New approaches for conducting surveillance for lymphatic filariasis elimination programmes and implications for other neglected tropical diseases

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel

von

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aus

Atlanta, Vereinigte Staaten von Amerika

Basel, 2018

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

auf Antrag von Prof. Dr. Jürg Utzinger und Dr. Jonathan D. King.

Basel, den 26. Juni 2018

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Philosophisch-Naturwissenschaftlichen Fakultät

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Acknowledgements

I would like to express my sincere thanks to my advisor, Prof. Dr. Jürg Utzinger, for his guidance and wisdom through this journey. From the taxi ride in the Philippines when we first started discussing the possibility of this collaboration, he has provided constant encouragement, scientific expertise and invaluable time to carefully review my work. I have been continually motivated by his optimistic enthusiasm.

There are no words to express the depth of gratitude I have for Dr. Patrick J. Lammie. Over the years he has offered unending encouragement and support and has provided professional opportunities that have shaped my career. He is an exemplary scientist and public health advocate, and his innovative thinking has truly advanced the field of neglected tropical diseases. He has been a respected advisor, mentor and collaborator, and has always pushed me to be thoughtful about and believe in the contributions I can make. This work would not have been possible without him. I'm sorry I ignored his subtle and not so subtle advice to pursue a PhD for so long. I thank him for not giving up.

I am grateful to Dr. Jonathan D. King for his willingness to be the co-referee for my thesis. His leadership at the World Health Organization (WHO) continues to drive the Global Programme to Eliminate Lymphatic Filariasis (GPELF) forward. I also thank him for setting the example by which this type of PhD programme could be done. His encouragement and advice gave me the confidence to know that I would succeed. I look forward to many more years of collaboration.

This PhD thesis project would not have been possible without the financial support of the Centers for Disease Control and Prevention (CDC), the United States Agency for International Development (USAID) and The Bill & Melinda Gates Foundation. I appreciate

the commitment of these institutions to provide resources to improve the lives of neglected populations.

I am deeply indebted to all of the partners, field teams and lab teams in American Samoa, The Gambia, Haiti and Kenya. They were the true hands and feet of the project. Their dedication to these activities resulted in high quality work that has contributed invaluably to the Global Programme to Eliminate Lymphatic Filariasis.

An important lesson I have learned in life and one that has been emphasised during this PhD journey is that success is never defined by the individual. Many of my colleagues have shared words of wisdom, experiences and guidance that have been essential elements in shaping my success. The following individuals have been influential in making completion of this thesis a reality:

Ben Arnold, Amanda Barry, Stephanie Bialek, Molly Brady, Tara Brant, Holly Chastain, Gretchen Cooley, Christine Dubray, Scott Elder, Katie Gass, Patricia Graves, Katy Hamlin, Kazuyo Ichimori, Angela Keller, Alaine Knipes, Colleen Lau, Andrew Majewski, Diana Martin, Sammy Njenga, Maurice Odiere, Eric Ottesen, Sonia Pelletreau, Jeff Priest, Maria Rebollo, Keri Robinson, Evan Secor, Ryan Wiegand, Caitlin Worrell and Aya Yajima.

I am deeply indebted to my friends for their support through this process. They always knew the perfect words of encouragement to provide motivation when needed. Importantly, they knew how to provide necessary distractions and humour when I needed a good laugh to keep things in perspective.

Finally, the utmost thanks goes to my family. Their unconditional love and support have been the firm foundation in my life. Without them this journey would not have been possible.

Summary

Background: Lymphatic filariasis (LF), a mosquito-transmitted parasitic disease caused by filarial worms, is a leading cause of disability worldwide. In 1997, at the 50th World Health Assembly, a resolution was passed to eliminate LF as a public health problem by 2020. To reach established elimination targets, LF programmes conduct annual community-wide mass drug administration (MDA). At the start of the Global Programme to Eliminate Lymphatic Filariasis (GPELF), an estimated 120 million individuals were infected, and approximately 1.4 billion people were at risk for filarial infection. Since then, mainly through MDA programmes, the number of people at risk of infection has been reduced to 856.4 million. By the end of 2016, MDA had been implemented in 63 of 72 LF-endemic countries. Demonstrating success of LF programmes depends on rigorous monitoring and evaluation (M&E) of programme activities. As prevalence declines, it is important to identify sensitive diagnostic tools and robust surveillance strategies to detect any possible recrudescence of infection as early as possible. Existing recommendations for LF surveillance are adequate for making the decision to stop MDA, but may not be sufficient for documenting that elimination endpoints have been met. Programme strategies need to be refined in order to establish a more robust M&E framework.

Goals and objectives: The overarching goal of this PhD thesis was to provide recommendations on approaches for conducting surveillance for LF elimination programmes. There were two interlinked objectives: (i) to determine the utility of serologic tools during the post-MDA surveillance period within communities that have received multiple rounds of LF MDA; and (ii) to determine the utility of the transmission assessment survey (TAS) and other activities during the post-MDA surveillance period as platforms for integrated disease surveillance. The specific aims were (i) to compare LF antigen and antifilarial antibody responses during the post-MDA surveillance period; (ii) to determine the appropriate age group(s) to monitor during the post-MDA period; (iii) to compare diagnostic tools for use during the surveillance period to determine the most appropriate diagnostic tool(s) to use for

LF surveillance; and (iv) to assess the feasibility of using existing disease programme infrastructures as platforms for multi-disease surveillance.

Methods: To compare the utility of parasitological and serological indicators for measuring LF programme endpoints, samples from participants (2-100 years old) in 10 sentinel sites in coastal Kenya were examined for circulating filarial antigen (CFA) and filarial antibodies. To evaluate the use of antibody responses as a way to measure the impact of MDA, serum samples collected at three time points from children 1-5 years of age in western Kenya were tested for antibody responses to two schistosome antigens by multiplex bead assay (MBA). In American Samoa, CFA and antibody results from children enrolled in LF TAS conducted 4 years apart were analysed to determine whether interruption of LF transmission has been achieved. A study was carried out in The Gambia among populations living in 15 villages with a history of high LF prevalence. Samples were collected and tested for CFA and filarial antibodies to evaluate the use of serological tools to confirm interruption of LF transmission. Published data from previously conducted studies in American Samoa were analysed to evaluate the relationship between human serological indicators and filarial DNA in mosquitoes. In Haiti, to evaluate the feasibility of using TAS as a platform to collect information about other tropical diseases, samples were collected to test for LF and malaria. In addition to LF testing, samples collected during the sentinel site surveys in coastal Kenya were used for the detection of antibodies against antigens from several parasitic infections as well as markers for immunity to vaccine-preventable diseases to determine the utility of integrated serosurveillance.

Results: The overall prevalence of filarial antigenaemia in coastal Kenya was low (1.3%). CFA prevalence among children under 10 years old was very low (<1%). However, quantitative antibody levels among children were higher in areas with suspected LF transmission. Antibodies to *Schistosoma* spp. antigens among children declined after MDA. There was a significant decrease in the proportion of 1-year olds with positive antibody responses from 33.1% in year 1 to 13.2% in year 3. In American Samoa, a total of 1,134 and 864 children (5-10 years old) were enrolled in TAS 1 (2011) and TAS 2 (2015), respectively.

Two CFA-positive children were identified in TAS 1, and one CFA-positive child was identified in TAS 2. In 2011, overall prevalence of antibody responses to Wb123, Bm14, and Bm33 was 1.0%, 6.8% and 12.0%, respectively. In 2015, overall prevalence of positive Bm14 and Bm33 responses declined significantly to 3.0% and 7.8%, respectively. However, there were persistent antibody responses in some schools. In The Gambia, a total of 2,612 dried blood spots (DBS) collected from individuals aged 1 year and above was tested for antibodies to Wb123 by enzyme-linked immunosorbent assay (ELISA). Overall, prevalence of Wb123 was low (1.5%). In seven of 15 villages, there were no Wb123-positive individuals identified. In American Samoa, there was a significant relationship between the presence of filarial DNA in mosquitoes and villages with individuals with responses to Wb123. It was feasible to add malaria testing to TAS in Haiti. A total of 16,655 children were tested for LF and 14,795 for malaria in 14 TAS. In Kenya, utilising a multiplex approach, antibody responses to 10 antigens representing six parasitic infections and three antigens to assess immunity to vaccine preventable diseases were generated from a single sample collected from each participant.

Conclusions: As prevalence declines, using parasitological indicators to determine LF programme endpoints becomes challenging and there is a need to identify alternative indicators to use during the surveillance period. Results from this PhD thesis support the use of antibody tools to determine the status of LF transmission and suggest that serological tools can have a role in guiding programmatic decision-making. The absence of antibody responses strongly suggests that LF transmission has been interrupted and, in contrast, the presence of antibody in children is an important indicator that programmes have not reached elimination. Finally, existing LF programme activities can provide a platform both to introduce the use of antibody testing into TAS and to conduct integrated assessments.

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List of abbreviations

Ab	Antibody
ADLA	Acute dermatolymphangioadenitis
AFRO	World Health Organization Regional Office for Africa
Ag	Antigen
ALB	Albendazole
aOR	Adjusted odds ratio
CDC	U.S. Centers for Disease Control and Prevention
CFA	Circulating filarial antigen
CI	Confidence interval
CL	Confidence limit
DA	Diethylcarbamazine + albendazole
DBS	Dried blood spot
DEC	Diethylcarbamazine
DNA	Deoxyribonucleic acid
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
EPG	Eggs per gram (of stool)
EU	Evaluation unit
FTS	Filariasis test strip
GLAMM	Generalized linear latent and mixed models
GLS	Geleralized least squares
GPELF	Global Programme to Eliminate Lymphatic Filariasis
GPS	Global positioning system
HRP	Horseradish peroxidase
ICT	Immunochromatographic card test
IDA	Ivermectin + diethylcarbamazine + albendazole

Ig	Immunoglobulin
IRB	Institutional review board
ITFDE	International Task Force for Disease Eradication
IU	Implementation unit
IVM	Integrated vector management
KEMRI	Kenya Medical Research Institutue
L3	3rd stage larvae
LF	Lymphatic filariasis
LIPS	Luciferase immunoprecipitation system
LLIN	Long-lasting insecticidal net
M&E	Monitoring and evaluation
MBA	Multiplex bead assay
MBZ	Mebendazole
MDA	Mass drug administration
MF	Microfilariae
MFI	Median fluoresence intensity
MMDP	Morbidity management and disability prevention
MOE	Minstry of education
MOH	Ministry of health
MSPP	Haitian Ministry of Public Health and Population
MX	Xenomonitoring
NGO	Non-governmental organisation
NPELF	National Programme for the Elimination of Lymphatic Filariasis
NTD	Neglected tropical disease
OD	Optical density
OR	Odds ratio
PacELF	Pacific Programme for the Elimination of Lymphatic Filariasis

PC	Preventive chemotherapy
PCR	Polymerase chain reaction
POC	Point-of-care
POC-CCA	Point-of-care circulating cathodic antigen
PRNT	Plaque reduction neutralization test
PSAC	Pre-school aged children
PZQ	Praziquantel
QC	Quality control
RDT	Rapid diagnostic test
RNA	Ribonucleic acid
ROC	Receiver operator characteristic
SAC	School aged children
SSB	Survey sample builder
STH	Soil-transmitted helminth
TAS	Transmission assessment survey
TMB	Tetramethylbenzidine
TPE	Tropical pulmonary eosinophilia
VPD	Vaccine-preventable disease
WHA	World Health Assembly
WHO	World Health Organization

1 Introduction

Neglected tropical diseases (NTDs), a diverse group of diseases, are widespread throughout the tropics and subtropics and affect more than 1 billion people worldwide (Herricks et al., 2017; WHO, 2018). These diseases are most prevalent among the poorest and most marginalized populations and can cause significant physical and emotional suffering (Engels and Savioli, 2006; Hotez et al., 2006b; 2006a; Utzinger et al., 2012; Houweling et al., 2016). NTDs impede the ability to work, impact quality of life, prevent communities from thriving, and contribute to significant economic loss in populations already living in fragile environments (Hotez et al., 2009; Conteh et al., 2010). Not only are these diseases co-endemic in countries, but individuals can often be affected by multiple NTDs (Keiser et al., 2002; Raso et al., 2004; Hürlimann et al., 2014; Madinga et al., 2017). While the impact of these diseases can be devastating, there has been considerable effort to control and in some cases eliminate these diseases (WHO, 2017a, 2017b). In 2005, the World Health Organization (WHO) called for the integration of NTD programmes, in order to take advantage of the common features of control and elimination strategies (WHO, 2006a). Some NTDs, lymphatic filariasis (LF), onchocerciasis, trachoma, schistosomiasis, and soil-transmitted helminthiasis (STH), can be effectively controlled and potentially eliminated using safe and effective drugs and other complementary interventions, making it feasible to implement large-scale preventive chemotherapy programmes. In 2012, governments, non-governmental organisations (NGOs), philanthropic groups, international coalitions, private donor foundations, and pharmaceutical companies formally committed to control or eliminate as many NTDs as possible by 2020 (NTDs, 2012). Toward this end, in 2016, 1.4 billion treatments were delivered to 1 billion individuals, a remarkable public health achievement (WHO, 2017a). While the number of treatments delivered is impressive, it is essential that programmes be monitored carefully in order to track and document progress. Inherent features of NTDs including the fact that most infections are asymptomatic and that diagnostic

tools are inadequate make surveillance complex, and hence, commonly used strategies need to be adapted to make them suitable for NTDs. Carefully designed epidemiological methods and appropriate use of diagnostic tools must be coupled with programmatic feasibility in order to provide actionable information for achieving control and elimination targets. Although there is general optimism that the global burden of NTDs can be reduced, it is critical to continually review existing knowledge and to adapt monitoring and surveillance strategies to fit programme needs.

This PhD thesis aims to explore approaches for conducting surveillance for LF, one of the preventive chemotherapy (PC) NTDs. Through large scale interventions, significant progress has been made towards the elimination of LF. However, as prevalence declines, it is essential to identify appropriate diagnostic tools and surveillance strategies to accurately assess population-wide LF status to document elimination targets have been met. As programmes near LF elimination endpoints, recommended strategies need to be refined in order to establish a robust framework for sustaining achievements made to date.

1.1 Aetiology of lymphatic filariasis

LF is a parasitic disease caused by three main species of filarial nematodes: *Wuchereria bancrofti*, responsible for more than 90% of all infections worldwide, *Brugia malayi*, and *B. timori* (Taylor et al., 2010). The parasite is transmitted to humans by multiple mosquito species including *Aedes* spp., *Anopheles* spp., *Culex* spp., and *Mansonia* spp. (Sasa, 1976), and the complete parasite life cycle requires development stages in both the definitive human host and the intermediate vector mosquito. During a blood meal, infective larvae penetrate into the bite wound and typically migrate to the lymphatic vessels and lymph nodes where they develop into adult worms. Upon maturation, female worms measure 80 mm to 100 mm in length while male worms are markedly shorter, measuring an average of 40 mm in length (Sasa, 1976). Adult worms often congregate in the lymphatics to form worm nests and can live for several years with fecundity lasting on average 6 to 8 years. Upon mating, female worms produce millions of microfilariae (MF) that migrate to the lymph and blood channels

where they can then be picked up by a mosquito taking a blood meal. After MF are ingested by the mosquito, they migrate through the midgut until they reach the thoracic muscles. The MF continue to develop into infective third stage larvae, but there is no multiplication or sexual reproduction of the parasite in the mosquito (Sasa, 1976). The infective larvae migrate through the haemocoele to the mosquito's proboscis upon which the mosquito can transmit the parasite to a person when it takes a blood meal. The complete life cycle of *W. bancrofti* is illustrated in Figure 1.1.

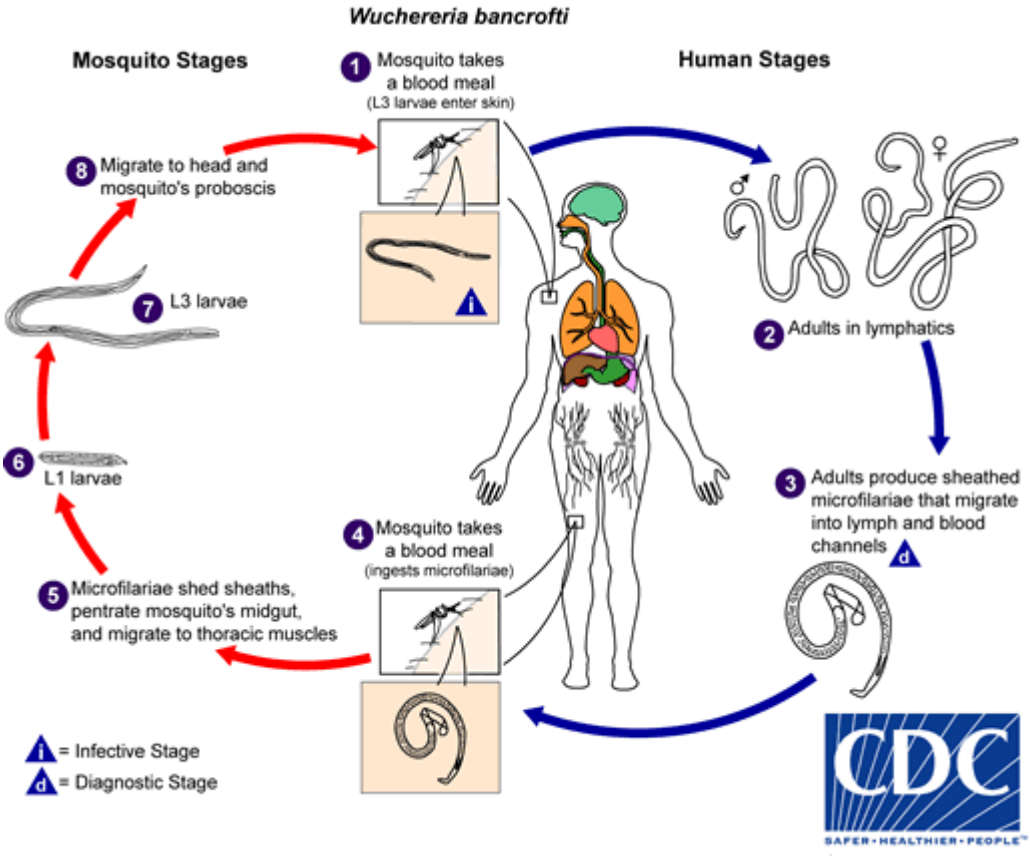

Figure 1.1 Life cycle of *Wuchereria bancrofti*

source: https://www.cdc.gov/parasites/lymphaticfilariasis/biology_w_bancrofti.html

1.2 Pathology of lymphatic filariasis

Filarial infection can cause a variety of clinical manifestations, most commonly lymphedema of the limbs and hydrocele among adult males (Addiss and Brady, 2007). However, most infections are asymptomatic, and the majority of infected individuals will never develop overt

clinical signs. Despite the absence of apparent clinical indications, nearly all infected persons have some degree of subclinical lymphatic damage that can eventually lead to permanent, chronic complications (Witt and Ottesen, 2001; Nutman, 2013). However, this subclinical damage is potentially reversible in children if infections are treated early (Shenoy et al., 2009; Shenoy and Bockarie, 2011; Kar et al., 2017).

1.2.1 Lymphedema

A proportion of infected individuals will develop lymphedema caused by improper functioning of the lymph system. In these individuals, adult worms cause lymphatic vessel dilatation resulting in lymphatic dysfunction that leads to collection of fluid and swelling in the areas of accumulation (Dreyer et al., 2000; Taylor et al., 2010). Swelling is generally gradual and observed primarily in the lower limbs. Although most lymphedema develops in the legs, it can also occur in the arms and breasts (Pani et al., 1990; 1991; Gyapong et al., 1994; Addiss and Brady, 2007). Swelling of the limbs is commonly unilateral, but bilateral involvement can occur. In such cases, swelling tends to be asymmetrical (Shenoy, 2008). In addition to swelling, lymphedema can be associated with skin changes including thickened skin, knobs, mossy lesions, and folds (Olszewski et al., 1993; Burri et al., 1996; Nutman, 2013) (Figure 1.2).

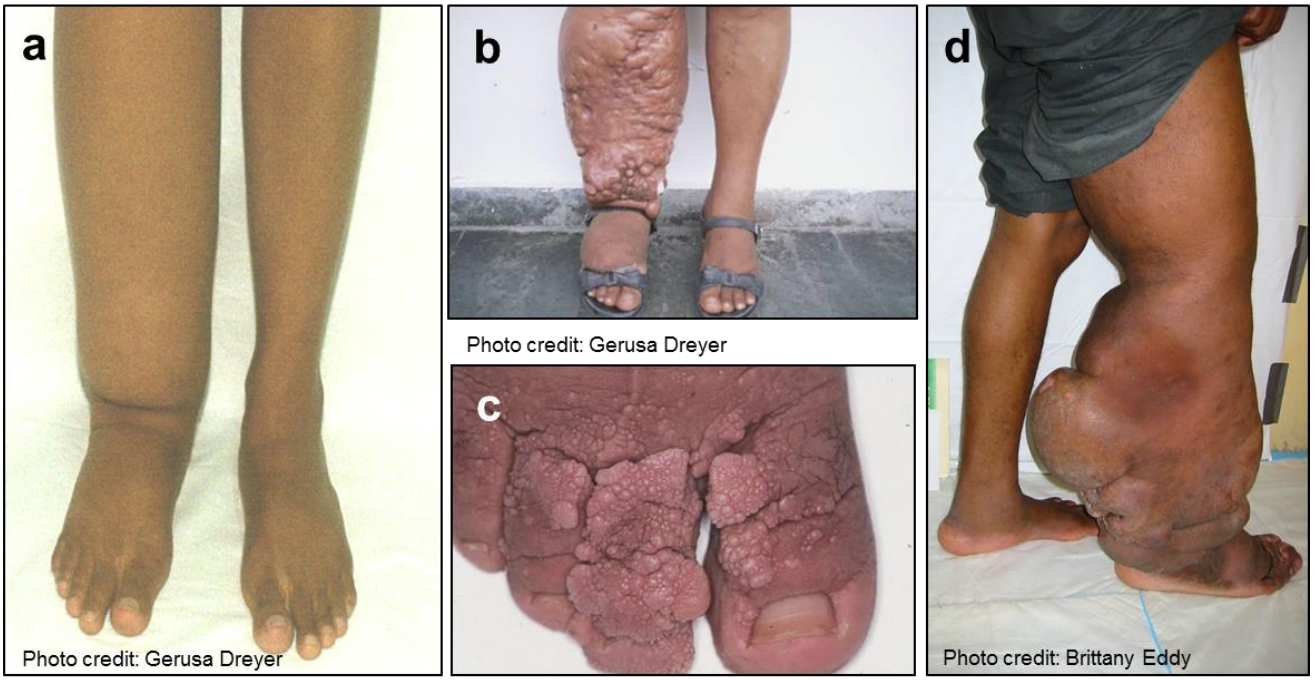

Figure 1.2 Skin changes associated with lymphedema: (a) unilateral swelling (b) knobs (c) mossy lesions (d) folds

Some individuals with lymphedema may experience progression to elephantiasis, extreme swelling and hardening of the skin, but the mechanism for progression from infection to lymphedema to elephantiasis is not well understood (Shenoy, 2008; Nutman, 2013). Of note, in most cases, individuals with lymphedema and elephantiasis do not have evidence of MF in the blood (Lammie et al., 1993; Addiss et al., 1995; Dissanayake, 2001). This is likely a result of the length of time required for disease progression to occur. Basic lymphedema management includes simple measures such as washing, skin care, and elevation of the affected limb(s) (McPherson et al., 2006; Ottesen, 2006).

1.2.2 Hydrocele

Among men infected with *W. bancrofti*, hydrocele is the most common clinical manifestation (Simonsen et al., 1995; Michael et al., 1996; Addiss and Brady, 2007; Njenga et al., 2007), but hydroceles among men with Brugian filariasis are uncommon (Nutman, 2013). Hydroceles result from the accumulation of fluid in the tunica vaginalis (Dreyer et al., 2000; Mand et al., 2011). Swelling may disappear after initial acute episodes (Noroes et al., 2003; Hussein et al., 2004), but over time the scrotum becomes enlarged and thickened due to excess fluid around the testicles, and the hydrocele becomes progressively larger. Onset is often silent, but may be preceded by an acute episode(s) of funiculitis (Estambale et al., 1994; Wamae et al., 1998). Similar to lymphedema, most cases are unilateral and bilateral involvement is often asymmetrical (Figure 1.3). Additionally, studies have shown that males living in endemic areas who have normal clinical examinations can have subclinical hydroceles (Simonsen et al., 2002; Mand et al., 2011). Rarely, a chylocele may form if lymph fluid from a ruptured lymphatic vessel enters the hydrocele (Dreyer et al., 2000). While unaddressed hydroceles can have significant impact on quality of life, simple surgery can be performed to drain and repair hydroceles (Addiss and Brady, 2007; Lim et al., 2015).

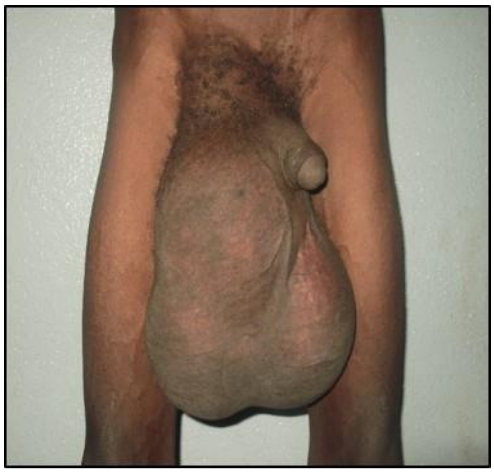

Photo credit: Tom Streit

Figure 1.3 Hydrocele

1.2.3 Acute dermatolymphangioadenitis

Lymphatic vessel damage and lymphatic dysfunction caused by LF often predispose individuals to recurrent bacterial infections (Olszewski et al., 1999; Esterre et al., 2000; Baird et al., 2002). These secondary infections elicit acute dermatolymphangioadenitis (ADLA), often referred to as acute attacks (Addiss and Brady, 2007). These episodes are common and likely play a role in the progression of lymphedema (Addiss and Brady, 2007). ADLA causes painful adenolymphangitis and cellulitis and is associated with swelling of the limbs, redness and warmth of the affected area and pain (Figure 1.4). During acute attacks, individuals often experience fever, headache, and general malaise (Olszewski et al., 1997; Dreyer et al., 1999). These symptoms can be managed by the use of analgesics and antipyretics to relieve pain and to reduce fever. Additionally, the use of first-line antibiotics is recommended (Shenoy, 2008; Mand et al., 2012). Frequency of acute attacks can be reduced by maintaining good hygiene that can be achieved by basic home care (Shenoy et al., 1995; Suma et al., 2002; Addiss et al., 2010; Jullien et al., 2011).

Figure 1.4 Acute dermatolymphangioadenitis (ADLA)

1.2.4 Tropical pulmonary eosinophilia

A small proportion of individuals experience hypersensitivity to filarial infection that results in a syndrome known as tropical pulmonary eosinophilia (TPE). The syndrome typically affects adult men (20 to 40 years old) living in Asian LF-endemic countries (Ottesen and Nutman, 1992; Ong and Doyle, 1998). TPE is characterised most commonly by hypereosinophilia (>3000/ml) and high levels of immunoglobulin (Ig) E in the blood (Neva and Ottesen, 1978; Ottesen et al., 1979; Hussain et al., 1981). Individuals with TPE generally do not have MF in the peripheral blood, but elevated antifilarial antibodies are often detectable (Ottesen and Nutman, 1992). Common symptoms include non-productive cough, shortness of breath, and

wheezing – clinical presentation that can be confused with asthma (Neva and Ottesen, 1978; Ottesen and Nutman, 1992; Ong and Doyle, 1998). Persons with TPE can also experience low-grade fever, weight loss and general malaise (Neva and Ottesen, 1978). TPE symptoms can last from a few days to a few weeks after which they can spontaneously resolve without treatment (Ottesen and Nutman, 1992). However, treatment with antifilarial drugs is recommended (Pinkston et al., 1987; Ottesen and Nutman, 1992).

1.3 Diagnosis

1.3.1 Adult worm

Definitive diagnosis of infection depends on identification of adult worms or MF. However, the ability to detect adult worms in the lymphatics of infected individuals is difficult and is not typically done. Although biopsies can be performed on suspicion of infection, they are impractical for routine use. Non-invasive techniques such as ultrasonography provide an alternative to biopsy and can be used to visualize adult worm nests in the lymphatics (Amaral et al., 1994; Dreyer et al., 1994; Noroes et al., 1996b; Fox et al., 2005). Active worms in these nests exhibit very distinct patterns of movement referred to as the “filarial dance sign” (Amaral et al., 1994). Additionally, subclinical lymphatic damage, dilatation of lymphatic vessels, can also be assessed by ultrasound (Suresh et al., 1997; Faris et al., 1998). While ultrasound examinations are relatively simple, they require specialized equipment and trained personnel and are not feasible to use on a regular basis in most LF-endemic settings.

1.3.2 Microfilariae

In contrast to the infrequent use of methods to identify adult worms, techniques used to detect MF have routinely been used to diagnosis filarial infection. MF in the peripheral blood can be observed by microscopically examining blood smears (20 µl to 60 µl whole blood) stained with Giemsa or hematoxylin and identifying distinct morphologic features to determine the species of filarial worm (Sasa, 1976). Giemsa stained images of the three species that cause LF are shown in Figure 1.5. Although blood smears are relatively simple and inexpensive to make, accurate diagnosis depends on skilled, experienced microscopists

correctly identifying MF. For increased sensitivity, larger volumes of blood (approximately 1 ml) can be collected for concentration techniques such as the Knott's technique (Rawlins et al., 1994; Oliveira et al., 2014) or membrane filtration (McMahon et al., 1979; Moulia-Pelat et al., 1992; Rawlins et al., 1994). More recently, polymerase chain reaction (PCR) assays have been developed to detect parasite DNA and are the most sensitive of all available techniques (Lizotte et al., 1994; Zhong et al., 1996; Rao et al., 2006a; 2006b). However, molecular methods are not commonly used as they are expensive and require sophisticated laboratory infrastructure. Regardless of the technique used, the number of MF or the amount of DNA detected provides a measure of intensity of infection and can serve as a useful indication of adult worm burden in an infected individual. However, in most parts of the world, tests to detect MF are limited by the nocturnal periodicity of the parasites. In these areas, accuracy of diagnosis by any of these techniques is dependent on both the volume of blood collected and the collection of blood at the appropriate time (22:00 to 02:00 hours). Furthermore, detection of MF is relatively insensitive when parasite density is low and few MF are circulating in the peripheral blood (Gass et al., 2012). Thus, the chance of missing MF increases when there are few viable adult worms producing MF.

1.3.3 Circulating filarial antigen

W. bancrofti ***B. malayi*** ***B. timori***

source: www.dpd.cdc.gov/dpdx

Figure 1.5 Giemsa stained images of microfilariae of the three species of filarial worms that cause lymphatic filariasis

When definitive diagnosis of the parasite is not possible or practical, the presence of circulating filarial antigen (CFA) can serve as a proxy for *W. bancrofti* infection. Currently,

there are no diagnostic tests available for the detection of CFA of *Brugia* spp. CFA tests recognise parasite antigen that is highly expressed in the cuticle and reproductive organs of adult worms and is released in relatively large quantities by living worms (Weil and Liftis, 1987). Consequently, CFA is commonly detectable in microfilaremic individuals (Weil and Liftis, 1987; Lammie et al., 1994; Weil et al., 1997). Additionally, CFA is often present in infected individuals who are amicrofilaremic and asymptomatic (Weil et al., 1988; 1996). Thus, results from these tests provide a more sensitive measure of infection compared to those used to identify MF. An added advantage is that CFA is detectable in peripheral blood at any time of day (Weil et al., 1986). As a result, the same restrictions for timely blood collection required for MF do not exist. CFA levels often decrease after treatment, but antigenaemia can persist after MF have been cleared from the blood (Eberhard et al., 1997; Ismail et al., 2001; El Setouhy et al., 2004; Simonsen et al., 2005; Helmy et al., 2006). While CFA tests afford advantages over parasitologic methods, positive results do not necessarily indicate the presence of viable adult worms capable of producing MF as worms past their reproductive lifespan can still produce detectable levels of CFA. Additionally, a viable female worm may be present in the absence of any male worms, making it impossible for MF to be produced.

Quantitative measures of CFA assessed by enzyme-linked immunosorbent assay (ELISA) can provide an indication of adult worm infection intensity (Weil et al., 1987; More and Copeman, 1990). While the ability to estimate levels of CFA can be useful to monitor infection, ELISAs cannot be performed without adequate laboratory infrastructure. Detection of CFA was simplified with the introduction of an immunochromatographic card test (ICT) (Weil et al., 1997). This rapid format, lateral flow, point-of-care (POC) test allowed for the detection of CFA from peripheral blood, serum or plasma outside the confines of a laboratory. As with the ELISA format, the ICT was found to be sensitive and highly specific for the detection of *W. bancrofti* adult worm antigen (Njenga and Wamae, 2001; Chandrasena et al., 2002; Pani et al., 2004). However, more recently, positive ICT results

have been reported among individuals infected with *Loa loa* but negative for *W. bancrofti*, thus making it difficult to discriminate between the two filarial infections (Wanji et al., 2015; Pion et al., 2016). Unlike the ELISA platform, the ICT can only provide a qualitative assessment of the presence or absence of CFA. While the ICT provides an alternative to laboratory-based assays, there are some limitations to its use. There is a narrow window in which the results can accurately be read and interpreted, and false positive results are common when the test is read after the recommended reading time. Additionally, the tests have a relatively short shelf life (approximately 3 months) when stored above 4°C. Furthermore, the relatively high cost of the tests make it prohibitive to use in under-resourced countries. In an effort to address these limitations, the ICT was reformatted, and the Filariasis Test Strip (FTS) was developed (Weil et al., 2013). In laboratory and field evaluations, the FTS was found to be slightly more sensitive than the ICT but overall very comparable (Weil et al., 2013; Yahathugoda et al., 2015; Chesnais et al., 2017). Currently, production of the ICT is being phased out, and is being replaced solely by the FTS.

1.3.4 Antifilarial antibody

Antifilarial antibody responses develop prior to CFA and MF, making them early markers of exposure and infection (Hamlin et al., 2012). Similar to CFA, antibody responses can be detected in both microfilaremic and amicrofilaremic individuals (Ottesen et al., 1982; Zhang et al., 1999; el Serougi et al., 2000) providing advantages over parasitologic methods. Although nearly all individuals who live in LF-endemic areas develop antifilarial antibody responses within the first few years of life (Witt and Ottesen, 2001), mechanisms of protective immunity are poorly understood. Early immunodiagnostic techniques included complement fixation, haemagglutination, and indirect fluorescent antibody to assess antibodies against MF and adult worms (Ridley, 1956; Kagan, 1963; Kagan et al., 1963; Yong, 1973). Over time, diagnostic techniques have shifted towards commonly used platforms such as ELISA to assess isotype-specific responses. Although all major immunoglobulin (Ig) classes have been targets for immunoassays, studies have shown that

IgG is produced in significant amounts in infected individuals (Hussain and Ottesen, 1985; Ottesen et al., 1985; Hitch et al., 1989). Furthermore, there is evidence that the IgG4 subclasses may distinguish active infection from chronic exposure (Hussain et al., 1987; Kwan-Lim et al., 1990; Kurniawan et al., 1993; Rahmah et al., 1994). However, because antibody responses persist after MF have cleared, it is often difficult to determine if responses are a result of current infection or past exposure. Limited evidence suggests that antifilarial responses are not lifelong and that individuals will eventually serorevert after infection has cleared (Wamae et al., 1992; Weil et al., 2008; Moss et al., 2011).

Early assays used native filarial parasite antigen, and although these assays were relatively sensitive, they suffered from poor specificity (Maizels et al., 1985; Muck et al., 2003; Fischer et al., 2005). Specificity of antibody assays was improved with the development of recombinant antigens such as Bm14 (Chandrashekar et al., 1994), WbSXP (Dissanayake et al., 1992), and BmR1 (Rahmah et al., 2001). Although cross-reactivity was reduced compared to when crude antigens were used, it was not eliminated (Weil et al., 2011). This made it challenging to use these assays in areas where multiple filarial parasites exist. Recently, Wb123 (Kubofcik et al., 2012), a highly specific recombinant antigen has been described as an early serologic marker for *W. bancrofti* infection, thus potentially eliminating some of the challenges encountered with previous recombinant and crude antigens.

1.3.5 Molecular xenomonitoring

Molecular xenomonitoring (MX), the use of molecular methods (e.g. PCR) to detect filarial DNA in mosquitoes, can provide an indirect measure of infection in the human population (Williams et al., 2002; Fischer et al., 2007; Weil and Ramzy, 2007). Prior to the introduction of molecular techniques, dissection and microscopic examination of vector mosquitoes was the standard method used to determine infection rates in mosquitoes. This method was time consuming, labor intensive, and impractical for large-scale assessments. Furthermore, the sensitivity for detecting parasites by dissection declined as infection prevalence declined

(Ramzy et al., 1997; Plichart et al., 2006; Chambers et al., 2009). With the introduction of MX, pools of mosquitoes could be examined with increased efficiency and sensitivity.

Collecting and examining mosquitoes provides a non-invasive alternative to sampling human populations. However, MX depends on the availability skilled persons to collect the mosquitoes, the ability to adequately sample the vector population, and laboratory infrastructure to test the mosquitoes. Additionally, the presence of filarial DNA in mosquitoes does not necessarily indicate the presence of infective parasites. RNA assays (Laney et al., 2008; 2010) to detect infective 3rd stage larvae (L3) larvae exist but not commonly used, as they require collection of mosquitoes into special media to preserve RNA. Importantly, the direct relationship between filarial DNA levels in vector mosquitoes and infection rates in humans is unclear. Hence, the value of MX may be limited to simply identifying areas where human infections exist.

1.4 Treatment

Currently, there are no drugs that specifically target adult worms. Diethylcarbamazine (DEC), developed in 1947 as a derivative of the antiparasitic drug, piperazine (Hewitt et al., 1947), is the preferred treatment. Although it has limited macrofilaricidal impact (1996a; Noroes et al., 1997), it is very effective at killing MF (Sasa, 1976; Hawking, 1979; Ottesen, 1985). Historically, a standard 12-day course of DEC (6 mg/kg/day) was recommended, but studies have documented comparable efficacy of a single dose (6 mg/kg) in achieving sustained reduction of MF of up to one year (Kimura and Mataika, 1996; Meyrowitsch et al., 1996; Noroes et al., 1997). DEC is relatively well tolerated but side effects such as headache, fever, and general malaise are common (Ottesen, 1985; Horton et al., 2000). Additionally, treated individuals can experience localized reactions and pain, likely due to the rapid killing of parasites (Ottesen, 1985).

Ivermectin, an avermectin derivative, was first introduced as a veterinary drug in the early 1980s and as a human drug later that same decade (Aziz et al., 1982; Campbell, 1982). Similar to DEC, a single dose (200 µg to 400 µg) is very effective at rapid clearing of MF for

periods of up to 12 months but has virtually no impact on adult worms (Cao et al., 1997; Brown et al., 2000). As an added benefit, ivermectin can be used to treat common intestinal worms and ectoparasites such as scabies and lice (Meinking et al., 1995; Bockarie et al., 2000; Kircik et al., 2016; Hardy et al., 2017). Individuals treated with ivermectin can experience similar systemic reactions as those treated with DEC (Horton et al., 2000).

Albendazole, a benzimidazole, was first introduced for human use in the early 1980s (Pene et al., 1982; Rossignol and Maisonneuve, 1983). At a standard dose of 400 mg, it is effective against several helminth infections including LF (Addiss et al., 1997; Ismail et al., 1998; Beach et al., 1999; Horton, 2000). Unlike DEC and ivermectin, the killing of MF after a single dose of albendazole is slow and gradual over 6 to 12 months (Gyapong et al., 2005). Systemic reactions to treatment are similar to the other antifilarial drugs (Horton et al., 2000).

1.5 Epidemiology

Historically, LF has been documented clinically or parasitologically in 104 countries in the tropics and sub-tropics (Sasa, 1976). MF of *W. bancrofti* were first described by Wucherer in 1866 in a urine sample from a patient in Bahia, Brazil suffering from urinary schistosomiasis (Sasa, 1976). Six years later, Lewis identified *W. bancrofti* MF in a peripheral blood sample from a patient in India (Sasa, 1976). The first adult *W. bancrofti* worm was isolated in 1876 from a lymphatic abscess on the arm of a Chinese patient in Brisbane, Australia (Sasa, 1976). MF of *Brugia malayi* were not described as distinctly different until Lichtenstein and Brug did so in 1927. MF of *Brugia timori* were not characterized until 1965 by David and Edeson in 1965 (Sasa, 1976). In 1947, prior to a clear distinction between *W. bancrofti* and *Brugia* spp., Stoll estimated 189 million people infected with *W. bancrofti* worldwide (Stoll, 1947).

Currently, primarily as an indirect result of economic development, change in environmental conditions, and in a few cases by intentional treatment and control efforts, the number of LF-endemic countries has been reduced to 72 (WHO, 2017c). Although LF is widespread across many regions, it is not homogenously distributed in the countries where it

is endemic. Distribution is focal (Michael et al., 1996; Boyd et al., 2010; Cano et al., 2014), and pockets of transmission become increasingly difficult to detect as prevalence declines. LF is strongly associated with poverty (Gyapong et al., 1996; Coreil et al., 1998; Ramaiah et al., 1998; Hotez, 2007) and transmission often occurs in environments where there is risk for multiple infectious diseases, compounding the impact LF can have on populations. Because there are multiple mosquito species capable of transmitting filarial parasites, LF exposure is not restricted to only rural or urban areas as suitable mosquito breeding environments exist in both settings. LF is one of the leading causes of disability worldwide and in 2016 was responsible for an estimated 1.19 million disability-adjusted life years (DALYs) (GBD, 2017).

Transmission of LF is influenced by a variety of factors including the number of infected persons, the density of MF in the blood of infected persons, and the density of mosquito vectors. Infection is usually acquired in childhood. Children born to microfilaremic mothers are more likely to be infected than those born to uninfected mothers (Lammie et al., 1991; 1998). However, MF infrequently cross the placental wall during pregnancy, suggesting infections are not acquired in utero, but instead that repeated exposure to the mother predisposes children to infection (Campello et al., 1993; Eberhard et al., 1993). In LF-endemic countries, prevalence of infection increases with age (Das et al., 1990; Lammie et al., 1994; Meyrowitsch et al., 1995; Kazura et al., 1997) and there are many more infected individuals than cases of overt clinical disease, thus making clinical diagnosis a significant underestimation of the number of persons affected by LF. Since repeated exposure to filarial parasites is needed to establish infection (Hati et al., 1989; Rwegoshora et al., 2005), short-term travel to endemic areas poses minimal risk for acquiring infection.

Because of the time required for MF to mature into adult worms, the ability to detect markers of infection is limited by the time required for patent infection to appear. Antifilarial antibody responses are the earliest marker of infection and typically develop within the first few months after exposure (Hamlin et al., 2012). CFA develops 6 to 12 months after antibodies (Hamlin et al., 2012). As MF are only produced after adult worms have mated, it is

the most lagging indicator. MF typically are not present for at least 12 to 18 months after CFA (Hamlin et al., 2012).

Although LF is widespread, several characteristics of the responsible filarial parasites make elimination feasible. The parasite does not multiply in the human host or the vector mosquito, and humans are the only definitive host for *W. bancrofti* (Sasa, 1976). Because sustained transmission is dependent on both humans and mosquitoes, there are multiple entry points for employing effective treatment and vector control strategies to disrupt the transmission cycle. Consequently, in 1993, an International Task Force for Disease Eradication (ITFDE) listed LF as one of six diseases that could feasibly be eradicated (CDC, 1993).

1.6 Global Programme to Eliminate Lymphatic Filariasis

In 1997, at the 50th World Health Assembly (WHA), a resolution was passed to eliminate LF as a public health problem by 2020 (WHA resolution 50.29) (WHO, 1997). Shortly thereafter, in 2000, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was organised to assist countries in achieving this goal (Ottesen, 2000). To reach established elimination targets, LF programmes set out to treat individuals in endemic areas through annual community-wide mass drug administration (MDA) for at least 5 years. At the start of GPELF, an estimated 120 million individuals were infected, and approximately 1.4 billion people were at risk for filarial infection (WHO, 2000). Since then, mainly through MDA programmes, the number of people at risk of infection has been reduced to 856.4 million (WHO, 2017c). By the end of 2016, MDA had been implemented in 63 of 72 LF-endemic countries, with a cumulative 6.7 billion treatments delivered since the launch of GPELF (WHO, 2017c).

The global LF elimination strategy has two main aims. First, to interrupt transmission through MDA; and second, to alleviate suffering caused by the disease through morbidity management and disability prevention (MMDP). WHO has established guiding principles to achieve these aims (WHO, 2011a, 2017d), and thus, all LF elimination programmes follow the basic programmatic steps.

1.6.1 Mapping and baseline

To address the first aim of GPELF, transmission interruption, programmes first conduct mapping to determine if intervention is necessary. At the start of GPELF, to take advantage of momentum surrounding the potential for elimination, there was a bias towards starting MDA. Treatment was sometimes initiated based on historic evidence alone or the presence of clinical disease (i.e. lymphedema and hydrocele). When parasitological assessments were conducted, district-wide treatment decisions were usually based on testing a convenience sample of 50 to 100 individuals. MDA was triggered when >1% of the tested individuals were positive for MF or CFA (WHO, 2011a). Currently, more rigorous, population-based assessments are recommended for mapping (Gass et al., 2017). After mapping, prior to initiating MDA, some programmes have chosen to conduct baseline assessments of MF or CFA. Although these surveys involve testing 300-500 people, they are frequently based on testing a convenience sample of the population and may not be a true representation of the entire area where MDA is implemented.

1.6.2 Treatment regimen

Recommended MDA treatment regimens depend on the local context. At the start of GPELF, co-administration of either DEC (6 mg/kg) plus albendazole (400 mg) (DA) or ivermectin (200 µg/kg) plus albendazole (400 mg) (IA) was recommended (WHO, 2001). Dual combination therapy was found to be safe and more effective at clearing MF than monotherapy with any of the three drugs (Horton et al., 2000; Gyapong et al., 2005). DA, the preferred combination, was recommended in all areas where LF and onchocerciasis were not co-endemic. Consequently, DA was used in most countries outside of Africa. Because of the risk of serious side effects induced by DEC when administered to individuals infected with *Onchocerca volvulus* (Bird et al., 1980; Aziz, 1986), the recommended drug combination in areas of co-endemicity was IA. This made IA the default MDA regimen in most of Africa. In select areas in Central and West Africa, in addition to *O. volvulus* and *W. bancrofti*, *L. loa* is present. Because there can be significant risk in treating loiasis with either DEC or ivermectin

(Carme et al., 1991; Gardon et al., 1997), monotherapy with albendazole is recommended for LF MDA (Pion et al., 2017; WHO, 2017d). Rapid scale up and implementation of MDA was possible in large part because of generous donations of albendazole from GlaxoSmithKline (then Smith Kline Beecham) and ivermectin from Merck and Company (Ottesen, 2000). Since then an additional donor of DEC, Easai Co., Ltd., has joined the effort of supporting the global need for MDA medicines (Naito, 2012).

Since all of the medicines have limited impact on adult worms, at least five annual rounds of dual combination MDA are administered during the reproductive lifespan of adult worms. The effectiveness of MDA depends on epidemiological coverage; an effective round is defined as more than 65% of the total population taking the medicines (WHO, 2017d). MDA is not recommended for pregnant women in the first trimester or severely ill persons. Because it is logistically challenging to weigh individuals during MDA, age or height are often used as proxies to determine dosing. DEC is not given to children under 2 years of age, and ivermectin is not recommended for individuals under 90 cm.

Recently, studies have been conducted to evaluate the safety and efficacy of triple drug combination of ivermectin, DEC, and albendazole (IDA). IDA was found to be extremely effective at clearing MF in infected patients and suppressing MF for up to two years after a single dose (Thomsen et al., 2016). As a result, in November 2017, WHO endorsed IDA for programmatic use (WHO, 2017d). Currently, WHO recommends IDA in areas eligible for DA MDA that have implemented fewer than four rounds MDA or have evidence of persistent transmission even after five rounds of MDA.

1.6.3 Monitoring and evaluation

In addition to ensuring high treatment coverage, assessments are needed to effectively monitor the impact of MDA. Large-scale surveys are not always feasible and cannot be done frequently. Instead, during the MDA implementation period, treatment impact is periodically monitored through sentinel and/or spot-check sites to provide information on trends of infection (WHO, 2011a, 2017d). Sentinel sites are established before the first round of MDA

and are recommended to remain constant throughout the duration of the programme. Sites are selected based on populations known to be or suspected to be at high risk. Ideally, sentinel sites have stable populations of at least 500 people so that a minimum of 300 individuals ≥5 years of age can be tested for MF or CFA during each survey. The general recommendation is to have a minimum of one sentinel site per 1 million population, but more sites are encouraged if resources allow. Because sentinel site populations are closely monitored, they often become more likely to adhere to programme activities. Results from these sites, therefore, may not be representative of the population over time. Spot-check sites have the same characteristics as sentinel sites other than the site locations are not fixed. Spot-check sites are selected in order to counter potential bias introduced by repeated assessments sentinel sites.

Ideally, sentinel and spot-check site surveys should be conducted as frequently as possible. Mid-term surveys, after the 3rd round of MDA, are useful to determine if the programme is on track or if programmatic activities need to be altered in any way. However, mid-term surveys are optional and are not routinely conducted. Currently, an assessment after the 5th round of effective MDA, known as a pre-transmission assessment survey (pre-TAS) is required as part of the criteria to determine eligibility for stopping MDA. Prevalence of CFA in *W. bancrofti* areas or antifilarial antibody in *Brugia* spp. areas must be <2% or MF prevalence <1% in sentinel and spot-check sites to qualify for the TAS (WHO, 2011a).

1.6.4 Transmission assessment survey

After multiple rounds of MDA, national LF elimination programmes must be able to determine whether transmission is likely no longer sustainable even in the absence of treatment. In 2011, WHO published guidance on conducting TAS, a statistically rigorous survey designed to help programme managers determine whether infection levels have been lowered below the threshold at which transmission is sustainable even in the absence of MDA (WHO, 2011a). The results of a TAS provide evidence for deciding whether to stop or continue MDA. The established threshold below which it is believed that transmission will no longer be self-

sustaining is <2% CFA prevalence in areas where *Anopheles* spp., *Culex* spp. and *Mansonia* spp. are the main vectors, and <1% CFA prevalence in areas where *Aedes* spp. is the main vector. In the absence of antigen detection tools suitable for *Brugia* spp., the same thresholds are applied for antifilarial antibody prevalence in *Brugia* areas.

The target population for TAS is young children (6 to 7 years old) because any evidence of infection in this population is an indication of relatively recent infection. If the total number of positive children identified in an evaluation unit (EU) is at or below a critical cutoff value, the EU "passes" and MDA can be stopped. If the number of positives exceeds the critical cutoff value, then the EU "fails" and MDA is continued. The TAS sample size and critical cutoff values are powered so that the EU has at least a 75% chance of passing if the true infection prevalence is half the threshold, and no more than a 5% chance of passing if the true prevalence is greater than or equal to the threshold (WHO, 2011a). After stopping MDA, TAS is repeated twice at 2- to 3-year intervals in order to confirm that transmission is still below the critical threshold.

1.6.5 Morbidity management and disability prevention

Although MMDP has been recommended since the inception of GPELF, progress toward providing adequate care has lagged behind the MDA arm. To meet the objectives of the 2nd aim of the global programme, programmes must ensure that all affected persons have access to a minimum package of care. These basic services including the ability to treat acute attacks, lymphedema management, and hydrocele surgery will help to reduce morbidity and prevent disability. However, integrating these activities into health systems has been challenging for programmes to do, and therefore, MMDP services have not scaled up as rapidly as needed. In many countries that have had successful MDA programs, MMDP services are still not available in all areas where they are required.

1.6.6 Validation of elimination of lymphatic filariasis

After all EUs in a country have successfully passed all required TAS, and access to MMDP services has been established where there is a need, the country has met the criteria

established by WHO for validation of elimination. In 2015, WHO formally endorsed a standardized process for formally acknowledging elimination of LF as a public health problem (WHO, 2017e). As part of this process, national programmes compile all relevant data into a dossier for review. To date, 11 countries (Cambodia, Cook Islands, Egypt, Maldives, Marshall Islands, Niue, Sri Lanka, Thailand, Togo, Tonga, and Vanuatu) have completed the current validation process and have been recognized by WHO for their achievements (Taleo et al., 2017; WHO, 2017f; Khieu et al., 2018). Post-validation surveillance activities have not yet been standardised. A schematic of the major stages of an LF elimination programme are illustrated in Figure 1.6.

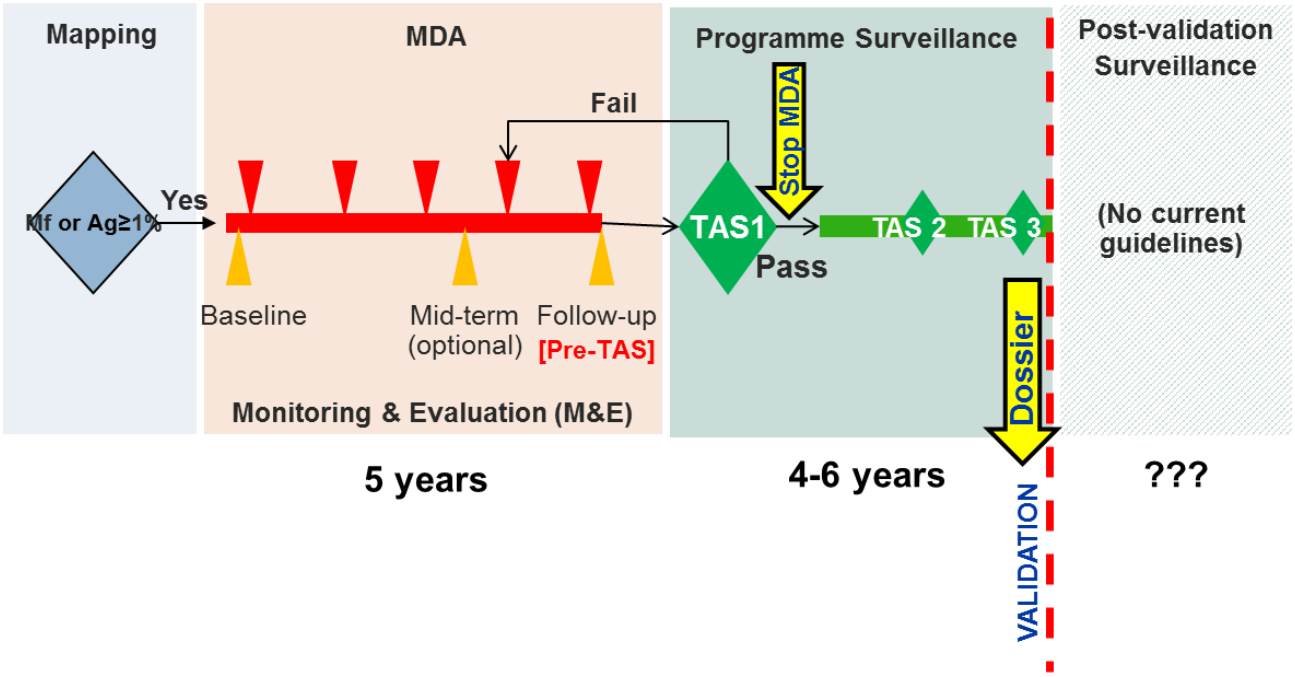

Figure 1.6 LF elimination programme life cycle

1.7 Surveillance and diagnostic needs

As of 2016, almost 1,100 TAS have been implemented globally with an overall pass rate of >90% (WHO, 2017c). As a result, there are 500 million people living in areas that no longer require MDA. Although there has been significant progress since the start of GPELF, it is clear that the original target of elimination by 2020 will not be reached since an estimated 856 million people in 52 countries are living in areas that still require MDA (WHO, 2017c). Of the countries where interventions are needed, 22 (42%) have not scaled up MDA to 100%

geographic coverage in all endemic implementation units (WHO, 2017c). Additionally, several countries have found that, despite completing the recommended number of treatment rounds, infection levels are still not below established thresholds for stopping MDA. Furthermore, some areas have passed TAS and stopped MDA despite undetected ongoing transmission (Lau et al., 2017; Rao et al., 2017).

At this critical juncture of GPELF, it is imperative to identify strategies to accelerate progress toward global elimination and to protect the advances made to date. The recently WHO-endorsed triple drug regimen will likely contribute toward the acceleration of programmes. Although this new strategy holds promise, it is unclear if the current monitoring and evaluation (M&E) framework will be appropriate for monitoring the impact of IDA, and it will be important to identify the most appropriate sampling strategies and diagnostic tools to use in this context.

1.7.1 Surveillance strategy gaps

As MDA begins to scale down in countries, it is important to develop robust strategies to establish a surveillance baseline and to detect any possible recrudescence of infection as early as possible. However, beyond TAS, post-MDA surveillance has not been standardised. During the surveillance period, programmes often face significant resource limitations. Therefore, it is essential for surveillance approaches to be simple and efficient yet sensitive and timely. Because of the lag between infection and the appearance of clinical signs, it is not feasible to rely on clinical indicators for surveillance. As a result, by definition, all surveillance for LF must involve active detection of infection. To maximise both efficiency and sensitivity to detect informative signals, it is critical to understand the most appropriate population(s) to monitor and what signal(s) to measure. In some cases it may be more appropriate to monitor incident infections among young children. In other cases it may be more appropriate to monitor decreasing trends of infection among older individuals. The TAS design, although statistically robust, is not powered to detect changes in CFA prevalence over time. Therefore, the results of TAS conducted during the post-MDA surveillance period

fail to document trends of antigenaemia. Collecting additional evidence using alternative indicators may provide important information needed during the surveillance period.

1.7.2 Deficiencies in diagnostic tools used for surveillance

In the early stages of GPELF, detection of MF in peripheral blood was used routinely for mapping and to monitor the impact of MDA (Ottesen et al., 1997; Ottesen, 2006; Weil and Ramzy, 2007). Detection of MF in thick blood films served as an indication of viable adult worm infections and provided evidence needed to make programmatic decisions. However, logistical challenges were encountered because of the requirement for night blood collections. Additionally, it was increasingly difficult to detect MF in populations after multiple rounds of MDA (Gass et al., 2012). Many of the limitations experienced with MF detection were addressed with the introduction of the ICT. The detection of CFA served as a reasonable proxy for infection, and importantly, could be conducted with blood collected any time of the day, thus eliminating the need for night blood collections. Although the ICT, and now the FTS, provides advantages over the detection of MF, there are some limitations to the use of CFA tests, especially during the post-MDA surveillance period. Similar to the observed decline in MF prevalence after treatment, antigenaemia also begins to decline and becomes increasingly difficult to detect in populations (Gass et al., 2012). Evidence suggests that detection of antifilarial antibodies provides the earliest indicator of filarial exposure (Hamlin et al., 2012). Therefore, monitoring filarial exposure through the assessment of antibody responses may provide a useful tool for detecting potential recrudescence. Many of the currently available LF antibody tests have been shown to be sensitive measures of exposure and infection but may lack the specificity needed to make important programmatic decisions (Muck et al., 2003; Lammie et al., 2004; Weil et al., 2011). With the recent development and availability of Wb123, the ability to use a highly specific antibody detection test as a surveillance tool may be within reach.

There is still a need to determine how best to use serologic responses during the post-MDA surveillance period in order to provide evidence for interruption of LF transmission.

To better understand the potential applicability of LF serology during the post-MDA surveillance period, antibody responses must first be characterised among populations that have received multiple rounds of treatment. Thereafter, it may be possible to identify the most appropriate surveillance strategy (e.g. population-based surveys, facility-based monitoring, etc.). Additionally, programmatic feasibility of diagnostic tool platforms should be considered. Currently, various platforms, including rapid tests, ELISA, and multiplex bead assays (MBAs) are used. Each of these platforms has advantages and disadvantages and therefore can have varying utility depending on the situation. Rapid tests provide a relatively simple platform that is not dependent on the constraints of laboratory infrastructure. However, these tests are often subject to inter-observer variability. For example, the Brugia Rapid™ (Reszon Diagnostics; Selangor, Malaysia) appears to have very good sensitivity in areas where *Brugia* spp. is endemic (Dewi et al., 2015), but there have been challenges with test interpretation because of the difficulty in determining the presence or absence of test lines. ELISA generally provides increased sensitivity compared to rapid tests, but it can be challenging to determine appropriate cutoffs needed to interpret results. Recently, MBAs have been developed as highly sensitive methods for simultaneous detection of antibody responses to multiple antigens (Priest et al., 2010; Moss et al., 2011; Hamlin et al., 2012; Lammie et al., 2012; Priest et al., 2016; Arnold et al., 2017). MBAs potentially allow for integrated assessments of multiple diseases providing an efficient approach to gathering information on diseases of public health importance, but MBAs require specialised equipment and sophisticated laboratory infrastructure. As LF prevalence declines, it is essential to identify sensitive tools capable of providing information on transmission status in order to guide programme decision-making.

2 Goal and objectives

2.1 Goal

The overarching goal of this PhD thesis is to provide recommendations on best approaches for conducting surveillance for lymphatic filariasis (LF) elimination programmes. There are two interlinked objectives:

- to determine the utility of serologic tools during the post-mass drug administration (MDA) surveillance period within communities that have received multiple rounds of LF MDA; and
- To determine the utility of the transmission assessment survey and other activities during the post-MDA surveillance period as platforms for integrated disease surveillance.

2.2 Specific objectives

The specific aims of this PhD thesis are

- to compare LF antigen and antifilarial antibody responses during the post-MDA surveillance period by establishing age-prevalence curves for these responses in communities that have received multiple rounds of treatment;
- to determine the appropriate age group(s) to monitor during the post-MDA period;
- to compare circulating filarial antigen and antibody diagnostic tools for use during the surveillance period in order to determine the most appropriate diagnostic tool(s) to use for LF surveillance; and
- to assess the feasibility of using existing disease programme infrastructures as platforms for multi-disease surveillance.

3 Assessment of lymphatic filariasis prior to restarting mass drug administration campaigns in coastal Kenya

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[This manuscript has been published in *Parasites & Vectors* (2017) 10(1):99

https://doi.org/10.1186/s13071-017-2044-5]

3.1 Abstract

Background: Lymphatic filariasis (LF) is a debilitating disease associated with extensive disfigurement and is one of a diverse group of diseases referred to as neglected tropical diseases (NTDs) which mainly occur among the poorest populations. In line with global recommendations to eliminate LF, Kenya launched its LF elimination program in 2002 with the aim to implement annual mass drug administration (MDA) in order to interrupt LF transmission. However, the program faced financial and administrative challenges over the years such that sustained annual MDA was not possible. Recently, there has been renewed interest to eliminate LF and the program, through support from World Health Organization (WHO), restarted annual MDA in 2015. The objective of this study was to evaluate the current status of LF infection in the endemic coastal region of Kenya before MDA campaigns were restarted.

Results: Ten sentinel sites in Kwale, Kilifi, Tana River, Lamu, and Taita-Taveta counties in coastal Kenya were selected for participation in a cross-sectional survey of LF infection prevalence. At least 300 individuals in each sentinel village were sampled through random house-to-house visits. During the day, the point-of-care immunochromatographic test (ICT) was used to detect the presence of *Wuchereria bancrofti* circulating filarial antigen in finger prick blood samples collected from residents of the selected sentinel villages. Those individuals who tested positive with the ICT test were requested to provide a night-time blood sample for microfilariae (MF) examination. The overall prevalence of filarial antigenaemia was 1.3% (95% CI: 0.9-1.8%). Ndau Island in Lamu County had the highest prevalence (6.3%, 95% CI: 4.1-9.7%), whereas sites in Kilifi and Kwale counties had prevalences <1.7%. Mean microfilarial density was also higher in Ndau Island (234 MF/ml) compared to sentinel sites in Kwale and Kilifi counties (<25 MF/ml). No LF infection was detected in Tana River and Taita-Taveta counties. Overall, more than 88% of the study participants reported to have used a bed net the previous night.

Conclusions: Prevalence of LF infection is generally very low in coastal Kenya, but there remain areas that require further rounds of MDA if the disease is to be eliminated as a public health problem in line with the ongoing global elimination efforts. However, areas where there was no evidence of LF transmission should be considered for WHO-recommended transmission assessment surveys in view of stopping MDA.

3.2 Background

In 2000, the World Health Organization (WHO) launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in response to World Health Assembly resolution WHA50.29, which urged Member States to initiate activities to eliminate lymphatic filariasis (LF), a goal subsequently targeted for 2020 (WHO, 2011a). The GPELF has two principal aims: (i) to interrupt LF transmission, and (ii) to manage morbidity and prevent disability. To interrupt transmission of LF infection, the GPELF recommends annual community-wide mass drug administration (MDA) of antifilarial tablets to entire at-risk populations aged two years and above for 4-6 years at adequate levels of coverage. Modeling studies have estimated adequate treatment coverage to be at least 65% of total population in endemic areas (Stolk et al., 2003; Michael et al., 2004).

In Kenya, LF is confined to the coastal region where ecological factors are suitable for its transmission (Moraga et al., 2015). The Kenyan Ministry of Health (MoH) launched its LF elimination program in 2002 when MDA was conducted in the then Kilifi District. Unlike in many other African countries, onchocerciasis is not endemic in LF-endemic coastal Kenya. Therefore, the recommended antifilarial treatment for MDA is single-dose annual mass treatment with diethylcarbamazine (DEC, 6 mg/kg) plus albendazole (400 mg). In 2003, the program was scaled up to include Kwale and Malindi Districts. Another two rounds of MDA were conducted in these districts in March 2005 and December 2008 and a further round was conducted in December 2011, when MDA was extended to Tana River and Lamu counties. Such intermittent MDA is not consistent with GPELF recommendations to provide annual MDA for 4-6 years and its impact on transmission is unclear.

Monitoring and evaluation is recognized as an essential activity during implementation of any disease control program. The current WHO guidelines for epidemiological monitoring of LF recommend selection of at least one sentinel site per 1 million people or implementation unit (IU) (WHO, 2011a). The selected villages should have at least 500 persons so as to enable sample collection of at least 300 specimens. Testing for circulating filarial antigen (CFA) using immunochromatographic test (ICT) and parasitological detection of microfilariae (MF) in blood have been the gold standard tests for monitoring the impact of LF elimination programs (WHO, 2011a).

Kenya's Ministry of Health NTD Unit successfully appealed to the World Health Organization Regional Office for Africa (WHO-AFRO) and other partners for support to re-establish the MDA program starting in 2015. Subsequently, the WHO Country Office selected the Eastern and Southern Africa Centre of International Parasitic Control (ESACIPAC), which is part of the Kenya Medical Research Institute (KEMRI), to conduct a comprehensive epidemiological assessment of LF infection before re-starting the MDA campaign in the coastal region of Kenya. The present paper reports results from this assessment and provides critical evidence that can be used for making decisions on MDA in addition to providing a basis for future monitoring of the LF program in coastal Kenya.

3.3 Materials and methods

3.3.1 Study design and survey sites

A cross-sectional survey was conducted in October 2015 in ten LF sentinel sites (villages) located across the coastal region in Taita-Taveta, Kwale, Kilifi, Tana River and Lamu counties. Five of the sites were those that were previously selected by the LF program – Ndau Island (Lamu), Kipini (Tana River), Masindeni and Jaribuni (Kilifi), and Makwenyeni (Kwale). Five new sentinels sites were selected in Tana-River (Mikinduni), Kilifi (Kinarani), Kwale (Mirihini and Mwadimu), and Taita-Taveta (Kimorigo) to represent implementation units (sub-counties) that were established after initial MDA implementation. The five earlier sentinel sites were selected according to estimated risk of LF as estimated from a previously

published report (Wijers, 1977). In the present study, health workers at the county level assisted in the selection of the 5 new sentinel sites. The new sites were purposively selected to participate in the survey based on the presence of cases of the diseases and/or environmental factors indicating that LF transmission is likely to occur as given in the WHO-AFRO guidelines for mapping of lymphatic filariasis (WHO, 2014).

3.3.2 Study population and sample size

The target population consisted of residents of the ten selected sentinel villages. The residents of villages in Taita-Taveta, Kwale, Kilifi and Tana River live in dispersed homesteads within their respective villages often located in the countryside. However, the residents of Ndau Island live in a relatively compact village with households being very close together. Typically, villages in the Kenyan coastal region have population of 600-900 persons (Njenga et al., 2011a). Following WHO guidelines that at least 300 persons be tested in each sentinel site, the target sample population for the survey was 3000 study participants. The sampling assumed that the average household size in coastal Kenya consists of 5 members per family and 3 individuals would agree to voluntarily participate in the survey. Thus, an estimated 100 households were to be visited in each village. Residents of the sentinel villages were recruited into the study if aged 2 years or more and not severely ill.

3.3.3 Survey strategy

The LF survey was conducted using a house-to-house approach by four teams. Each team consisted of two laboratory technologists, two data collectors, a driver and a team leader. Additionally, the village chairman and a local volunteer in each selected village joined the survey team to assist with mobilization of community members. Individuals in each sentinel village were sampled through random house-to-house visits. Refusal to participate in the survey was encountered but the target sample was achieved in most sentinel sites.

A survey questionnaire was programmed onto mobile smartphones (Samsung Galaxy Trend S7560) and used to collect data from consenting participants (or parent/guardian in case of children). The data collected using the mobile smartphones included information on

age, history of previous residence, use of deworming tablets, and long lasting insecticide-treated net (LLIN) ownership and use. Data on blood collection and results of the ICT test were also recorded onto the questionnaire. Additionally, the smartphones were used to collect global positioning system (GPS) coordinates of each study household.

3.3.4 Laboratory procedures

Blood collection

The middle finger of consenting individuals was cleaned using a cotton ball soaked in 70% isopropyl alcohol. After drying, the tip of the finger was pricked using a sterile lancet and blood immediately collected using capillary tubes for ICT test (100 µl) and preparation of dry blood spots (DBS) on TropBio filter paper (60 µl). Serological tests will be performed later and described elsewhere. Any individual who tested positive for filarial antigens by ICT test, if consenting, was also tested for MF. Details of each laboratory procedure are given below.

Immunochromatographic test (ICT)

Prior to survey initiation, quality control (QC) of the ICT test kits (BinaxNow® Filariasis, Alere Inc., USA) received for the survey was performed in KEMRI-ESACIPAC Regional NTD Reference Laboratory using well characterized serum samples. All the test kits assessed passed the QC analysis. In the field, 100 µl of the blood was used for the ICT test. After application of a whole blood sample to the ICT card, the results were read exactly at 10 minutes as recommended by the manufacturer. An additional 60 µl of finger prick blood samples were collected from participants and applied onto TropBio filter paper (TropBio Pty Ltd, Townsville, Qld, Australia) for future serological studies.

Microfilariae detection

Individuals who tested positive by ICT test were invited for further testing for MF in night time blood samples collected between 20:00 hours and 24:00 hours. The counting chamber method was used for examination and enumeration of *Wuchereria bancrofti* microfilariae in the night blood specimens (McMahon et al., 1979). Briefly, 100 µl of blood was mixed with 900 µL of 3% acetic acid and the samples transported to KEMRI-ESACIPAC regional NTD

reference laboratory in Nairobi where MF were examined and counted under a light microscope.

3.3.5 Data management and analysis

Participants' responses were captured electronically into Open Data Kit (www.opendatakit.org/), which included in-built data quality checks to prevent data entry errors.

Filarial infection was defined as a positive ICT result. Observed overall prevalence of filarial infection was calculated at sentinel site and county levels. 95% confidence intervals (CIs) were obtained by binomial logistic regression, taking into account clustering by households. Prevalence by sex and age group was calculated and 95% CIs determined using a Generalized Least Squares (GLS) random effects model that adjusts for household clustering. For purposes of this analysis, the following age groups were used: < 10, 10-17 and ≥ 18 year olds. The overall and village level proportion estimates of reported LLIN use were estimated and 95% CIs were determined using Generalized Linear Latent and Mixed Models (GLLAMM) adjusted for clustering by households. Overall, cross-county analysis of the impact of LLIN use on participant infection status was analysed, first using univariable analysis allowing for factors associated with filarial infection (i.e. age group and gender) and described as odds ratios (OR), using mixed effects logistic regression at both household and county levels. For multivariable analysis, adjusted OR (aOR) were obtained by mutually adjusting all minimum generated variables using multivariable mixed effects logistic regression at 95% CI taking into account both household and county levels.

The mean coordinates of all households sampled in each village were used to obtain geographic locations of the sentinel sites that were mapped using Arc GIS Desktop version 10.2.2 software (Environmental Systems Research Institute, Inc., Redlands, CA). All statistical analyses were carried out using STATA version 14.0 (STATA Corporation, College Station, TX, US).

3.4 Results

3.4.1 Sentinel site surveillance

Ten sentinel sites (villages) were surveyed between 8th and 18th October 2015 in Kwale, Kilifi, Tana River, Lamu and Taita-Taveta counties in Coastal Kenya (Figure 3.1). A total of 2,996 participants agreed to be registered for the survey, but 20 individuals (0.67%) either withdrew or did not provide a blood sample, hence final analysis was done for the remaining 2,976 participants. Samples for CFA testing using ICT test and dry blood spots (DBS) for serological assays were obtained and prepared for 2,976 participants and 2,972 participants, respectively. The reported age of individuals ranged from 2 to 100 years, with a median of 18 years (IQR = 31 years). Of the enrolled participants, 1,260 (42.3%) were male.

Table 3.1 provides the projected population of the five counties (Statistics, 2009), demographic characteristics of the study participants, overall LF infection prevalence by ICT test in each county, and the adjusted odds ratios for the factors associated with the LF infection. Overall, 38 of 2,976 (1.3%; 95% CI: 0.9–1.8) individuals were found to be CFA positive using the ICT test. There was no significant difference in the prevalence of CFA positive individuals by sex (p=0.148). Age-group classification was arbitrarily assigned for younger children (<10 year olds), older children (10–17 year olds), and adults (≥18 year olds). The odds of CFA among persons aged 18 years and above was significantly higher than those among younger persons (OR = 3.12; 95% CI: 1.16–8.43; p=0.024). The overall prevalence of CFA positive persons in Kilifi and Kwale counties was 0.9% (95% CI: 0.4–1.8) and 1.1% (95% CI: 0.6–2.1), respectively, but there were villages where the prevalence was up to 1.7%. There was no evidence of LF infection in the sentinel sites in Tana River and Taita-Taveta counties.

Table 3.2 and Figure 3.1 present the prevalence of CFA positive individuals by sentinel site. Ndau Island/village in Lamu County had the highest percentage of CFA positive persons, with 20 of 320 (6.3%; 95% CI: 4.1–9.7) individuals found to be antigen positive.

Infection in Ndau Island was also observed in young children with 6 of the 20 (30%) CFA positive individuals being children aged 10 years and below.

Out of the 38 persons found to be positive for LF infection by ICT test, 33 (86.8%) provided a night-time blood sample for examination of MF. Assuming that all the individuals that were CFA negative by the ICT test were also negative for microfilaraemia, the prevalence of MF was highest in Ndau Island in Lamu County (1.9%; 95% CI: 0.9–4.1), but below 1% in three sentinel sites found to have CFA positive individuals in Kwale and Kilifi counties. The mean intensity of microfilaremia among MF positive persons in Ndau Island was also higher (234 MF/ml; 95% CI: 62–880) than in the other sentinel sites (Table 3.3).

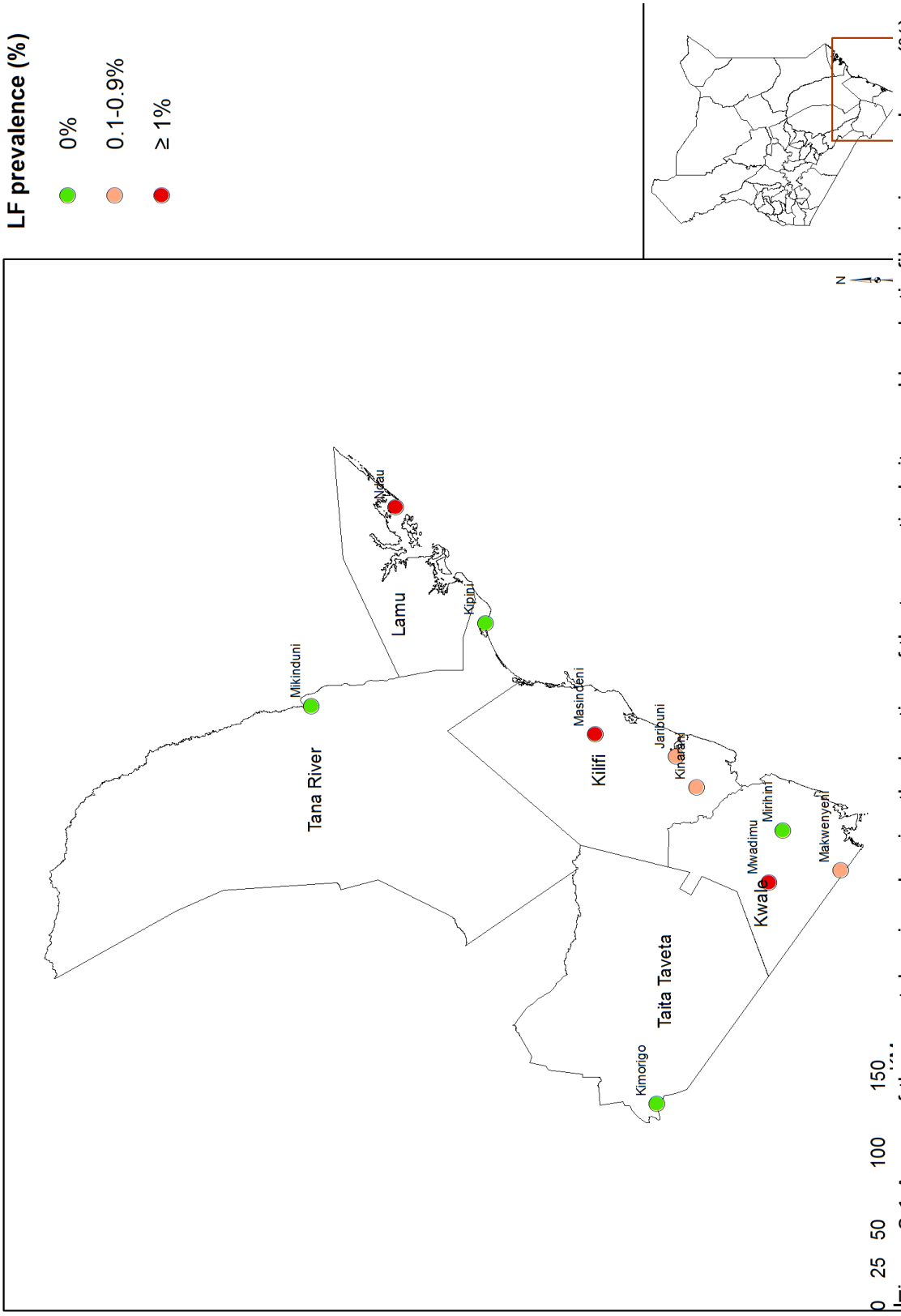

Figure 3.1 A map of the coastal region showing the location of the ten sentinel sites and lymphatic filariasis prevalence (%) levels by immunochromatographic test. The highest prevalence of lymphatic filariasis infection was detected in Ndau Island in Lamu County

Table 3.1 Demographic characteristics and filarial prevalence (%) by ICT test in 10 sentinel sites, coastal Kenya, October 2015

Demographic	2015 Population projections	Sentinel sites	*n* (%)	CFA prevalence (%) (95% CI)	Multivariable logistic	
					aOR (95% CI)[a]	*P*-value
County						
Kwale	792,698	3	877 (29.5)	1.1 (0.6–2.1)	–	–
Kilifi	1,307,185	3	911 (30.6)	0.9 (0.4–1.8)	–	–
Tana River	292,885	2	593 (19.9)	0	–	–
Lamu	123,842	1	320 (10.8)	6.3 (4.1–9.7)	–	–
Taita-Taveta	347,195	1	275 (9.2)	0	–	–
All counties	2,863,805	10	2,976	1.3 (0.9–1.8)	–	–
Sex						
Male	–	10	1,260 (42.3)	1.5 (0.9–2.4)	1.58 (0.85–2.95)	0.148
Female	–	10	1,716 (57.7)	1.1 (0.7–1.7)	Reference	
Age group						
< 10	–	10	865 (29.1)	0.7 (0.3–1.7)	Reference	
10–17	–	10	609 (20.5)	0.2 (0–1.2)	0.23 (0.03–2.05)	0.188
≥ 18	–	10	1,502 (50.5)	2.1 (1.5–2.9)	3.12 (1.16–8.43)	0.024*
LLIN use						
Yes	–	10	2,647 (88.9)	1.1 (0.8–1.6)	0.40 (0.19–0.86)	0.019*
No	–	10	329 (11.1)	2.7 (1.4–5.2)	Reference	

[a]Adjusted odds ratios (aOR) were obtained by mutually adjusting all minimum generated variables using multivariable mixed effects logistic regression at 95% CI taking into account households and county levels

*$P < 0.05$

Table 3.2 Surveyed households and sentinel site level circulating filarial antigen (CFA) prevalence (%), coastal Kenya, October 2015

County/village	Households	No. CFA positive/No. examined	Prevalence (%) (95% CI)
Kwale County			
Makwenyeni	69	5/297	1.7 (0.7-3.9)
Mwadimu	67	5/290	1.7 (0.7-4.0)
Mirihini	52	0/290	0
Kilifi County			
Kinarani	94	1/307	0.3 (0-2.4)
Jarubuni	93	2/298	0.7 (0.2-2.6)
Masindeni	96	5/306	1.7 (0.7-3.9)
Tana River County			
Mikinduni	75	0/294	0
Kipini	83	0/299	0
Lamu County			
Ndau	105	20/320	6.3 (4.1-9.7)
Taita-Taveta County			
Kimorigo	94	0/275	0
All villages	828	38/2976	1.3 (0.9-1.9)

Table 3.3 Sentinel site microfilariae prevalence (%) and mean intensity (MF/ml), coastal Kenya, October 2015

Village	No. CFA positive/No. examined	No. examined for MF[a]	No. MF positive	Mean intensity[b] (MF/ml) (95% CI)	MF prevalence[c] (95% CI)
Kwale County					
Makwenyeni	5/297	5	1	22 (3–156)	0.3 (0–2.4)
Mwadimu	5/290	4	1	10 (1–71)	0.3 (0–2.4)
Mirihini	0/290	0	0	0	0
Kilifi County					
Kinarani	1/307	0	0	0	0
Jaribuni	2/298	1	0	0	0
Masindeni	5/306	4	1	5 (1–35)	0.3 (0–2.4)
Tana River County					
Mikinduni	0/294	0	0	0	0
Kipini	0/299	0	0	0	0
Lamu County					
Ndau	20/320	19	6	234 (62–880)	1.9 (0.9–4.1)
Taita Taveta County					
Kimorigo	0/275	0	0	0	0
All villages	38/2,976	33	9	140 (39–502)	0.3 (0.2–0.6)

3.4.2 Bed nets and deworming

Table 3.4 summarizes bed net ownership and usage among the 10 sentinel villages. Overall, 97.6% (95% CI: 96.6-98.5%) of the respondents reported owning at least one LLIN, with 88.8% (95% CI: 87.0-90.7%) reporting to have slept under a bed net the previous night. However, bed net usage was observed to be lower in Mwadimu village (73.3%; 95% CI: 63.8-82.7) in Kwale County and Ndau Island (75.0%; 95% CI: 67.9-82.1) in Lamu County. There was a significantly lower risk of LF infection among participants who reported bed net use compared to those who did not use a bed net (*Table 3.1*, OR = 0.40, 95% CI: 0.19-0.86, p = 0.019).

Of 2,950 responses about deworming, 1,184 individuals (40%) reported receiving deworming drugs during the last six months prior to the study with 68.6% and 21.0% receiving the treatment at school and home, respectively.

Table 3.4 Bed net ownership and usage by sentinel village, coastal Kenya, October 2015

Village	Proportion possessing at least one LLIN % (95% CI)	LLIN usage, previous night% (95% CI)
Makwenyeni	99.7 (99.0–100)	89.2 (84.0–94.4)
Mwadimu	95.1 (90.8–99.3)	73.3 (63.8–82.7)
Mirihini	91.5 (84.2–98.7)	89.5 (82.0–96.9)
Kinarani	97.4 (91.2–99.6)	89.6 (83.9–95.4)
Jaribuni	99.5 (98.6–100)	92.7 (88.1–97.3)
Masindeni	98.4 (93.0–99.1)	88.1 (82.8–93.5)
Mikinduni	99.0 (95.8–100)	93.6 (89.5–97.7)
Kipini	100 (98.6–100)	99.5 (98.5–100)
Ndau	98.7 (96.7–100)	75.0 (67.9–82.1)
Kimorigo	96.7 (94.2–99.3)	96.7 (94.4–99.0)
All villages	97.6 (96.6–98.5)	88.8 (87.0–90.7)

3.5 Discussion

The results of the current survey suggest that transmission of LF infection in Tana River and Taita-Taveta counties may be absent and could be used to request WHO-AFRO to support the Kenyan LF program to conduct transmission assessment surveys in these counties. Kenya's LF elimination program was launched in 2002, but has however, seen inconsistent treatment delivery coupled with challenges that resulted in MDA campaigns not being conducted every year as recommended by the GPELF (Table 3.5). A renewed commitment to re-start the LF elimination program in Kenya attracted support from the WHO-AFRO Regional Office and other partners and an MDA campaign was conducted in October 2015. This study was undertaken to provide the status of LF infection in the Kenyan coastal region, which is required in order to inform decisions on MDA campaigns. Overall, ICT positivity in most sentinel sites ranged between 0 and 1.7%. However, the LF infection data in sentinel sites in Lamu, Kilifi and Kwale counties indicate that transmission is still ongoing in these counties, thus justifying additional rounds of MDA in the three counties. These data, therefore, could allow the program to focus the currently available resources in areas that have empirical evidence of LF infection.

Table 3.5 MDA implementation in coastal Kenya showing overrall treatment coverage (%), 2002-2015

County	2002	2003	2005	2008	2011	2015
Kalifi	MDA	MDA	MDA	MDA	MDA	MDA
(Malindi)		MDA	MDA	MDA	MDA	MDA
Kwale		MDA	MDA	MDA	MDA	MDA
Tana River					MDA	MDA
Lamu					MDA	MDA
Taita-Taveta						
Program (drug) coverage	81.2	79.5	72.3	62.7	58.3	54.3

The original IUs have been revised due to several changes in administrative structures. Malindi is currently a sub-county in Kilifi County. Source: WHO preventive chemotherapy database (WHO/PCT databank) http://www.who.int/neglected_diseases/preventive_chemotherapy/lf/en/ Accessed 06/11/2016

Ndau Island in Lamu County had a relatively higher infection rate (6.3%) compared to the sentinel sites on the mainland. The microfilarial density among MF positive persons was also relatively higher in Ndau Island compared to the other sentinel

sites. Additionally, about 30% of LF infections on this island was detected in children aged ten years and below. Therefore, Ndau Island appears to be a hotspot of LF transmission and could be an indication of a similar situation in the other neighboring islands. A previous study conducted in Ndau Island four years after a pilot MDA campaign found MF prevalence to be 13.7% (Wijers and Kaleli, 1984). A survey conducted by our team in 2011, prior to the first MDA in Lamu County under the LF elimination programme, found an MF prevalence of 11.6% (MoH, unpublished). The results of the current study, however, demonstrate that the MDA campaign conducted in 2011 may be associated with a reduced prevalence of LF infection in the Island. Nonetheless, further epidemiological studies in Ndau Island should be considered to identify factors responsible for continued transmission of LF infection. A study in Leogane, Haiti examined factors that could contribute to continued transmission of LF infection and found that MDA non-compliance was significantly associated with infection (Boyd et al., 2010).

The current study found that most households possessed at least one bed net and the majority of people interviewed reported that they used the nets regularly. The high bed net possession was corroborated by observation of many new bed nets (some still unopened) during the current study because the national malaria control program had conducted a mass LLIN distribution a few weeks prior to the LF survey. Vector control is increasingly being recognized as a possible complementary strategy for LF elimination (Sunish et al., 2007; Bockarie et al., 2009; Ichimori et al., 2014). A previous study found that vector control in Africa had increased significantly since 2005, with a three-fold increase in LLIN ownership and IRS coverage (Kelly-Hope et al., 2013). A few countries where there has been high LLIN coverage have reported the possibility of LF elimination in the absence of a MDA program. For example, the Gambia has historical evidence of LF transmission (McGregor et al., 1952; McFadzean, 1954; Hawking, 1977), a long history of large scale bed net distribution (Snow et al., 1988; Cham et al., 1996), and recent reports suggest that LF is no longer a public health problem in the country (Rebollo et al., 2015). The current study

observed significantly lower LF prevalence and risk of infection among individuals who reported bed net use thus suggesting that LLINs may have played a complementary role in reducing LF infection in the endemic Kenyan coastal region.

A study on the impact of permethrin-impregnated bed nets on LF vector mosquitoes in villages in Kwale County reported that LF is transmitted by both culicine and anopheline mosquitoes. Of the LF vector species collected before implementation of the intervention, 33.6% were members of *An. gambiae* complex [with more than 98% being *An. gambiae* (*sensu stricto*)], 30% were *An. funestus*, and 36.4% were *Culex quinquefasciatus* (Bogh et al., 1998). A malaria entomologic study reported that the primary vectors of malaria along the coast of Kenya include *An. funestus* and *An. gambiae* complex: *An. gambiae* (*s.s.*), *An. arabiaensis*, and *An. merus* (Mbogo et al., 2003). The WHO promotes integrated vector management (IVM) to improve the cost effectiveness of vector-control operations, and to strengthen the capacity of programmes, partnerships and intersectoral collaboration in their efforts to control, eliminate or eradicate vector-borne diseases (WHO, 2008). In areas with overlapping geographical distribution of LF and malaria, particularly where both infections are transmitted by the same species of mosquito vectors, the IVM approach is recommended as useful and appropriate for jointly managing control activities for the two diseases (WHO, 2011b). Although pyrethroid resistance has become widespread among anopheline and culicine mosquitoes (Corbel et al., 2007; N'Guessan et al., 2007; Oxborough et al., 2010), the sustained use of insecticide-treated bed nets has been associated with significant decrease in number of culicine mosquitoes in houses (Lindblade et al., 2006), which should therefore contribute to a reduction in LF transmission.

According to the 2010–2020 strategic plan of the GPELF, the strategic aim is to provide access to MDA and other measures to interrupt transmission in all endemic areas (WHO, 2010). The current study provided further evidence that LLINs against malaria can indeed have complementary impact against LF and thus significantly contribute towards the goal to interrupt transmission of infection. This finding could be used to strengthen the call to

adopt IVM approach which requires coordinated control of both malaria and lymphatic filariasis so that the two programs could benefit from each program's activities, thus enhancing their overall impact on public health (WHO, 2008, 2011b). Therefore, the Kenyan LF and malaria programs should consider jointly undertaking mosquito vector control in the coastal region so as to enhance their overall impact on public health. This way, any residual LF transmission is likely to be completely eliminated.

Albendazole is a broad spectrum anthelmintic and is also used to treat LF infection, although the evidence on its efficacy when used alone is conflicting; studies in India demonstrated significant effects on both MF and antigenaemia (Hoti et al., 2010), but a study in Ghana reported minimal efficacy (Dunyo et al., 2000). The current study found substantial use of deworming drugs, which could be due to the ongoing national school-based deworming program that provides annual albendazole for the treatment of soil-transmitted helminths (Mwandawiro et al., 2013). A recent study conducted in an informal settlement area in Nairobi revealed that there are many non-governmental organizations and religious organizations that also provide albendazole to school-age children in Kenya (Harris et al., 2015). Nonetheless, the results of the current study are similar to those from previous work in a historically high LF endemic area in Malindi sub-County in Kilifi County, which reported sustained reduction in LF infection despite missing MDA rounds (Njenga et al., 2011a). Taken together, the data suggest that LLIN use and deworming may have contributed to reduce LF infection despite the irregular implementation of MDA.

A number of tests are currently available for diagnosis of *W. bancrofti* infection but thick blood smear microscopy for detection of MF and ICT for testing for CFA were chosen for monitoring and evaluation of LF elimination programs (WHO, 2011a). Previous evaluation of the ICT test in the coastal Kenya setting, before start of MDA campaigns, found the diagnostic tool to be 100% sensitive and specific for LF (Njenga and Wamae, 2001). However, a study in Cameroon has reported loss of sensitivity of ICT test in low prevalence settings and raised concern regarding the use of this tool for monitoring and evaluation of LF

elimination programs (Gounoue-Kamkumo et al., 2015). Additionally, results of studies carried out in Central Africa have shown cross-reactivity of ICT test with *Loa loa* and *Onchocerca ochengi* infections and raised some doubts to the reliability of LF mapping data particularly in areas of *L. loa* co-endemicity (Wanji et al., 2015; Wanji et al., 2016). Therefore, the use of ICT test as the gold standard diagnostic tool in this study may be considered as a limitation that may significantly impact on the conclusions. Nonetheless, there are studies suggesting that antifilarial antibody testing could provide a more sensitive and specific measure of exposure to *W. bancrofti* in carefully selected populations in endemic areas and thus, may also be valuable as a tool for monitoring and evaluation of LF elimination programs (Joseph et al., 2011; Hamlin et al., 2012). Therefore, it might be useful to conduct operational research using strategies that complement CFA testing with the sensitive and specific antibody detection diagnostic assays to provide further information on current LF transmission in these counties.

3.6 Conclusion

The current study suggests that LF transmission may be absent in Taita-Taveta and Tana River counties in coastal Kenya and therefore transmission assessment surveys (TAS) should be considered with a view to stopping MDA. By contrast, ongoing transmission in Kwale, Kilifi, and Lamu counties indicates the need for further MDA rounds in these counties.

3.7 Acknowledgements

The authors would like to thank the County Health Departments of Taita-Taveta, Kwale, Kilifi, Tana River and Lamu for supporting the survey, including provision of laboratory technicians and local transportation for the survey teams. The communities of the selected sentinel sites and their local leaders are sincerely thanked for the cooperation and assistance. The WHO-AFRO office through the WHO country office, Kenya is thanked for excellent support. The Kenya Medical Research Institute (KEMRI) provided scientific leadership and oversight for this study.

4 Multiplex serologic assessment of schistosomiasis in western Kenya: antibody responses in preschool aged children as a measure of reduced transmission

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[This manuscript has been published in *American Journal of Tropical Medicine and Hygiene* (2017) 96(6):1460-1467 https://doi.org/10.4269/ajtmh.16-0665]

4.1 Abstract

Currently, impact of schistosomiasis control programs in *Schistosoma mansoni* endemic areas is monitored primarily by assessment of parasitologic indicators only. Our study was conducted to evaluate the use of antibody responses as a way to measure the impact of schistosomiasis control programs. A total of 3,612 serum samples collected at three time points from children 1-5 years of age were tested for antibody responses to two schistosome antigens (SEA and Sm25) by multiplex bead assay. The overall prevalence of antibody responses to SEA was high at baseline (50.0%). After one round of mass drug administration (MDA) there was minimal change in odds of SEA positivity (OR=1.02, CI=0.79-1.32, p=0.89). However, after two rounds of treatment, there was a slight decrease in odds of SEA positivity (OR=0.80, CI=0.63-1.02, p=0.08). In contrast to the SEA results, prevalence of antibody responses to Sm25 was lowest at baseline (14.1%) and higher in years 2 (19.8%) and 3 (18.4%). After one round of MDA, odds of Sm25 positivity increased significantly (OR=1.51, CI=1.14-2.02, p=0.005) and remained significantly higher than baseline after two rounds of MDA (OR=1.37, CI=1.07-1.76, p=0.01). There was a significant decrease in the proportion of 1-year olds with positive SEA responses from 33.1% in year 1 to 13.2% in year 3 and a corresponding decrease in the odds (OR=3.25, CI=1.75-6.08, p<0.001). These results provide preliminary evidence that schistosomaisis program impact can be monitored using serologic responses.

4.2 Introduction

Schistosomiasis, caused by infection with *Schistosoma* spp., affects more than 200 million people worldwide (WHO, 2015a). Prevalence and intensity of infection with *Schistosoma mansoni* peak between 10 and 15 years of age and gradually decline with age. In children, chronic schistosomiasis is associated with anemia and malnutrition and can compromise growth and cognitive development (King and Dangerfield-Cha, 2008). Because of the influence school aged children (SAC) have on transmission of schistosomiasis, mass treatment of this age group with praziquantel (PZQ) has been the cornerstone of schistosomiasis control activities (WHO, 2011c). Until recently, disease burden and morbidity among pre-school age children (PSAC) has remained understudied. However, recent research has shown that first infection is often acquired at a very young age (Sousa-Figueiredo et al., 2010; Stothard et al., 2011; Verani et al., 2011; Ekpo et al., 2012; Stothard et al., 2013), and there is growing evidence that the burden of disease among PSAC may warrant global attention. Although schistosomiasis-associated morbidity among PSAC is still not well defined, documented effects include fecal occult bleeding (Betson et al., 2010; Betson et al., 2012), anemia (Green et al., 2011; Magalhaes and Clements, 2011) and ultrasound abnormalities (Davis et al., 2015); however, discriminating these symptoms from other potential infectious causes remains a challenge. Despite mounting evidence for the need, PSAC are not routinely screened or included in schistosomiasis mass treatment programs in large part due to the need for better diagnostic tools and the lack of a pediatric formulation of PZQ.

Currently, program impact in *S. mansoni* endemic areas is monitored primarily by assessment of parasitologic indicators only. This is traditionally done by monitoring changes in prevalence and intensity of infection using the Kato-Katz stool examination method (WHO, 1991), which has long been the primary diagnostic tool used for *S. mansoni* and soil-transmitted helminth (STH) control programs. While this method allows for relatively simple assessment of prevalence and intensity of infection, there are known limitations with its use.

Logistical challenges are introduced with the short time needed to collect and process samples, and quality results are dependent on trained microscopists who can correctly identify eggs. Furthermore, as prevalence of *S. mansoni* and STH infection decreases, the sensitivity of the Kato-Katz often decreases in parallel (Nikolay et al., 2014; Mwinzi et al., 2015). Recent development of a urine based point of care circulating cathodic antigen test (POC-CCA) for *S. mansoni* has addressed some of the limitations with the Kato-Katz. A number of studies have compared the POC-CCA to Kato-Katz and found that it is more sensitive than the traditional stool based test (Tchuem Tchuente et al., 2012; Colley et al., 2013). However, there are still some questions about the specificity of the test, especially in low prevalence settings (Foo et al., 2015). While there has been significant emphasis placed on using stool and urine based diagnostic tools to monitor the impact of treatment programs, less emphasis has been placed on the utility of antibody detection tools as a way to measure impact. Reduced transmission of schistosomiasis can be assessed, in principle, by documenting a lower prevalence of infection-specific antibody. Although there may be limitations to using antibody responses among older age groups, documenting reduced infection incidence among cohorts of young children can be one of the most powerful measures of program impact. However, this measure has not been incorporated into most monitoring and evaluation strategies. Newly-developed multiplex bead assays (MBA) to detect antibodies against multiple antigens could make it possible to monitor the effect of treatment on infections, and these assays could potentially be used as an additional measure of program impact (Lammie et al., 2012). Our study was conducted to evaluate the use of antibody responses as a way to measure the impact of schistosomiasis control programs.

4.3 Methods

4.3.1 Study site

The study was conducted from 2012-2014 in Mbita sub-county, which borders Lake Victoria in western Kenya. The majority of residents are subsistence farmers, although fishing is the main commercial activity in villages near the lake. In addition to fishing, the lake is used for

other occupations such as car washing and sand harvesting, and daily activities such as washing clothes and bathing. High rates of *S. mansoni* infection and malaria have been documented in the area (Odiere et al., 2012; Minakawa et al., 2015). Prior to the start of the study, malaria interventions had been in place for several years, but no mass drug administration (MDA) for schistosomiasis had been conducted. A single round of MDA for STH infections had been conducted in 2009 for SAC by the Kenya National school-based deworming program.

4.3.2 Study design

The study was part of a multi-country project designed to evaluate the impact of integrated neglected tropical disease (NTD) control programs. In Mbita, SAC from schools within 5km of Lake Victoria were screened to identify communities with *S. mansoni* prevalence ≥25% (Odiere et al., 2012). Thirty villages that met the selection criteria were randomized into two study arms to compare different MDA strategies for schistosomiasis and STH programs. Fifteen villages were randomized to a community-wide treatment arm and the remaining 15 villages were randomized to a school-based treatment arm. In each of the 30 study villages, we aimed to enroll 100 PSAC (1-5 years) and their mothers or guardians. Additionally, we aimed to enroll 100 individuals ≥ 6 years (with no upper age limit) to give us a total target sample size of 300 individuals per study village. In both study arms, parasitologic and serologic indicators were monitored at baseline (year 1) and annually following treatment. All monitoring was done in cross-sectional surveys in the selected villages.

4.3.3 Ethical considerations

The study was approved by the Scientific Steering and Ethics Review Committees of the Kenya Medical Research Institute (KEMRI, SSC No. 2185) and of the Institutional Review Board of the U.S. Centers for Disease Control and Prevention (protocol #6249) through a reliance agreement with KEMRI. The study was explained to potential participants and written informed consent was obtained from persons who agreed to participate. Parents or guardians provided consent for children <18 years of age. Additionally, children between 7-

17 years were asked to provide verbal assent for their participation. All identifiable information was kept confidential and maintained by using a secure database with access restricted to essential study personnel.

4.3.4 Data collection

All study villages were visited between May and July of each study year. Community leaders were sensitized to the study details at least one week prior to the arrival of the field teams. On the day of sample collection, residents of the community were asked to come to a central location within the village. The study was explained, and potential participants were given an opportunity to ask questions. After obtaining informed consent, participants were assigned a unique identifier and asked to provide basic demographic information such as age and sex. Additionally, information about the length of residence within the study village and bednet usage was collected. A single global-positioning system coordinate per village was recorded at the site of data collection. All data were collected on smartphones (Motorola Milestone XT720, Motorola, Chicago, IL) through a modified version of the OpenDataKit application and uploaded to a secure SQL server.

4.3.5 Stool and urine collection and diagnostic tests

For each participant, an attempt was made to collect a single stool sample to be processed by the Kato-Katz method. Two slides were prepared from each stool sample, read independently by trained microscopists and examined for the presence of *S. mansoni* and STH (*Ascaris lumbricoides, Trichuris trichiura*, hookworm) eggs. Arithmetic means of the results from the duplicate slides were calculated and expressed as eggs per gram (EPG) of stool. Results for *S. mansoni* were categorized as light (1-99 EPG), moderate (100-399 EPG) and heavy (≥400 EPG) intensity infections according to the current WHO thresholds.(WHO, 2011c) A single urine sample was collected and tested by dipstick (URiSCAN, YD Diagnostics in year 1 and Hemastix, Siemens Healthcare Diagnostics in years 2 and 3) to assess hematuria as a proxy for *S. haematobium* infection. In year 2 and year 3, urine

samples positive for hematuria were filtered and examined for the presence of *S. haematobium* eggs.

4.3.6 Blood collection and diagnostic tests

Blood was collected via a single fingerstick. To assess anemia, hemoglobin levels were measured using a portable, battery operated hemoglobinomteter (HemoCue, Angelholm, Sweden) according to manufacturer's specifications. Anemia was defined according to the Kenyan clinical guidelines: <10.0 g/dL for children <5 years old, <11.0 g/dL for children 5 to 8 years old and <12.0 g/dL for individuals ≥9 years old; anemia was categorized as mild if hemoglobin was >8.0 g/dL, moderate if 5.0 to 8.0 g/dL and severe if <5.0 g/dL (Health, 2002) after adjusting for altitude (WHO, 2011d). Malaria infection status was determined by preparing thick blood films and using standard Giemsa staining techniques. Slides were examined by trained microscopists to determine malaria parasitemia, and positive infection was defined by the presence of one or more malaria parasites in 300 high-powered fields. Approximately 100µL of blood was collected into a serum capillary collection tube (Ram Scientific, Yonkers, NY) and transported back to the laboratory where serum samples were separated by centrifugation. Serum was stored at -20°C in the field laboratory in Homabay until transported monthly to the main KEMRI NTD laboratory located in Kisumu. In Kisumu, samples were stored at -80°C until sent to a KEMRI laboratory in Nairobi where samples were tested for antibody responses to a panel of antigens by multiplex bead assay (MBA) (described in the Multiplex bead assay section below).

4.3.7 Treatment

All eligible individuals in the community-wide treatment arm were offered annual treatment with single doses of PZQ (40mg/kg) and albendazole (ALB)(400 mg) approximately two months after data collection. In the school-based treatment arm, the current WHO-recommended strategy to treat only SAC was followed (WHO, 2011c). In both study arms, because no current guidelines exist for the inclusion of PSAC in schistosomiasis control programs, only PSAC identified as positive for *S. mansoni* infection by Kato-Katz were

treated with crushed PZQ under the supervision of a medical professional. Coartem (artemether [20mg/dose] and lumefantrine [120 mg/dose]) was provided to individuals with malaria. Treatment with iron supplementation was provided for persons with mild and moderate anemia, and individuals with severe anemia were referred to the sub-county hospital according to the Kenya National Clinical Guidelines for Nutritional and Hematologic Conditions (Health, 2002).

4.3.8 Multiplex bead assay

An MBA was used in order to analyze antibody responses to multiple antigens at one time from a single serum sample (Lammie et al., 2012). The following schistosome antigens were included in the panel for our study: *S. mansoni* soluble egg antigen (SEA)(Carter and Colley, 1978) and Sm25, an integral glycoprotein found in microsomal preparations of *S. mansoni* adult worms (GeneBank Accession M37004.1) (Tsang et al., 1983; Ali et al., 1991). The Sm25 gene was cloned into BD BaculoGold ^{TM} pAcSecG2T Baculovirus Transfer Vector (BD 554797, Fisher Scientific, Waltham, MA), and the expressed Sm25 recombinant proteins from Sf-9 insect cells were purified using glutathione agarose beads. SEA was coupled to SeroMap microsphere beads (Luminex Corp., Austin, TX) in PBS at pH 7.2 using 120 micrograms protein for 12.5 x 10^6 beads, and Sm25 was coupled in PBS buffer at pH 7.2 using 12 micrograms of protein / 12.5 x 10^6 beads as previously described (Moss et al., 2011). Test sera were diluted 1:400 in PBS buffer (pH 7.2) containing 0.3% Tween-20, 0.02% sodium azide, 0.5% casein, 0.5% polyvinyl alcohol (PVA), 0.8% polyvinylpyrrolidone (PVP), and 3 µg/ml *E. coli* extract. Duplicate samples were tested as previously described (Moss et al., 2011; Hamlin et al., 2012). Samples having a coefficient of variation of >15% between duplicate wells for >3 positive antibody responses were repeated. Cutoff values of 713.5 median fluorescence intensity (MFI)-background (bg) units for SEA (sensitivity = 97.5%, specificity = 100%) and 52.5 MFI - bg units for Sm25 (sensitivity = 93.5%, specificity = 97.3%) were calculated at CDC from receiver operator characteristic curves using sera from 46 stool positive *S. mansoni* patients, presumed negative sera from 65 adult US citizens

with no history of foreign travel, and presumed negative sera from 45 US children. Cutoffs were adjusted for instrument differences between CDC and KEMRI using a 2-fold serial dilution of a strong positive serum pool to generate a standard curve. The adjusted cutoffs for the KEMRI instrument were 965 MFI – bg units for SEA and 38 MFI – bg units for Sm25. Additional antigens for malaria, *Strongyloides*, *Ascaris*, *Giardia*, tetanus and diphtheria were included in the MBA. Results from the additional antigens will be described elsewhere.

4.3.9 Data analysis

Statistical analyses were performed in SAS software version 9.3 (SAS Institute Inc., Cary, NC) and used the 5% level of significance. Frequencies and proportions were compared using either the Rao-Scott Chi-squared statistic (Rao JN, 1984), which incorporates a design correction into the analyses; logistic regression with variance estimates by a Taylor series expansion (DA, 1983) to account for cluster sampling; or in two analyses of *S. mansoni* classification, a standard Pearson Chi-squared because a design effect could not be estimated. For logistic regression, odds ratios (ORs) and 95% confidence intervals are reported. Unless otherwise stated, results in this paper are for PSAC only and analyses are restricted to children with MBA results only.

4.4 Results

A total of 4,611 PSAC were enrolled in the study between 2012 and 2014. Of those enrolled, serum samples were available from 3,612 (78.3%) children and were tested by MBA. Mean age of enrollment at baseline was 3.0 years, decreased slightly to 2.8 years in year 2 and remained at 2.8 years in year 3. In each year of the study, the youngest (one year old) and oldest age (five years old) groups were somewhat underrepresented. The distribution of PSAC in each age group is shown in Table 4.1. Malaria prevalence as determined by thick blood smear increased significantly from year 1 to year 2 (OR=1.80, CI=1.03-3.16, p=0.04) and remained elevated in year 3 (Table 4.2). Approximately one-third of all PSAC had anemia at baseline. Prevalence of anemia increased to 40.9% in year 2, resulting in increased odds of being anemic (OR=1.35, CI=1.08-1.68, p=0.01) with approximately 32% of

anemic children classified as having moderate to severe anemia (Table 4.2). Anemia was significantly associated with malaria (Rao-Scott $\chi^2_{(1)}$=129.60, p<0.001) and older age (Rao-Scott $\chi^2_{(4)}$=109.09, p<0.001) but was not associated with *S. mansoni* infection determined by either Kato-Katz (Rao-Scott $\chi^2_{(1)}$=0.40, p=0.53) or MBA (Rao-Scott $\chi^2_{(1)}$=0.08, p=0.77). The proportion of children with hematuria was high at baseline (33.8%) but was significantly lower in years 2 (1.5%, OR=0.03, CI=0.02-0.06, p<0.001) and 3 (3.9%, OR=0.08, CI=0.04-0.15, p<0.001; Table 4.2). None of the filtered urine samples was positive for *S. haematobium* eggs.

Table 4.1 Age and sex distribution of pre-school aged children enrolled and tested by multiplex bead assay in each study year.

		Year											
		1 (baseline)				**2**				**3**			
Years	(months)	n	(%)	female	(%)	n	%	female	(%)	n	%	female	(%)
1	(12-23)	154	14.0	79	51.3	181	15.4	92	50.8	235	17.6	124	52.8
2	(24-35)	225	20.4	134	59.6	259	22.1	126	48.6	296	22.2	156	52.7
3	(36-47)	274	24.8	142	51.8	284	24.2	163	57.4	332	24.9	165	49.7
4	(48-59)	354	32.1	169	47.7	427	36.4	233	54.6	433	32.4	218	50.3
5	(60-71)	96	8.7	46	47.9	23	2.0	13	56.5	39	2.9	24	61.5

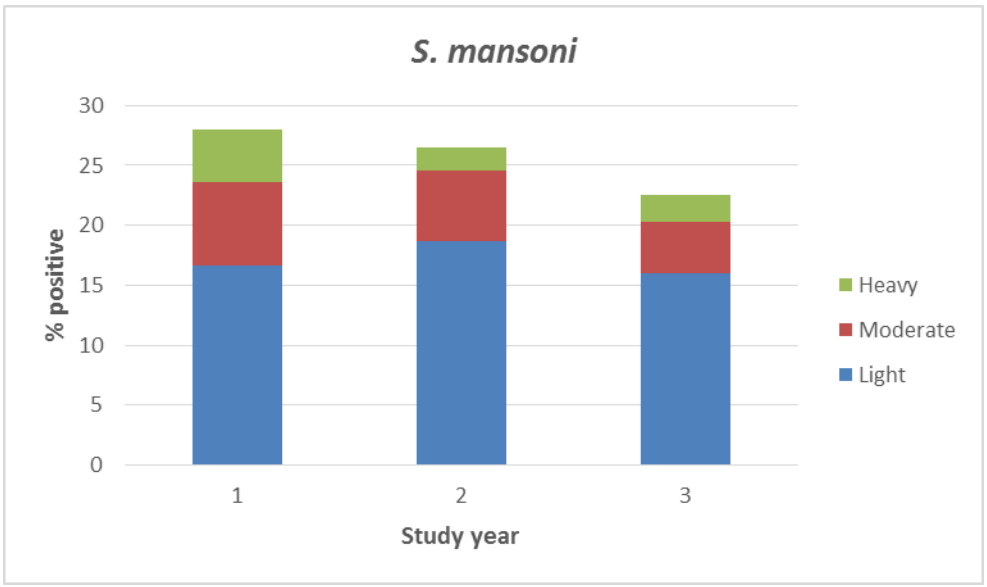
Table 4.2 Prevalence of malaria, anemia and hematuria among pre-school aged children in each study year.

	Year								
	1 (baseline)			**2**			**3**		
	n	positive	%	n	positive	%	n	positive	%
Malaria	727	91	12.5	1,160	238	20.5	1,306	268	20.5
Anemia	1,096	371	33.9	1,170	478	40.9	1,323	517	39.1
mild		*250*	*67.4*		*345*	*72.2*		*351*	*67.9*
moderate		*113*	*30.5*		*131*	*27.4*		*152*	*29.4*
severe		*8*	*2.2*		*2*	*0.4*		*14*	*2.7*
Hematuria	1,025	346	33.8	1,103	17	1.5	1,241	48	3.9

At baseline, the overall prevalence of *S. mansoni* infection by Kato Katz was 28.0%, with 40.3% of infections classified as moderate or heavy intensity. *S. mansoni* infection significantly increased with age (Rao-Scott $\chi^2_{(4)}$=58.69, p<0.001). After one round of MDA in the study villages, there was minimal change in odds of *S. mansoni* infection among PSAC (OR=0.93, CI=0.70-1.23, p=0.61), but there was a significant decrease in the percentage of

moderate and heavy intensity infections from 11.3% to 7.9% and the odds of moderate or heavy intensity infection (OR=0.67, CI=0.47-0.97, p=0.03). By year 3, after two rounds of treatment, overall prevalence was still >20% and nearly 30% of infections were classified as moderate or heavy intensity (Figure 4.1). In contrast to *S. mansoni* infection, very low rates of STH infection were observed. Prevalence of any STH infection was <3% in each study year (data not shown).

Figure 4.1 Prevalence and intensity of *Schistosoma mansoni* infection measured by Kato Katz among pre-school aged children in each study year.



The overall prevalence of PSAC with antibody responses to SEA was high at baseline (50.0%) (Figure 4.2). After one round of MDA there was minimal change in odds of SEA positivity (OR=1.02, CI=0.79-1.32, p=0.89). However, after two rounds of treatment, there was a slight decrease in odds of SEA positivity (OR=0.80, CI=0.63-1.02, p=0.08). In contrast to the SEA results, prevalence of PSAC with antibodies to Sm25 was lowest at baseline (14.1%) and higher in years 2 (19.8%) and 3 (18.4%) (Figure 4.2). After one round of MDA, odds of Sm25 positivity increased significantly (OR=1.51, CI=1.14-2.02, p=0.005) and remained significantly higher than baseline after two rounds of MDA (OR=1.37, CI=1.07-1.76, p=0.01). There was a significant association between dichotomized anti-SEA antibody response and intensity of infection measured by Kato-Katz (Pearson $\chi^2_{(3)}$=230.22, p<0.001,

Figure 4.3a). Similarly, there was a significant association between dichotomized Sm25 responses and intensity of infection (Pearson $\chi^2_{(3)}$=129.43, p<0.001, Figure 4.3b).

Figure 4.2 Prevalence of antibody responses to SEA and Sm25 by study year measured by multiplex bead assay.

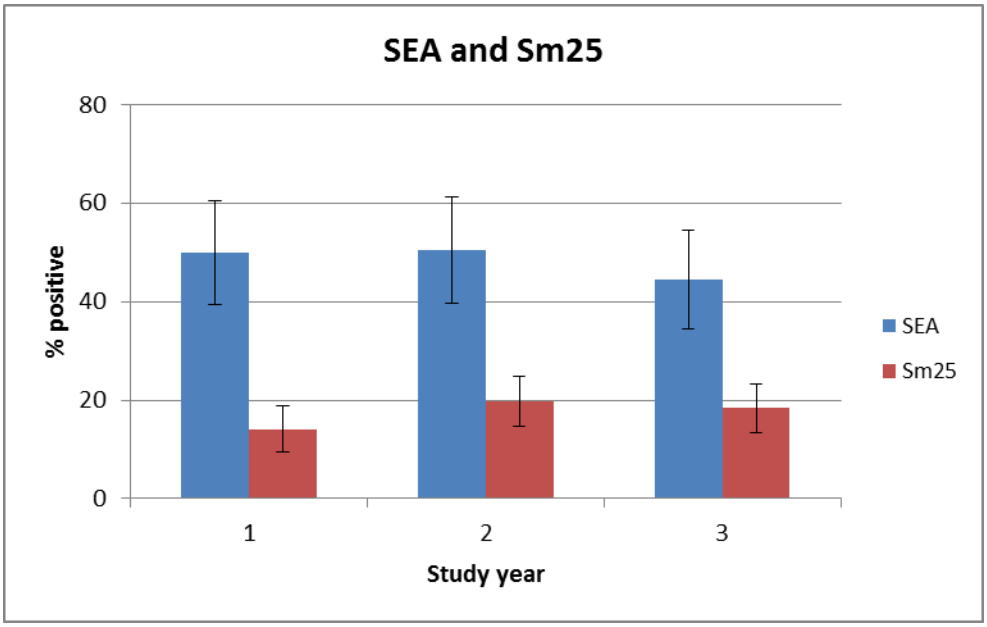
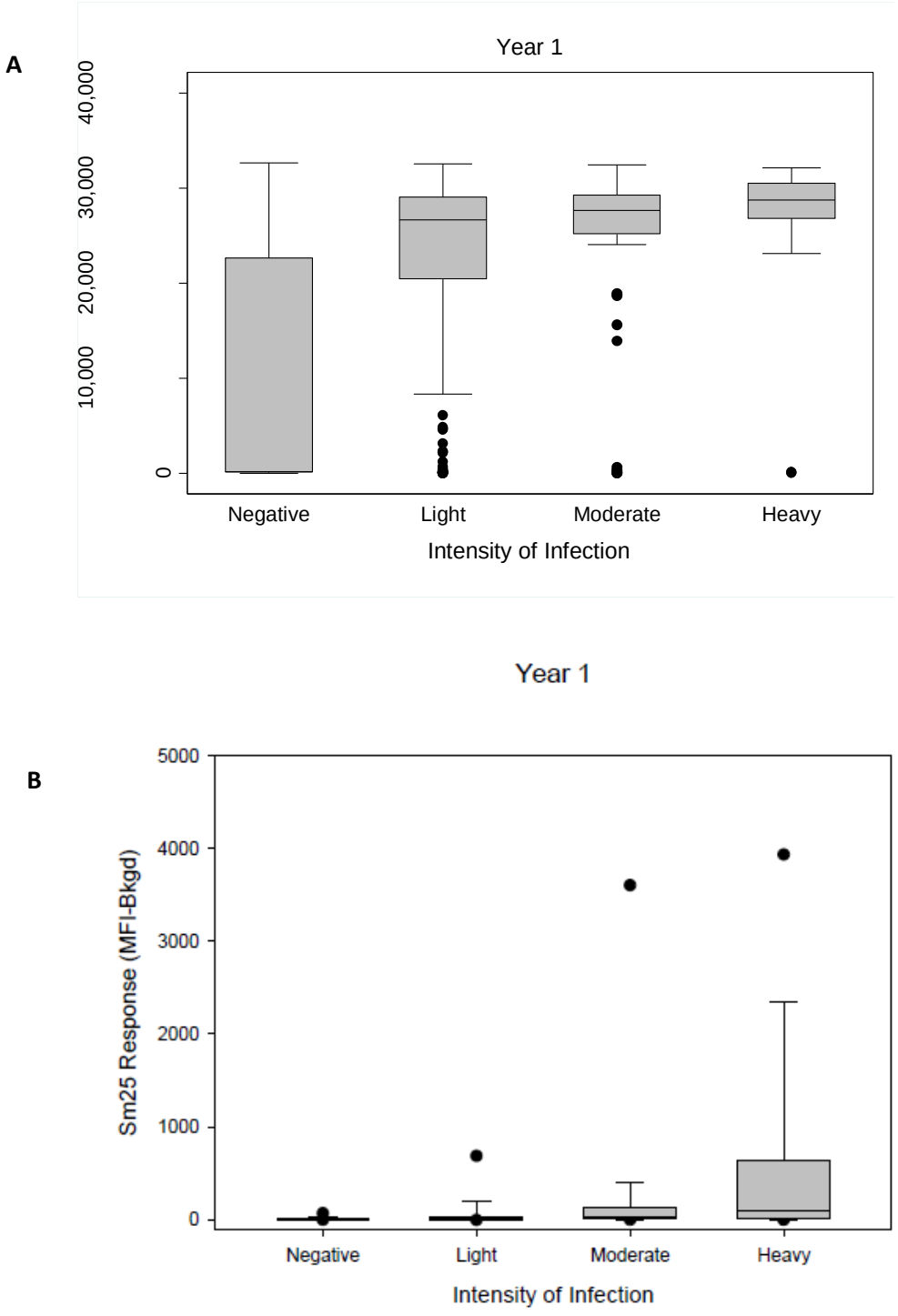


Figure 4.3 Antibody responses to (**A**) soluble egg antigen (SEA) and (**B**) Sm25 were significantly associated (p<0.001) with intensity of infection measured by Kato-Katz. Boxes enclose 25th and 75th percentile. Lines inside the boxes represent median MFI values.

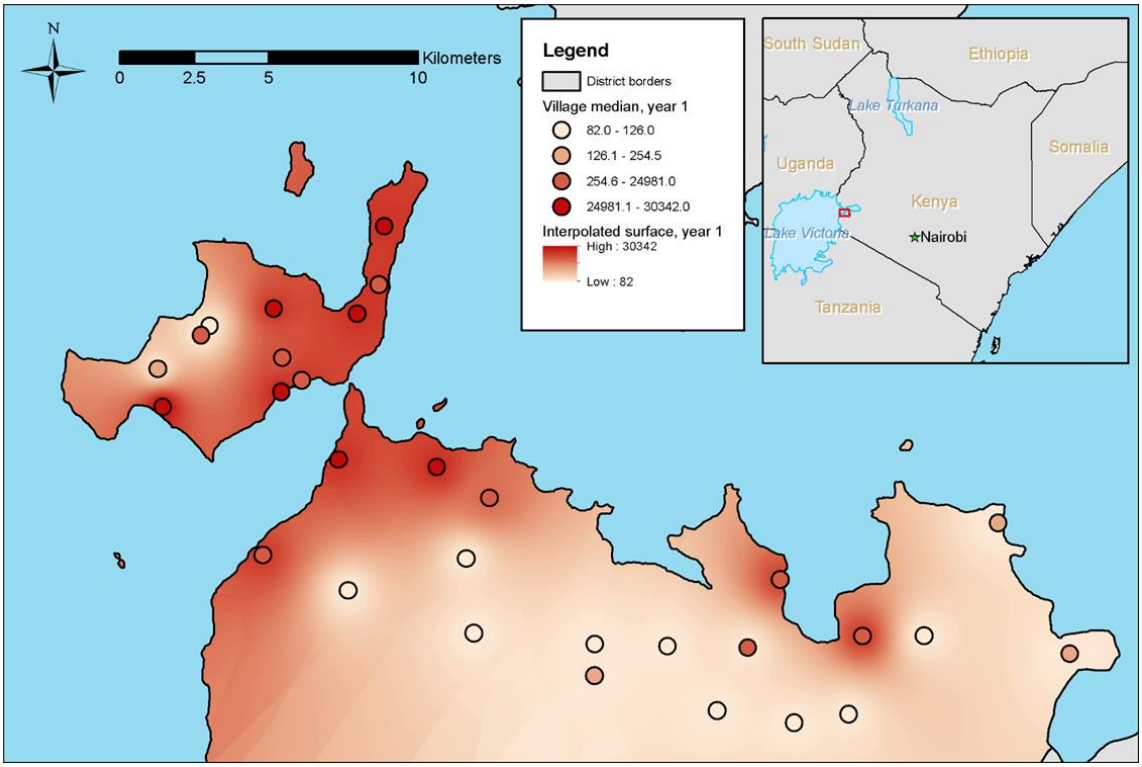


Although all of the study villages were located relatively close (<5km) to Lake Victoria, a gradient of antibody responses to SEA was observed. Median SEA responses for the villages at baseline ranged from 4 to 32,685 MFI. The highest responses were observed on

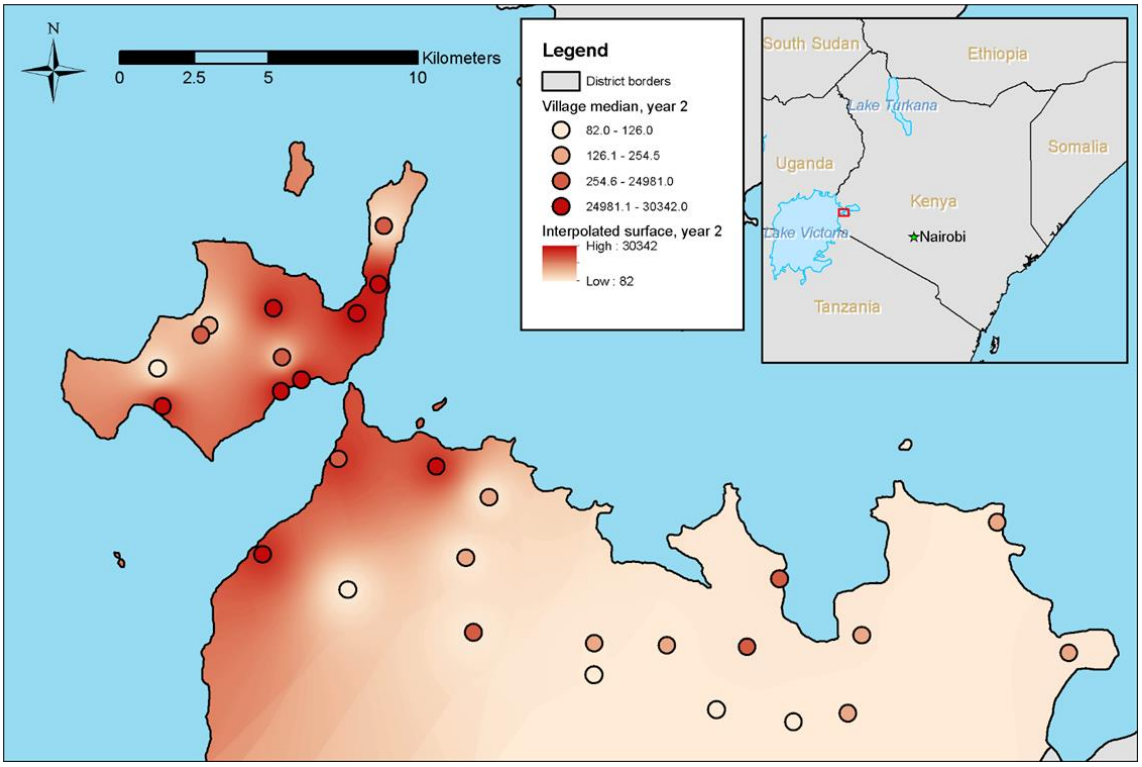
the island of Rusinga in the northwest corner of the sub-county and there was a significant decrease in the odds of a positive SEA response with each additional kilometer away from the lake (OR=0.22, CI=0.08-0.62, p=0.004, Figure 4.4a) after controlling for study year. After each round of treatment, median SEA responses remained very high on Rusinga, but decreased in some villages on the mainland closest to the lake (Figure 4.4b and Figure 4.4c). In any study year, there were no differences in stool results or antibody responses between the two study treatment arms.

Figure 4.4 (**A**) Year 1: significant decrease (p=0.004) in the odds of a positive soluble egg antigen (SEA) response was observed with each additional kilometer away from Lake Victoria. (**B**) Year 2: after one round of treatment, median SEA responses remained high on Rusinga Island, but decreased in some villages on the mainland closest to the lake. (**C**) Year 3: after two rounds of treatment, median SEA responses remained high on Rusinga Island, but continued to decrease in some villages on the mainland closest to the lake.

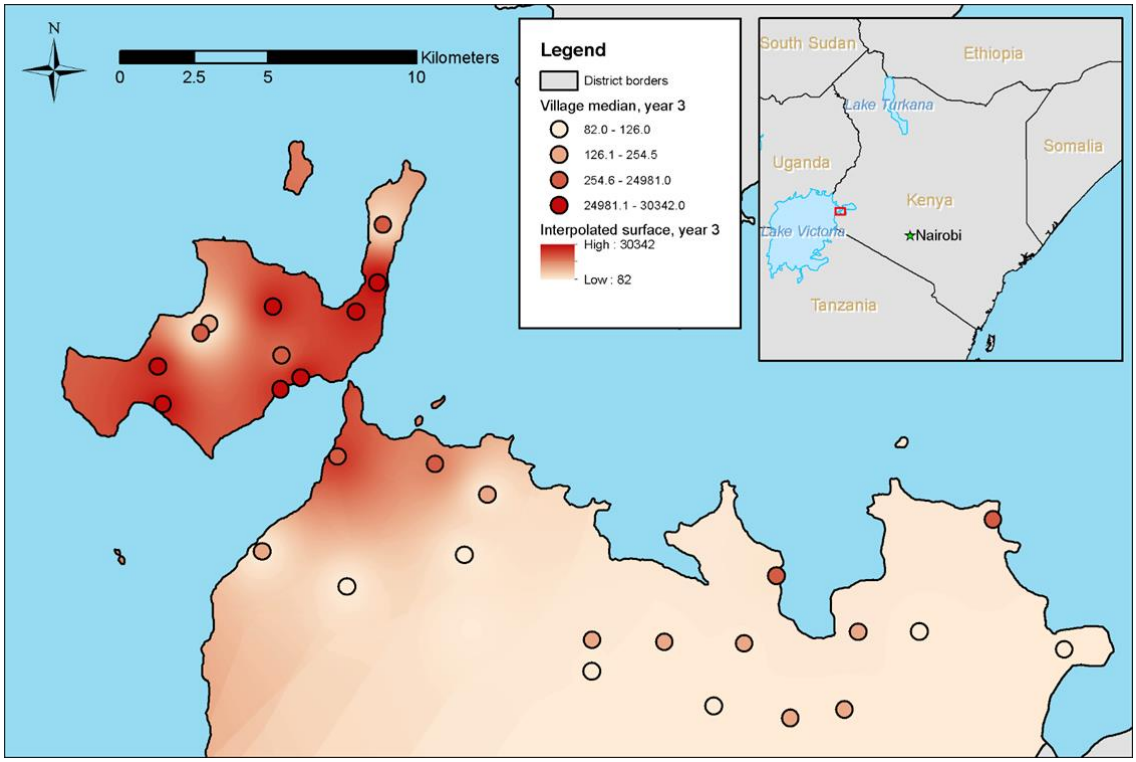
A



B



c



There was no significant change in prevalence of *S. mansoni* infection by age after two rounds of treatment as determined by stool examination (All $\chi^2_{(2)}<3.11$, $p>0.21$), and prevalence was >20% every year for older PSAC (Figure 4.5). In contrast to the egg data, there was a decrease in the proportion of 1-year olds with positive SEA responses from 33.1% in year 1 to 13.2% in year 3 (OR=3.25, CI=1.75-6.08, $p<0.001$). Furthermore, there was a significant reduction in the median SEA MFI values among 1-year olds after two rounds of MDA ($p<0.05$) (Figure 4.6). The same reduction was not observed in any other age group.

Figure 4.5 *Schistosoma mansoni* prevalence by age and study year measured by Kato-Katz.

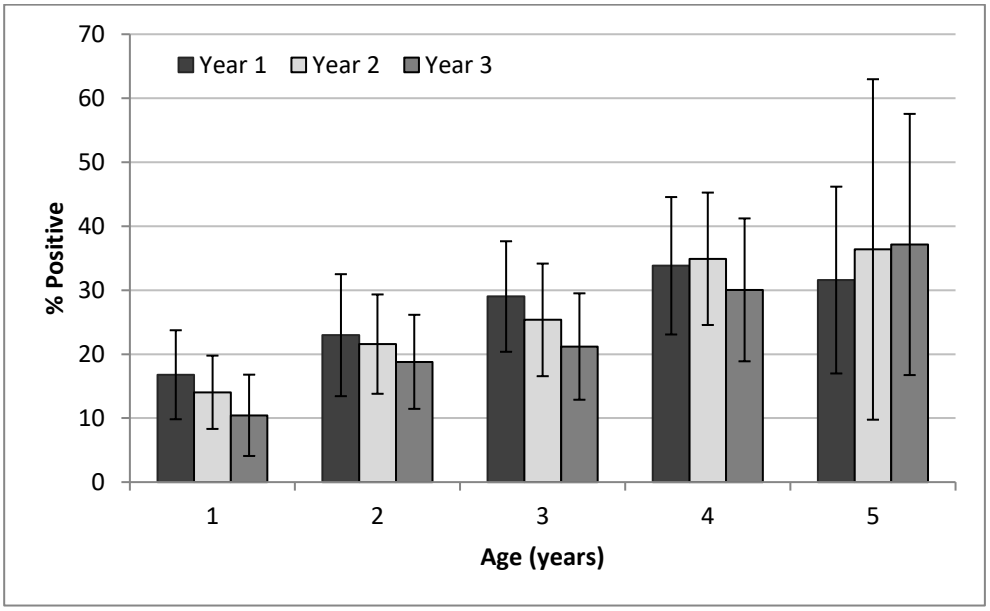
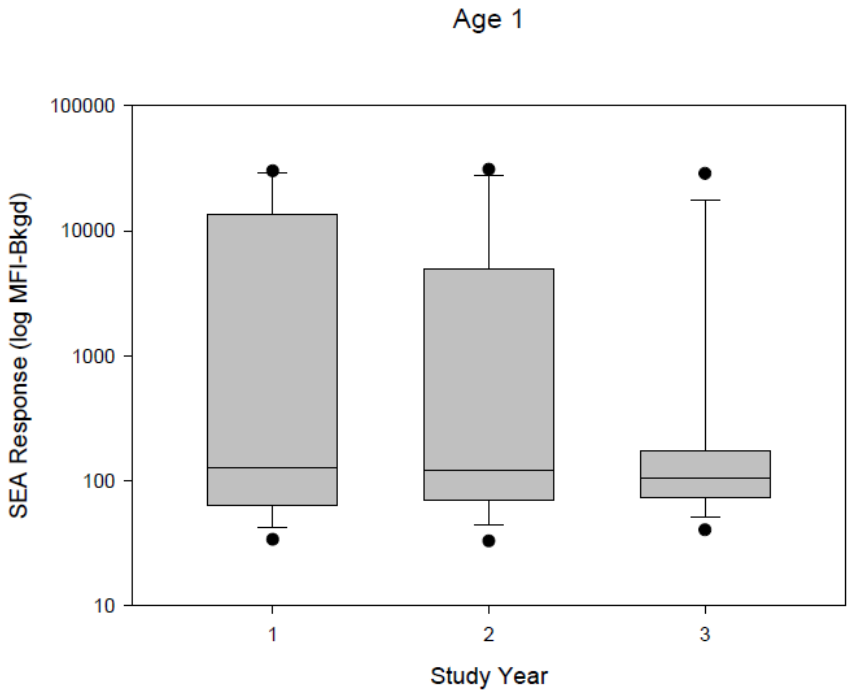


Figure 4.6 A significant reduction ($p<0.05$) in the median soluble egg antigen (SEA) MFI values among 1-year olds after two rounds of MDA was observed. Boxes enclose 25th and 75th percentile. Lines inside the boxes represent median MFI values.



4.5 Discussion

In our prospective, cluster randomized trial in Mbita sub-county, western Kenya, *S. mansoni* infection prevalence was high among a group of young children. Although our findings were consistent with previous reports of high rates of *S. mansoni* infection among SAC in this sub-county (Odiere et al., 2012), this study provides unique data on PSAC. At baseline, using the relatively insensitive Kato-Katz method on a single stool sample, nearly 30% of PSAC were identified as infected with a parasite that is often considered to be of little public health importance in this age group. As expected, prevalence *of S. mansoni* infection as determined by stool examination increased with age. However, our results highlight that young children were being exposed to contaminated water early in life, in some cases up to three years or more before they would be eligible for inclusion in MDA programs. Our findings add to the growing body of evidence that children are at risk for schistosomiasis at a very early age.

Despite a high rate of *S. mansoni* infection in our study population, there was a relatively low prevalence of STH infection in the same group. Although the environmental conditions in Mbita sub-county were conducive to STH transmission and despite the lack of improved water, sanitation and hygiene interventions, the prevalence of STH infection was much lower than expected. Prior to the study there was one MDA for STH in 2009 and there have been anecdotal reports of unprogrammed deworming with ALB for STH. These factors may have contributed to the low prevalence of STH observed in our study.

Schistosomiasis control program strategies have traditionally aimed to reduce prevalence of moderate and heavy intensity infections. More recently, additional strategies are being considered to interrupt transmission. Attaining program goals are therefore dependent on diagnostic tools that can adequately measure prevalence and intensity of infection. Although it is commonly believed that lower intensity infections do not have significant impact on morbidity due to the disease, there is growing recognition that even light intensity infections can have considerable impact on the health of children (King, 2015). In our study, after two rounds of MDA, traditional parasitologic methods showed little change in

prevalence of *S. mansoni* infection in PSAC. There was a significant decrease in heavy intensity infections after the first round of treatment in our study, but approximately 29% of infections were still classified as moderate or heavy intensity after two rounds of MDA. Our results support the growing concern that a single annual round of MDA in high prevalence areas may not be sufficient to achieve program goals (Secor, 2015).

In our study, stool exams were less sensitive than serology. It is possible that prevalence of *S. mansoni* infection was underestimated by only performing a single stool exam, but other studies have shown that in high prevalence areas, multiple stool exams conducted on consecutive days performed no better than a single exam (Verani et al., 2011). In complex and resource-constrained program settings, it is not feasible to collect multiple stool samples over consecutive days. We aimed to provide information that could be compared to current programmatic approaches. Additionally, it is unlikely that antibody results were significantly influenced by *S. haematobium* infection. Despite high rates of hematuria at baseline, there was no visible blood in any urine sample. High rates of hematuria were not observed in years 2 and 3. The use of different brands of urine dipsticks in year 1 and year 2 may have impacted hematuria results, but the absence of *S. haematobium* eggs upon urine filtration supports the claim that antibody results were likely attributable to *S. mansoni* infections . Although there have been reports of few isolated foci of *S. haematobium* in areas adjacent to Mbita (Sang et al., 2014), none have been identified in our study area. Furthermore, it is possible that cutoff values for the MBA were inaccurate, leading to incorrect prevalence estimates. The ability to define robust cutoffs for serological assays can be challenging and is often limited by the availability of well characterized panels of samples to determine appropriate cutoffs. Despite potential limitations, it is clear that a high proportion of children in our study were exposed to *S. mansoni* at an early age. Our results showed good correlation between antibody responses to SEA and Sm25 and intensity of infection measured by Kato-Katz. Additionally, we observed a significant association between antibody responses and distance to Lake Victoria. This inverse gradient relationship

has been observed with stool results (Handzel et al., 2003; Woodhall et al., 2013), but to our knowledge has not previously been documented by serology.

While there are limitations to using serology to distinguish between present and past schistosome infections, longitudinal monitoring of antibody responses could provide useful information on possible changes in exposure and may provide an advantage over traditional parasitologic methods. In addition to parasitologic methods, schistosomiasis control programs often include morbidity markers such as anemia to assess program impact. However, these markers are often difficult to measure and are not unique to infection with *Schistosoma* spp. In our study, we observed high rates anemia that were associated with malaria and not *S. mansoni* infection. As control programs successfully implement interventions, reduced transmission of schistosomiasis will result in fewer infections and lower prevalence of infection-specific antibody in cohorts of young children. Malaria control programs have described the use of seroincidence among young children born after control measures have been put in place as a way to measure current and historical transmission within communities (Drakeley et al., 2005; Cook et al., 2010; Arnold et al., 2014). Recently, in an lymphatic filariasis program setting, antibody responses were used to distinguish areas where programs had been implemented and successful, suboptimally implemented and not implemented at all (Dewi et al., 2015). This type of information would be useful for understanding how effective control programs have been. The use of serologic markers has most often been used in the context of low prevalence settings or surveillance. However, our results showed a decline in antibody responses among young children in an area where transmission was clearly ongoing. Although further studies are needed to support this finding, these results provide preliminary evidence that program impact can be monitored using serologic responses.

Despite the limitations of this study, we believe the ability to use serologic assays to monitor schistosomiasis control programs could potentially provide advantages over the current stool based approach. It is often easier to collect blood samples versus stool and an

additional advantage is the ability to directly observe the collection of fingerstick blood whereas the same opportunity does not exist for stool or urine collection. Furthermore, very small quantities of blood or dried bloodspots can be used in MBAs that can simultaneously test for a variety of diseases of public health importance at the same time. As there is often overlap of many of these diseases, this opens opportunities for integrated program monitoring, including vaccine coverage surveys. As schistosomiasis programs consider the feasibility of transitioning from control to elimination, the ability to document reduced seroincidence strengthens the evidence of elimination of transmission.

4.6 Acknowledgments

The authors are grateful to Delynn Moss and Amanda Barry at CDC for coupling the beads used for the MBA. This paper is published with the permission of the Director of the Kenya Medical Research Institute. We thank the local administration of Mbita sub-county; the assistant chiefs, village heads and community health workers for help with community mobilization. We are especially grateful to the residents of Mbita sub-county for their participation and cooperation throughout the study. The study was funded by the Bill and Melinda Gates Foundation.

5 Comparison of antigen and antibody responses in repeat lymphatic filariasis transmission assessment surveys in American Samoa

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[This manuscript has been published in *PLoS Neglected Tropical Diseases* (2018) 9;12(3) e0006347 https://doi.org/10.1371/journal.pntd.0006347]

5.1 Abstract

Background: Current WHO recommendations for lymphatic filariasis (LF) surveillance advise programs to implement activities to monitor for new foci of transmission after stopping mass drug administration (MDA). A current need in the global effort to eliminate LF is to standardize diagnostic tools and surveillance activities beyond the recommended transmission assessment survey (TAS).

Methodology: TAS was first conducted in American Samoa in 2011 (TAS 1) and a repeat TAS was carried out in 2015 (TAS 2). Circulating filarial antigen (CFA) and serologic results from both surveys were analyzed to determine whether interruption of LF transmission has been achieved in American Samoa.

Principal findings: A total of 1,134 and 864 children (5-10 years old) were enrolled in TAS 1 and TAS 2, respectively. Two CFA-positive children were identified in TAS 1, and one CFA-positive child was identified in TAS 2. Results of both surveys were below the threshold for which MDA was warranted. Additionally, 1,112 and 836 dried blood spots from TAS 1 and TAS 2, respectively were tested for antibodies to Wb123, Bm14 and Bm33 by luciferase immunoprecipitation system (LIPS) assay and multiplex bead assay. In 2011, overall prevalence of responses to Wb123, Bm14, and Bm33 was 1.0%, 6.8% and 12.0%, respectively. In 2015, overall prevalence of positive Bm14 and Bm33 responses declined significantly to 3.0% ($p<0.001$) and 7.8% ($p=0.013$), respectively.

Conclusions/significance: Although passing TAS 1 and TAS 2 and an overall decline in the prevalence of antibodies to Bm14 and Bm33 between these surveys suggests decreased exposure and infection among young children, there were persistent responses in some schools. Clustering and persistence of positive antibody responses in schools may be an indication of ongoing transmission. There is a need to better understand the limitations of current antibody tests, but our results suggest that serologic tools can have a role in guiding programmatic decision making.

5.2 Author summary

Lymphatic filariasis (LF), endemic in 72 countries, is a debilitating mosquito-transmitted parasitic disease caused by filarial worms. The Global Program to Eliminate Lymphatic Filariasis (GPELF) aims to interrupt transmission through mass drug administration (MDA) and to reduce suffering caused by the disease. At the start of GPELF in 2000 it was estimated that approximately 1.4 billion people were at risk for infection. By the end of 2016, primarily through successful MDA programs, the global number of people requiring interventions was reduced to 856.4 million. Current recommendations by the World Health Organization for LF surveillance advise programs to implement activities to monitor for new foci of transmission after stopping MDA. A current need in the global effort to eliminate LF is to standardize diagnostic tools and surveillance activities beyond the recommended transmission assessment survey (TAS). Two TAS were conducted in American Samoa; first in 2011 (TAS 1) and repeated in 2015 (TAS 2). In our evaluation, circulating filarial antigen and serologic results from both surveys were analyzed to determine whether interruption of LF transmission has been achieved in American Samoa. Despite passing TAS 1 and TAS 2, clustering and persistence of positive antibody responses in schools may be an indication of ongoing transmission. Results from our evaluation suggest that serologic tools can have a role in guiding programmatic decision-making.

5.3 Introduction

Lymphatic filariasis (LF), endemic in 72 countries, is a debilitating mosquito-transmitted parasitic disease caused by filarial worms (*Wuchereria bancrofti* and *Brugia* spp.) (Taylor et al., 2010). In 1997, at the 50th World Health Assembly (WHA), a resolution was passed to eliminate LF as a public health problem by 2020 (WHO, 1997). Shortly thereafter, in 1999, the Pacific Program for the Elimination of Lymphatic Filariasis (PacELF) was established to eliminate the disease in the Pacific Region through a strategy of annual rounds of mass drug administration (MDA) (Pacific, 2006). The following year, the Global Program to Eliminate Lymphatic Filariasis (GPELF) was established to assist all LF-endemic countries in achieving this elimination goal through the same MDA strategy. At the start of GPELF it was estimated that approximately 1.4 billion people were at risk for infection. By the end of 2016, MDA had been implemented in 66 of 72 LF-endemic countries, with a cumulative total of 6.7 billion treatments delivered since the start of GPELF (WHO, 2017c).

After multiple rounds of MDA, LF elimination programs must be able to determine when it is appropriate to stop treatment. The World Health Organization (WHO)-recommended transmission assessment survey (TAS) was designed as a decision-making tool to determine when transmission of LF is presumed to have reached a level low enough that it cannot be sustained even in the absence of MDA (WHO, 2011a). In areas where *W. bancrofti* is the principal LF pathogen, infection is assessed in the TAS by measuring circulating filarial antigen (CFA). Since its integration into national programs in 2011, TAS has successfully been implemented across LF endemic countries, and based on the results, MDA has been discontinued in multiple locations. The global number of people requiring MDA has been reduced from 1.4 billion in 2000 to 856.4 million in 2016 (WHO, 2017c).

Effective M&E is not only necessary during the MDA period but important throughout the lifespan of the LF program, including after MDA has stopped. Current WHO recommendations for post-MDA surveillance include periodic surveys: repeating TAS twice at 2- to 3-year intervals after stopping MDA. Beyond the TAS, post-MDA surveillance guidance

has not been standardized. Current WHO recommendations for surveillance advise programs to implement activities to monitor for new foci of transmission through the assessment of microfilaremia, antigenemia, or antibodies (WHO, 2011a). After effective MDA, microfilaremia and antigenemia begin to decline in populations and become increasingly difficult to detect (Gass et al., 2012). Detection of antifilarial antibodies appears to provide the earliest indicator of filarial exposure (Hamlin et al., 2012), and the absence of detectable antibody responses may provide evidence that transmission has been interrupted.

Surveys conducted in 1999 indicated that 17% of residents in 18 villages in American Samoa were infected with *W. bancrofti* (Pacific, 2006). This established American Samoa as one of the areas with the highest filarial infection levels in the Pacific Region and the only U.S. territory endemic for LF. The American Samoa Department of Health (DOH) started MDA in 2000. Annual MDA coverage was low (<50%) prior to 2003. After reassessment and modification of the communication and distribution strategies, the program treated an estimated 70% and 65% of the population in 2003 and 2004, respectively (King et al., 2011). Results from surveys in four sentinel sites showed an overall decline in CFA levels from 13% in 2003 to 0.95% in 2006 (Liang et al., 2008). An island-wide survey was conducted in 2007, and CFA prevalence was 2.3%, with the majority of the antigenemia detected in adults (Coutts et al., 2017). Because LF was presumed to be at very low levels, minimal programmatic activities were conducted from 2008-2010.

In accordance with WHO recommendations, TAS 1 was conducted in American Samoa in 2011 and was repeated in 2015 (TAS 2). The DOH opted to include antifilarial antibody testing in both surveys to complement antigen testing. In this paper we report CFA and serologic results from the two TAS that were conducted to determine whether or not interruption of LF transmission has been achieved in American Samoa.

5.4 Methods

5.4.1 Ethics statement

The surveys were approved by the DOH Institutional Review Board (IRB) and the U.S. Centers for Disease Control and Prevention (CDC) as program evaluation, non-research. In preparation for the TAS, survey details were described in a written document distributed to school officials and parents or guardians of potential participants. In accordance with DOH and Department of Education policies, parents or guardians provided written permission for participation of children. Additionally, children ≥7 years of age were asked to provide oral assent for their participation on the day of the survey. All data were collected electronically, and identifiable information was kept confidential and maintained by using a secure database with access restricted to essential survey personnel.

5.4.2 Survey site and design

American Samoa, a U.S. territory, is located in the South Pacific comprising of seven small islands and atolls. More than 90% of the total population live on the main island of Tutuila with the remainder of the residents dispersed on the adjacent island of Aunu'u and the outer Manu'a islands of Ta'u, Ofu and Olosega. Tutuila and Aunu'u comprised the evaluation unit for TAS. TAS 1 was carried out in February 2011 and TAS 2 was conducted in April 2015. Surveys were implemented according to WHO guidelines for conducting TAS in areas where *Aedes* spp. are the main LF vectors (WHO, 2011a). Because of high school enrollment rates (>95%), school-based surveys were conducted at both time points, and grades 1 and 2 were used as a proxy for the recommended age (6-7 years). Systematic sampling was recommended for both surveys, but due to low rates of consent, all children with signed consent forms were enrolled. The target sample sizes in 2011 and 2015 were 1,042 and 1,014, respectively. The critical cutoff, the maximum number of observed positive results that is consistent with a threshold of < 1%, for both surveys was six antigen-positive children.

5.4.3 Blood collection and examination

For both surveys approximately 160 µL of blood was collected via a single finger stick into an EDTA-coated blood collection tube (Ram Scientific, Yonkers, NY). One hundred microliters of blood was used for the detection of CFA by immunochromatographic card test (ICT) (Alere; Scarborough, ME). The cards were read at 10 min and marked as either positive or negative according to the manufacturer's instructions. The remaining 60 µL of blood (10 µL per extension x 6 extensions) was spotted onto filter paper (Cellabs, Sydney, Australia), dried and stored at -20°C until shipped to National Institutes of Health (NIH) for antifilarial antibody testing by luciferase immunoprecipitation system (LIPS) assay (Kubofcik et al., 2012) or CDC for testing by multiplex bead assay (MBA) (Moss et al., 2011; Lammie et al., 2012; Priest et al., 2016) (described below).

5.4.4 LIPS

In TAS 1, IgG responses to Wb123 were determined by previously described LIPS assay (Kubofcik et al., 2012). One modification was made to accommodate the use of dried blood spots (DBS) instead of serum. DBS were eluted in 200 µl of PBS, and 40 µl of the eluted material was used for the assay. Cutoff values were calculated from receiver operator characteristic (ROC) curves using sera from *W. bancrofti*-infected patients and presumed negative sera from North Americans with no history of foreign travel.

5.4.5 MBA

Antifilarial antibody responses to Bm14 (Chandrashekar et al., 1994) and Bm33 (Dissanayake et al., 1993) for samples collected during TAS 1 and responses to Wb123, Bm14 and Bm33 for samples collected during TAS 2 were determined by previously described MBA (Moss et al., 2011; Hamlin et al., 2012; Lammie et al., 2012; Priest et al., 2016). Briefly, DBS were eluted to yield a sample dilution of 1:400 in PBS buffer (pH 7.2) containing 0.3% Tween-20, 0.02% sodium azide, 0.5% casein, 0.5% polyvinyl alcohol (PVA), 0.8% polyvinylpyrrolidone (PVP), and 3 µg/ml *Escherichia coli* extract. *E. coli* extract was added to the buffer to absorb antibodies to any residual *E. coli* proteins that may not have

been eliminated in the antigen purification process. Samples having a coefficient of variation of >15% between duplicate wells for ≥2 positive LF antibody responses were repeated. The average of the median fluorescent intensity (MFI) values from the duplicate wells minus the background (bg) fluorescence from the buffer-only blank was reported as MFI-bg. Cutoff values were calculated from ROC curves using sera from *W. bancrofti*-infected patients and presumed negative sera from US citizens with no history of foreign travel.

5.4.6 Treatment

Parents or guardians of individuals who were ICT positive were notified of test results, and the children were offered a standard single dose of diethylcarbamazine (DEC) (6 mg/kg) and albendazole (400 mg).

5.4.7 Statistical analysis

Analyses were performed in R version 3.3.0 (Team, 2016) with the survey package (Lumley, 2004) using a 5% level of significance. Because a high percentage of the American Samoa population of 1st and 2nd graders participated, samples from TAS 1 and TAS 2 were treated as clustered samples with a finite population correction. Differences in frequencies were evaluated with a Rao-Scott X^2 statistic (Rao JN, 1984). Confidence intervals for proportions utilize the incomplete beta function (Korn EL, 1998). Changes in MFI were evaluated with the complex sampling version of Mood's test for differences in medians (Lumley, 2013).

5.5 Results

5.5.1 TAS 1

A total of 1,134 children from 25 of 26 public and private elementary schools were enrolled in TAS 1; 50.6% were male, and the mean age was 6.8 years (range 5-10 years). Because written informed parental consent was required for participation, systematic sampling of children could not be applied as intended. All children with signed consent forms from parents/guardians were enrolled in the survey. One small private school (St. Theresa) was not sampled because of school officials refusal to participate. Demographic information was not available for 57 students from Tafuna Elementary. For 197 (17.4%) children enrolled, no

blood sample was collected or the quantity of blood collected was insufficient for testing by ICT. Of the samples tested, 2/937 (0.2%, 95% upper confidence limit (CL) 0.8%) were antigen positive. Both positive children were from the same school, Lupelele Elementary. Demographic information and number of samples tested by ICT are summarized by school in Table 5.1.

5.5.2 TAS 2

By 2015, two elementary schools that had existed in 2011 were closed and six new schools had opened. A total of 864 children from all 30 public and private elementary schools were enrolled in TAS 2; 48.4% were male, and the mean age was 7.0 years. As with TAS 1, written parental consent was required for participation and all children with signed consent forms were enrolled in the survey. For 96 (11.1%) children enrolled, no blood sample was collected or the quantity of blood collected was insufficient for ICT testing. Of the samples tested, 1/768 (0.1%, 95% CL 0.3%) was positive. The antigen-positive child was from Lupelele Elementary, the same school where the two antigen-positive children were identified in TAS 1 four years earlier. Demographic information and number of samples tested by ICT for TAS 2 are given in Table 5.1, stratified by school.

Table 5.1 All public and private elementary schools in American Samoa included in TAS 1 and TAS 2. Age and sex distribution of children enrolled in TAS 1 and TAS 2 and number of samples tested for circulating filarial antigen by ICT are summarized by survey year.

	TAS 1 (February 2011)						TAS 2 (April 2015)					
School	Total enrollment in grades 1 and 2	Number enrolled	Number of males	Mean Age in years	Number tested by ICT	% tested by ICT	Total enrollment in grades 1 and 2	Number enrolled	Number of males	Mean Age in years	Number tested by ICT	% tested by ICT
A P Lutali	21	18	11	6.7	16	88.9	23	12	6	7.0	12	100.0
Afonotele	24	16	11	6.8	12	75.0	14	7	3	7.4	6	85.7
Alataua	76	40	19	7.0	38	95.0	64	32	15	6.7	29	90.6
Alofau	34	24	16	6.8	18	75.0	38	16	9	6.9	13	81.3
Aoa	30	27	16	6.7	26	96.3						
Aua	114	59	27	6.9	47	79.7	88	25	10	7.2	14	56.0
Coleman	210	71	36	6.7	63	88.7	143	41	16	7.2	37	90.2
Kanana Fou	35	10	3	6.7	10	100.0	82	45	19	6.8	40	88.9
Lauli'i	43	32	15	6.6	25	78.1	37	19	13	6.6	18	94.7
Le'atele							20	11	7	6.7	4	36.4
Leone Midkiff	269	130	77	6.6	113	86.9	195	76	45	7.0	76	100.0
Lupelele	203	106	60	6.8	92	86.8	167	87	37	7.0	85	97.7
Manulele	249	119	69	6.7	90	75.6	250	51	20	7.4	44	86.3
Manumalo	165	63	32	7.2	48	76.2	126	45	21	6.8	44	97.8
Masefau	19	16	7	6.6	13	81.3	21	13	5	7.3	9	69.2
Matafao	174	37	21	6.6	34	91.9	124	55	23	7.1	35	63.6
Matatula	38	14	8	6.8	14	100.0	39	26	16	6.8	26	100.0
Mt. Alava							18	12	6	6.8	10	83.3
Olomoana							19	7	6	7.4	6	85.7
Pacific Horizon							16	1	0	7.0	1	100.0
Pavaiai	294	158	83	6.5	107	67.7	278	96	49	6.9	89	92.7
Peteli							15	8	3	6.8	8	100.0
Samoa Baptist	38	4	1	7.0	3	75.0	35	13	7	7.5	12	92.3
SDA	32	17	9	6.9	11	64.7	18	12	4	7.0	10	83.3
Siliaga	59	31	19	7.0	28	90.3	41	16	7	6.8	14	87.5
South Pacific Academy	35	13	2	6.9	7	53.8	29	13	7	7.0	9	69.2
SPICC	78	30	14	6.6	25	83.3	66	27	17	7.1	27	100.0
St. Francis	41	29	12	6.7	28	96.6	29	10	4	7.0	10	100.0
St. Theresa	34						43	18	12	7.0	18	100.0
Tafuna	216	57	N/A	N/A	57	100.0	197	68	31	6.9	60	88.2
Ta'iala							11	2	0	8.0	2	100.0
Vatia	16	13	6	6.8	11	84.6						
TOTALS	**2,547**	**1,134**	**574**	**6.8**	**937**	**82.6**	**2,246**	**864**	**418**	**7.0**	**768**	**88.9**

5.5.3 Antibody responses

In 2011, a total of 1,112 DBS were prepared for antibody testing. Overall prevalence of responses to Wb123, Bm14, and Bm33 was 1.0%, 6.8% and 12.0%, respectively. There was at least one Wb123 antibody-positive child in 6/25 (24.0%) schools. Distribution of responses to Bm14 and Bm33 responses was more widespread than responses to Wb123 with at least one antibody-positive child identified in 88.0% and 80.0% of schools, respectively. In 2015, a total of 836 DBS were collected for antibody testing. Overall prevalence of Bm14 and Bm33 responses declined significantly to 3.0% ($p<0.001$) and 7.8% ($p=0.013$), respectively. The prevalence of Wb123 responses was 3.6% in TAS 2, but results were not directly compared to those from TAS 1 because of the different testing platform used. The distribution of Wb123 responses was more widespread in TAS 2 than TAS 1 with at least one antibody-positive child identified in 12/30 (40.0%) schools. Distribution of Bm14 responses was more focal in 2015 than in 2011 with at least one antibody-positive child identified in 12/30 (40.0%) schools; only one of these 12 schools did not have any Bm14-positive children in TAS 1. Distribution of positive Bm33 responses was the most widespread (56.7% of schools) of the three markers assessed, but was still more focally distributed in 2015 compared to 2011. Antibody responses are summarized by school in Figure 5.1 and Table 5.2. Change in MFI-bg for Bm14 and Bm33 was compared for the 22 schools included in both surveys. There were significant declines in the median quantitative MBA responses for Bm14 and Bm33 in 21/22 (95.5%) schools. Median MFI-bg values are summarized in Table 5.3, stratified by school.

Figure 5.1 Distribution of antibody responses to Bm14, Bm33, and Wb123 by school for TAS 1 (2011) and TAS 2 (2015) in American Samoa. Responses to Wb123 in TAS 1 were assessed by luciferase immunoprecipitation system (LIPS) assay. All other responses were assessed by multiplex bead assay.

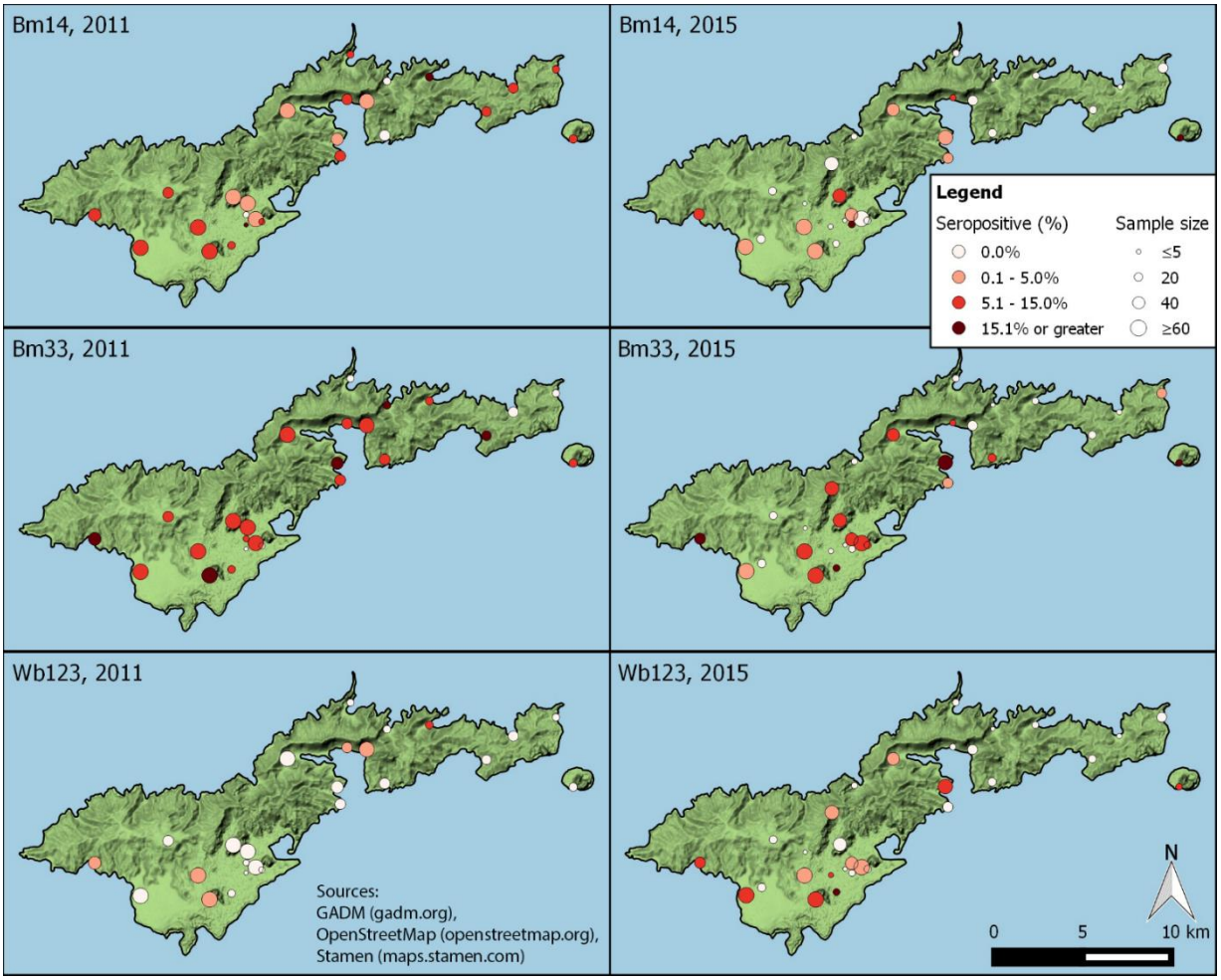

Table 5.2 Distribution of antibody responses to Wb123, Bm14, and Bm33 in all elementary schools located on the main island of Tutuila in American Samoa. Responses to Wb123 in TAS 1 were assessed by LIPS. All other responses were assessed by multiplex bead assay.

	TAS 1 (February 2011)							TAS 2 (April 2015)						
School	Number DBS* tested	Wb123 positive	% positive	Bm14 positive	% positive	Bm33 positive	% positive	Number DBS tested	Wb123 positive	% positive	Bm14 positive	% positive	Bm33 positive	% positive
A P Lutali	18	0	0.0	1	5.6	2	11.1	11	1	9.1	2	18.2	2	18.2
Afonotele	15	0	0.0	0	0.0	4	26.7	5	0	0.0	0	0.0	0	0.0
Alataua	40	2	5.0	5	12.5	8	20.0	31	3	9.7	4	12.9	6	19.4
Alofau	24	0	0.0	2	8.3	5	20.8	16	0	0.0	0	0.0	0	0.0
Aoa	27	0	0.0	4	14.8	0	0.0							
Aua	56	1	1.8	1	1.8	3	5.4	25	0	0.0	0	0.0	0	0.0
Coleman	70	0	0.0	2	2.9	8	11.4	40	1	2.5	2	5.0	3	7.5
Kanana Fou	10	0	0.0	0	0.0	1	10.0	41	1	2.4	2	4.9	3	7.3
Lauli'i	30	0	0.0	0	0.0	3	10.0	18	0	0.0	0	0.0	1	5.6
Le'atele								10	0	0.0	0	0.0	0	0.0
Leone Midkiff	129	0	0.0	10	7.8	12	9.3	75	4	5.3	1	1.3	2	2.7
Lupelele	105	5	4.8	15	14.3	27	25.7	85	5	5.9	1	1.2	11	12.9
Manulele	118	0	0.0	5	4.2	13	11.0	48	1	2.1	0	0.0	5	10.4
Manumalo	62	0	0.0	2	3.2	5	8.1	45	0	0.0	4	8.9	3	6.7
Masefau	16	1	6.3	3	18.8	2	12.5	12	0	0.0	0	0.0	0	0.0
Matafao	37	0	0.0	1	2.7	7	18.9	53	6	11.3	1	1.9	9	17.0
Matatula	14	0	0.0	1	7.1	0	0.0	25	0	0.0	0	0.0	1	4.0
Mt. Alava								12	0	0.0	0	0.0	0	0.0
Olomoana								7	0	0.0	0	0.0	0	0.0
Pacific Horizon								1	0	0.0	0	0.0	0	0.0
Pavaiai	155	1	0.6	11	7.1	13	8.4	94	4	4.3	4	4.3	9	9.6
Peteli								7	1	14.3	0	0.0	0	0.0
Samoa Baptist	4	0	0.0	1	25.0	0	0.0	12	0	0.0	2	16.7	0	0.0
SDA	15	0	0.0	1	6.7	1	6.7	12	2	16.7	0	0	2	16.7
Siliaga	29	0	0.0	2	6.9	3	10.3	16	0	0.0	0	0.0	0	0.0
South Pacific Academy	10	0	0.0	1	10.0	0	0.0	13	0	0.0	0	0.0	1	7.7
SPICC	29	0	0.0	2	6.9	4	13.8	27	0	0.0	0	0.0	1	3.7
St. Francis	28	1	3.6	4	14.3	4	14.3	10	0	0.0	1	10.0	1	10.0
St. Theresa								18	0	0.0	1	5.6	0	0.0
Tafuna	58	0	0.0	1	1.7	8	13.8	65	1	1.5	0	0.0	5	7.7
Ta'iala								2	0	0.0	0	0.0	0	0.0
Vatia	13	0	0.0	1	7.7	0	0.0							
TOTALS	**1112**	**11**	**1.0**	**76**	**6.8**	**133**	**12.0**	**836**	**30**	**3.6**	**25**	**3.0**	**65**	**7.8**

Table 5.3 Median fluorescence intensity minus background (MFI-bg) values by school for the 22 schools included in both TAS 1 (February 2011) and TAS 2 (April 2015) in American Samoa. Minimum and maximum MFI-bg values within each school are indicated in parentheses.

			Bm14			Bm33		
School	N, 2011	N, 2015	2011	2015	p	2011	2015	p
A P Lutali	18	11	45 (18, 379)	16 (4, 24,429)	0.001	347 (190, 1,611)	140 (12, 5,252)	<0.001
Afonotele	15	5	42 (20, 144)	16 (5, 23)	<0.001	329 (193, 3,452)	96 (58, 342)	0.039
Alataua	39	31	46 (23, 16,379)	14 (3, 25,424)	<0.001	451 (152, 26,090)	104 (12, 26,761)	<0.001
Alofau	24	16	46 (21, 333)	15 (1, 120)	<0.001	365 (109, 5,397)	90 (5, 381)	<0.001
Aua	56	25	39 (13, 274)	10 (2, 79)	<0.001	253 (108, 6,881)	58 (10, 251)	<0.001
Coleman	70	40	45 (16, 455)	12 (3, 7,014)	<0.001	244 (119, 16,449)	152 (71, 10,725)	<0.001
Kanana Fou	10	41	37 (23, 73)	10 (0, 2,162)	<0.001	169 (102, 1,273)	80 (-28, 30,402)	<0.001
Lauli'i	30	18	53 (20, 157)	20 (6, 148)	<0.001	401 (151, 2,992)	124 (54, 664)	<0.001
Leone Midkiff	128	75	50 (15, 872)	11 (-2, 844)	<0.001	454 (99, 5,373)	90 (-4, 24,291)	<0.001
Lupelele	103	85	43 (13, 24,135)	14 (5, 11,059)	<0.001	536 (107, 30,886)	158 (34, 4,563)	<0.001
Manulele	116	48	49 (16, 859)	14 (3, 150)	<0.001	384 (132, 4,033)	141 (15, 23,015)	<0.001
Manumalo	62	45	39 (13, 317)	18 (7, 11,800)	<0.001	288 (109, 4,137)	124 (37, 7,536)	<0.001
Masefau	16	12	54 (25, 761)	9 (2, 147)	<0.001	356 (118, 5,354)	139 (0, 219)	<0.001
Matafao	37	53	40 (20, 299)	13 (2, 15,237)	<0.001	272 (100, 8,988)	151 (-38, 24,914)	0.001
Matatula	14	25	39 (32, 172)	15 (7, 71)	<0.001	309 (127, 649)	114 (26, 1,652)	<0.001
Pavaiai	153	94	43 (9, 1,847)	13 (4, 25,471)	<0.001	304 (55, 15,131)	158 (-15, 14,071)	<0.001
Samoa Baptist	4	12	26 (17, 1,821)	14 (6, 4,680)	0.231	179 (167, 753)	68 (8, 244)	<0.001
SDA	15	12	51 (18, 3,644)	11 (4, 86)	0.002	282 (148, 8,449)	175 (74, 1,148)	0.377
Siliaga	29	16	42 (16, 300)	6 (2, 24)	<0.001	340 (139, 2,275)	68 (47, 355)	<0.001
South Pacific Academy	10	13	27 (13, 169)	11 (5, 35)	<0.001	216 (100, 495)	124 (80, 696)	0.001
SPICC	28	27	35 (14, 877)	19 (4, 360)	0.004	281 (97, 13,161)	83 (9, 1,477)	<0.001
St. Francis	28	10	44 (19, 5,180)	15 (5, 393)	<0.001	223 (105, 2,732)	131 (52, 904)	0.051

All three ICT-positive children identified in the surveys had positive antibody responses to Wb123, Bm14 and Bm33. However, concordance of individual antibody responses for antigen-negative children was relatively poor at both time points. Only 21/161 (13.0%) antibody-positive children in TAS 1 and 20/73 (27.4%) positive children in TAS 2 had positive responses to at least two markers. Similar discordance was also observed for samples tested where the antigen status was unknown. Test concordance for antigen negative children is summarized in Table 5.4. Test concordance for children whose antigen status was unknown is summarized in Table 5.5.

Table 5.4 Antibody test concordance among antigen negative children in TAS 1 (February 2011) and TAS 2 (April 2015) in American Samoa.

			Concordance with positive index		
	Index	**total # positive/N (%)**	**Wb123**	**Bm14**	**Bm33**
TAS 1	**Wb123**	7/935 (0.75)		6/7 (85.7)	7/7 (100.0)
	Bm14	63/935 (6.7)	6/63 (9.5)		20/63 (31.7)
	Bm33	118/935 (12.6)	7/118 (5.9)	20/118 (16.9)	
TAS 2	**Wb123**	26/743 (3.5)		11/26 (42.3)	16/26 (61.5)
	Bm14	22/743 (3.0)	11/22 (50.0)		15/22 (68.2)
	Bm33	56/743 (7.5)	16/56 (28.6)	15/56 (26.8)	

Table 5.5 Antibody test concordance among children with unknown antigen status in TAS 1 (February 2011) and TAS 2 (April 2015) in American Samoa.

			Concordance with positive index		
	Index	**total # positive/N (%)**	**Wb123**	**Bm14**	**Bm33**
TAS 1	**Wb123**	2/175 (1.1)		2/2 (100.0)	1/2 (50.0)
	Bm14	11/175 (6.3)	2/11 (18.2)		4/11 (36.4)
	Bm33	13/175 (7.4)	1/13 (7.7)	4/13 (30.8)	
TAS 2	**Wb123**	3/92 (3.3)		0/3 (0.0)	2/3 (66.7)
	Bm14	2/92 (2.2)	0/2 (0.0)		1/2 (50.0)
	Bm33	8/92 (8.7)	2/8 (25.0)	1/8 (12.5)	

5.6 Discussion

The TAS is used to determine when transmission of LF is low enough that MDA can safely be stopped. The attraction of the TAS design is that it facilitates decision-making and has proven feasible to implement yet is standardized and incorporates a statistically rigorous design. After stopping MDA, WHO recommends repeating TAS twice at 2- to 3-year intervals and conducting additional surveillance activities to confirm that transmission has been interrupted. However, beyond the TAS, post-MDA surveillance guidance has not been standardized. A current need in the global effort to eliminate LF is reliable diagnostic tools that can be used to guide programmatic decisions, especially decisions made in the final stages of the program (Solomon et al., 2012). In principle, because of the greater sensitivity of detection of antibody responses compared to antigen testing, antibody testing could provide an earlier signal of recrudescence or decreased transmission over time. Antibody testing could be included in the TAS without any modification of the survey design. The inclusion of antifilarial antibody testing in both TAS 1 and TAS 2 conducted in American Samoa in 2011 and 2015, offered an opportunity to determine whether antibody testing would provide evidence that interruption of LF transmission had been achieved.

Results of TAS 1 conducted in 2011 met criteria for stopping MDA, and results from TAS 2 carried out in 2015 were below the threshold for which MDA was recommended. The outcomes from these surveys indicated that LF transmission had been reduced below the threshold at which transmission was thought to be sustainable. Although there was a decrease in the absolute number of antigen-positive children identified from TAS 1 to TAS 2, it was not possible to determine if antigen prevalence had changed between the two surveys since TAS is not statistically powered to detect changes over time. This illustrates a key challenge in post-MDA monitoring – as transmission declines and programs near elimination endpoints, it becomes increasingly difficult to rely on microfilariae (mf) and antigen markers.

Ideally, during the post-MDA surveillance period, trends could be measured to provide information on LF status in order to assist programs to take appropriate action as

necessary. Since measures of antibody responses are more sensitive than mf and antigen detection (Gass et al., 2012), it is conceivable that changes in antibody prevalence could be used in the context of TAS to complement antigen testing. Although antigen prevalence in TAS 1 was <1%, responses to Wb123, Bm14, and Bm33 were 1.0%, 6.8%, and 12.0%, respectively. Similarly, antigen prevalence was low in TAS 2, but antibody prevalence was greater than antigen prevalence by all three markers. While there are limitations to using antigen markers during the surveillance period, the ability to monitor LF transmission status may be facilitated by using more sensitive antibody markers.

There were, however, differences among the three antibody markers. In TAS 1, the distribution of positive responses to Wb123 was relatively focal with antibody positive children in <25% of schools. In contrast, the distribution of positive Bm14 and Bm33 responses was more widespread with at least one antibody-positive child identified in >80% of schools. The more widespread distribution of Wb123 responses seen in TAS 2 may have been in part a function of using a different assay platform in 2015. Responses to Bm14 in TAS 2 were also fairly widespread, but less common than in TAS 1. However, the schools with Bm14-positive children were not necessarily the same ones in which Wb123-positive children were identified. Distribution of positive Bm33 responses was the most widespread of the three antibody markers assessed in TAS 2, detected in children in more than half of the schools, but was still more focally distributed than in TAS 1. It is possible that the three antibody markers used were measuring different LF exposure or infection patterns, but it is unclear how results of antibodies to a single marker should be interpreted.

There was good concordance of antibody responses among antigen-positive children. As expected, presence of CFA was associated with positive antibody responses to all three markers; Wb123, Bm14, and Bm33. However, among antigen-negative children, concordance of the antibody responses was poor, and a similar pattern was observed among children whose antigen status was unknown. These differences in antibody responses could be a reflection of the differences between antibody responses triggered by larval (Wb123)

and adult worm (Bm14 and Bm33) exposures, but further research is needed to characterize LF antibody responses, especially during the post-MDA surveillance period.

In principle, as LF programs successfully implement MDA, reduced transmission of LF will result in lower prevalence of infection-specific antibody in young children and eventually an absence of detectable antibodies in the population. Overall, on the main island of American Samoa, there was a significant decline in antibody responses to Bm14 and Bm33 from 2011 to 2015 suggesting LF transmission was declining in the area. Furthermore, in every school included in both surveys, there was a significant decrease in the intensity of antibody responses from TAS 1 to TAS 2 providing additional support that LF exposure and infection had decreased during this period.

Although the overall decline in Bm14 and Bm33 prevalence suggested lower exposure and infection among young children, the results should be interpreted with caution. Positive antibody responses to these antigens were relatively widespread across the island in 2011. Even though responses to Bm14 and Bm33 were more focal in 2015, there were persistent responses in some schools. Furthermore, there was an apparent increase in Wb123 prevalence, but results of the two surveys could not be directly compared because of the different testing platforms used. Antibody responses in these schools could represent focal areas of persistent or recurrent LF transmission, residual seropositivity following interruption of transmission, or false-positive results. Bm14 and Bm33 are known to cross-react with closely related filarial parasites (Lammie et al., 2004; Weil et al., 2011), but these parasites are not known to be in circulation in American Samoa. It is also possible that the cutoff values for the MBA were inaccurate. The ability to define robust cutoffs for serological assays can be challenging and is often limited by the availability of well characterized panels of samples to determine appropriate cutoffs. If the responses represent residual seropositivity after interruption of transmission, then seroprevalence will continue to decline, and future surveys can be conducted to confirm the downward trend. While there may have been issues with defining antibody assay parameters, the possibility that persistent antibody signals

represented true ongoing focal transmission cannot be excluded. Although the duration of antibody responses to Bm14 and Bm33 is unclear, presumably, positive signals among children in the target age range for TAS (6-7 years) indicate relatively recent exposure.

All three CFA-positive children identified in TAS 1 and TAS 2 were from the same school (Lupelele Elementary), and there were antibody-positive children in this school at both time points. These results may have been an indication of LF status in the communities in which the children lived. Yet, since the primary vector, *Aedes polynesiensis*, is a day-biting mosquito, it is difficult to determine if LF exposure took place in the community or elsewhere. In areas of diurnal LF periodicity, there is a need to better understand the relationship between results from school-based surveys and community transmission.

At present, it is unclear how to interpret persistent antibody responses in TAS 1 and TAS 2 in American Samoa. Although CFA results were below the threshold for which MDA was warranted, persistence of positive antibody signals is a potential cause for concern. In an island-wide vector study conducted in parallel to TAS 1, a large sample of mosquitoes was collected and tested for the presence of filarial DNA (Schmaedick et al., 2014). Results from the xenomonitoring survey indicated widespread, PCR-positive mosquitoes across the island. The presence of filarial DNA in mosquitoes raised suspicion that LF transmission was ongoing, but in the absence of established thresholds for programmatic action, no additional interventions were conducted. Additionally, in a serosurvey conducted just prior to TAS 1 there was evidence of possible clustering of antigen-positive adults in some communities (Lau et al., 2014b). Although the potential impact of antigen-positive clusters on transmission is unclear, it is possible that infection levels are high enough in these areas to sustain transmission. Furthermore, results from a recent study in American Samoa demonstrated that PCR-positive pools of LF vector mosquitoes were statistically significant predictors of seropositivity for Wb123 but not for Bm14 (Lau et al., 2016), suggesting Wb123 could be an indicator of ongoing transmission. Although Wb123 prevalence was low in both TAS, it may have been an indication of recent larval exposures and the potential for ongoing

transmission. This also highlights the importance of understanding how to appropriately interpret responses to each of the antibody markers.

It is unclear why there was an apparent disconnect between declining antibody prevalence in TAS and evidence of ongoing transmission in other survey results. It is possible that the school-based cluster survey approach was not sensitive enough to reflect LF status in communities. Although all primary schools were included in the TAS, children from multiple villages attended each school. The transmission status of individual communities may have been more accurately reflected if a certain proportion of children from each village was tested. There is a need to better understand the significance of spatial distribution of CFA and antibody signals. Additionally, there may have been potential bias introduced in the way the TAS were conducted. The requirement for written informed consent resulted in lower than desired participation in surveys, and a true random sample of children could not be obtained. This highlights one of the challenges in areas where survey participation is dependent on intensive sensitization of communities.

Currently, there is no clear guidance on how to investigate CFA- or antibody-positive children identified during TAS, but utilizing survey results may allow programs to identify areas where additional interventions may be needed. In American Samoa, there was an overall decline in antibody prevalence, but there were indications that raised concerns of ongoing transmission. All antigen-positive children were from the same school, but no specific follow up activities took place. Furthermore, there were schools in which there were persistent antibody signals from TAS 1 to TAS 2, which may have been an indication of focal transmission. There are challenges in utilizing cluster-based survey methodologies to assess focal diseases especially when prevalence is assumed to be low. There have been activities conducted in Sri Lanka to determine relationships among various indicators, including CFA, antifilarial antibody, and the presence of filarial DNA in *Culex* mosquitoes (Rao et al., 2014; 2017). However, there is a pressing need to conduct similar activities in *Aedes* spp. and *Anopheles* spp. settings.

As GPELF continues to make progress, it is critical to identify strategies for effective monitoring and evaluation to determine if transmission has been interrupted. Making incorrect programmatic decisions can have major political and financial implications. Despite passing TAS 1 and TAS 2, clustering and persistence of positive antibody responses in schools may be an indication of ongoing transmission in American Samoa. Although there is a clear need to better understand the limitations of current antibody tests, our results suggest that serologic tools can have a role in guiding programmatic decision making.

5.7 Acknowledgments

The authors are grateful to Amanda Barry at CDC for coupling the beads used for the 2015 MBA. We would like to thank Angela Keller at CDC and Brian Chu at the Task Force for Global Health for their assistance with logistics, planning and field implementation of TAS. The authors are grateful to Faaui Vaitautolu and Puna Tanielu of the American Samoa Department of Education for their assistance in coordinating school visits. We would like to thank the field workers for their effort and dedication to the survey activities. The authors are especially grateful to the children of American Samoa for their participation and cooperation throughout the surveys.

6 Use of antibody tools to provide serologic evidence of elimination of lymphatic filariasis in The Gambia

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[This manuscript has been published in *American Journal of Tropical Medicine and Hygiene* (2018) 98(1):15-20 https://doi.org/10.4269/ajtmh.17-0371]

6.1 Abstract

A current need in the global effort to eliminate lymphatic filariasis (LF) is the availability of reliable diagnostic tools that can be used to guide programmatic decisions, especially decisions made in the final stages of the program. This study carried out in The Gambia aimed to assess antifilarial antibody levels among populations living in historically highly LF endemic areas, and to evaluate the use of serologic tools to confirm interruption of LF transmission. A total of 2,612 dried blood spots (DBS) collected from individuals aged 1 year and above from 15 villages was tested for antibodies to Wb123 by enzyme-linked immunosorbent assay (ELISA). A subset of DBS (n=599) was also tested for antibodies to Bm14 by ELISA. Overall, prevalence of Wb123 was low (1.5%, 95% confidence interval (CI) 1.1-2.1%). In 7 of 15 villages (46.7%), there were no Wb123-positive individuals identified. Individuals with positive responses to Wb123 ranged in age from 3 to 100 years. Overall, Bm14 prevalence was also low (1.5%, 95% CI 0.7-2.8%). Bm14 positivity was significantly associated with older age ($p<0.001$). The low levels of antibody responses to Wb123 observed in our study strongly suggest that sustainable LF transmission has likely ceased in The Gambia. Additionally, our results support the conclusion that serologic tools can have a role in guiding programmatic decision making and supporting surveillance.

6.2 Introduction

Lymphatic filariasis (LF) is a mosquito-transmitted parasitic disease caused by three main species of filarial worms (*Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*) (Taylor et al., 2010). In 1997, at the 50th World Health Assembly (WHA), a resolution was passed to eliminate LF as a public health problem by 2020 (WHA resolution 50.29) (WHO, 1997). Shortly thereafter, in 2000, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was organized to assist countries in achieving this goal (Ottesen, 2006). At the onset of GPELF, it was estimated that 120 million individuals were infected, and that approximately 1.3 billion people throughout the tropics and subtropics were at risk for filarial infection (Ottesen, 2006). To reach established elimination targets, LF programs set out to treat individuals in endemic areas through annual community-wide mass drug administration (MDA) for at least 5 years. By the end of 2015, MDA had been implemented in 63 of 73 LF-endemic countries, with a cumulative total of 6.2 billion treatments delivered since the launch of GPELF (WHO, 2016a).

Typically, LF programs first conduct mapping surveys to identify areas to target for treatment, then carry out multiple rounds of MDA and finally conduct transmission assessment surveys (TAS) designed to determine if infection levels are low enough to stop MDA (WHO, 2011a). Although most LF endemic countries have successfully followed this approach, some countries have yet to scale up programmatic activities. While there is a clear need to implement MDA in some of these areas, others have a history of high microfilariae (mf) prevalence but recent surveys have failed to confirm the presence of infection and appropriate programmatic action is unclear. A current need in the global effort to eliminate LF is reliable diagnostic tools that can be used to guide programmatic decisions, especially decisions made in the final stages of the program. Additionally, validated tools are needed to confirm the absence of LF transmission in situations where the requirement for program implementation is unclear.

In the early stages of the global LF program, detection of mf in peripheral blood was used routinely to monitor the impact of MDA (Ottesen et al., 1997; 2006; Weil and Ramzy, 2007). In most LF-endemic areas in the world where the parasite is nocturnally periodic, logistic challenges were encountered because of the requirement to collect blood at night between 22:00 and 02:00 hours. Furthermore, it became increasingly difficult to detect mf in populations after multiple rounds of MDA (Gass et al., 2012). Many of the limitations experienced with mf detection were addressed with the introduction of the immunochromatographic card test (ICT) to detect circulating filarial antigen (CFA) (Weil et al., 1997). Importantly, the ICT could be conducted with blood collected any time of the day, eliminating the need for night blood collections. Based on results of a multi-country comparison, the ICT was the diagnostic tool recommended for TAS (Gass et al., 2012). Currently, the recently introduced Filariasis Test Strip (FTS) (Weil et al., 2013) is the official diagnostic tool recommended for TAS. As production of the ICT is being phased out, it is acceptable for LF programs to use either the ICT or FTS in the interim.

Although tools to detect CFA have been and continue to be useful for the global LF program, there are some limitations to their use. Similar to the observed decline in mf prevalence after treatment, antigenemia also begins to decline and becomes increasingly difficult to detect in populations that have been subjected to multiple rounds of MDA (Gass et al., 2012). Additionally, as infection prevalence declines, the prevalence and magnitude of serologic responses shifts and operational sensitivity of the assays will decline compared to the lab-defined sensitivity. Recent evidence suggests that detection of antifilarial antibodies provides the earliest indicator of filarial exposure (Hamlin et al., 2012), and the absence of detectable antibody responses may provide evidence that transmission has been interrupted. As control programs move ahead, there will be fewer infection-specific antibody responses in populations, and increasingly, only residual antibody responses in older individuals will be observed. Many of the currently available LF antibody tests have been shown to be sensitive measures of exposure and infection but may lack the specificity needed to make important

programmatic decisions (Muck et al., 2003; Lammie et al., 2004; Weil et al., 2011). However, the identification of a highly specific recombinant antigen, Wb123 (Kubofcik et al., 2012), as an early serologic marker for filarial infection provides a new surveillance tool (Steel et al., 2013) that may be useful to confirm interruption of LF transmission.

In the 1950s, mf prevalence in The Gambia was reported to be approximately 50% (McFadzean, 1954), among the highest in the world. Surveys conducted in the 1970s showed that mf prevalence had declined significantly in the absence of any LF-specific interventions (Knight, 1980). In 2001, stored serum samples from individuals living in historically highly LF endemic areas were tested for the presence of CFA, a more sensitive diagnostic marker than mf (Gass et al., 2012), and results indicated an even further decline in LF prevalence compared to results from the 1970s (Rebollo et al., 2015). In 2003, the Gambian Ministry of Health and Social Welfare (MOHSW) carried out a national LF mapping survey with the intent of identifying areas in need of MDA. Interestingly, the results indicated MDA was not necessary (Rebollo et al., 2015). In 2013, the MOHSW conducted TAS and found no evidence of LF transmission among young children (Rebollo et al., 2015). Although The Gambia has not followed the traditional approach for LF elimination, it appears that current criteria used as the operational definition of elimination have been achieved. The absence of antigenemia among children was likely an indicator of interrupted transmission, but there was no information collected from older age groups. In the absence of LF-specific interventions, the Gambian MOHSW felt it was important to assess LF status among older age groups to complement the TAS results. The current study aimed to assess antifilarial antibody levels among communities living in historically highly LF endemic areas of The Gambia, and to use serologic tools to determine whether or not interruption of LF transmission has been achieved.

6.3 Methods

6.3.1 Study site and design

The study took place in February 2015 in The Gambia, a small African country with the Atlantic Ocean to the west and all of its land borders shared with Senegal. It is the smallest country on the mainland of the continent and has an estimated population of 1.9 million people. The country is divided into five divisions (Central River, Lower River, North Bank, Upper River, and Western) and one city (Banjul). Fifteen villages (Dampha Kunda, Jappineh, Jambanjelly, Jiboro Koto, Kafuta, Kamanka, Kembujeh, Keneba, Kololi, Latrikunda Sabiji, Mandinaring, Marakissa, Sare Opatah Jawa/Dar Silameh, Sikon Batabu Kantora, and Tambasansang) with the highest historic evidence of LF were purposely selected for this study. The villages were located in four of the five divisions and in Banjul. No villages in the Central River Division were included in the study since LF prevalence in this area was low in the 1970s, and no evidence of infection was found in recent surveys. Following World Health Organization (WHO) guidance for monitoring LF in sentinel sites,(WHO, 2011a) a convenience sample of approximately 300 individuals (≥1 year old) in each village was enrolled in the study.

6.3.2 Ethical considerations

The study was approved by the Gambia Government/MRC Joint Ethics Committee. The Institutional Review Board of the U.S. Centers for Disease Control and Prevention (CDC) determined CDC to be a non-engaged research partner. Study details were explained to potential participants and written informed consent was obtained from persons who agreed to participate. Parents or guardians provided permission for participation of children <18 years of age. Additionally, children aged between 7 and 17 years were asked to provide verbal assent for their participation. All identifiable information was kept confidential and maintained by using a secure database with access restricted to essential study personnel.

6.3.3 Data collection

On the day of sample collection, residents of the community were asked to come to a central location within the village. Upon obtaining informed consent, participants were assigned a unique identifier and asked to provide basic demographic information such as age and sex. All data were collected on Android-platform smartphones (BLU; Miami, FL) using the LINKS application (Pavluck et al., 2014) and uploaded to a secure SQL server.

6.3.4 Blood collection and antigen testing

Approximately 100 µL of blood was collected via finger stick and used for the detection of CFA by ICT (Alere; Scarborough, ME). The cards were read at 10 min and marked as either positive or negative according to the manufacturer's instructions. An additional 60 µL of blood (10 µL per extension x 6 extensions) was collected onto filter paper (Cellabs; Sydney, Australia), dried and stored for antifilarial antibody testing. The dried blood spots (DBS) were stored at -20^{o}C until shipped to CDC for testing.

6.3.5 Antibody testing by enzyme-linked immunosorbent assay (ELISA)

DBS were tested with the Filaria Detect^{TM} IgG4 ELISA (InBios; Seattle, WA), a direct enzyme immunoassay that detects IgG4 antibodies to the recombinant Wb123 antigen. This test was performed according to the protocol provided by the manufacturer with minor modifications. Briefly, one blood spot extension (10 µL whole blood) was eluted overnight in 250 µL of sample dilution buffer provided in the kit to yield an approximate 1:50 serum dilution. The following day, samples were tested in duplicate by adding 100 µL of eluate to each well. Kit-provided positive and negative controls were also tested in duplicate at a 1:50 dilution. Additionally, two internal positive controls (H3, H19; not provided in the kit) available at CDC were tested in duplicate at a 1:1500 (H3) and 1:900 (H19) dilution on each plate. These internal controls were used to standardize results across plates. Plates were incubated at 37°C for 30 min and then washed with kit-provided wash buffer. Mouse anti-human IgG4 conjugated with horseradish peroxidase (HRP) was added to each well at a 1:100 dilution and incubated at 37°C for 30 min. After washing, 100 µL of tetramethylbenzidine (TMB) was

added to each well and plates were developed at room temperature in the dark for 9 min. Reactions were stopped by adding 50 µL of kit provided stop solution to each well, and plates were read at 450 nm. To compare optical density (OD) values between Wb123 ELISA plates, mean OD values for each sample were divided by the mean OD of the H3 positive control to normalize results.

All available samples from two villages (Kololi and Tambansansang) were tested with a non-commercial ELISA (CDC; Atlanta, GA) for IgG4 antibodies against the recombinant Bm14 antigen (Chandrashekar et al., 1994). This assay has relatively high sensitivity (92%) and specificity (99%) and was determined to be an appropriate alternative to a commercially available Bm14 ELISA. Greiner Microlon High Binding plates (Greiner Bio-One; Monroe, NC) were coated with Bm14 at a concentration of 0.3 µg/mL in antigen sensitizing buffer (0.5 M Tris/HCl, pH 8.0 + 0.3 M KCl + 2 mM EDTA) and incubated overnight at 4°C. One blood spot extension was added to 250 µL of dilution buffer (PBS pH 7.2 + 0.3% Tween20 + 5% milk) and incubated overnight at 4°C. Positive control serum samples were diluted 1:50 in dilution buffer and used to construct a 9-point standard curve and to serve as two calibrator positive controls for each plate. All controls were held at 4°C overnight. The following day, diluted samples and controls were tested in duplicate by adding an aliquot of 100 µL to each well. Plates were incubated at room temperature on a shaker for 30 min. Mouse anti-human IgG4 conjugated with HRP (cloneHP6025; Southern Biotech; Birmingham, AL) was diluted 1:2000 and 100 µL was added to each well. Plates were incubated at room temperature with shaking for 30 min. TMB (100 µL) was added to each well and plates were developed at room temperature for 2 min. One hundred microliters of 1 M H_2SO_4 was added to each well to stop the reaction, and plates were immediately read at 450 nm. Plates were washed between each step with PBS + 0.3% Tween20.

6.3.6 Cutoff determination for ELISAs

Cutoff values for Wb123 and Bm14 were calculated at CDC from receiver operator characteristic (ROC) curves using sera from *W. bancrofti* mf positive patients and presumed negative sera from adult US citizens with no history of foreign travel to LF-endemic countries.

6.3.7 Statistical analysis

Statistical analyses were performed in Stata version 14.1 (StataCorp LP; College Station, TX) and used the 5% level of significance. Chi-squared tests and logistic regression were used to identify associations between seropositivity and other factors.

6.4 Results

A total of 4,481 individuals (aged 1-100 years) from the 15 villages were enrolled in the study. Of those enrolled, a total of 2,612 (58.2%) DBS from all 15 villages were tested for antibodies to Wb123. There was no difference in age or sex between individuals not included for serologic testing and individuals with antibody results. Demographic information was not available for 161 (6.2%) samples with antibody results. Antibody prevalence for individuals with missing demographic data was not different than prevalence for those with available demographic information. There were no individuals who were antigen positive by ICT. Overall, prevalence of positive Wb123 responses was low (1.5%, 95% confidence interval (CI) 1.1-2.1%). In 7 of 15 villages (46.7%), there were no antibody positive individuals identified. Of the eight villages with at least one person with a positive Wb123 result, six (75%) were located in the Western Division (Figure 6.1). Individuals with positive responses to Wb123 ranged in age from 3 to 100 years. Wb123 results by community are summarized in Table 6.1. There was no statistically significant difference in Wb123 prevalence among study villages once adjusted for age, sex, and clustering by village.

Figure 6.1 Location of the 15 study villages in The Gambia and Wb123 antibody status in 2015.

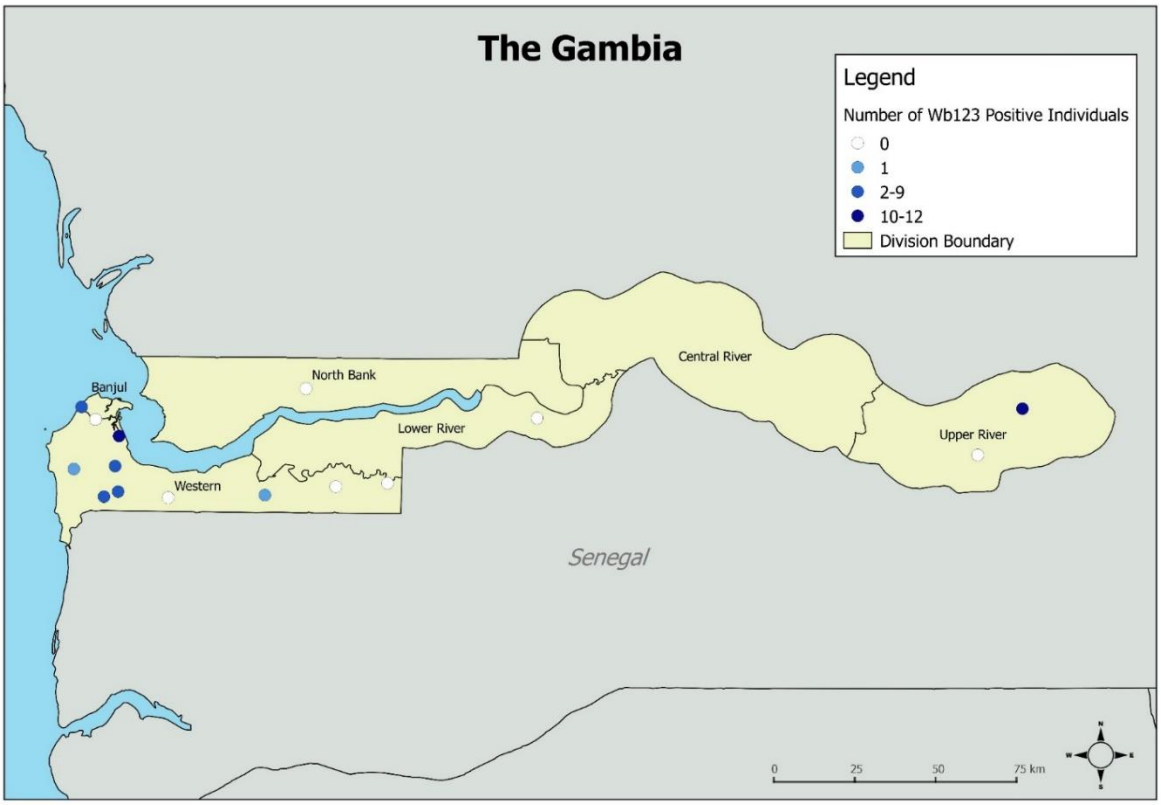

Table 6.1 Wb123 antibody prevalence by community in The Gambia in 2015.

Division	Community	Total enrolled	Total tested	Median age in years (range)	Wb123 positive	% positive [95% CI]
Banjul	Kololi	335	307	10 (1-70)	2	0.7 [0.1, 2.3]
Banjul	Latrikunda Sabiji	305	124	17 (1-70)	0	0.0 [0, 2.9*]
Lower River	Jappineh	308	123	10 (1-80)	0	0.0 [0, 3.0*]
Lower River	Keneba	305	124	10 (1-80)	0	0.0 [0, 2.9*]
North Bank	Sare Opatah Jawa/Darsilameh	309	118	11 (1-90)	0	0.0 [0, 3.1]
Upper River	Dampha Kunda	342	124	8 (1-89)	0	0.0 [0, 2.9*]
Upper River	Tambasansang	294	292	12 (1-100)	10	3.4 [1.7, 6.2]
Western	Jambanjelly	235	90	11 (1-70)	1	1.1 [0, 6.0]
Western	Jiboro Koto	231	90	11 (1-70)	3	3.3 [0.7, 9.4]
Western	Kafuta	305	124	9.5 (1-70)	0	0.0 [0, 2.9]
Western	Kamanka	305	123	12 (2-80)	0	0.0 [0, 3.0]
Western	Kembujeh	303	125	10 (1-80)	4	3.2 [0.9, 8.0]
Western	Mandinaring	299	296	10.5 (1-100)	12	4.1 [2.1, 7.0]
Western	Marakissa	302	275	6 (1-90)	4	1.5 [0.4, 3.7]
Western	Sikon Batabu Kantora	303	116	13 (1-90)	1	0.9 [0, 4.7]
#N/A (missing demographic data)			161	N/A	3	1.9 [0.4, 5.3]
	TOTAL	**4,481**	**2,612**	**11 (1-100)**	**40**	**1.5 [1.1, 2.1]**

*one-sided, 97.5% confidence interval

All available samples from Kololi and Tambasansang were also tested by Bm14 ELISA. Overall, Bm14 prevalence was low (1.5%, 95% CI 0.7-2.8%) in these two villages. In Kololi, there were two Wb123 positive individuals, but there were no positive Bm14 responses in this community. In Tambasansang, there were 10 (3.4%) Wb123 positive individuals ranging in age from 4 to 65 years. Although a similar number of Bm14 positive persons was identified (9/292; 3.1%), all Bm14-positive individuals were over the age of 50 years. Bm14 positivity was significantly associated with older age ($p<0.001$). The results of antibody testing and historic mf results are summarized in Table 6.2.

Table 6.2 Microfilariae prevalence in selected villages of The Gambia in the 1970s (Knight, 1980) and antifilarial responses to Wb123 and Bm14 in the same villages in 2015.

	Kololi					Tambasansang				
	1974-1976		2015			1974-1976		2015		
Age	n	Mf [95% CI]	n	Wb123 [95% CI]	Bm14 [95% CI)	n	Mf [95% CI]	n	Wb123 [95% CI]	Bm14 [95% CI]
0-5	n/a		52	0.0 [0, 6.8*]	0.0 [0, 6.8*]	n/a		61	3.3 [0.4, 11.3]	0.0 [0, 5.9*]
6-15	44	22.7 [11.5, 37.8]	16 9	1.2 [1.4, 4.2]	0.0 [0, 2.2]	10 7	17.8 [11.0, 26.3]	12 1	2.5 [0.5, 7.1]	0.0 [0, 3.0]
16+	38	26.3 [13.4, 43.1]	86	0.0 [0, 4.2*]	0.0 [0, 4.2]	66	25.8 [15.8, 38.0]	11 0	4.5 [1.5, 10.3]	8.2 [3.8, 15.0]

* one-sided, 97.5% confidence interval

6.5 Discussion

The results of TAS conducted in 2013 in The Gambia indicated there was no LF transmission among 6- and 7-year-old children and, in 2016, The Gambia was removed from WHO's official list of LF endemic countries (WHO, 2016a). Although the absence of antigenemia among children in The Gambia was likely an indicator of interrupted transmission, there was no information collected from older age groups. Our study aimed to assess antifilarial antibody levels among populations living in historically highly LF endemic areas in The Gambia, and to evaluate the use of serologic tools to confirm the absence of LF transmission.

Overall, no antigenemia was detected, and the prevalence of antibodies to Wb123 was low in the 15 villages included in the survey conducted in 2015. Although there was clear evidence of LF transmission in the 1970s (Knight, 1980), results from the current survey suggest that little to no transmission of LF was occurring in these areas, consistent with the results of TAS implemented in the Gambia in 2013. The dramatic decline in LF prevalence over a 50-year period has been observed with increasingly sensitive diagnostic markers, and strongly suggests that sustainable LF transmission likely ceased in The Gambia during this period. The decrease in LF prevalence has been mainly attributed to a reduction in mosquito density due to changes in climate, improved standard of living, and the use of bednets for protection from mosquito bites since no LF-specific interventions have taken place (Knight, 1980). The rapid scale-up of insecticide-treated nets (ITNs) used for malaria control since 2000 has likely further contributed to the decline (Ceesay et al., 2008; Noor et al., 2009). Antibody responses are generally considered an early and sensitive indicator of transmission, and while there is incomplete information about the duration of antibody responses, they do appear to fall as transmission declines. In a study conducted in the Cook Islands, samples collected in the mid-1970s were analyzed for antibodies to Wb123 and compared to results from samples collected from the same island in 1992, five years after a single round of MDA against LF. Results indicated a significant decrease in Wb123 antibody

positivity, suggesting LF transmission had significantly decreased (Steel et al., 2012). Recently, in Indonesia, antibody responses were used to successfully distinguish areas where programs had been implemented and successful, sub-optimally implemented, and not implemented at all (Dewi et al., 2015). Although there is a need to gain more practical experience to operationalize the use of antibody assays, including determining the most appropriate diagnostic platform (e.g. rapid test, ELISA), our results have added information on the utility of serologic tools in an area where MDA was never conducted.

Although seroprevalence was low, positive responses were not completely absent. Antibody responses could represent very focal areas of persistent or recurrent LF transmission, residual seropositivity following interruption of transmission, cross-reactivity, or false-positive results; the detected Wb123 responses may have different implications than the detected responses to Bm14. Approximately half of the villages had at least one positive Wb123 response. Six of eight villages with at least one antibody positive individual were located in the Western Division, where mf rates were in excess of 50% in the 1950s (McFadzean, 1954). However, the absence of positive antigen tests in these village makes it less likely that these results reflect focal transmission.

If positive serology reflected persistence following interruption of transmission, an association with age would be expected (Mladonicky et al., 2009; Gass et al., 2012; Shawa et al., 2013). Antibodies to Bm14 can persist for years, but the expectation is that seroreversion will occur at some point (Ramzy et al., 2006; Moss et al., 2011). However, currently, there is insufficient data available on the rates of antibody decay to accurately predict when filarial infection cleared. An association between seropositivity and age was seen for the responses to Bm14, but not to Wb123; in Tambasansang, Bm14 positive individuals were all older than 50 years of age, and could have been exposed to infected mosquitoes before transmission had ceased in the country.

The recombinant Bm14 antigen has also been reported as a highly sensitive marker for the assessment of filarial antibodies, but it is also known to cross react with closely

related filarial parasites (Lammie et al., 2004; Weil et al., 2011). Wb123, used on various diagnostic platforms including ELISAs, is reported to have high sensitivity and specificity for distinguishing *W. bancrofti* infection from closely related filarial infections (Kubofcik et al., 2012). However, a possible explanation for the detected positive Wb123 responses is lower than expected Wb123 specificity. It is possible that the cutoff values for the ELISAs were inaccurate. The ability to define robust cutoffs for serological assays can be challenging and is often limited by the availability of well characterized panels of samples to determine appropriate cutoffs.

As the GPELF continues to make progress, it is critical to identify strategies for reaching stated goals. Our results strongly suggest that LF transmission has likely ceased in The Gambia and that no programmatic intervention is required. Although there is a clear need to better understand the limitations of current antibody tests, to develop appropriate sampling strategies, and to determine optimal age groups to define antibody thresholds in order to provide robust evidence of the absence of transmission, our results also support the use of antibody tools to determine the status of LF transmission and suggest that serologic tools can have a role in guiding programmatic decision making.

6.6 Acknowledgments

The authors are grateful to Caitlin Worrell at CDC for creating the Gambia map. We would like to thank the field workers for their effort and dedication to the study activities. We are especially grateful to the residents of the villages for their participation and cooperation throughout the study. The study was funded by the Bill & Melinda Gates Foundation (OPP1053230).

7 Lymphatic filariasis elimination in American Samoa: evaluation of molecular xenomonitoring as a surveillance tool in the endgame

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[This manuscript has been published in *PLoS Neglected Tropical Diseases* (2016) 1;10(11) e0005108 https://doi.org/10.1371/journal.pntd.0005108]

7.1 Abstract

The Global Programme to Eliminate Lymphatic Filariasis has made significant progress toward interrupting transmission of lymphatic filariasis (LF) through mass drug administration (MDA). Operational challenges in defining endpoints of elimination programs include the need to determine appropriate post-MDA surveillance strategies. As humans are the only reservoirs of LF parasites, one such strategy is molecular xenomonitoring (MX), the detection of filarial DNA in mosquitoes using molecular methods (PCR), to provide an indirect indicator of infected persons nearby. MX could potentially be used to evaluate program success, provide support for decisions to stop MDA, and conduct post-MDA surveillance. American Samoa has successfully completed MDA and passed WHO recommended transmission assessment surveys in 2011 and 2015, but recent studies using spatial analysis of antigen (Ag) and antibody (Ab) prevalence in adults (aged ≥ 18 years) and entomological surveys showed evidence of possible ongoing transmission. This study evaluated MX as a surveillance tool in American Samoa by linking village-level results of published human and mosquito studies. Of 32 villages, seropositive persons for Og4C3 Ag were identified in 11 (34.4%), for Wb123 Ab in 18 (56.3%) and for Bm14 Ab in 27 (84.4%) of villages. Village-level seroprevalence ranged from 0–33%, 0–67% and 0–100% for Og4C3 Ag, Wb123 Ab and Bm14 Ab respectively. PCR-positive *Aedes polynesiensis* mosquitoes were found in 15 (47%) villages, and their presence was significantly associated with seropositive persons for Og4C3 Ag (67% vs 6%, $p<0.001$) and Wb123 Ab (87% vs 29%, $p = 0.001$), but not Bm14 Ab. In villages with persons seropositive for Og4C3 Ag and Wb123 Ab, PCR- positive *Ae*. *polynesiensis* were found in 90.9% and 72.2% respectively. In villages without seropositive persons for Og4C3 Ag or Wb123 Ab, PCR-positive *Ae*. *polynesiensis* were also absent in 94.1% and 70.6% of villages respectively. Our study provides promising evidence to support the potential usefulness of MX in post-MDA surveillance in an *Aedes* transmission area in the Pacific Islands setting.

7.2 Author summary

Lymphatic filariasis (LF) is caused by infection with filarial worms that are transmitted by mosquito bites. Globally, 36 million are disfigured and disabled by complications such as severe swelling of the legs (elephantiasis) or scrotum (hydrocele). The Global Programme to Eliminate LF (GPELF) aims to interrupt disease transmission through mass drug administration (MDA), and to control illness and suffering in affected persons. Significant progress has been made toward eliminating LF from many parts of the world, including the Pacific Islands. Current challenges of the GPELF include identification of any residual hotspots of ongoing transmission, and effective strategies for early identification of any resurgence of infections. As humans are the only reservoirs of LF parasites and mosquitoes have short flight ranges, one such strategy is to monitor LF infection in mosquitoes as an indicator of ongoing transmission nearby. Mosquito monitoring could potentially be used to evaluate program success, provide support for decisions to stop MDA, and conduct post-MDA surveillance. Our study evaluated mosquito monitoring as a surveillance tool in American Samoa by linking village-level results of published studies of LF in humans and mosquitoes, and provides promising evidence to support the potential usefulness of mosquito monitoring in post-MDA surveillance the Pacific Islands.

7.3 Introduction

Lymphatic filariasis (LF) is a parasitic infection caused by *Wuchereria* or *Brugia* species of helminth worms, and transmitted by mosquito vectors including *Aedes*, *Anopheles*, *Culex* and *Mansonia* species. Globally, an estimated 68 million people are currently affected, including 36 million microfilaraemic persons and 36 million who are disabled or disfigured with complica- tions such as severe lymphoedema, including elephantiasis and scrotal hydrocoeles (Ramaiah and Ottesen, 2014). The Global Programme to Eliminate LF (GPELF) aims to eliminate the disease as a public health problem by 2020 using two main strategies: i) to interrupt transmission through mass drug administration (MDA) and ii) to control morbidity and disability of affected persons. In the Pacific Islands, the Pacific Programme to Eliminate LF (PacELF) was formed in 1999 as part of GPELF to focus on 22 Pacific Island Countries and Territories (PICTs), which include >3000 islands and 8.6 million people (Ichimori and Crump, 2005).

The sustained success of elimination programs requires cost-effective assessment and monitoring after successful completion of MDA to determine whether there are any residual foci of infection, and to detect potential resurgence in a timely manner. The WHO currently recommends post-MDA surveillance using transmission assessment surveys (TAS), which use critical cut-off values of numbers of antigen-positive children aged 6–7 years to determine whether transmission has been interrupted in defined evaluation units (WHO, 2011a). In *Brugia* transmission areas, antibody positivity is used for TAS. Cut-off thresholds for passing TAS vary depending on population size of the target group and the local species of filarial parasites and mosquito vectors. For example, where *W*. *bancrofti* is endemic, the target cut-off value is estimated based on upper 95% confidence interval of <1% antigen prevalence if *Aedes* is the principal vector, or <2% antigen prevalence if *Anopheles* or *Culex* predominate. TAS typically involve school-based or community-based testing of 6–7 year old children. Community-based surveys are often logistically challenging, particularly in developing countries with limited financial and human resources. In some areas (e.g. most of

the Pacific Islands), difficult access to dispersed populations in remote islands provides additional challenges. Also, TAS typically use rapid antigen detection tests (Filarial Immunochromatographic Test (ICT) cards or Filarial Test Strips (Weil et al., 2013)), which might have reduced sensitivity after many rounds of MDA (Njenga et al., 2008; Gounoue-Kamkumo et al., 2015). TAS has been widely used to inform important programmatic decisions including stopping or restarting MDA, but recent studies suggest that in some settings (including American Samoa), TAS might not be sufficiently sensitive for determining whether transmission has been interrupted (Lau et al., 2014a; Rao et al., 2014).

As elimination programs reach the endgame phases and antigen prevalence drops to very low levels, increasingly sensitive tools and strategies will be required to efficiently detect any evidence of ongoing transmission or resurgence. The WHO and GPELF have identified a number of operational challenges and unanswered questions for elimination programs, including the significance of residual microfilaraemia and antigenaemia in communities where the target threshold level has been achieved through MDA, identification of residual high-prevalence areas and strategies for managing them, and the need for development of cost-effective strategies for post-MDA surveillance (WHO, 2016b). As humans are the only reservoir for *W*. *bancrofti* parasites, one such strategy is to monitor mosquitoes for evidence of LF larval stages (Bockarie, 2007; Mladonicky et al., 2009). Dissection and microscopic examination of mosquitoes is time consuming and labor-intensive, and cannot be routinely recommended for post-MDA surveillance for practical reasons. With recent technological advances, LF molecular xenomonitoring (MX), the use of molecular methods (PCR) to detect filarial DNA in mosquitoes, has been explored and promising results are emerging (Farid et al., 2007; Pedersen et al., 2009). PCR-positive mosquitoes provide an indirect indicator of the presence of infected humans and possible ongoing transmission (Williams et al., 2002; Goodman et al., 2003; Plichart et al., 2006; Bockarie, 2007). For example, considering that the flight ranges of *Ae*. *polynesiensis* mosquitoes are on the order of a hundred meters (Jachowski, 1954), detection of PCR-positive mosquitoes in areas where these are the main

vectors indicates that infected persons are or were recently nearby. Molecular methods are also more sensitive than manual dissection for detecting infections (Chambers et al., 2009). Studies have reported the ability of PCR to detect one microfilaria in pools of 50–100 mosquitoes (Nicolas et al., 1996), and for at least 2 weeks after mosquitoes (both vector and non-vector) ingest microfilaria-positive blood (Fischer et al., 2007), which is close to the average life span of most mosquito species.

Molecular xenomonitoring has been found to be a potentially useful indicator of human LF infections with different species of mosquito vectors in diverse settings including American Samoa (Mladonicky et al., 2009), French Polynesia (Plichart et al., 2006), Egypt (Farid et al., 2001; 2007), Sri Lanka (Rao et al., 2014), Sierra Leone (de Souza et al., 2015), and Ghana (Owusu et al., 2015). Molecular xenomonitoring is therefore potentially useful for evaluating the success of elimination programs, providing support for decisions to stop MDA, and conducting ongoing post-MDA surveillance (Weil and Ramzy, 2007). Compared to TAS, MX has the advantages of being non-invasive to humans, and potentially more cost-effective in some settings. However, MX requires entomological expertise for trapping and processing mosquitoes, and laboratories capable of conducting large scale molecular diagnostics. In addition, there are currently unanswered questions about sampling strategies, limited evidence to inform the translation of MX results into operational strategies, and no clear guidelines on the thresholds of DNA prevalence that should be used to indicate likely ongoing transmission. Cut-off points of 0.25%, 0.5%, and 1% have been suggested for *Culex* areas (Michael et al., 2006; Farid et al., 2007; Rao et al., 2014), and 0.085% for L3 and 0.65% for any larval stage for *Anopheles* areas (Pedersen et al., 2009). There are currently no clear recommendations for *Aedes* areas, but a provisional threshold of <0.1% has been suggested (WHO, 2006b). The lower the estimated cut-off points, the larger the sample sizes of mosquitoes that will be required for MX.

As part of the PacELF, American Samoa has made significant progress toward reducing LF infection rates. After seven rounds of MDA from 2000 to 2006, antigen

prevalence in humans dropped from 16.5% (N = 3018) in the 1999 baseline assessment to 2.3% (N = 1881) in 2007 in a community cluster survey (DoPTS, 2013). American Samoa passed TAS in 2011–2012 and again in 2015, but recently published studies using spatial analysis of antigen prevalence in adults (Lau et al., 2014a) and molecular xenomonitoring (Schmaedick et al., 2014) showed evidence of possible ongoing transmission. By linking the results of the published human and mosquito studies, we aim to evaluate MX as a surveillance tool in the post-MDA setting in American Samoa, an *Aedes* transmission area in the Pacific Islands.

7.4 Methods

7.4.1 Study location and setting

American Samoa is a US Territory in the South Pacific, consisting of a group of small tropical islands with a total population of 56,000 (Commerce, 2012) living in 67 villages. Over 90% of the population live in small villages on the main island of Tutuila, and the remainder on the adjacent island of Aunu'u and the remote Manu'a group of islands (Ta'u, Ofu, and Olosega). *W*. *bancrofti* is the only species of human filarial worm known to be present in American Samoa. The main vector is the highly efficient day-biting *Ae*. *polynesiensis*, and other vectors include *Ae*. *samoanus* (night-biting), *Ae*. *tutuilae* (night-biting), and *Ae*. *upolensis* (day-biting) (Ramalingam and Belkin, 1964; Ramalingam, 1968; Samarawickrema et al., 1987).

7.4.2 Human infection data

Data were obtained from a published study on the seroprevalence and spatial epidemiology of lymphatic filariasis in American Samoa (Lau et al., 2014a). The study used samples from a serum bank collected from May to August 2010 for a leptospirosis study; the study design has been published previously (Lau et al., 2012a; 2012b). Briefly, the study included 807 adults (aged 18 to 87 years, 52.4% males) from 659 households in 55 villages on all five inhabited islands of American Samoa. Sampling was designed to provide a representative sample of the adult population in American Samoa, in both age and geographic distribution. Using these 2010 samples, a seroprevalence study was conducted in 2013 (Lau et al.,

2014a), and found that 3.2% were seropositive for Og4C3 Ag, and 8.1% and 17.9% were seropositive for Wb123 Ab and Bm14 Ab, respectively (Lau et al., 2014a). The study also found significant spatial clustering of Ag-positive persons; average cluster size was 1,498m in diameter for those with Og4C3 Ag >32 units, and the proportion of the variation explained by geographic proximity was 62%. Higher infection rates were found in males and recent migrants to American Samoa. Antigen (Og4C3) positivity indicates the presence of adult worm antigen but does not provide information on the viability, e.g. the worm could be alive or dead, or there could be a single sex worm infection or sterile worm infection. The presence of Og4C3 Ag represents current or recent infection. The presence of antibodies represents current or past infection, possibly many years in the past.

For our study, human data were summarized by village and the following variables were generated:

- Total number of people sampled from each village
- Village-level seroprevalence (point estimates and 95% CI) for Og4C3 Ag, Wb123 Ab and Bm14 Ab,
- Seropositive village for Og4C3 Ag, Wb123 Ab, and Bm14 Ab (defined as villages with at least one seropositive person)

A village-level summary of the human serological data is provided in Table S 7.1.

7.4.3 Molecular xenomonitoring data

Schmaedick et al conducted a MX study in American Samoa from February to June 2011, approximately 9 months after the above human serum specimens described above were collected. Detailed descriptions of the study and its findings have been published (Schmaedick et al., 2014), and a village-level summary is provided in Table S 7.1. Briefly, mosquitoes were collected from 34 randomly selected villages on the island of Tutuila, the only village on Aunu'u, all five villages on the Manu'a Islands, and the village of Ili'ili (on Tutuila) where two ICT-positive children were identified during the 2011 TAS. Up to 10 traps

were placed in each village for 24 to 48 hours, and mosquitoes were removed from traps twice daily. The study collected a total of 22,014 female mosquitoes of *Aedes* and *Culex* genera that were sorted into 2,629 pools of 20 mosquitoes (range 1 to 20) for PCR testing. Real-time PCR was conducted using primers designed to amplify a fragment of *W*. *bancrofti* (Rao et al., 2006a). A positive PCR result indicates the presence of filarial worm DNA, but does not provide any information on whether the worms are alive or transmissible. Each pool included only one mosquito species, except for the *Ae*. *(Finlaya)* group of species (*Ae*. *oceanicus*, *Ae*. *samoanus*, and *Ae*. *tutuilae*) which were combined for PCR testing because of morphological similarities. The MX study calculated maximum likelihood point estimates of the prevalence of PCR-positive *Ae*. *polynesiensis* for each village or village group using PoolScreen software (version 2.0.3), which takes into account the average number of mosquitoes per pool and the proportion of pools that were PCR-positive. Point estimates of village-level prevalence of PCR-positive *Ae*. *polynesiensis* ranged from 0% to 2.8% for villages on Tutuila and Aunu'u, and was 0% for all villages in the Manu'a islands. The findings indicated widespread presence of filarial DNA in the mosquito population, suggesting persistent low-level transmission of LF on Tutuila and Aunu'u.

For our study, mosquito data were summarized for each village for i) *Ae*. *polynesiensis*, and ii) other mosquito species (all species apart from *Ae*. *polynesiensis*), and iii) any mosquito species. Entomological data available by village included number of traps used; number of females and pools of each mosquito species; number of PCR-positive pools of each species; and estimated prevalence of PCR-positive *Ae*. *polynesiensis* (using PoolScreen software).

7.4.4 Ethical considerations

This study used de-identified data from the two previously published studies described above (Lau et al., 2014a; Schmaedick et al., 2014). The human study only included adults, and written informed consent was obtained from each participant. The American Samoa

Institutional Review Board (IRB) provided approval for the use of the human serum bank for lymphatic filariasis research.

7.4.5 Data analysis

In the MX study, some small adjacent villages were combined into groups of two to four villages for trapping and analyses, and human data were grouped accordingly to match the entomological data. Human data were not available for three of the villages included in the MX study. For this study, analyses were limited to the villages or village groups where both human data and MX data were available for 32 locations: 23 individual villages on Tutuila, 3 village groups (of two villages each) on Tutuila, the only village on Aunu'u, and all five villages on the Manu'a Islands. The 32 villages and village groups will be referred to as "villages" from here for ease of reference.

Chi-squared tests and logistic regression were used to identify associations between seropositive humans and PCR-positive mosquito pools, and answer the following operational questions:

- Is the presence of PCR-positive pools of *Ae*. *polynesiensis* in a village a useful indicator of a seropositive village? If so, how accurate are PCR-positive pools for predicting seropositive villages for Og4C3 Ag, Wb123 Ab, and Bm14 Ab?
- Is the presence of PCR-positive pools of *Ae. polynesiensis* a better indicator of seropositive villages than PCR-positive pools of other mosquito species, or PCR-positive pools of any mosquito species? In other words, do the time, effort, and expertise required to separate mosquitoes into species-specific pools improve the accuracy of the predictions?
- Is the estimated prevalence of PCR-positive *Ae. polynesiensis* (calculated by PoolScreen) a better indicator of the above measures? In other words, is it necessary to estimate prevalence using PoolScreen, or does the presence/absence of PCR-positive pools provide equally accurate predictions?

7.5 Results

Serological results from 376 persons residing in 32 villages were included in the analyses. The average number of persons per village was 13.9 (range 2–73) for Tutuila and Aunu'u, and 14.0 (range 11–16) for the Manu'a Islands.

Table 7.1 provides a summary of the number of seropositive persons and village-level seroprevalence for each serological marker in humans, and the entomological data used in this study. Of the 32 villages included in this study, 11 (34.4%) had residents who were seropositive for Og4C3 Ag, 18 (56.3%) for Wb123 Ab, and 27 (84.4%) for Bm14 Ab. On Tutuila and Aunu'u, village-level seroprevalence ranged from 0% to 33.3% for Og4C3 Ag, 0% to 66.7% for Wb123 Ab, and 0% to 100% for Bm14 Ab. In the Manu'a Islands, no individuals were seropositive for Og4C3 Ag, and village-level seroprevalence ranged from 0% to 18.8% for Wb123 Ab, and 13.3% to 27.3% for Bm14 Ab. On Tutuila and Aunu'u, the MX study identified PCR-positive pools of *Ae. polynesiensis* in 15 (55.6%) of the 27 villages included in this study, of other mosquito species in 7 (25.9%) villages, and of mosquitoes of any species in 17 (63.0%) of the villages. In the five villages on the Manu'a Islands, no PCR-positive pools of mosquitoes were identified during the MX study.

Table 7.1 Summary of human and entomological data from the 32 villages included in this study.

Islands	Total number of study villages in island group	Number of villages with persons sero-positive for Og4C3 Ag (%)	Number of villages with persons sero-positive for Wb123 Ab (%)	Number of villages with persons sero-positive for Bm14 Ab (%)	Mean village-level sero-prevalence of Og4C3 Ag (range)	Mean village-level sero-prevalence of Wb123 Ab (range)	Mean village-level sero-prevalence of Bm14 Ab (range)	Mean number of mosquito pools per village (range)	Villages with ≥1 PCR-positive pool of *Ae. polynesiensis* N (%)	Villages with ≥1 PCR-positive pool of other mosquito species N (%)	Villages with ≥1 PCR-positive pool of any mosquito species N (%)	Mean point estimates of prevalence of PCR-positive *Ae. polynesiensis* * (range)
Tutuila & Aunu'u	27	11 (40.7%)	15 (55.6%)	22 (81.5%)	4.8% (0–33.3%)	9.3% (0–66.7%)	18.4% (0–100%)	80.5 (12–133)	15 (55.6%)	7 (25.9%)	17 (63.0%)	0.5% (0–2.8%)
Manu'a	5	0 (0%)	3 (60.0%)	5 (100%)	0% (N/A)	8.6% (0–18.8%)	21.4% (13.3–27.3%)	84.0 (44–119)	0 (0%)	0 (0%)	0 (0%)	0% (0%)
Total	32	11 (34.4%)	18 (56.3%)	27 (84.4%)	4.0%	9.2%	18.8%	81.1	15 (46.9%)	7 (21.9%)	17 (53.1%)	0.4% (0–2.8%)

* Calculated using PoolScreen software in molecular xenomonitoring study (Schmaedick et al., 2014)

7.5.1 Association between PCR-positive mosquito pools and seropositive villages

Associations between the presence of PCR-positive pools of mosquitoes and seropositive villages and are shown in Table 7.2, with analyses stratified for i) *Ae. polynesiensis* only, ii) for all other mosquito species and iii) for mosquitoes of any species. Chi-squared tests of association showed that PCR-positive pools of *Ae. polynesiensis* ($p < 0.001$) and PCR-positive pools of any species ($p = 0.002$), but not pools of other species, were significantly associated with seropositive villages for Og4C3 Ag and Wb123 Ab, but not Bm14 Ab.

Figure 7.1 shows that the presence of at least one PCR-positive pool of *Ae. polynesiensis* or of any species was associated with a significantly higher probability of identifying a village with inhabitants seropositive for Og4C3 Ag ($p < 0.001$ and $p = 0.002$) and Wb123 Ab ($p = 0.001$ and $p = 0.002$). In the 15 villages with at least one PCR-positive pool of *Ae. polynesiensis*, 10 (67%) were seropositive for Og4C3 Ag and 13 (87%) were seropositive for Wb123 Ab, compared to 6% and 29% of villages respectively, where PCR-positive pools were not identified. Similarly, in the 17 villages where at least one PCR-positive pool of any species were identified, 11 (59%) had inhabitants who were seropositive for Og4C3 Ag and 14 (82%) with persons seropositive for Wb123 Ab, compared to 7% and 27% of villages respectively, with no PCR-positive pools. The presence of PCR-positive pools was not significantly associated with seropositivity for Bm14 Ab. PCR-positive pools of other mosquito species were not significantly associated with seropositive villages for any of the serological markers.

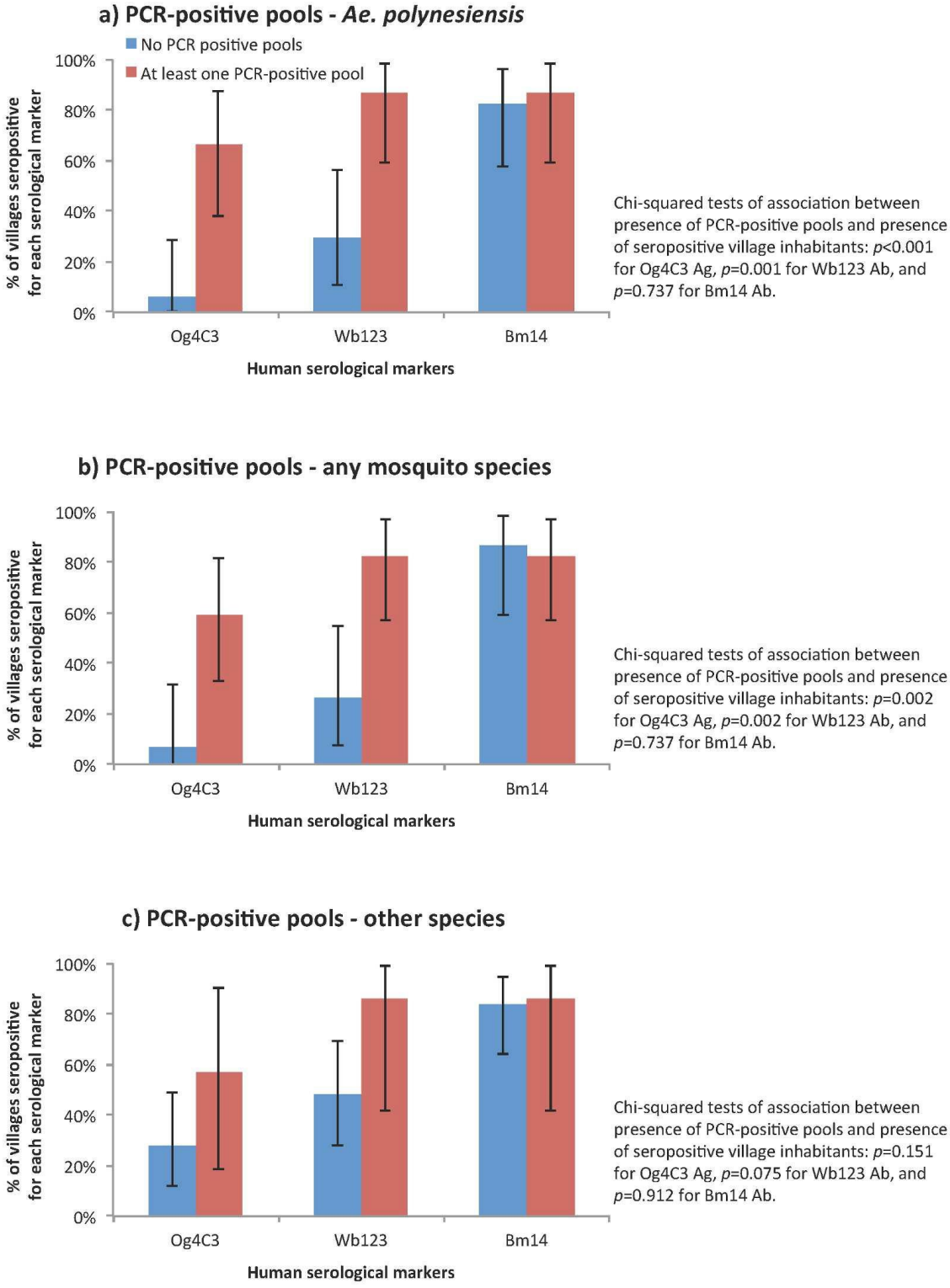
Table 7.2 Association between PCR-positive pools of mosquitoes and seropositive villages for Og4C3 Ag, Wb123 Ab, and Bm14 Ab.

Antigen/antibody status of villages[#]	**Number of villages (% of total)**	**Villages with PCR- positive pools of *Ae. polynesiensis***		**Villages with PCR-positive pools of other species**		**Villages with PCR- positive pools of any species**	
		N (%)	***p* value***	**N (%)**	***p* value***	**N (%)**	***p* value***
Total villages	32 (100%)	**15 (100%)**		**7 (100%)**		**17 (100%)**	
Seropositive for Og4C3 Ag	11 (34.4%)	10 (66.7)	**<0.001**	4 (57.1)	0.151	10 (58.8)	**0.002**
Seronegative for Og4C3 Ag	21 (65.6%)	5 (33.3)		3 (42.9)		7 (41.2)	
Seropositive for Wb123 Ab	18 (56.3%)	13 (86.7)	**0.001**	6 (85.7)	0.075	14 (82.4)	**0.002**
Seronegative for Wb123 Ab	14 (48.8%)	2 (13.3)		1 (14.3)		3 (17.6)	
Seropositive for Bm14 Ab	27 (84.4%)	13 (86.7)	0.737	6 (85.7)	0.912	14 (82.4)	0.737
Seronegative for Bm14 Ab	5 (15.6%)	2 (13.3)		1 (14.3)		3 (17.6)	

[#] A seropositive village is defined as a village with at least one seropositive person for the antigen or antibody. A seronegative village is defined as a village with no seropositive persons.

*Chi-squared tests comparison of villages with presence/absence of PCR-positive mosquito pools and presence/absence of seropositive persons. Statistically significant results highlighted in bold.

Figure 7.1 Probabilities of identifying seropositive villages for Og4C3 Ag, Wb123 Ab and Bm14 Ab based on the presence of PCR-positive pools of **a**) *Ae. polynesiensis*, **b**) any mosquito species, and **c**) other mosquito species.



7.5.2 Predicting the location of seropositive villages by using the presence of PCR-positive pools of mosquitoes

Table 7.3 provides a summary of the accuracy of PCR-positive mosquito pools for predicting seropositive villages for each antigen and antibody. PCR-positive pools of *Ae*. *polynesiensis* provide a sensitivity of 90.9% and specificity of 76.2% for identifying villages with seropositive persons for Og4C3 Ag, with a high negative predictive value of 94.1% (i.e. absence of PCR-positive pools was a good indicator of the absence of seropositive persons). PCR-positive pools of any mosquito species provide the same sensitivity (90.9%) but a lower specificity (66.7%), and a negative predictive value of 93.9%.

For Wb123 Ab, PCR-positive pools of *Ae*. *polynesiensis* provide a sensitivity of 72.2% and specificity of 85.7%, while PCR-positive pools of any mosquito species provide a sensitivity of 77.8% and specificity of 78.6% for identifying seropositive villages. For Bm14 Ab, PCR-positive pools of *Ae*. *polynesiensis* and any species had poor sensitivities (48.1% and 51.9%) and specificities (60.0% and 40.0%) for predicting seropositive villages. PCR-positive pools of *Ae*. *polynesiensis* or any mosquito species were statistically significant predictors of villages with residents seropositive for Og4C3 Ag (odds ratios of 32.0 and 20.0) and Wb123 Ab (odds ratios of 15.6 and 12.8), but not for Bm14 Ab. The correlation between PCR-positive pools of *Ae*. *polynesiensis* and seropositive villages for Og4C3 Ag and Wb123 Ab are shown for each village in Tutuila and Aunu'u in Figure 7.2, and the Manu'a Islands in Figure 7.3.

Table 7.3 PCR-positive pools of mosquitoes as predictors of villages with inhabitants seropositive for Og4C3 Ag, Wb123 Ab, and Bm14 Ab.

	Any PCR-positive pools	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Odds ratio* (95% CI)	*p* value*
a) Seropositive villages for Og4C3 Ag	*Ae. polynesiensis*	90.9%	76.2%	66.7%	94.1%	32.0 (3.2–315.3)	**0.003**
	Other mosquito species	36.4%	85.7%	57.1%	72.0%	3.43 (0.6–19.4)	0.163
	Any mosquito species	90.9%	66.7%	58.8%	93.3%	20.0 (2.1–189.2)	**0.009**
b) Seropositive villages for Wb123 Ab	*Ae. polynesiensis*	72.2%	85.7%	86.7%	70.6%	15.6 (2.5–96.1)	**0.003**
	Other mosquito species	33.3%	92.9%	85.7%	52.0%	6.5 (0.7–62.1)	0.104
	Any mosquito species	77.8%	78.6%	82.4%	73.3%	12.8 (2.4–69.7)	**0.003**
c) Seropositive villages for Bm14 Ab	*Ae. polynesiensis*	48.1%	60.0%	86.7%	17.6%	1.4 (0.2–9.7)	0.738
	Other mosquito species	22.2%	80.8%	85.7%	16.0%	1.1 (0.1–12.2)	0.912
	Any mosquito species	51.9%	40.0%	82.4%	13.3%	0.7 (0.1–5.0)	0.738

*Odds ratio of seropositive village if PCR-positive mosquitoes were identified (logistic regression), and associated *p* value (statistically significant results highlighted in bold).

Figure 7.2 Associations between PCR-positive pools of *Ae. polynesiensis* and seropositive villages for Og4C3 Ag and Wb123 Ab on Tutuila and Aunu'u.

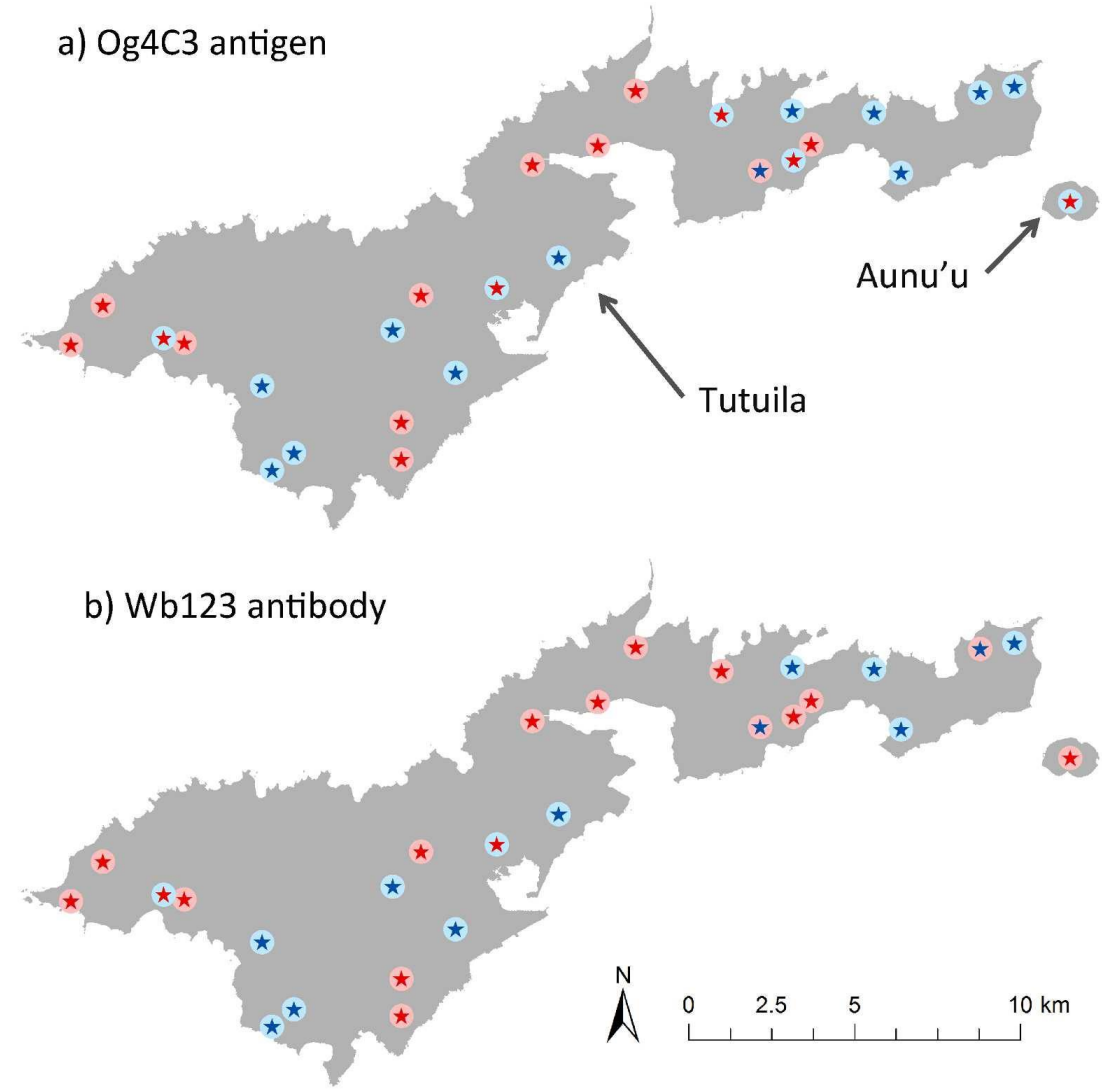
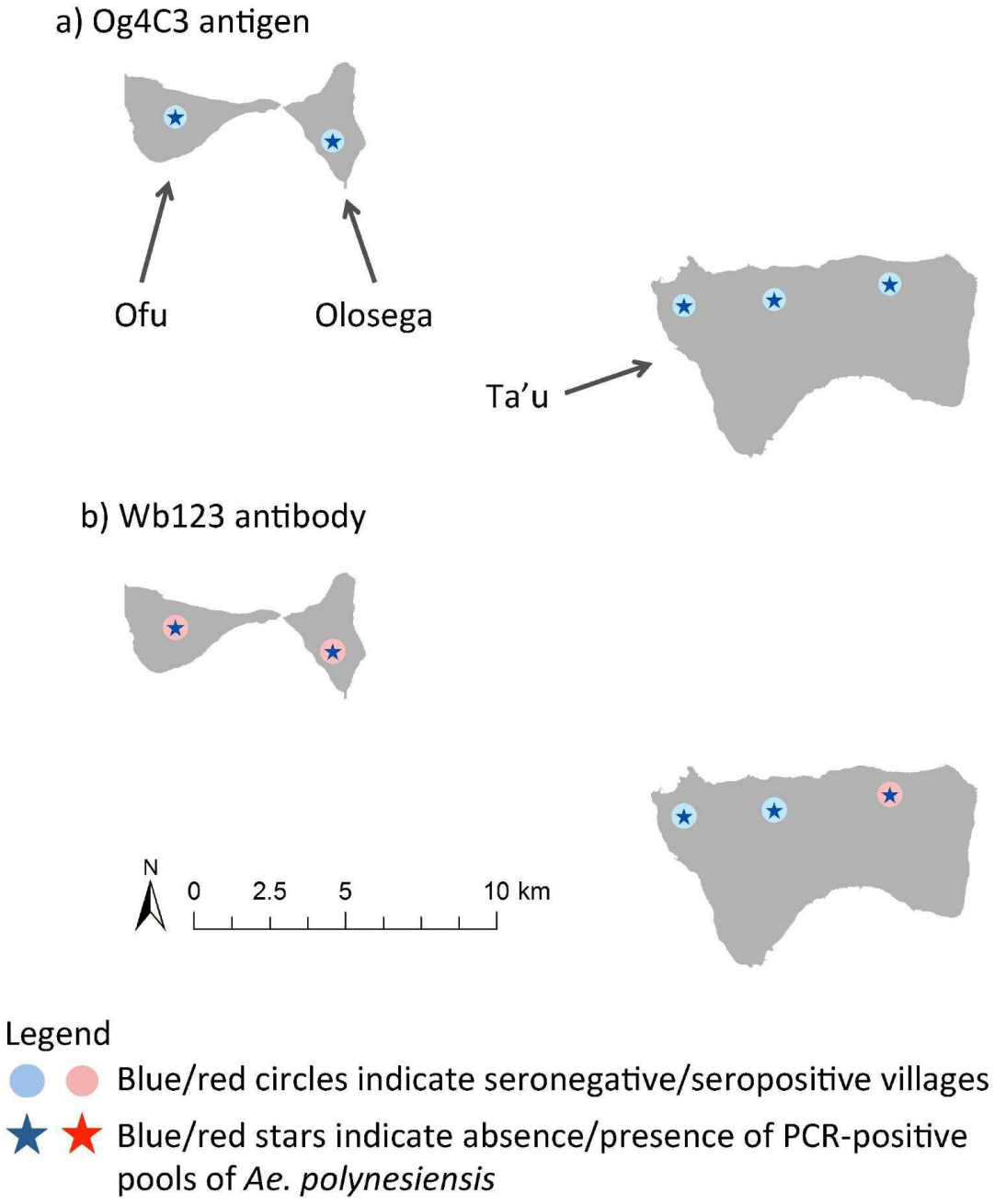


Figure 7.3 Association between PCR-positive pools of *Ae. polynesiensis* and seropositive villages for Og4C3 Ag and Wb123 Ab on the Manu'a Islands.



7.5.3 Predicting the location of seropositive villages by using the estimated prevalence of PCR-positive *Ae. polynesiensis*

In the MX study, the estimated prevalence of PCR-positive *Ae*. *polynesiensis* ranged from 0% (95% CI 0–0.1%) to 2.8% (0.5–7.9%) on Tutuila and Aunu'u, and was 0% for all villages in the Manu'a islands. Table 7.4 shows that a higher estimated prevalence of PCR-positive

Ae. *polynesiensis* (as a continuous variable) was associated with increased odds of a seropositive village for Og4C3 Ag and Wb123 Ab, but the findings were not statistically significant with this sample size and the level of precision inherent in PoolScreen predictions based on pooled mosquito samples.

Table 7.4 Association between estimated prevalence of PCR-positive *Ae. polynesiensis* (using PoolScreen) and seropositive villages for Og4C3 Ag, Wb123 Ab, and Bm14 Ab.

Seropositive villages for	Odds ratio*	95% CI	*p* value
Og4C3	1.76	0.51–6.03	0.367
Wb123	1.85	0.46–7.34	0.384
Bm14	0.42	0.11–1.64	0.211

* Odds ratio on logistic regression, per 1% increase in estimated prevalence of PCR-positive *Ae*. *polynesiensis* (calculated using PoolScreen).

7.6 Discussion

Our results show that MX is a potentially useful tool for post-MDA surveillance of lymphatic filariasis in American Samoa. The presence of PCR-positive pools of *Ae*. *polynesiensis* was found to be a good predictor of villages with persons seropositive for Og4C3 Ag and Wb123 Ab, but not Bm14 Ab. Bm14 Ab can persist for many years or decades after initial infection but does not necessarily persist for life, and antibody levels can also decline or be cleared after MDA (Helmy et al., 2006; Weil et al., 2008). Wb123 Ab can also persist for many years after initial infection, and declines after MDA (Steel et al., 2012). Currently, there is insufficient data on the relative rates of antibody decay or clearance, but it is thought that Wb123 Ab responses decay more rapidly than Bm14 Ab. Biologically, Wb123 Ab responses might increase earlier because they are against a larval antigen and therefore also more likely to be associated with mosquito exposure. In our study, the lack of association between PCR-positive pools of mosquitoes and Bm14 Ab was therefore not unexpected, but the association with Wb123 Ab could be related to earlier appearance or faster disappearance of Wb123 Ab than Bm14 Ab during and after active infections, respectively (Hamlin et al., 2012; Kubofcik et al., 2012; Steel et al., 2013).

In American Samoa, where ~75% of mosquitoes collected in the entomology study (using BG Sentinel traps) were *Ae*. *polynesiensis*, the presence of PCR-positive pools of either *Ae*. *polynesiensis* alone or any mosquito species provided similar predictive accuracy of identifying villages with residents seropositive for Og4C3 Ag or Wb123 Ab. Our study shows that in this setting, separation of mosquito species for MX did not improve the predictive accuracy for identifying villages with seropositive inhabitants. However, it is important to point out that our results would have been different if we had used traps with a different level of selectivity for *Ae*. *polynesisensis*. MX studies in locations with other vector species and employing different traps may require sorting of mosquito species to achieve optimal results.

The presence of PCR-positive pools of *Ae*. *polynesiensis* or mosquitoes of any species had high sensitivity and high negative predictive value (both >90%) for correctly identifying villages with antigen-positive persons. These are both important for post-MDA surveillance because tests should have a high probability of identifying residual foci of transmission (when prevalence is very low) and low probability of missing these foci. In this study, the estimated prevalence of PCR-positive *Ae*. *polynesiensis* (using PoolScreen) was no more useful than the presence/absence of PCR-positive pools, but the sampling design (small number of persons in some villages) might have limited the ability to detect significant associations.

Previous studies in three sentinel villages in American Samoa showed that MX could be a useful tool in post-MDA surveillance (Chambers et al., 2009; Mladonicky et al., 2009); our larger study of 32 villages corroborates those conclusions. Our findings also suggest that in American Samoa, it is appropriate to conduct post-MDA surveillance at the village level. This is biologically plausible considering that the main vector, *Ae*. *polynesiensis*, has a relatively short flight range of about 100 meters (Jachowski, 1954), and village residents are generally quite mobile within their own village, e.g. visiting homes of family and friends, sharing outdoor spaces, attending school and church, and shopping at local stores. Further

interventions (e.g. further targeted MDA or a test and treat approach) could also be conducted at the village or even sub-village level.

The results should be considered in light of the study's limitations. The study was based on serological data from humans; microfilaria results were not available because the study was conducted using a pre-existing serum bank. Human serological data and entomological data were sourced from previously published studies, and there was a time lag of approximately nine months between the human and entomology studies. Sampling of the human seroprevalence study was designed to maximize spatial dispersion for the purposes of predictive risk mapping for the original leptospirosis study (Lau et al., 2014a), resulting in small numbers of subjects in some villages and wide confidence intervals for the village-level LF seroprevalence estimates. The serum bank only included samples from adults (aged ≥ 18 years); a study that focused on or only included children might produce different results regarding the usefulness of serological markers, e.g. there could be significant associations between PCR-positive mosquitoes and Bm14 Ab in children. *Ae*. *polynesiensis*, the primary vector in American Samoa, is a day-biting mosquito; our human data were summarized by village of residence, and it is possible that PCR-positive mosquitoes acquired infections from residents of other villages who visited during day time.

Despite the study's limitations, we were able to identify statistically significant associations between MX data and human seroprevalence data at the village level. Further studies specifically designed to assess the usefulness of MX in the post-MDA setting might produce results with even stronger associations. With higher resolution data, it is also potentially possible to determine thresholds for the prevalence of PCR-positive *Ae*. *polynesiensis* at which further interventions (e.g. repeating MDA or more intensive surveillance) are recommended. In American Samoa, LF transmission is dominated by *Ae*. *polynesiensis*; studies in other countries with a different mix of vector mosquitoes will be needed to determine whether separation of mosquitoes by species is necessary for MX.

This exploratory study provides promising evidence to support the potential usefulness of MX in post-MDA surveillance in an *Aedes* transmission area in a Pacific Island setting to predict sub-national areas where LF transmission may still be occurring. Although American Samoa has successfully completed MDA and passed two TAS of 6–7 year old children, there is evidence of ongoing low-level transmission of LF. Our findings demonstrated that in this setting, MX was useful for localizing residual areas of focal transmission and could potentially be used to inform the need for additional elimination activities. Our study also highlights that assessment of antigen prevalence in adults in post-MDA surveillance could complement TAS and provide valuable information for informing programmatic decisions in the endgame.

7.7 Acknowledgments

We would like to thank Professor Tom Burkot at James Cook University for providing constructive feedback on data analysis and interpretation of results.

Table S 7.1 Village-level human serological data for Og4C3, Wb123 and Bm14

Village ID	Total number of people sampled	Seroneg for Og4C3	Seropos for Og4C3	No result for Og4C3	Village-level seroprevalence for Og4C3 (%)	Seroneg for Bm14	Seropos for Bm14	No result for Bm14	Village-level seroprevalence for Bm14 (%)	Seroneg for Wb123	Seropos for Wb123	No result for Wb123	Village-level seroprevalence for Wb123 (%)
1	14	13	1	0	7.14	8	6	0	42.86	11	3	0	21.43
2	3	3	0	0	0.00	2	1	0	33.33	2	1	0	33.33
3	8	7	1	0	12.50	4	4	0	50.00	6	2	0	25.00
4	9	9	0	0	0.00	8	1	0	11.11	9	0	0	0.00
5	10	9	1	0	10.00	7	3	0	30.00	8	2	0	20.00
6	5	4	1	0	20.00	5	0	0	0.00	4	1	0	20.00
7	16	16	0	0	0.00	14	2	0	12.50	14	2	0	12.50
8	3	2	1	0	33.33	0	3	0	100.00	1	2	0	66.67
9	10	10	0	0	0.00	9	1	0	10.00	9	1	0	10.00
10	13	9	4	0	30.77	7	6	0	46.15	9	4	0	30.77
11	10	10	0	0	0.00	10	0	0	0.00	10	0	0	0.00
12	15	14	0	1	0.00	13	2	0	13.33	15	0	0	0.00
13	16	15	0	1	0.00	11	4	1	25.00	12	3	1	18.75
14	23	19	4	0	17.39	14	9	0	39.13	18	5	0	21.74
15	20	20	0	0	0.00	17	3	0	15.00	20	0	0	0.00
16	28	27	1	0	3.57	25	3	0	10.71	25	3	0	10.71
17	14	14	0	0	0.00	12	2	0	14.29	14	0	0	0.00
18	2	2	0	0	0.00	2	0	0	0.00	2	0	0	0.00
19	7	7	0	0	0.00	6	1	0	14.29	7	0	0	0.00
20	5	5	0	0	0.00	4	1	0	20.00	5	0	0	0.00
21	2	2	0	0	0.00	2	0	0	0.00	2	0	0	0.00
22	11	11	0	0	0.00	8	3	0	27.27	10	1	0	9.09
23	14	14	0	0	0.00	11	3	0	21.43	12	2	0	14.29
24	17	17	0	0	0.00	15	2	0	11.76	14	3	0	17.65
25	73	71	2	0	2.74	60	13	0	17.81	69	4	0	5.48
26	21	21	0	0	0.00	19	2	0	9.52	21	0	0	0.00
27	14	14	0	0	0.00	13	1	0	7.14	14	0	0	0.00
28	14	14	0	0	0.00	11	3	0	21.43	14	0	0	0.00
29	26	26	0	0	0.00	25	1	0	3.85	26	0	0	0.00
30	2	2	0	0	0.00	2	0	0	0.00	2	0	0	0.00
31	7	6	1	0	14.29	6	1	0	14.29	6	1	0	14.29
32	14	13	1	0	7.14	11	3	0	21.43	13	1	0	7.14

8 Partnering for impact: integrated transmission assessment surveys for lymphatic filariasis, soil transmitted helminths and malaria in Haiti

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[This manuscript has been published in *PLoS Neglected Tropical Diseases* (2017) 16;11(2) e0005387 https://doi.org/10.1371/journal.pntd.0005387]

8.1 Abstract

Background: Since 2001, Haiti's National Program for the Elimination of Lymphatic Filariasis (NPELF) has worked to reduce the transmission of lymphatic filariasis (LF) through annual mass drug administration (MDA) with diethylcarbamazine and albendazole. The NPELF reached full national coverage with MDA for LF in 2012, and by 2014, a total of 14 evaluation units (48 communes) had met WHO eligibility criteria to conduct LF transmission assessment surveys (TAS) to determine whether prevalence had been reduced to below a threshold, such that transmission is assumed to be no longer sustainable. Haiti is also endemic for malaria and many communities suffer a high burden of soil-transmitted helminths (STH). Heeding the call from WHO for integration of neglected tropical diseases (NTD) activities, Haiti's NPELF worked with the national malaria control program (NMCP) and with partners to develop an integrated TAS (LF-STH-malaria) to include assessments for malaria and STH.

Methodology/principle findings: The aim of this study was to evaluate the feasibility of using TAS surveys for LF as a platform to collect information about STH and malaria. Between November 2014 and June 2015, TAS were conducted in 14 evaluation units (EUs) including 1 TAS (LF-only), 1 TAS- STH-malaria, and 12 TAS-malaria, with a total of 16,655 children tested for LF, 14,795 tested for malaria, and 298 tested for STH. In all, 12 of the 14 EUs passed the LF TAS, allowing the program to stop MDA for LF in 44 communes. The EU where children were also tested for STH will require annual school-based treatment with albendazole to maintain reduced STH levels. Finally, only 12 of 14,795 children tested positive for malaria by RDT in 38 communes.

Conclusions/significance: Haiti's 2014–2015 Integrated TAS surveys provide evidence of the feasibility of using the LF TAS as a platform for integration of assessments for STH and or malaria.

8.2 Author summary

Lymphatic filariasis and malaria are mosquito-borne parasitic infections that are endemic in Haiti. Soil-transmitted helminths are also present in Haiti, infecting large numbers of people every year. Since 2001, Haiti's National Program for the Elimination of Lymphatic Filariasis (NPELF) has worked to reduce the transmission of LF through annual mass drug administration with the aim of reducing LF prevalence in the population below a threshold, such that transmission is assumed to be no longer sustainable. By treating the entire population of Haiti with a combination of drugs, the elimination program has made tremendous progress towards eliminating the disease. By 2014, Haiti's NPELF had met the World Health Organization eligibility criteria to conduct LF transmission assessment surveys (TAS) and decided to use the LF TAS as a platform to collect information about STH and malaria. The WHO has called for the integration of program activities in the field, and the TAS is a platform that allows for such integration. In Haiti the integrated TAS reduced the burden of repeated surveys on communities by minimizing site visits and benefited all three disease programs by sharing the responsibilities of field data collection.

8.3 Introduction

Globally, lymphatic filariasis (LF), soil transmitted helminths (STH) and malaria are frequently co-endemic, presenting opportunities for integration of programs targeting their control and elimination. The WHO Global Program to Eliminate LF (GPELF) was launched in 2000 with a commitment to the elimination of LF as a public health problem by 2020 through mass drug administration (MDA) (WHO, 2012a). By 2013, more than 4.4 billion treatments of diethylcarbamazine or ivermectin plus albendazole (DEC+ALB or IVM+ALB) had been distributed in 56 countries, achieving an estimated 46% reduction in the population at risk of LF from 1.46 billion to 789 million people (WHO, 2012a, 2013; Hooper et al., 2014). In 2012, an estimated 1.5 billion people were infected with STH globally (WHO, 2012b). In 2014, an estimated 269 million pre-school-aged (PSAC) and 576.6 million school-aged children (SAC) were living in areas endemic for STH, which WHO recommends be addressed with periodic administration of ALB or mebendazole (MBZ) preventive chemotherapy (PC) (WHO, 2012b). Globally, the WHO target is to treat at least 75% of children living in STH endemic countries with PC by 2020 (WHO, 2015b). Approximately 3.2 billion people live in areas where they are at risk for malaria transmission (WHO, 2015c). The WHO global technical strategy for malaria (2016–2030) aims to ensure universal access to malaria prevention, diagnosis and treatment, to accelerate efforts towards elimination and attainment of malaria-free status, and to transform malaria surveillance into a core intervention (WHO, 2015d). Occurring in the tropical and subtropical zones, LF and malaria are both transmitted by mosquito vectors, and in certain areas, by the same species.

The island of Hispaniola is the only remaining Caribbean island that is endemic for both malaria and LF (Raccurt, 2004), with Haiti bearing the greater burden of both diseases. In 2001, Haiti's National LF elimination program (NPELF) determined that nearly all communes were endemic for LF (Beau de Rochars et al., 2004) and began administration of MDA (DEC + ALB) in select areas. Full national treatment coverage (140 communes) was achieved by 2012 (Lemoine et al., 2016). In 2014, Haiti was one of the 25 countries in the

Americas where PC was needed for STH, and one of seven in the region that achieved the ≥ 75% national coverage target (WHO, 2015b). Haiti's malaria prevalence is low (0.4% in 2011) (Lucchi et al., 2014), though 17,662 confirmed cases were reported in 2014 (WHO, 2015c). Documented asymptomatic parasitemia (Lindblade et al., 2013; Elbadry et al., 2015) highlights the need for surveillance strategies to identify remaining high transmission foci. To attain elimination of LF and malaria in Haiti, and control of STH, the three disease programs sought to identify remaining foci of disease transmission by integration of surveillance activities.

In 2011, the World Health Organization (WHO) recommended the Transmission Assessment Survey (TAS), a standardized and statistically rigorous survey for measuring LF prevalence (WHO, 2011a). WHO recommends that LF elimination programs conduct the TAS in areas that have: 1) received 5 or more effective annual rounds of MDA; and 2) where spot-check and sentinel site surveys indicate microfilaria (mf) prevalence is less than 1% or antigenemia prevalence is less than 2%. The WHO has called for integration of neglected tropical diseases (NTD) activities, and in 2015, released a protocol to integrate LF-TAS with an assessment for STH (WHO, 2015e). LF-TAS have successfully been integrated with STH assessments in various countries (Chu et al., 2014; Gunawardena et al., 2014; Drabo et al., 2016). Results from these integrated surveys can be used to determine the frequency of school-based STH treatment needed after community-wide LF MDA stops. Haiti's NPELF, National Malaria Control Program (NMCP) and partners saw an opportunity to synergize efforts for the LF and STH surveys in order to collect community-level information on malaria, as an integrated TAS for all three diseases ('TAS-STH-malaria'). The decision for the LF and malaria programs to work together was facilitated by the fact that the both programs are led by the same director, and are housed within the same office at the Ministry of Public Health and Population (MSPP). To our knowledge, this was the first time the LF TAS was used as a platform for also assessing both STH and malaria. The aim of this study was to evaluate the

feasibility of using TAS surveys for LF as a platform to collect information about STH and malaria.

8.4 Methods

8.4.1 Ethics statement

Transmission assessment surveys were conducted according to study protocols approved by institutional review board (IRB) of the United States Centers for Disease Control and Prevention and the Ethics Committee of the Haitian Ministry of Public Health and Population (MSPP). Following ministry policy, MSPP and IMA World Health staff recruited designated schools to participate in the survey and contacted the schools' headmasters in advance to advise them of the purpose of the survey and to request that they notify parents. Children provided verbal assent at the time of the survey.

8.4.2 Study site

At the time of the surveys, Haiti's 10 departments were divided into 140 communes. Communes with the lowest baseline LF antigen prevalence based on the 2001 national survey were combined to form evaluation units (EUs); these EUs ranged from three or more communes to an entire department (maximum of 10 communes, Sud Est) (Table 8.1). Communes with greatest LF antigen prevalence at baseline were evaluated individually (n = 8 EUs). Altogether, surveys were conducted in 14 EUs, composed of 47 communes in 6 departments, where TAS eligibility requirements had been met (Figure 8.1). Between November 2014 and June 2015, TAS (LF-only) (EU 1), TAS-STH-malaria (EU 2) and TAS-malaria (EUs 3–14) were conducted. Due to the ambitious TAS schedule for 2014–2015, MSPP approved piloting of the three disease protocol (TAS-STH-Malaria) in one EU for 2015.

Table 8.1 Evaluation Units.

EU #	TAS type	Department	Number of communes in Department	Number of Communes per EU	Baseline LF antigen Prevalence (2001)
1	LF	Sud Est	10	10	0–4.9%
2	LF + malaria + STH	Nippes	11	10 [A]	0–4.9%
3	LF + malaria	Centre	12	1 [B]	0–4.9%
4	LF + malaria	Nord Est	13	2 [C]	5–9.9%
5	LF + malaria	Nord Est	13	1 [D]	10–45%
6	LF + malaria	Nord Est	13	9 [E]	0–4.9%
7	LF + malaria	Nord Ouest	10	1 [F]	7.0%
8	LF + malaria	Nord Ouest	10	7 [G]	2.9%
9	LF + malaria	Nord	19	1 [H]	14.0%
10	LF + malaria	Nord	19	1 [I]	30.0%
11	LF + malaria	Nord	19	1 [J]	37.4%
12	LF + malaria	Nord	19	1 [K]	45.0%
13	LF + malaria	Nord	19	1 [L]	28.0%
14	LF + malaria	Nord	19	1 [M]	19.0%

[A] = Anse à Veau, Arnaud, Asile, Fonds des Nègres, Grand Boucan, Miragoâne, Paillant, Petit Trou des Nippes, Petite Rivière des Nippes, Plaisance du Sud

[B] = Saut d'Eau

[C] = Saint Suzanne, Trou du Nord, Terrier Rouge

[D] = Caracol

[E] = Carice, Capotille, Ferrier, Fort Liberté, Ounaminthe, Mombin Crouchu, Mont Organise, Perches, Vallieres

[F] = Chansolme

[G] = Jean Rabel, Bombardopolis, Baie-de-Henne, Mole Saint Nicolas, Saint Louis du Nord, Bassin Bleu, Anse à Foleur

[H] = Dondon

[I] = Plaisance

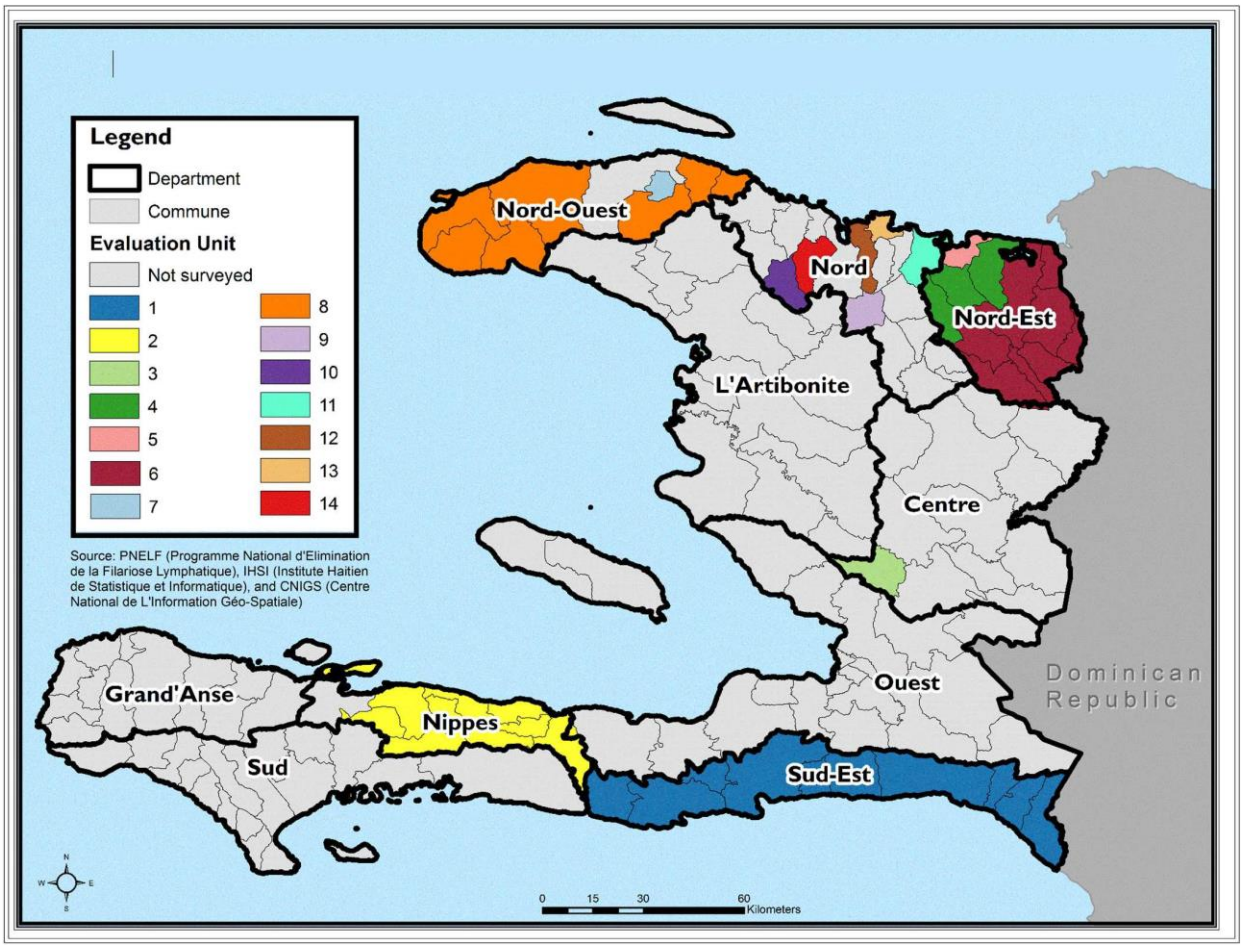
[J] = Limonade

[K] = Plaine du Nord

[L] = Cap Haitien

[M] = Limbe

Figure 8.1 Integrated Transmission Assessment Surveys. Haiti 2014–2015.



8.4.3 Study design

The TAS is a school- or community-based survey which employs a sampling strategy (cluster, systematic or census) determined by the total number of children in the target age group (six and seven years old), number of clusters (schools or census enumeration areas), primary school enrollment rate, and vector and parasite species in predetermined EUs (Table 8.2). The TAS uses a critical cutoff for antigen prevalence in children, below which transmission is assumed to be no longer sustainable, even in the absence of MDA. When the number of LF positive cases among six and seven year olds is at or below the established threshold, the EU ‘passes’ the TAS and LF programs can decide to stop MDA. Surveys were designed using Survey Sample Builder (SSB) (Health) with survey design for STH and malaria assessments the same as for TAS.

Table 8.2 Transmission Assessment Survey Design by Evaluation Unit.

EU number #	TAS- type	Number of Schools in EU	Number of Targeted Schools	Estimated number of Children Aged 6 & 7 Years in the EU	Target Sample Size	Critical Cutoff of LF positives	Survey Design C = Cluster; S = Systematic (Sampling Fraction, Interval)	Sampling by Age or Grade	Assumed Absentee Rate
1	TAS	721	36	35,357	1,556	18	C (1.0, 1.0)	Grade	10%
2	TAS-STH-malaria	367	43	14,813	1,548	18	C (1.0, 1.0)	Grade	10%
3	TAS-malaria	67	38	2,442	1,228	14	C (1.0, 1.0)	Age	10%
4	TAS-malaria	120	30	6,821	1,524	18	C (1.0, 1.0)	Age	10%
5	TAS-malaria	17	17	707	365	4	S (0.57, 1.74)	Age	10%
6	TAS-malaria	333	31	18,977	1,552	18	C (1.0, 1.0)	Age	10%
7	TAS-malaria	25	25	1,597	530	6	S (0.39, 2.56)	Age	15%
8	TAS-malaria	441	39	20,883	1,552	18	C (1.0, 1.0)	Age	15%
9	TAS-malaria	26	29	754	365	4	S (0.57, 1.76)	Age	15%
10	TAS-malaria	34	34	1,679	594	7	S (0.42, 2.4)	Age	15%
11	TAS-malaria	42	42	1,336	780	9	C (0.96, 1.04)	Age	15%
12	TAS-malaria	48	31	1,634	891	11	C (1.0, 1.0)	Age	15%
13	TAS-malaria	199	39	9,299	1,532	18	C (1.0, 1.0)	Age	15%
14	TAS-malaria	74	30	4,038	1,380	16	C (1.0, 1.0)	Age	15%

8.4.4 Survey teams

The LF-only TAS teams were composed of a total of four people: (i) facilitator (responsible for identifying and organizing eligible children); (ii) enroller (responsible for enrollment); (iii) laboratory technician; and (iv) reader (responsible for reading laboratory test and recording result).

Some of the TAS-malaria teams had an additional laboratory technician, for a total of 5 team members. The TAS-STH-malaria teams had two additional individuals to collect and process the stool specimens and to perform Kato Katz, for a total of six or seven team members. Team members received intensive training prior to the surveys.

8.4.5 Population and school data

The target population for all three survey parts was children aged six and seven years. This age group is selected based on the assumption that this age group of children would have been born just before or during the annual MDA campaigns for LF, and therefore, they should not have been exposed to bites of mosquitoes carrying infective larvae.

Standard projection methods were used to estimate the population in the 14 EUs using data from Haiti's most recent national census (2003).

Since comprehensive lists of school enrollment were not available from the Ministry of Education (MOE), trained community-level workers visited schools within the EU and survey areas to generate lists of all schools with the number and ages of children enrolled. The numbers of children (of the targeted ages) enrolled in school were compared with the projected population of children in the target age group for each EU to estimate percent school enrollment rates and to inform survey design.

8.4.6 Sampling

Based on school enrollment rates, all 14 EUs were eligible to conduct school-based TAS (WHO, 2011a). The Haitian Ministry of Education indicated that 1st and 2nd grade students served as a reasonable proxy for six and seven year old children, therefore, selection criteria were set a priori to be conducted amongst 1st and 2nd grade students across the 14 EUs.

At each school, eligible children were selected to receive an LF test according to the sample interval prescribed by SSB (Table 8.2). In the EUs where TAS-malaria was conducted (2–14), all children selected for LF testing were also tested for malaria. In the 1 EU in which LF-malaria- STH TAS was conducted, an additional sampling interval was defined by SSB for selecting a subset of children to be tested for STH.

8.4.7 Sample collection and field diagnostics (LF, STH and malaria)

All diagnostic tests for the integrated TAS were carried out in the field. The diagnostic tests used for LF, STH and malaria were the BinaxNOW Filariasis immunochromatographic test (ICT) (Alere, Maine), Kato Katz (Vestergaard-Frandsen, Denmark), and First Response

Malaria Histidine-Rich Protein II (HRP2) (II3FRC30) (Premier Medical Corporation, New Jersey) rapid diagnostic test (RDT), respectively. Dried blood spots (DBS) were collected on calibrated filter paper (Cellabs, Australia) for subsequent serological testing.

From each enrolled child, technicians collected approximately 175 µL of blood from a single finger stick. Immediately following blood collection, 100 µL of blood was applied to the ICT and results were read at 10 minutes according to manufacturer instructions. Five µL of blood and 2 drops (60 µL) buffer were applied to the RDT and read at 20 minutes, according to manufacturer instructions. Sixty µl of blood was applied to calibrated filter paper and dried individually.

Serological assays are currently underway at CDC in Atlanta, GA, and results will be reported separately.

8.4.8 Sample collection and field diagnostics (STH)

Stool cups were distributed at the time of enrollment. Stool samples were immediately processed on site and two slides were prepared and examined from each sample.

8.4.9 Data collection and analysis

All enrollment information and diagnostic results were recorded directly into Blu® smart phones and uploaded to the cloud-based LINKS (Pavluck et al., 2014) server using software developed and supported by the NTD Support Center.

At the conclusion of the survey at each school visit, teachers and administration were given the diagnostic results.

Data were downloaded from the server in Microsoft Excel. They were cleaned, merged and analyzed using SAS 9.3 at CDC.

8.4.10 Treatment

Children in whom any of the three diseases were detected were provided treatment according to national guidelines, using medications provided by the survey team. Those found to have LF or STH were given DEC+ALB or ALB, respectively. Children found to have malaria were referred to the nearest health public facility to receive chloroquine and

primaquine treatment free of charge, in compliance with the MSPP's antimalarial first-line drug recommendation for treating uncomplicated malaria.

8.5 Results

Haiti's NPELF and partners completed 14 TAS surveys between November 2014 and June 2015 (Figure 8.1 and Table 8.1). In all, 16,655 children were tested for LF, 14,795 for malaria, and 298 for STH (Table 8.3).

Table 8.3 Results: Transmission Assessment Survey.

EU #	# Schools Visited	Total Tested by ICT	% Female	% 6 Years	% 7 Years	# Positive ICT	# Negative ICT	# Positive RDT Malaria	# Negative RDT Malaria	TAS Pass/ Fail
1	36	1,494	46.3%	19.0%	21.8%	0	1,494	-	-	PASS
2	45	1,662	44.7%	25.8%	22.3%	3	1,659	0	1,667	PASS
3	53	1,233	52.8%	38.8%	61.2%	2	1,231	7	1,191	PASS
4	45	1,528	50.0%	52.3%	47.7%	0	1,528	1	1,531	PASS
5	16	365	50.6%	56.0%	43.7%	1	364	0	365	PASS
6	42	1,619	48.8%	46.3%	53.7%	2	1,617	1	1,628	PASS
7	25	551	50.7%	45.1%	54.0%	0	551	0	545	PASS
8	47	1,589	55.9%	37.2%	62.8%	2	1,587	1	1,569	PASS
9	24	587	53.7%	47.0%	53.0%	0	587	0	585	PASS
10	30	672	50.7%	47.5%	52.5%	0	672	1	671	PASS
11	31	877	51.6%	41.2%	58.7%	19	858	1	856	FAIL
12	37	1,052	48.5%	38.0%	62.0%	15	1,037	0	1,073	FAIL
13	32	2,002	39.0%	33.8%	66.2%	18	1,984	1	2,004	MARGINAL
14	33	1,424	53.5%	43.0%	56.9%	10	1,414	0	1,417	PASS
Total	**460**	**16,655**	**49.1%**	**40.6%**	**53.8%**	**72**	**16,583**	**12**	**14,783**	

Haiti's first TAS (LF only) was conducted in November 2014 in Sud Est department (EU 1). Teams visited 36 schools and found that zero (0%) of 1,494 children tested positive for LF by ICT.

The second TAS (TAS-STH-malaria) was conducted in February 2015 in Nippes department (EU 2). Teams visited 45 schools and found that 3 (0.2%) of 1,662 children tested positive for LF by ICT, 0 (0%) of 1,667 tested positive for malaria by RDT, and 46 (15.4%) of 298 children tested positive for STH (Kato Katz).

In the first two EUs, school grades poorly approximated age, with children enrolled in first and second grades ranging in age from five to 18 years. Despite testing children as old as 18 years of age, who could be more likely to have contracted LF before the start of MDA campaigns, only 3 children tested positive for LF in EU 2. In subsequent EUs, only six and seven year old children (from any grade) were identified and eligible for testing.

From February to June 2015, TAS-malaria surveys were carried out in Nord, Nord Est, Nord Ouest and Centre departments (EUs 3–14). In total, teams visited 379 schools and tested more than 13,000 children for LF and malaria. In all, nine EUs passed the TAS. One (EU 13) marginally passed TAS with 18 positive for LF, which met, but did not surpass the critical threshold for continuation of LF MDA. Two EUs (11 and 12) failed the TAS with 19 and 15 children testing positive for LF antigen by ICT, surpassing their respective thresholds of 9 and 11, respectively. Twelve of 13,128 children tested had positive RDT results.

8.5.1 Estimated survey costs

Team members spent a total of 316 working days (working day = 1 person working for 1 day) in the field to accomplish the TAS in 14 EUs. The LF-only TAS, the TAS-malaria and the TAS-STH-malaria required an average of 22, 22.5, and 27 working days per EU, respectively. In terms of productivity, LF-only TAS, TAS-malaria and TAS-STH-malaria teams completed activities at the same rate of 1.6 schools/day, accomplished by the additional personnel for the integrated TAS surveys. The cost of the TAS-malaria evaluation was an estimated 15% higher than the cost for the LF-only evaluation. The cost of the TAS-STH-

malaria evaluation was 49% higher than the cost for the LF-only evaluation. The additional costs resulted from resources required for additional personnel and their transportation.

8.6 Discussion

Integrated surveys have the potential to both optimize resource utilization (human and financial) and generate useful data across programs using a robust survey platform. The Integrated TAS-STH-malaria survey was found to be feasible and generated useful information for all three programs.

Twelve of the 14 EUs passed the LF TAS, allowing the program to stop MDA for LF in 12 EUs, or 45 communes. In 36 of the communes that were able to stop MDA, baseline LF prevalence was low; however, the NPELF also succeeded in reducing LF prevalence sufficiently to pass TAS in 8 communes where LF prevalence was moderate or high at the time of mapping in 2000–2001. This achievement underscores the quality of the MDA activities and the ability of the program to achieve adequate participation of the population. In the two EUs that failed TAS (EU 12, 13), MSPP and partners will continue MDA for an additional two rounds, before re-evaluating in sentinel and spot check sites. Though EU 13 technically passed the TAS with 18 children positive out of 2,002 tested, the EU is surrounded by other areas of ongoing transmission, and thus MSPP and partners took the conservative decision to continue MDA there for an additional two rounds.

According to the WHO TAS-STH manual, the 46 positive STH results from EU 2 would categorize the area as having a prevalence range of 10% to <20% (WHO, 2015e). This represents a decrease in STH prevalence from the 2002 national survey, but leads to the programmatic recommendation to conduct annual, school-based treatment for STH in that EU to maintain reduced STH levels.

In all, malaria was detected by RDT in only 12 of 14,795 children in 38 communes. The inclusion of malaria RDTs in the LF TAS confirms that malaria prevalence in Haiti is low, and provides additional evidence in support of the decision to continue current programs and the development of new strategies in surveillance toward malaria elimination. Although few

malaria RDT-positive individuals were identified amongst a limited population, antibody assays should provide a cumulative history of exposure and potentially define transmission foci for both diseases.

The integrated TAS strategy has several advantages. Most importantly, the integrated TAS-STH-malaria enabled partners to make programmatic decisions for stopping LF MDA and for deworming frequency for STH. As the LF program nears elimination, new approaches to STH monitoring and control, including the integrated TAS which produces actionable results for STH, should be incorporated into work plans.

Second, the integrated TAS establishes a potential framework for integrated surveillance and a more coordinated approach to community-based intervention strategies. By testing for multiple infections, we were able to generate actionable results for more than one program. The results also informed thinking about the testing needed to support malaria elimination. In a single, integrated and carefully planned survey, we are able to maximize returns on the investment of field activities while also reducing burden on both field teams and communities. These synergies are especially important for diseases in the elimination phase, since surveillance efforts will be resource intensive for increasingly rare conditions.

Third, this activity fostered collaboration between ministries of education (MOE) and health (MSPP), and across disease programs within MSPP. The integrated TAS marks the first time in which field activities for the three disease programs were combined in such a manner in Haiti. The NPELF and partners conveyed the common goals of each public health activity to encourage school administrators to allow field teams to conduct integrated TAS in schools. The support of school administrators will be particularly important as STH control moves towards school-based deworming following the end of LF MDA. The LF, malaria and STH programs and field teams worked well together in this mutually beneficial and informative activity, thereby developing and strengthening the new working relationship.

Fourth, the integrated survey represented an overall cost savings, compared with performing similar assessments independently. The addition of the malaria assessment to

the TAS (TAS-malaria) cost an estimated 15% more in Haiti than the LF-only TAS, due to the addition of a team member for processing and reading of malaria RDTs. As teams become more familiar with the workflow, it is anticipated an additional team member might not be necessary to perform the malaria RDT. The integrated TAS-STH-malaria costs an estimated 49% more than the LF-only TAS, due to the addition of two team members for processing of stool by Kato Katz. Obtaining the same information through separate surveys conducted by each independent program would have incurred substantially more expense. The integrated survey platform creates options to include additional tests to expand the utility of the survey.

Lastly, the integrated TAS represented less of an intrusion for the communities than would three independent surveys. By performing multiple diagnostic tests in one coordinated visit, the amount of time that children were kept from classes was minimized. The overall activity was less time consuming, in that the school administration was approached only once on behalf of the three programs, and consent was obtained for all diagnostics concurrently. The one visit proved to take only slightly longer than the TAS-only activity, and provided immediate results to communities and treatment for infected children.

This study identified a few challenges to performing the TAS in Haiti. First, the age of the children enrolled in first and second grades varies greatly, so the program was unable to rely on grades as a proxy for age. Testing children older than seven years (i.e. born prior to the start of MDA), provides a more conservative estimate of LF transmission since it includes children were born before MDA began, however it fails to answer the question about the effect of MDA on recent transmission. For this reason, the decision was made to identify children by age in EUs 3–14, rather than continuing to use grade as a proxy for age in order to better keep with the TAS protocol guidelines.

Second, the estimate of student enrollment frequently exceeded actual child attendance on the day of the surveys. More schools needed to be visited in order to reach the predetermined sample size, which posed additional logistical burden on the field teams. The TAS survey guidelines rely on accurate estimations of the number of children of the

target ages living in each EU as well as school enrollment rates, to ensure that survey design yields representative and appropriately distributed samples from across the targeted population. Prior to the survey, current school enrollment rates were not available from the MOE, so partners obtained school data directly from the schools in each EU.

Third, field teams had to manage several logistical challenges including limited access to cold chain for storage of ICT cards before TAS and dried blood spots after TAS. Limited cellular service, accessibility and difficult terrain of some selected schools, and school holidays were a challenge for field teams impacting sample size. Finally, sensitization was not always sufficient, leading to refusals in some instances.

There were also limitations associated with the malaria program. First, although testing more than 14,700 six and seven year old children confirmed the low prevalence of malaria in the surveyed areas, the rates of RDT positivity was low and consequently, the results of the malaria antibody testing planned for specimens collected during this study are likely to be more informative for programmatic decisions than the RDT results. Second, the integrated TAS survey design, including geographic distribution of EUs, was based on historical LF mapping data. Use of malaria distribution as the basis for the creating EUs would have likely influenced the choice of how to combine communes to form EUs.

8.7 Conclusions

The activities reported here from 14 TAS surveys provide evidence of the feasibility of using the LF TAS as a platform for integration of assessments for STH and or malaria.

In 2014, Haiti's LF elimination program achieved eligibility for administration of TAS in 47 communes and after conducting TAS was able to stop MDA campaigns for approximately1,981,920 people in 44 communes. Intensified activities in the next five years, including progressive implementation of TAS, stopping MDA, and phasing in post-MDA surveillance, will be essential to achieving the country's elimination goal by 2020 (Ichimori et al., 2014). With this in mind, the NPELF and partners plan to conduct TAS (including TAS-STH-malaria and TAS-malaria) in 58 additional communes in 2016 and 39 additional

communes before the end of 2017. Integrating assessment of STH infections will enable program managers to determine the effectiveness of proposed school-based STH programs, while Integrating malaria into the TAS platform will provide additional national data on recent malaria history, which is important for targeting malaria elimination efforts and ensuring progress towards a malaria-free Haiti. Despite experiencing many challenges, including the 2010 earthquake and cholera outbreak, integrated TAS results support the assertion that Haiti is on track to meeting the WHO's 2020 LF global elimination targets. Although malaria elimination is admittedly a more ambitious goal, development of integrated surveillance strategies will help to achieve this goal.

8.8 Acknowledgments

Haiti's National Program for the Elimination of Lymphatic Filariasis and partners want to acknowledge the participation and support of the communities, as well as the technical and support staff at the MSPP and national lab who assisted with training of field staff and. Authors thank Caitlin Worrell for producing the integrated TAS map.

Finally, we would like to acknowledge the field teams who visited the schools, tested and comforted the children, all the while adhering to a demanding implementation schedule. This work would not have been possible without them.

9 Multiplex serologic testing within a cross-sectional lymphatic filariasis sentinel site survey in coastal Kenya reveals community-level differences in IgG antibody responses to parasitic diseases and vaccines

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[This manuscript is under review at *PLoS Neglected Tropical Diseases*]

9.1 Abstract

Accurate, cost-effective measurement of the burden of co-endemic infections would enable public health managers to identify opportunities for implementation of integrated control programs. Dried blood spots (DBS) collected during a cross-sectional lymphatic filariasis sentinel site survey in the coastal Kenyan counties of Lamu, Tana River, Kilifi, Kwale, and Taita-Taveta were used for the integrated detection of serologic IgG antibodies against antigens from several parasitic infections (*Wuchereria bancrofti*, *Schistosoma mansoni*, *Plasmodium* spp., *Ascaris lumbricoides*, and *Strongyloides stercoralis*) as well as markers for immunity to vaccine-preventable diseases (measles, diphtheria, and tetanus) on a multiplex bead assay (MBA) platform. High heterogeneity was observed in antibody responses by pathogen and antigen across the sentinel sites. Antibody seroprevalence against Wb123, Bm14, and Bm33 recombinant filarial antigens were generally higher in Ndau Island ($p<0.001$), which also had the highest prevalence of filarial antigenemia compared to other communities. Antibody responses to the *Plasmodium* species antigens CSP and MSP-1_{19} were higher in Kilifi and Kwale counties, with Jaribuni community showing higher overall mean seroprevalence ($p<0.001$). Kimorigo community in Taita-Taveta County was the only area where antibody responses against *Schistosoma mansoni* Sm25 recombinant antigen were detected. Seroprevalence rates to *Strongyloides* antigen NIE ranged between 3% and 26%, and there was high heterogeneity in immune responses against an *Ascaris* antigen among the study communities. Differences were observed between communities in terms of seroprevalence to vaccine-preventable diseases. Seroprotection to tetanus was lower in all three communities in Kwale County compared to the rest of the communities. This study has demonstrated that the MBA platform holds promise for rapid integrated monitoring of trends of infections of public health importance in endemic areas, and assessing the effectiveness of control and elimination programs.

9.2 Author summary

Establishment of successful private-public partnerships in the recent past has led to an increase in resources available for control and elimination of malaria and Neglected Tropical Diseases (NTDs). Implementation of control and elimination programs and their subsequent monitoring and evaluation would be greatly facilitated by development of new tools and strategies for rapid identification of areas of transmission so that interventions could be prioritized to regions where they were most needed. Since development of antibody responses in a host depend on exposure to an infectious agent, assessment of such serologic markers provides a sensitive way to measure differences between populations in pathogen exposure. Our study applied a state-of-the-art multiplex bead assay platform to perform integrated measurement of antibody responses to multiple parasitic diseases and immunizing antigens for vaccine-preventable diseases (VPDs) in ten lymphatic filariasis sentinel sites across the Kenyan coastal region. A community-level analysis of age-specific and overall mean seroprevalence fit using a flexible model ensemble provided an improved understanding about the distributions of the various parasitic infections and seroprotection to VPDs. This study provides an important proof of concept for how we could dramatically increase the value of existing surveillance activities using small volumes of blood collected on filter paper and analyzed using a single multiplex laboratory assay and novel data analysis techniques.

9.3 Introduction

Persons living in tropical and subtropical areas are often faced with enormous health challenges resulting from the co-endemicity of HIV/AIDS, tuberculosis, and malaria. In addition, several other infectious diseases found in sub-Saharan Africa including some Neglected Tropical Diseases (NTDs) are common, particularly among the poor (Molyneux et al., 2005; Brooker et al., 2006; Hotez et al., 2006a). Past studies in the region have identified subgroups who are polyparasitized with soil-transmitted helminth (STH) infections, filarial parasites, and malaria (Keiser et al., 2002; Raso et al., 2004; Hürlimann et al., 2014). Lymphatic filariasis (LF) caused by *Wuchereria bancrofti* is principally confined to the coastal region of Kenya where ecological factors are suitable for its transmission (Moraga et al., 2015), and LF co-occurs with other infectious diseases such as STH infections, schistosomiasis, lower respiratory infections, and malaria (Okiro et al., 2007; Njenga et al., 2011b; Munywoki et al., 2013).

In the past, lack of resources and competing health priorities in sub-Saharan Africa have led to insufficient commitments to control NTDs. More recently, implementation of successful public-private partnerships (PPPs) for health have availed resources for control and/or elimination of NTDs as public health problems. In 2000 the World Health Organization (WHO) Global Programme to Eliminate Lymphatic Filariasis (GPELF), launched in response to World Health Assembly resolution WHA50.29, urged Member States to initiate activities to eliminate LF as a public health problem, a goal subsequently targeted for 2020 (WHO, 2011a). Community-wide mass drug administration (MDA) of antifilarial drugs for 4-6 years is recommended for LF elimination, and modeling studies have estimated adequate treatment coverage to be at least 65% of total population in endemic areas (Michael et al., 1996; Stolk et al., 2003). Substantial progress has been made towards elimination of LF, with Togo being the first country in sub-Saharan Africa to be recognized by WHO for eliminating the disease as a public health problem (Sodahlon et al., 2013; WHO, 2017f). The Kenyan Ministry of Health launched an LF elimination program in 2002, but the program did not sustain MDA

campaigns annually as per GPELF recommendations (Njenga et al., 2011a; Njenga et al., 2017). In 2015, the Ministry of Health successfully appealed to World Health Organization Regional Office for Africa (WHO-AFRO) and other partners for support to re-establish annual MDA campaigns. Subsequently, the WHO Country Office selected the Eastern and Southern Africa Centre of International Parasitic Control (ESACIPAC), which is part of the Kenya Medical Research Institute (KEMRI), to conduct a comprehensive epidemiological assessment of LF infection before re-starting MDA.

Antibody levels can provide valuable information about exposure to infections and can be helpful for characterizing pathogen transmission dynamics to help identify where interventions are needed the most. As some parasite antigens are known to elicit an immunoglobulin G (IgG) response that can be detected for a long period of time, serological analysis of young children provide an estimate of more recent exposure (Wipasa et al., 2010; Hamlin et al., 2012). A state-of-art multiplex bead assay (MBA) serological platform that enables simultaneous detection of antibodies against multiple antigens using a small volume of blood sample dried on filter paper [10 µL dried blood spots (DBS)] has been developed as a tool for integrated biomarker surveys (Priest et al., 2010; Moss et al., 2011; Lammie et al., 2012). The MBA has successfully been used to simultaneously measure antibody responses to multiple parasitic diseases of public health importance as part of a vaccine-preventable disease serological survey in Cambodia (Priest et al., 2016). The platform has also been used to simultaneously assess IgG responses to a panel of malaria antigens (Arnold et al., 2014; Rogier et al., 2017). In the current study, the MBA platform was used for multiplex serosurveillance of diseases of public health importance by testing for antibodies against LF and several other parasitic diseases (malaria, schistosomiasis, ascariasis, strongyloidiasis) as well as seroprevalence to selected vaccine-preventable diseases (measles, diphtheria, and tetanus).

9.4 Methods

9.4.1 Study design and samples

The DBS samples used in this study were collected during a cross-sectional LF survey conducted in October 2015 in ten sentinel sites located across the coastal region in Taita-Taveta, Kwale, Kilifi, Tana River and Lamu counties as previously described (Njenga et al., 2017). Briefly, 300 persons aged 2 years or more in each sentinel site were targeted for the LF survey as recommended in the WHO guidelines (WHO, 2011a). The characteristics of the study participants are described in Njenga et al. (Njenga et al., 2017). The middle finger of consenting individuals was cleaned using a cotton ball soaked in 70% isopropyl alcohol. After drying, the tip of the finger was pricked using a sterile lancet and blood was collected into capillary tubes for detection of circulating filarial antigen (CFA) by immunochromatographic card test (ICT) and onto filter paper for preparation of dried blood spots (6 spots of 10 µl each; Tropbio Pty Ltd, Queensland, Australia) which were used for the MBA.

9.4.2 Ethics statement

The study received ethical approval from Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit. In the study villages, chiefs and assistant chiefs arranged for community mobilization meetings during which the purpose of the survey and procedures to be followed were explained. Written informed consent was obtained from every individual who agreed to participate in this study; parents or legal guardians consented on behalf of children below 17 years. All of the community acquired samples were assayed in the KEMRI-ESACIPAC laboratory in Nairobi, Kenya.

9.4.3 Recombinant antigens and coupling to microsphere beads

Recombinant *Schistosoma mansoni* glutathione-*S*-transferase (GST) protein was expressed from pGEX 4T-2 plasmid (GE Healthcare, Piscataway, NJ) and purified as previously described [26]. GST fusion proteins that included protein sequences from *Brugia malayi* [Bm33 (Moss et al., 2011); and Bm14 (Hamlin et al., 2012)], *Strongyloides stercoralis* [NIE (Rascoe et al., 2015)], and *Plasmodium falciparum* 3D7 strain [$MSP1_{19}$ (Won et al., 2017)]

were expressed and purified as previously described. A *W. bancrofti* Wb123-GST fusion protein was a kind gift from T. Nutman (NIH, Bethesda, MD). These proteins were coupled to SeroMap beads (Luminex Corp., Austin TX) using the protein quantities and buffer conditions previously described (Priest et al., 2016). *S. mansoni* native soluble egg antigen (SEA) was a kind gift of E. Secor (CDC, Atlanta, GA), and recombinant *S. mansoni* Sm25 antigen was expressed using the Baculovirus system previously described (Won et al., 2017). Both proteins were coupled to SeroMap beads using the protein quantities and buffer conditions previously described.

Tetanus toxoid (Massachusetts Biological Laboratories, Boston, MA), diphtheria toxoid from *Corynebacterium diphtheriae* (List Biological Laboratories, Campbell, CA), and recombinant measles nucleoprotein (MV-N, Meridian Life Sciences, Memphis, TN) (Hummel et al., 1992) were purchased from commercial sources. Tetanus toxoid was coupled to SeroMap beads as previously described (Scobie et al., 2016). Diphtheria toxoid was coupled in buffer containing 50 mM 2-(N-morpholinoethanesulfonic acid (MES) at pH 5.0 with 0.85% NaCl at a concentration of 60 µg of protein per 1.25 x 10^7 beads in 1 ml final volume. In order to decrease background reactivity, measles MV-N was purified by chromatography on a MonoQ HR 5/5 strong anion exchange column (GE Healthcare, Piscataway, NJ) prior to use. Protein (0.75 mg) was loaded onto the column at a flow rate of 1 ml/ min and washed with 4 ml of 25 mM Tris buffer at pH 8.0. This was followed by a 10 ml linear gradient to 0.25 M NaCl in Tris buffer, then by a 5 ml linear gradient to 1 M NaCl in Tris buffer. The majority of antibody-reactive MV-N eluted in the high salt fractions between 0.4 and 0.7 M NaCl. These fractions were pooled, concentrated using a Centricon-30 centifugal filter device (Millipore Corporation, Bedford, MA), and exchanged into buffer containing 10 mM sodium phosphate with 0.85% NaCl at pH 7.2 (PBS). Approximately 115 mg of protein was recovered (BCA micro assay, Pierce, Rockford, IL). MonoQ purified MV-N was coupled in buffer containing 50 mM MES at pH 5.0 with 0.85% NaCl at a concentration of 6 µg of protein per 1.25 x 10^7 beads in 1 ml final volume.

Purified native hemoglobin (Hb) from *Ascaris suum* worms was a kind gift from P. Geldhof (Ghent University, Belgium) (Vlaminck et al., 2012; 2016). This antigen was coupled to 1.25 x 10^7 SeroMap beads in PBS buffer (pH 7.2) at a concentration of 120 µg/ ml.

Cloning of the *P. malariae* $MSP1_{19}$ coding sequence from China I parasite strain is described elsewhere (Priest et al., in preparation). This antigen was coupled to 1.25 x 10^7 SeroMap beads in 50 mM MES buffer at pH 5.0 with 0.85% NaCl at a concentration of 30 µg/ ml. The glutaraldehyde protocol of Benitez et al. (Benitez et al., 2011) was used to cross-link a synthetic 20 amino acid peptide [$(NANP)_5$-amide] corresponding to the carboxy-terminal repeat of the *P. falciparum* circumsporozoite protein (PfCSP) (Dame et al., 1984; Ballou et al., 1985) to purified GST protein. Bead coupling conditions for this antigen were identical to those described above for the *P. malariae* $MSP1_{19}$ protein.

9.4.4 Multiplex bead assay

One bloodspot from each person, corresponding to about 10 µl of whole blood, was eluted overnight at 4ºC with 200 microliters of PBS containing 0.05% Tween-20 and 0.05% sodium azide (1:40 serum dilution assuming a 50% hematocrit). A further dilution of 50 microliters of eluate into 450 µl of PBS containing 0.5% casein, 0.3% Tween 20, 0.02% sodium azide, 0.5% polyvinyl alcohol (PVA), and 0.8% polyvinylpyrrolidone (PVP) (designated as PBN1) with 3 micrograms/ml *Escherichia coli* extract was made for a final serum dilution of 1:400. Serum dilutions were centrifuged at maximum speed to pellet the *E. coli* extract particulates immediately before use. Bloodspot dilutions were assayed in duplicate with antigen-coupled microsphere beads using a BioPlex 200 system platform (Bio-Rad, Hercules, CA) as previously described (Moss et al., 2011; Priest et al., 2016; Rogier et al., 2017). The average of the median fluorescent intensity values from the duplicate wells *minus* the background fluorescence from the buffer-only blank was reported as the “median fluorescence intensity *minus* background” (MFI-bg). Samples having a coefficient of variation of >15% for ≥2 positive responses between the duplicate wells were repeated.

9.4.5 Cutoff determinations

WHO International Standard reference sera for tetanus (TE-3; 120 IU/ml) and diphtheria (10/262; 2 IU/ml) purchased from the National Institute for Biological Standards and Control (NIBSC) (Potters Bar, Hertfordshire, United Kingdom) were used to identify MFI-bg cutoff values corresponding to immunoprotection. A tetanus TE-3 value of 10 mIU/ml (Kristiansen et al., 1997; Borrow et al., 2006) corresponded to a tetanus toxoid MBA response of 118 MFI-bg units. A diphtheria toxoid MBA response of 4393 MFI-bg units corresponded to the 0.1 IU/ml threshold for complete protection (Scheifele and Ochnio, 2009), and an MBA response of 183 MFI-bg units corresponded to the 0.01 IU/ml threshold for partial protection. Others have shown good concordance between the 'gold standard' assays for tetanus and diphtheria and assays using the multiplex bead format (van Gageldonk et al., 2011; Scobie et al., 2016). Although a WHO reference standard is available for the quantitation of measles virus-neutralizing antibody responses using the whole virus Plaque Reduction Neutralization Test (PRNT) (NIBSC 97/648; 3 IU/ml), the standard has not been calibrated for use in ELISA format assays (Bentley et al., 2006), and our MBA only detects IgG antibodies to the measles MV-N protein. In independent work using the specific bead set from this study Coughlin et al. (in preparation) determined that an ROC-optimized MFI-bg cutoff value of 178 MFI-bg units provided good sensitivity and specificity compared to the 'gold standard' PRNT.

MBA cutoff estimates for the *S. stercoralis* NIE assay and for the three LF antigens (Bm33, Bm14, and Wb123) were assigned using a panel of 94 presumed negative sera donated by anonymous adult US citizens with no history of foreign travel. Test values greater than the mean *plus* three standards deviations of the presumed negative sample values were considered to be positive. For the *P. malariae* and *P. falciparum* $MSP1_{19}$ assays, log transformed data were used for the mean *plus* three standard deviation calculation, and the panel used for the *P. falciparum* cutoff included only 65 of the original 94 US adult volunteers. A ROC curve using sera from 41 stool-confirmed, anonymous ascariasis patients, 65 of the adult US citizen volunteers and sera from 45 anonymous US children was used to

identify the cutoff for the *Ascaris* Hb MBA. All of the parasitic disease cutoff values were adjusted to account for differences between the instrument used for cutoff determination at the CDC in Atlanta, GA, and the instrument used to assay the Kenyan sample set at KEMRI in Nairobi, Kenya. Two-fold serial dilutions of the same strong positive sera were assayed on both instruments to generate standard curves for cutoff value adjustment.

S. mansoni SEA and Sm25 coupled beads were used in an earlier study, and the adjusted, ROC-assigned cutoff values have been reported elsewhere (965 and 38 MFI-bg units, respectively) (Won et al., 2017).

We also estimated seropositivity cutoff points for malaria, LF, and helminth antibody responses using the mean plus three standard deviations of a seronegative distribution estimated from the study measurements using finite Gaussian mixture models with two components (Benaglia et al., 2009).

9.4.6 Statistical analysis

Mean antibody levels (MFI-bg) were analyzed on the $\log_{10}$ scale due to skewness in their distribution. We estimated age-dependent mean antibody levels and seroprevalence for each study community using cross-validated, ensemble machine learning, with a library that included the simple mean, linear models, locally weighted regression (loess), and smoothing splines with 2 to 10 degrees of freedom, selected using 10-fold cross-validation (Arnold et al., 2017). We estimated age-adjusted geometric mean antibody levels and seroprevalence for each community using targeted maximum likelihood estimation with influence curve-based standard errors (Arnold et al., 2017). In cases where seroprevalence approached zero, we estimated exact binomial confidence intervals. Analyses were conducted using R version 3.3.1, and full replication files (data, scripts) are available through the Open Science Framework (https://osf.io/taknp).

9.5 Results

Antibody measurements were obtained from 2,837 individuals (range 271 – 297 per community) (Figure S 9.1). Antibody distributions varied by pathogen and antigen, and

overall there was good concordance between seropositivity cutoff values for malaria, LF and helminth antibody responses derived through ROC curve analysis or mean *plus* 3 standard deviation calculations and those derived by Gaussian mixture model analysis (Figure 9.1). We therefore relied on cutoff values derived from the Gaussian mixture model antibody responses for comparability to future studies that may not have access to positive and negative control specimens. Age-dependent patterns and community-level estimates of mean antibody levels and seroprevalence were highly consistent (Figure S 9.2, Figure S 9.3, Figure S 9.4, Figure S 9.5, Figure S 9.6), so we report results based on mean antibody levels in supporting information.

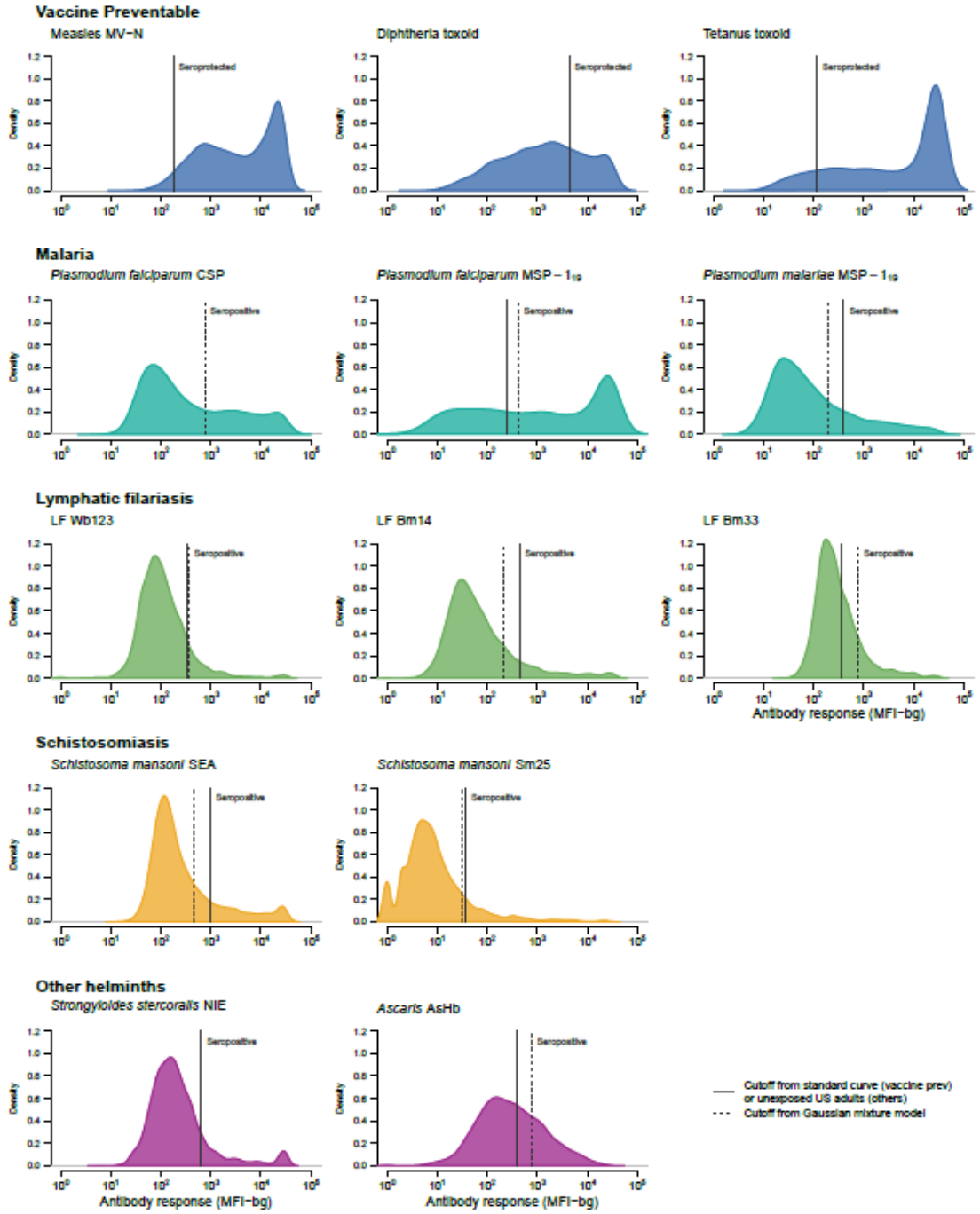


Figure 9.1 Distribution of quantitative antibody levels measured in 10 communities in Kenya's coastal region, 2015. Antibody response measured in multiplex using median fluorescence units minus background (MFI-bg) on a Bio-Rad Bio-Plex platform. Seroprotection cut points for measles, diphtheria, and tetanus estimated using standard curve from WHO reference standards. Seropositive cut points for other antigens estimated using negative control serum samples (solid) and finite Gaussian mixture models (dashed). There was no negative control cut point determined for the *P. falciparum* CSP antigen. Table S 9.1 includes cutoff values. The script that created the figure is here: https://osf.io/d9jrc.

9.5.1 Antifilarial antibody measurements

Individuals who tested positive for LF infection by ICT had higher mean levels of antibody responses against the 3 recombinant filarial antigens (Figure S 9.7). Antibody seroprevalence against all 3 recombinant filarial antigens were significantly higher in Ndau Island compared to other communities and the difference in seroprevalence in Ndau compared to other communities was greater among persons less than 30 years old (Figure 9.2). Antifilarial antibody responses against Bm14 antigen continued to increase with age in all communities. For Wb123, seroprevalence gradually increased with age in Ndau and increased from around the age of 30 - 35 years in Mwadimu community. Compared to the other communities, Jaribuni had slightly elevated mean antibody responses against Wb123 and Bm33 antigens ($p<0.0001$), but not for Bm14 antigen ($p=0.08$). Amongst the youngest children, quantitative antibody levels differentiated communities more clearly than seroprevalence owing to high variability in seroprevalence estimates from the small sample sizes in the youngest age strata (Figure S 9.8). Elevated antibody levels among young children in Ndau, and possibly Jaribuni, were consistent with ongoing LF transmission.

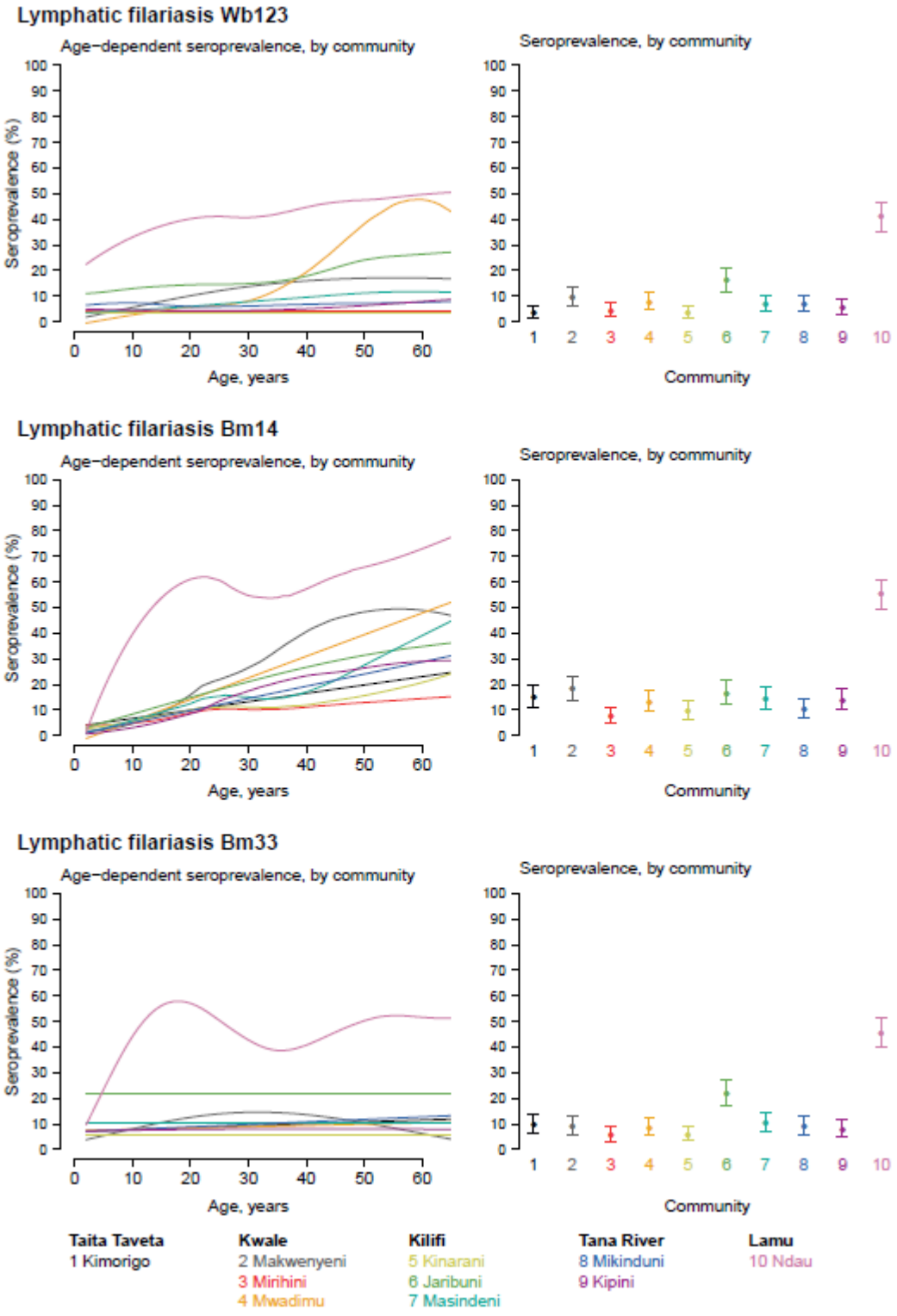


Figure 9.2 Lymphatic filariasis antibody age-dependent seroprevalence and overall means, stratified by community in Kenya's coastal region, 2015. Community-level mean seroprevalence is age-adjusted and error bars represent 95% confidence intervals. Figure S 9.2 is an extended version of this figure that also includes quantitative antibody levels. The script that created this figure is here: https://osf.io/5zkxw.

9.5.2 Antibody responses to other parasite antigens

Antibody responses to the *P. falciparum* CSP and MSP-1_{19} antigens and to the *P. malariae* MSP-1_{19} antigen increased with age in communities in Kilifi and Kwale counties, with higher seroprevalence in Jaribuni community compared to other communities in Kilifi ($p<0.0001$, Figure 9.3). Mean antibody responses against *P. malariae* MSP-1_{19} antigen also increased with age and were highest in Jaribuni ($p<0.0001$), but very low in Ndau Island and Kipini communities ($p<0.0001$ for difference with other communities).

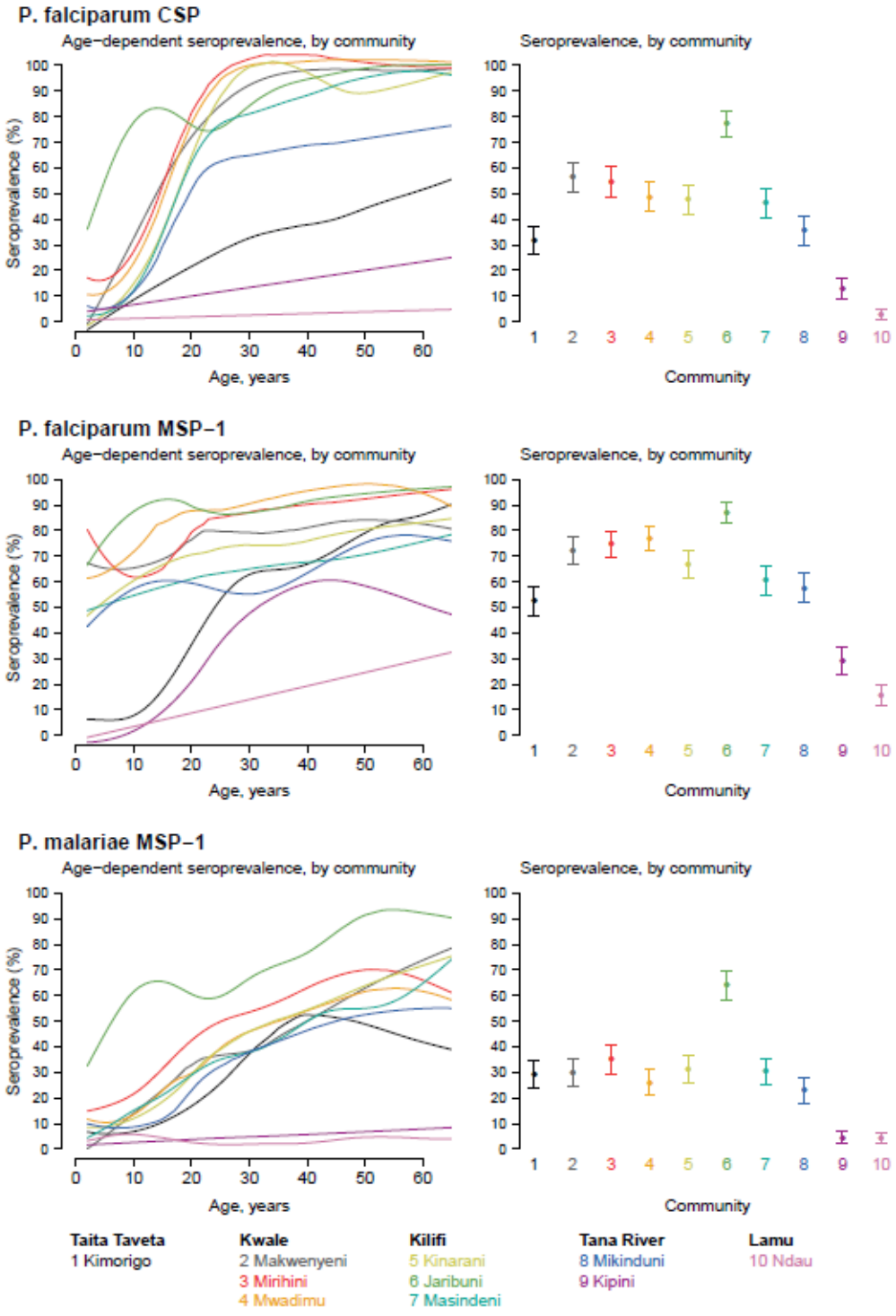


Figure 9.3 Malarial antibody age-dependent seroprevalence and overall means, stratified by community in Kenya's coastal region, 2015. Community-level mean seroprevalence is age-adjusted and error bars represent 95% confidence intervals. Figure S 9.3 is an extended version of this figure that also includes quantitative antibody levels. The script that created this figure is here: http://osf.io/kzfd3.

Antibody responses against *Schistosoma mansoni* Sm25 recombinant antigen were primarily detected in Kimorigo community, and the seroprevalence increased gradually with age, reaching a peak at around 25 years of age (Figure 9.4). However, although antibody responses to *S. mansoni* SEA antigen also increased with age in Kimorigo community and mean seroprevalance was higher, there were some responses against this antigen in many other communities.

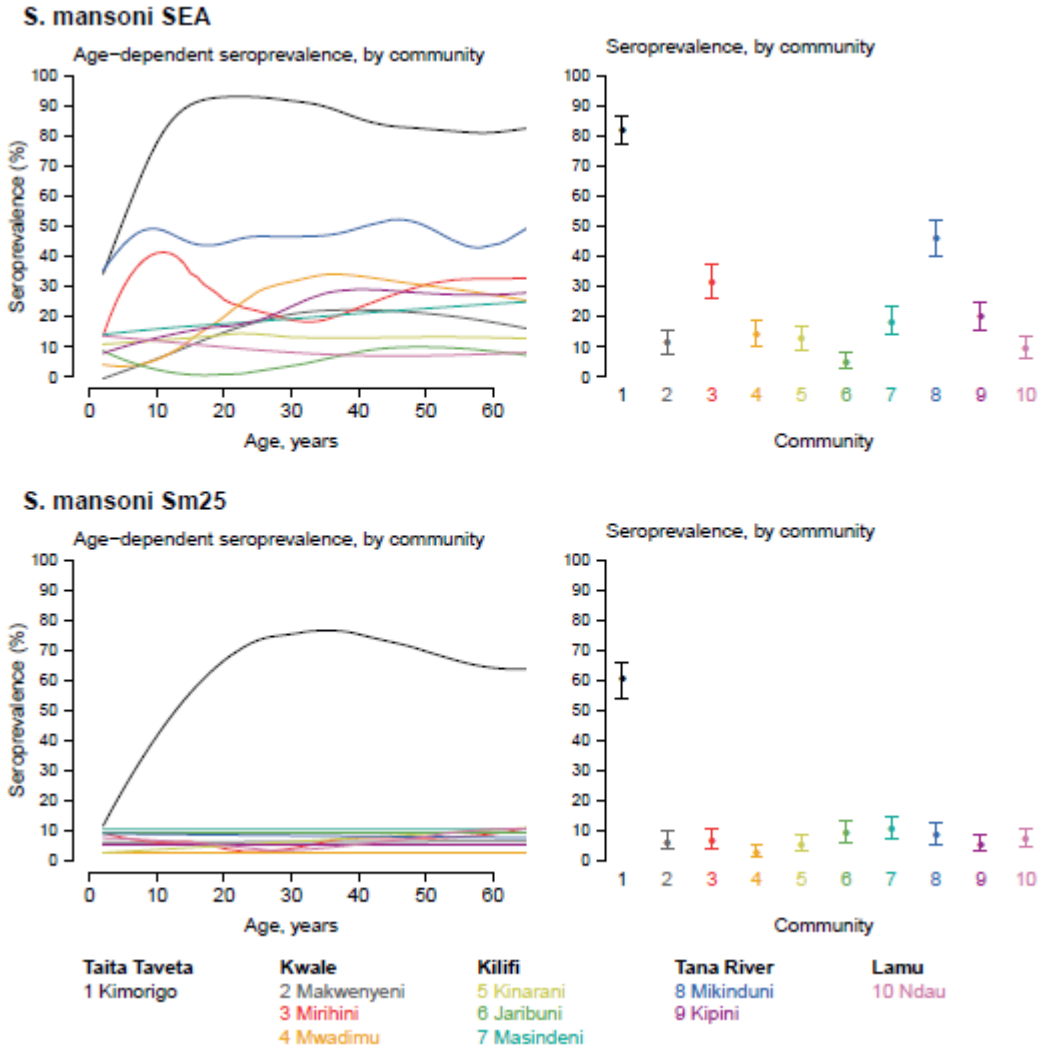


Figure 9.4 Schistosomiasis antibody age-dependent seroprevalence and overall means, stratified by community in Kenya's coastal region, 2015. Community-level mean seroprevalence is age-adjusted and error bars represent 95% confidence intervals. Figure S 9.4 is an extended version of this figure that also includes quantitative antibody levels. The script that created this figure is here: https://osf.io/tpcg7.

Steady increases in *S. stercoralis* NIE seroprevalence with age were observed and community level mean seroprevalence ranged between 3% and 26% (Figure 9.5). There was heterogeneity in age-dependent *Ascaris* Hb seroprevalence patterns across communities, with seroprevalence increasing with age in some communities and decreasing with age in others (Figure 9.5).

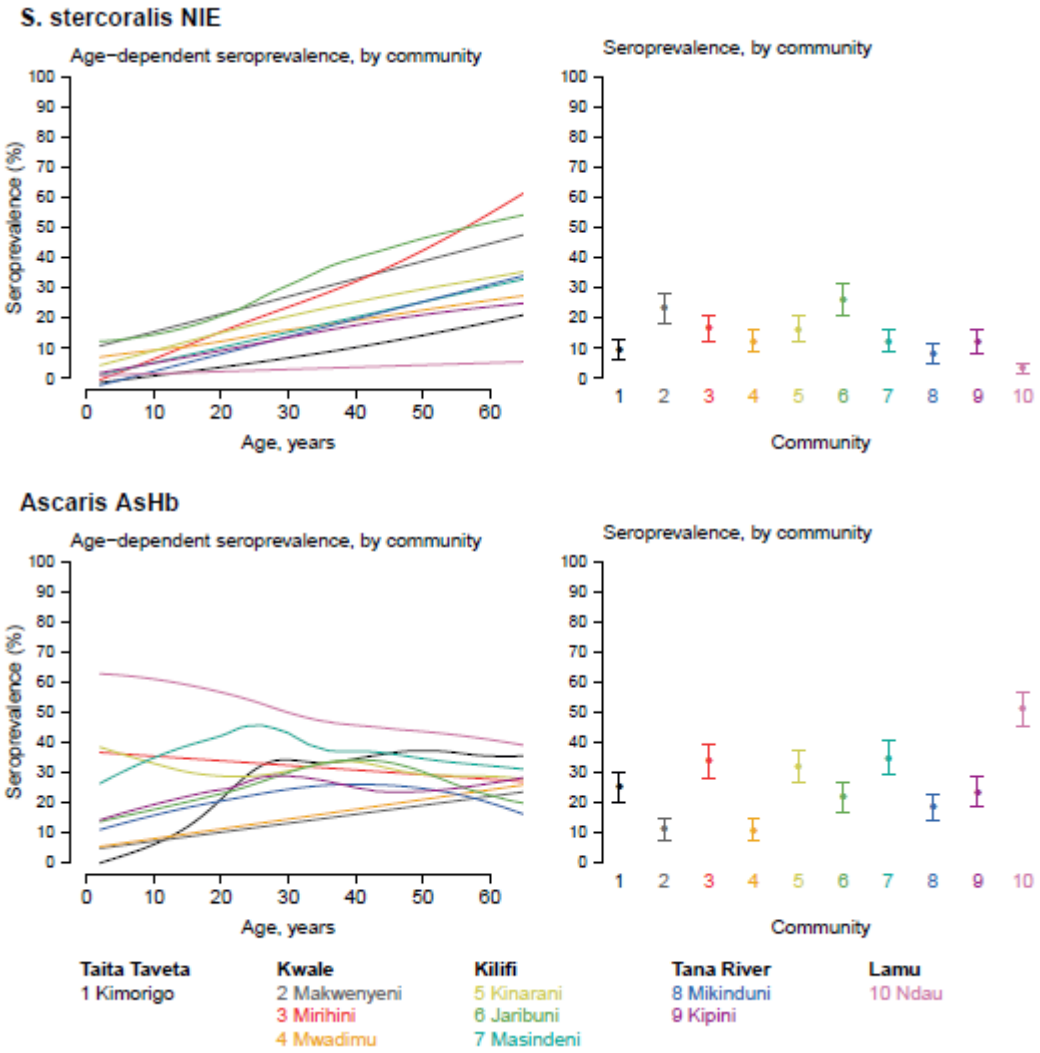


Figure 9.5 Age-dependent seroprevalence and overall means for antibodies to *S. stercoralis* and *A. lumbricoides*, stratified by community in Kenya's coastal region, 2015. Community-level mean seroprevalence is age-adjusted and error bars represent 95% confidence intervals. Figure S 9.5 is an extended version of this figure that also includes quantitative antibody levels. The script that created this figure is here: https://osf.io/j7uxz.

9.5.3 Immune responses to vaccine preventable diseases

Immune response against measles MV-N antigen increased with age, but two communities in Kwale County (Mirihini and Mwadimu) had <90% seroprotection (Figure 9.6). Immune responses to diphtheria toxoid were relatively higher among children, but waned slightly around the ages of 30-40 years before increasing slightly. Generally, diphtheria seroprotection ranged between 22-44% across communities, and partial protection (defined as responses of 0.01-0.099 IU/ml) ranged between 70-88% across communities. Immune responses against tetanus toxoid decreased by age in all communities until around 15 years when the levels increased again. Tetanus seroprotection was lower in all 3 communities in Kwale County.

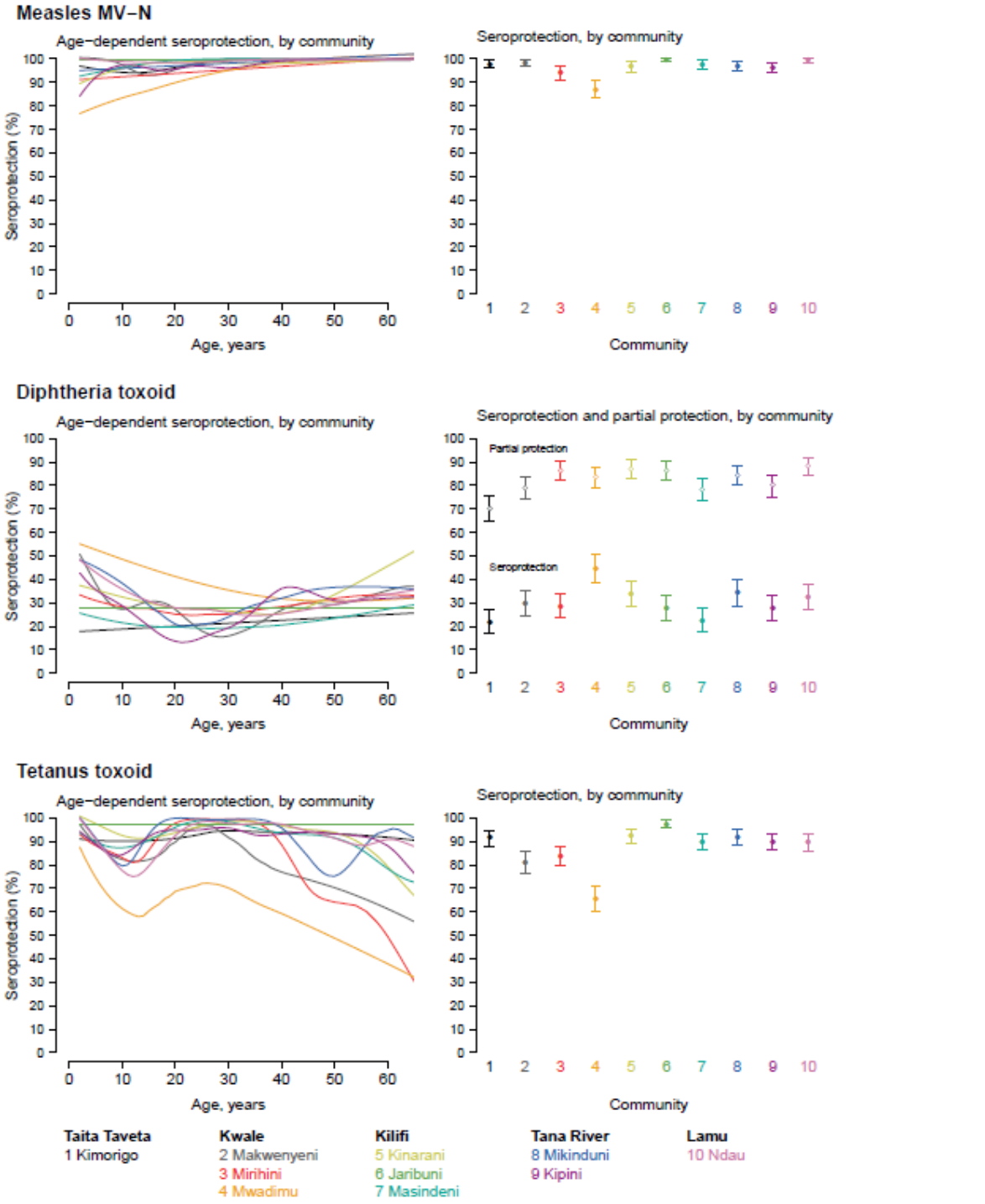


Figure 9.6 Age-dependent seroprotection and overall seroprotection for measles, diphtheria, and tetanus stratified by community in Kenya's coastal region, 2015. Community-level seroprotection is age-adjusted and error bars represent 95% confidence intervals. For diphtheria, we included separate community level estimates of seroprotection (MFI > 4393 corresponding to 0.1 IU/ml) and partial protection (MFI > 183 corresponding to 0.01 IU/ml). Figure S 9.6 is an extended version of this figure that also includes quantitative antibody levels. The script that created this figure is here: https://osf.io/qrkhm.

9.6 Discussion

Many established national parasitic disease control and elimination programs conduct routine surveillance to monitor and evaluate the impact of their targeted interventions. Epidemiologic surveillance systems that enable integrated surveillance and monitoring of co-endemic diseases and public health interventions could provide cost-effective synergy to support public health programs (Lammie et al., 2012). Antibodies can provide valuable information about past exposure to pathogens, and can be helpful for characterizing transmission dynamics in an area to help prioritize where and what interventions are needed the most (Lau et al., 2014a; Priest et al., 2016).

The LF survey in coastal region of Kenya, which provided the opportunity to collect information for this study, demonstrated that Ndau Island in Lamu County had the highest prevalence of CFA by ICT (Njenga et al., 2017). The antifilarial antibody measurements assessed by MBAs closely aligned with the CFA results. Ndau Island had the highest levels of antibody responses to all three recombinant antifilarial antigens, which confirms the observation that LF transmission is currently higher in Ndau Island compared to the other communities. Previous studies have demonstrated a spatial relationship between antibody-positive individuals and infected persons (Joseph et al., 2011). The high seroprevalence rates in Ndau, especially among children, are consistent with the conclusion that transmission is ongoing and not yet halted by the MDA campaign.

Antibody responses against Bm14 antigen continued to increase with age in all villages, which may have been an indication of cumulative exposure to *W. bancrofti*, and also likely reflects historic transmission. Generally, antibody responses against the three recombinant filarial antigens were higher among with CFA-positive individuals than in CFA-negative persons although the difference was relatively smaller for Bm33 (see Figure S 9.7). Results from a recent study in American Samoa demonstrated that PCR-positive pools of LF vector mosquitoes were statistically significant predictors of seropositivity for Wb123 but not Bm14, suggesting Wb123 could be an indicator of ongoing transmission (Lau et al., 2016).

Longitudinal studies in areas of intense LF transmission have shown that children acquire infections early in life (Lammie et al., 1998; Witt and Ottesen, 2001). Additionally, previous studies have demonstrated that antibody response against infective stage filarial larvae antigen Wb123 is a specific measure of *Wuchereria bancrofti* infection, and reduction in both antibody prevalence and transmission is seen most clearly in young children (Kubofcik et al., 2012; Steel et al., 2012). Quantitative antifilarial antibody responses among youngest children (2-5 yr, 6-10 yr) provided much higher resolution distinctions between communities compared with seroprevalence using the same antigens or the ICT test (Figure S 9.8) – a result consistent with a recent analysis across diverse pathogens in low transmission settings where seropositive individuals are rare (Arnold et al., 2017). The higher resolution of quantitative antibody responses compared with seroprevalence, particularly when measured in small sampling clusters, suggests that quantitative antibody levels could serve as an important and more sensitive indicator of recent exposure in sentinel populations of young children, and may be valuable tool for surveillance in the context of lymphatic filariasis elimination programs (Hamlin et al., 2012). Thus, combined measurement of these markers may be suitable for characterization of LF transmission settings particularly towards the end of the program when the infection prevalence is very low.

There was high heterogeneity in malaria seroprevalence among the study communities with Kwale and Kilifi counties generally showing relatively higher malaria transmission compared to the other 3 counties. The community mean seroprevalence values suggested that both *P. falciparum* and *P. malariae* transmission were highest in Jaribuni community in Kilifi County. These differences may reflect environmental heterogeneity in malaria larval breeding sites. A previous study in Kilifi and Kwale counties identified the primary vectors of malaria along the coast of Kenya to include *Anopheles funestus* and three members of the *An. gambiae* complex: *An. gambiae s.s.*, *An. arabiensis*, and *An. merus* (Mbogo et al., 2003). The study also showed that relatively high malaria parasite prevalence can occur at low and even non-detectable levels of entomological inoculation rates (EIR),

suggesting that measurement of EIR may be a relatively insensitive indicator of malaria transmission in some settings. Although malaria parasite prevalence and/or EIR have traditionally been used for reporting malaria transmission intensity (Pothin et al., 2016), serological markers have increasingly been recognized as useful indicators for estimating malaria transmission intensity, which is key for assessing the impact of control interventions (Drakeley et al., 2005; Cook et al., 2010; Wong et al., 2014; Dewasurendra et al., 2017). Because of the longevity of the specific antibody response, seroprevalence reflects cumulative exposure and thus is less affected by seasonality or unstable transmission (Badu et al., 2012).

In Kenya, *Schistosoma haematobium* is highly endemic along the coast where human exposure occurs primarily at pond and stream snail habitats (Clennon et al., 2004; Njenga et al., 2011b; Njaanake et al., 2016). The absence of *S. mansoni* from most of the Kenyan coastal region is attributable to the absence of the *Biomphalaria* spp. intermediate-host snails (Brown et al., 1981). In Mikinduni Community, along the lower Tana River, crude antigen SEA antibody responses were observed, but *S. mansoni*-specific Sm25 responses were lacking. In contrast, Taveta area in Taita-Taveta County is known to be endemic for both *S. haematobium* and *S. mansoni* infections (Thiongo and Ouma, 1987; Gouvras et al., 2013), and this is reflected in the high SEA and Sm25 antibody responses we observed in Kimorigo, a community located on the banks of the shallow freshwater Lake Jipe. The absence of *S. mansoni* species-specific antibody responses to Sm25 recombinant antigen in all of the communities except Kimorigo confirms that *S. mansoni* infection is likely absent from the lower coastal areas. Thus, *S. mansoni* Sm25 recombinant antigen seems to be an excellent antigen for measuring antibody responses to *S. mansoni* infection (Tsang et al., 1983), and SEA antigen likely detects antibody responses caused by both *Schistosoma* species by virtue of cross-reactivity.

Presence of responses to *S. stercoralis* NIE antigen is noteworthy because there has been little information on the geographic distribution of this helminth in Kenya due to

diagnostic limitations. Copromicroscopic diagnostic methods commonly used in soil-transmitted helminthiasis prevalence studies are inadequate for *S. stercoralis* detection (Steinmann et al., 2007), and thus its distribution in many areas is unknown. Concentration methods, namely the Baermann technique and Koga agar plate culture, have better but still unsatisfactory sensitivity (Glinz et al., 2010). A study employing NIE serology in Argentina found no cross-reactivity between *S. stercoralis* and infections with *A. lumbricoides*, hookworms, or *H. nana*, and the presence of other helminths in the stool did not affect the *S. stercoralis*-specific antibody responses (Krolewiecki et al., 2010). A study comparing five serologic tests identified NIE- Luciferase Immunoprecipitation System to be the most accurate assay for the diagnosis of *S. stercoralis* infection (Bisoffi et al., 2014). Previous studies using the recombinant NIE have documented high seroprevalence of *S. stercoralis* infection in remote Australian Indigenous communities and suggest that collection of dried blood spots may be a useful approach for field diagnosis of *S. stercoralis* seroprevalence (Mounsey et al., 2014; Kearns et al., 2017). This study, therefore, provides evidence for possible low-level transmission of *S. stercoralis* in coastal Kenya as the seroprevalence varies from community to community. Community mean antibody responses to the *Ascaris* Hb native antigen and seroprevalence exhibited high heterogeneity among the study communities. A population-based study in Indonesia has shown that an assay for antibodies to *Ascaris* Hb is useful for assessing transmission of *Ascaris* infections, and community antibody rates decreased rapidly following MDA of anthelmintic drugs. The decrease was also found to reflect reduced egg excretion at the community level (Vlaminck et al., 2016).

Vaccination is one of the most one of the most cost-effective public health interventions available, and the epidemiology and burden of vaccine-preventable diseases vary by country and by region partly because of differences in vaccine uptake (Brenzel et al., 2006). This multiplex integrated serosurveillance study identified heterogeneity in serologic antibody levels against measles, diphtheria, and tetanus antigens. Our study demonstrates a

need for regularly monitoring serological responses to vaccination programs in resource-poor settings where coverage may be low.

Some of the limitations of this study are somewhat similar to those highlighted previously (Priest et al., 2016). Serological studies are traditionally faced with the challenge of establishing diagnostic cutoff points especially when well-characterized positive and negative serum samples are not available. Finite Gaussian mixture models applied in this study led to cutoff values that were very similar to those derived through ROC curves or from mean *plus* 3 standard deviation calculations for malaria, LF and helminth antibody responses (*Figure 9.1*). This result is consistent with a recent, multi-country comparison of cutoff methodology for trachoma antibodies (Migchelsen et al., 2017), and supports the use of finite mixture models to identify seropositivity cutoffs in studies without access to panels of known positive and negative specimens. For pathogens where cutoff values fall in the centre of a unimodal distribution and it is more difficult to distinguish seropositive and seronegative groups (e.g., *A. suum* Hb in Figure 9.1), the use of community mean antibody levels avoids the requirement of choosing a cutoff, and observed antibody response patterns were very consistent with seroprevalence estimates across all of the antibodies tested in this study (Figure S 9.2, Figure S 9.3, Figure S 9.4, Figure S 9.5, Figure S 9.6). Another limitation of this study is potential for antibody cross-reactivity. Since the coastal area has a typical tropical climate, it is likely that a plethora of pathogens are coincident, some with potentially cross-reactive antigens. A previous study reported that cross-reactivity of the *Ascaris* Hb native antigen with hookworm and possibly *S. stercoralis* and *Toxocara* spp. limited its value in serology if one is interested in ascariasis alone (Vlaminck et al., 2016). Thus, further studies are required to identify sensitive and specific recombinant antigens that could be used with more confidence in serological assays.

In spite of these limitations this study employed a single multiplex integrated serological assay and analysis methodology to measure antibody levels against several pathogens. There was no need to run separate assays for each pathogen, and we did not

need to develop different mathematical models for each pathogen in order to compare exposure across communities and counties. The study highlighted overlap in pathogen burden that would not necessarily have been detected through single-disease surveillance. For example, Ndau Island was found to have the highest LF seroprevalence, but it also had highest *Ascaris* seroprevalence, thus supporting integrated control of these two helminths. Interestingly, Ndau had almost no evidence for *P. falciparum* malaria transmission. On the other hand Jaribuni community was found to stand out in terms of malaria, LF, and *Strongyloides*. Multiplex, integrated surveillance has the potential to enable us to look across diseases for opportunities for integrated control, thus providing synergy to global public health initiatives.

9.7 Conclusion

This study highlighted the utility of the MBA platform for integrated serosurveillance of biomarkers of diseases of public health importance. The multiplex integrated serologic assay has the potential to become an invaluable tool for integrated monitoring of trends in endemicity of diseases of public health importance and the effectiveness of public health control programs.

9.8 Acknowledgements

The authors would like to thank the County Health Departments of Taita-Taveta, Kwale, Kilifi, Tana River and Lamu for supporting the survey, including provision of laboratory technicians and local transportation for the survey teams. The communities of the selected sentinel sites and their local leaders are sincerely thanked for the cooperation and assistance. We acknowledge WHO-AFRO office through the WHO country office, Kenya and Dr. Simon Brooker for great help during implementation of the LF field survey which provided the opportunity to collect specimens used in this study. Dr. Patrick Lammie (CDC) is thanked for useful comments and suggestions. We wish to thank members of the Vaccine Preventable Disease Branch (CDC) including Sun Bae Sowers for sharing measles PRNT data. The Kenya Medical Research Institute (KEMRI) provided scientific leadership and oversight for

this study. BFA was supported by National Institute of Allergy and Infectious Disease grant K01-AI119180.

9.9 Supplemental Figures

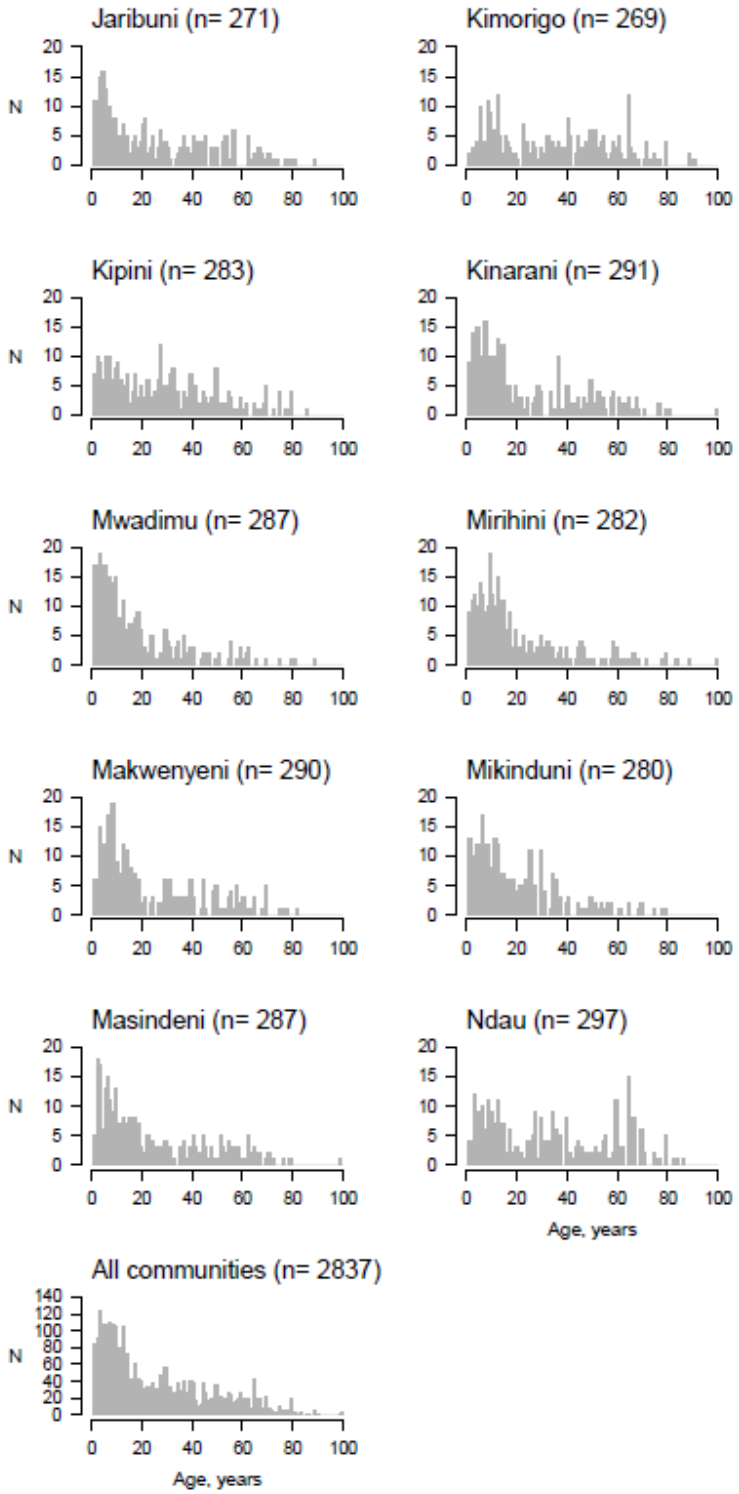
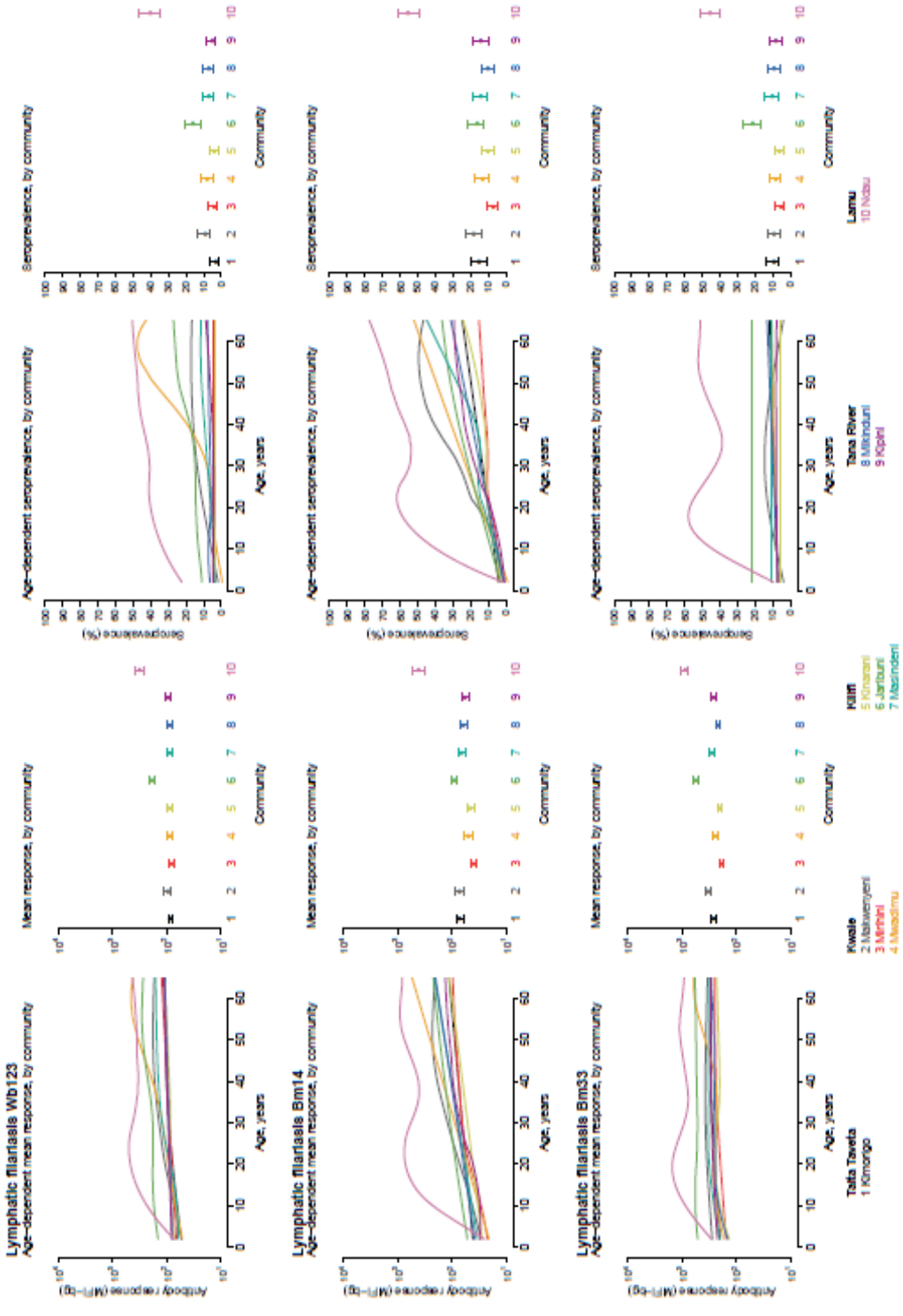


Figure S 9.1 Community-level sample size and age distribution. The script that created this figure is here: https://osf.io/7jxmn.



Lymphatic filariasis antibody age-dependent mean response and seroprevalence, stratified by community in Kenya's coastal region, 2015. Community-level mean antibody response and seroprevalence are age-adjusted and error bars represent 95% confidence intervals. Antibody response measured in median fluorescence units minus background (MFI-bg) on a Bio-Rad Bio-Plex platform. The script that created this figure is here: https://osf.io/c79rw.

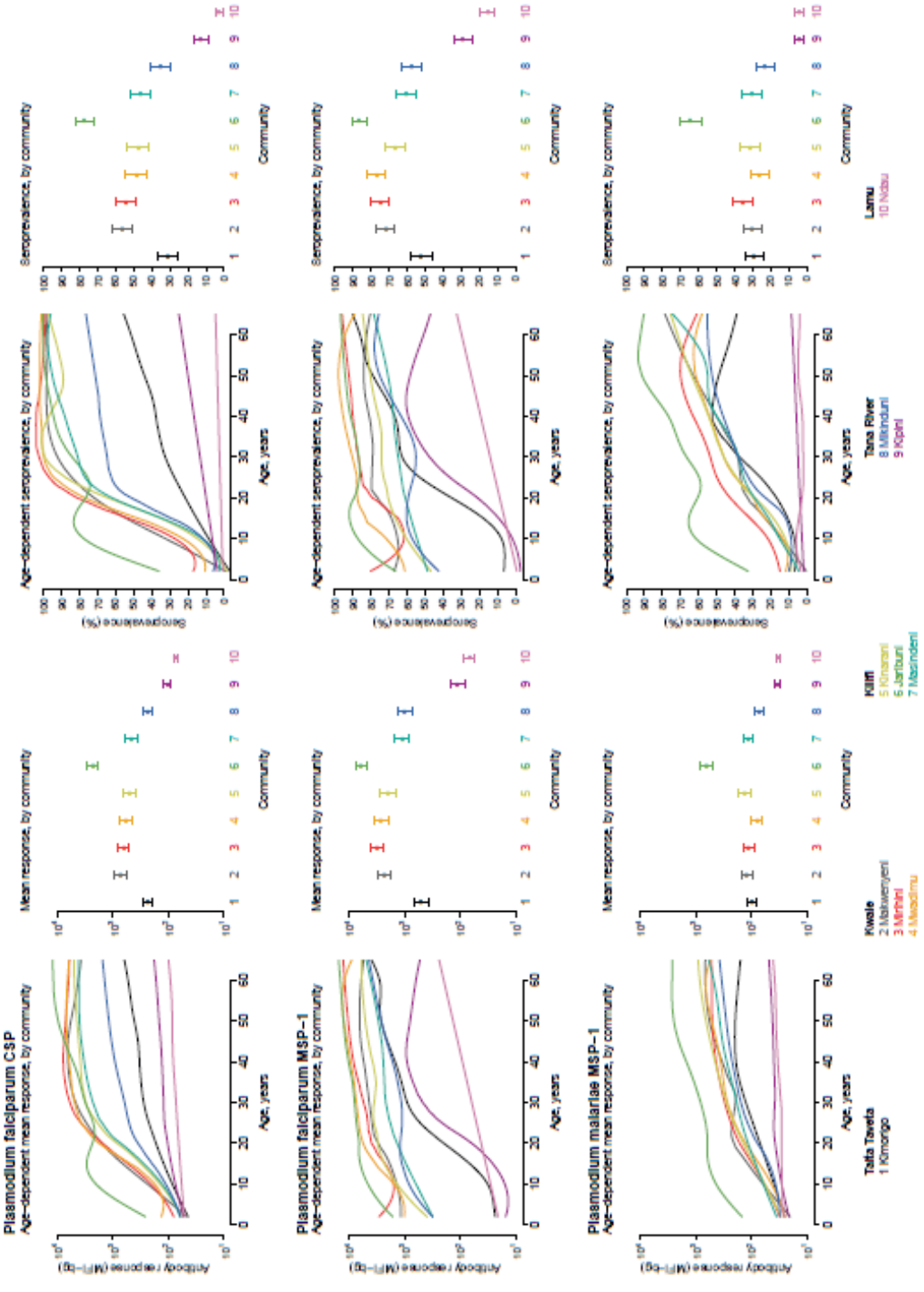


Figure S 9.3 Malarial antibody age-dependent mean response and seroprevalence, stratified by community in Kenya's coastal region, 2015. Community-level mean antibody response and seroprevalence are age-adjusted and error bars represent 95% confidence intervals. Antibody response measured in median fluorescence units minus background (MFI-bg) on a Bio-Rad Bio-Plex platform. The script that created this figure is here: https://osf.io/nhrc2.

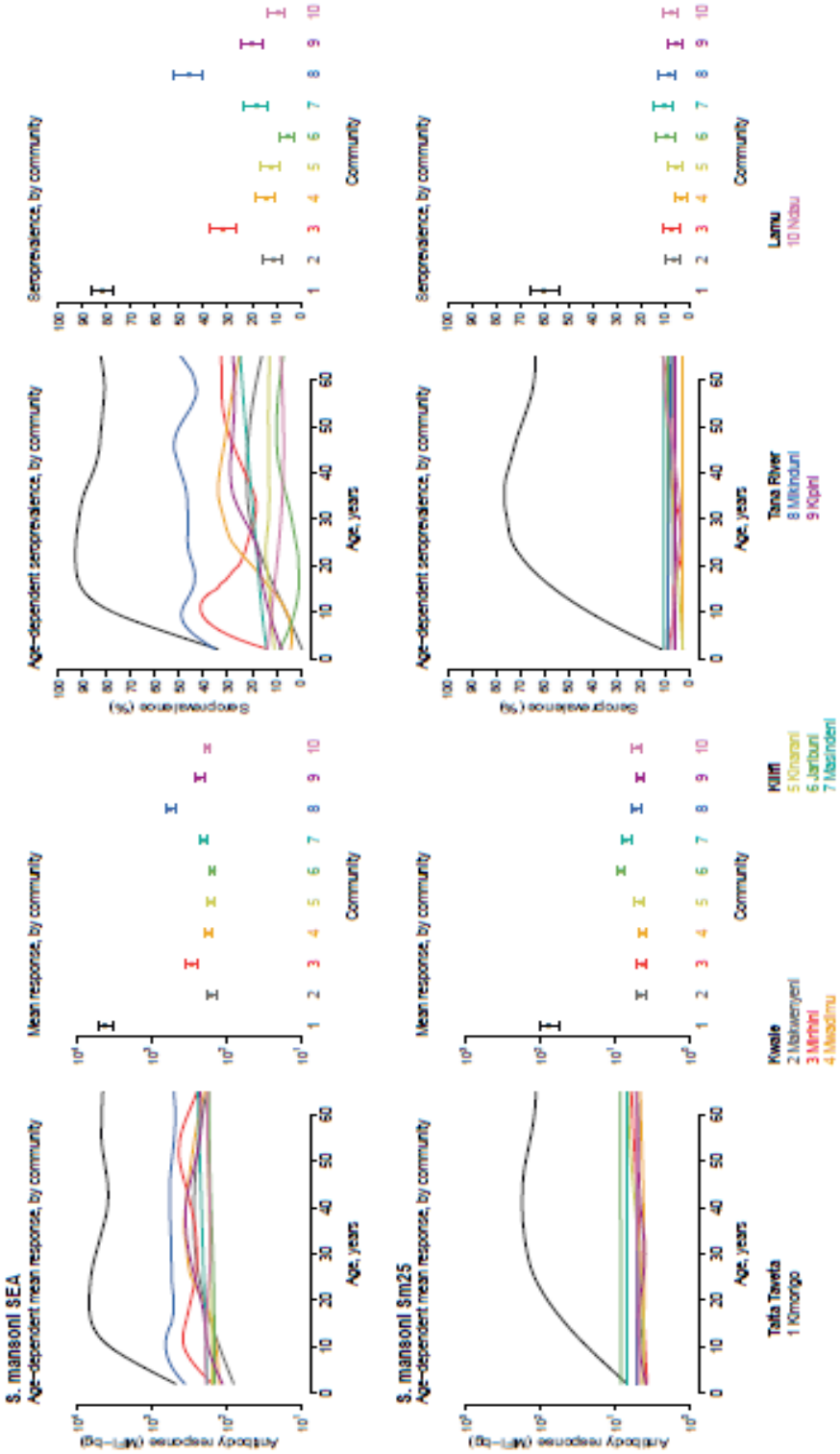


Figure S 9.4 Schistosomiasis antibody age-dependent mean response and seroprevalence, stratified by community in Kenya's coastal region, 2015. Community-level mean antibody response and seroprevalence are age-adjusted and error bars represent 95% confidence intervals. Antibody response measured in median fluorescence units minus background (MFI-bg) on a Bio-Rad Bio-Plex platform. The script that created this figure is here: https://osf.io/z8v4n.

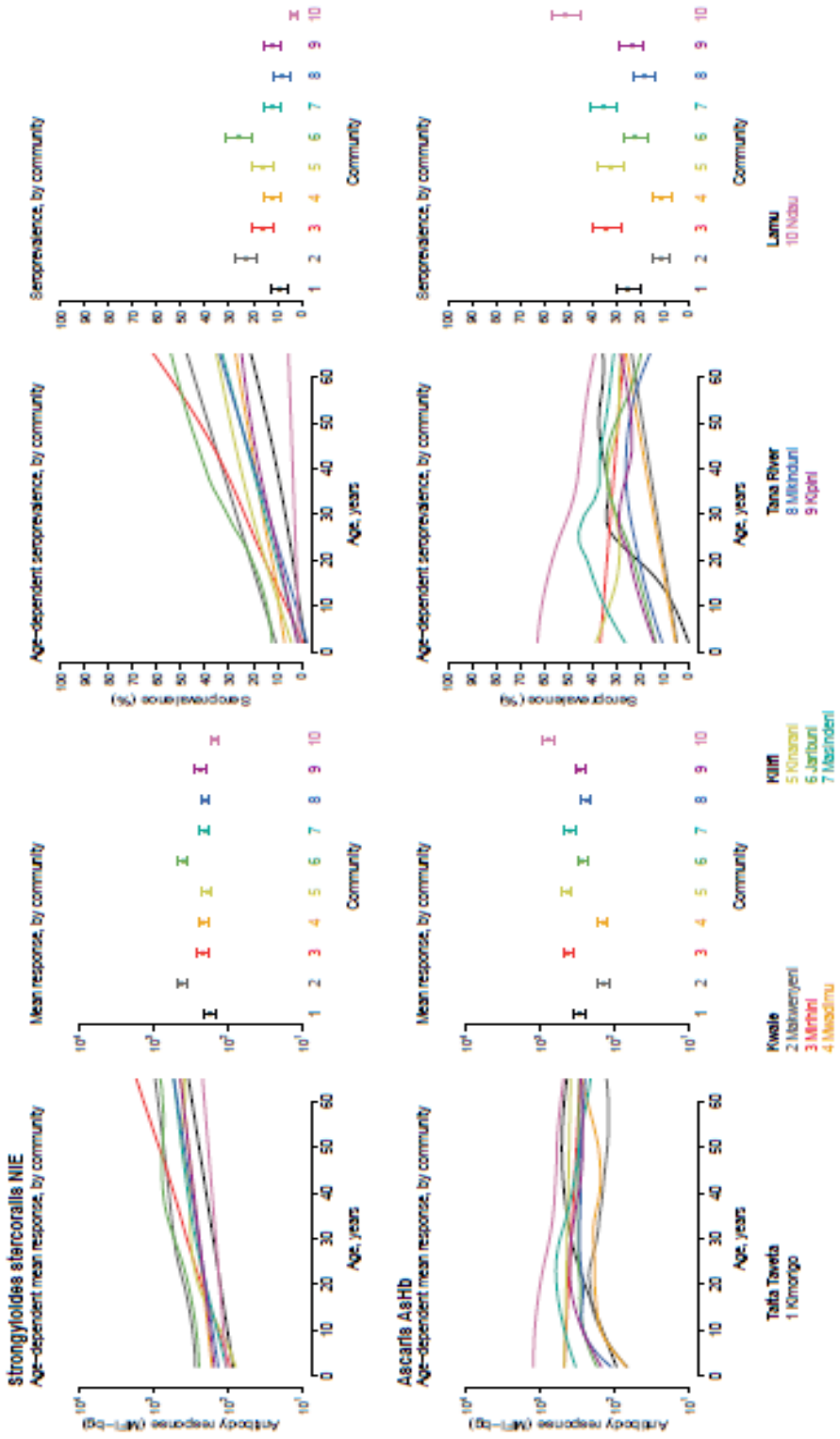


Figure S 9.5 Age-dependent mean response and seroprevalence antibodies to *S. stercoralis* and *A. lumbricoides*, stratified by community in Kenya's coastal region, 2015. Community-level mean antibody response and seroprevalence are age-adjusted and error bars represent 95% confidence intervals. Antibody response measured in median fluorescence units minus background (MFI-bg) on a Bio-Plex platform. The script that created this figure is here: https://osf.io/spnvx.

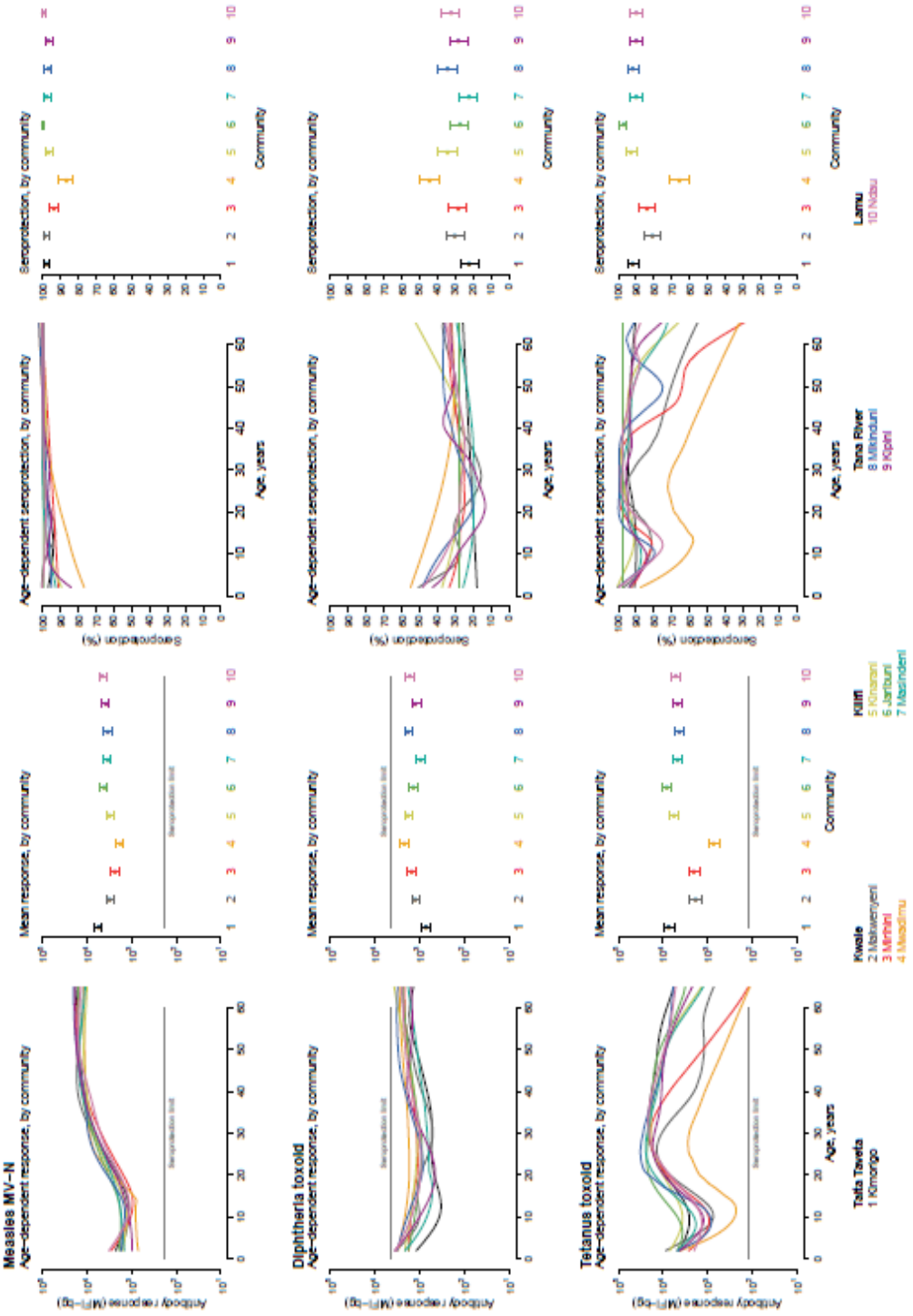


Figure S 9.6 Age-dependent mean response and seroprotection for measles, diphtheria, and tetanus stratified by community in Kenya's coastal region, 2015. Community-level mean antibody response and seroprotection are age-adjusted and error bars represent 95% confidence intervals. Antibody response measured in median fluorescence units minus background (MFI-bg) on a Bio-Rad Bio-Plex platform. The script that created this figure is here: https://osf.io/uy5bf.

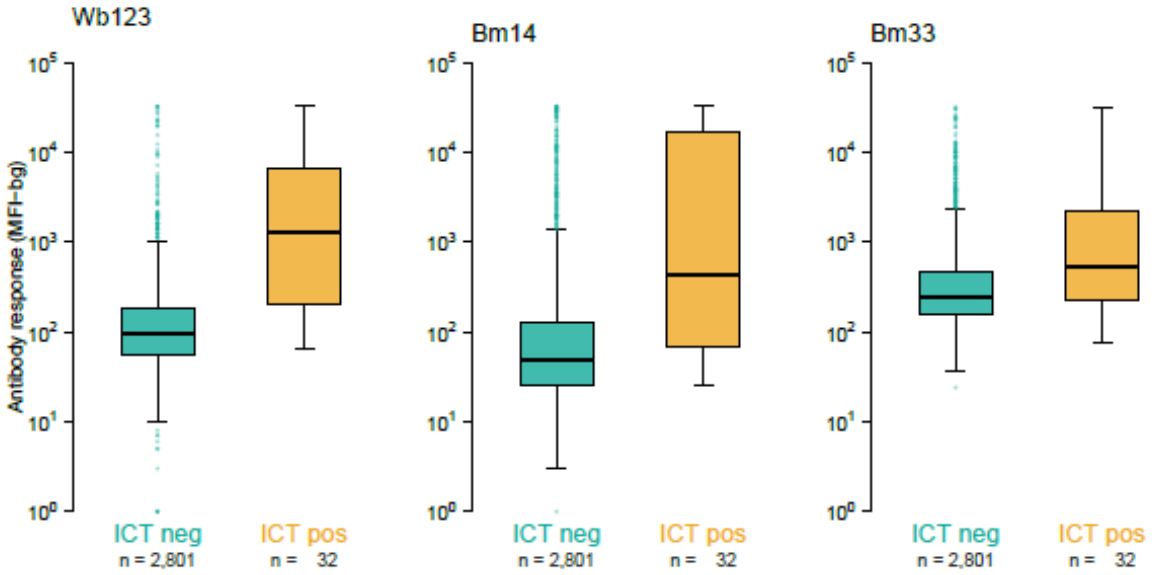


Figure S 9.7 Distribution of three lymphatic filariasis antibodies, stratified by rapid antigen immunochromatographic card test (ICT) results. Boxes mark the median and interquartile range of the distributions. Antibody response measured in median fluorescence units minus background (MFI-bg) on a Bio-Rad Bio-Plex platform. Mann-Whitney U-test $p < 0.0001$ for differences in antibody responses between ICT negative and positive individuals. The script that created this figure is here: https://osf.io/k9tms.

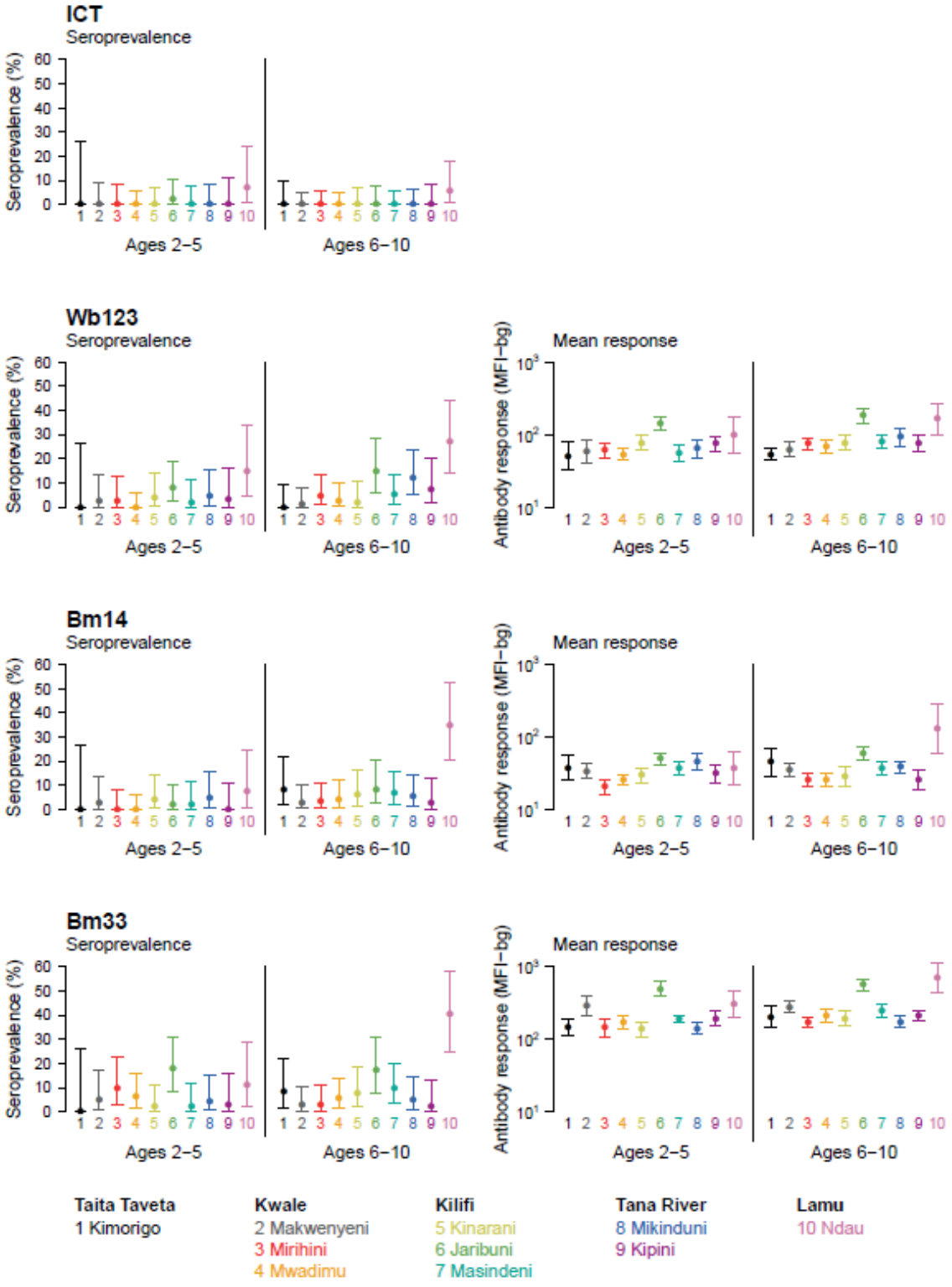


Figure S 9.8 Community-level estimates of lymphatic filariasis seroprevalence and geometric mean antibody levels among children ages 2-5 and 6-10 years old. Child blood samples were tested by immunochromatographic card test (ICT) and three antigens (Wb123, Bm14, Bm33) measured in median fluorescence units minus background (MFI-bg) on multiplex Bio-Rad Bio-Plex platform. The mean number of specimens tested per community within each age stratum was 47 (median=47; interquartile range=39, 58; range 12, 70).

10 Discussion

Prior to the start of GPELF, LF had been documented in 104 countries (Table 10.1) (Sasa, 1976). LF disappeared in more than 30 countries, primarily as an indirect result of economic development, change in environmental conditions, and in a few cases by intentional treatment and control efforts. At the inception of GPELF, the number of LF-endemic countries has been reduced to 81 and subsequently, 9 more countries were determined to have no evidence of transmission. There is little debate that considerable progress has been made during the GPELF era from 2000 to 2016. The number of people at risk for infection was reduced from 1.4 billion in 2000 to 856.4 million in 2016, primarily through MDA programs (WHO, 2017c).By 2016, 20 of 72 LF-endemic countries no longer required MDA. Additionally, 30 of the remaining 52 countries had scaled up MDA to 100% geographic coverage of the areas in need (Table 10.2) (WHO, 2017c). Much of the success can be attributed to the commitment of national governments and donors, but it is important to recognise that these accomplishments were facilitated by the existence of a well-defined M&E framework. With clear guidelines, national programmes are better able to plan activities to reach established targets and to measure the impact of these efforts. While it is important to recognise the progress of these programmes, it is critical to identify challenges and obstacles that may impede reaching the ultimate goal of global LF elimination. The majority of countries and sub-national areas that lag behind are those that were anticipated to be the most difficult from the start of GPELF. These include areas affected by civil strife which prevents consistent implementation of programme activities, complex epidemiological settings where programme activities may need to be modified, and areas with persistent transmission despite multiple rounds of MDA. The recently endorsed IDA MDA regimen (WHO, 2017d) is an example of a new strategy that will likely play an important role in accelerating the progress of programmes. Results from clinical trials demonstrated superiority of IDA in sustaining clearance of MF over a 24-month period compared to the standard two-drug regimens

(Thomsen et al., 2016). However, in a programmatic context, it is impractical to identify all MF-positive individuals in order to monitor clearance of parasites. Furthermore, surveys that are designed to measure changes in MF prevalence require large sample sizes that are not feasible in most programme settings. It is important to understand if alternative indicators can be used that offer more programmatic feasibility. At present, it is unclear if CFA or antibody responses can be used to monitor the impact of IDA, but studies should be undertaken to determine if the current M&E framework needs to be adapted for monitoring the impact of IDA.

Table 10.1 Countries with clinical or parasitologic evidence of LF prior to GPELF

Americas	**Africa**	**Europe**	**Asia**	**Pacific**
Barbados	Algeria	Bosnia and Herzegovina	Bangladesh	American Samoa
Brazil	Benin	Croatia	Cambodia	Australia
Colombia*	Burkina Faso	Hungary	China	Cook Islands
Costa Rica	Cameroon	Italy	India	Fiji
Cuba	Cape Verde	Macedonia	Indonesia	French Polynesia
Dominica*	Chad	Montenegro	Japan	Guam
French Guyana	Comores	Serbia	South Korea	Kiribati
Guadeloupe	DR Congo	Slovenia	Laos	Marianas
Guatemala*	Egypt	Turkey	Malaysia	Marshall Islands
Guyana	Ethiopia		Maldives	Nauru
Martinique	Gambia		Myanmar	New Caledonia
Mexico*	Guinea		Nepal	Niue
Montserrat*	Guinea Bissau		Philippines	Palau
Puerto Rico	Ivory Coast		Singapore	Papua New Guinea
St. Kitts	Kenya		Sri Lanka	Pitcairn
St. Lucia*	Liberia		Thailand	Samoa
Surinam	Madagascar		Timor Leste	Solomon Islands
Trinidad*	Malawi		Vietnam	Tokelau
United States	Mali			Tonga
Venezuela	Mauritius			Tuvalu
Virgin Islands	Mozambique			Vanuatu
	Niger			Wallis and Futuna
	Nigeria			
	Reunion			
	Sao Tome and Principe			
	Senegal			
	Seychelles			
	Sierra Leone			
	Sudan			
	Tanzania			
	Tunisia			
	Uganda			
	Zambia			
	Zimbabwe			

*by clinical signs only

Table 10.2 Status of LF-endemic countries in 2016

MDA not started	MDA started but not at scale	MDA scaled up to all endemic areas	Post-MDA surveillance	Elimination of LF as a public health problem
Equatorial Guinea	Angola	Benin	American Samoa	Cambodia
Eritrea	Cameroon	Brunei Darussalam	Bangladesh	Cook Islands
Gabon	Central African Republic	Burkina Faso	Brazil	Egypt
New Caledonia	Chad	Côte d'Ivoire	Kiribati	Maldives
Sao Tome and Principe	Comoros	Dominican Republic	Malawi	Marshall Islands
South Sudan	Congo	Fiji	Palau	Niue
	DR Congo	French Polynesia	Vietnam	Sri Lanka
	Ethiopia	FSM	Wallis and Futuna	Thailand
	Guinea	Ghana	Yemen	Togo
	Guinea-Bissau	Haiti		Tonga
	Guyana	India		Vanuatu
	Indonesia	Kenya		
	Madagascar	Lao PDR		
	Nigeria	Liberia		
	Papua New Guinea	Malaysia		
	Sudan	Mali		
		Mozambique		
		Myanmar		
		Nepal		
		Niger		
		Philippines		
		Samoa		
		Senegal		
		Sierra Leone		
		Tanzania		
		Timor-Leste		
		Tuvalu		
		Uganda		
		Zambia		
		Zimbabwe		

As MDA begins to scale down in countries where programmes have successfully met established targets, robust strategies are needed to establish a surveillance baseline and to detect any possible recrudescence of infection as early as possible. Since most LF infections are asymptomatic and because of the long delay between infection and the appearance of clinical signs, it is not possible to rely on clinical indicators for surveillance. Therefore, all surveillance for LF must involve active detection of infection. The TAS, currently the only recommended activity during the post-MDA surveillance period, is statistically robust but is not powered to detect changes in CFA prevalence over time. Therefore, the results of TAS 2 and TAS 3 fail to document trends of antigenaemia. Collecting additional evidence using alternative indicators may provide important information that can be used to strengthen monitoring during the surveillance period.

As prevalence declines, it is no longer practical to rely on parasitological indicators, and the necessity to adapt to the changing needs of the programme becomes increasingly important. During the surveillance period it is critical to balance limited resources with practical solutions without compromising the integrity of the programme. Although the detection of CFA serves as a reasonable proxy for infection, antigenaemia becomes increasingly difficult to detect in populations during the post-MDA period (Gass et al., 2012). Evidence suggests that detection of antifilarial antibodies provides the earliest indicator of filarial exposure (Hamlin et al., 2012). Therefore, monitoring filarial exposure through the assessment of antibody responses may provide a useful signal for detecting potential recrudescence.

At present, there are opportunities to refine the M&E framework to adapt to the complex and changing needs of GPELF. As programmes continue to make progress towards elimination endpoints, there is an urgent need to identify sensitive diagnostic tools and robust strategies that can be used to guide programmatic decision making. To address the existing needs, the overarching goal of this PhD thesis was to provide recommendations on best approaches for conducting surveillance for LF elimination programmes. The studies

conducted as part of this thesis were designed to elucidate the two interlinked objectives of determining 1) the utility of serologic tools during the post-MDA surveillance period; and 2) the utility of the TAS and other activities during the post-MDA surveillance period as platforms for integrated disease surveillance. The activities included in this body of work were undertaken in an effort to provide meaningful contribution to GPELF. The studies were conducted in a way that aligned with the guiding principles of Swiss Tropical and Public Health Institute (Swiss TPH) to achieve significant improvements of human health and well-being through research that sought solutions through innovation, validation, and application. Table 10.3 summarises the main findings and contributions of this PhD thesis within the context of the Swiss TPH guiding principles. While the specific outcomes and limitations have been discussed in the respective chapters, a more comprehensive view shall be discussed in this final chapter.

Table 10.3 Overview of the contributions of the studies implemented in this PhD thesis categorized by the guiding principles of Swiss Tropical and Public Health Institute

Chapter	Title	Innovation	Validation	Application
3	Assessment of lymphatic filariasis prior to restarting mass drug administration campaigns in coastal Kenya	Described the challenge of using parasitologic indicators to determine LF programme endpoints and identified the need to identify alternative indicators	Documented the need to resume MDA in some areas	
4	Multiplex serologic assessment of schistosomiasis in western Kenya: antibody responses in preschool aged children as a measure of reduced transmission	Utilised a multiplex bead assay (MBA) to demonstrate that sensitive serologic tools can be used to more accurately monitor the impact of schistosomiasis interventions	Comparison of standard parasitologic methods and MBA over a 3-year period in a programmatic setting; documented high prevalence of schistosomiasis in pre-school aged children	Parasite-specific antibodies among children declined, demonstrating antibody responses can be used to measure the impact of MDA
5	Comparison of antigen and antibody responses in repeat lymphatic filariasis transmission assessment surveys in American Samoa	Utilised antibody tools to determine LF transmission status	Comparison of circulating filarial antigen (CFA) and antibody responses	Antibody results led to the concern that focal areas of transmission existed; the same concern was not raised when examining CFA results alone
6	Use of antibody tools to provide serologic evidence of elimination of lymphatic filariasis in The Gambia			Absence of antibody responses strongly suggests antibody responses will no longer be detectable in populations after transmission has been interrupted; contributed to documenting the elimination of LF in the country
7	Lymphatic filariasis elimination in American Samoa: evaluation of molecular xenomonitoring as a surveillance tool in the endgame	Analysed relationship between presence of filarial DNA in mosquitoes and antibody responses in humans		Responses to Wb123 related to filarial DNA in mosquitoes suggesting that Wb123 can be used as a marker of transmission for surveillance
8	Partnering for impact: integrated transmission assessment surveys for lymphatic filariasis, soil transmitted helminths and malaria in Haiti	Integrated malaria assessment into TAS	Feasible to add malaria testing into existing LF programme platform	TAS can be considered as a platform for integrated surveillance; the malaria programme in Haiti continues to use TAS as a platform
9	Multiplex serologic testing within a cross-sectional lymphatic filariasis sentinel site survey in coastal Kenya reveals community-level differences in IgG antibody responses to parasitic diseases and vaccines	Utilised a single blood sample from a single survey to assess antibody responses to multiple antigens of public health importance	Utilising a multiplex approach allows the generation of more comprehensive assessment of disease burden compared to results from single-disease surveillance	Integrated serosurveillance could greatly facilitate prioritizing areas where interventions are most needed

10.1 Establishing the need for serologic tools for lymphatic filariasis programmes

10.1.1 Determining lymphatic filariasis status in areas of complex epidemiology

The global LF elimination strategy is predicated on the assumption that if antifilarial medicines are delivered consistently over a defined period of time, transmission will be reduced to a level at which it is no longer sustainable even in the absence of MDA. A clear threat to LF elimination is the inability to accurately assess if intervention is warranted. While the WHO-recommended programmatic steps are straightforward, there are settings where the standard methods cannot be applied as designed. In areas where *W. bancrofti* and *L. loa* are co-endemic, standard practice is to treat with albendazole only (Pion et al., 2017; WHO, 2017d). Because of the risk of severe adverse events, ivermectin is administered only after individuals are tested for *L. loa* infection and confirmed to be eligible for treatment. This requires collecting blood at the appropriate time (10:00 to 14:00 hours) (Sasa, 1976) to rule out high *L. loa* MF density. Test-and-treat programmes are resource-intense and are difficult to implement, but this approach is recommended in filarial-endemic areas where there is risk of *L. loa* infection. The inability to include ivermectin as part of the LF MDA package, will likely prolong the time needed to reduce transmission below the target thresholds. Currently, there is a need to clearly map and define the geographic expanse of loiasis and LF, especially the areas where they overlap. One challenge in confirming the presence of LF is the apparent lack of specificity of CFA tests when *L. loa* is present (Wanji et al., 2015; Pion et al., 2016). Consequently, there is a critical need to identify an alternative test that can be used. While detection of MF is an option, low sensitivity of the method coupled with logistical constraints as well as the challenge in distinguishing species of MF make it a poor choice for an alternative platform. In principle, species-specific serologic tests for *W. bancrofti* and *L. loa* could be used to rule in or rule out areas for treatment and reduce the areas where expensive test and treat programmes are needed. Recently, the development of a highly specific recombinant antigen, Wb123, has been shown to be an early marker for *W. bancrofti* infection and may provide the ability to use antibody detection for mapping in *L. loa* areas.

10.1.2 Assessing programme outcomes in areas with irregular programme delivery

In areas where LF programme implementation is not complicated by *L. loa*, a different challenge is introduced when the MDA strategy recommended by WHO is not followed as intended. When there is inconsistent implementation of interventions, it is difficult to discern the underlying reasons for unsuccessful programme outcomes. In some cases, irregular programme activities may be contributing to the inability to reduce transmission below established thresholds. There is evidence that missing a single annual cycle of MDA can reverse progress made to that point (Won et al., 2009). However, perhaps an issue of greater concern is the uncertainty around the apparent absence of transmission when MDA has not been consistently carried out. Since there are recognised risks associated with missing rounds of MDA, ensuring that all epidemiological targets have been met in these settings is paramount. As illustrated in our sentinel site surveys in Kenya (chapter 3), despite irregular implementation of MDA, transmission appeared to be below target thresholds in some areas. It is possible that widespread use of long-lasting insecticidal nets (LLIN) distributed primarily for malaria control may have provided ancillary benefit beyond their intended use. In principle, use of LLINs can reduce exposure to infective mosquito bites (Bockarie et al., 2002; Pedersen and Mukoko, 2002; Odermatt et al., 2008), thus contributing to the reduction and possible interruption of LF transmission.

In the surveys carried out in chapter 3, the absence of CFA among adults in in Kenya suggests transmission had been interrupted, but concerns were raised knowing that the sensitivity of the ICT may be inadequate in low prevalence settings (Gass et al., 2012). This highlights the potential challenge of using traditional parasitologic methods to determine programme endpoints. In a programmatic setting in Sri Lanka, despite meeting WHO-established CFA targets among children and stopping MDA, there was evidence of transmission among older individuals that was supported by the detection of filarial DNA in mosquitoes (Rao et al., 2014). In this setting, CFA prevalence among schoolchildren was well below the threshold (<2%) considered necessary for sustainable transmission. However,

antibody prevalence was >5% in some schools, and elevated antibody levels were found in communities where parasite DNA was detected in mosquitoes. In this case, the use of an antibody indicator instead of an antigen indicator may have led to a decision to conduct targeted treatment or MDA. The ability to use a serologic indicator may provide the necessary evidence to definitively document the presence or absence of transmission. In Kenya, where no antigen- or MF-positive individuals were identified in some sentinel sites, absence of infection-specific antibody responses in children would strengthen the conclusion that interventions were not warranted. As described in chapter 9, quantitative antibody levels among children were higher in areas where CFA-positive adults were identified, whereas assessing transmission status using CFA results in children was less clear because so few children under 10 years of age were antigen-positive. The next step in Kenya will be to implement TAS in the areas where transmission appears to have been interrupted. Since CFA prevalence is expected to be very low, consideration should be given to including antibody testing at that time to ensure MDA is not warranted.

10.2 Demonstrating the utility of antibody tools for neglected tropical disease programmes

10.2.1 Advantages of serology compared to traditional diagnostic methods

Currently, of the NTDs amenable to preventive chemotherapy, only onchocerciasis elimination programmes routinely use a serologic indicator to assess programme performance (WHO, 2016c). The other PC NTD programmes, including LF, face similar challenges in relying on parasitological or clinical indicators that are often insensitive and inadequate for use during the late stages of programmes. As prevalence declines, tools with greater sensitivity and specificity are needed to detect residual infections and will allow more conclusive demonstration that transmission has been interrupted. Additionally, more robust tools will provide early signals of any recrudescence that may arise during the surveillance period. As a result, there is increasing effort to investigate the use of more sensitive serologic tools for programme decision-making. For example, trachoma elimination programmes

currently rely on the use of a clinical indicator to determine the need for MDA. This requires expertise in examining eyelids for follicular inflammation caused by infection with *Chlamydia trachomatis* (Solomon et al., 2004). As prevalence declines, it becomes increasingly difficult for graders to maintain the skills needed to accurately identify disease-related pathology. Furthermore, it is possible for clinical signs of trachoma to persist after infection has cleared (Martin et al., 2015; Macleod et al., 2016; Butcher et al., 2018). Reliance on a non-specific clinical indicator may unnecessarily initiate or prolong MDA in areas where it is not needed. However, there is evidence that measuring antibody responses may provide a more objective way to assess the need for treatment. In Tanzania, children with more intense ocular pathology had higher antibody responses than others, and 44% of children with no apparent ocular pathology had antibody responses to trachoma antigens (Goodhew et al., 2012). Additionally, antibody responses appear to decline after treatment (Goodhew et al., 2014), providing opportunities to evaluate changes in antibody responses to monitor the impact of MDA.

Similar deficiencies and needs exist for schistosomiasis control programmes. Currently, programmes are monitored primarily by assessing parasitological indicators. However, as prevalence declines, there are similar declines in sensitivity of diagnostic methods that are routinely used (Bergquist et al., 2009; Nikolay et al., 2014; Mwinzi et al., 2015; Utzinger et al., 2015) making it arduous to monitor the impact of MDA. In chapter 4, our findings in western Kenya provide another example of the potential to use serologic responses to guide programme activities. In this setting, significant changes in antibody prevalence were observed among the youngest children, and suggest that exposure from infection with *Schistosoma mansoni* had been reduced. Although the children enrolled in the study were too young to be eligible for routine MDA programmes, they served as an appropriate age group to monitor for incident infections. Our results documented that MDA led to reduced parasite-specific antibodies among children, demonstrating the value of using serologic tools for surveillance as programmes reduce infection prevalence. Additionally, the

relative ease of collecting finger stick blood samples compared to the difficulty of collecting stool samples provides another reason to further explore the use of antibody tools, especially when testing young children. In the examples of schistosomiasis control and trachoma elimination, similarities across the programmes illustrate the limitations of traditionally used indicators and provide support for the general principle that sensitive serologic tools can be used to more accurately monitor the impact of interventions.

10.2.2 Using serology to determine transmission status

LF programmes also face similar challenges with available diagnostic tools that become much more apparent as prevalence declines. Although CFA is likely an appropriate indicator for making decisions to stop MDA through TAS, the inability to measure changes in antigen prevalence in subsequent TAS (TAS 2 and TAS 3) highlights the need to identify more sensitive diagnostic tools. Failing TAS conducted after stopping MDA presents significant challenges to national programmes as they are forced to mobilize resources to restart treatment. Since WHO guidance for surveillance does not exist beyond TAS 3, these surveys represent opportunities to collect information needed to detect ongoing transmission and to support the programmatic decision to re-start MDA, if needed. As presented in chapter 5, in American Samoa, all antigen-positive children identified during TAS had detectable antibody responses to all three markers (Bm14, Bm33, and Wb123) used, but there were also antigen-negative children who had positive antibody responses. It is possible that antibody-positive children were born before transmission was interrupted and that positive results represented a slow rate of decay of residual antibodies; alternatively, these responses may reflect ongoing transmission. In areas endemic for *W. bancrofti*, there is evidence that responses to Bm14 decline slowly after treatment. In Egypt, asymptomatic microfilaraemic adults were tested for antibody responses to Bm14 after repeated annual treatment with DEC and albendazole. Antibody levels cleared in 20%, 35%, 39%, and 53% of individuals after 12, 24, 36, and 48 months, respectively (Helmy et al., 2006). However, results from a separate study of children in Egypt showed that Bm14 responses among children had nearly cleared

after five rounds of MDA (Ramzy et al., 2006). Similar findings from children in Haiti support the observations from Egypt. Bm14 responses significantly declined after treatment with DEC or DEC + albendazole, whereas there was no significant change among children given a placebo (Moss et al., 2011).

Although no MDA was ever conducted in The Gambia, our findings in chapter 6 support the conclusion that antibody responses will no longer be detectable in populations after transmission has been interrupted. Responses to Bm14 were nearly absent among most age groups, suggesting LF transmission had stopped in The Gambia. However, we were unable to determine the exact timeframe when transmission had ceased. Since the only evidence of Bm14 positivity was among individuals aged 50 years and above, we assume transmission had been interrupted sometime after these individuals were born. Furthermore, the overall prevalence of positive Wb123 responses was low (1.5%), and there were no antibody-positive individuals identified in 7 of 15 villages. While the absence of seropositive individuals in populations strongly suggests the absence of transmission, assessing antibody responses in adults may underestimate the impact of interventions as antibody responses may persist for years after transmission has been broken. The slow clearance of Bm14 responses in adult populations has important implications with respect to the suitability of using this marker to monitor changes.

Because Wb123 was first described in 2011, limited information exists on the characteristics of responses to this marker in programme settings. As observed in the Cook Islands, results from samples collected 17 years apart indicated a significant decrease in Wb123 antibody positivity, suggesting LF transmission had significantly decreased (Steel et al., 2012). While there is limited information about the persistence of Wb123 responses, it appears that individuals will serorevert after clearing infections. Ideally, antibody markers used for surveillance will appear soon after exposure to parasites and will decline quickly after infection clears. While there is still a need to better define relative rates of decay of Wb123 responses, our results in chapter 7 indicated a significant relationship between the

presence of filarial DNA in mosquitoes and villages with individuals with responses to Wb123. However, the same relationship was not observed between positive mosquitoes and Bm14-positive individuals. Thus, responses to Wb123 may be an indication of ongoing transmission. In chapter 9, Wb123 prevalence in Kenya was low in sites that had an absence of CFA-positive individuals. In contrast, in other sites, elevated antibody levels among children was consistent with evidence of ongoing transmission. Furthermore, although it was not possible to directly compare Wb123 results from TAS 1 and TAS 2 in American Samoa (chapter 5), the apparent increase in Wb123 positivity added to the concern that focal areas of transmission existed. The same concern was not raised when examining CFA results alone. In this setting, antibody responses in young children may have been an early warning signal that was not detected by CFA. This concern was substantiated when results from TAS 3 conducted in 2016 indicated antigen prevalence was above the threshold at which transmission is sustainable. Regardless of whether the TAS 3 results represent premature stopping of MDA or a recrudescence of transmission, the effort to restart MDA will carry significant political and financial consequences. It is possible that this outcome could have been avoided if antibody responses were taken into consideration at the time of TAS 1 and TAS 2.

Based on our results from The Gambia, Kenya, and American Samoa, there is likely a role for antibody tools during the surveillance period. In these settings, the absence of antibody responses strongly suggested interruption of transmission, and persistence of responses among children indicated potential risk of transmission. In all cases, antibody tools appeared to be more sensitive than antigen tools. Furthermore, if seroreversion can be expected after clearing infection, there are options to design surveillance strategies that incorporate serologic tools. Because seroreversion is expected to take a long time in adults, children may be the appropriate population to monitor during surveillance. However, it is important to note that the absence of seropositive adults is a strong indication, if not definitive evidence that transmission has been interrupted.

10.3 Platforms for integrated disease surveillance

Although the focus of this thesis was concentrated primarily on LF, our results should be considered in a broader context and not just that of a single disease. Often, NTD and other public health programmes face significant resource constraints, especially during the surveillance period. Integration of surveillance activities may provide cost-effective approaches for monitoring multiple programmes, and our work in Haiti (chapter 8) and Kenya (chapter 9) highlighted the potential value of conducting integrated surveillance.

The LF TAS represents one potential platform for integrated activities, and this is facilitated because these surveys are currently being implemented globally as part of a standardised WHO framework. The ability to leverage resources to conduct a single survey represents a responsible way for programmes to gather information needed for public health interventions. For example, in the Pacific Islands where populations are scattered across multiple islands, the cost and effort needed to reach these populations is significant. When activities are coordinated, there is the potential to reduce the number of visits required to specific communities. Furthermore, the ability to collect a single sample (e.g. blood) will not only reduce cost, but will also be less disruptive to the target population.

WHO guidance already exists for including soil-transmitted helminthiasis (STH) assessments in TAS. Since albendazole is delivered as part of the LF MDA package and is also used by STH control programmes, multiple rounds of MDA have likely had an impact on the prevalence of STH infections. As successfully passing TAS results in stopping MDA, it is important to understand the burden of STH that remains. While coordinated LF and STH assessments provide useful programmatic information, there are some drawbacks to the co-implementation of these surveys. The different sample types needed, blood versus stool, add complexity to the planning and logistics of the survey. More feasible opportunities may exist around the ability to use a single sample type. In Haiti (chapter 8), the common goal of elimination led to the natural collaboration of the LF and malaria programmes. By collecting a

single blood sample both programmes were able to leverage a single survey to collect information for both diseases.

Areas co-endemic for LF and onchocerciasis illustrate another example where integrated TAS may be advantageous. Since ivermectin is delivered through MDA by both programmes, the TAS presents an opportunity to assess the impact the interventions have had on both diseases. In principle, the ability to discriminate between onchocerciasis- and LF-specific antibody responses could enable programmes to design programme activities as appropriate. Additionally, historically, onchocerciasis control programmes focused treatment efforts primarily in areas of high transmission only. As a result, areas of low transmission have routinely been excluded from programmatic activities. However, some of these areas may have been inadvertently treated through the LF MDA. As the goal of onchocerciasis programmes has shifted from morbidity control to elimination of transmission, there is an urgent need to identify all areas that require treatment. The inclusion of onchocerciasis-specific antibody tests in the TAS may provide insight into areas where the onchocerciasis status was previously unknown and may help to define the treatment requirements for these settings.

TAS represents just one opportunity to integrate programmatic assessments. While this platform should be considered for multi-disease activities, it is important to recognize that this structure is not permanent. As LF programmes continue to meet criteria to reach validation of elimination, fewer TAS will be conducted globally each year and will eventually stop altogether. As a result, LF programmes should begin to explore opportunities for integration with other public health programmes and systems. In Cambodia, a nationally representative survey to assess immunity to vaccine preventable diseases (VPD) was used as a platform to integrate serologic assessment of multiple diseases including LF (Priest et al., 2016). In chapter 9, samples collected in Kenya were tested by MBA for detection of antibodies against multiple antigens from several parasitic infections as well as markers for immunity to VPDs. While the multiplex technology that was used is unlikely to be feasible for

routine surveillance activities, our results demonstrated the value of integrating assessments for multiple diseases. Results from these types of activities could greatly facilitate prioritizing areas where interventions are most needed. With the appropriate diagnostic tools, routine sample collection in public health activities could provide the access to populations that could be a surveillance platform for LF. For example, malaria indicator surveys or routine demographic health surveys are two existing platforms that provide access to representative populations.

10.4 Future considerations and research needs

The work presented in this thesis outlines the utility and potential value of using antibody tools when conducting surveillance for LF. However, some knowledge gaps were identified during the course of activities conducted to date. The following issues should be considered when planning and conducting future studies:

- **Kinetics of antibody responses** - our results did not allow us to clearly define the kinetics of antibody responses. This will likely be important as programmes adopt the newly WHO-endorsed IDA strategy. At present, it is unclear if the standard CFA indicator used for TAS will be appropriate for decision-making in IDA settings, and there may be a role for antibody tools in this context. It is important to recognize that seroreversion will occur after transmission has been interrupted, but the rate at which this occurs is not well understood. To further refine recommendations for the use of antibody tools, future work should be conducted to characterize the rate at which antibody responses can be expected to decline in populations after MDA.
- **Relationship between antibody responses and other indicators** - there is a need to clearly define the relationship between antibody responses in populations to other indicators including CFA, MF and filarial DNA in mosquitoes. This thesis has described the limitations of using parasitologic indicators when prevalence is low, so it is important to identify alternative indicators that can feasibly and reliably be used for

surveillance. It is unlikely that sustainable platforms can be built on the collection and testing of mosquitoes, so tools to assess infection in humans will be necessary.

- **Defining antibody thresholds for programme decision making -** our results suggest that Wb123 may be a transmission marker, thus making this the ideal candidate for a surveillance tool. If the relationship between Wb123 prevalence and other indicators can be clearly defined, antibody thresholds can be established below which transmission in not expected to be sustainable.
- **Defining spatial distribution of antibody responses** - although the TAS design is robust, there are known limitations when using cluster-based sampling for focal diseases. However, there may be opportunity to use spatial distribution of antibody responses to identify hotspots of transmission. Future research should include spatial analyses to understand if antibody responses can be used to define areas of risk.
- **Optimal diagnostic tool formats** - in addition to identifying the appropriate indicator(s), it is important to consider the format and performance characteristics of the test(s) to be used. While the MBA platform presented in this thesis has provided examples of the value of its use, alternative platforms are needed to ensure programmatic feasibility across all settings. More field-friendly simplified platforms will likely be required. While there are some settings where laboratory-based tests like ELISA can be relatively easily incorporated, the availability of a sensitive and specific RDT will be important to support the programmes. The ability to generate recommendations at the point of contact not only assists the programme in timely decision-making, but also builds trust in the population as samples are not removed from communities with a significant time lapse before results are provided back to the community. As another research need, the ability to accurately define sensitivity and specificity of serologic assays can be challenging and is often limited by the availability of well-characterized panels of samples. Cutoff values are routinely determined using sera from infected individuals and presumed negative sera from

persons with no known exposure to LF. However, positive samples used to define the performance characteristics of assays are usually collected pre-MDA or during the period of intervention and may not necessarily represent the same antibody profiles as samples collected post-MDA. Efforts should be made to collect samples from the appropriate programmatic stage to validate diagnostic tools intended for that stage.

- **Identifying new markers of infection** - there is opportunity to investigate new candidate antigens that can complement or replace existing tools. At a minimum, an additional tool could be used as a confirmatory test which would strengthen the basis on which decisions are made. Target product profiles will be needed to define the characteristics required of a confirmatory test, and consideration should be given to identify the most appropriate isotypes and isotype subclasses to use for surveillance. Previous assumptions have been made on the use of IgG4 as the most appropriate surveillance target. However, it known that IgG4 responses develop later than some other isotypes and IgG subclasses. In the surveillance period, it is important to use sensitive and specific tools that will provide the earliest detection of potential recrudescence.

Addressing these limitations will help to define and refine optimal approaches for conducting LF surveillance.

10.5 Policy implications

To sustain progress that has been made to date and to reach the ultimate goal of LF elimination, it is imperative that the M&E framework be adapted to the changing needs of the LF elimination programme. It is important to recognise that investments in field and laboratory research over the years have supported the development and refinement of important programme policies and guidance since the start of GPELF (Weil et al., 1997; Gass et al., 2012; Chu et al., 2013; Weil et al., 2013; Gass et al., 2017; Lammie et al., 2017). Despite the limitations of our work, our findings can help to reshape and strengthen the existing M&E framework. Potential policy implications related to our results include:

- **Recommendation to include antibody tools in existing programme activities (e.g. TAS)** - our results demonstrated the value of using serologic tools for surveillance as programmes reduce infection prevalence. Sensitive serologic tools can be used to more accurately monitor the impact of interventions.
- **Recommendation to conduct integrated surveillance when feasible** – our work in Haiti and Kenya illustrated the feasibility of utilising the LF programme platform to conduct integrated assessments, reducing the need to carry out single-disease surveillance activities.

11 Conclusions

Although there is a need to better understand existing limitations, our results support the use of antibody tools to determine the status of LF transmission and suggest that serologic tools can have a role in guiding programmatic decision making. Furthermore, existing LF programme activities can provide a platform to conduct integrated assessments. Specifically, the conclusions substantiated through the findings presented in this thesis are as follows:

- As prevalence declines, using parasitologic indicators to determine LF programme endpoints becomes challenging and there is a need to identify alternative indicators to use during the surveillance period.
- Antibodies to *Schistosoma* spp. antigens among children declined after MDA, demonstrating antibody responses can be used to measure the impact of treatment.
- The absence of antibody responses in The Gambia strongly suggests LF transmission has been interrupted.
- The presence of antibody responses among children in American Samoa led to the concern that focal areas of LF transmission existed; the concern was confirmed with a TAS 3 failure in 2016.
- TAS can be considered as a platform for integrated surveillance; the malaria programme in Haiti continues to use TAS as a platform.
- Antibody responses to Wb123 among adults in American Samoa related to the presence of filarial DNA in mosquitoes, suggesting that Wb123 can be used as a marker of transmission during the surveillance period.
- It was feasible to add malaria testing to TAS in Haiti indicating that TAS can be considered a viable platform for integrated surveillance.
- In Kenya, utilising a multiplex approach, antibody responses to 10 antigens representing six parasitic infections and 3 antigens to assess immunity to vaccine preventable diseases were generated from a single sample collected from each

participant; integrated serosurveillance could greatly facilitate prioritizing areas where public health interventions are most needed.

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