (HDA) and the same given twice-yearly for a period of 2 years (HDT).

Study drugs were taken orally under direct observation by field staff to ensure compliance and to record any immediate side effects in the 30 minutes post-ingestion. The study team made scheduled visits once a day for 7 days to a pre-defined place within the village to review any individual who reported adverse events post-treatment. Adverse events were self-reported and assessed according to pre-defined clinical criteria. These were recorded on an adverse event form and, if considered severe or life threatening, was referred appropriately to the nearest health facility.

3.3.5 Follow up

Participants were followed-up every 6 months for 2 years, underwent clinical assessment, venepuncture for full blood count and night blood microfilarial counting and received subsequent study drug administration as appropriate. In addition to the clinical assessment done at each follow up visit, information was collected on filarial related clinical manifestations such as fever, lymphoedema, lymphangitis, hydrocoele and skin rash (Appendix 5 – Filariasis Dosage Study Follow up Form). Individuals with missed appointments remained in the study until 24 months and were actively traced at each follow up appointment.

3.3.6 Sample size

The primary study endpoint was a difference in the clearance rate of *Wuchereria bancrofti* microfilaraemia, expressed as microfilariae per ml of blood (mf/ml), at 12 months. Based on data comparing single and multi-dose regimens for the treatment of lymphatic filariasis [9,

114], the study assumed that the standard annual therapy would clear microfilaraemia in approximately 25% of subjects at 1 year whereas multi-dose therapy should give 75% clearance. The sample size needed to detect this difference by Fisher's exact test with two-sided alpha level of 0.05 and power equal to 80% is 20 per group. Taking 15% attrition at each visit into account, the study planned to recruit 30 subjects per each study arm.

3.3.7 Data management and statistical analyses

Details of the overall statistical methods can be found in section 3.6. For this study participant information was collected onto paper record forms. Forms were transported to the project headquarters in Chilumba, Karonga district, where they were checked, coded, double entered and verified using Microsoft Access 2007 software. All statistical analyses were done using STATA 12.0 (Stata Corporation, USA). The difference in clearance proportions between the intervention treatment arms and the comparison group was examined using the Fisher's exact test and the log rank test where appropriate.

The analysis used the last observation carried forward and intention-to-treat approaches to deal with participants who did not take all the intended treatments during the study.

3.3.8 Ethical Approval

The study was registered with ClinicalTrials.gov (NCT01213576) and approved by the National Health Sciences Research Committee of the Malawi Ministry of Health (protocol number 495) and the Ethical Committee of the London School of Hygiene and Tropical Medicine (protocol number 5344). Community permission to undertake the study in the area was sought from village and community leaders.

3.4 Filarial Research Study 3: The impact of MDA on circulating filarial antigenaemia by HIV status and ITN ownership

This study set out to investigate the impact of MDA on circulating filarial antigenaemia (CFA) by HIV status and bed net ownership using available stored follow up blood samples and data collected as part of the whole adult population HIV sero-surveys that were undertaken in the Karonga Health and Demographic Surveillance Site (KHDSS).

3.4.1 Study design and links to parent studies

This was a longitudinal assessment of the impact of MDA on circulating filarial antigenaemia by HIV status and ITN ownership that was nested within a comprehensive population-based HIV sero-survey. Repeated rounds of data and sample collection spanned the time periods before and after the introduction of MDA. Baseline samples were taken from HSS round 1 (September 2007 to October 2008) as this pre-dated MDA and follow up samples were taken

from HSS rounds 3 and 4 (November 2009 to October 2011) covering the period 2-3 years after MDA had reached all areas. Round 3 samples were preferred but if unavailable then round 4 was investigated and if samples on that individual were available these were used instead. Individuals HIV profile was obtained from the HSS dataset while baseline demographic data, MDA usage, number and timing of doses and household bed net ownership were derived from a self-reported questionnaire and were linked with the test results via unique study identifiers.

3.4.2 Study participants and selection of control samples

Study participants were members of the KHDSS who participated in the annual census and HIV sero-survey and provided an EDTA blood sample for storage and further testing in the KPS laboratory archive. All individuals with samples from HSS round one were included in the baseline assessment.

Follow up assessments included all individuals who tested positive for CFA in the baseline assessment and had stored plasma samples available for retesting. HIV sero-converters were excluded from the analysis.

In addition to this primary cohort, a randomly selected control group of individuals who were negative for LF antigen at baseline was established (LF negative group) and these were assessed in subsequent samples to investigate natural acquisition of LF antigenaemia during a period of MDA administration. To limit the size and cost of the LF negative group, participants were selected by frequency matching on age with the LF positive group in a 2:1 ratio. The age groups used for frequency matching were 15-40 & >40 years of age. The selection was done in a random way by assigning a number to each LF negative participant and listing in a random number generator. The selection was made in sequence until the required number of LF negatives was achieved. To avoid selection bias, the identified individuals were included whether they were known to have a follow up EDTA sample or not in the same way that LF positive individuals were identified.

3.4.3 Study Procedures

Plasma samples derived from EDTA blood were selected for assessment of CFA. Follow up samples were selected first from round 3. If no follow up samples were identified in round 3 then round 4 was checked to see if there was an available sample from the same participant.

3.4.4 Sample size assumptions

With an estimated LF prevalence of 10% and HIV prevalence of 10% and assuming low levels of ART use in this population, the study expected to have 80 HIV and LF co-infected individuals

and 720 HIV-uninfected and LF-positive individuals after screening all individuals who participated in the HSS round 1 and with baseline samples stored in the KPS laboratory (approximately 8000 individuals).

Table 1: Estimates of power to show given effects based on available sample size

Expected Difference in proportion Ag^+ post-MDA	Expected proportion Ag^+ in HIV- post-MDA		
	50%	60%	70%
10%	34.9	35.9	40.3
15%	68.8	72.3	81.4
20%	92.3	95.3	99.1
25%	99.3	100	100

Assuming post-MDA LF antigenaemia proportion of 0.5 in the HIV-uninfected individuals, the study expected to demonstrate a 15% difference in antigenaemia with 69% power (Table 1).

Primary outcome:

Difference in CF titre between pre-MDA and post-MDA LF antigenaemia in HIV-infected and HIV-uninfected individuals

Secondary outcomes:

1. Association of HSS round 1 LF antigenaemia with key predictors: age, gender, HIV, bed net usage and location of residence.
2. Measurement of the acquisition of LF antigenaemia in LF negative control group.
3. Cross-sectional prevalence of LF and HIV co-infections and LF antigenaemia by ITN use.

3.4.5 Data management and statistical analysis

Details of the overall statistical methods can be found in section 3.6. For this study sub analysis adjusted for number of MDA treatments and adjusted for potential confounders such as age, gender, location of residence and bed net usage.

3.5 Laboratory Methods

These included the ICT card test, microfilarial counting and HIV testing.

3.5.1 Immunochromatographic (ICT) Card Test

The ICT card test (Figure 7) is a commercially available assay for detecting filarial antigen manufactured by Binax (Maine, USA). It was used as the screening test for LF infection in the

Songwe filariasis clinical trial. It is a portable, rapid, point-of-care test suitable for LF screening in non-laboratory settings at any time of the day and was used in accordance with manufacturer's instructions. In brief, a 100 µL finger prick blood sample was added to the sample application pad and the card was closed. The test result was read after 10 minutes and if two lines were visible in the viewing window that individual was recorded positive for CFA. Individuals whose samples produced faint lines were recorded as weakly positive and deemed as positive individuals for subsequent investigations. The test result was read independently by a field worker and was verified by a second field worker who served as a field supervisor.

Figure 7: The ICT Card Test for Lymphatic Filariasis

3.5.2 Microfilaria counting

Microfilaria counting on night blood samples from the Songwe filariasis clinical trial was done by the Nucleopore membrane filtration technique. In brief, this involved filtering a measured volume of venous blood through a 5µM pore size Nucleopore filter. After filtration, the filter was removed, placed on a glass slide and mounted on a light microscope for examination and counting of microfilariae.

3.5.3 HIV testing

In all the studies, HIV testing used whole blood rapid diagnostic tests according to Malawian national guidelines with demonstrated high accuracy in community settings in Karonga [107]. . The parallel HIV testing algorithm was used for individuals tested until May 2008 and the serial testing algorithm for individuals tested after May 2008. The initial screening test was with Determine TM HIV-1/2 (Abbott Japan Co Ltd, Japan) and confirmatory testing was done with UniGold TM HIV-1/2 (Trinity Biotech PLC, Ireland). Samples with a non-reactive screening test were considered negative and those with a reactive screening and confirmatory test were considered positive. Where the screening and confirmatory tests were discordant, a 'tie breaker' using a third rapid test, (SD Bioline, Korea) was used. The results of the HIV test were recorded on a form by the field personnel (Appendix 6 – Rapid Test Form). After field HIV testing, the venous blood sample was transported in a cool-box to the KPS laboratory for further laboratory-based HIV testing and quality control. Quality control of the rapid HIV testing was done by retesting all positive samples, all inconclusive samples, and a random selection of 1 in 10 negative samples.

3.5.4 Additional filarial research laboratory methods – Og4C3 ELISA

Sample selection and preparation

The plasma samples stored in cryotubes to be processed were first pulled from the freezers, placed on a rack and left at room temperature until they thawed.

LF antigenaemia detection and quantification

LF antigenaemia detection and quantification was done in all the studies using the W. bancrofti Og4C3 antigen-capture enzyme-linked immunosorbent assay (TropBio, James Cook University, Townsville, Queensland, Australia) following the manufacturer's instructions as described in the Appendix 7 - Trop Bio ELISA kit manual.

Plate Reading

The microtitre plate was read using a Dynex revelation plate reader at single wavelength (405nm) and dual wavelength (405 and 492nm). The optical density (OD) was determined by the ELISA plate reader and in addition to a positive/negative response the TropBio ELISA gave a graded response expressed in antigen units or titre group. Using seven control samples provided in the ELISA plates, the test samples were allocated into eight titre groups according to manufacturer's instructions. The antigen units present in each titre group were indicated in the manufacturer's instructions and ranged from <10 units to 32,000 units.

Quality control

If the optical density for the high titre control (Standard No 7) was less than 1.1 or the optical density for the negative control (Standard No 1) is more than 0.3, the test results were regarded as unreliable and the test was repeated.

3.6 Data Management and Statistical Methods

All individual data was collected on paper-based questionnaires and returned to the KPS data coding office. The questionnaires were checked, coded, double-entered and verified using Microsoft Access 2007 software and subsequently exported into STATA statistical software version 12 (StataCorp, Texas, USA) for statistical analyses.

All statistical analyses were done using STATA 12.0 (Stata Corporation, Texas, USA). Basic descriptive statistics explored the data and described characteristics of the study population. Comparison between categorical data were made using Pearson's Chi-square, Fisher's exact test and the log rank test where appropriate. Logistic regression was used to calculate odds ratios (OR) for risk factors associated with the outcomes of interest.

For the Songwe clinical trial, the difference in clearance proportions between the intervention treatment arms and the comparison group was examined using the Fisher's exact test and the log rank test. The analysis used the last observation carried forward and the intention-to-treat approaches to deal with participants who did not take all the intended treatments during the study.

For the cross-sectional assessment of the relationship of LF and HIV infections, and the follow up cohort study, continuous variables were log transformed prior to analysis to achieve an approximate normal distribution. Linear regression was used for crude and adjusted analyses with results expressed as geometric mean ratios (GMR) and their 95% confidence intervals. The association of age, gender and CFA status with HIV positivity was estimated using Chi-square tests for crude analyses and a logistic regression model for adjusted Odds Ratios. In a risk factor analysis for CFA positivity, logistic regression was used to estimate crude and adjusted ORs. Variables were retained in the model if significant associations were identified in the unadjusted estimates. Geographic identifiers for groups of survey villages were also incorporated in the models to adjust for geographic confounding as previous surveys had indicated heterogeneity of CFA prevalence across the region. Rather than a binary variable, HIV was treated as a categorical variable in the model with the HIV-negative group and two HIV-positive groups based on ART use at the time of blood sampling. A sub-group analysis to

investigate the effect of cotrimoxazole and duration of ART use on CFA prevalence was performed on the HIV-positive group only.

Logistic regression models were used to estimate odds ratios. Difference in CFA prevalence with increased use of ART was investigated with a Chi-square test for linear trend with odds ratios derived from a 2xn table. Adjusted odds ratios were also estimated with logistic regression.

3.7 Ethical considerations

The National Health Sciences Research Committee of the Malawi Ministry of Health (protocol numbers 495 and 419) and the Ethical Committee of the London School of Hygiene and Tropical Medicine (application numbers 5344 and 5081) gave ethical clearance for both the Songwe filariasis clinical trial and the HIV sero-survey. Study participants in both studies were consented for storage and later testing of samples at the time of enrolment. This covered testing for HIV and other diseases of local significance. The National Health Sciences Research Committee (protocol number 908) and the Ethical Committee of the Liverpool School of Tropical Medicine (protocol number 11.77) approved the additional analysis conducted on stored samples.

Chapter 4 Results

This chapter presents the study findings of each of the three filarial research studies that make up this thesis. In section 4.1 the cross-sectional analysis is described that underpins the findings on the relationship between LF and HIV. The characteristics of the two study populations from which samples were drawn are described in detail. Results of the HIV and filarial antigen testing are summarised with details of HIV rapid test results, circulating filarial antigen, microfilarial testing and LF quantification. Samples sourced from the KHDSS are linked to the data from the detailed household survey that included information on bed net ownership and ART. Section 4.2 describes the Songwe clinical trial participants and the microfilarial clearance in the different treatment arms. In section 4.3 results of samples taken before and after a period of mass drug administration are presented. It presents participant characteristics at baseline and follow up, CFA clearance rates and an analysis of the impact of mass drug administration and bed net ownership. An HIV subgroup analysis is also included in this section.

4.1 Filarial Research Study 1: Cross-sectional Assessment of the relationship of HIV and markers of lymphatic filariasis infection

In this section, the study presents findings from the analysed markers of lymphatic filariasis infection, by HIV infection status, among adults in two geographically separate sites in Karonga district, northern Malawi. Serum samples for this were drawn from two source studies at KPS: 1) screening samples from the Songwe Clinical Trial, results of which are presented in section 4.2, and 2) samples from the KHDSS whole population baseline HIV sero-survey as described in Chapter 3, section 3.1.2.

4.1.1 Samples sourced from Songwe Clinical Trial

4.1.1.1 Characteristics of the study population

From the estimated total population of 36,643 adults from 44 target villages in the study area, 1,851 individuals from 16 villages were eligible and consented to participate. The 16 villages were chosen because they were historically known to be highly endemic for LF and had easier access of recruitment. There were more females (57.8%) than males. Mean age was 32.6 years (SD 10.4) and the majority were aged between 18 and 30 years old (Table 2).

Table 2: Age and sex distribution of the study population

Age Category	Females N (%)	Males N (%)	Total N (%)
All	1069 (57.8)	782 (42.2)	1851 (100)
18-29 years	495 (46.3)	348 (44.5)	843 (45.5)
30-39 years	302 (28.3)	241 (30.8)	543 (29.3)
≥ 40 years	272 (25.4)	193 (24.7)	465 (25.2)

4.1.1.2 LF and HIV coinfection

Of the 1,851 individuals screened for LF antigen by the ICT card test, 447 (24.2%) were CFA positive, 1402 (75.7%) were CFA negative while 2 (0.1%) had an invalid ICT test result. A total of 1,656 individuals accepted HIV testing and 169 (10.2%) of these were HIV positive (Figure 8). HIV positive individuals tended to be older but there was no difference in HIV prevalence by sex (Table 3). CFA positivity was present in 43 (25.6%) of HIV positive and 351 (23.6%) of HIV negative (crude OR 1.11, 95% CI 0.77–1.60) with an LF/HIV co-infection prevalence rate

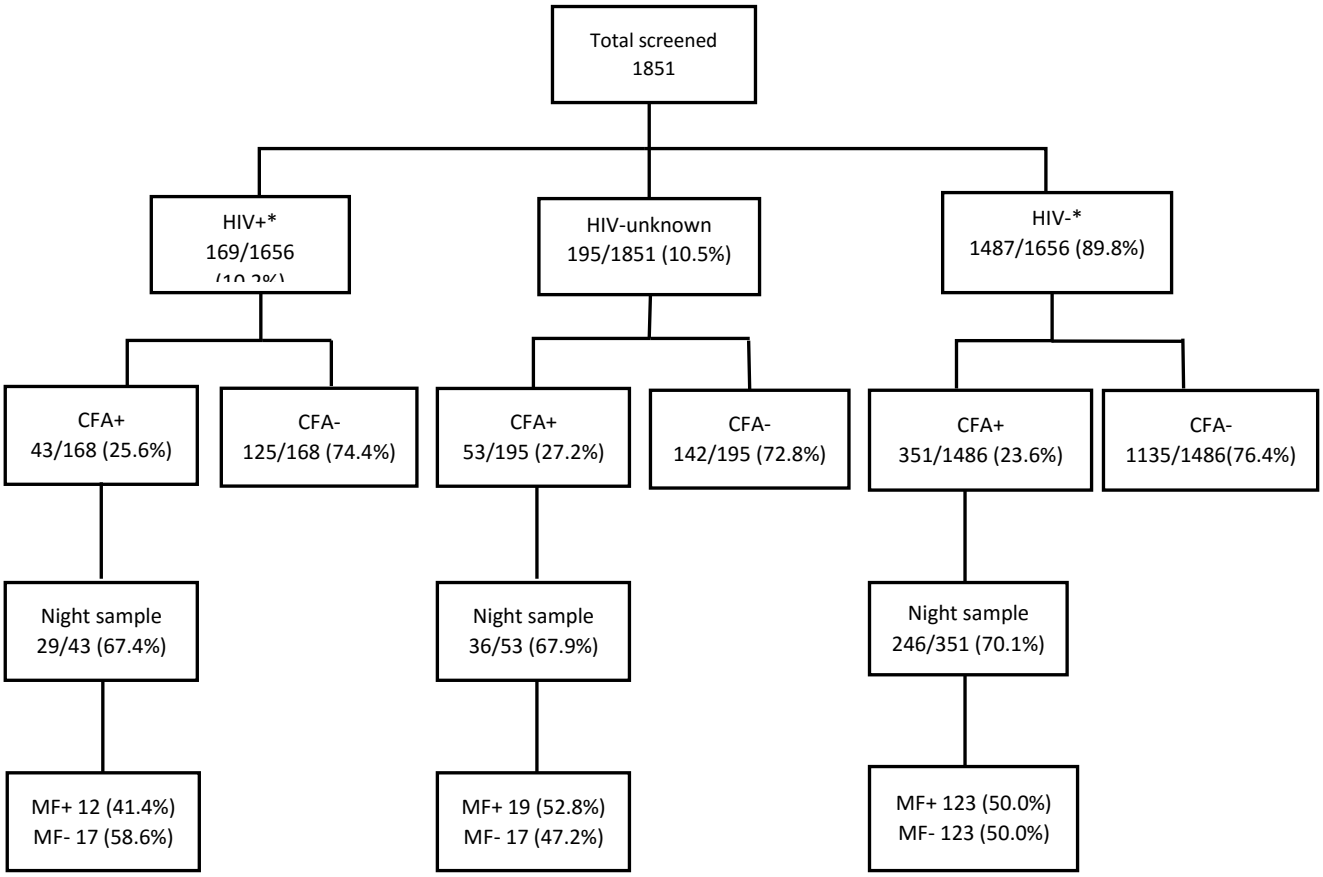


Figure 8: Flow Chart Detailing the Breakdown of Individuals by HIV Status, Circulating Filarial Antigen (CFA) Status By Immunochromatographic Card (ICT) Test and Microfilarial Counts

*2 individual had an invalid ICT test. MF—microfilaria.

Table 3: Baseline characteristics of the participants by HIV status

A				
Characteristic	**HIV-positive (n=169)**	**HIV-negative (n=1487)**	**OR (95% CI†)**	**Adjusted OR (95% CI)#**
Age group				
18 - 29 years	43 (25.4%)	727 (48.9%)	-	-
30 - 39 years	68 (40.2%)	406 (27.3%)	**2.83 (1.90 – 4.23)**	**2.83 (1.90 – 4.23)**
40 years and above	58 (34.3%)	354 (23.8%)	**2.77 (1.83 – 4.19)**	**2.69 (1.77 – 4.08)**
Sex				
Male	66 (39.0%)	618 (41.6%)	-	-
Female	103 (61.0%)	869 (58.4%)	1.11 (0.80 – 1.54)	1.14 (0.82 – 1.59)
CFA status				
Negative	125 (74.4%)	1135 (76.4%)	-	-
Positive	43 (25.4%)	351 (23.6%)	1.11 (0.77 – 1.60)	1.13 (0.78 – 1.65)
MF status				
Positive	17 (58.6%)	123 (50.0%)	-	-
Negative	12 (41.4%)	123 (50.0%)	0.71 (0.32 – 1.54)	0.81 (0.35 – 1.85)
B				
Characteristic	**HIV-positive**	**HIV-negative**	**GMR (95% CI)**	**Adjusted GMR (95% CI)#**
CFA GMC Ag/ml (95% CI)	859 (231 – 3193)	1660 (1198 – 2302)	0.85 (0.49 – 1.50)	0.91 (0.55 – 1.51)
C				
Characteristic	**HIV-positive**	**HIV-negative**	**P value**	
MF count, median (IQR), mf/ml	0 (0 – 22)	1 (0 – 93)	0.13	

A) The association of age group, gender and circulating filarial antigen (CFA) with HIV positivity. Adjusted Odds Ratio (OR) derived from logistic regression model. **B)** Geometric mean concentration (GMC) of CFA in those CFA positive. Geometric mean ratio (GMR) derived from linear regression model. **C)** Microfilaria (MF) count in those CFA positive expressed as median and interquartile range, difference assessed by rank sum testing owing to the skewed nature of data.

#adjusted for age, sex and village location; †CI – confidence interval

of 2.6%. The prevalence of LF infection was not affected by HIV infection status and age but more males than females were CFA positive (Table 5). There was heterogeneity in the prevalence of CFA by village location (Figure 9), median 24.4%, range 15.7–33.3% (Pearson $\chi 2$ 22.7, $p<0.01$, 9 degrees of freedom). Data on the use of antiretroviral and cotrimoxazole was incomplete in the context of this study.

4.1.1.3 Microfilaria counting

Microfilaria counting was done in 311 (69.6%) LF antigen positive individuals who were eligible and gave consent for night blood sampling. The remainder either refused or left before follow up. HIV prevalence in those lost to follow up was broadly similar to those sampled (10.3% vs. 9.3% respectively, $\chi 2$ test $p = 0.90$). Microfilariae were present in 49.5% of the 311 sampled individuals. Twelve (41.4%) of HIV positive individuals and 123 (50.0%) of HIV negative individuals had microfilaraemia but this difference was not statistically significant (Figure 8 and Table 3). Microfilarial count levels had a trend to lower levels in HIV positive than HIV negative individuals did, but similarly without any statistical significance (Table 3).

4.1.1.4 LF antigen quantification

Of 311 stored baseline night blood plasma samples that were positive for CFA by the ICT card test, 290 (93.2%) were also CFA positive using the Og4C3 antigen-capture ELISA. CFA was positive in 26 (89.7%) of HIV positive individuals, 231 (93.9%) of the HIV negative individuals and 33 (97.1%) of HIV-unknown individuals respectively ($p = 0.47$). The geometric mean CFA concentration levels by HIV status were 859 and 1660 for HIV positive and HIV negative respectively (GMR 0.85, 95% CI 0.49–1.50). CFA and MF counts showed reasonable positive correlation (Pearson correlation coefficient $r = 0.56$, $p<0.01$) and majority of individuals (53%) that were CFA positive by Og4C3 ELISA were also MF positive (Table 4).

Table 4: Characteristic features of individuals by Og4C3 ELISA status

	All	CFA Negative	CFA Positive	P Value
N	309	19	290	
MF status				
MF negative	155 (50.2%)	18 (94.7%)	137 (50.2%)	-
MF positive	154 (49.8%)	1 (5.3%)	153 (49.8%)	**<0.001**
Gender				
Female	141 (45.6%)	14 (73.7%)	127 (43.8%)	-
Male	168 (54.4%)	5 (26.3%)	163 (56.2%)	**0.011**
Age group				
18-29 years	137 (44.3%)	7 (73.7%)	130 (44.8%)	0.715
30-39 years	90 (29.1%)	7 (26.3%)	83 (28.6%)	
≥40 years	82 (26.5%)	5 (26.3%)	77 (26.6%)	
HIV status				
HIV negative	246 (79.6%)	15 (78.9%)	231 (79.7%)	
HIV positive	29 (9.4%)	3 (15.8%)	26 (9.0%)	0.474
HIV unknown	34 (11.0%)	1 (5.3%)	33 (11.4%)	
Village CFA prevalence				
Low	197 (63.8%)	11 (57.9%)	186 (64.1%)	-
High	112 (36.2%)	8 (42.1%)	104 (35.9%)	0.583

Table 5: Baseline characteristics of the participants by CFA status

Characteristic	CFA positive (N %)	CFA negative (N %)	OR (95% CI)	Adjusted OR (95% CI)*
Age group				
18-29 years	205 (45.9)	638 (45.5)	-	-
30-39 years	129 (28.9)	414 (29.5)	0.97 (0.75-1.25)	0.91 (0.69-1.20)
40 years and above	113 (25.2)	350 (25.0)	1.00 (0.77-1.31)	0.99 (0.74-1.32)
Sex				
Male	234 (52.3)	547 (39.0)	-	-
Female	213 (47.7)	855 (61.0)	**0.58 (0.47-0.72)**	**0.55 (0.44-0.70)**
HIV status				
HIV negative	351 (89.1)	1135 (90.1)	-	-
HIV positive	43 (10.9)	125 (9.9)	1.11 (0.77-1.60)	1.15 (0.78-1.68)

*Adjusted for age, sex, HIV status and village

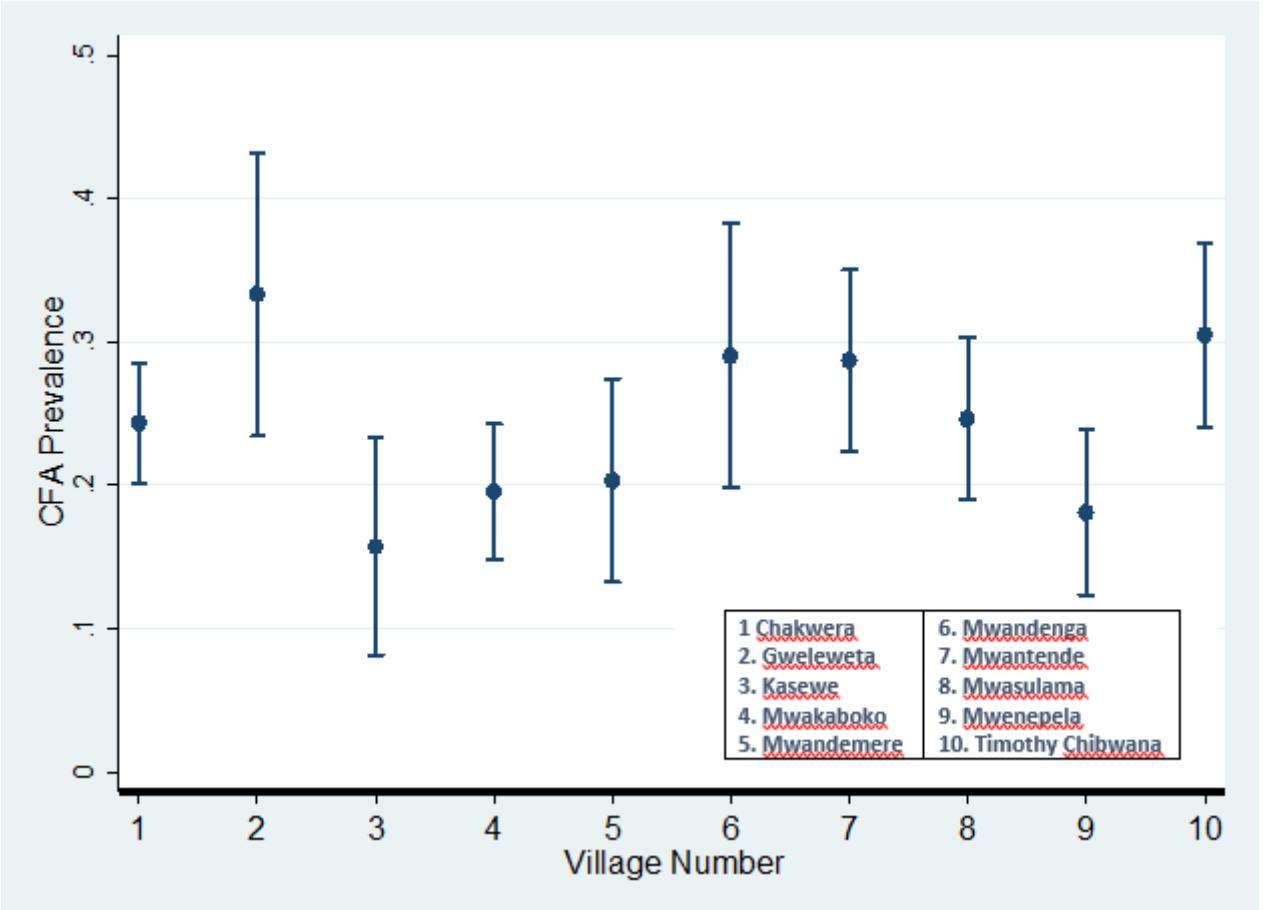


Figure 9: CFA Prevalence by Village Location

4.1.2 Samples sourced from KHDSS

4.1.2.1 LF antigen and HIV testing

The total eligible population of 15-year-olds and older in the KDHSS at the baseline survey was 11,756. From this group, 7,863 (66.9%) underwent HIV testing and consented to storage of their blood sample. A total of 1,875 (23.9%) individuals were CFA positive by the Og4C3 ELISA. HIV infection was identified in 411 (5.2%) participants. HIV positive adults tended to be older and more likely to be female (Table 6). CFA positivity was present in 86 (20.9%) of HIV positive and 1789 (24.0%) of HIV negative (crude OR 0.84, 95% CI 0.66–1.07) with an HIV/LF co-infection prevalence rate of 4.6%. In the female participants, CFA positivity was present in 17.8% of the HIV-positive and 19.8% of the HIV negative (OR 0.88, 95% CI 0.64–1.21) and in the male participants, 26.8% of the HIV positive and 29.4% of the HIV negative (OR 0.88, 95% CI 0.60–1.28) respectively. CFA concentration levels had a right skewed distribution with most values concentrated towards the lower end of the range and progressively fewer values towards the top of the range (Figure 10). Overall geometric mean CFA concentration was measured at 828 Ag/ml (95% CI 789-869) and was lower in the HIV positive individuals by 25% although this association was weakened when adjusted for age and sex (Table 6).

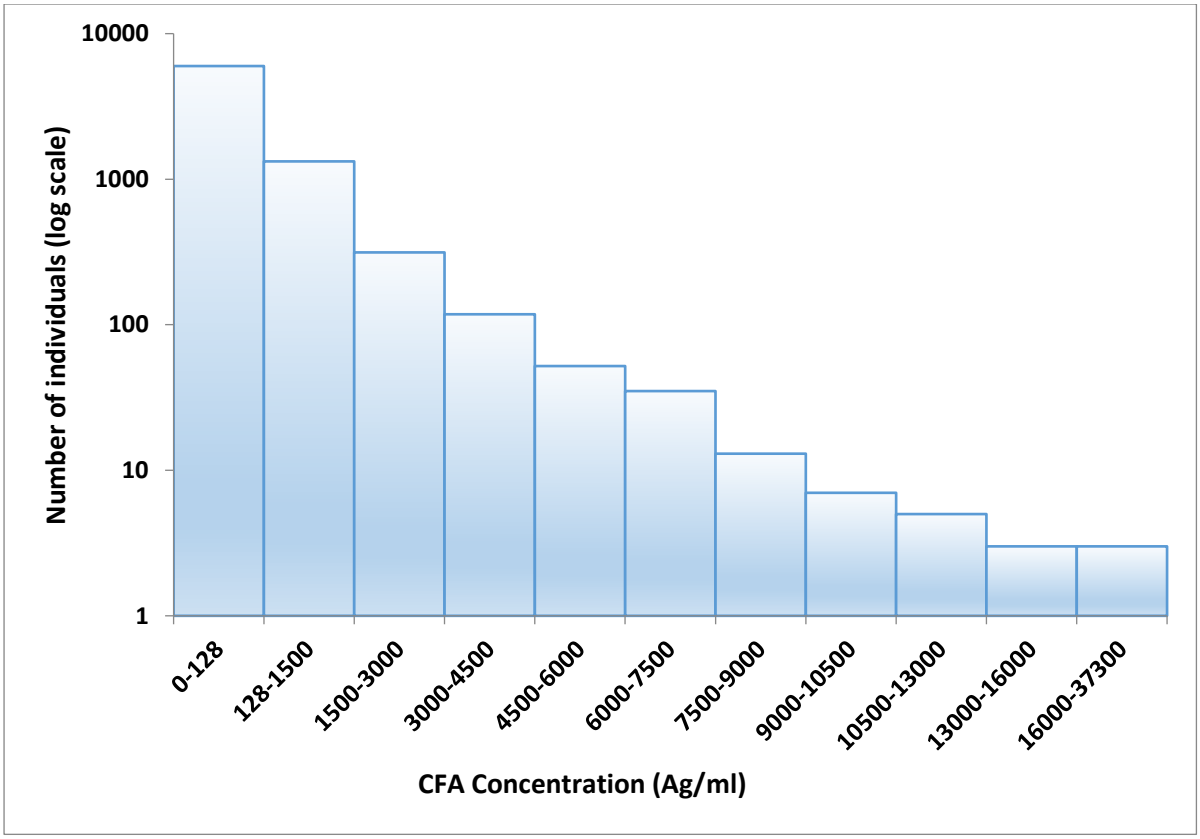


Figure 10: Histogram of Geometric Mean CFA Concentration

4.1.2.2 HIV, antiretroviral therapy and LF antigenaemia

Several risk factors were associated with an increased prevalence of CFA (Table 7). These included male gender, age between 30 and 39 and lower quality housing, whilst decreased CFA prevalence was associated with higher levels of education ($p<0.01$, $\chi 2$ for linear trend) the availability of piped tap water or the use of lake water and the use of antiretroviral drugs. Bed net ownership was high, however ownership or the number of nets owned in the household was not associated with CFA prevalence. Data on actual bed net use was not collected for this study. Individuals were found in all 21 reporting groups of the KHDSS, with a median of 314 participants (range 129–820). There was considerable heterogeneity in the prevalence of CFA by reporting group (Figure 11 and Table 7), median 23.2%, range 5.7–37.2% (Pearson $\chi 2$ 354.7, $p<0.01$, 20 degrees of freedom). The reporting group CFA prevalence was lowest in reporting groups 6, 7, 4 and 5, and highest in reporting groups 18, 12, 19, 3, 1, 15 and 20.

Table 6: Baseline Characteristics of the Participants by HIV status

A				
Characteristic	**HIV-positive (n= 411)[#]**	**HIV-negative (n= 7452)[#]**	**OR (95% CI†)**	**Adjusted OR (95% CI)[*]**
Age group				
15 - 29 years	77 (18.7%)	3793 (50.9%)	-	-
30 - 39 years	159 (38.7%)	1480 (19.9%)	**5.29 (4.00 – 6.99)**	**5.35 (4.04 – 7.07)**
40 years and above	175 (42.6%)	2179 (29.2%)	**3.96 (3.01 – 5.20)**	**4.02 (3.06 – 5.29)**
Sex				
Female	269 (65.5%)	4201 (56.4%)	-	-
Male	142 (34.5%)	3251 (43.6%)	**0.68 (0.55 – 0.84)**	**0.72 (0.58 – 0.89)**
CFA status				
Negative	325 (79.1%)	5663 (75.9%)	-	-
Positive	86 (20.9%)	1789 (24.0%)	0.84 (0.66 – 1.07)	0.86 (0.67 – 1.10)
B				
Characteristic	**HIV-positive**	**HIV-negative**	**GMR (95% CI)**	**Adjusted GMR (95% CI)[*]**
CFA GMC, Ag/ml (95% CI)	630 (511 – 778)	839 (799 – 882)	**0.75 (0.60 – 0.94)**	0.62 (0.38 – 1.02)

A) The association of age group, gender and circulating filarial antigen (CFA) with HIV positivity. Adjusted Odds Ratio (OR) derived from logistic regression model. **B)** Geometric mean concentration (GMC) of CFA in those CFA positive, Geometric Mean Ratio (GMR) derived from linear regression model.

[#]2 HIV-positive and 26 HIV-negative with incomplete data; [†]CI – confidence interval; *Adjusted for age, sex, and reporting group

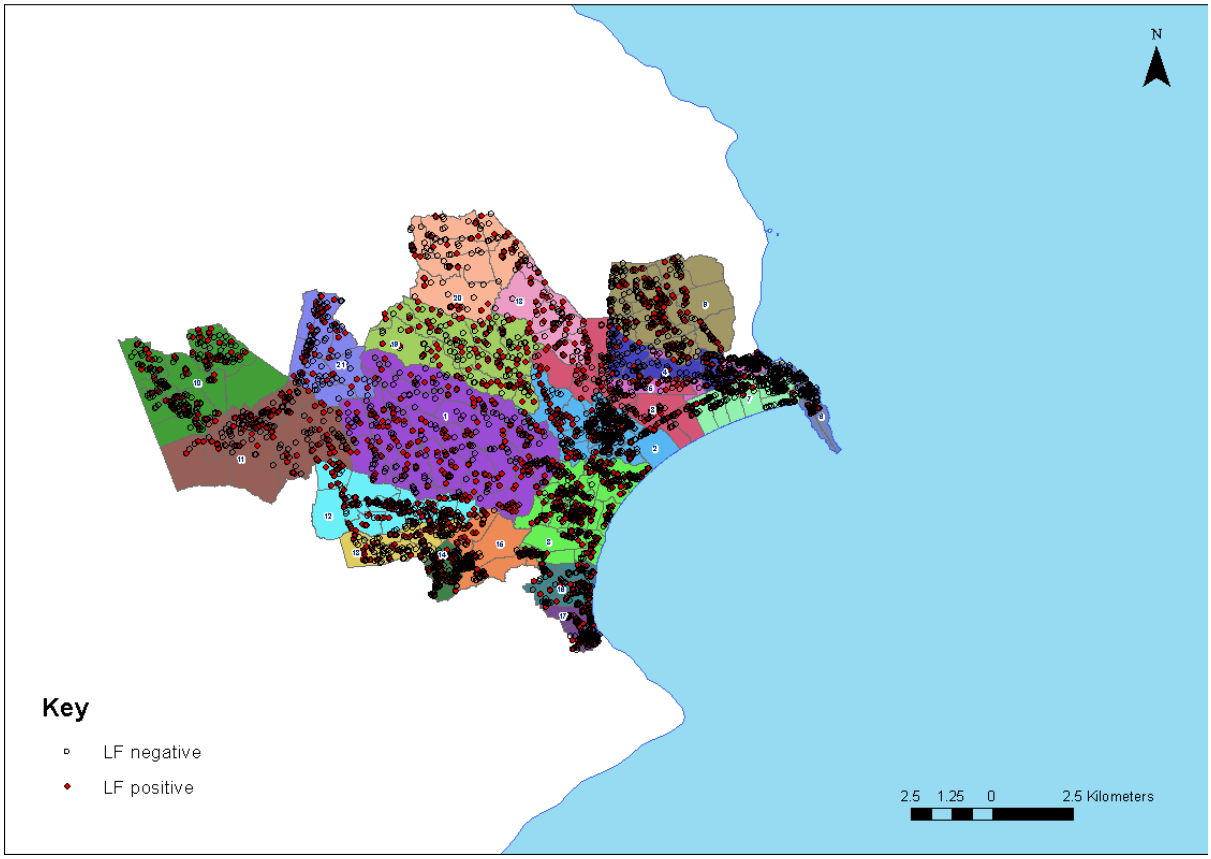


Figure 11: Map of KHDSS Showing Distribution of Baseline CFA Positive Individuals by Reporting Group

Table 7: Reporting Group Population, CFA Positive Individuals and Baseline CFA Prevalence

Reporting Group	Reporting Group Population	CFA Positive Individuals	Baseline CFA Prevalence (%)	95% Confidence Interval
1	624	204	32.7	29.0 - 36.5
2	672	144	21.4	18.4 - 24.7
3	820	279	34.0	30.8 – 37.4
4	313	25	8.0	5.2 – 11.6
5	365	36	9.9	7.0 – 13.4
6	314	18	5.7	3.4 – 8.9
7	386	27	7.0	4.7 – 10.0
8	252	57	22.6	17.6 – 28.3
9	624	152	24.4	21.0 – 28.0
10	470	107	22.8	19.1 – 26.8
11	360	89	24.7	20.4 – 29.5
12	391	144	36.8	32.0 – 41.8
13	279	57	20.4	15.9 – 25.6
14	311	72	23.2	18.6 – 28.2
15	278	82	29.5	24.2 – 35.2
16	301	67	22.3	17.7 – 27.4
17	260	56	21.5	16.7 – 27.0
18	129	48	37.2	28.9 – 46.2
19	318	109	34.3	29.1 – 39.8
20	189	54	28.6	22.2 – 35.6
21	207	48	23.2	17.6 – 29.5
Total	**7863**	**1875**		

Of the 411 HIV positive adults, 142 (34.5%) were taking antiretroviral therapy (ART) and 117 (28.5%) were using cotrimoxazole prophylaxis (CTX) with only four of the 117 taking CTX without ART at the time of sampling (Figure 12). In six of the 411 individuals, information on ART and/or CTX use at the time of sampling was unavailable. ART consisted of Lamivudine, Stavudine and Nevirapine (Triomune-30) in 94% of cases with Zidovudine or Efavirenz substitutions in the remainder. No protease inhibitors were in use.

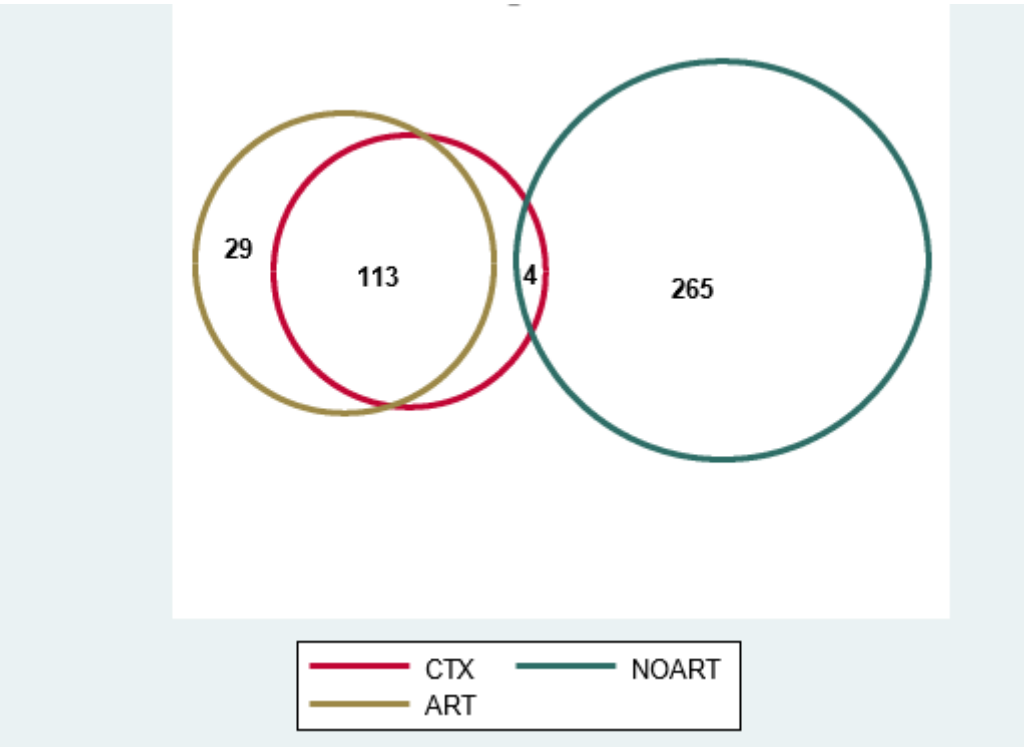


Figure 12: Venn Diagram Showing Use of ART and CPT by the 411 HIV Positive Individuals

In the HIV positive group, ART use was associated with a lower prevalence of CFA when compared to those not on ART [12.7% vs. 25.3% (OR 0.43, 95% CI 0.24–0.76)]. Similarly, CTX use was associated with lower CFA prevalence [12.8% vs. 24.1% (OR 0.46, 95% CI 0.25–0.85)].

In a multivariable model incorporating ART and CTX use along with age, sex and geographical location, the adjusted odds ratio for ART use was 0.47 (95% CI 0.17–1.31) and for CTX use 0.92 (95% CI 0.31–2.71). When the ART treated group were further sub-divided by year since treatment started, there was a significant trend to decreased prevalence of CFA with increasing time on treatment, $p<0.01$ $\chi 2$ for linear trend (Figure 13). This relationship persisted after adjustment for age, gender and reporting group. In the HIV positive individuals with

detectable CFA, the geometric mean concentration of CFA was not significantly different between those off and on ART, 647 vs. 512 Ag/ml respectively, GMR 1.27, 95% CI 0.76–2.08 (Figure 14), nor did the GMC differ by ART duration category 647, 392, 762, 516 & 0 Ag/ml for no treatment, year 1, 2, 3 & 4 of treatment respectively.

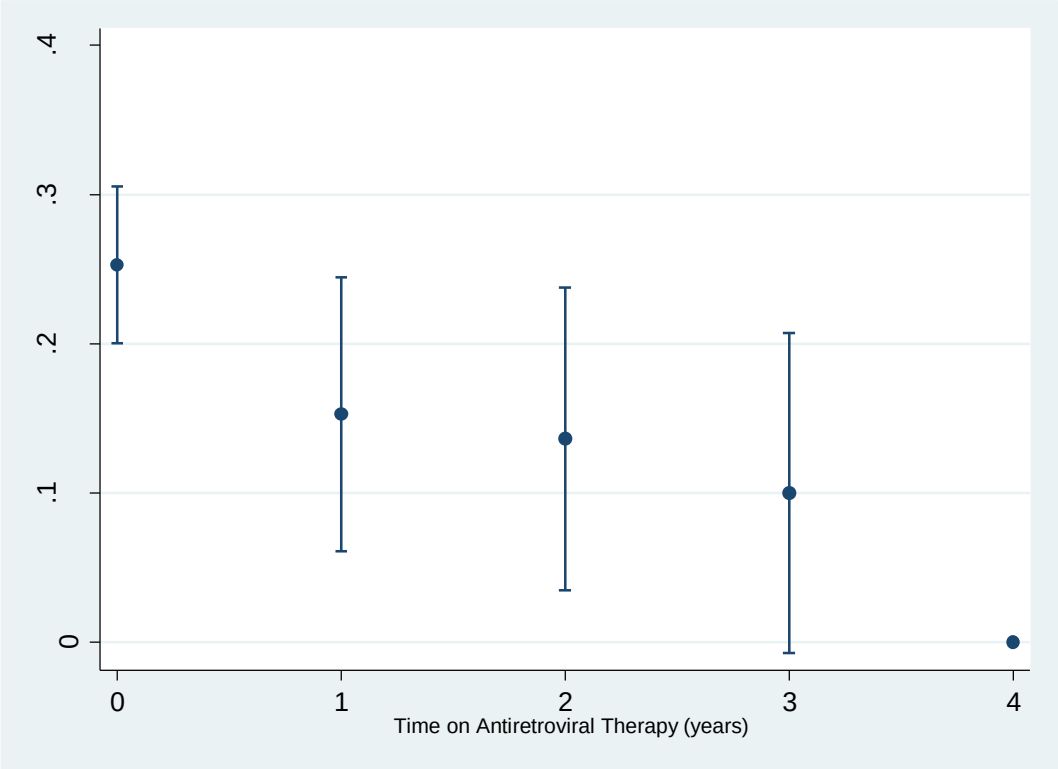
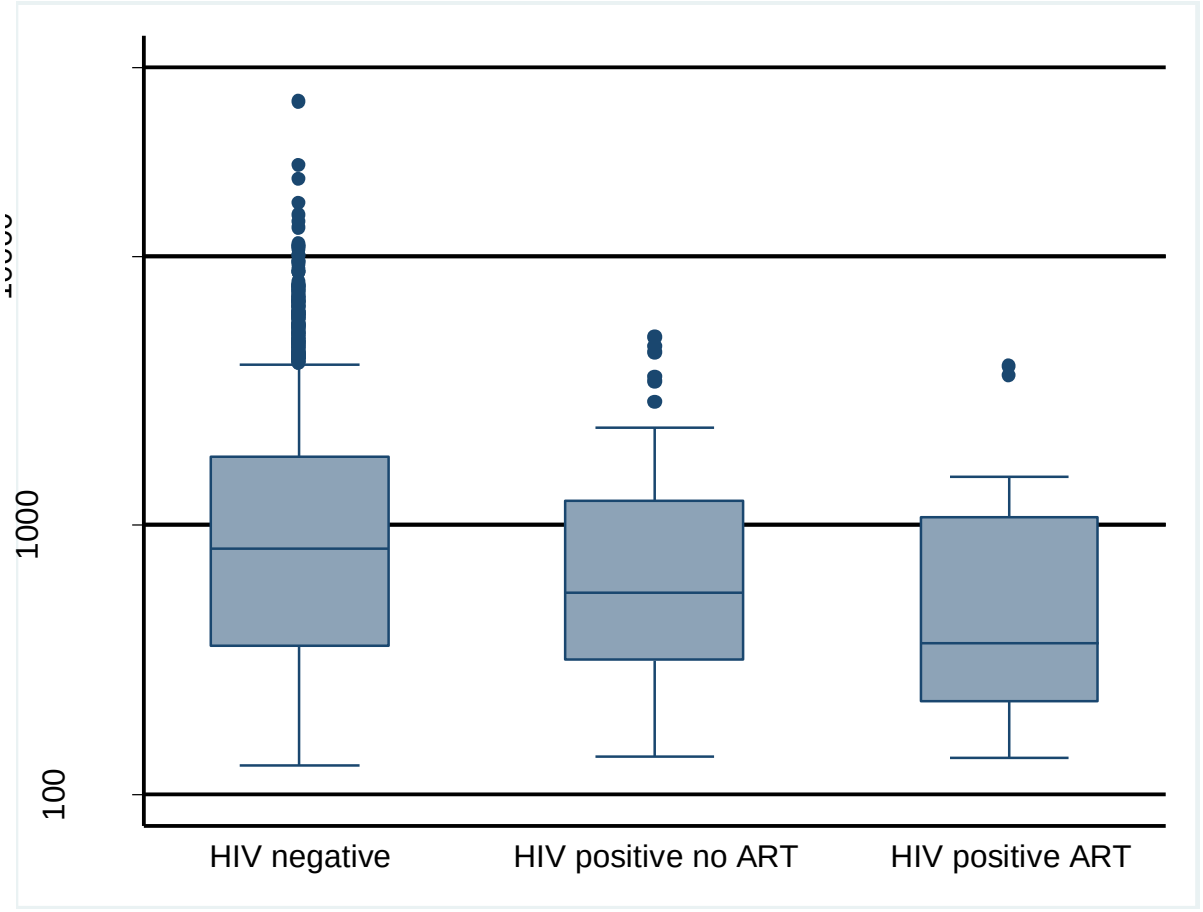


Figure 13: Relationship of CFA Prevalence and Time on Antiretroviral Therapy

Table 8: The association of circulating filarial antigenaemia (CFA) prevalence with HIV and antiretroviral therapy (ART) status and major potential confounding socio-demographic characteristics in the 7,863 KHDSS participants

Characteristic	N (%)	CFA prevalence (%)	OR (95% CI[#])	aOR (95% CI)
HIV and ART status				
HIV-negative	7452 (94.8)	1789 (24.0)	Ref.*	Ref.
HIV-positive – no ART	265 (3.4)	67 (25.3)	1.07 (0.81-1.42)	1.20 (0.90-1.62)
HIV-positive - ART	142 (1.8)	18 (12.7)	**0.46 (0.28-0.76)**	**0.50 (0.30-0.84)**
Gender				
Male	3392 (43.1)	995 (29.3)	**1.69 (1.52-1.88)**	**1.77 (1.59-1.98)**
Female	4470 (56.9)	880 (19.7)	Ref.	Ref.
Age group				
Age 15 – 29 years	3870 (49.2)	896 (23.2)	Ref.	Ref.
Age 30 – 39 years	1639 (20.9)	440 (26.7)	**1.22 (1.07-1.39)**	**1.24 (1.08-1.43)**
Age 40 years and above	2354 (29.9)	539 (22.9)	0.99 (0.87-1.11)	0.95 (0.84-1.09)
Mosquito net ownership				
0	197 (2.5)	46 (23.4)	Ref.	-
1	945 (12.0)	254 (26.9)	1.21 (0.84-1.73)	-
2	1817 (23.1)	461 (25.4)	1.12 (0.79-1.58)	-
3	1835 (23.3)	430 (23.4)	1.00 (0.71-1.42)	-
≥4	2854 (36.3)	640 (22.4)	0.95 (0.67-1.34)	-
Unknown	215 (2.7)	-	-	-
Educational achievement				
Nil	277 (3.6)	83 (30.0)	Ref.	Ref.
Primary	5493 (71.3)	1379 (25.1)	0.78(0.60-1.02)	**0.71 (0.54-0.93)**
Secondary	1886 (24.5)	377 (20.0)	**0.58 (0.44-0.77)**	**0.55 (0.41-0.75)**
Tertiary	45 (0.6)	8 (17.8)	0.51 (0.23-1.13)	0.52 (0.22-1.19)
Unknown	2 (0.0)	-	-	
Water supply				
Bore hole	3755 (47.7)	1037 (27.6)	Ref.	Ref.
Tap to house	1082 (13.8)	105 (9.7)	**0.28 (0.23-0.35)**	**0.30 (0.24-0.37)**
Shared tap	835 (10.6)	127 (15.2)	**0.47 (0.38-0.57)**	**0.48 (0.39-0.59)**
Covered well	999 (12.7)	303 (30.3)	1.14 (0.98-1.33)	1.16 (0.99-1.35)
Open well	572 (7.3)	167 (29.2)	1.08 (0.89-1.31)	1.06 (0.87-1.30)
Lake	456 (5.8)	106 (23.3)	**0.79 (0.63-1.00)**	**0.77 (0.61-0.97)**
Unknown	11 (0.1)	-	-	-
Housing type				
Burnt brick	5750 (73.1)	1328 (23.1)	Ref.	Ref.
Unburnt brick	678 (8.6)	169 (24.9)	1.11 (0.92-1.33)	1.00 (0.83-1.21)
Mud	1087 (13.8)	299 (27.5)	**1.26 (1.09-1.46)**	1.03 (0.88-1.20)
Grass/bamboo	167 (2.1)	49 (29.3)	1.38 (0.98-1.94)	1.14 (0.81-1.62)
Other	18 (0.2)	2 (11.1)	0.42 (0.10-1.81)	0.43 (0.10-1.91)
Unknown	163 (2.1)	-	-	-

Crude and adjusted Odds Ratios (OR) derived from a logistic regression model. Data on reporting group are not shown in the table but adjusted models include this as a potential confounder along with the other significant variables in the crude analysis.
[#]CI – confidence interval: *Reference category



Boxes show the median value and the interquartile range. Whiskers include all values within 1.5 times the interquartile range with outliers shown as points.

Comparison of CFA concentrations by group HIV- vs. HIV +/ART- (p = 0.22): HIV- vs. HIV+/ART+ (p = 0.05): HIV+/ART- vs. HIV+/ART+ (p = 0.28), derived from a linear regression model adjusted for age, gender and reporting group

Figure 14: Box plot of CFA Concentration Distribution (on logarithmic scale) by HIV and ART Status for the KHDSS Participants

4.2 Filarial Research Study 2: Songwe Filariasis Clinical Trial

In this section, results of the clinical trial that was undertaken in Songwe area in the northern part of Karonga district of northern Malawi (Figure 1) are presented. The study took place from January 2009 to March 2012 and it was a randomised, controlled, four-arm, open-label clinical trial that compared three modified treatment groups with increased dose and/or frequency to standard MDA dosage of ivermectin and albendazole in adults with confirmed circulating LF antigen and microfilaria.

4.2.1 ICT card testing, randomisation and follow up

A total of 1,851 individuals were screened and 70 participants were found to be eligible for randomisation into the four treatment arms of the study (Figure 16). Rates of missed appointments were similar in all the treatment groups overall. In the twice-yearly arms, this led to five missed treatments in SDT and one in HDT in the first year. Recruitment ceased before the planned sample size was achieved after the national MDA programme for lymphatic filariasis commenced in the study location in October 2009 [17]. Demographic and baseline characteristics were similar between the treatment groups except for higher baseline microfilarial count levels in the annual standard dose treatment group (Table 7).

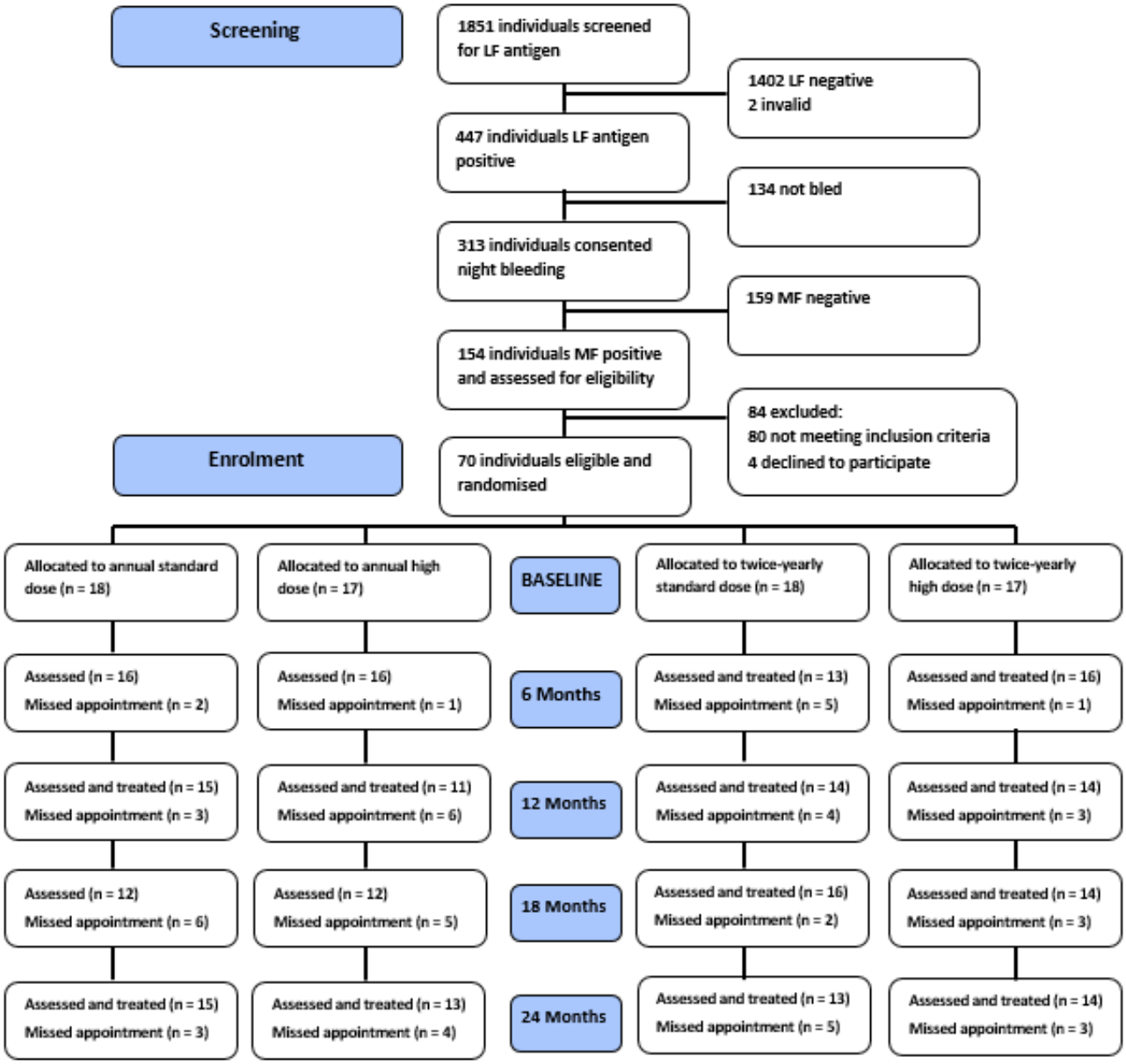


Figure 15: Flow diagram of Study Participants of the Songwe Filariasis Clinical Trial

Table 9: Baseline demographic and clinical characteristics for each treatment group in the clinical trial

Characteristic	Annual standard dose (n=18)	Annual high dose (n=17)	Twice-yearly standard dose (n=18)	Twice-yearly high dose (n=17)
Age, median (range), years	32.5 (21 - 50)	31 (20 - 53)	27.5 (18 - 55)	35 (20 - 54)
Male sex (%)	15 (83.3)	11 (64.7)	11 (61.1)	10 (58.8)
Microfilarial count, geometric mean (95% CI), mf/ml	464.7 (250.1 - 863.4)	338.7 (198.7 - 577.4)	204.5 (145.4 -287.8)	264.7(175.0 - 400.4)
Eosinophil count, geometric mean (95% CI), x 10^3 cells/uL	1.3 (1.0 – 1.7)	1.4 (1.0 – 1.9)	1.6 (1.1 – 2.1)	1.1 (0.9 – 1.5)
Haemoglobin level, geometric mean (95% CI), g/dL	13.7 (13.1 – 14.2)	13.2 (12.7 – 13.6)	13.2 (12.1 – 14.4)	12.4 (11.6 – 13.4)

4.2.2 Microfilarial clearance

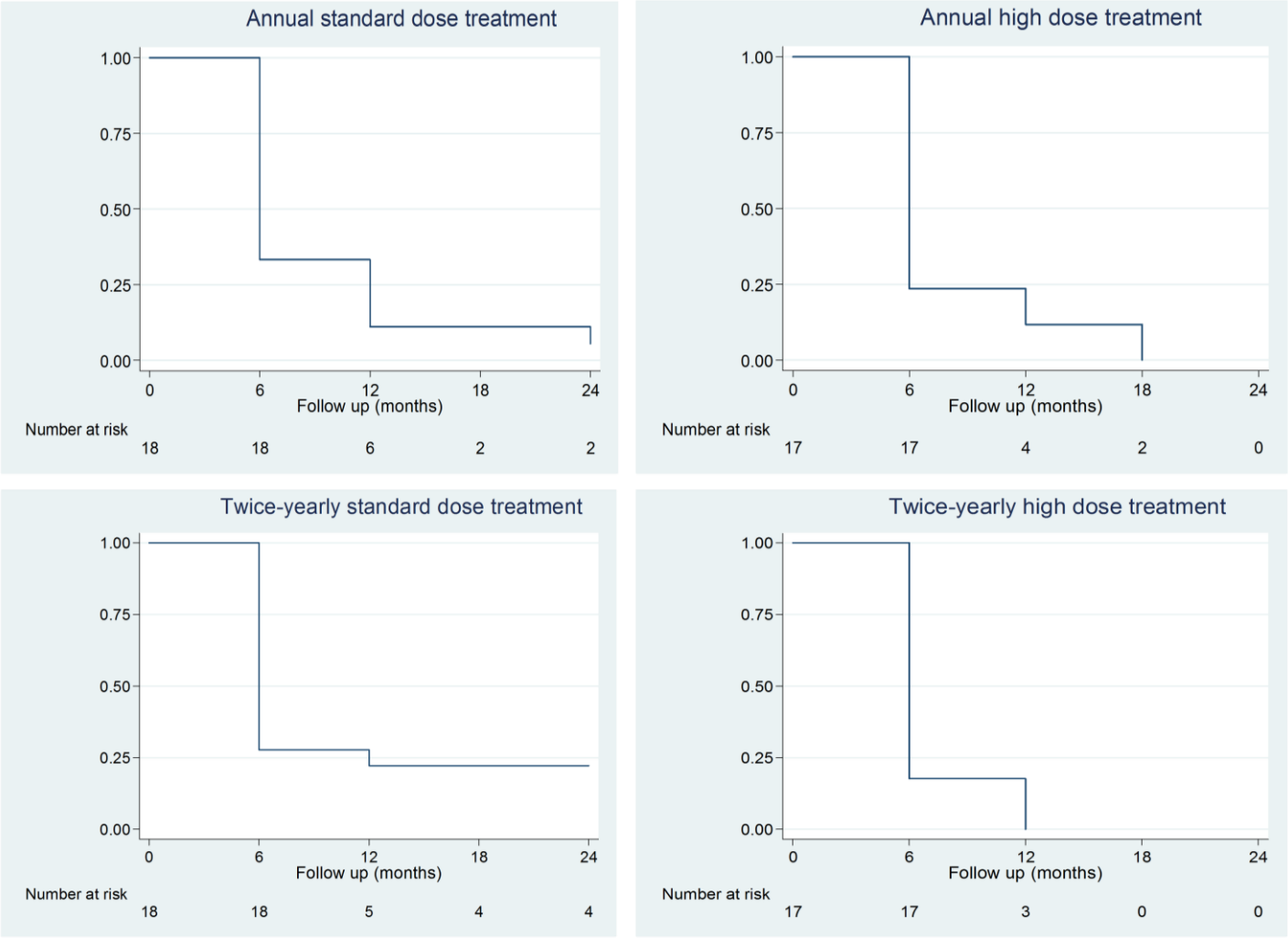
In the intention to treat analysis, all the treatment groups achieved significant reduction of microfilariae levels after albendazole and ivermectin treatment by 12 months of follow up. Microfilarial clearance was 83% (15/18) for SDA, 82% (14/17) for HDA, 72% (13/18) for SDT and 100% (17/17) for HDT treatment doses, respectively. There was no statistically significant difference between high versus standard dose or between twice-yearly versus once-yearly dosing of albendazole and ivermectin (Table 8). In a post-hoc analysis comparing all participants with available data at 12 months who had received a single dose (i.e., those in the single dose arms plus those in the twice-yearly arms who failed to receive their second dose) versus those who received two doses. In total, 35 received one dose, 29 received two doses and six did not have 12 months' data available. Microfilarial clearance was observed in 31 of 35 (89%) and 28 of 29 (97%), respectively. This was not significantly different, p=0.38 (Fisher's exact test).

Table 10: Number of participants with complete clearance of microfilaraemia by treatment group and month of follow up

Treatment Group	6 months[1]	12 months[2]	18 months	24 months
Annual Standard Dose	12/18 (66.7%)	**15/18 (83.3%)**	15/18 (83.3%)	**17/18 (94.4%)**
Annual High Dose	13/17 (76.5%)	**14/17 (82.4%)**	17/17 (100%)	**17/17 (100%)**
Twice-yearly Standard Dose	13/18 (72.2%)	**13/18 (72.2%)**	14/18 (77.8%)	**14/18 (77.8%)**
Twice-yearly High Dose	14/17 (82.4%)	**17/17 (100%)**	17/17 (100%)	**17/17 (100%)**

[1]P = 0.42 – high versus standard dose regimens at 6 months; [2]P = 1.00 – twice annual versus single annual dose regimens at 12 months (Fisher's exact test)

While the difference in W. bancrofti microfilarial clearance rate tended towards significance in the high dose twice-yearly group at 12 months, this again did not achieve statistical significance. The high dose twice-yearly treatment group achieved complete clearance of microfilaraemia at 12 months of follow up without any subsequent recurrence of microfilaraemia, while the other treatment doses took up to 18 months to achieve complete clearance of microfilaraemia (Figure 16).



P=0.38 - annual standard versus annual high dose; P=0.40 - annual standard versus twice-yearly standard dose

Figure 16: Kaplan-Meier Plots for the Four Treatment Arms of the Songwe Clinical Trial

4.2.3 Adverse events

Twenty-two participants (31%) reported seven different adverse events at various times in all treatment groups especially after the first round of treatment. Most of these were mild to moderate in intensity and included fever, headache, joint pains and abdominal pains (all typically associated with treatment of microfilaraemia). Two cases of malaria, one of pneumonia and a case of acute bacterial meningitis, which resulted in the death of the patient, were admitted to hospital for treatment, and were not considered treatment related. All other symptoms were treated conservatively and did not result in hospital admission.

4.2.4 LF clinical manifestations

At baseline, filarial related clinical manifestations were reported in 8 (11%) of the participants for lymphoedema, 6 (9%) for lymphangitis, 12 (17%) for fever, 4 (6%) for hydrocoele and none for elephantiasis and skin rash, respectively. There was no difference in these clinical manifestations between subjects in the four treatment groups of the study. During follow up, there were no new cases of lymphoedema, lymphangitis, elephantiasis and skin rash. Four individuals (6%) at 6 months and one individual (1%) reported fever at 12 months, while two new cases of hydrocoele were reported at 6 and 12 months, respectively. Eosinophilia (absolute eosinophil count .500/ml) was present in 68 (97%) patients (see Table 7) at baseline and no patient was found with eosinophilia during follow up, indicating a significant reduction after treatment.

4.3 Filarial Research Study 3: The Impact of Mass Drug Administration on Circulating Filarial Antigenaemia by HIV Status and ITN Ownership

This section presents results from a longitudinal cohort study that utilised stored blood samples to investigate the impact of MDA on circulating filarial antigenaemia by HIV status and bed net ownership. It was nested within comprehensive annual population-based HIV sero-surveys that were undertaken at the Karonga Health and Demographic Surveillance Site (KHDSS) and used available stored blood samples and data collected as part of the HIV sero-surveys. Baseline samples were taken between September 2007 and October 2008 before introduction of MDA in the study area and follow up samples were taken between October 2009 and October 2011, 2-3 years after MDA had reached all areas.

4.3.1 Characteristics of the follow up cohort

The follow up cohort was derived from the 1875 individuals with a positive baseline CFA and 5988 with a negative baseline CFA (Figure 17) from filarial research study 1. Of the 1875 individuals who were CFA positive at baseline, 1722 individuals (91%) had a follow up blood sample during the MDA distribution period and were reassessed for CFA. The remainder of the individuals who were CFA positive at baseline were not included because they did not have a stored blood sample available for retesting in the laboratory archive despite consenting to blood drawing and storage on at least one follow up HIV sero-survey round (Figure 17).

Of the 5988 baseline CFA negative individuals, a random sample of 938 individuals (15.7% or 1 in 6) was chosen and selected for CFA reassessment during follow up to investigate the natural acquisition of LF antigenaemia during a period of MDA administration. Of the 938 baseline CFA negative individuals selected, 766 individuals had samples available and selected from the third annual round of the HIV sero-survey. The remaining 172 individuals were not available from the third annual round of the HIV sero-survey and were selected from the fourth annual round of the HIV sero-survey. Demographic and baseline characteristics of the individuals who were CFA negative at baseline and retested at follow up were broadly similar when compared to those not retested at follow up (Table 9).

Table 10 shows the features of the 1722 and 938 individuals who were CFA positive and CFA negative at baseline respectively and were retested for CFA after a period of MDA administration. The selected follow up cohort had similar baseline characteristics to the overall study population.

A total of 1464 (55.0%) individuals were followed up after 1-2 years from the time of baseline sample collection, 869 (32.7%) after 2-3 years and 327 (12.3%) after 3-4 years. Follow up time ranged from 414 days to 1391 days (mean 787 days, SD 150 days, IQR 709-751 days).

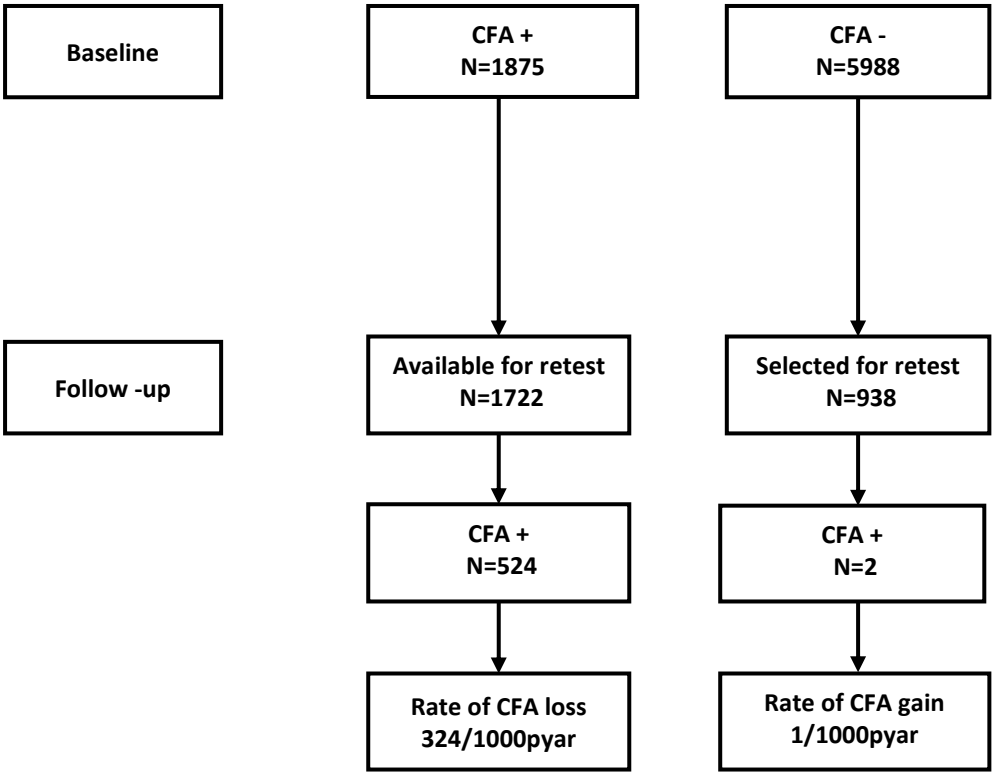
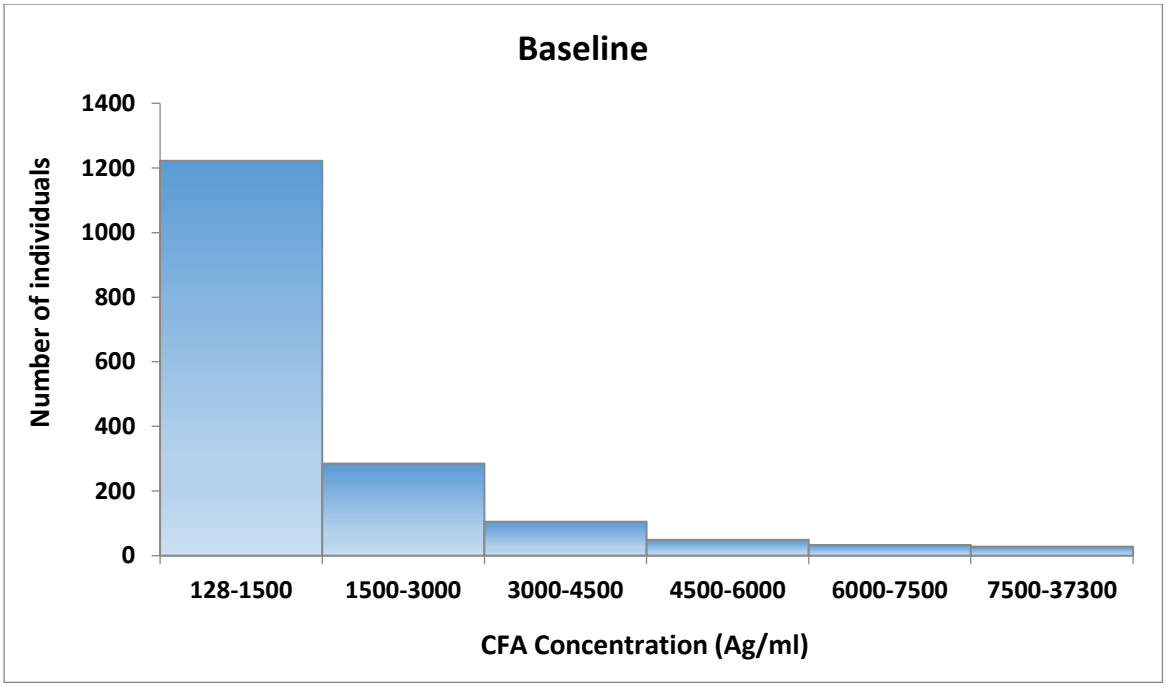


Figure 17: Flow diagram of CFA Assessment of the Follow up Cohort

4.3.2 Circulating filarial antigenaemia at follow up

Of the 1722 individuals who were CFA positive at baseline and were reassessed for CFA during follow up, 524 (30.4%) remained CFA positive (Figure 18) translating to a calculated CFA clearance of 69.6% and clearance rate of 324 cases per 1000 person-years at risk. Of the 938 individuals who were CFA negative at baseline, all but 2 (0.2%) remained CFA negative at follow up translating to a calculated CFA gain of 0.2% and rate of CFA acquisition of one case per 1000 person-years at risk (Figure 17). At baseline, geometric mean CFA concentration was measured at 828 Ag/ml (95% CI 789-869). At follow up in those that were positive, geometric mean CFA concentration was 1124 Ag/ml (95% CI 998-1265). Similar to the baseline CFA concentration distribution, follow up CFA concentration had a right skewed distribution with most values concentrated towards the lower end of the range and

progressively fewer values towards the top of the range (Figure 18). Figure 19 shows the distribution of baseline and follow up level of CFA prevalence in the KHDSS by reporting group. CFA prevalence was lowest in reporting groups 6, 7, 4 and 5 respectively, and highest in reporting groups 18, 12, 19, 3, 1 and 15. These reporting groups are adjacent areas for both the highest and lowest CFA prevalence categories.



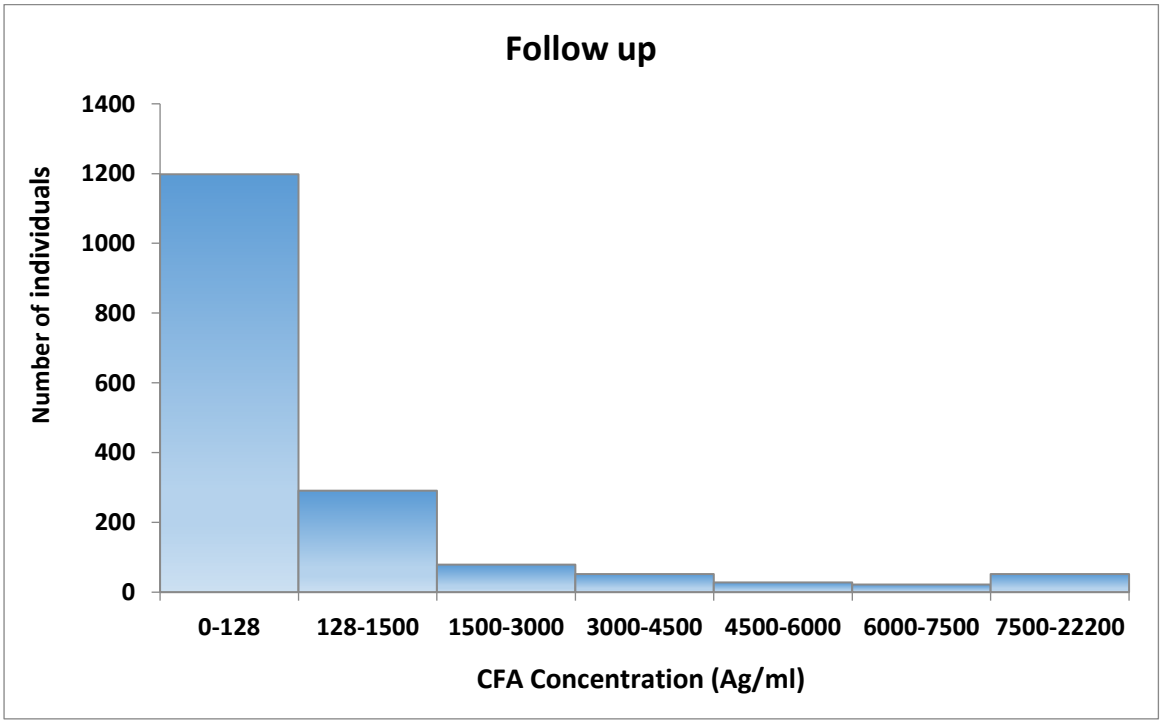


Figure 18: Histograms of Geometric Mean CFA Concentration of the 1722 individuals who were CFA positive at baseline

In the two individuals who were CFA negative at baseline and became CFA positive at follow up, CFA concentration was measured at 141 and 318 Ag/ml respectively at follow up. They were both males from reporting groups 2 and 3 and aged 26 and 29 years respectively, HIV negative and both had received only one MDA dose. The first individual had follow up time of 714 days between collection of the baseline and follow up samples, and according to the timing of MDA administration in the study area, this individual could have received a maximum of one MDA dose during this period. The second individual had follow up time of 1106 days between collections of the baseline and follow up samples and could have received a maximum of one MDA dose during this period. It is hard to interpret if any of these characteristics could be linked to CFA acquisition because of the small numbers.

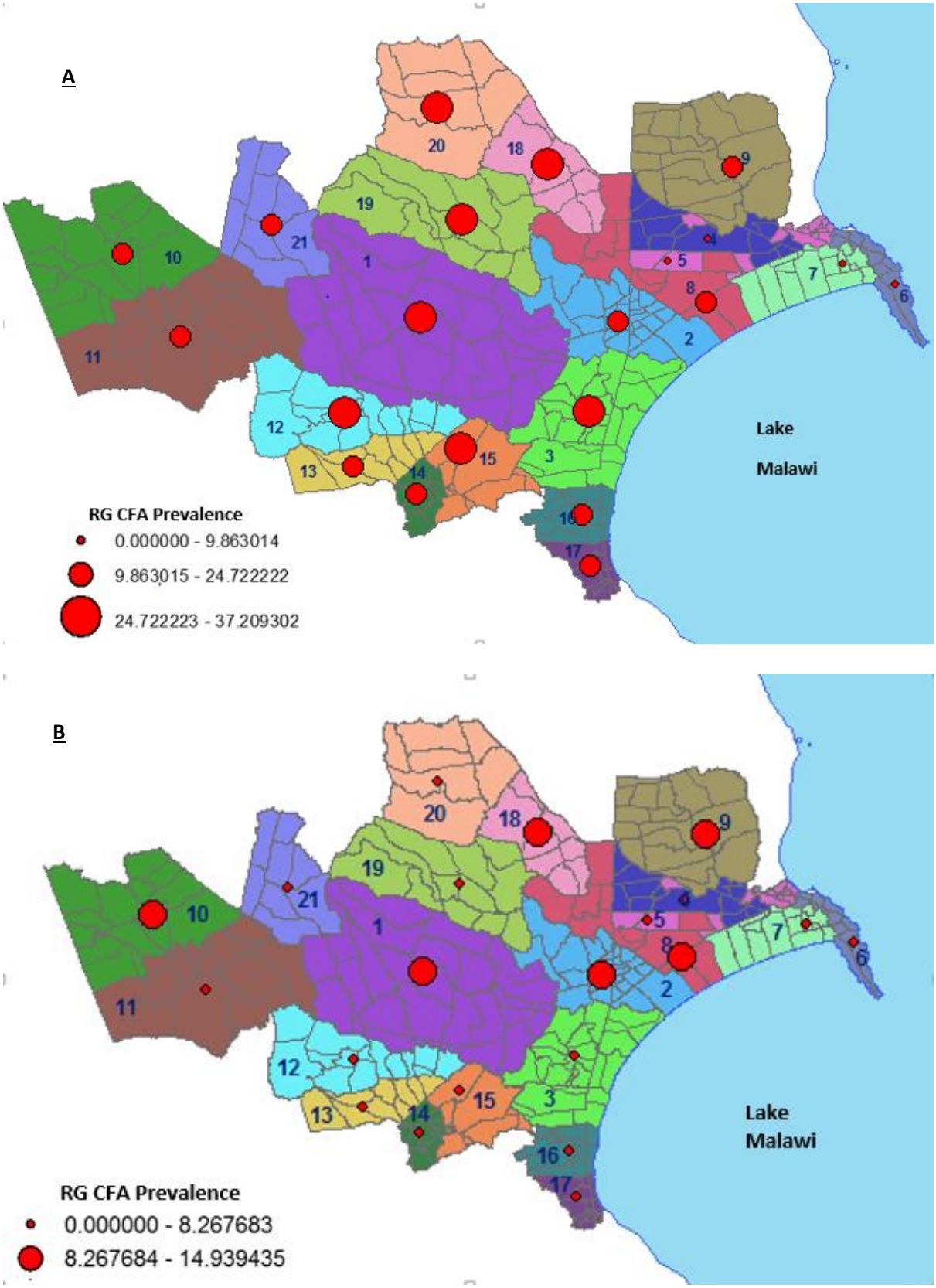


Figure 19: Maps of KHDSS Showing Distribution of Baseline (A) and Follow up (B) CFA Prevalence by Reporting Group

Table 11: Baseline characteristic features of LF negative individuals retested and those not retested

Characteristic	LF-negative not retested (n=4542)	LF-negative retested (n=939)	OR (95% CI)
Age group			
15-29 years	2271 (50.0%)	459 (48.9%)	Ref.
30-39 years	887 (19.5%)	202 (21.5%)	1.13 (0.94-1.35)
40 years and above	1384 (30.5%)	278 (29.6%)	0.99 (0.84-1.17)
Sex			
Female	2735 (60.2%)	574 (61.1%)	Ref.
Male	1807 (39.8%)	365 (38.9%)	0.96 (0.83-1.11)
HIV and ART status‡			
HIV negative	4302 (94.8%)	878 (93.5%)	Ref.
HIV positive - no ART	146 (3.2%)	34 (3.6%)	1.14 (0.78-1.67)
HIV positive - ART	91 (2.0%)	27 (2.9%)	1.45 (0.94-2.25)
Mosquito net use			
None	116 (2.6%)	20 (2.1%)	Ref.
Any bed net use	4334 (95.4%)	895 (95.3%)	1.20 (0.74-1.94)
Unknown	92 (2.0%)	24 (2.6%)	-
Mosquito net number			
0	116 (2.6%)	20 (2.1%)	Ref.
1	538 (11.8%)	98 (10.4%)	1.06 (0.63-1.78)
2	1002 (22.1%)	234 (24.9%)	1.35 (0.83-2.22)
3	1063 (23.4%)	229 (24.4%)	1.25 (0.76-2.05)
≥4	1708 (37.6%)	327 (34.8%)	1.11 (0.68-1.81)
Unknown	115 (2.5%)	31 (3.3%)	-
CPT use in those HIV positive†			
None	166 (69.2%)	35 (57.4%)	Ref.
Any CPT use	72 (30.0%)	26 (42.6%)	1.69 (0.97-2.95)
MDA use			
None	687 (15.1%)	144 (15.3%)	Ref.
Any MDA use	3283 (72.3%)	701 (74.7%)	1.02 (0.84-1.24)
Unknown	572 (12.6%)	94 (10.0%)	-
MDA doses			
0	687 (15.1%)	144 (15.3%)	Ref.
1	1133 (24.9%)	228 (24.3%)	0.96 (0.76-1.20)
2	2150 (47.3%)	473 (50.4%)	1.05 (0.85-1.29)
Unknown	572 (12.6%)	94 (10.0%)	-

‡3 LF-negative not retested individuals have missing HIV and ART status

†2 LF-negative not retested individuals have missing CPT status

Table 12: Characteristic features of the follow up cohort

Characteristic	CFA positive (n=1722)	CFA negative (n=938)	OR (95% CI)	Adjusted OR (95% CI)*
Age group				
15-29 years	668 (38.8%)	393 (41.9%)	Ref.	Ref.
30-39 years	477 (27.7%)	216 (23.0%)	**1.30 (1.06-1.59)**	1.57 (0.52-4.74)
40 years and above	577 (33.5%)	329 (35.1%)	1.03 (0.86-1.24)	1.69 (0.55-5.26)
Sex				
Male	915 (53.1%)	364 (38.8%)	Ref.	Ref.
Female	807 (46.9%)	574 (61.2%)	**0.56 (0.48-0.66)**	0.62 (0.31-1.27)
HIV and ART status				
HIV negative	1643 (95.4%)	877 (93.5%)	Ref.	Ref.
HIV positive - no ART	61 (3.5%)	34 (3.6%)	0.96 (0.62-1.47)	0.89 (0.23-3.42)
HIV positive - ART	18 (1.1%)	27 (2.9%)	**0.36 (0.19-0.65)**	0.46 (0.11-1.87)
Mosquito net ownership				
None	60 (3.5%)	23 (2.4%)	Ref.	
Any bed net use	1642 (95.3%)	906 (96.6%)	0.69 (0.43-1.13)	-
Unknown	20 (1.2%)	9 (1.0%)	-	-
Mosquito net number				
0	60 (3.5%)	23 (2.4%)	Ref.	-
1	171 (9.9%)	100 (10.7%)	0.66 (0.38-1.13)	-
2	427 (24.8%)	206 (22.0%)	0.79 (0.48-1.32)	-
3	470 (27.3%)	251 (26.8%)	0.72 (0.43-1.19)	-
≥4	561 (32.6%)	341 (36.3%)	0.63 (0.38-1.04)	-
Unknown	33 (1.9%)	17 (1.8%)	-	-
CPT use in those HIV positive				
None	64 (81.0%)	35 (57.4%)	Ref.	Ref.
Any CPT use	15 (19.0%)	26 (42.6%)	**0.38 (0.19-0.77)**	0.60 (0.18-1.95)
MDA use				
None	278 (16.1%)	144 (15.4%)	Ref.	-
Any MDA use	1265 (73.5%)	699 (74.5%)	0.94 (0.75-1.17)	-
Unknown	179 (10.4%)	95 (10.1%)	-	-
MDA doses				
0	278 (16.1%)	144 (15.4%)	Ref.	-
1	448 (26.0%)	227 (24.2%)	1.02 (0.79-1.32)	-
2	817 (47.4%)	472 (50.3%)	0.90 (0.71-1.13)	-
Unknown	179 (10.4%)	95 (10.1%)	-	-

*Adjusted for age group, sex, ART use, CPT use and reporting group

4.3.3 MDA use and CFA clearance

Participants were interviewed during two consecutive annual survey rounds if they had previously received MDA drugs during the annual MDA treatment campaigns for LF in the area. Of the 2660 individuals that were followed up, 1964 (73.8%) had received at least one MDA dose during the follow up period, 422 (15.9%) did not receive any MDA drugs while 274 (10.3%) had unknown MDA use. Of the 1964 individuals who received at least one MDA dose, 1289 (65.6%) received two MDA doses while 675 (34.4%) received one MDA dose.

The characteristics of the1722 individuals who were CFA positive at baseline are shown in Table 11 broken down into those who became negative and those who persisted with detectable CFA. In a univariable analysis, the clearance of CFA was not associated with age, HIV and ART status, educational achievement, water supply and housing type. Any MDA use did not appear to associate with CFA clearance, however, when MDA was categorised into number of doses, two doses of MDA were associated with a 34% reduction in CFA positivity compared to no doses (Table 11). However, a single dose of MDA was associated with no evidence of clearance and a borderline association with increased CFA positivity at follow up, but this association disappeared in the multivariable analysis. CFA concentration in individuals with persisting CFA at follow up also showed a similar relationship when analysed by MDA treatment (Figure 20), with no apparent impact by any MDA use and slightly reduced CFA concentration in those who had two doses of MDA.

Among the other characteristics that were associated with CFA clearance, clearance increased with increasing duration of follow up, females were more likely to clear CFA than males while individuals from high CFA prevalence areas were more likely to clear CFA than individuals from low CFA prevalence areas.

The characteristics of the1722 individuals who were CFA positive at baseline by number of MDA doses is shown in Table 12. All the characteristics except HIV status, and educational achievement, were significantly associated with MDA dosing.

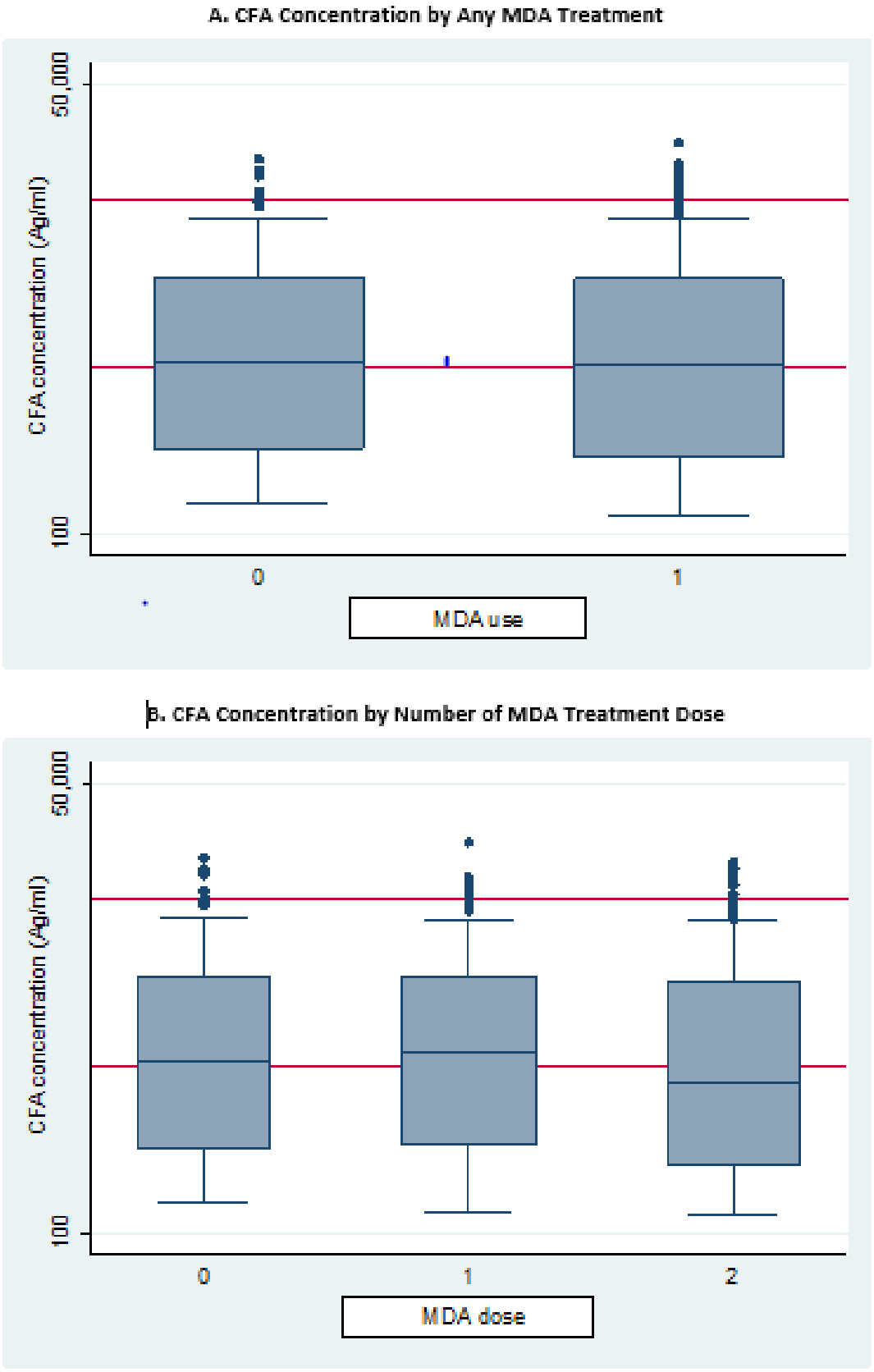


Figure 20: Box Plots of CFA Concentration in Individuals Who Persisting CFA Positivity by MDA Treatment

4.3.4 Bed net ownership per household

Of the 2,660 individuals aged 15 years and above reassessed for CFA at follow up, 2,548 (95.8%) reported possessing at least one mosquito net in the household; 83 (3.1%) indicated that they did not possess a mosquito net while the remaining 29 (1.1%) had an unknown bed net ownership status. The number of mosquito nets per household ranged from 0 to 9 nets (median 3, IQR 2-4). When bed net ownership per household was categorised into number of mosquito nets per household, 83 (3.1%) had no mosquito net in the household, 271 (10.2%) had one, 633 (23.8%) had two, 721 (27.1%) had three while 902 (33.9%) had four or more mosquito nets per household. The remaining 50 (1.9%) individuals had an unknown mosquito net number per household. Data on actual bed net use and bed use among children were not collected as part of this study.

Table 15 shows the characteristics of the 2,660 individuals aged 15 years and above who had follow up samples available for CFA testing and were reassessed for CFA at follow up. There was no clear association between mosquito net ownership and CFA clearance. The only characteristics that were associated with bed net ownership among the individuals aged 15 years and over were the level of education which showed strong evidence that bed net ownership increased with increasing level of educational achievement and reporting group CFA prevalence at baseline which showed that individuals from medium reporting group CFA prevalence areas were more likely to own a mosquito net than that individuals from low reporting group CFA prevalence areas. This was also the case when comparing individuals from high reporting group CFA prevalence areas and low reporting group CFA prevalence areas although this was not statistically significant.

4.3.5 HIV subgroup analysis

In a subgroup analysis of follow up CFA status of 79 individuals who were CFA positive and HIV positive at baseline (Table 16), 54 (68.3%) cleared CFA while 25 (31.7%) remained CFA positive at follow up. In a univariate analysis, all the characteristics examined did not yield any statistical significance. This may suggest that there is loss of statistical power because of very small numbers in this subgroup. However, the tendency of the relationship of the characteristics with CFA clearance was similar to that of the overall group except MDA use which had odds ratios in the opposite direction to that of the overall group and suggesting increased CFA clearance in individuals who had no MDA than individuals who had any MDA use.

Table 13: Comparison of the characteristics of the 1722 CFA positive follow up cohort and association with CFA clearance

Characteristic	CFA negative n=1198	CFA positive n=524	OR 95%CI	Adjusted OR (95%CI)
MDA use				
None	190 (68.3%)	88 (31.7%)	Ref.	-
Any MDA use	895 (70.8%)	370 (29.2%)	0.89 (0.67-1.18)	-
Unknown	113 (63.1%)	66 (36.9%)	-	-
MDA doses				
0	190 (68.3%)	88 (31.7%)	Ref.	Ref.
1	270 (60.3%)	178 (39.7%)	**1.42 (1.04-1.95)**	1.37 (0.98-1.93)
2	625 (76.5%)	192 (23.5%)	**0.66 (0.49-0.90)**	**0.72 (0.52-0.99)**
Unknown	113 (63.1%)	66 (36.9%)	-	-
Duration of follow up				
1-2 years	626 (62.8%)	371 (37.2%)	Ref.	Ref.
2-3 years	395 (76.6%)	121 (23.4%)	**0.52 (0.41-0.66)**	**0.52 (0.39-0.69)**
3-4 years	177 (84.7%)	32 (15.3%)	**0.31 (0.20-0.45)**	**0.23 (0.15-0.35)**
Age group				
15-29 years	454 (68.0%)	214 (32.0%)	Ref.	-
30-39 years	327 (68.6%)	150 (31.4%)	0.97 (0.76-1.25)	-
40 years and above	417 (72.3%)	160 (27.7%)	0.81 (0.64-1.04)	-
Sex				
Male	607 (66.3%)	308 (33.7%)	Ref.	Ref.
Female	591 (73.2%)	216 (26.8%)	**0.72 (0.59-0.89)**	**0.66 (0.52-0.83)**
HIV and ART status				
HIV negative	1144 (69.6%)	499 (30.4%)	Ref.	-
HIV positive - no ART	41 67.2%)	20 (32.8%)	1.12 (0.65-1.93)	-
HIV positive - ART	13 (72.2%)	5 (27.8%)	0.88 (0.31-2.49)	-
Mosquito net use				
None	40 (66.7%)	20 (33.3%)	Ref.	-
Any bed net use	1143 (69.6%)	499 (30.4%)	0.87 (0.51-1.51)	-
Unknown	15 (75.0%)	5 (25.0%)	-	-
Mosquito net number				
0	40 (66.7%)	20 (33.3%)	Ref.	-
1	116 (67.8%)	55 (32.2%)	0.95 (0.51-1.77)	-
2	311 (72.8%)	116 (27.2%)	0.75 (0.42-1.33)	-
3	330 (70.2%)	140 (29.8%)	0.85 (0.48-1.50)	-
≥4	378 (67.4%)	183 (32.6%)	0.97 (0.55-1.70)	-
Unknown	23 (69.7%)	10 (30.3%)	-	-
CPT use in those HIV positive				
None	47 (70.1%)	20 (29.9%)	Ref.	-
Any CPT use	10 (66.7%)	5 (33.3%)	1.18 (0.36-3.88)	-
Unknown	3 (75.0%)	1 (25.0%)	-	-
Reporting group CFA prevalence				
Low	145 (60.7%)	94 (39.3%)	Ref.	Ref.
Medium	486 (61.1%)	310 (38.9%)	0.98 (0.73-1.32)	0.97 (0.68-1.38)
High	567 (82.5%)	120 (17.5%)	**0.33 (0.24-0.45)**	**0.37 (0.25-0.54)**

Educational achievement				
Nil	51 (68.0%)	24 (32.0%)	Ref.	-
Primary	893 (70.4%)	376 (29.6%)	0.89 (0.54-1.48)	-
Secondary	227 (66.2%)	116 (33.8%)	1.09 (0.64-1.85)	-
Tertiary	6 (75.0%)	2 (25.0%)	0.71 (0.13-3.77)	-
Unknown	21 77.8%)	6 (22.2%)	-	-
Water supply				
Bore hole	674 (70.3%)	284 (29.7%)	Ref.	Ref.
Tap to house	62 (62.0%)	38 (38.0%)	1.45 (0.95-2.23)	1.05 (0.64-1.73)
Shared tap	64 (56.1%)	50 (43.9%)	**1.85 (1.25-2.75)**	1.37 (0.86-2.17)
Covered well	223 (79.1%)	59 (20.9%)	**0.63 (0.46-0.86)**	0.73 (0.51-1.05)
Open well	94 (67.6%)	45 (32.4%)	1.14 (0.78-1.66)	1.24 (0.79-1.96)
Lake	61 (59.8%)	41 (40.2%)	**1.60 (1.05-2.43)**	1.18 (0.74-1.90)
Unknown	20 (74.1%)	7 (25.9%)	-	-
Housing type				
Burnt brick	857 (70.0%)	367 (30.0%)	Ref.	-
Unburnt brick	105 (67.7%)	50 (32.3%)	1.11 (0.78-1.59)	-
Mud	186 (68.9%)	84 (31.1%)	1.05 (0.79-1.40)	-
Grass/bamboo	29 (63.0%)	17 (37.0%)	1.37 (0.74-2.52)	-
Other	2 (100.0%)	0	-	-
Unknown	19 (76.0%)	6 (24.0%)	-	-

Separate regression models used for mosquito net use and mosquito net number

*Adjusted for MDA dose, duration of follow up, sex, water source, and reporting group CFA prevalence

Table 14: Characteristics of individuals by number of MDA doses

Characteristic	No MDA dose n=278	One MDA dose n=443	Two MDA dose n=823	Unknown MDA dose n=178
Duration of follow up				
≤2 years	162 (16.3%)	256 (25.6%)	480 (48.1%)	99 (9.9%)
2-3 years	79 (15.3%)	111 (21.5%)	265 (51.4%)	61 (11.8%)
3-4 years	37 (11.7%)	76 (36.4%)	78 (37.3%)	18 (8.6%)
Age group				
15-29 years	110 (13.4%)	203 (24.7%)	407 (49.6%)	101 (12.3%)
30-39 years	56 (13.9%)	116 (28.9%)	192 (47.8%)	38 (9.4%)
40 years and above	112 (22.4%)	124 (24.8%)	224 (44.9%)	39 (7.8%)
Sex				
Female	103 (12.8%)	225 (27.9%)	405 (50.2%)	74 (9.2%)
Male	175 (19.1%)	218 (23.8%)	418 (45.7%)	104 (11.4%)
HIV status				
HIV negative	262 (94.2%)	419 (94.6%)	792 (96.2%)	170 (95.5%)
HIV positive	16 (20.2%)	24 (30.4%)	31 (39.2%)	8 (10.1%)
Mosquito net use				
None	14 (23.0%)	15 (24.6%)	15 (24.6%)	17 (27.9%)
Any bed net use	262 (16.0%)	419 (25.5%)	807 (49.2%)	153 (9.3%)
Unknown	2 (0.7%)	9 (2.0%)	1 (0.1%)	8 (4.5%)
Reporting group CFA prevalence				
Low	63 (18.0%)	125 (35.7%)	115 (32.9.0%)	47 (13.4%)
Medium	81 (14.5%)	183 (32.7%)	229 (41.0%)	66 (11.8%)
High	134 (16.5%)	135 (16.6%)	479 (58.9%)	65 (8.0%)
Educational achievement				
Nil	231 (17.2%)	342 (25.4%)	629 (46.8%)	142 (10.6%)
Primary/Secondary	42 (12.0%)	98 (27.9%)	177 (50.4%)	34 (9.7%)
Unknown	5 (18.5%)	3 (11.1%)	17 (63.0%)	2 (7.4%)

Table 15: Characteristics of the follow up cohort and association with bed net ownership

Characteristic	No bed net n=83	Bed net n=2548	Unknown n=29	Crude OR (95% CI)	Adjusted OR (95% CI)
Age group					
15-29 years	33 (3.1%)	1015 (95.7%)	13 (1.2%)	Ref.	Ref.
30-39 years	18 (2.6%)	669 (96.5%)	6 (0.9%)	1.21 (0.67-2.16)	1.37 (0.74-2.52)
40 years and above	32 (3.5%)	864 (95.4%)	20 (1.1%)	0.88 (0.54-1.44)	1.06 (0.63-1.78)
Sex					
Female	44 (3.2%)	1321 (95.7%)	16 (1.2%)	Ref.	Ref.
Male	39 (3.1%)	1227 (95.9%)	13 (1.0%)	0.95 (0.62-1.48)	1.19 (0.75-1.89)
HIV status					
HIV negative	80 (3.2%)	2403 (95.8%)	26 (1.0%)	Ref.	-
HIV positive	3 (2.0%)	145 (96.0%)	3 (2.0%)	1.61 (0.50-5.16)	-
CFA status					
CFA negative	63 (3.0%)	2047 (95.9%)	24 (1.1%)	Ref.	-
CFA positive	20 (3.8%)	501 (95.2%)	5 (1.0%)	0.77 (0.46-1.29)	-
Reporting group CFA prevalence					
Low	22 (4.5%)	466 (94.7%)	4 (0.8%)	Ref.	Ref.
Medium	33 (2.6%)	1207 (96.1%)	16 (1.3%)	**1.73 (1.00-2.99)**	**1.83 (1.04-3.21)**
High	28 (3.1%)	875 (95.9%)	9 (1.0%)	1.48 (0.83-2.61)	1.62 (0.91-2.89)
Educational achievement					
Nil	9 (8.3%)	98 (90.7%)	1 (0.9%)	Ref.	Ref.
Primary	59 (3.1%)	1823 (95.8%)	21 (1.1%)	**2.84 (1.37-5.89)**	**2.90 (1.35-6.23)**
Secondary/Tertiary	12 (2.0%)	580 (96.8%)	7 (1.2%)	**4.44 (1.82-10.81)**	**4.67 (1.81-12.07)**
Unknown	3 (6.0%)	47 (94.0%)	0 (0.0%)	-	
Water supply					
Bore hole	43 (3.1%)	1328 (95.8%)	15 (1.1%)	Ref.	-
Tap to house	8 (3.1%)	240 (93.8%)	8 (3.1%)	0.97 (0.45-2.09)	-
Shared tap	8 (3.4%)	226 (96.2%)	1 (0.4%)	0.91 (0.42-1.97)	-
Covered well	9 (2.4%)	367 (96.6%)	4 (1.0%)	1.32 (0.64-2.73)	-
Open well	10 (5.0%)	189 (94.5%)	1 (0.5%)	0.61 (0.30-1.24)	-
Lake	2 (1.3%)	148 (98.7%)	0 (0.0%)	2.40 (0.57-9.99)	-
Unknown	3 (5.7%)	50 (94.3%)	0 (0.0%)	-	-
Housing type					
Burnt brick	53 (2.8%)	1831 (96.2%)	19 (1.0%)	Ref.	-
Unburnt brick	9 (3.7%)	230 (95.0%)	3 (1.2%)	0.74 (0.36-1.52)	-
Mud	17 (4.3%)	375 (94.0%)	7 (1.7%)	0.64 (0.37-1.12)	-
Grass/bamboo	1 (1.5%)	64 (98.5%)	0 (0.0%)	1.85 (0.25-13.6)	-
Other	0 (0.0%)	3 (100.0%)	0 (0.0%)	1	-
Unknown	3 (6.2%)	45 (93.8%)	0 (0.0%)	-	-

***Adjusted for sex, age group, reporting group CFA prevalence and educational achievement**

Table 16: Characteristics of the 79 individuals who were CFA positive and HIV positive at baseline and association with CFA clearance

Characteristic	CFA negative (n=54)	CFA positive (n=25)	OR (95% CI)
MDA use			
None	12 (75.0%)	4 (25.0%)	Ref.
Any MDA use	36 (65.4%)	19 (34.6%)	1.58 (0.45-5.59)
Unknown	6 (75.0%)	2 (25.0%)	-
MDA doses			
0	12 (75.0%)	4 (25.0%)	Ref.
1	12 (48.0%)	13 (52.0%)	3.25 (0.82-12.88)
2	24 (80.0%)	6 (20.0%)	0.75 (0.18-3.17)
Unknown	6 (75.0%)	2 (25.0%)	-
Duration of follow up			
≤2 years	30 (60.0%)	20 (40.0%)	Ref.
2-3 years	13 (81.2%)	3 (18.8%)	0.35 (0.09-1.37)
3-4 years	11 (84.6%)	2 (15.4%)	0.27 (0.05-1.36)
Age group			
15-29 years	4 (57.1%)	3 (42.9%)	Ref.
30-39 years	26 (68.4%)	12 (31.6%)	0.62 (0.12-3.19)
40 years and above	24 (70.6%)	10 (29.4%)	0.56 (0.10-2.95)
Sex			
Male	22 (61.1%)	14 (38.9%)	Ref.
Female	32 (74.4%)	11 (25.6%)	0.54 (0.21-1.41)
ART status			
HIV positive - no ART	41 (67.2%)	20 (32.8%)	Ref.
HIV positive - ART	13 (72.2%)	5 (27.8%)	0.79 (0.25-2.52)
Reporting group CFA prevalence			
Low	9 (69.2%)	4 (30.8%)	Ref.
Medium	21 (52.5%)	19 (47.5%)	2.03 (0.54-7.71)
High	24 (92.3%)	2 (7.7%)	0.19 (0.03-1.21)

Chapter 5 Discussion

This chapter reiterates the overall study objective and presents a short summary of the main findings of the study. It examines and gives details of these findings and discusses these in the light of the existing evidence from elsewhere. It also provides alternative explanations of the findings and summarises and acknowledges the main limitations of the study. Finally, it draws generalisations from the important findings and implications for public health, makes recommendations for LF control programmes and outlines areas for further research.

5.1 Introduction

This study set out to investigate the relationship between HIV and LF in the context of on-going MDA treatment for LF elimination and presents data from two geographically separate studies undertaken in Karonga district, rural northern Malawi. In both studies a high LF and HIV prevalence was measured with HIV co-infection rates of 2.6% and 4.6% among those who were CFA positive and 15 years and older. There was no evidence that HIV is associated with an increased risk of LF infection but there was a tendency to lower CFA prevalence in the HIV positive group, attributable to significantly lower CFA prevalence in the ART treated sub-group. This persisted following adjustment for key potential confounders and showed a significant trend to lower CFA prevalence with duration of ART use.

In an associated randomised controlled open-label clinical trial comparing double dose vs. standard dose and once-yearly vs. twice-yearly dosing, all four treatment groups achieved a significant reduction of microfilaraemia by 12 months, with additional clearance in all arms at 24 months. Doubling the standard dose and giving it twice yearly showed a non-significant tendency towards faster and more complete clearance of microfilaraemia compared to the WHO-recommended annual standard dose albendazole 400 mg single dose and ivermectin 200 mg/kg treatment regimen, without serious adverse reactions.

At follow up, two doses of MDA treatment effectively reduced CFA prevalence and worm burden and its effectiveness was unaffected by HIV co-infection and ART status. During follow up a very low rate of new infections was measured, and this could not be directly linked to an MDA effect.

5.2 LF, HIV and antiretroviral therapy

This study did not find any evidence that HIV has any impact on the prevalence of LF antigenaemia but found evidence to support significantly lower CFA prevalence in ART treated HIV positive individuals.

5.2.1 No interaction between LF and HIV infection

Previous studies on the association between LF and HIV infections have been few with small samples from selected populations and have reported divergent findings. A positive association of LF and HIV infections, with increased LF infection levels in HIV positive individuals, was reported in a cross-sectional study of 907 adults undertaken in Tanga coastal region, north-eastern Tanzania [21]. A further evaluation of the same study population in a subgroup comprising of 59 individuals infected with HIV and/or *W. bancrofti* did not support any association between HIV and *W. bancrofti* infection [22]. No association between HIV and LF infections in terms of quantitative difference in *W. bancrofti* CFA levels by HIV status was also reported in another cross-sectional study of 432 HIV positive and 99 HIV negative patients in urban southern India [24]. A recent study from the same cohort in India found no differences in HIV replication and disease progression in HIV positive patients after treatment with DEC and albendazole [115].

In contrast to these studies, this study had a larger sample taken from a whole population survey, including a high proportion of the at-risk population in an area with high prevalence rates of LF and HIV infections. Findings in this thesis are supported by a recently published study that was undertaken in Kyela and Mbeya districts, southwestern Tanzania, a neighbouring country to Malawi and about 200km to the north of the KHDSS [116]. In a cross-sectional analysis of LF antigenaemia and HIV infection, the study reported no difference in the prevalence of LF infection in HIV positive and HIV negative adults. Similar to the study in this thesis, this study had a larger sample taken from a whole population survey that was conducted in an area with high prevalence rates of LF and HIV infection and with no previous LF treatment campaigns with MDA or other antifilarial drugs. However, their sample was smaller than the study sample in this thesis and did not include ART use data in HIV positive patients but included children below 18 years of age.

Both source studies in this thesis had some degree of selection bias, but it is unlikely that this has fundamentally altered the findings in this thesis. In filarial research study 1 component sourced from the Songwe Clinical Trial, villages known historically to have a high prevalence of LF infection were targeted [117, 118]. If participation by HIV positive individuals was reduced because of perceived stigma associated with an HIV test, this study may have had reduced power to identify an association between LF and HIV. However, a similar finding in the much larger filarial research study 1 component sourced from the KHDSS provides consistency. In this study, it is known that HIV positive adults were under-represented. Adults who knew their status from earlier HIV testing studies or through routine service provision in the district, declined participation [99]. However, it is difficult to see a mechanism whereby LF

co-infection would disproportionately lead to non-participation by HIV positive adults and, ART treated HIV positive adults, thereby obscuring the true association. As with all observational studies, it is possible that unaccounted for confounding has limited our ability to observe a true difference. We may be partially reassured by the follow up component of this study which did not demonstrate any impact of HIV modifying the effects of MDA treatment, which may have been expected if HIV modifies the control of the host response to filarial.

5.2.2 Antiretroviral therapy associated with reduced LF prevalence

The findings in relation to ART use are novel and only one other study that investigated whether ART is associated with reduced prevalence of helminth infections among HIV positive adults attending a primary HIV clinic in a semi-rural area in Gabon reported similar findings [119]. However, in contrast to the study findings in this thesis in relation to ART use, the Gabon study reported no association between ART use and helminths infections. However, the helminths related outcomes of interest that were investigated in this study were positive stool for intestinal helminths (*T. trichiura, A. lumbricoides, N. americanus, S. stercoralis)*, and presence of *Loa loa* microfilaraemia on microscopy. In addition, the study was clinic based and reported very low numbers of infections, which may have masked a more discrete effect of ART which the study also acknowledges among its major limitations. Another explanation of the contrasting results to these findings may be that because this study measured *W. bancrofti* CFA and therefore investigated the macrofilaricidal effects of ART while the Gabon study measured *Loa loa* microfilaraemia and investigated the microfilaricidal effects of ART.

In this study, individuals receiving ART may represent a select group of the HIV positive population who have better health seeking behaviour, may be more educated, live in better accommodation and/or may live near health providers. However, as this work was undertaken in the context of an ongoing demographic surveillance survey and annual HIV cross-sectional surveys [103, 107], it was able to investigate these potential confounders by adjusting for reported educational status, housing quality, access to clean water and geographic location. ART treated individuals may survive longer but at the expensive of increased use of non-ART medications and therapies with the potential for antihelminthic effects. More detailed information on antibiotic usage and other “over the counter” treatments was unavailable and would represent an area for further study going forward.

Residual confounding or an unrecognised selection bias remains possible but seems unlikely given the highly significant trend to lower CFA prevalence with duration of ART therapy i.e a strong dose response relationship. If LF infection adversely impacts on the success of ART therapy, then over time the prevalence of CFA positivity in this group will reduce as the LF/HIV

co-infected die. There is no evidence from the Malawi national HIV programme that outcomes from ART treatment are worse in regions of the country endemic for LF compared to those with low LF prevalence. Helminth infections have been linked to increased viral load in non-ART treated individuals [23] but not to evidence of faster HIV progression [120]. Similarly, LF infection had no significant effect on HIV disease progression in a study of *W. bancrofti* and HIV coinfections in southern India [115]. Altered diagnostic accuracy of the Og4C3 ELISA in the presence of ART has not been reported. ART has been rarely linked to false negative HIV results in children and adults, but this is more likely to be due to low levels of virus and/or antibody than a direct inhibitory effect. The reduction in CFA prevalence by ART treatment duration and the antigen capture nature of the Og4C3 ELISA would be difficult to explain by ART inhibition of the assay. Immune reconstitution as a result of ART does not adequately explain the findings in this thesis either as there is a similar prevalence of CFA in the HIV negative and the HIV positive untreated. There is no precedent for immune recovery following ART leaving the immune system in a more competent state than that of an HIV negative person. ART treatment is an imprecise proxy marker of duration of HIV infection. If the natural history of LF in the HIV positive is a steady fall in antigenaemia, could this explain the association? The study did not have accurate HIV sero-conversion dates for the majority of this population so it was not possible to fully consider this possibility. However, with ART use, the “natural history” of HIV is dramatically altered and it might be expected that any tendency to lower antigenaemia with time would also be altered and this would be inconsistent with the findings in this thesis.

The most plausible explanation for the finding of lower CFA prevalence in ART treated HIV positive individuals is a direct filaricidal activity of the major ART agents. Further evaluation of these molecules or their metabolites as antihelminthics would be appropriate as the underlying mechanism of the antifilarial effects of ARTs remains less clear. The Anti-Wolbachia (AWOL) Consortium based at Liverpool School of Tropical Medicine (LSTM) aims to develop new drugs against onchocerciasis and lymphatic filariasis and has developed assays to screen various drugs for antifilarial activity [121]. The ART drugs, Lamivudine, Stavudine and Nevirapine, were included in the AWOL drug screening process and the results have shown that these drugs have no direct effect on microfilariae, adult female worms or Wolbachia levels as single drugs. Nevirapine had some modest activity against male worms, with a slow decline in motility over a 14-day period, but this was not interpreted as displaying typical direct anti-filarial activity. It remains possible that these drugs may show some synergism in combination but not as single drugs (Professor Mark Taylor, personal communication).

One possible explanation is through mitochondrial toxicity of the ART on the worms which is a well-known adverse effect of many ART drugs [119]. Few other previous studies that have investigated the effects of ART drugs on intestinal parasites and found significant reductions of helminths infections in ART treated HIV positive individuals, also speculate on the antifilarial effects of the ART drugs [122-124]. Another possible way in which the ART drugs can work against filarial infections is by a process of activation of autophagy in the adult filarial worms and this action has been shown to produce bactericidal activity against the *Wolbachia* endobacteria and consequent death of the adult filarial worm [125]. This has not been tested in laboratory conditions and may be interesting to see ongoing research in this area. ART drugs may also produce antifilarial effects through their metabolites and screening of these as part of existing drug screening strategies currently investigating possibilities for repurposing approved drugs for use in may provide further insight to their antifilarial effects.

5.2.3 Co-trimoxazole preventive therapy in HIV patients and LF

Cotrimoxazole preventive therapy (CPT) is part of the standard package of preventive services that are provided to all HIV positive patients to help reduce and prevent serious HIV-related diseases according to the Malawi clinical guidelines for the management of HIV/AIDS [93]. CPT is provided to all HIV positive adults for life as a daily dose of one 960 mg cotrimoxazole tablet. In this study, 28.5% of HIV positive patients were on CPT. A crude association of CTX therapy with lower CFA prevalence was observed but no significant effect of CTX therapy on CFA prevalence when analysed in a multivariable model. The crude association of CTX with lower CFA prevalence seems adequately explained by concomitant use of ART, and data to support either sulphonamides or trimethoprim, the components of CTX, as effective antifilarial agents is limited and less clear. A recently published study from Gabon reported a significant reduction of *Loa loa* microfilaraemia with CTX therapy in HIV-positive adults and suggested a direct antihelminthic effect of CTX through interference with folic acid biosynthesis and metabolism in the worms [119]. This is in contrast to the study findings in this thesis which reported no significant effect of CTX therapy on CFA prevalence after controlling for known potential confounders. Nevertheless, given the strong collinearity between ART use and CTX use, from a purely epidemiological and mathematical perspective we are not able to firmly conclude that CTX has no effect. Thus further experimental investigation of this agent may be merited.

5.3 Factors associated with LF positivity in cross-sectional analysis

Of the factors associated with CFA positivity in this study, which included gender, age, quality of housing, level of education and source of water, all except ART use have been reported

previously, providing reassurance that the epidemiology of LF disease in Karonga is not unique and results are generalizable to other similar regions.

One surprise was the lack of association with bed net ownership. However, most households possessed bed nets limiting the power of any comparison as a consequence of major imbalances in group sizes. Moreover, this survey did not specifically ask about usage, or condition of the nets, thus limiting the value of this finding. More detailed evaluation of this will be needed in future work with careful attention to reliable methods for determining true bed net usage and protection.

There were more females than males amongst the study participants in both source studies. This may represent the easier access to females at the time of recruitment since females are more likely to be at home and males are often out in the field for work and recreation activities. In most communities including the study area, significantly more males than females are infected with LF and more females than males are infected with HIV. Although it is known that men are more likely to be infected with LF in this population, this under-representation may not have meaningfully affected the LF/HIV association. Sub-group analyses showed similar odds ratios for the LF/HIV association by gender in filarial research study 1 component sourced from the KHDSS, suggesting no major effect modification.

5.4 MDA dosing regimens and frequency

This study found high levels of microfilarial clearance from both the standard MDA treatment and experimental arms after 12 months, with additional clearance in all arms at 24 months. The twice-yearly high dose albendazole (800 mg dose) and ivermectin (400 mg/kg) treatment regimen had a non-significant tendency to faster clearance of microfilaraemia compared to the WHO-recommended annual standard dose albendazole 400 mg single dose and ivermectin 200 mg/kg treatment regimen, without any increase in adverse events.

These findings are consistent with available historic and contemporary trials of anti-filarial agents in other settings [10-12, 126]. A randomised controlled clinical trial conducted in Mali just prior to this study also reported that the higher dose twice-yearly treatment regimen was more effective than the standard dose annual regimen [12]. Another randomised controlled trial of anti-filarial agents recently reported from India showed that higher and more frequent doses of albendazole were more effective than the standard regimen in producing clearance of microfilaria and reductions in antigenemia [126]. The Mali trial was conducted in an area where there had been considerable previous exposure to ivermectin for onchocerciasis as well as a number of rounds of MDA for LF elimination and infected subjects with adequate microfilarial levels were difficult to find. Similarly, the study area in the India trial had seen

intermittent MDA activity initially using DEC alone and later DEC and albendazole [126]. This study endorses, and adds to, the findings in Mali and India since it had three intervention groups compared with the standard regimen and was conducted in a setting of high LF-endemicity with no known previous exposure to LF treatment or MDA for LF elimination.

The response to standard treatment was much better than the expected 25% and 75% at 12 and 24 months, respectively and this was a contributory reason for failure to achieve a definitive outcome in this study. The sample size calculations were based on findings emerging from data comparing single and multi-dose regimens for the treatment of LF [9, 114]. The reduced response to standard treatment in the Mali study may be due to the selection of less sensitive strains as a consequence of previous exposure to LF treatment. With the excellent response to standard treatment in this study, a much larger cohort will be needed to investigate these dosing schedules further. However, future studies like this are likely to prove increasingly difficult to undertake with the introduction and increased coverage of MDA.

The principal limitation of this study was the failure to recruit adequate numbers of participants. Recruitment did not reach the protocol target due to lower numbers of potential subjects with the target microfilaraemia of .80 microfilariae/ml and only 70 were randomised into the study. Recruitment was also discontinued following the rollout of the albendazole/ivermectin national MDA programme in the study area in October 2009. Thus the study was under powered to confirm a true positive effect of the non-standard interventions. Other limitations of the study were a relatively high rate of missed follow up appointments compounded by a series of earthquakes in late 2009 that affected the ability of field workers to reach potential subjects. The study was non blinded in the field. This should not have affected the outcome assessment as this was undertaken blind in the laboratory. However, there is the potential for participants to seek additional therapies if they believed they were being under treated. Although this was felt to be unlikely and questioning was done to seek evidence of additional anti filarial medications, it is not possible to fully exclude this possibility as an explanation for the better than expected performance in the standard arm.

5.5 Test sensitivity and specificity

The measurement of exposure (HIV) and outcome endpoints (LF status) in this study were based on accurate and well described tests and these are not believed to have introduced significant bias into the study. Both parallel and serial rapid HIV testing strategies were used during the period of data collection for the annual HIV surveys that were used as source studies for this study. Under both testing strategies, Determine HIV-1/2 (Abbott Japan Co Ltd, Tokyo, Japan) was combined with Uni-Gold HIV (Trinity Biotech PLC, Bray, Ireland) as the

first and second rapid tests respectively, while SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc, Kyonggi-do, Korea) was used as a third tie-breaker. All three rapid tests used in Malawi have been reported to have sensitivities and specificities of, respectively, 100% and 99.4% (Determine™), 100% and 100% (Uni-Gold™), and 100% and 99.3% (SD Bioline) when evaluated under standard operational conditions [107]. Laboratory-based quality control at KPS was conducted by systematically re-testing plasma from all samples that were positive, inconclusive or for which the initial two rapid tests were discordant using the parallel testing strategy, and from every tenth negative sample collected [107].

The study used different tests for assessment of circulating filarial antigen in the two studies with different sensitivities and specificities, the ICT card test with sensitivity and specificity reportedly close to 100% and the Og4C3 ELISA test with 100% sensitivity and specificity of at least 94% [127-129]. There was some disparity between these two tests identified in filarial research study 1 component sourced from the Songwe Clinical Trial. This is consistent with previous studies that have reported overall agreement between the ICT and Og3C4 tests but different sensitivities and specificities [127, 128]. MF counts were not assessed in the filarial research study 1 component sourced from the KHDSS due to the use of a stored sample collection. Whilst this study cannot categorically rule out an association between MF density and HIV, data from study 1 showed a positive correlation between CFA levels and MF density. Previous studies have also shown a positive correlation between CFA levels and MF density [129, 130]. This implies that the CFA relationship will broadly apply to MF counts.

5.6 Declining LF incidence and rise of malaria vector control programmes

This study found very low rates of CFA incidence in individuals who were CFA negative and demonstrated a natural background loss of CFA in individuals who were CFA positive and reported no MDA use. This demonstrates that there are other drivers of the decline in lymphatic filariasis infection in the study area besides annual MDA treatment campaigns and demonstrates a declining problem of LF generally in the population.

A reduction in LF incidence was observed in the study area for the Songwe clinical trial. Prior work in the area had shown a high prevalence of LF antigenaemia (46%) and microfilaraemia (30.8%) some 10 years previously in 2000-2001[80]. During the screening process for the clinical trial, the prevalence of LF antigenaemia was 24%, 22% lower than previously observed in earlier LF studies in the same area [80], suggesting a reduction in the incidence of new infections during this period. A similar trend was observed in Zambia, a neighbouring country to Malawi, where LF prevalence decreased from 33.3 to 14.8% between 2003 and 2011 [131].

This was attributed to a decrease in LF transmission following the scaling up of malaria vector control activities since the anopheline mosquito vector that transmits malaria in these areas also transmits LF and these control activities could also be contributing to the decline in LF incidence.

Bed net distribution is one such malaria vector control activity that has been scaled up in Malawi, including this study's area, with 55% of households in rural areas reported owning at least one insecticide-treated mosquito net [132]. In contrast to this study, several studies have reported a significant effect of use of insecticide-treated nets in lowering the prevalence and transmission of LF. Some studies have shown how ITN use alone without the need for combination with MDA can reduce LF infection and transmission; a prospective study conducted in south-eastern Nigeria which reported a significant decrease in LF infection and infectivity in mosquitoes from areas which had full coverage of LLIN distribution ; a study of three filariasis surveys in The Gambia conducted over a period of 17 years which attributed a significant decline in LF endemicity and transmission to widespread use of ITNs [133]; and a longitudinal study in coastal regions of Kenya which reported significant reductions in LF infection prevalence and intensity despite the fact that the study villages missed MDA in some of the years [134]. A cross-sectional study from south-eastern Tanzania which reported a marked reduction in *W. bancrofti* CFA in young school children is one of the studies which have provide evidence on how ITN use for malaria control complements MDA in achieving an accelerated and increased reduction of LF infection and transmission is [135].

Thus, long term use of bed nets may be the most likely explanation for the long term downward trend in filarial infection and the downward trend noted in the follow up study. Contrary to the expectations of this study and findings from previous studies, bed net results from this thesis did not demonstrate a significant association with CFA clearance. As noted in section 5.3 above, this study was not able to measure bed net use directly and did not ask about frequency of use or condition of the nets and whether all household members slept under nets. Bed net ownership was used as a proxy of bed net use. To ensure efficiency of field work, a time limitation was placed on the length of any household interview and detailed questions on this topic were avoided. This was in hindsight a limitation of the study and further, more detailed evaluation of this is needed to understand ownership and use.

Several studies have reported changes in mosquito biting patterns including shifts from outdoor to outdoor feeding with malaria transmission occurring both indoors and outdoors [136-138]. Since the same mosquito vectors transmit both malaria and LF in Malawi, this could also apply to LF transmission additionally occurring outdoors and therefore reducing the impact of bed nets on LF transmission. A change in the principal mosquito vector and

consequently biting patterns may explain a change in epidemiology. However, if this was away from the night biting anopheline vector to a daytime biting species an increase in infections might be expected and would not be in keeping with the findings from this study.

Another malaria vector control strategy that may account for the LF decline observed in the area of this study is IRS. In Malawi, IRS was initiated in 2007 as a pilot in the lakeshore district of Nkhotakota in the Central Region and scaled up to a further six districts in 2010 including Karonga district. However, the programme was discontinued between 2011 and 2012 due to logistical problems including evidence of emerging insecticide resistance until 2013 when it was resumed [29]. This means that the study area had only one IRS round during the study duration and the IRS intervention is less likely to have been as effective as anticipated because of this and the reports of emerging insecticide resistance. The contribution of MDA over the period of follow up for this study is less easy to establish, but the recorded low rate of new infections suggests that MDA superimposed on effective vector control is highly effective at limiting new infections and leading to filarial elimination.

Several factors were considered as possible explanations for failure to see a lack of bed net effect on CFA clearance. Leading amongst these was the evidence of minimal LF transmission and acquisition and a natural background loss of CFA occurring in the study area. An already much reduced transmission limits the power of this study to identify an impact, 2 per 1000 person-years at risk would need a very large study to show even a 50% effect size of bed nets. Moreover, most households possessed bed nets and the almost universal ownership limits further the statistical power of any comparison by virtue of the small group sizes in those without nets. Misclassification of bed net ownership, particularly if bidirectional would reduce the effect of the study towards the null. Although over-reporting of bed nets might be possible or ownership without use, under-reporting of ownership seems unlikely and given that the vast majority of households reported ownership this is most unlikely. Bed nets in households have also been reported to be mainly used by children and pregnant women [139]. The study sample did not include children, and this might also have affected the examination of the association between bed net use and CFA clearance.

5.7 MDA treatment in routine use

Two doses of MDA were shown to significantly increase the reduction in CFA prevalence when compared to those who received no doses. This would be expected from previous studies confirming the impact of MDA. A single dose of MDA was associated with a borderline slower decrease in CFA prevalence when compared to those who were not treated. This is a difficult

finding to explain and although the effect size lost significance in the adjusted analysis, the point estimate remained very similar. The individuals who recorded receiving only a single dose of MDA showed a greater proportion of individuals who had been followed up for 3 or more years, which might associate with a more mobile or chaotic life style, greater propensity to be put at risk of biting and a lower likelihood of being sampled in consecutive years. Adjusting for length of follow up in the overall analysis did not significantly adjust the MDA effect sizes and longer follow up was associated with a reduction in CFA. Thus, it is not clear that heterogeneity in follow up explains these findings. Individuals who received a single dose of MDA were more likely to reside in a medium prevalence CFA reporting group, unlike the no dose and two dose groups who resided predominantly in high prevalence areas. Residence in a high prevalence area may be associated with greater use of mosquito nets if biting intensity is higher, and its questioning in this study would not be sensitive to this finding. This could explain the relatively higher prevalence in the individuals receiving just a single dose if the benefits of bed net usage are greater than the impact of MDA. In the overall adjusted clearance analysis, residence in the highest prevalence areas was associated with the greatest CFA reduction lending some support to this idea. Alternatively, a by chance finding is a possible explanation for this unexpected association.

5.8 Implications for LF control programme and community health

A primary research question of this work was whether HIV would impact on the effect of MDA on LF control. No effect of HIV infection was noted in the overall clearance analysis and in the analysis of the HIV-infected subset alone and these findings were similar to the follow up cohort as a whole. This is the first investigation of this magnitude into HIV and LF co-infection in Malawi and it adds significantly to existing knowledge in the field. MDA effectively reduces CFA prevalence and worm burden and the effectiveness of MDA treatment is unaffected by HIV co-infection and ART status. Thus, the study concludes that there is no interaction between HIV infection status and success of MDA treatment, an important conclusion for filarial control programmes in regions of high HIV prevalence. This provides assurance to the Malawi national LF programme and countries with high prevalence rates of both infections that HIV coinfection will not interfere with the current LF control and elimination strategies.

In addition, the findings from the clinical trial indicate that standard treatment may be adequate for national LF programmes in settings with similar epidemiology and treatment history to Malawi. Doubling the standard MDA dose and giving it twice yearly have the potential to achieve a successful and accelerated outcome of mass treatment programmes which could be at a lower overall cost to national programmes by achieving critical programmatic milestones much earlier.

5.9 Recommendations for future research

A significant association of ART use with decreased CFA prevalence merits further investigation to understand the potential of antiretrovirals as molecules with antihelminthic properties and to exclude any adverse impact of LF on HIV. Although the AWOL Consortium initial screening of these drugs as single antifilarial agents has shown that these drugs have no direct effect on microfilariae, adult female worms or Wolbachia levels, it remains possible that these drugs may show some synergism in combination. A significant association of ART use with lower CFA prevalence merits further investigation to understand this apparent beneficial impact of ART.

This study confirms that there is no evidence that HIV infection has an impact on LF epidemiology that will interfere with LF control measures and that MDA effectively reduces CFA prevalence and worm burden and the effectiveness of MDA treatment is unaffected by HIV co-infection and ART status. However further studies with a longer duration of follow up time of MDA coverage are also needed to validate the effectiveness of MDA treatment in HIV endemic settings.

With the excellent response to standard treatment in this study and the finding of a non-significant tendency to faster clearance of microfilaraemia in the twice-yearly high dose albendazole (800 mg dose) and ivermectin (400 mg/kg) treatment regimen compared to the WHO-recommended annual standard dose albendazole 400 mg single dose and ivermectin 200 mg/kg treatment regimen, much larger cohorts will be needed to further investigate the benefit of higher and more frequent regimens definitively. Higher annual doses or more frequent dosing regimens might have the potential to achieve a successful and accelerated outcome of mass treatment programmes. This could be at a lower overall cost to national programmes in countries with limited resources by achieving critical programmatic milestones much earlier. However, future studies like this are likely to be increasingly challenging and expensive to undertake at the required scale with the introduction and increased coverage of MDA.

More detailed evaluation of malaria vector control interventions that impacts on LF transmission are also needed. This can include trials investigating impact of bed net use rather than ownership.

5.10 Conclusion

This PhD thesis described the relationship between HIV and LF in Karonga district, rural northern Malawi, both before and after introduction of MDA treatment for LF elimination in the

area. There was a high prevalence of LF and HIV infections among the study participants, but no evidence was observed of a negative impact of HIV coinfection on LF epidemiology. A significant reduction of LF prevalence and worm burden was observed after a period of MDA treatment and its effectiveness appeared to be unaffected by HIV coinfection and ART status. In addition, all regimens were effective in clearing microfilaraemia with a tendency to faster and more complete clearance when the standard dose was doubled and given twice yearly.

In conclusion, the findings from this PhD thesis confirms that there is no negative interaction between LF and HIV infections in co-infected individuals that can impair the effectiveness of MDA treatment for LF elimination. The current treatment regimen for MDA may be adequate and doubling the standard dose and giving it twice yearly has the potential to achieve successful and accelerated outcomes for LF control and elimination programmes.

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Appendices

KARONGA PREVENTION STUDY CONSENT FORM FOR LYMPHATIC FILARIASIS STUDY (SCREENING)

EFFECT OF ALBENDAZOLE AND IVERMECTIN DOSE ON *WUCHERERIA BANCROFTI* MICROFILARIAL CLEARANCE IN MALAWI: A RANDOMIZED, OPEN LABEL STUDY

Consent to Participate in Screening for Filariasis

Investigators: _____

Site:_____

Volunteer's Name_____
First Middle Last

Identification Number __ __ __-__ __ __ __ Age _____ years

We invite you to take part in a research study being undertaken by Karonga Prevention Study/LEPRA on behalf of the Global Programme for the Elimination of Lymphatic Filariasis. It is important that you understand several general principles that apply to all that take part in this study:

1. Participation in the study is entirely voluntary.
2. Personal benefit to you may not result from taking part in the study, but knowledge may be gained that will benefit others.
3. You may withdraw from the study at any time.

Lymphatic filariasis (LF) is a disease caused by infection of the blood with very small worms called *Wuchereria bancrofti*. These worms are spread by Mosquitoes. LF can cause swellings in the arms, legs, breast and genitals. It can also progress to a permanent swelling of the legs or arms called elephantiasis. Usually, infection with the worms causes no illness at all. When a person with LF is treated with the right medicines, the risk of developing elephantiasis is lower. If an entire community could be treated at one time, the risk of getting infected in that area would be reduced because the mosquitoes that spread the infection could no longer become infected. Doctors hope to eliminate this infection in your community soon by treating everyone with medicines but research is required to find out the right quantity and type of medicine to give. At present medicines must be given yearly for 4-6 years. If the medicines were better at lowering the numbers of worms in the blood of infected people, less time might be needed to eliminate the infection. This could make it easier for countries like Malawi to succeed in eliminating this disease.

Study Design

We would like to see if treatments given once or twice a year with either the usual dose of albendazole and ivermectin (the two drugs used to treat LF in Africa) or with a higher dose of

albendazole are better at getting rid of the worms from the blood than the current standard treatment. In order to do this, we plan to treat some people with filarial worms in the blood with the medicines at the normal dose once a year. A second group of people with filarial worms in the blood will receive the higher dose of albendazole once a year, a third group will receive the normal dose twice a year and a fourth group will receive the higher albendazole dose twice a year. The treatments will be given either every 6 months (total of 4 treatments) or yearly (total of two treatments) and the study will last for 2 years.

Before we give any treatment we need to see if a person is infected. To do this we will take a sample of blood from a finger prick (like a malaria test). If this test shows the presence of the filarial worm we would then wish to take a sample of blood (10ml or a tablespoon in quantity) at nighttime. The blood needs to be taken at nighttime as this is when the worms appear. If this test confirms there are a large number of worms we will ask you to participate in the study of different treatments, however we will ask for your consent again before going any further. If you have a small infection of worms and are not suitable for the treatment study we will give you the current standard treatment if you wish to be treated. In all, we plan to screen 1000 people and expect to find 120 people to take part in the treatment part of the study.

We are looking for healthy people, ages 18 to 55, who are not pregnant to be in the study. We will ask you some questions about your health, including whether or not you have been treated for filariasis .You will be told whether or not you are infected with filarial worms and you may choose, based on this or any other information, not to take part in the study. All study visits and blood drawing will take place at home.

Risks/benefits

The risks associated with this screening study are few. Drawing blood may cause discomfort and occasional bruising at the site.

The benefit from this study is that you will be tested for filarial infection. Filarial infections can cause swelling of arms, legs, scrotum or breasts and this may be prevented or lessened by treatment**.** If you are infected, you will be offered and provided the medicines to treat filarial infection even if you do not wish to participate in our treatment study.

Alternatives to Participation and Exclusion from the Study

You do not have to take part in this study and may refuse to take part at any time. If you are infected with the worm that causes lymphatic filariasis but do not wish to take part in our treatment study, you will still be treated for this infection if you wish.

Reporting of Findings and Confidentiality

Although your study records and medical information will be kept confidential, personnel from GPELF, Ministry of Health or other supervisory body may need to review information. All unanticipated uses of your medical information will be reported to the Institutional Review Board in Malawi**.**

Do you have any questions about participation in this study?

If you have questions or concerns at a later date, you may speak with one of our staff or you can call The Karonga Prevention Study Office on 01364256 or 09971860 and speak with Dr Bagrey Ngwira

If you agree to participate in this study, please put your signature or thumb or fingerprint where indicated below.

_____	_____	_____
Signature, Finger or Thumb Print	**Date**	**Name**
_____	_____	_____
Witness Signature	**Date**	**Name**
_____	_____	_____
Physician Signature	**Date**	**Name**

KARONGA PREVENTION STUDY CONSENT FORM FOR LYMPHATIC FILARIASIS STUDY (TREATMENT)

EFFECT OF ALBENDAZOLE AND IVERMECTIN DOSE ON *WUCHERERIA BANCROFTI* MICROFILARIAL CLEARANCE IN MALAWI: A RANDOMIZED, OPEN LABEL STUDY

Consent to Participate in a Filariasis Treatment Study

Investigators: _____

Site:_____

Volunteer's Name_____
First Middle Last

Identification Number __ __ __-__ __ __ __ Age _____ years

You have already kindly participated in a study to see if you are infected with the lymphatic filariais worm. We would now like to invite you to take part in a further research study being undertaken by Karonga Prevention Study/LEPRA on behalf of the Global Programme for the Elimination of Lymphatic Filariasis. It is important that you understand several general principles that apply to all that take part in this study:

1. Participation in the study is entirely voluntary.
2. Personal benefit to you may not result from taking part in the study, but knowledge may be gained that will benefit others.
3. You may withdraw from the study at any time.

Introduction

Lymphatic filariasis (LF) is a disease caused by infection with very small worms called *Wuchereria bancrofti*. Mosquitoes spread these worms. LF can cause swellings in the arms, legs, breast and genitals. It can also progress to a permanent swelling of the legs or arms called elephantiasis. Usually, infection with the worms causes no illness at all. When a person with LF is treated with the right medicines, the risk of developing elephantiasis is lower. Treatment also eliminates the parasites from the blood. If an entire community could be treated at one time, the risk of getting infected in that area would be reduced because the mosquitoes that spread the infection could no longer become infected. Doctors hope to eliminate this infection in your community soon by treating everyone with medicines.

One of the problems with eliminating lymphatic filariasis is the cost of the treatment program. Medicines must be given yearly for 4-6 years. If the medicines were better at lowering the numbers of worms in the blood of infected people, less time might be needed to eliminate the infection. This could lower the cost of the program and make it easier for countries like Malawi to succeed in eliminating this disease.

Study Population/Study Design

Our study is designed primarily to see whether higher or more frequent doses of albendazole and ivermectin is better at lowering the numbers of *Wuchereria bancrofti* worms in the blood than the standard dose of albendazole given yearly with ivermectin. We would also like to study the effects of these two treatments on the adult worms, which live in the lymphatics. The reason you have been invited to participate in this study is because you were identified by our screening study as being in good health, and it is known that you have filarial worms in your blood. You should have already been informed that you are infected, and you may decide, based on this or any other information, not to participate further. In all, up to 120 people will be selected to participate in the treatment study.

In order to participate in the treatment part of this study, you must:

1) be between the ages of 18 and 55
2) be in good health
3) not be pregnant
4) not be allergic to the drugs being used (albendazole, mebendazole) or ivermectin
5) not drink alcohol to excess (more than 1 beer or other alcohol-containing drink/day)
6) have no laboratory evidence of low red blood cells (Haemoglobin)
7) sign informed consent
8) have a minimum number of worms in your blood (80mf/ml)
9) be willing to have an HIV test performed

For the treatment portion of the study you will be assigned to one of four groups:

1. albendazole (400 mg) and ivermectin (150 mcg/kg) given once a year
2. albendazole (400 mg) and ivermectin (150 mcg/kg) given twice a year
3. albendazole (800 mg) and ivermectin (150 mcg/kg) given once a year
4. albendazole (800 mg) and ivermectin (150 mcg/kg) given twice a year

During the study, you may not take albendazole or ivermectin except when it is given to you by or discussed with the study team. Other medicines are allowed.

You will be assigned to a treatment group using a method similar to flipping a coin. The morning that you receive the treatment, we will measure your temperature and ask you some questions about how you feel. If you are a woman of child-bearing age, we will also check your urine to make sure that you are not pregnant. Then, if you are not pregnant, you will be given the medicines. The visit will take approximately 30 minutes. If you develop symptoms at any time during the treatment part of the study, you are encouraged to let the study doctors know. Additional medicines (including paracetamol, antihistamines and antibiotics) will be available from the study physicians at no charge to you to treat symptoms due to the study medicines or procedures.

If you agree to participate, an ultrasound test will be performed to look for filarial worms in your body. This test involves putting some gel on your groin or chest and using a small machine to look inside your body with sound waves. The test takes about 15 minutes. It does not hurt and is not harmful in any way.

Six months after the beginning of the treatment part of the study, you will be asked to come to the clinic for a short history and physical examination. We will also draw one tube of blood (less

than10 cc or 2 tablespoons) at nighttime to check for worms and to repeat the testing done at the start of the study. If you are a woman of childbearing age, we will also check your urine to make sure that you are not pregnant. If you are not pregnant and in the group that is receiving medicine every 6 months, you will be given a second dose of medicine at this time. The morning that you receive the treatment, we will measure your temperature and ask you some questions about how you feel.

One year after the beginning of the study, you will be asked to come to the clinic again for a short history and physical examination. We will also draw one tube of blood (less than10 cc or 2 tablespoons) at nighttime to check for worms and to repeat the testing done at the start of the study. If you are a woman of childbearing age, we will also check your urine to make sure that you are not pregnant. Then if you are not pregnant, you will be given a second (or third) dose of medicine at this time. The morning that you receive the treatment, we will measure your temperature and ask you some questions about how you feel. If you had adult worms detected by ultrasound at the beginning of the study, the ultrasound test will be repeated to see if the worms are still alive.

Eighteen months after the beginning of the treatment part of the study, you will be asked to come again to the clinic for a short history and physical examination. We will also draw one tube of blood (less than10 cc or 2 tablespoons) at nighttime to check for worms and to repeat the testing done at the start of the study. If you are a woman of childbearing age, we will also check your urine to make sure that you are not pregnant. If you are not pregnant and in the group that is receiving medicine every 6 months, you will be given a fourth dose of medicine at this time. The morning that you receive the treatment, we will measure your temperature and ask you some questions about how you feel.

Two years after the beginning of the study, we will ask you to return to the clinic a final time for a short history and physical examination. , We will also draw one tube of blood (less than10 cc or 2 tablespoons) at nighttime to check for worms and to repeat the testing done at the start of the study. If you had adult worms detected by ultrasound at the beginning of the study, the ultrasound test will be repeated to see if the worms are still alive.

Risks

The risks associated with this study are few. Drawing blood may cause discomfort and occasional bruising at the site. Rarely, fainting or infection occurs as a result of drawing blood. We will clean your arm with alcohol before taking blood and will use new, sterile needles every time.

We expect some of those who are treated with albendazole and ivermectin to develop symptoms from the body reacting to dying worms (fever, headache, soreness in muscles or joints, tiredness, weakness, itching, diarrhea or nausea). We do not expect any serious reactions, and we expect all of them to resolve after a few days. Both of the medicines (albendazole and ivermectin) also kill many types of gut worms. You may pass some worms in your stool or develop small lumps from dead worms under your skin during the days after treatment.

Other than the reactions to dying worms, side effects from these medicines in the doses we are using are very rare. People who take albendazole every day occasionally develop abdominal pain, diarrhea, nausea, vomiting, dizziness, hair loss, fever and headaches. Albendazole may

damage babies in the mother if taken whilst pregnant. Laboratory tests for pregnancy will be carried out before each dose of albendazole is given. In addition, women of childbearing age are advised not to have sex or to use a contraceptive method of their choice for one month after taking albendazole. Pregnant women will not be given albendazole, but will continue to be followed until the end of the study.

Benefits

Albendazole and ivermectin are medicines that kill a variety of intestinal and tissue parasites. Although everyone in the community will eventually be eligible for treatment with albendazole and ivermectin as part of the campaign to eliminate lymphatic filariasis, if you have a parasite infection, including but not restricted to lymphatic filariasis, you will benefit from earlier treatment of these infections. If taking part in this study causes you any medical problems or makes you feel bad in any way, you will receive appropriate medical treatment at no cost to you.

Alternatives to Participating in the Study.

If you were found to be infected with the filarial parasite that causes lymphatic filariasis by the screening blood test but do not wish to participate in the treatment study, you will be offered treatment with albendazole and ivermectin. These are the same two medicines that will eventually be distributed by the government as part of a program to eliminate lymphatic filariasis in Malawi that is planned to start sometime in the next several years.

Early Withdrawal

You may withdraw from the study at any time. In addition, if you become pregnant, develop a serious medical illness, or if the study physician feels that it is not in your best interest to remain in the study, you may be withdrawn.

Reporting of Findings and Confidentiality

The findings of this study may be reported at scientific meetings or in medical journals, but your name will not be used in the report. Although your study records and medical information will be kept confidential, personnel from the Task Force for Child Survival or the GPELF or their representatives may review the study records. All unanticipated uses of your medical information will be reported to the Institutional Review Board in Malawi.

Do you have any questions about participation in this study?
If you have questions or concerns at a later date, you may speak with one of our staff or you can call The Karonga Prevention Study Office on 01364256 or 09971860 and speak with Dr Bagrey Ngwira

If you agree to participate in this study, please put your signature or thumb or fingerprint where indicated below.

Signature, Finger or Thumb Print	**Date**	**Name**
Witness Signature	**Date**	**Name**
Physician Signature	**Date**	**Name**

FEDE –ELIGIBILITY CRITERIA FOR FILARIA DOSAGE STUDY				
1	Fed subject ID:	|__|__|__|-|__|__|__|		fedid
	Subject's initials:	|___|___|		
2	Interview date:	|___|____|_____|		intdat
3	Spectset number			specset
	Inclusion Criteria (All answers must be "YES")			
3	Is the subject 18 to 55 years of age?	**Y**	N	inage
4	Is the *Wb* count above ≥80mf/mL?	**Y**	**N**	wbcnt
	Exclusion criteria (All answers must be "NO")			
7	Is the subject less than 18 years of age or more than 55 years of age?	**Y**	**N**	Excage
8	Is the subject a pregnant female?	**Y**	**N**	preg
9	Does the subject have Hgb less than 9g/dL?	**Y**	**N**	hgb
10	Does the subject use alcohol heavily (more than seven beers or other alcohol containing drink per week)?	**Y**	**N**	alcoh
11	Does the subject have a temperature ≥ 37.5 C or other serious medical condition?	**Y**	**N**	temp
12	Does the subject have a history of benzimidazole allergy?	**Y**	**N**	alballgy
13	Does the subject have a history of ivermectin allergy?	**Y**	**N**	Iverallgy
14	Has the subject used albendazole or ivermectin within the past six months?	**Y**	**N**	Iver/alb
	ENROLLMENT INFORMATION			
15	Is the subject eligible for enrolment?	**Y**	**N**	Enrol
16	Did the subject sign the treatment informed consent?	**Y**	**N**	Icf
17	Date subject signed	|___|____|_____|		consdat
	Assignment of study group			
18	Annual standard Dose	**Y**	**N**	annsd
19	Annual High Dose	**Y**	**N**	annhd
20	standard Dose Semiannual	**Y**	**N**	Semsd
21	High Dose Semiannual	**Y**	**N**	semhd
	Staff code …….. Signature……. Date…… coder…….. Checker…….			*Rcdr Cdr chkr*

	FEDF – FILARIASIS DOSAGE STUDY FOLLOW-UP FORM						
1	Fed subject ID:					__|__|__|-|__|__|__|	fedid
	Subject's initials:					___|___|	
2	Interview date:					___|____|_____|	intdat
3	Month of follow-up (0, 06, 12, 18, 24)					____|	mnthf
4	Specset number						specset
	Medical History (please tick that applies)						
	Symptom	**Now**	**≤ 1 month**	**>1 month**	**Never**	**comments**	
4	Lymphedema						lymphe
	If yes, where? (*LLE RLE LUE RUE other)*						whlym
5	Lymphangitis						lympha
6	Cellulitis						cellu
7	Elephantiasis						eleph
	If yes, where? (*LLE RLE LUE RUE other)*						whelep
8	Hydrocele						hydro
9	Hematuria						hematu
10	Chyluria						chylur
11	Pruritus						prurit
12	Fever/chills						fever
13	Headaches						heada
14	Vomiting						vomit
	If now, # of episodes in last 24 hrs						episod
15	Diarrhoea						diarr
	If now, blood?					**Y** **N**	blood
16	Abdominal pain						abdpai
17	Weight loss >5kg						wtloss
18	Jaundice						jaund
19	Respiratory symptoms						Resp
20	**If now, cough?**					**Y** **N**	cough
21	Other……						other
22	Heavy alcohol use						*alcohol*
	VITAL SIGNS						
23	Weight					__|__|__|.|__|	*wt*
24	Axillary temperature					__|__|.|__|	*temp*
25	Pulse/min					__|__|__|	*pulse*
26	Respiration rate/min					__|__|	*resp*
	PHYSICAL EXAM						
	Physical Sign	**Yes**	**No**	**Comments**			
27	Dehydration						*dehyd*
28	Icterus/jaundice						*jaund*
29	Lung Exam Abnormal **If yes add comments**						*lungs*
30	Cardiac Exam Abrnomal **If yes add comments**						*cardiac*
31	Hepatomegaly						*hepato*
33	Hydrocele						*hydro*
34	Skin rash **If yes add comments**						*skin*

35	Pitting edema				*edema*
36	Lymphedema			*Grade:* ***1 2 3 4 5*** *Site:* ***LLE RLE LUE RUE other***	*lymphe*
37	Lymphadenitis			*Site:* ***LLE RLE LUE RUE other***	*adenitis*
38	Elephantiasis			*Site:* ***LLE RLE LUE RUE other***	*elepha*
39	Other symptoms and signs **If yes, add comments**				
	LABORATORY TESTS				
40	At what time was venipuncture performed?			|__|__|: |__|__|	*venitm*
41	*Pregnancy Test*	**Y**	**N**	**NA**: reason……..	*preg*
42	*White Blood cell count*			……**cells/mm3**	*wbc*
43	*Eosinophil Count*			……**%**	*eosin*
44	*Heamoglobin*			……**g/dl**	*hb*
	CALIBRATED THICK SMEAR (NIGHTTIME)				
45	*W . bancrofti*	**Y**	**N**	If yes ……mf/mL	*wban*
46	*M. perstans*	**Y**	**N**	If yes ……mf/mL	*mpers*
	SPECIMEN COLLECTION				
47	*Was serum stored for other research tests?*	**Y**	**N**	If no, reason……	plasma
48	*Was whole blood for other research tests?*	**Y**	**N**	If no, reason……	wbloo
	DRUG ADMINISTRATION				
49	*Was drug administered?*			***Y*** ***N***	*drug*
	If yes, select the assigned study group and dosages given				
	Annual standard dose (albendazole (400mg)+ Ivermectin (200µg/kg) x wt(……kg)= … ***……..µg/1000= ……..mg*** ***Ivermectin tablets administered ……***			***Y*** ***N***	Annsd iver
	Annual high dose (albendazole (800mg)+ Ivermectin (400µg/kg) x wt(……..kg)= ***……µg/1000 = ……..mg*** ***Ivermectin tablets administered ……***			***Y*** ***N***	Annhd iver
	semiannual standard dose (albendazole (400mg)+ Ivermectin (200µg/kg) x ***wt(……kg)= … ……..µg/1000= ……..mg*** ***Ivermectin tablets administered ……***			***Y*** ***N***	Semsd iver
	semiannual high dose (albendazole (800mg)+ Ivermectin (400µg/kg) x wt(……..kg)= … ***……..µg/1000= ……..mg*** ***Ivermectin tablets administered ……***			***Y*** ***N***	Semhd iver
54	Lot Number: Albendazole				*lotalb*
55	Lot Number: Ivermectin				*lotivr*
	Staff code ……. Signature…….. Date…… coder……. Checker……				*Rcdr Cdr chkr*

FEDR- ICT RESULT SHEET

1	**FED- ID**						fedid
2	Initials						
3	Specset number						specset
4	Lab number						labno
5	**ICT test**	Date	……dd/mm/yyyy				datetest
		Time set	……**:**……Hrs				timeset
		Time read	……**:**……Hrs				timeread
6	**ICT result**	Test 1	**P**ositive	**N**egative	**F**aint	**I**nvalid	ictresult1
		Test 2 (**If test 1 = invalid**)	**P**ositive	**N**egative	**F**aint	**I**nvalid	ictresult2
7	Field staff code…….		Coder……		Checker……		Rcdr Codr Chkr

FEDI –FILARIASIS DOSAGE STUDY- IDENTIFIER

1.	FED – ID			fedid
	Identity			
2.	Name			name ident
3.	Sex	M male F female		sex
4.	Date of birth Birth decade estimate	<1910 1910-19 1920-29 1930-39 1940-49 1950-59 1960-69 1970-79 1980-89 1990-99 B C D E F G H I J K		birthest birthmm birthyea
5.	Name of mother			idmoth
	if alive elsewhere, name head of HH and village	HH: vge.:	**A**live **D**ied in year:	mothvita mothdyr
6.	Name of father			idfath
	if alive elsewhere, name head of HH and village	HH: vge.:	**A**live **D**ied in year:	fathvita fathdyr
7.	Village of birth			bvil
	Household and Residence			
8.	Current Household			Nameho
10.	Current Village			currvll
11.	GPS South **S**		GPS East **E**	gpssdeg gpsedeg

Additional identifiers

12	Have you been known by any other names in the past?			
13	Specify previous contact: **LEP1** **LEP2** **HC** **Control HH** year:			
14	Name HH & village or HC where last seen:			
15	If *not* seen before, why? **born** after LEP's **un**known **came** from: in year:			
16	List siblings alive in Karonga If no siblings are known alive in Kga.: List other members of the HH where last seen	*tick if same:* mother	father	
	(1)	☐	☐	
	(2)	☐	☐	
	(3)	☐	☐	

Field staff code…… Coder…….. Checker…….. Date……

Rcdr
Codr
Chkr
recdat

Fill in the non-marked sections for non-study samples only

GSP - SPECIMEN FORM – KPS (*One form per specimen*)

1a.	Name		name ident
1b.	Initials only		
2.	Sex	M male F female	sex
3.	Date of birth *or* Birth decade estimate	<1910 B; 1910-19 C; 1920-29 D; 1930-39 E; 1940-49 F; 1950-59 G; 1960-69 H; 1970-79 I; 1980-89 J; 1990-99 K	
4.	Name of mother		
5.	Head of household		
6.	Village		
7.	Study number		studyno
8.	Set (first) number + Specimen number		specset specno
9.	Where collected		hccode
10.	Accompanying form the form that this specimen form *belongs to* – this might not be the form that it is returned to the lab with		accform

11. **TB SPECIMENS ONLY** if sputum is collected for both TB work and bacteriology then fill 2 specimen forms, one for each test

11a. C CSF; F fresh/frozen lymph node biopsy; H formalin lymph node biopsy; N needle aspirate (unspecified); P pleural fluid; S sputum; U pus; W needle aspirate (Washing); Z needle aspirate (Z-N slide); other (specify) …… — spectype

11b. *Reason* 1 suspect 2 TB admission 3 scheduled review *2 4 6 mo* 4 Other …… — reascoll monthsrev

11c. TB lab no. — tblabno

12.. **OTHER SPECIMENS ONLY**

12a. *Collection Reason:*

ART	ART cohort	HSS	HIV Sero-survey	THI	TB HIV Immunlogy (incl control fup)
BIB	BCG Immunology Baby	LEP	Leprosy Suspect	TBX	TB Case only
BIM	BCG Immunology Mother	PSU	Paediatric Cohort	TXI	TB case & immunology
EUI	EU Immunology	PVC	Pneumo carriage study	TBO	TB Control only
ESC	EU sick control	ROP	Regular Outpatient	TOI	TB control & immunology
GBS	Group B strep study	GSC	Gates sick control	THP	Gates follow-up (TB case)
		FED	Filariasis study	LIS	Long term infection

reascoll — **B**aseline / Followup — basefoll

Is this a repeat sample? **Y N** — Rptsample

12b. *Specimen type: Refrigerate*
C Fresh Stool; D Naso-pharyngeal aspirate; E EDTA blood; G Sputum (bacteriology); T Throat swab; M Cheek cell swab; N Serum; P Paxgene; R Fluid (including CSF); V Saliva; W Per nasal swab; U Fresh Urine; Other _____

Specimen type: Ambient
A Finger prick; I EDTA immunology; H Heparin blood; L Blood culture; J Skin smear

— spectype

12c. Volume ml — vene

12d Cord blood **Y N** — cordblood

12e. Finger-prick taken **Y N** — fpind

12f. Lab no. — labno

12g. *Tests required; if not specified by study*_____ — testreq1/2/3

13. Post counselling required **Y N** — postcoun

14. If female, pregnant? (*relevant for prescribing)* **Y N** — preg

15. Date collected Time produced Time refrigerated/preserved Staffcode — dt/timecol timepres staff

16. Date received Time received in lab Staffcode receiver Checker — dateinlab labstaffco checker

KARONGA PREVENTION STUDY

WELLCOME TRUST LEPRA
PO Box 46, Chilumba (tel:08 263001)

Consent Form for HIV testing in the Filariasis study:

The Karonga Prevention Study is carrying out a study to determine the best dosage and schedule for the control of lymphatic filariasis. From research studies done on Malaria there is evidence that suggests impairment in clearance of malaria parasites following efficacious treatment in HIV infected individuals. It is plausible that this may also happen with LF. The KPS is therefore planning to investigate this in the current study

1. WHY ME?

You are being asked to give consent to have an HIV test as you have accepted to participate in the LF dosage study being undertaken by the KPS.

2. WHAT IS KPS TRYING TO LEARN?

As mentioned above KPS would like to find out how HIV affects clearance of microfilaria following treatment. Our current scientific knowledge suggests that HIV interfers with clearance of malaria parasites following therapy. It is possible that this might also happen in filariasis. This has not been tested before anywhere in the world. The results of this study will help the Malawi Government and others (with high HIV prevalence and endemic for filariasis) in deciding the best approach for the control filariasis.

3. WHAT WILL IT MEAN TO TAKE PART?

If you agree to have an HIV test, we will take a blood sample at the same time as we test for LF. Your HIV as well as LF result will be given to you on the spot. If you are HIV positive you will be referred to Kaporo ART clinic for staging and further management

6. WHAT ARE THE BENEFITS OF THIS PART OF THE STUDY?

You will know your HIV status. If you have HIV we will counsel you on how to look after yourself and keep strong, as well as how to prevent passing HIV on to other people. We will also refer you to Kaporo ART clinic where you can find out whether you need treatment.

7. IS THERE ANY RISK TO BEING IN THE STUDY?

Taking a blood sample may cause a little pain but this will soon go away. You may be worried about knowing you HIV status, but our trained counselors can help with this.

8. WHAT IF I DO NOT WANT TO TAKE PART?

You participation is voluntary. You are free to choose to be tested for LF only, or choose not to participate at all.

9. WHO WILL SEE THE INFORMATION THAT WE COLLECT?

Information about your HIV and treatment status is confidential. We will conduct interviews in private, we will not record your name on confidential information, and we will keep your records secure. KPS will tell the Government and local health services what they find, and will also publish these findings as scientific papers, but they will never let people know your name.

We would like you to answer the following to see if you will participate:

	The information has been read to me; I have had the opportunity to ask questions, and my questions have been answered satisfactorily. I understand that the information about HIV and access to care services is confidential and I understand that I can withdraw from the study at any time.		
1	I **AGREE** voluntarily to take part in this study. (**Y**es, **N**o)	**Y N**	consent
	I understand that I will have blood taken for HIV and LF testing by KPS. The results of the test will be confidential.		
3	I **AGREE** to have blood taken for HIV testing. (**Y**es, **N**o)	**Y N**	consbl
4	I **WANT** to know the result. (**Y**es, **N**o)	**Y N**	wantres

(Signature/thumbprint of participant)	counsellor signature rcdr..... .....codr chckr

Fill in only the marked sections for laboratory testing

RTF - RAPID TEST FORM – KPS

Version 5a
10/10/2008

9.	Date of interview	*(DD/MM/YYYY)*		intdate
10.	Reason for form	BIM BCG Imm Mother; TBX TB Case Only; FED Filariasis HSS HIV Serosurvey; TXI TB Case & Imm; LIS Latent TB EUI EU Imm / ESC; TBO TB Control only; PSU Under 5s THI TB HIV Imm.; TOI TB Control & Imm; PVC Pneumo vaccine TBR TB Review sputum; TBF TB Annual F'up	SEX	Reasrt sex
3a.	Study number			studyno
3b.	Specimen set number			specset

ARV and PROPHYLAXIS

4a.	Have you ever had an HIV test?	**Y**es **N**o **U**nknown **R**efuse to answer	**Y N U R**	evertest
4b.	***If yes***, what was the result?	**P**ositive **N**egative **U**nknown **R**efuse to answer	**P N U R**	testout0
5.	Have you ever been referred for ARV therapy?	**Y**es **N**o **U**nknown **R**efuse to answer	**Y N U R**	Refever
6a.	Have you ever taken anti-retrovirals? (ARV, e.g. Triommune, Duovir, Nevirapine)	**Y**es **N**o Yes, **O**nly during pregnancy **R**efuse to answer	**Y N O R**	Arvever
	If Yes 6b. When did you start taking ARVs? *(DD/MM/YYYY)*			Arvstart
	6c. Do you still take ARVs?		**Y N**	Arvcurr
	6d. ***If No,*** when did you stop taking ARVs? *(DD/MM/YYYY)*			arvstop
7a.	Have you ever taken co-trimoxazole prophylaxis?		**Y N U**nknown	cotriever
	If Yes 7b. When did you start taking co-trimoxazole prophylaxis? *(DD/MM/YYYY)*			cotristart
	7c. Do you still take co-trimoxazole prophylaxis?		**Y N**	cotricurr
	7d. ***If No,*** when did you stop taking co-trimoxazole prophylaxis? (DD/*MM/YYYY)*			cotristop
8a.	Have you ever taken isoniazid prophylaxis?		**Y N U**nknown	INHever
	If Yes 8b. When did you start taking isoniazid prophylaxis? *(DD/MM/YYYY)*			INHstart
9.	Informant	Self / Other; Relationship		relinform

RAPID TEST RESULTS

10.	Has the participant accepted testing? (***Z*** *when Not Applicable*)				**Y N Z**	acceptrtr
11.	**TEST 1 (Determine)**	**R**eactive	**N**on-reactive	**I**nvalid	**R N I**	testout1
12.	**TEST 2 (Unigold)**	**R**eactive	**N**on-reactive	**I**nvalid *(not done if test 1 is N)*	**R N I**	testout2
13.	**RAPID TEST RESULT**	**P**ositive (Both tests reactive)	**N**egative (First test non-reactive)	**I**nvalid (Both tests invalid)	**D**iscrepant (Any other combination, go to test 3: tie-breaker)	**P N I D**	Rtr1
14.	**TEST 3 (Bioline)** Tie-breaker *only for **D**iscrepant results*	**R**eactive	**N**on-reactive	**I**nvalid	**R N I**	testout3
15.	**TIE-BREAK RESULT:**	**P**ositive (2 out of 3 tests reactive)	**N**egative (2 out of 3 tests non-reactive)	**I**nvalid (2 out of 3 tests invalid)	**D**iscrepant (Any other combination)	**P N I D**	Rtr2
16.	Date of testing *(DD/MM/YYYY)*					*Rtrdate*
17a.	Was participant given result?				**Y N**	Rtrgiven
17b.	Was participant referred to HIV clinical services?				**Y N**	refserv

Staff code		Lab staff code		Coder/Checker	*Rcdr labsc chkr codr*

Date of interview	__/ __ / ____ intdate
Was subject present?	Y N present
hssstudyno (pink):	**specset (white)**

Round rd

KARONGA PREVENTION STUDY

WELLCOME TRUST LEPRA
PO Box 46, Chilumba (te:08 263001)

HIV SEROSURVEY & ADULT BEHAVIOUR SURVEY: KPS CONSENT FORM

1. WHY ME?

We are asking you to take part in a study being done by the Karonga Prevention Study ("LEPRA") to help improve the health of your community. The study is about HIV.

2. WHAT IS HIV?

HIV causes AIDS, but now there is free treatment called "antiretroviral (ARV) therapy". The treatment helps people stay healthy for longer.

3. WHAT IS KPS TRYING TO LEARN?

Firstly, we would like to find out how many people are affected by HIV and whether this number is changing. Secondly, we want to find out more about the sexual behaviours that may put people at risk, and about whether and when people want to have children. Finally, we want to find out who needs treatment. This will help improve services for your community.

4. WHAT WILL IT MEAN TO TAKE PART IN THE STUDY?

If you are 15 years or older and agree to take part in our study, we will first ask you a few questions about marriage, other sexual relationship, your use of condoms, and your plans for children. Then we will discuss the HIV test with you, and what it means to know your result. If you agree to a test we will take a little blood from your arm. If you prefer we can do this with a finger-prick. If you want to know the result we will tell you immediately.

5. What will Happen to my blood?

We will take the blood sample to the laboratory so we can do further HIV-related tests at KPS to confirm your test result, and tell us more about the HIV virus, and another infection called HSV-2 that may be important in the spread of HIV in the community. If we understand HIV better then we can help stop the infection spreading, so even if you do not want to know the result of your HIV test we would still like a blood sample.

We would also like to store your blood for possible later testing for other diseases related to HIV or of importance in Karonga District. Further testing will only be done if approved by the Malawi Ministry of Health. We will let you know if you could benefit from the results of these tests. You can choose at any time to have your stored blood destroyed.

6. What are the benefits to being in the study?

There are many advantages to knowing whether or not you have HIV (your HIV status). If your test shows us you have HIV we will counsel you on how to look after yourself and keep strong, as well as how to prevent passing HIV on to other people. We will also refer you to the hospital where you can find out whether you need treatment – a trained clinician will help you there. Not everyone needs treatment immediately; but for pregnant women this is especially important, as there is also treatment to help prevent their babies from getting HIV. If you do not have HIV you will have more reason to take care and remain negative.

7. Is there any risk to being in the study?

Taking a blood sample may cause a little pain but this will soon go away. You may be worried, but our trained counselors can help. We strongly encourage everyone to be tested, since knowing the results can help them protect themselves and their partners from infection, and also help them plan for their own and their families' future.

8. What if I do not want to take part?

The choice is yours

9. Who will see the information that we collect?

Information about your HIV and treatment status is confidential. We will conduct interviews in private, we will not record your name on confidential information, and we will keep your records secure. KPS will use the information to find out more about HIV and tell the health services what they find, but they will never let people know your name.

10. Support Available for People Affected by HIV/AIDS

We will talk to you about support available for you and your family regardless of what your results show. Your KPS counsellor can also tell you about local HIV/AIDS prevention, care and support activities and where you can find help locally. If you want an HIV test but not as part of our study you can go to Chilumba Rural Hospital VCT Centre; people who are HIV positive will also be seen there by a trained medical professional at Mwabi Clinic who will ensure you have the necessary care. Staff at the VCT centre and at Mwabi will tell you what to do next. Ask your counsellor or telephone us if you want to find out more.

We would like you to answer the following to see if you will participate:

	The information has been read to me; I have had the opportunity to ask questions, and my questions have been answered satisfactorily. I understand that the information you collect is confidential and I understand that I can withdraw from the study at any time without it affecting my right to appropriate medical care.		
1	I **AGREE** voluntarily to take part in this study. (**Y**es, **N**o)	**Y N**	consent
	I understand that, I will be asked about marriage, other types of sexual relationship, my use of condoms, and my plans for children so that KPS can find out more about the behaviours that put people at risk of becoming infected. The answers are confidential.		
2	I **AGREE** to take part in the KPS behavioural study. (**Y**es, **N**o, **I**neligible)	**Y N I**	consbeh
	I understand that I will have blood taken for HIV and related testing by KPS. The results of the test will be confidential. I understand that I can choose whether I am told the result. If I am found to be HIV positive I understand that I will be helped further and will receive treatment if necessary.		
3	I **AGREE** to have blood taken for HIV and related testing. (**Y**es, **N**o)	**Y N**	consbl
4	I **WANT** to know the result. (**Y**es, **N**o)	**Y N**	wantres
	I understand that my blood will be stored for possible later testing for other diseases related to HIV or of importance in Karonga District. I understand that further testing will only be done if approved by the Malawi Ministry of Health, and that I can choose at any time to have my stored blood destroyed.		Rsnbloo d
5	I **AGREE** to my blood being stored for later testing (**Y**es, **N**o)	**Y N**	consblstore

Crsno:	repgp	cluster	hhlistno	member	visitor

Name:	**Sex:**	ident

(Signature/thumbprint of participant)	counsellor signature
	rcdr..... .....codr chckr

If consent to give blood is refused ask the subject the main reason for refusal

01	*no reason*	***06***	*already tested & knows status*
02	*family/community decision*	***07***	*thinks knows status*
03	*confused/incapable of consenting*	***08***	*prefers clinis*
04	*afraid of needles/fingerprick*	***09***	*does not think he/she is at risk*
05	*afraid for other reasons*	***10***	*other*

SEI – INDIVIDUAL SOCIO-ECONOMIC SURVEY – KPS

22/09/2010 Version 5

1.1.	**Round**	**4**	**Interview Date** (DD/MM/YYYY)			rd intdate
1.2.				RG	Sess:	repgp sess

Identity

1.3.	GHHID			ghhid
1.4.	CRS number			crsnum
1.5.	Name:			name ident
1.6.	Sex	**M** **F**	Birth date	sex birthdat birthyr
1.7.	**Informant type**	**S**elf **P**arent **1** spouse, sibling, child **2** grandparent, grandchild, other relative **N**on-relative	**S** **P** **1** **2** **N**	infmtype
1.8.	**Name of informant** **(If Self, write Self)**			informtid

Current household

1.9.	Relationship to household head	**1** self **2** spouse **3** child **4** grand-child **5** niece/nephew **6** sibling **7** cousin **8** parent **9** aunt/uncle **13** step-child **14** step grand-child **15** parent-in-law **16** grandparent **10** family friend **11** other relative **12** other non-relative	relhh
1.10.	Was subject seen?	**Y** **N**	seen
1.11.	When was (s)he last here?	**0** today; **1** yesterday; **2** in last 7 days; **3** in last 4 wks; **4** more than 4 wks ago	when

Parents survival status and education (ask only of individuals aged ≤30 years old)

1.12.	Is your father alive? **If no,** when did he die?	**Y** **N** **DK**	Is your mother alive? **If no,** when did she die?	**Y** **N** **DK**	fathvita mothvita
1.13.		Year died		Year died	fathdyr mothdyr
1.14.	Did your father go to school? **If yes,** highest level attended by father	**N**one **P**rim **S**ec **T**ert **U**nknown	Did your mother go to school? **If yes,** highest level attended by mother	**N**one **P**rim **S**ec **T**ert **U**nknown	fschltype mschltype

IF SUBJECT IS AGED UNDER 5 YEARS THEN SKIP → Q39, IF SUBJECT IS AGED 5-11 THEN SKIP → Q22

Marital Status (ask only if aged 12+ years old)

1.15.	N never married M married D divorced/septd W widowed		If never married skip→ **Q22**	marital
1.16.	How many spouses do you have now?			spousenum

Spouses

For individuals who are currently married, use Columns 1-4 to contain information on <u>current</u> spouses.

For individuals who are divorced/separated, use Column 1 for the most recently divorced/separated spouse.

For individuals who are widow(er)s, use Column 1 for the spouse who died most recently.

For individuals who are divorced/separated, or widowed, fill Q17 (name), Q18 (NA if widowed), and Q20. Fill Q19 (the spouse CRS number) only if the spouse was previously seen in the CRS in <u>this</u> household.

		Column 1		Column 2	Column 3	Column 4	
1.17.	Name						spname1-4
1.18.	Residency in CRS area	**Y** **N** **DK** **NA**		**Y** **N** **DK**	**Y** **N** **DK**	**Y** **N** **DK**	sparea1-4
1.19.	crsno						spcrsno1-4
1.20.	Year marriage start and end	Start	End	Start	Start	Start	spmarstrt1-4 spmarend1-4
1.21.	ident						spident1-4

Fill spouse ident in office - use GP form for <u>current</u> spouse if not co-resident and not in CRS area, and attach (tick)

Education and Occupation and Economic activities

1.22.	Have you ever been to school?	**N**ever **E**ver **C**urrent	schlever
1.23.	What is the highest level of education attended?	**P**rim **S**ec **T**ertiary — form/standard	schltype schlstd
1.24.	What is your main occupation? (**Specify** occupation with a salary or if unsalaried the occupation with most income)	Emp / Occ	emp occ

Ask Q25-26 only of individuals aged 5-20 years old

1.25.	During the last 4 weeks, did you participate in any economic activities? For example farming, fishing, gathering natural products, piece work, preparing and selling food or beverages, selling goods manufactured by this household, providing a service?	**Y N**	hhecyn
1.26.	If yes, which ones? (record the two most important activities, in order of importance)	ec1 / ec2	ecactivity1 ecactivity2

For individuals >30 years old, skip → Q46. For individuals who have never been to school (Never), skip →Q46
For individuals who have been to school, but are not currently enrolled in school (Ever), skip →Q27
For individuals who are currently enrolled in school, skip → Q28 and then continue with Q29-38

1.27.	What is the reason that you left school? (record the two most important reasons, in order of importance) *Do not read out possible answers. Record the reasons given, without "prompting".* ***Now continue with Q28-Q30***	rsn1 / rsn2	rsnleft1 rsnleft2
1.28.	What is the highest educational qualification you have acquired?		edqual
1.29.	Age, or year, first started primary school	(age) / (year) / don't know	aschstart yschstart
1.30.	Age, or year, left school (Z if still in school)	(age) / (year) / don't know / Z	aschleft yschleft

Now ask Q31-38 if individual is currently enrolled in school. If not currently enrolled in school, skip → Q37

1.31.	Name of school		schlname
1.32.	Who are the fees primarily paid by?	**1 parent**/step-parent **2** aunt/uncle **3** Sibling **4** Grandparent **5** Other relative **6** Non-relative **7** Organisation **8** Not applic **9** DK **10** Self **11** Government	feespaid
	Have you attended school during the last 4 weeks that your school was in session?	**Y N**	schlattd
1.33.	If yes: During the last 4 weeks that your school was in session, how many school days / weeks did you miss? ***(now skip → Q35 if 0 days missed. If has not attended school in last 4 weeks must still ask Q34)***	days / wks	schlday schlwk
1.34.	What were the 2 most important reasons that you missed school, during the last 4 weeks that your school was in session?	rsn1 / rsn2	rsnattd1 rsnattd2
1.35.	At any time in the last 12 months, did you ever miss >2 weeks of school at one time (consecutive)?	**Y N**	schlbrk
1.36.	Have you attended your current standard/form before? If yes, how many times (including this year)?	**Y N**	cstdrep cstdnum
1.37.	Did you ever leave school for at least 12 months, and later return?	**Y N**	schleave
1.38.	**If yes (to Q37):** Age first left	Form / Standard when first left: P S T _____ Number of years absent: (years) — Reason first left	leftage lefttype leftstd lftyrs ceftrsn
1.39.	**Vaccine history for children <5 years old. If 5+ years old, *skip →Q46***	**Health passport available? Y N**	cardseen

		Vaccines recvd.	Vacc. date	Vacc. HC			Vacc. date	Vacc. HC	
1.40.	BCG	**Y N**			Polio0	**Y N**			bcgdate p0date
1.41.	Penta1	**Y N**			Polio1	**Y N**			dpthh1date p1date
1.42.	Penta2	**Y N**			Polio2	**Y N**			dpthh2date p2date
1.43.	Penta3	**Y N**			Polio3	**Y N**			dpthh3date p3date
1.44.	Measles	**Y N**			No of doses Vit A				Measdate vitados
1.45.	Record dates of the first two doses vit A		**1st**				**2nd**		Vitadat1 Vitadat2

Health and Caring (ask of individuals aged > = 10 years old)

1.46.	During the last 3 months, for how many days/weeks have you cared for a sick **adult** (aged 15 or above) at home or at a health facility? (record in EITHER days or weeks, but not both)	(days) / (weeks)	sdaycare3 sweekcare3
1.47.	During the last 3 months, for how many days/weeks have you been unable to work and/or attend school due to your own illness? (record in EITHER days or weeks, but not both)	(days) / (weeks)	illdays illweeks

Clothing expenditure (money spent by this household on clothes and shoes, including school uniform. Gifts not included)

1.48.	Over the last 12 months, how much money has been spent on clothing **for you**?	**0** K 0 **1** K 1-500 **2** K 501-1000 **3** K 1001-2000 **4** K 2001-5000 **5** K 5001-10000 **6** K >10000 **7** DK	clothexp
1.49.	Over the last 12 months, how much money has been spent on footwear **for you**? (incl. slippers)	**0** K 0 **1** K 1-250 **2** K 251-500 **3** K 501-1000 **4** K 1001-2000 **5** K 2001-5000 **6** K >5000 **7** DK	shoesexp
1.50.	**Mass drug treatment for worms**: Did you receive tablets for filariasis during the mass treatment campaign in Oct/Nov2009?	**Y N U**	massfila
1.51.	**Measles – ask if >8months &<15 years old**: Did you receive measles vaccine during the mass vaccine campaign in 2010?	**Y N U**	massmeas
1.52.	**TB case finding, if individual is seen: do you have a cough?** **Y N** — Duration of cough (weeks)		cough couweeks
1.53.	Haemoptysis? **Y N** *If cough >3 weeks / haemoptysis / TB suspect on other grounds fill GP form and collect sputum*	tbsuspect **Y N**	haemopt tbsuspect

END Field-Staffcode Coder /L1 Checker rcdr codr,chkr

Trop-Ag *W. Bancrofti*

Boiling Treatment

Five plate kit

ELISA KIT FOR DETECTING AND QUANTIFYING *Wuchereria bancrofti* ANTIGEN in SERUM or PLASMA

480 test Kit

Catalogue No 03-010-01

Lymphatic filariasis

An antigen detection assay has been developed by **TropBio** and James Cook University of North Queensland for the detection of *Wuchereria bancrofti* (Bancroftian filariasis) infection in man. This assay has been marketed to fulfil a perceived need in the testing of human patients suspected of being infected with this parasite.

Lymphatic filariasis in man is caused by infection with the filarial parasites *W. bancrofti and Brugia spp.* These parasites inhabit the lymphatics and cause disease by obstruction and secondary inflammatory changes to lymph vessels. The disease is transmitted by mosquitoes which ingest microfilariae during feeding on an infected host and transmit the infective larvae to other individuals at a subsequent feeding.

Throughout the world, more than 90 million people are affected by lymphatic filariasis. Most live in the humid tropics in areas such as Africa (south of the Sahara), Egypt, the Indian subcontinent, South-East Asia, China, Madagascar, Papua New Guinea, the Pacific Islands, the Philippines and Central and South America (World Health Organisation, 1984).

Acute symptoms of lymphatic filariasis primarily involve lymphadenitis and lymphangitis. Recurring fever and pain of affected lymph nodes are the normal sequelae. In some patients, symptoms may be less specific, with fever and malaise being the only symptoms. Some infected individuals are asymptomatic.

Lymphoedema becomes apparent after repeated episodes of lymphadenitis, and swelling of the limbs or scrotum may occur. Lymphoedema and elephantiasis may affect the leg, arm and scrotum and occasionally the vulva and breasts, but they are usually restricted to the leg below the knee.

With brugian filariasis, more severe inflammatory changes are noted in the lymphatics, whereas bancroftian filariasis has a more extensive swelling of the entire limb.

Filariasis in previously unexposed migrants to an endemic area has a similar clinical course, but can be manifested earlier (6 - 8 weeks) than the normal 7 - 8 month clinical incubation period. Microfilaraemia is an uncommon finding in these migrant individuals, reflecting an intense immunological reaction to the parasite (Partono *et al.*, 1977).

References

More, S.J., and Copeman, D.B. (1990) A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. *Tropical Medicine and Parasitology* **41:** 403- 406

Partono, F., Oernijati, S. and Hudojo, I. (1977) Malayan filariasis in Central Sulawesi (Celebes), Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health* **8:** 452-458

Turner P., Copeman B., Gerisi, D. and Speare R (1993) A comparison of the OG4C3 antigen capture ELISA, the Knott test, an IgG_4 assay and clinical signs, in the diagnosis of Bancroftian filariasis. *Tropical Medicine and Parasitology* **44:** 45-48

World Health Organisation (1984) Lymphatic Filariasis. *Fourth Report of the WHO Expert Committee on Filariasis*, Geneva. WHO Technical Services 702

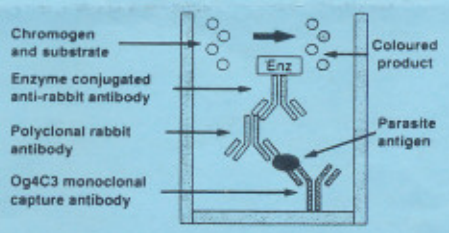
Notes on the assay

This kit has been designed to be used in conjunction with serum or plasma samples. Samples are boiled in an EDTA solution and centrifuged. This treatment has been shown to dissociate antigen/antibody complexes. The target antigen is heat stable and is retained in the supernatant. Following treatment there can be up to a fourfold rise in antigen titre.

Test samples can be handled in a 96 well microtitre format using racked tubes (Catalogue No 05-002-13). The racks are modified to allow them to be placed into a boiling water bath. Subsequently the racks can be centrifuged in a rotor with microtitre buckets at approximately 2,000 *g* for 15 minutes. Additional tubes are available in bags of 1,000 (Cat No: 05-002-03).

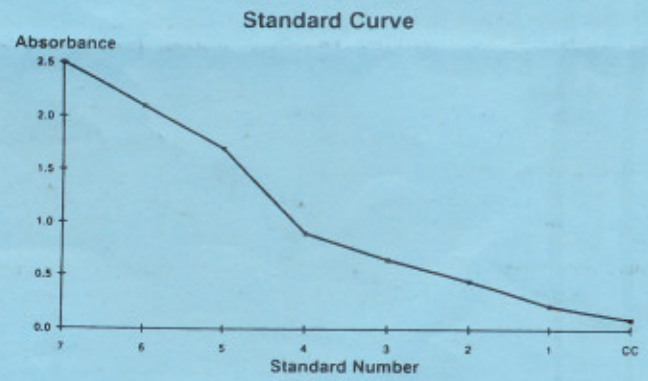
The 96 well microtitre plates supplied are coated with a monoclonal antibody(Og4C3) which has been shown to specifically recognise only *Wuchereria bancrofti* antigen in human sera. Og4C3 will not cross react with human sera infected with *Onchocerca volvulus*, *Brugia malayi*, *Brugia timori*, *Loa loa*, *Mansonella perstans*, *Strongyloides stercoralis*, *Dracunculus medinensis* or *Ascaris lumbricoides*. The cattle parasite *Onchocerca gibsoni* is recognised by this monoclonal antibody and is used to standardise the ELISA.

ELISA configuration (antigen detection) for *W. bancrofti*



The indicator antibody is produced by vaccinating rabbits with purified *Onchocerca gibsoni* antigen. Finally the rabbit globulin is detected by goat anti-rabbit globulin conjugated to horseradish peroxidase. The substrates solution which contains the chromogen ABTS produces a green colour which has maximum absorption at 414 nm.

A typical standard curve for the seven standard antigens is shown below. These values are used to allocate the test samples into one of eight titre groups according to the table shown below.



ELISA KIT FOR DETECTING AND QUANTIFYING *Wuchereria bancrofti* ANTIGEN in serum or plasma

Boiling pretreatment

Catalogue No. 03-010-01

Manufactured by TropBio Pty Ltd
James Cook University, Townsville
Queensland, Australia 4811
ABN: 97 051 617 424

This kit contains sufficient reagents for 480 tests.

CONTENTS

1. **Microtitre Plates** (in Silver foil pouches)
   Five U-bottom polystyrene microtitre plates pre-coated with Og4C3 monoclonal antibody.

2. **Sample diluent** (Clear solution)
   One 120 mL bottle of diluent at working strength for adding to samples prior to boiling.

3. **Antibody and conjugate diluent**
   (Blue solution) One 100 mL bottle.

4. **Standard antigens (1-7)** (Orange cap)
   Seven dilutions of *Onchocerca gibsoni* antigen. Each vial contains 800 µL of standard.

5. **Rabbit anti-onchocerca antibody**
   One 350 µL bottle (Yellow cap).

6. **Anti-rabbit HRPO Conjugate**
   One 350 µL bottle (Purple cap).

7. **ABTS Chromagen** (Amber bottle)
   One 60 mL bottle of single component ABTS ready to use.

8. **Washing buffer** (Twin-neck bottle)
   One 250 ml ×20 concentrate.

9. **Wash Bottle** 500 ml (optional)

METHOD

All steps carried out at room temperature.
Ensure that all reagents and the microtitre plates are at room temperature before use.

Quantities indicated below refer to those required for the use of **ONE** plate.

To prepare wash buffer, dispense **TWO** full measures (or 25 ml) of x20 wash buffer from the dispensing bottle into a 500 ml wash bottle and fill to the mark with distilled water

1. **Preparation of serum/plasma samples.**

   Add 100 µL of each test sample to 300 µL of sample diluent in a suitable tube. Eg racked tubes in a 96 well microtitre format (Cat. No 05-002-13).

   Alternatively, Eppendorf microcentrifuge tubes can be used (pierce the lid with a fine needle to allow air to escape during boiling).

   Place the tubes into a 100°C boiling water bath for five minutes.

   After boiling, centrifuge the samples at 2,000g for 15 minutes (racked tubes) or 10,000g for five minutes (Eppendorf tubes). The clear supernatant fluid contains the heat stable antigen.

Add 50 µL aliquots of supernatant fluid to a test well. Up to 80 samples can be tested per plate (see the plate layout diagram).

2. **Adding Standard Antigens and Conjugate Control to columns 11 & 12**

Following the plate diagram shown below, add 50 µL per well of each standards (1-7) in duplicate.
Do not dilute or boil.
For conjugate control (CC), add 50 µL of the sample diluent to wells A11 and A12.

3. **Incubation of Test Samples**

Place the plate in a humid container and incubate for at least 1.5 hours at room temperature. Plates may be incubated overnight to increase sensitivity.

4. Wash the plate three times with wash buffer, invert and tap gently to remove residual droplets.

5. **Addition of Rabbit anti-*Onchocerca* Antibody**

Dilute by adding 50 µL of rabbit anti-*Onchocerca* antibody (Yellow cap) to 6 mL of antibody diluent (Blue solution).
Add 50 µL of diluted rabbit antibody to all wells and incubate for one hour.

6. Wash the plate three times as before.

7. **Addition of Anti-rabbit HRPO**

Dilute by adding 50 µL of Anti-rabbit HRPO conjugate (Purple cap) to 6 mL of antibody diluent.
Add 50 µL of diluted conjugate to all wells and incubate for one hour.

8. Wash the plate three times as before.

9. **Addition of ABTS Chromagen**

Add 100 µL of ABTS (**do not dilute**) to each well and incubate for one hour.

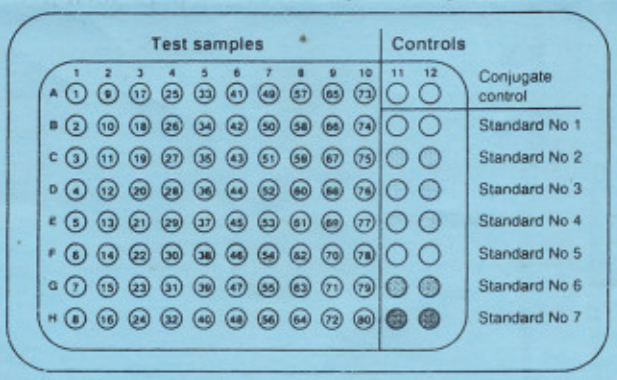
10. **To Read the Reaction**

Plates can be read with a spectrophotometer at a wavelength between 405 - 414 nm, or dual wavelengths of 405 - 414 and 492 nm.

Blank the plate reader on wells containing conjugate control or a row of wells containing substrate in a separate blanking plate.

This kit is to be used for *in vitro* testing purposes only. All components must be disposed of by autoclaving at the completion of the testing.

Standard ELISA plate layout



INTERPRETATION OF RESULTS

If the optical density for the high titre control is less than 1.1 or the optical density for the negative control (Standard No 1) is more than 0.3 the test results should be regarded as unreliable and the test should be repeated.

Please note that there can be quite large differences between plate readers. The result will also be influenced by the choice of filter (a 414 nm filter will produce the highest results). The absorbance indicated by the plate reader may also change as the filters deteriorate with age.

Seven control samples are used in duplicate on all plates. These samples are produced using parasite antigen extracted from *Onchocerca gibsoni* nodules. Control sample No 1 contains no parasite antigen. Control samples No 2 to No 7 all contain parasite antigen.

Sample No 2 is at the limit of the sensitivity of the assay. However, it will consistently produce an absorbance higher than the control sample No 1. Very few serum samples will react with a higher absorbance than control sample No 7.

Using these seven control samples it is possible to allocate the test samples into eight titre groups according to the following table. The titre groups are very useful for population studies. Moore and Copeman (1990) allocated antigen units to the seven controls.

Allocation of samples to titre groups			
Titre group	**Absorbance**	**Standard No**	**Antigen units**
1	< Control sample No 1	1	<10
2	≤ Control sample No 2	2	32
3	≤ Control sample No 3	3	128
4	≤ Control sample No 4	4	512
5	≤ Control sample No 5	5	2,048
6	≤ Control sample No 6	6	8,192
7	≤ Control sample No 7	7	32,000
8	> Control sample No 7		

A total of 308 samples from uninfected patients from the Townsville region reacted with the following distribution. Mean OD = 0.149. Standard deviation = 0.019.

308 Australian samples	
Titre group	**Number of samples**
1	306
2	2
3 or more	0

The test samples allocated to titre groups 1 and 2 can be considered to be non-reactors (negative). Samples allocated to titre groups 4 to 8 can be considered to be reactors (positive).

Samples allocated to titre group 3 can be considered to be equivocal or suspect reactors. In a previous study in New Guinea, Ghana, the Philippines and India this group represented up to 10% of the test samples. None of the Australian samples from an uninfected population were allocated to group 3. It is very likely that samples allocated to group 3 are reacting in the assay. Further data will be collected on this group to determine their status.

Further Reading:

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Lalitha, P., Ravichandran, M., Suba, S., Kalilaj, P., Narayanan, R.B. and Jayaraman, K. (1998) Quantitative assessment of circulating antigens in human lymphatic filariasis: a field evaluation of monoclonal antibody-based ELISA using blood collected on filter strips. *Tropical Medicine and International Health* 3:41-45

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Expiry Date

FEB 2012

LI_BoilPlate 08.wpd_Nov 2008

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Human Immunodeficiency Virus, Antiretroviral Therapy and Markers of Lymphatic Filariasis Infection: A Cross-sectional Study in Rural Northern Malawi

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Citation: Tafatatha T, Taegtmeyer M, Ngwira B, Phiri A, Kondowe M, Piston W, et al. (2015) Human Immunodeficiency Virus, Antiretroviral Therapy and Markers of Lymphatic Filariasis Infection: A Cross-sectional Study in Rural Northern Malawi. PLoS Negl Trop Dis 9(6): e0003825. doi:10.1371/journal.pntd.0003825

Editor: Thomas B. Nutman, National Institutes of Health, UNITED STATES

Received: October 13, 2014

Accepted: May 12, 2015

Published: June 4, 2015

Data Availability Statement: The data for this study comes from the Karonga Prevention Study (KPS) which has been conducting research in Karonga district, rural northern Malawi for more than 30 years. KPS is fully committed to making its data fully accessible and available to other researchers and fully support the PLOS data sharing policy. To this effect, arrangements for setting up an institutional data repository are in the process through funding from the Wellcome Trust for a four-year project. This will make our extensive data (on ~300,000 individuals

Abstract

Background

Lymphatic filariasis (LF) and human immunodeficiency virus (HIV) are major public health problems. Individuals may be co-infected, raising the possibility of important interactions between these two pathogens with consequences for LF elimination through annual mass drug administration (MDA).

Methodology and Principal Findings

We analysed circulating filarial antigenaemia (CFA) by HIV infection status among adults in two sites in northern Malawi, a region endemic for both LF and HIV. Stored blood samples and data from two geographically separate studies were used: one a recruitment phase of a clinical trial of anti-filarial agent dosing regimens, and the other a whole population annual HIV sero-survey. In study one, 1,851 consecutive adult volunteers were screened for HIV and LF infection. CFA prevalence was 25.4% (43/169) in HIV-positive and 23.6% (351/1487) in HIV-negative participants (p=0.57). Geometric mean CFA concentrations were 859 and 1660 antigen units per ml of blood (Ag/ml) respectively, geometric mean ratio (GMR) 0.85, 95%CI 0.49-1.50. In 7,863 adults in study two, CFA prevalence was 20.9% (86/411) in HIV-positive and 24.0% (1789/7452) in HIV–negative participants (p=0.15). Geometric mean CFA concentrations were 630 and 839 Ag/ml respectively (GMR 0.75, 95%CI 0.60-0.94). In the HIV-positive group, antiretroviral therapy (ART) use was associated with a lower CFA prevalence, 12.7% (18/142) vs. 25.3% (67/265), (OR 0.43, 95%CI 0.24-0.76). Prevalence of CFA decreased with duration of ART use, 15.2% 0-1 year (n=59), 13.6% >1-2 years (n=44), 10.0% >2-3 years (n=30) and 0% >3-4 years treatment (n=9), p<0.01 χ^2 for linear trend.

and > 1 million participant contacts) for the whole project more accessible, and will ensure that, as we continue to add to this unique resource, it remains usable, flexible and available to local and international researchers. This is a large undertaking and involves making major changes to the data structure, the user interface and the way new data are collected, whilst maintaining the integrity and relationships in the existing database. Meanwhile, a procedure for data sharing is in place, which requires the consent of the programme director in consultation with senior scientific staff. Applications to access the data can be made to the Deputy Director/ Scientific Programme Manager: mia.crampin@lshtm.ac.uk (http://www.lshtm.ac.uk/eph/ide/research/kps/index.html). No reasonable data sharing requests are turned down and the programme is committed to ensuring as much access as possible to the data, while maintaining full confidentiality.

Funding: Study 1 was supported by an award from the Task Force for Child Survival, Emory University (Grant No 43922) and Study 2 was supported by a grant from The Wellcome Trust (Grant No 079828/Z/06/Z). Additional analysis conducted on stored samples from Study 1 and 2 was funded by the Centre for Neglected Tropical Diseases (CNTD), Liverpool School of Tropical Medicine, with a grant from the Department for International Development. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Conclusions/Significance

In this large cross-sectional study of two distinct LF-exposed populations, there is no evidence that HIV infection has an impact on LF epidemiology that will interfere with LF control measures. A significant association of ART use with lower CFA prevalence merits further investigation to understand this apparent beneficial impact of ART.

Author Summary

Lymphatic filariasis (LF) and HIV are both major public health problems worldwide and where they co-exist have the potential to interact. The main strategy for LF elimination is annual mass drug administration (MDA). A particular concern is whether HIV, through its impact on the immune system, will interfere with the effectiveness of this approach to control and eliminate LF. We report findings from cross-sectional studies in two separate populations in northern Malawi where both HIV and LF are common. One group (1,851 individuals) were studied at enrolment into a trial of anti-LF treatments, whilst the other study used samples stored from adult participants in a whole population HIV survey (7,863 individuals). Between 5–10% of the study participants were HIV-positive and 24% were LF-infected. We found no evidence that LF infection was more or less common in HIV-positive adults in either population. However, we identified robust evidence that antiretroviral therapy use was associated with lower LF prevalence rates. We have no evidence to suggest HIV will have a detrimental effect on LF control. On the contrary, the evidence suggests that antiretroviral therapy may have beneficial effects and merits further careful evaluation of the anti-filarial properties of these compounds.

Introduction

Human immunodeficiency virus (HIV) and parasitic infections affect widely overlapping populations in sub-Saharan Africa. Of the estimated 35 million people infected with HIV worldwide at the end of 2013, about 70% were from sub-Saharan Africa [1]. Parasitic infections, including lymphatic filariasis (LF) are also widespread in sub-Saharan Africa, raising the possibility of clinically significant interactions between the two pathogens. It has been suggested that HIV and parasitic co-infections may have bidirectional deleterious interactions by affecting susceptibility to HIV, impacting on HIV progression and potentially worsening clinical outcomes of filarial infection [2]. Previous in-vitro studies have shown helminth infections to increase susceptibility of peripheral blood mononuclear cells to HIV infection [3]. In addition deworming can result in increases in CD4+ cells and reduction in plasma HIV-1 RNA concentrations [4]. Derangements in the immune response associated with HIV-infection might also be expected to alter susceptibility to, or complications from, filarial infection or other helminths such as *Strongyloides* [5]. To date, there are few studies that have investigated LF and HIV co-infection and to our knowledge, none have been on a large population scale. A cross-sectional study of 907 adults undertaken in Tanga region of Tanzania reported increased circulating filarial antigen (CFA) concentration in HIV-positive persons [6], although a further evaluation of this group of individuals did not support any association between HIV and *Wuchereria bancrofti* infection [7]. Similarly, in urban southern India, no quantitative difference in *W. bancrofti* CFA levels by HIV status was found in a study of 432 HIV-positive and 99 HIV-negative patients [8].

Malawi embarked on a programme of mass drug administration (MDA) for LF control and elimination in 2009 [9]. Concerns that the programme may be less effective in areas of high HIV and LF prevalence prompted this study in Karonga, a district in the northern region of Malawi which was known to be highly endemic for LF infection [10]. Karonga is bordered by Lake Malawi to the east, the Songwe river to the north (which also forms the boundary with Tanzania), and by the Nyika plateau and escarpment to the west and south. The population is rural and dependent on subsistence agriculture including rice growing and fishing from the lake.

Two previous studies had been undertaken by co-authors in the district and both had serum samples stored with approval for later testing. The first was the recruitment phase of a randomised controlled clinical trial that investigated alternative schedules and dosing regimens of ivermectin and albendazole use in MDA programmes (study 1). Findings from this clinical trial are reported elsewhere [11]. The second study was nested within a comprehensive population-based HIV survey which enabled a longitudinal assessment of CFA in a whole adult population (study 2). In this paper we report the prevalence and relationship of LF and HIV infections from these two studies.

Methods

Study 1 used samples and data collected as part of the screening phase of a clinical trial of the effectiveness of increasing the dose and frequency of albendazole and ivermectin as antifilarial agents for clearing LF microfilaraemia (clinical trials registration number NCT01213576) [11]. It was undertaken between January 2009 to March 2012 in the northern portion of Karonga district along the Tanzanian border and Songwe river delta. Villages in this area had previously been shown to have a high prevalence of filarial antigenaemia and chronic manifestations of LF [10]. No mass treatment interventions had been undertaken in the area at the time the study was started.

Enrolment to the clinical trial required individuals to have a microfilarial count of >80 microfilariae per ml of blood. Consequently a population-based screening process was undertaken to identify suitable participants for enrolment in the therapeutic trial. The estimated total adult population of the target villages was 36,643 [12]. Sensitization meetings were held with community members, the Traditional Authority (TA) and all the village headmen and their aides who are administratively responsible for the study area. At these meetings the aims and procedures of the study were explained. Following verbal approval by the community leaders, a team of field workers went house-to-house seeking written consent and recruiting individual participants. All households in selected villages were visited in sequence before moving on to the next village. This screening phase was planned to continue until 120 eligible individuals with appropriate microfilarial levels were recruited into the trial. However, screening and recruitment into this study was discontinued following the rollout of the albendazole/ivermectin national MDA programme in the study area as further recruitment into the clinical trial became impractical. At the end of the recruitment phase individuals from 16 villages were included. For analysis purposes smaller villages were combined in a geographically appropriate way to produce 10 village location categories with suitable numbers of participants.

Individuals were eligible if they provided written informed consent, were residents of the area and aged between 18 and 55. At each home visit, the study was introduced and explained to all members and individual participants were asked to provide written informed consent by signing (or thumb printing if they were illiterate) the informed consent forms that were translated in the local language (Tumbuka). A questionnaire was administered to capture personal details. Eligible individuals were screened for CFA by the immunochromatographic (ICT) card test and for HIV by trained counsellors following the national HIV rapid testing algorithm.

CFA positive individuals were asked to provide a night blood sample between 22:00 and 02:00 hours when a 5ml sodium citrate sample was collected for microfilaria counting and later stored in the project laboratory archive at -20°C. All individuals who were CFA positive but declined to participate in the clinical trial or did not meet the eligibility criteria for the trial were offered standard dose antifilarial therapy with albendazole and ivermectin. Individuals who were HIV positive were referred for HIV treatment and care. Assessment of HIV clinical stage, CD4 count and viral load were not performed on these individuals as a part of the study protocol.

Study 2 used samples and data from an annual whole adult population survey. Repeated rounds of data and sample collection spanned the time periods before and after the introduction of MDA. It was undertaken in the Karonga Health and Demographic Surveillance Site (KHDSS) and nested within a comprehensive population-based HIV sero-survey [13]. The KHDSS area is mapped and divided into geographically defined clusters of 20–30 households which are further aggregated into 21 geographically distinct reporting groups. The KHDSS was established between August 2002 and August 2004 to serve as a sampling frame for on-going epidemiological and clinical studies. Unlike study 1, data from study 2 included detailed socio-demographic information allowing for inclusion of these factors in statistical analysis. Since its establishment, the initial population of the KHDSS has been under continuous demographic surveillance. In addition, between September 2007 and October 2011 four annual HIV serological surveys have been conducted in all individuals aged 15 or more years using rapid point-of-care HIV tests on finger-prick whole blood samples [14]. Community sensitisation meetings to explain the aims and procedures of the study were held in each village and were followed by house-to-house visits by counsellors to recruit participants. The counsellors were trained and certified by Malawi Ministry of Health staff to perform HIV counselling and testing and referral using standard procedures [15]. Written informed consent was obtained as in study 1. In addition, all consenting adults were asked to provide a 5ml blood sample for quality control and storage for further laboratory analysis including for other diseases of importance in Karonga district. Plasma samples from consenting participants were stored in the project laboratory archive at -20°C. Samples from the first surveillance round, which took place between September 2007 and October 2008, prior to MDA introduction, were included in our study. Viral load, CD4 counts and clinical staging were not measured as a part of the survey.

Common laboratory methods for both studies

The ICT card test (Binax, Portland, ME) [16] was only used as the screening test for LF infection in study 1. This is a portable rapid point-of-care test suitable for screening in non-laboratory settings and was used in accordance with manufacturer's instructions. Microfilaria counting was done on ICT card test positive individuals in study 1 by the nucleopore membrane filtration technique [17]. This involves filtering a measured volume of venous blood through a 5μM pore size nucleopore filter. After filtration, the filter is removed, placed on a glass slide and mounted on a light microscope for examination and counting of microfilariae. Filarial antigenaemia was quantified in all plasma samples in both study 1 and study 2 by means of the Og4C3 antigen-capture ELISA (TropBio, Australia) [18]. Samples were analysed in accordance with the manufacturer's instructions and expressed as antigen units per ml of blood (Ag/ml) based on control samples supplied with the ELISA kits. Samples with CFA concentration more than 128Ag/ml were considered clear positives for CFA. Samples with CFA concentration of 32Ag/ml and below were considered negatives. Samples with a titre between 32 and 128Ag/ml were considered indeterminate.

In both studies HIV testing used whole blood rapid diagnostic tests according to Malawian national guidelines [15] with demonstrated high accuracy in community settings in Karonga

[14]. The initial screening test was with Determine TM HIV-1/2 (Abbott Japan Co Ltd, Japan) and confirmatory testing was done with UniGold TM HIV-1/2 (Trinity Biotech PLC, Ireland). Samples with a non-reactive screening test were considered negative and those with a reactive screening and confirmatory test were considered positive. Where the screening and confirmatory tests were discordant, a tie breaker using a third rapid test, (SD Bioline, Korea) was used.

Common data management and statistical methods for both studies

Study forms were checked, coded, double entered and verified using Microsoft Access software. Statistical analysis was done in Stata 12 software (StataCorp, Texas, USA). Continuous variables were log transformed prior to analysis to achieve an approximate normal distribution. Linear regression was used for crude and adjusted analyses with results expressed as geometric mean ratios (GMR) and their 95% confidence intervals. The association of age, gender and CFA status with HIV positivity was estimated using χ^2 tests for crude analyses and a logistic regression model for adjusted Odds Ratios (OR). In a risk factor analysis for CFA positivity, logistic regression was used to estimate crude and adjusted ORs. Variables were retained in the model if significant associations were identified in the unadjusted estimates. Geographic identifiers for groups of survey villages were also incorporated in the models to adjust for geographic confounding as previous surveys had indicated heterogeneity of CFA prevalence across the region [19]. Rather than a binary variable, HIV was treated as a categorical variable in the model with the HIV—negative group and two HIV-positive groups based on ART use at the time of blood sampling. A sub-group analysis to investigate the effect of cotrimoxazole and duration of ART use on CFA prevalence was performed on the HIV-positive group only. Logistic regression models were used to estimate odds ratios. Difference in CFA prevalence with increased use of ART was investigated with a χ^2 test for linear trend with odds ratios derived from a 2xn table. Adjusted odds ratios were estimated with logistic regression.

Ethics

The National Health Sciences Research Committee of the Malawi Ministry of Health (protocol numbers 495 and 419) and the Ethical Committee of the London School of Hygiene and Tropical Medicine (protocol numbers 5344 and 5081) gave ethical clearance for both studies 1 and 2. Study participants in both studies were consented for storage and later testing of samples at the time of enrolment. This covered testing for HIV and other diseases of local significance. The National Health Sciences Research Committee (protocol number 908) and the Ethical Committee of the Liverpool School of Tropical Medicine (protocol number 11.77) approved the additional analysis conducted on stored samples.

Results

The overall population of Karonga district during the study period was 272,789 [12] with the KHDSS population accounting for 33,500. HIV prevalence in the KHDSS in the 2007–2008 survey year was measured at 7.4% but estimated to be 10.4% when adjusted for non-testing by those who already knew they were HIV-positive [20]. At baseline 54.8% of those aged 15 years or more reported previous HIV testing.

Study 1

From the estimated total of 36,643 adults of the target villages, 1,851 individuals were eligible and consented to participate. Of these 1,851 individuals screened for LF antigen by the ICT card test, 447 (24.2%) were CFA positive (Fig 1). A total of 1,656 individuals accepted HIV

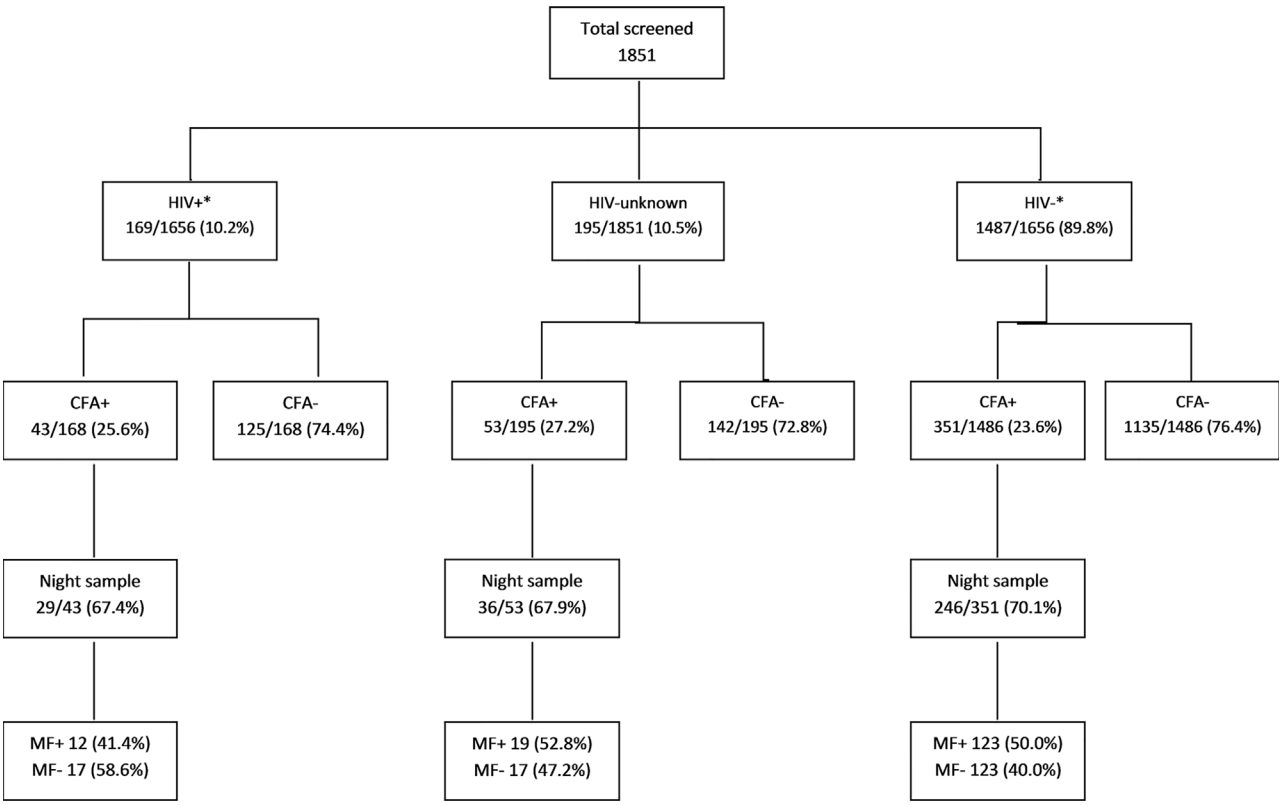

Fig 1. Study 1 flow chart detailing the breakdown of individuals by HIV status, circulating filarial antigen (CFA) status by immunochromatographic card (ICT) test and microfilarial counts. *2 individual had an invalid ICT test. MF—microfilaria.

doi:10.1371/journal.pntd.0003825.g001

testing and 169 (10.2%) of these were HIV-positive. HIV-positive individuals tended to be older (Table 1). CFA positivity was present in 43 (25.6%) of HIV-positive and 351 (23.6%) of HIV-negative (crude OR 1.11, 95% CI 0.77–1.60) with an LF/HIV co-infection prevalence rate of 2.6%. CFA positivity did not differ by HIV infection status (Table 1). There was heterogeneity in the prevalence of CFA by village location, median 24.4%, range 15.7–33.3% (Pearson χ^2 22.7, $p<0.01$, 9 degrees of freedom). Data on the use of antiretroviral and cotrimoxazole was incomplete in the context of this study.

Microfilaria counting was done in the 311 (69.6%) LF antigen positive individuals who were eligible and gave consent for night blood sampling. The remainder either refused or left before follow up. HIV prevalence in those lost to follow up was broadly similar to those sampled (10.3% vs. 9.3% respectively, χ^2 test $p = 0.90$). Microfilariae were present in 49.5% of the 311 sampled individuals. Microfilarial detection and levels did not differ by HIV infection status (Fig 1 and Table 1).

Of 311 stored baseline night blood plasma samples, 290 (93.2%) were CFA positive using the Og4C3 antigen-capture ELISA. CFA was positive in 26 (89.7%) of HIV-positive individuals, 231 (93.9%) of the HIV-negative individuals and 33 (97.1%) of HIV-unknown individuals respectively ($p = 0.47$). The geometric mean CFA concentration levels by HIV status were 859 and 1660 for HIV-positive and HIV-negative respectively (GMR 0.85, 95% CI 0.49–1.50). CFA and MF counts showed reasonable positive correlation (Pearson correlation coefficient $r = 0.56$, $p<0.01$).

Table 1. Baseline characteristics of Study 1 participants by HIV status.

		A		
Characteristic	**HIV-positive (n = 169)**	**HIV-negative (n = 1487)**	**OR (95% CI†)**	**Adjusted OR (95% CI)#**
Age group				
18–29 years	43 (25.4%)	727 (48.9%)	-	-
30–39 years	68 (40.2%)	406 (27.3%)	**2.83 (1.90–4.23)**	**2.83 (1.90–4.23)**
40 years and above	58 (34.3%)	354 (23.8%)	**2.77 (1.83–4.19)**	**2.69 (1.77–4.08)**
Sex				
Male	66 (39.0%)	618 (41.6%)	-	-
Female	103 (61.0%)	869 (58.4%)	1.11 (0.80–1.54)	1.14 (0.82–1.59)
CFA status				
Negative	125 (74.4%)	1135 (76.4%)	-	-
Positive	43 (25.4%)	351 (23.6%)	1.11 (0.77–1.60)	1.13 (0.78–1.65)
MF status				
Positive	17 (58.6%)	123 (50.0%)	-	-
Negative	12 (41.4%)	123 (50.0%)	0.71 (0.32–1.54)	0.81 (0.35–1.85)
		B		
Characteristic	**HIV-positive**	**HIV-negative**	**GMR (95% CI)**	**Adjusted GMR (95% CI)#**
CFA GMC Ag/ml (95% CI)	859 (231–3193)	1660 (1198–2302)	0.85 (0.49–1.50)	0.91 (0.55–1.51)
		C		
Characteristic	**HIV-positive**	**HIV-negative**		**P value**
MF count, median (IQR), mf/ml	0 (0–22)	1 (0–93)		0.13

A) The association of age group, gender and circulating filarial antigen (CFA) with HIV positivity. Adjusted Odds Ratio (OR) derived from logistic regression model. **B)** Geometric mean concentration (GMC) of CFA in those CFA positive. Geometric mean ratio (GMR) derived from linear regression model. **C)** Microfilaria (MF) count in those CFA positive expressed as median and interquartile range, difference assessed by rank sum testing owing to the skewed nature of data.

†CI—confidence interval;

adjusted for age, sex and village location

doi:10.1371/journal.pntd.0003825.t001

Study 2

The total eligible population of 15 year olds and older in the KDHSS at the baseline survey was 11,756. From this group, 7,863 (66.9%) underwent HIV testing and consented to storage of their blood sample. Of these, 1,875 (23.9%) individuals were CFA positive by the Og4C3 ELISA. HIV infection was identified in 411 (5.2%) participants. HIV-positive adults tended to be older and more likely to be female (Table 2). CFA positivity was present in 86 (20.9%) of HIV-positive and 1789 (24.0%) of HIV-negative (crude OR 0.84, 95% CI 0.66–1.07) with an HIV/LF co-infection prevalence rate of 4.6%. In the female participants, CFA positivity was present in 17.8% of the HIV-positive and 19.8% of the HIV-negative (OR 0.88, 95% CI 0.64–1.21) and in the male participants, 26.8% of the HIV-positive and 29.4% of the HIV-negative (OR 0.88, 95% CI 0.60–1.28) respectively. Geometric mean CFA concentration was lower in the HIV-positive individuals by 25% although this association was weakened when adjusted for age and sex (Table 2).

Several risk factors were associated with an increased prevalence of CFA (Table 3). These included male gender, age between 30 and 39 and lower quality housing, whilst decreased CFA prevalence was associated with higher levels of education ($p<0.01$, χ^2 for linear trend) the availability of piped tap water or the use of lake water and the use of antiretrovirals. Bed net ownership was high, however ownership or the number of nets owned in the household was

Table 2. Baseline characteristics of study 2 participants by HIV status.

		A		
Characteristic	**HIV-positive (n = 411)[#]**	**HIV-negative (n = 7452)[#]**	**OR (95% CI[†])**	**Adjusted OR (95% CI)***
Age group				
15–29 years	77 (18.7%)	3793 (50.9%)	-	-
30–39 years	159 (38.7%)	1480 (19.9%)	**5.29 (4.00–6.99)**	**5.35 (4.04–7.07)**
40 years and above	175 (42.6%)	2179 (29.2%)	**3.96 (3.01–5.20)**	**4.02 (3.06–5.29)**
Sex				
Female	269 (65.5%)	4201 (56.4%)	-	-
Male	142 (34.5%)	3251 (43.6%)	**0.68 (0.55–0.84)**	**0.72 (0.58–0.89)**
CFA status				
Negative	325 (79.1%)	5663 (75.9%)	-	-
Positive	86 (20.9%)	1789 (24.0%)	0.84 (0.66–1.07)	0.86 (0.67–1.10)
		B		
Characteristic	**HIV-positive**	**HIV-negative**	**GMR (95% CI)**	**Adjusted GMR (95% CI) ***
CFA GMC, Ag/ml (95% CI)	630 (511–778)	839 (799–882)	**0.75 (0.60–0.94)**	0.62 (0.38–1.02)

A) The association of age group, gender and circulating filarial antigen (CFA) with HIV positivity. Adjusted Odds Ratio (OR) derived from logistic regression model. **B)** Geometric mean concentration (GMC) of CFA in those CFA positive, Geometric Mean Ratio (GMR) derived from linear regression model.

[#] 2 HIV-positive and 26 HIV-negative with incomplete data;

[†]CI—confidence interval;

* Adjusted for age, sex, and reporting group

doi:10.1371/journal.pntd.0003825.t002

not associated with CFA prevalence. Individuals were found in all 21 reporting groups, with a median of 314 participants (range 129–820). There was considerable heterogeneity in the prevalence of CFA by reporting group, median 23.2% range 5.7–37.2% (Pearson χ^2 354.7, $p<0.01$, 20 degrees of freedom).

Of the 411 HIV-positive adults, 142 (34.5%) were taking antiretroviral therapy (ART) and 117 (28.5%) were using cotrimoxazole prophylaxis (CTX) with only 4 of the 117 taking CTX without ART at the time of sampling. In 6 of the 411 individuals, information on ART and/or CTX use at the time of sampling was unavailable. ART consisted of Lamivudine, Stavudine and Nevirapine (Triomune-30) in 94% of cases with Zidovudine or Efavirenz substitutions in the remainder. No protease inhibitors were in use. In the HIV-positive group, ART use was associated with a lower prevalence of CFA when compared to those not on ART [12.7% vs. 25.3% (OR 0.43, 95% CI 0.24–0.76)]. Similarly, CTX use was associated with lower CFA prevalence [12.8% vs. 24.1% (OR 0.46, 95% CI 0.25–0.85)]. In a multivariable model incorporating ART and CTX use along with age, sex and geographical location, the adjusted odds ratio for ART use was 0.47 (95% CI 0.17–1.31) and for CTX use 0.92 (95% CI 0.31–2.71). When the ART treated group were further sub-divided by year since treatment started, there was a significant trend to decreased prevalence of CFA with increasing time on treatment; 25.3% no treatment (n = 265), 15.2% year 1 treatment (n = 59), 13.6% year 2 treatment (n = 44), 10.0% year 3 treatment (n = 30) and 0% year 4 treatment (n = 9), ($p<0.01$ χ^2 for linear trend). This relationship persisted after adjustment for age, gender and reporting group. In the HIV-positive individuals with detectable CFA, the geometric mean concentration of CFA was not significantly different between those off and on ART, 647 vs. 512 Ag/ml respectively, GMR 1.27, 95% CI 0.76–2.08 (Fig 2), nor did the GMC differ by ART duration category 647, 392, 762, 516 & 0 Ag/ml for no treatment, year 1, 2, 3 & 4 of treatment respectively.

Table 3. The association of circulating filarial antigenaemia (CFA) prevalence with HIV and antiretroviral therapy (ART) status and major potential confounding socio-demographic characteristics in the 7,863 study 2 participants.

Characteristic	N (%)	CFA prevalence (%)	OR (95% CI[#])	aOR (95% CI)
HIV and ART status				
HIV-negative	7452 (94.8)	1789 (24.0)	Ref*	Ref
HIV-positive—no ART	265 (3.4)	67 (25.3)	1.07 (0.81–1.42)	1.20 (0.90–1.62)
HIV-positive—ART	142 (1.8)	18 (12.7)	**0.46 (0.28–0.76)**	**0.50 (0.30–0.84)**
Gender				
Male	3392 (43.1)	995 (29.3)	**1.69 (1.52–1.88)**	**1.77 (1.59–1.98)**
Female	4470 (56.9)	880 (19.7)	Ref	Ref
Age group				
Age 15–29 years	3870 (49.2)	896 (23.2)	Ref	Ref
Age 30–39 years	1639 (20.9)	440 (26.7)	**1.22 (1.07–1.39)**	**1.24 (1.08–1.43)**
Age 40 years and above	2354 (29.9)	539 (22.9)	0.99 (0.87–1.11)	0.95 (0.84–1.09)
Mosquito net ownership				
0	197 (2.5)	46 (23.4)	Ref	-
1	945 (12.0)	254 (26.9)	1.21 (0.84–1.73)	-
2	1817 (23.1)	461 (25.4)	1.12 (0.79–1.58)	-
3	1835 (23.3)	430 (23.4)	1.00 (0.71–1.42)	-
≥4	2854 (36.3)	640 (22.4)	0.95 (0.67–1.34)	-
unknown	215 (2.7)	-	-	-
Educational achievement				
Nil	277 (3.6)	83 (30.0)	Ref	Ref
Primary	5493 (71.3)	1379 (25.1)	0.78(0.60–1.02)	**0.71 (0.54–0.93)**
Secondary	1886 (24.5)	377 (20.0)	**0.58 (0.44–0.77)**	**0.55 (0.41–0.75)**
Tertiary	45 (0.6)	8 (17.8)	0.51 (0.23–1.13)	0.52 (0.22–1.19)
Unknown	2 (0.0)	-	-	
Water supply				
Bore hole	3755 (47.7)	1037 (27.6)	Ref	Ref
Tap to house	1082 (13.8)	105 (9.7)	**0.28 (0.23–0.35)**	**0.30 (0.24–0.37)**
Shared tap	835 (10.6)	127 (15.2)	**0.47 (0.38–0.57)**	**0.48 (0.39–0.59)**
Covered well	999 (12.7)	303 (30.3)	1.14 (0.98–1.33)	1.16 (0.99–1.35)
Open well	572 (7.3)	167 (29.2)	1.08 (0.89–1.31)	1.06 (0.87–1.30)
Lake	456 (5.8)	106 (23.3)	**0.79 (0.63–1.00)**	**0.77 (0.61–0.97)**
Unknown	11 (0.1)	-	-	-
Housing type				
Burnt brick	5750 (73.1)	1328 (23.1)	Ref	
Unburnt brick	678 (8.6)	169 (24.9)	1.11 (0.92–1.33)	1.00 (0.83–1.21)
Mud	1087 (13.8)	299 (27.5)	**1.26 (1.09–1.46)**	1.03 (0.88–1.20)
Grass/bamboo	167 (2.1)	49 (29.3)	1.38 (0.98–1.94)	1.14 (0.81–1.62)
Other	18 (0.2)	2 (11.1)	0.42 (0.10–1.81)	0.43 (0.10–1.91)
Unknown	163 (2.1)	-	-	-

Crude and adjusted Odds Ratios (OR) derived from a logistic regression model. Data on reporting group are not shown in the table but adjusted models include this as a potential confounder along with the other significant variables in the crude analysis.

[#]CI—confidence interval:

*Reference category

doi:10.1371/journal.pntd.0003825.t003

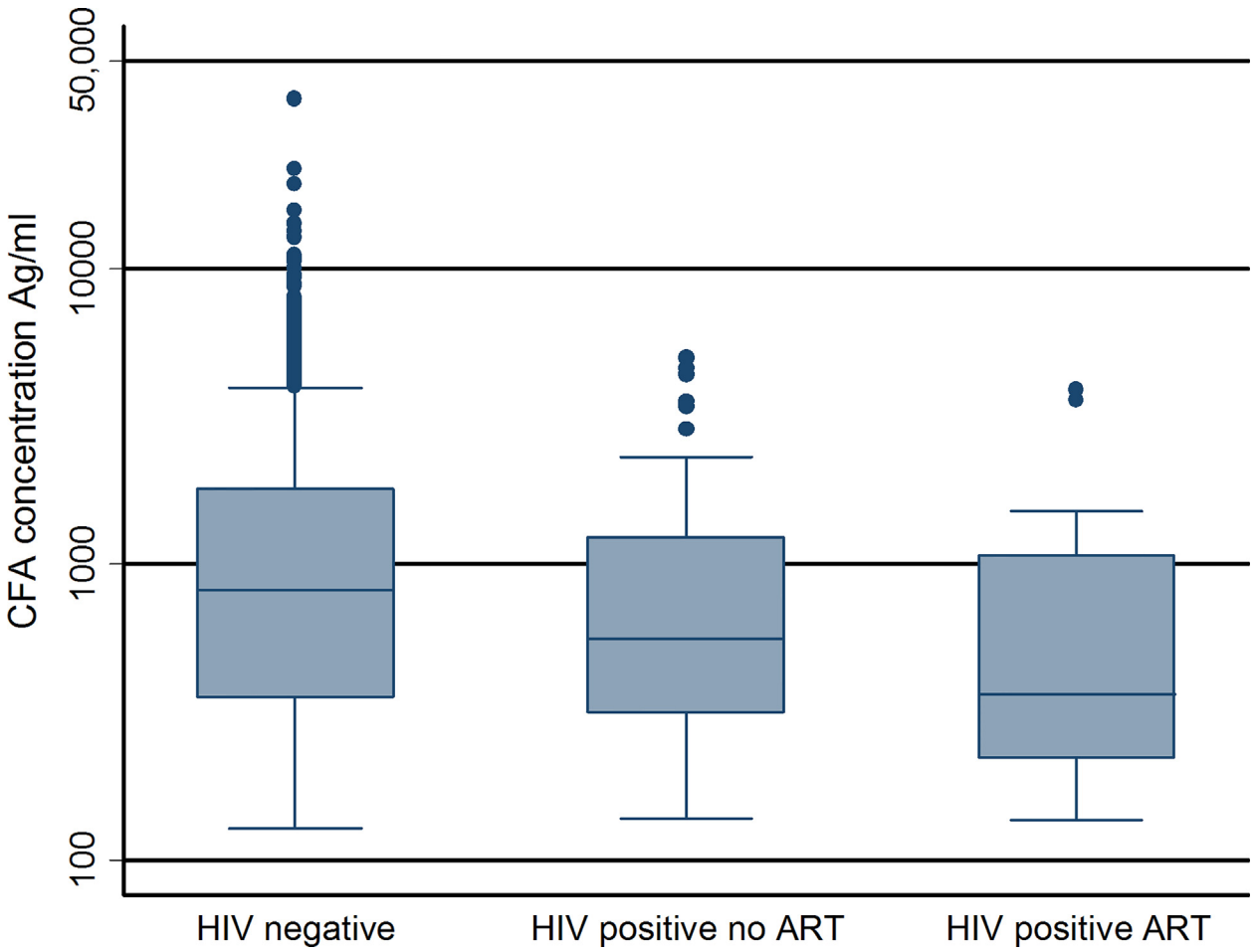

Fig 2. Box plot of Circulating Filarial Antigen (CFA) concentration distribution (on logarithmic scale) by HIV and antiretroviral treatment (ART) status for study 2 participants. The boxes show the median value and the interquartile range. The whiskers include all values within 1.5 times the interquartile range with outliers shown as points. Comparison of CFA concentrations by group HIV- vs. HIV +/ART- ($p = 0.22$): HIV- vs. HIV+/ART+ ($p = 0.05$): HIV+/ART- vs. HIV+/ART+ ($p = 0.28$), derived from a linear regression model adjusted for age, gender and reporting group.

doi:10.1371/journal.pntd.0003825.g002

Discussion

We present data from two separate studies undertaken in Karonga district, northern Malawi. In both studies a high LF and HIV prevalence was measured with HIV co-infection rates of 2.6% and 4.6% among those who were CFA positive and 15 years and older. We found no evidence that HIV is associated with an increased risk of LF infection. Initial findings from study 1, a clinical trial not powered to definitively test the impact of HIV on LF infection, revealed a tendency to lower CFA and microfilarial density in the HIV-positive adults. Subsequent investigation of these parameters in the much larger population sample revealed a tendency to lower CFA prevalence in the HIV-positive group, attributable to significantly lower CFA prevalence in the ART treated sub-group, a finding that persisted following adjustment for key potential confounders and showed a significant trend to lower CFA prevalence with duration of ART use. There was no significant effect of CTX therapy, when analysed in a multivariable model. CFA concentration was also persistently lower in the HIV-positive group although at a level of uncertain clinical or public health significance.

Previous studies have reported divergent findings with some showing an association between LF and HIV infections but these have tended to be small samples and in selected populations [6–8]. In contrast to these studies, our second study had a larger sample taken from a whole population survey, including a high proportion of the at-risk population in an area with high prevalence rates of LF and HIV. The findings in relation to ART use are novel and we are unaware of other studies that have investigated this association. Individuals receiving ART may represent a select group of the HIV-positive population who have better health seeking behaviour, may be more educated, live in better accommodation and/or may live in close proximity to health providers. However, as this work was undertaken in the context of a demographic survey we were able to investigate these potential confounders by adjusting for reported educational status, housing quality, access to clean water and geographic location. The finding of ART associated with a lower CFA prevalence appears robust.

An explanation for these findings remains less clear and merits further work. Residual confounding or an unrecognised selection bias remains possible, but seems unlikely given the highly significant lower CFA prevalence with duration of ART therapy. The crude association of CTX with lower CFA prevalence seems adequately explained by concomitant use of ART, and there is no evidence to support either sulphonamides or trimethoprim, the components of CTX, as effective antifilarial agents. If LF infection adversely impacts on the success of ART therapy, then over time the prevalence of CFA positivity in this group will reduce as the LF/HIV co-infected die. There is no evidence from the Malawi national HIV programme that outcomes from ART treatment are worse in regions of the country endemic for LF compared to those with low LF prevalence. Helminth infections have been linked to increased viral load in non-ART treated individuals [21] but not to evidence of faster HIV progression [22]. Similarly, LF infection had no significant effect on HIV disease progression in a study of *W. bancrofti* and HIV coinfections in south India [23]. Altered diagnostic accuracy of the Og4C3 ELISA in the presence of ART has not been reported. ART has been rarely linked to false negative HIV results in children and adults but this is more likely to be due to low levels of virus and/or antibody than a direct inhibitory effect. The reduction in CFA prevalence by ART treatment duration and the antigen capture nature of the Og4C3 ELISA would be difficult to explain by ART inhibition of the assay. Immune reconstitution as a result of ART does not adequately explain our finding either as there is a similar prevalence of CFA in the HIV-negative and the HIV-positive untreated. There is no precedent for immune recovery following ART leaving the immune system in a more competent state than an HIV-negative person. ART treatment is an imprecise proxy marker of duration of HIV infection. If the natural history of LF in the HIV-positive is a steady fall in antigenaemia could this explain the association? We do not have accurate seroconversion dates for the majority of this population so are not able to fully consider this possibility. However with ART use the "natural history" of HIV is dramatically altered and it might be expected that any tendency to lower antigenaemia with time would also be altered and this would be inconsistent with our findings. The most plausible explanation for this finding is a direct filaricidal activity of the major ART agents. We are unaware of any data on the effect of Lamivudine, Stavudine or Nevirapine on helminths. Further evaluation of these molecules as antihelminthics would be appropriate.

Of the other factors associated with CFA positivity all have been reported previously, providing reassurance that the epidemiology of LF disease in Karonga is not unique and results are generalizable to other similar regions. One surprise was the lack of association with bed net ownership. However most households possessed bed nets limiting the power of any comparison, and during this survey we did not specifically ask about usage, or condition of the nets, thus limiting the value of this finding. More detailed evaluation of this will be needed in future work.

Both of our studies had some degree of selection bias, but it is unlikely that this has fundamentally altered our findings. In study 1, we targeted villages known historically to have a high prevalence of LF infection. If participation by HIV-positive individuals was reduced because of perceived stigma associated with an HIV test, we may have had reduced power to identify an association between LF and HIV. However, a similar finding in the much larger study 2 provides consistency. In study 2, we know HIV-positive adults were under-represented. Adults who knew their status from earlier HIV testing studies or through routine service provision in the district, declined participation [20]. However it is difficult to see a mechanism whereby LF co-infection would disproportionately lead to non-participation by HIV-positive adults and in particular ART treated HIV-positive adults thereby obscuring the true association.

More females than males were included in both studies. This may represent the easier access to females at the time of recruitment since females are more likely to be at home. Although we know men are more likely to be infected with LF in this population, we do not think this under-representation has meaningfully affected the LF/HIV association. Sub-group analyses showed similar odds ratios for the LF/HIV association by gender in study 2 suggesting no major effect modification.

The measurement of our exposure (HIV) and outcome endpoints (LF status) were based on accurate and well described tests and we do not believe these have introduced significant bias into the study. We used different tests for assessment of circulating filarial antigen in the two studies with different sensitivities and specificities, the ICT card test with sensitivity and specificity reportedly close to 100% and the Og4C3 ELISA test with 100% sensitivity and specificity of at least 94% [24–26]. There was some disparity between these two tests identified in study 1. This is consistent with previous studies that have reported overall agreement between the ICT and Og3C4 tests but different sensitivities and specificities [24,25]. In study 2, we were not able to assess MF counts due to the use of a stored sample collection. Whilst we cannot categorically rule out an association between MF density and HIV, data from study 1 showed a positive correlation between CFA levels and MF density. Previous studies have also shown a positive correlation between CFA levels and MF density [26,27]. This implies that the CFA relationship will broadly apply to MF counts.

In summary, we did not demonstrate a significant detrimental association between LF and HIV in these studies that will have a negative impact on plans to eliminate lymphatic filariasis. However ART treated adults had significantly lower CFA prevalence, a finding that merits further careful evaluation to exclude an adverse impact of LF on HIV, or the potential of antiretrovirals as molecules with antihelminthic properties.

Supporting Information

S1 Checklist. STROBE Checklist.
(DOCX)

Acknowledgments

We thank the people of Chilumba and Songwe areas for their participation in this study. We also thank the traditional leaders for their support of the study, and KPS field, laboratory, data coding office, administrative and support staff for their work.

Author Contributions

Conceived and designed the experiments: NF JH BN AC TT. Performed the experiments: TT AP MK WP. Analyzed the data: TT MT BN NF. Contributed reagents/materials/analysis tools:

MT NF. Wrote the paper: TT BN MT AP MK WP AM NK JH NF OK AC. Read, gave comments and approved final version of the manuscript: MT AM OK AC JH NF TT.

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Trans R Soc Trop Med Hyg 2015; **109**: 393–399
doi:10.1093/trstmh/trv027 Advance Access publication 15 April 2015

Randomised controlled clinical trial of increased dose and frequency of albendazole and ivermectin on *Wuchereria bancrofti* microfilarial clearance in northern Malawi

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Received 14 October 2014; revised 5 March 2015; accepted 6 March 2015

Background: In Africa, albendazole and ivermectin are currently used in combination for annual mass drug administration (MDA) for lymphatic filariasis (LF) elimination. Rapid and sustained clearance is desirable for public health impact and elimination of LF. Increasing the dose and/or frequency of albendazole and ivermectin treatment may be more effective in clearing microfilariae than standard MDA.

Methods: We conducted a randomised controlled open label trial in northern Malawi comparing three modified treatment groups to standard dosage of ivermectin and albendazole in adults with confirmed circulating LF antigen and microfilaria. Participants were followed-up every 6 months for 2 years for repeat microfilarial counts and safety assessments.

Results: A total of 1851 adults were screened and 70 with microfilarial counts >80 microfilariae/ml were randomised. All treatment groups achieved a significant reduction of microfilariae levels by 12- and 24-months of follow-up. Doubling the standard dose and administering it twice yearly showed a non-significant tendency towards faster and more complete clearance. There were no serious adverse reactions.

Conclusions: In this small study, all regimens effectively cleared microfilaria. Standard treatment may be adequate in settings like Malawi but not in all endemic settings and larger studies are required to demonstrate benefit of higher dosages.
[ClinicalTrials.gov identifier: NCT01213576].

Keywords: Albendazole, Clinical trial, Ivermectin, Lymphatic filariasis, Malawi, Microfilaria

Introduction

Lymphatic filariasis (LF) remains one of the leading causes of disability in tropical areas.[1] Most cases of LF, and all cases in Africa, are caused by the filarial nematode, *Wuchereria bancrofti*, with more than 120 million persons affected globally and up to one billion persons at risk of infection.[2] In response to this, WHO launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000, aiming to eliminate LF through annual mass drug administration (MDA) using single-dose albendazole (400 mg) and ivermectin (150–200 μg/kg) annually for 4–6 years to affected populations in Africa.[3,4] Rapid and sustained clearance is desirable for public health impact and the ideal annual dosing regimens remain unclear with documented 'hot spots' of new transmission remaining in areas that have received MDA and reports of individuals who remain microfilaria (mf) positive despite treatment.[5]

Higher annual doses or more frequent dosing regimens might have the potential to achieve a successful and accelerated outcome of mass treatment programmes. This could be at a lower overall cost to national programmes by achieving critical programmatic milestones much earlier.[6] However, available data to guide and support such a large programmatic shift are limited. A randomised clinical trial conducted in Giza, Egypt reported that multi-dose diethylcarbamazine and albendazole treatment was more effective than single dose treatment for reducing and clearing LF microfilaraemia.[7] In a study that compared repeated annual and semi-annual doses of ivermectin and diethylcarbamazine for prevention of *W. bancrofti* filariasis in French

Polynesia, the efficacy of ivermectin was shown to be much higher when given twice-a-year rather than annually.[8] Another study conducted in Haiti suggested that high doses of ivermectin may have longer lasting suppressive effects on LF microfilarial levels.[9] Similarly, in Mali, a high dose, biannual treatment with albendazole and ivermectin was shown to be more effective than the standard dose regimen in suppressing microfilariae.[10]

In Malawi, the districts of Karonga in the north, and Chikwawa and Nsanje in the south are historically known to be endemic for LF[11,12] and recent surveys have shown that this infection has spread more widely than previously thought.[13] We undertook a study in Malawi to investigate whether higher and/or more frequent doses of albendazole and ivermectin were more effective in eliminating *W. bancrofti* microfilariae than the current WHO–approved regimen in an African setting where previous exposure to LF treatment regimens had been limited or absent.

Materials and methods

This was a randomised, four-arm, open-label, clinical trial. The study took place from January 2009 to March 2012 in 16 villages in a pre-defined study area close to the Songwe River in the north of Karonga district of northern Malawi. This area was known to be highly endemic for *W. bancrofti* infection and no mass treatment intervention had been undertaken prior to commencement of this study. The trial was registered with Clinical trial.gov, number NCT01213576.

Study participants

Eligible participants were all adults aged between 18 and 55 years living within the study area who understood and signed the informed consent, were willing to undergo night time blood drawing every 6 months for 2 years, had haemoglobin levels $\geq$9 g/dl and microfilarial levels >80 microfilariae/ml. Exclusion criteria were pregnancy, lactation and history of taking albendazole or ivermectin for any reason within the previous 6 months or a known allergy to either drug.

Study procedures

At the initiation of the study, field study personnel explained procedures at village sensitisation meetings. Recruitment was then done through house-to-house visits. Consenting, eligible individuals were initially screened for circulating filarial antigenaemia (CFA) using the Immunochromatographic (ICT) card test (Binax, Portland, ME, USA).[14] The ICT card test was administered according to the manufacturer's instructions with measurements read at 10 minutes and if two lines were visible in the viewing window that individual was recorded positive for CFA. Individuals whose samples produced faint lines were recorded as weakly positive and deemed as positive individuals for subsequent investigations.

Individuals who were positive for CFA by the ICT card test and consented to provide a night blood sample were evaluated with a brief medical history and physical examination and visited at their households between 22:00 and 02:00 hours for collection of two venous 'night blood' samples. One EDTA sample was used for full blood count, including eosinophils and the other, a sodium citrate sample, was used for microfilaria measurement by the Nucleopore membrane filtration technique[15] expressed as density of microfilariae (mf) per ml of blood.

Randomisation

Participants who fulfilled the eligibility criteria underwent further informed consent and were assigned to study treatment groups using a computer-generated randomisation list. An independent investigator pre-generated the list, which had an allocation ratio of 1:1:1:1 and restricted block sizes of four. Study field personnel enrolled and allocated participants to the treatment groups using allocation letters on the randomisation list consecutively for each individual recruited into the study. The study field personnel kept the allocation list in a field site locker and no blinding was employed since the trial design was open-label.

Interventions

Eligible participants were randomly allocated to the four treatment study arms (see Figure 1): the standard regimen of single dose albendazole (400 mg) and ivermectin (200 μg/kg) once-yearly (control group [SDA]), a twice-yearly standard regimen (SDT), a high-dose regimen of albendazole (800 mg) and ivermectin (400 μg/kg) given either once-yearly (HDA) and the same given twice-yearly for a period of 2 years (HDT).

Study drugs were taken orally under direct observation by field staff to ensure compliance and to record any immediate side effects in the 30 minutes post-ingestion. The study team made scheduled visits once a day for 7 days to a pre-defined place within the village to review any individual who reported adverse events post-treatment. Adverse events were self-reported and assessed according to pre-defined clinical criteria. These were recorded on an adverse event form and, if considered severe or life threatening, was referred appropriately to the nearest health facility.

Follow-up

Participants were followed-up every 6 months for 2 years, underwent clinical assessment, venepuncture for full blood count and night blood microfilarial counting and received subsequent study drug administration as appropriate. In addition to the clinical assessment done at each follow-up visit, information was collected on filarial related clinical manifestations such as fever, lymphoedema, lymphangitis, hydrocoele and skin rash. Individuals with missed appointments remained in the study until 24 months and were actively traced at each follow-up appointment.

Sample size

The primary study endpoint was a difference in the clearance rate of *W. bancrofti* microfilaraemia, expressed as microfilariae per ml of blood (mf/ml), at 12 months. Based on data comparing single and multi-dose regimens for the treatment of lymphatic filariasis,[7,16] we assumed that the standard annual therapy would clear microfilaraemia in approximately 25% of subjects at 1 year whereas multi-dose therapy should give 75% clearance. The sample size needed to detect this difference by Fisher's exact test with two-sided alpha level of 0.05 and power equal

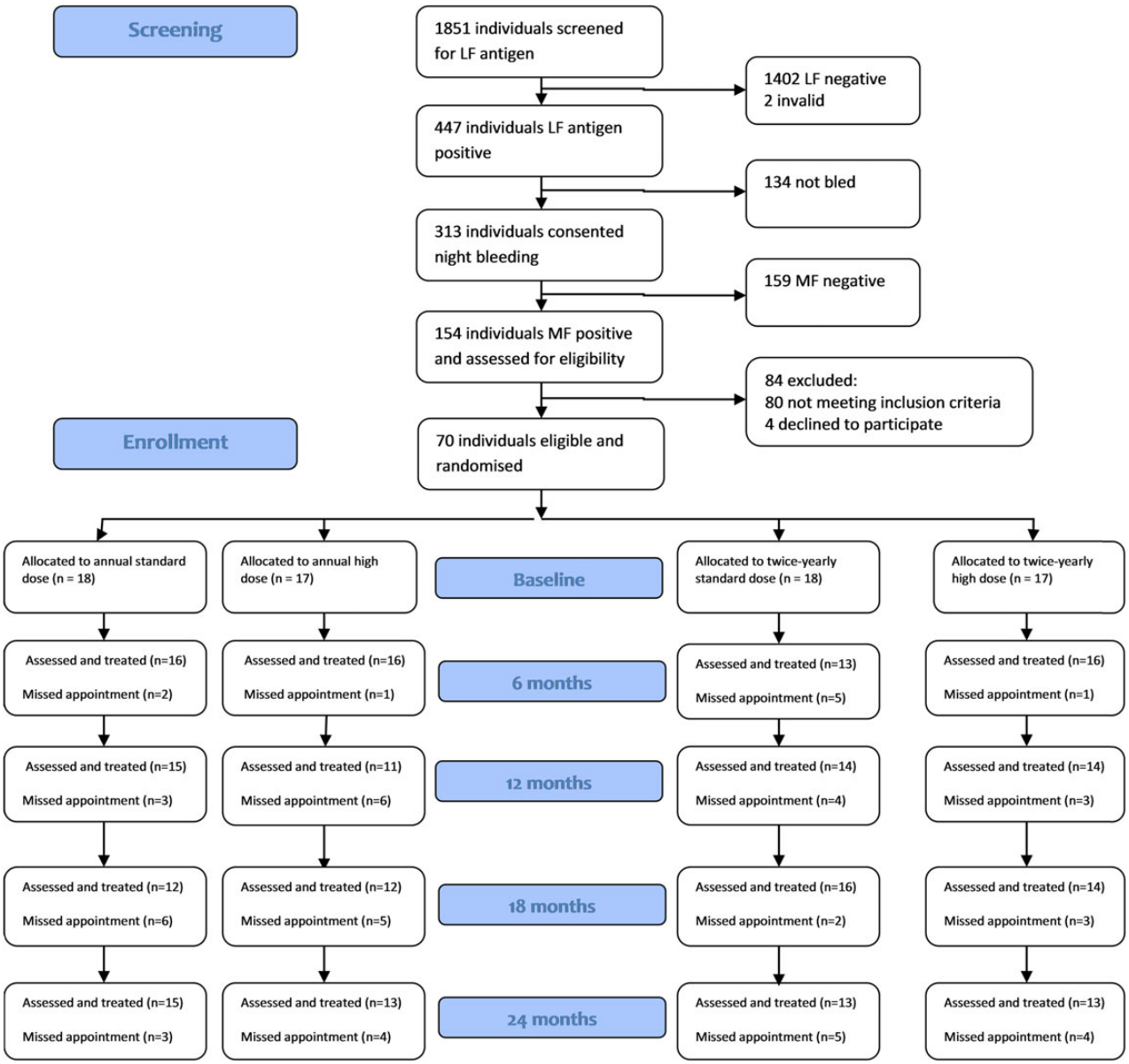

Figure 1. Flow diagram of study participants of the clinical trial. This figure is available in black and white in print and in colour at Transactions online.

to 80% was 20 per group. Taking 15% attrition at each visit into account, we planned to recruit 30 subjects per each study arm (120 planned in total).

Data management and statistical analyses

Participant information was collected onto paper record forms. Forms were transported to the project headquarters in Chilumba, Karonga district, where they were checked, coded, double entered and verified using Microsoft Access 2007 software. All statistical analyses were done using STATA 12.0 (Stata Corporation, TX, USA). The difference in clearance proportions between the intervention treatment arms and the comparison group was examined using the Fisher's exact test and the log rank test where appropriate.

The analysis used the last observation carried forward and intention-to-treat approaches to deal with participants who did not take all the intended treatments during the study.

Results

A total of 1851 individuals were screened and 70 participants were found to be eligible for randomisation into the four treatment arms of the study (Figure 1). Rates of missed appointments were similar in all the treatment groups overall. In the twice-yearly arms, this led to five missed treatments in SDT and one in HDT in the first year. Recruitment ceased before the planned sample size was achieved after the national MDA programme for lymphatic filariasis commenced in the study location in October 2009.[17]

Table 1. Baseline demographic and clinical characteristics for each treatment group in the clinical trial

Characteristic	Annual standard dose (n=18)	Annual high dose (n=17)	Twice-yearly standard dose (n=18)	Twice-yearly high dose (n=17)
Age, median (range), years	32.5 (21–50)	31 (20–53)	27.5 (18–55)	35 (20–54)
Male sex (%)	15 (83.3)	11 (64.7)	11 (61.1)	10 (58.8)
Microfilarial count, geometric mean (95% CI), mf/ml	464.7 (250.1–863.4)	338.7 (198.7–577.4)	204.5 (145.4–287.8)	264.7 (175.0–400.4)
Eosinophil count, geometric mean (95% CI),$\times 10^3$ cells/ul	1.3 (1.0–1.7)	1.4 (1.0–1.9)	1.6 (1.1–2.1)	1.1 (0.9–1.5)
Haemoglobin level, geometric mean (95% CI), g/dl	13.7 (13.1–14.2)	13.2 (12.7–13.6)	13.2 (12.1–14.4)	12.4 (11.6–13.4)

Table 2. Number of participants with complete clearance of microfilaraemia by treatment group and month of follow-up

Treatment group	6 months[a]	12 months[b]	18 months	24 months
Annual standard dose	12/18 (67%)	15/18 (83%)	15/18 (83%)	17/18 (94%)
Annual high dose	13/17 (77%)	14/17 (82%)	17/17 (100%)	17/17 (100%)
Twice-yearly standard dose	13/18 (72%)	13/18 (72%)	14/18 (78%)	14/18 (79%)
Twice-yearly high dose	14/17 (82%)	17/17 (100%)	17/17 (100%)	17/17 (100%)

[a] p=0.42: high versus standard dose regimens at 6 months.
[b] p=1.00: twice annual versus single annual dose regimens at 12 months (Fisher's exact test).

Demographic and baseline characteristics were similar between the treatment groups except for higher baseline microfilarial count levels in the annual standard dose treatment group (Table 1).

In the intention to treat analysis, all the treatment groups achieved significant reduction of microfilariae levels after albendazole and ivermectin treatment by 12 months of follow-up. Microfilarial clearance was 83% (15/18) for SDA, 82% (14/17) for HDA, 72% (13/18) for SDT and 100% (17/17) for HDT treatment doses, respectively. There was no statistically significant difference between high versus standard dose or between twice-yearly versus once-yearly dosing of albendazole and ivermectin (Table 2). In a post-hoc analysis, we compared all participants with available data at 12 months who had received a single dose (i.e., those in the single dose arms plus those in the twice-yearly arms who failed to receive their second dose) versus those who received two doses. In total, 35 received one dose, 29 received two doses and 6 did not have 12 months data available. Microfilarial clearance was observed in 31 of 35 (89%) and 28 of 29 (97%), respectively. This was not significantly different, p=0.38 (Fisher's exact test).

While the difference in *W. bancrofti* microfilarial clearance rate tended towards significance in the high dose twice-yearly group at 12 months, this again did not achieve statistical significance. The high dose twice-yearly treatment group achieved complete clearance of microfilaraemia at 12 months of follow-up without any subsequent recurrence of microfilaraemia, while the other treatment doses took up to 18 months to achieve complete clearance of microfilaraemia (Figure 2).

Twenty-two participants (31%) reported seven different adverse events at various times in all treatment groups. Most of these were mild to moderate in intensity and included fever, headache, joint pains and abdominal pains (all typically associated with treatment of microfilaraemia). Two cases of malaria, one of pneumonia and a case of acute bacterial meningitis, which resulted in the death of the patient, were admitted to hospital for treatment, and were not considered treatment related. All other symptoms were treated conservatively and did not result in hospital admission.

At baseline, filarial related clinical manifestations were reported in 8 (11%) of the participants for lymphoedema, 6 (9%) for lymphangitis, 12 (17%) for fever, 4 (6%) for hydrocoele and none for elephantiasis and skin rash, respectively. There was no difference in these clinical manifestations between subjects in the four treatment groups of the study. During follow-up, there were no new cases of lympoedema, lymphangitis, elephantiasis and skin rash. Fever was reported by four individuals (6%) at 6 months and one (1%) at 12 months, while two new cases of hydrocoele were reported at 6 and 12 months, respectively. Eosinophilia (absolute eosinophil count >500/μl) was present in 68 (97%) patients (see Table 1) at baseline and no patient was found with eosinophilia during follow-up, indicating a significant reduction after treatment.

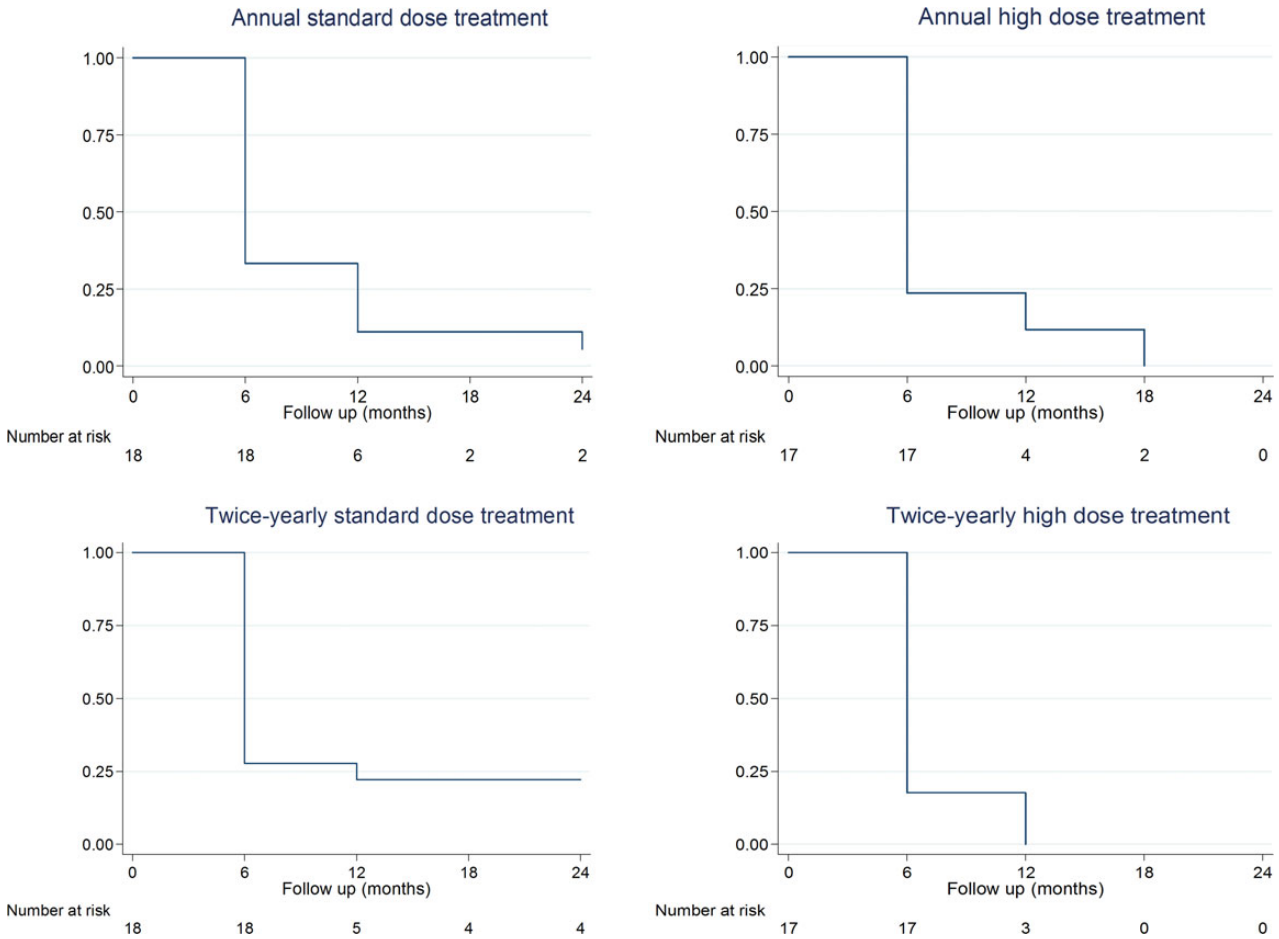

Figure 2. Kaplan-Meier plots for the four treatment arms of the Songwe clinical trial. p=0.38: annual standard versus annual high dose; p=0.40: annual standard versus twice-yearly standard dose; p=0.16: annual standard versus twice-yearly high dose (log rank test). This figure is available in black and white in print and in colour at Transactions online.

Discussion

In this study, we found high levels of microfilarial clearance from both the standard MDA treatment and experimental arms after 12 months, with additional clearance in all arms at 24 months. The twice-yearly high dose albendazole (800 mg dose) and ivermectin (400 μg/kg) treatment regimen had a non-significant tendency to faster clearance of microfilaraemia compared to the WHO-recommended annual standard dose albendazole 400 mg single dose and ivermectin 200 μg/kg treatment regimen, without any increase in adverse events.

Our findings are consistent with available historic and contemporary trials of anti-filarial agents in other settings.[8–10] A randomised controlled clinical trial conducted in Mali just prior to our study also reported that the higher dose twice-yearly treatment regimen was more effective than the standard dose annual regimen.[10] This trial was conducted in an area where there had been considerable previous exposure to ivermectin for onchocerciasis as well as a number of rounds of MDA for LF elimination and infected subjects with adequate microfilarial levels were difficult to find. Our study endorses, and adds to, the findings in Mali since it had three intervention groups compared with the standard regimen and was conducted in a setting of high LF-endemicity with no known previous exposure to LF treatment or MDA for LF elimination.

The response to standard treatment was much better than the expected 25% and 75% at 12 and 24 months, respectively and this was a contributory reason for failure to achieve a definitive outcome in this study. The sample size calculations were based on findings emerging from data comparing single and multi-dose regimens for the treatment of LF.[7,16] The reduced response to standard treatment in the Mali study may be due to the selection of less sensitive strains as a consequence of previous exposure to LF treatment. With the excellent response to standard treatment in our study, a much larger cohort will be needed to investigate these dosing schedules further. However, future studies similar to this are likely to prove increasingly difficult to undertake with the introduction and increased coverage of MDA.

The principal limitation of this study was the failure to recruit adequate numbers of participants. Recruitment did not reach the protocol target due to lower numbers of potential subjects with the target microfilaraemia of >80 microfilariae/ml and only 70 were randomised into the study. Recruitment was also discontinued following the rollout of the albendazole/ivermectin national MDA programme in the study area in October 2009.

Other limitations of the study were a relatively high rate of missed follow-up appointments compounded by a series of earthquakes in late 2009 that affected the ability of field workers to reach potential subjects.

A reduction in LF incidence is likely to have contributed to lower than expected recruitment. The study site in the Songwe River area of Malawi was chosen because prior work in the area had shown a high prevalence of LF antigenaemia (46%) and microfilaraemia (30.8%) some 10 years previously.[18] During the screening process for this study, the prevalence of LF was lower, suggesting a reduction in the incidence of new infections during this period. A similar trend was observed in Zambia, a neighbouring country to Malawi, where LF prevalence decreased from 33.3 to 14.8% between 2003 and 2011.[19] This was attributed to a decrease in LF transmission following intensive vector control activities, including the distribution of bed nets. Our study area had not been subject to MDA for LF and there was no previous distribution of albendazole for soil-transmitted nematodes. Migration of patients from the area can also be discounted, as can significant changes in climatic conditions and vegetation cover. However, as described in Zambia, there has been a scale-up of malaria control interventions in Malawi with 55% of households in rural areas reported owning at least one insecticide-treated mosquito net.[20] Thus vector control may account for some, but not all, of the LF decline observed in our study area. We were unable to further confirm this through entomological data or through reports of a decline in filarial-related clinical manifestations. It is possible that the decline in LF has been ongoing for many years unnoticed, and that the disease may already have been declining before the implementation of MDA.

Conclusions

All four treatment doses were effective in clearing lymphatic filariasis microfilariae and these findings are consistent with results from historic and contemporary trials of anti-filarial agents. Although these findings did not reach statistical significance due to low numbers, they provide additional high quality data on the treatment of LF from an LF-endemic area and indicate that standard treatment may be adequate for national LF programmes in settings with similar epidemiology and treatment history to Malawi. Publication of these results will make them accessible for a future meta-analysis. While larger studies would be required to demonstrate the benefit of higher and more frequent regimens definitively, these are likely to be increasingly challenging and expensive to undertake at the required scale.

Authors' contributions: NF, JH and BMN conceived and designed the study; TTT, AJP, LGB, TPW and WNP performed the experiments; TTT, OK, MT, BMN and NF analysed and interpreted the data; TTT, NF and MT drafted the manuscript. All authors read and approved the final manuscript. TTT and NF are guarantors of the paper.

Acknowledgments: We thank the people of the Songwe area in northern Malawi for their participation in this study. We also thank the traditional leaders for their support of the study, and KPS field, laboratory, data coding office, administrative and support staff for their valuable contributions to the study.

Funding: The study was funded through a Wellcome Trust grant to the Karonga Prevention Study [079828/Z/06/Z] and an award from the Taskforce for Child Survival and Development, Atlanta, Georgia as part of the Bill & Melinda Gates Foundation Grant [#43922].

Competing interests: None declared.

Ethical approval: The National Health Sciences Research Committee of the Malawi Ministry of Health [protocol number 495] and the Ethical Committee of the London School of Hygiene and Tropical Medicine [protocol number 5344] approved the study. Community permission to undertake the study in the area was sought from village and community leaders.

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