Assessing The Role of Anti-Filarial Antibody As A Community Infection Indicator In Areas Treated With Double- or Triple- Mass Drug Administration In Quartier Morin Haiti

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ABSTRACT

ASSESSING THE ROLE OF ANTI-FILARIAL ANTIBODY AS A COMMUNITY INFECTION INDICATOR IN AREAS TREATED WITH DOUBLE- OR TRIPLE- MASS DRUG ADMINISTRATION IN QUARTIER MORIN HAITI

By

KERI LYNN ROBINSON

DECEMBER 13, 2019

In the global effort to eliminate lymphatic filariasis (LF) additional treatment regimens like triple drug therapy and diagnostic tools like Wb123 anti-filarial antibody have become increasingly available. This study aimed to look at two components, time and treatment regimen, in order to assess prevalence of anti-filarial antibody and its programmatic potential in the evolving Global Programme to Eliminate Lymphatic Filariasis (GPELF) setting. At year 0, 2,394 (47.4%) samples were collected from participants before mass drug administration (MDA) was administered and 2,656 (52.6%) were collected 12 months later (year 1). Blood samples from 4,939 (97.8%) participants 5 years of age and older were tested for antigen status by filariasis test strip (FTS) and dried blood spots (DBS) from 4,740 (93.9%) participants were tested for Wb123 antibodies. Older individuals were more likely to be both antigen and antibody positive than younger children. Our results show overall and across most age groups that antibody prevalence was higher than antigen prevalence for both drug arms. Individuals living in localities treated with the double drug regimen had 66% greater chance of demonstrating declining antibody prevalence than those living in localities treated with the triple drug regimen. The results of this investigation support treatment with both MDA regimens and the addition of antibody testing as a surveillance tool. Our results have contributed to the evidence base surrounding population-level antibody data and help to inform programmatic decision making around treatment strategies.
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1.0 INTRODUCTION

1.1 Background

Endemic in 72 countries, lymphatic filariasis (LF) is a mosquito transmitted parasitic disease with more than 1.3 billion people at risk globally, making it the second most common vector borne disease behind malaria.\(^1\)\(^2\) Transmitted via bites from *Anopheles, Culex, Aedes* or *Mansonia* mosquitoes, LF is a parasitic infection caused by three filarial nematodes *Wuchereria bancrofti, Brugia malayi* and *Brugia timori*.\(^3\) Adult worms reside in the lymph system of human hosts and infection can lead to lymphedema, elephantiasis and hydrocele or breast/vulva enlargement, making LF the second leading cause of long-term disability and responsible for an estimated 1.19 million DALYs.\(^2\)\(^4\)\(^5\)\(^6\) Once mature, adult female worms release microfilariae (Mf) into the bloodstream allowing for the proliferation of the disease in the individual as well as transmission among the population when Mf are picked up by mosquitoes during blood meals from infected individuals.

In 1997 at the 50\(^{th}\) World Health Assembly, a resolution was passed to eliminate LF as a public health problem by 2020.\(^7\)\(^8\) To accomplish elimination goals across the 72 countries declared LF-endemic, the Global Program to Eliminate Lymphatic Filariasis (GPELF) was formed. From program inception in 2000 through the end of 2018, annual mass drug administrations (MDA) have been implemented in 69 countries delivering more than a cumulative 7.7 billion treatments.\(^1\) GPELF has made significant progress; MDA has stopped in 24 countries of which 14 countries have met all criteria for validation of elimination of LF by the World Health Organization (WHO).\(^1\)

As 2020 nears, there are several countries that have faced challenges completing MDA and will not achieve established elimination criteria by the target date;\(^9\) A new treatment strategy could help accelerate progress towards elimination of LF as a public health problem. Currently, MDAs are conducted using a two-drug regimen. In areas potentially co-endemic with onchocerciasis, ivermectin and albendazole are provided. In areas without risk of onchocerciasis, diethylcarbamazine (DEC) and albendazole (DA) are provided. Recent clinical trials in non-onchocerciasis endemic countries have indicated providing all three drugs may reduce the number of annual MDAs needed to reach elimination. A single dose of three drugs (ivermectin, DEC and albendazole (IDA)) in combination yields complete clearance of microfilaremia from the blood for at least one year among more treated individuals than the standard double drug (DEC and albendazole) regimen.\(^10\)\(^11\) Adopting the IDA regimen likely means fewer rounds of MDA would be necessary to reach elimination, due to sustained reduction of Mf.\(^12\)
These studies’ conclusions have led to the revision of WHO guidelines for LF MDA to include triple drug treatment.\textsuperscript{13}

This is particularly relevant for Haiti, a country that has faced decades of adversity that has delayed LF elimination. The Ministry of Health and Population (MSPP) in Haiti began The National Program to Eliminate LF (NPELF) with the first MDA in 2000 and five successful rounds in the following consecutive years; however civil strife and lack of funding prevented additional rounds of MDA, necessary in highly endemic regions.\textsuperscript{14, 15} Sentinel site surveys in 2007 indicated that gap in MDA set Haiti’s program back by at least two years, as evident by significant recrudescence.\textsuperscript{14} As Haiti began to scale up MDA to include more communes, a devastating earthquake occurred in 2010 that killed more than 300,000 people and led to a nationwide cholera outbreak.\textsuperscript{15} It was not until 2012 that Haiti achieved 100\% geographic coverage with MDA, though by this time it was clear five rounds may not be enough to interrupt transmission in high prevalence areas, and new methods should be investigated to improve the effectiveness of MDA.\textsuperscript{15} By 2015, the endemic commune Quartier Morin, located in the Northern Department, had received seven consecutive rounds of MDA but still had not met the threshold for stopping treatment. In 2016 it was the location of a clinical trial to assess the safety of triple drug MDA.\textsuperscript{16} This clinical trial included a cross sectional study collecting baseline data on lymphatic filariasis biomarkers of infection in the community. In 2017, a cross sectional survey was conducted in the same communities as the prior year in order to monitor the impact of triple drug therapy relative to double drug therapy provided one year earlier during the safety trial.

Currently, programmatic decision-making regarding transmission interruption and stopping MDA is determined by measuring two biomarkers, Mf and circulating filarial antigen (CFA) antigenemia. Presence of Mf in peripheral blood is biological evidence of active infection with viable reproducing adult worms, however detecting Mf is programmatically difficult for several reasons. In most LF-endemic countries, Mf circulate with nocturnal periodicity, meaning blood collection must take place between 10pm and 2am. Additionally, Mf are identified by examining stained blood smears, a technique that requires highly trained microscopists. Measuring Mf is the most specific tool available but the least sensitive;\textsuperscript{17, 18} negative Mf smears do not indicate absence of infection.\textsuperscript{19, 20} As transmission declines, infected individuals are expected to have lower density [Mf] infections, further decreasing sensitivity of detection.

The other recommended biomarker, CFA, is measured using a field-deployable rapid diagnostic lateral flow assay test, the filariasis test strip (FTS) (Abbott, Lake Forest, IL). Measuring CFA alleviates
the need for both highly trained microscopists and inconvenient night blood collections, however presence of CFA is only a proxy for infection. The presence of CFA does not guarantee there is a mating pair of worms producing Mf. Furthermore, antigenemia has been shown to persist in individuals long after treatment and sustained Mf clearance; making interpretation of antigenemia more challenging when making decisions about stopping IDA-MDA due to fewer necessary rounds of treatment. Additionally, the FTS is currently the only WHO recommended antigen test and has proven problematic during field implementation. It is frequently unreliable, difficult to obtain, and subjective to interpret further complicating the ability to generate high quality data.

The benefits and drawbacks of each test alone are concerning, but when used in concert they can be implemented with a reasonable expectation of quality data generation. Expanding the program tool portfolio to include antibody testing may strengthen the information collected and lead to higher quality data. While not currently routinely used in LF elimination programs, anti-filarial antibody tools have been shown to be useful in low-transmission settings.\textsuperscript{21,22} Identifying a steady downward trend in antibody prevalence among a population is a strong indication that transmission is declining. However, with the implementation of triple drug MDA and the potential subsequent decrease in the number of years of treatment, it is important to assess the role of anti-filarial antibody responses at the population level to determine if it is an appropriate marker to use to make programmatic decisions.

This study aims to look at two components, time and treatment regimen, in order to assess prevalence differences of anti-filarial antibody and its programmatic potential in the evolving GPELF setting. Using data collected in Haiti from both the baseline pre-IDA study in 2016 and the follow up survey conducted 12 months later, we will have two time points, before and after MDA. The MDA conducted in 2016 as part of the clinical trial included localities treated with both the standard double drug regimen (DA) and the new triple drug regimen (IDA), allowing us to directly compare similar populations receiving different treatment regimens. The results of this analysis will add to the knowledge base surrounding population-level antibody data and help inform programmatic decision making around treatment strategies.
1.2 Research Question:

Using two repeated cross-sectional studies from communities in Quartier Morin, Haiti, compare anti-filarial antibody to other current biomarkers (circulating filarial antigen antigenemia) in order to determine its suitability to assess population prevalence differences between double- and triple- drug mass drug administration one year after treatment.

1.3 Expected Results:

The a priori hypotheses for this analysis address three variables: drug treatment arm, time, and participant age. First, the population prevalence is expected to be different across the two drug treatment arms: triple-drug IDA treated localities and double-drug DA treated localities. Next, it is expected that one year will not be enough time to see a significant change in anti-filarial antibody prevalence at the population level. Last, antibody prevalence will be associated with age.

2.0 REVIEW OF THE LITERATURE

2.1 Causative Agents and Biology

Lymphatic filariasis (LF), a human helminthiasis infection, is caused by three different parasites, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* are thread-like worms called nematodes that live in the human lymphatic system. The parasites are transmitted person to person through the bite of an infected mosquito. Four main genera of mosquitoes are responsible for LF transmission, *Aedes*, *Anopheles*, *Culex* and *Mansonia*. The life cycle of LF parasites is relevant to the epidemiology of disease transmission. When infected mosquitoes take a blood meal they deposit the infective stage of filarial larvae into the bite wound of the human. The larvae then develop into adult worms and reside primarily in the lymphatic system. Adult worms live for approximately 6-8 years; throughout their life span, male and female worms mate and produce millions of sheathed microfilariae (Mf). The Mf circulate with nocturnal periodicity (except in the South Pacific) in peripheral blood. At this point, a mosquito taking a blood meal can ingest sheathed Mf. The life cycle continues in the mosquito as the Mf lose their sheaths and migrate towards the midgut of mosquito. In the midgut the Mf develop from first-stage larvae (L1) through third-stage larvae (L3), the infective stage. L3 larvae then migrate to the mosquito proboscis allowing for the life cycle to begin again as the mosquito takes another blood meal and deposits the
infective larvae into the human bite wound.\textsuperscript{17} Because the life cycle requires development in both vector and host for the parasite to mature, the effectiveness of transmission is determined by the agent/vector combination.

Understanding the relationship between agent and vector provides insight into how transmission propagates in different areas of the world. Bancroftian filariasis is responsible for 90\% of LF infections worldwide; \textit{W. bancrofti} parasites are transmitted primarily via \textit{Culex} mosquitos in urban and semi-urban settings, \textit{Anopheles} mosquitos in rural settings of Africa and the Americas and \textit{Aedes} mosquitos in the Pacific islands. Brugian filariasis makes up the remaining 10\% of infections in the geographically limited region of South Asia; primarily India, Indonesia, Malaysia and Thailand.\textsuperscript{17,23} \textit{Brugia} spp. are primarily transmitted by \textit{Mansonina} mosquitos and occasionally \textit{Anopheles}.\textsuperscript{23} \textit{Aedes} mosquitos are aggressive day biters, potentially exposing individuals to LF parasites in their workplace or school. \textit{Culex} mosquitos are night biters, possibly increasing risk of infection for individuals without a bed net in their home. \textit{Anopheles} mosquitos bite primarily at dusk and dawn with some night time activity.

The south pacific provides a unique example of this relationship. \textit{Aedes} is the common vector, which bites during the day. If Mf there exhibited typical nocturnal periodicity, they would not be circulating in the peripheral blood at the time the mosquito takes a blood meal, the life cycle would not complete, and the disease would die out. Because of this, the south pacific is the only region in the world where there is diurnal periodicity. Knowledge of these patterns and vector behaviors have contributed to the construction of program thresholds for interrupting transmission, discussed further in the following sections.

\textbf{2.2 Public Health Significance}

Lymphatic filariasis (LF) is a neglected tropical disease (NTD) affecting millions around the world. Endemic in 72 countries across 5 continents, more than 1.3 billion people have been at risk for LF. It is the second leading cause of vector borne diseases behind malaria and the second leading cause of long-term disability behind leprosy. Despite having effective preventive chemotherapy (PC), LF is still considered a leading cause of avoidable disability.\textsuperscript{1} In 2000, over 120 million people were infected; 40 million of them disfigured and incapacitated by the disease.\textsuperscript{23} It is this high morbidity that made LF responsible for over 1.19 million disability-adjusted life years (DALYs) in 2016.\textsuperscript{6}

After a 1997 World Health Assembly resolution to eliminate LF as a public health problem by 2020, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was formed and new and
increased efforts were made to combat the disease. GPELF has two goals: interruption of disease transmission – stopping the spread of infection through large-scale annual treatment of all eligible people in an area or region where infection is present, and morbidity management and disability prevention (MMDP) - alleviating the suffering caused by lymphatic filariasis through provision of a recommended basic package of care. Significant progress has been made throughout GPELF’s tenure; more than 7.7 billion treatments have been delivered across 69 countries. Currently, 597 million people no longer require preventive chemotherapy; however 893 million people in 49 countries are still at risk for LF.

Different treatment strategies have been implemented including DEC-fortified salt distribution to annual mass drug administration (MDA), the current WHO recommendation. Three drugs are available for LF treatment: diethylcarbamazine (DEC), albendazole, and ivermectin; each is a highly effective microfilaricide. In combination, some macrofilaricidal effect is suspected. The ability to administer a combination of these drugs in MDA is dependent on the regions’ co-endemicity with other filarial diseases. DEC is contraindicated in regions with onchocerciasis, so the standard MDA therapy is ivermectin plus albendazole in these areas, while in onchocerciasis free regions the standard therapy is DEC plus albendazole. Both DEC and ivermectin are contraindicated in regions with loiasis. Using this strategy, 14 countries have met all criteria to be validated by WHO as having eliminated LF.

Countries without loiasis and onchocerciasis are in a unique position; recent evidence has shown co-administering all three drugs together can safely and successfully clear almost all Mf from the blood in less than one week, an improvement over the longer duration for complete clearance when only two drugs are administered. With WHO formally recommending triple-drug therapy MDA in 2017, (where appropriate) national programs potentially have a shorter road to elimination.

GPELF’s strategy for elimination programs consist of five stages: mapping, treatment, post treatment surveillance, validation and post validation surveillance. Implementation of triple drug therapy is promising for many reasons. Triple drug therapy has led to both a shorter and more effective MDA treatment stage. Fewer rounds of required MDA means programs will shift into post-treatment surveillance sooner, and hopefully eliminate LF in less time and with more confidence there will be no recrudescence. If programs can reach elimination in fewer years, countries will see many benefits. Fewer individuals will be newly infected and individuals may be at a lower risk for developing severe complications, disfigurements and disability. Economic benefits are twofold, programs will cost less to operate saving countries money, while the health of their workforce will improve, increasing the growth
and earning capacity.\textsuperscript{24} For developing countries, contributing to the reduction of poverty is as important as eliminating the disease itself.

2.3 Available Assessment Tools

The stages of LF elimination programs can be grouped into three components: mapping, treatment + monitoring and evaluation, and surveillance.\textsuperscript{18} Each component has diagnostic tests associated with it chosen based on balancing test performance, availability, and program feasibility. These tests include demonstrating presence of Mf with blood smears, detecting circulating filarial antigen (CFA) using the filariasis test strip (FTS) (Abbott, Lake Forest, IL) and detecting anti-filarial antibody using the Brugia Rapid\textsuperscript{TM} (Reszon Diagnostics International Sdn. Bhd., Selangor, Malaysia).\textsuperscript{25}

Blood smears and the FTS are used to map regions with suspected or unknown endemicity and determine if the prevalence of infection is high enough to sustain transmission and therefore should proceed to the treatment phase with MDA.\textsuperscript{25} They are also used during the treatment phase to conduct impact assessments to monitor the effectiveness of treatment, to appropriately assess when infection has been reduced to levels at which transmission is unsustainable and treatment can be stopped.\textsuperscript{25} During transmission assessment surveys (TAS), the bridge from treatment to surveillance, FTS and Brugia Rapid\textsuperscript{TM} are used, depending on which species of parasite(s) is present.\textsuperscript{25} In areas with \textit{W. bancrofti}, FTS is used. \textit{Brugia spp.} are detected using Brugia Rapid\textsuperscript{TM}. Once TAS are passed, the same two tests are used throughout the post-treatment surveillance phase.\textsuperscript{25}

Blood smears are prepared by collecting fingerstick whole blood during the period of time Mf are known to circulate in peripheral blood – typically between 10pm and 2am; this required collection time represents an immediate challenge for program implementation. Once collected, that blood is then spread as a thick smear, in three parallel 20 \( \mu \text{L} \) lines down the length of the slide then stained with Giemsa for viewing. This method is both labor and time intensive; it takes two days to have the slide ready for examination. Once prepared, examining slides to identify and calculate worm load density requires significant time and skill. As scientific research moves away from microscopy, highly trained parasite microscopists are increasingly difficult to identify and train. This often leads to health programs having to outsource slide reading, making Mf smears even more logistically complicated and slowing the turnaround time from collection to result. In addition, the test parameters for Mf smears are not ideal. While the test is highly specific, sensitivity for detection of Mf can be low and variable.\textsuperscript{17, 18} To be ‘positive’ by Mf smear, LF microfilariae must be seen on the slide. This means individuals who have
amicrofilaremic infections or have very low mf counts will likely be classified negative even though they can contribute to future transmission. Historically, it has been treated as the gold standard diagnostic out of necessity, but identification of a tool with a better combination of higher sensitivity, specificity and programmatic feasibility is necessary.

The FTS is a rapid diagnostic, point of care, lateral flow assay antigen test. It is more sensitive than Mf smears, easier and faster to conduct, and because it detects antigen, can be used on blood collected any time regardless of Mf periodicity. While the FTS alleviates many difficulties associated with conducting Mf smears, there are other limitations to its use. It detects adult *W. bancrofti* CFA which serves as a proxy for infection, and only for bancroftian filariasis. FTS cannot be used in areas where there is brugian filariasis. Care must be taken when interpreting CFA presence (FTS positive) because it can be detected in several situations that will not lead to transmission. To propagate the parasite life cycle and produce Mf to be picked up by mosquitos, there must be a mating pair of adult worms. Since adult worms of both sexes produce CFA, it is possible to be FTS positive but not produce any Mf. A dying worm would also produce CFA; as would a sterile adult worm. The drugs for LF treatment are not inherently macrofilaricidal, but some sterilization effect has been seen when used in combination. Once CFA is released it persists for quite a long time; treated individuals may remain antigen positive for years, even after sustained clearance of microfilaremia. For these reasons, FTS should be interpreted with caution in programs attempting to monitor progress after MDA has begun and not recommended to be the only tool used in these settings.

In settings where FTS is biologically appropriate, there are still logistical and programmatic concerns. The FTS is the second iteration of the ICT card test (immunochromatographic card test, BinaxNOW). The ICT card test had three primary issues leading to the transition to the FTS; short shelf life, cost per test and cold chain requirements, all of which impeded widespread programmatic implementation. To address these, the ICT was reformulated and subsequently the FTS was developed. It utilizes the same monoclonal antibody and test mechanics but in order to reduce test cost the test strip now comes without a cassette; used in field settings, function of this unprotected strip is increasingly unreliable. Interpreting the qualitative test is done by observing the presence of a pink test line at the designated read time. Delayed readings can result in false positives from reagent overdevelopment and observing the often faint test line is difficult and subjective. These constraints further complicate the ability to generate high quality data.
In areas with brugian filariasis the Brugia Rapid™ is the only rapid test recommended for use by WHO. Unlike the FTS, it is an antibody test that detects IgG4 antibodies to BmR1 filarial antigen. This test is less user friendly and more expensive compared to other rapid diagnostic lateral flow assay tests. In operational settings its performance has been unreliable due to mechanical malfunction of the cassette and parts. Due to the unique cassette design, false negative results are possible (unpublished data). Given these concerns, additional tests should be investigated for use in brugian endemic areas.

Tools that detect Mf and CFA are integral to LF elimination programs and have played a significant role in global progress to date. However, the current tests have limitations that could be compensated for with the inclusion of antibody tools, especially in programs using them to monitor the impact of MDA. Of the three biomarkers, antibody is the earliest marker of infection. What is less clear is which indicator persists the longest. It has been established Mf clears quickly once treatment is provided and that CFA persists for years; research on anti-filarial antibody persistence is limited. Anti-filarial antibody is known to persist for several years but not provide lifelong immunity. The eventual clearance of anti-filarial antibody suggests that it could serve as a monitoring tool. The multiplex bead assay (MBA) is a laboratory-based assay that utilizes fluorescent microspheres conjugated with protein ligands and a flow cytometer to provide quantitative determinations of bound antibody. As sensitive as conventional ELISAs with the added ability of multiplexing up to 100 unique antigens, this total IgG assay is well suited for integrated NTD surveillance programs.

With double-drug MDA, programs spend many years in the treatment stage, making persistent CFA responses troublesome but not a deterrent preventing their use. As triple-drug MDA gains programmatic acceptance, using CFA to monitor impact may not be effective due to the significantly shorter program lifespan. Antibody tests could be an acceptable alternative or a useful complement if there is a better understanding of how quickly a meaningful decline in the population can be identified. Absence or decline of detectable antibody responses could indicate transmission has been interrupted in turn allowing for programs to stop treatment.

2.4 Lymphatic Filariasis in Haiti

Haiti is a small Caribbean country situated on the island of Hispaniola to the west of the Dominican Republic. Fifty nine percent of the population of more than 10 million people live below the poverty line, making Haiti the lowest income country in the western hemisphere. There are four countries left in the Americas with ongoing LF transmission; Haiti has the largest population at risk with
60% living in rural settings and the remaining 40% living throughout four primary urban areas. In Haiti, LF is transmitted by *Culex* mosquitos carrying *W. bancrofti* parasites. This combination means transmission primarily occurs at night. As per GPELF standards and WHO guidelines, the threshold for believed interruption of transmission and eligibility for stopping treatment in a *Culex* predominant area is <2% antigenemia.

Public health research for LF in Haiti has been ongoing for decades. As far back as the 1980s epidemiologic studies were conducted that both provided useful information on LF transmission (such that an infection as low as 1 Mf/mL of blood can still infect mosquitos), and established precedent for successful field work, paving the way for future research and intervention. Historically, the standard treatment for LF consisted of a 12-day course of DEC provided at 6 mg/kg body weight. When mass treatment campaigns first began in Haiti compliance was very low, and for those who did comply, directly observed treatment was not feasible for treatment teams. These logistical issues made scaling up treatment to the community level difficult. To address this, research was done on alternative dosing strategies, including weekly doses of DEC. While a superior method for clearing Mf, this was no better for logistical scaling up. Next Haiti was included in a multi-country study investigating single-dose DEC. The success of that study was a critical turning point for mass treatment campaigns gaining traction as a valuable and practical public health intervention for LF. More research in Haiti led to the addition of single dose albendazole to the DEC treatments and ultimately established the framework for MDAs as they are known today, to be adopted by GPELF and used beyond Haiti.

Because LF research studies are so well established in Haiti, it has often been used to pilot new diagnostic tools as they become available. These pilot diagnostic studies led to a better understanding of the relationship between preventive chemotherapy (PC) and antigen status, including that PC leads to slow and partial reductions in antigenemia, and that most individuals with severe morbidity including lymphedema and elephantiasis are actually antigen negative. These conclusions indicate that PC is useful for interrupting transmission (GPELF goal 1) but is not a beneficial tool for chronic morbidity management (GPELF goal 2). When the ICT test was first implemented it meant countries could finally conveniently map regions with suspected or unknown endemicity. As Haiti did so, widespread transmission was uncovered, and the country declared national action should be taken.

The LF research in Haiti has led to many successful public health interventions. The Haitian Ministry of Health and Population (MSPP) began The National Program to Eliminate Lymphatic Filariasis (NPELF) in 2000 aligning their goals with those of GPELF and adding a third – to encourage
positive health behaviors. Early on, the primary goal was interrupting transmission. This involved categorizing communes by endemicity level and beginning MDA in geographically disperse areas with the intention of full national coverage within three years. Due to considerable financial and logistic constraints this goal was modified to better maximize resources and the decision was made to start MDA in highly endemic ‘zone rouge’ communes.

The early MDAs consisted of DEC and albendazole. While the combination was deemed safe and efficacious, Haiti’s national program made the decision to exclude all women of child bearing age from the standard MDA and provide them only DEC to ensure any first trimester unrecognized pregnancies were not inadvertently affected by albendazole. After two years, impact assessments showed a clear difference in the men and children receiving both drugs compared to women of child bearing age receiving DEC only. Recognizing this impact on program progress, this assessment led to MSPP revising their policy making women eligible for both drugs.

As NPELF expanded and MDAs began to operate smoothly, outside factors hindered progress. Full scale-up of all communes took many years due to interruptions in funding, civil strife and multiple natural disasters. A standard double drug MDA regimen is expected to be delivered for five to six years consecutively. These situations in Haiti unintentionally demonstrated that interrupting or skipping those annual MDAs can hinder programs substantially. After civil strife halted external funding in late 2005, the 2006 MDA was not conducted. Eventually funding was restored but a follow up survey in 2007 indicated that the program was set back by at least two years. As the program got back on track four hurricanes hit Haiti in 2008 followed by a devastating earthquake in 2010 that killed 300,000 people and displaced one million. Almost immediately after the earthquake a nationwide cholera epidemic killed almost 1,000 citizens and affected tens of thousands more. In the wake of these disasters partners and donors pledged support and MDA was finally able to scale up to nationwide coverage; by 2012 all 140 communes had received at least one year of MDA. While this is itself a victory for NPELF and the people of Haiti, there is much work to be done in zones rouge and other communes that have conducted more than six rounds of MDA but still have clear evidence of persistent transmission.

Research on using DEC-fortified salt as an MDA alternative has been sprinkled throughout Haiti’s history. Pilot studies on its use were conducted in Haiti in 2001 and were considered a ‘exceptionally effective intervention’. However, as MDA became the primary intervention, large scale intervention with DEC-salt remained relatively small scale. Approximately 500 tons of the fortified salt is distributed annually to Leogane, one of the urban settings, and nearby communities. As MDA
fatigue sets in in areas with many years of implementation, the argument has been made multiple times that expanding fortified salt distribution more broadly could help shorten the duration of MDA programs.\textsuperscript{14, 15} Whereas this intervention struggles to gain traction across both Haiti and the global program, triple drug MDA consisting of ivermectin, DEC and albendazole has quickly become widely accepted. Triple drug treatment has been implemented in clinical trials, safety and efficacy trials and is now officially recommended by WHO for implementation in appropriate settings.\textsuperscript{10, 11, 12, 13, 16, 37}

By 2015, Quartier Morin one of the highly endemic zone rouge communes, had received 7 consecutive rounds of double drug MDA but still had not met the threshold for stopping treatment. In 2016 it was the location of a clinical trial to assess the safety of triple drug MDA.\textsuperscript{16} This clinical trial included a cross sectional study collecting baseline data on lymphatic filariasis biomarkers of infection in the community. In 2017, a cross sectional survey was conducted in the same communities as the clinical trial in order to monitor the impact of triple drug therapy relative to double drug therapy provided one year earlier during the safety trial. Analyzing the antibody data collected from these two studies and comparing it to antigen and Mf data collected is essential for understanding the role of antibody data in stopping-MDA decision making in triple drug MDA settings.

3.0 MANUSCRIPT

3.1 Abstract

In the global effort to eliminate lymphatic filariasis (LF) additional treatment regimens like triple drug therapy and diagnostic tools like Wb123 anti-filarial antibody have become increasingly available. This study aimed to look at two components, time and treatment regimen, in order to assess prevalence of anti-filarial antibody and its programmatic potential in the evolving Global Programme to Eliminate Lymphatic Filariasis (GPELF) setting. At year 0, 2,394 (47.4\%) samples were collected from participants before mass drug administration (MDA) was administered and 2,656 (52.6\%) were collected 12 months later (year 1). Blood samples from 4,939 (97.8\%) participants 5 years of age and older were tested for antigen status by filariasis test strip (FTS) and dried blood spots (DBS) from 4,740 (93.9\%) participants were tested for Wb123 antibodies. Older individuals were more likely to be both antigen and antibody positive than younger children. Our results show overall and across most age groups that antibody prevalence was higher than antigen prevalence for both drug arms. Individuals living in localities treated with the double drug regimen had 66\% greater chance of demonstrating
declining antibody prevalence than those living in localities treated with the triple drug regimen. The results of this investigation support treatment with both MDA regimens and the addition of antibody testing as a surveillance tool. Our results have contributed to the evidence base surrounding population-level antibody data and help to inform programmatic decision making around treatment strategies.

### 3.2 Introduction

Endemic in 72 countries, lymphatic filariasis (LF) is a mosquito transmitted parasitic disease with more than 1.3 billion people at risk globally, making it the second most common vector borne disease behind malaria.\(^1\),\(^2\),\(^3\),\(^2\),\(^3\) Transmitted via bites from *Anopheles*, *Culex*, *Aedes* or *Mansonia* mosquitos, LF is a parasitic infection caused by three filarial nematodes *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*.\(^3\),\(^9\) Adult worms reside in the lymph system of human hosts and infection can lead to lymphedema, elephantiasis and hydrocele or breast/vulva enlargement, making LF the second leading cause of long-term disability and responsible for an estimated 1.19 million DALYs.\(^2\),\(^4\),\(^5\),\(^6\)

In 1997 at the 50\(^{th}\) World Health Assembly, a resolution was passed to eliminate LF as a public health problem by 2020.\(^7\),\(^8\) To accomplish elimination goals across the 72 countries declared LF-endemic, the Global Program to Eliminate Lymphatic Filariasis (GPELF) was formed. From program inception in 2000 through the end of 2018, annual mass drug administrations (MDA) have been implemented in 69 countries delivering more than a cumulative 7.7 billion treatments.\(^1\) GPELF has made significant progress; MDA has stopped in 24 countries of which 14 countries have met all criteria for validation of elimination of LF by WHO.\(^1\)

As 2020 nears, there are several countries that have faced challenges completing MDA and will not achieve established elimination criteria by the target date.\(^9\) Currently, MDAs are conducted using a two-drug regimen. In areas potentially co-endemic with onchocerciasis, ivermectin and albendazole are provided. In areas without risk of onchocerciasis, diethylcarbamazine (DEC) and albendazole (DA) are provided. Recent clinical trials in non-onchocerciasis endemic countries have indicated providing all three drugs may reduce the number of annual MDAs needed to reach elimination. A single dose of three drugs (ivermectin, DEC and albendazole (IDA)) in combination yields complete clearance of microfilaremia from the blood for at least one year among more treated individuals than the standard double drug (DA) regimen.\(^10\),\(^11\) Adopting the IDA regimen likely means fewer rounds of MDA would be necessary to reach elimination, due to sustained reduction of Mf.\(^12\) These studies’ conclusions have led to the revision of WHO guidelines for LF MDA to include triple drug treatment.\(^13\)
This is particularly relevant for Haiti, a country that has faced decades of adversity that has delayed LF elimination. The Ministry of Health and Population (MSPP) in Haiti began The National Program to Eliminate LF (NPELF) with the first MDA in 2000 and five successful rounds in the following consecutive years; however civil strife and lack of funding prevented additional rounds of MDA, necessary in highly endemic regions.\textsuperscript{14, 15} Sentinel site surveys in 2007 indicated that gap in MDA set Haiti’s program back by at least two years, as evident by significant recrudescence.\textsuperscript{35} As Haiti began to scale up MDA to include more communes, a devastating earthquake occurred in 2010 that killed more than 300,000 people and led to a nationwide cholera outbreak.\textsuperscript{15} It was not until 2012 that Haiti achieved 100% geographic coverage with MDA.\textsuperscript{15} By 2015, the endemic commune Quartier Morin, located in the Northern Department, had received 7 consecutive rounds of MDA but still had not met the threshold for stopping treatment. In 2016 it was the location of a clinical trial to assess the safety of triple drug MDA.\textsuperscript{16} This clinical trial included a cross sectional study collecting baseline data on LF biomarkers of infection in the community. In 2017, a cross sectional community survey was conducted there in order to monitor the impact of triple drug therapy relative to double drug therapy provided one year earlier during the safety trial.

Currently, programmatic decision-making regarding transmission interruption and stopping MDA is determined by measuring two biomarkers, Mf and circulating filarial antigen (CFA) antigenemia. Presence of Mf in peripheral blood is biological evidence of active infection with viable reproducing adult worms, however detecting Mf is programmatically difficult for several reasons. In most LF-endemic countries, Mf circulate with nocturnal periodicity, meaning blood collection must take place between 10pm and 2am. Additionally, Mf are identified by examining stained blood smears, a technique that requires highly trained microscopists. Measuring Mf is the most specific tool available but the least sensitive;\textsuperscript{17, 18} negative Mf smears do not indicate absence of infection.\textsuperscript{19, 20} As transmission declines, infected individuals are expected to have lower density [Mf] infections, further decreasing sensitivity of detection.

The other recommended biomarker, CFA, is measured using a field-deployable rapid diagnostic lateral flow assay test, the filariasis test strip (FTS) (Abbott, Lake Forest, IL). Measuring CFA alleviates the need for both highly trained microscopists and inconvenient night blood collections, however presence of CFA is only a proxy for infection. The presence of CFA does not guarantee there is a mating pair of worms producing Mf. Furthermore, antigenemia has been shown to persist in individuals long after treatment and sustained Mf clearance; making interpretation of antigenemia more challenging.
when making decisions about stopping IDA-MDA due to the shorter duration of treatment. Currently, the FTS is the only WHO recommended antigen test and has proven problematic during field implementation. It is frequently unreliable, difficult to obtain, and subjective to interpret further complicating the ability to generate high quality data.

The benefits and drawbacks of each test alone are concerning, but when used in concert they can be implemented with a reasonable expectation of quality data generation. Expanding the program tool portfolio to include antibody testing may strengthen the information collected and lead to higher quality data. While not currently routinely used in LF elimination programs, anti-filarial antibody tools have been shown to be useful in low-transmission settings. Identifying a steady downward trend in antibody prevalence among a population is a strong indication that transmission is declining. However, with the implementation of triple drug MDA and the potential subsequent decrease in the number of years of treatment, it is important to assess the role of anti-filarial antibody responses at the population level to determine if it is an appropriate marker to use to make programmatic decisions.

This study aimed to look at two components, time and treatment regimen, in order to assess prevalence of anti-filarial antibody and its programmatic potential in the evolving GPELF setting. Using data collected in Haiti from both the baseline pre-IDA study in 2016 and the follow up survey conducted 12 months later, information at two time points, before and after MDA, was collected. The MDA conducted in 2016 as part of the clinical trial included localities treated with both the standard double drug (DA) MDA regimen, and the new triple drug (IDA) MDA regimen, allowing us to directly compare similar populations that received different treatment regimens.

3.3 Methods

Study Site and Design

The safety and efficacy study (year 0) took place in October 2016-February 2017, in Haiti, a small Caribbean country, bordered to the east by the Dominican Republic on the island of Hispaniola. It is made up of 140 communes with more than 10 million people. The commune of Quartier Morin located in the Northern Department of Haiti was purposely selected for this study because of its high baseline antigen prevalence of 39%. An open-label, cluster-randomized community study design was utilized, and each of the 10 localities in Quartier Morin was randomly assigned to receive double drug (DA) MDA regimen, or triple drug (IDA) MDA regimen, five in each arm. Each household in the 10 localities listed on the July 2016 census was visited and all eligible residents aged 5 years or older were
asked to participate. The total number of eligible participants was approximately 6,000 (3,000 per drug arm).

The community prevalence study (year 1) took place in January-February 2018. The target sample size was 1000 individuals residing in the 10 localities treated with the DA and IDA (2000 total). Recent census data from Quartier Morin were used to identify and recruit participants into the study. Households were systematically randomized for inclusion and all family members home 5 years or older were asked to participate. More households were randomized than necessary to reach the study sample in order to account for refusals, absence and presence of children under the age of five. The study team sequentially visited each selected household and continued until they reached the study sample target desired. Once consent was obtained, 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 SDK application and uploaded to a secure SQL server.

For this analysis, year 0 samples were randomly selected to match overall sample size with year 1. This resulted in approximately 1,000 samples from each drug arm, from each time point.

Ethical Considerations

The Haitian National Ethics Committee approved this study. The Institutional Review Board of the U.S. Centers for Disease Control and Prevention (CDC) determined CDC to be a nonengaged 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. In addition, children aged between 7 and 17 years were asked to provide verbal assent for their participation. All identifiable information was kept confidential and maintained using a secure database with access restricted to essential study personnel.

Blood Collection and Examination

At each time point, approximately 250 µL of whole blood was collected from each participant by finger stick into a lithium heparin capillary tube in the participant’s home. In a centrally located lab 75 µL was used for the detection of CFA by FTS (Abbott, Lake Forest, IL). The tests were read at 10 minutes and marked positive or negative according to the manufacturer’s instructions. Next 60 µL of whole blood (10 µL per extension x 6 extensions) was collected onto filter paper (Cellabs, Sydney, Australia) then dried and stored for anti-filarial antibody testing. The dried blood spots (DBS) were stored at -20°C until shipped to the CDC for testing. Remaining blood, if available, was used for
repeating FTS in the event the initial test was invalid. Participants with positive FTS results were tested for Mf with three-line thick smears prepared with a measured 60 µL quantity of whole blood from a second finger stick collected between 10:00 pm and midnight. Slides were fixed, stained with Giemsa, and examined by microscopy with a 10x objective according to WHO guidance for monitoring MDA. The species of Mf present were determined by using morphological criteria.

**Treatment**

FTS positive individuals, or parents of positive children, were notified of test results and offered treatment by the study team with the same regimen according to randomized study arm. Those living in double-drug treated communities received diethylcarbamazine (DEC) (6 mg/kg) and albendazole (400 mg) while those in triple-drug communities also received ivermectin (200 µg/kg) in addition to DEC and albendazole.

**Antibody testing by multiplex bead assay (MBA)**

Anti-filarial antibody (Ab) responses to Wb123, along with antibody responses to 7 other diseases, were determined using MBA as previously described. 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. Previously tested sera were combined into pools to create positive and negative external controls. Shewhart rules were applied to each control and samples on affected plates were repeated accordingly. The median fluorescent intensity (MFI) values minus the background (bg) fluorescence from the buffer-only blank was reported as MFI-bg. Results for non-LF antigens will be reported elsewhere.

**Cutoff determination for MBA**

MBA semi-quantitative results were dichotomized into ‘positive’ or ‘negative’ using a median fluorescence intensity minus background (MFI-bg) cutoff generated by a receiver operator characteristic curve (ROC) analysis. The ROC panel used consisted of 160 known positives, determined by positive Mf blood smears and 86 presumed negatives, determined by personal history of being born and residing in the United States, non-endemic for LF, with no history of foreign travel. The cutoff was selected by applying the smallest absolute difference [abs(Se-Sp)] to compensate for testing parameters; this method
more heavily weighted sensitivity because the truth overvalues specificity. The ROC curve area was 0.87 and the final cutoff was 223 MFI-bg.

**Statistical Analysis**

Statistical analyses were performed in SAS version 9.4 (SAS Institute Inc, Cary, NC). Chi square and multivariate logistic regressions were used to identify associations between seropositivity and other factors. All analyses used a 5% level of significance.

**3.4 Results**

A total of 5,050 individuals aged 5 years or older participated in this study; at year 0, 2,394 (47.4%) samples were collected, and 2,656 (52.6%) were collected 12 months later as part of the community prevalence study (year 1). Overall, 53.8% were female and the mean age was 24.9 years (range 5 – 102 years). The five localities in the double drug arm consisted of 2,614 (51.8%) individuals and the five localities in the triple drug arm consisted of 2,436 (48.2%) individuals. Blood samples from 4,939 (97.8%) participants were tested for antigen status by FTS, and DBS from 4,740 (93.9%) participants were tested for Wb123 antibodies. Of the total participants tested at year 1, 40.5% did not participate in the MDA at baseline; participation was also significantly different between drug arms, with 8.8% less participation in the triple drug arm (OR 0.69; 95% CI 0.59-0.81) (Table 1).

Antibody prevalence was significantly different across drug arms at both baseline (OR 1.91; 95% CI 1.59–2.31) and follow up (OR 1.28; 95% CI 1.06-1.55). Antibody results are summarized by drug arm and time point in Table 2. Antibody prevalence in the double drug arm decreased 10.6% from year 0 to year 1 (OR 1.66; 95% CI 1.40-1.98); the triple drug arm decreased 1.8% (Table 3). The double drug regimen was 8.8% more effective at demonstrating a reduction in antibody prevalence one year post-treatment than the triple drug regimen (Table 3).

Overall, older individuals were more likely to be both antigen and antibody positive than younger children (Table 2). There was not a consistent pattern across age groups and time points for either drug arm. Participants in the double drug arm had highest baseline prevalence of both biomarkers in the oldest age group and the lowest prevalence of both biomarkers at follow up in the youngest age group. Antibody prevalence in the triple drug arm was similar across all age groups at both time points while antigen prevalence had a larger range across age groups at both time points (Table 2).
### 3.5 Tables

Table 1: Mass drug administration (MDA) participation rates of antibody positive individuals by study arm at year 1 follow up in Quartier Morin, Haiti.

<table>
<thead>
<tr>
<th></th>
<th>Double Drug</th>
<th>Triple Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%) of population</td>
<td>N+ (%+) Antibody</td>
</tr>
<tr>
<td>participated in MDA at year 0</td>
<td>845 (63.8%)</td>
<td>208 (26.9%)</td>
</tr>
<tr>
<td>did not participate in MDA at year 0</td>
<td>480 (36.2%)</td>
<td>94 (21%)</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of antibody and antigen biomarkers across all age groups by study arm before and after treatment with double- or triple- drug mass drug administration in Quartier Morin, Haiti.

<table>
<thead>
<tr>
<th></th>
<th>Year 0 Baseline (2016-2017)</th>
<th>Year 1 Follow Up (2018)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Double Drug</td>
<td>Triple Drug</td>
</tr>
<tr>
<td></td>
<td>&lt;10</td>
<td>10-19</td>
</tr>
<tr>
<td>Antibody total N</td>
<td>228</td>
<td>345</td>
</tr>
<tr>
<td>Number positive</td>
<td>52</td>
<td>123</td>
</tr>
<tr>
<td>% positive</td>
<td>22.8%</td>
<td>35.7%</td>
</tr>
<tr>
<td>Antigen total N</td>
<td>227</td>
<td>354</td>
</tr>
<tr>
<td>Number positive</td>
<td>34</td>
<td>51</td>
</tr>
<tr>
<td>% positive</td>
<td>15.0%</td>
<td>14.4%</td>
</tr>
</tbody>
</table>

Table 3: Change in prevalence of antibody and antigen biomarkers one year after treatment with double- or triple- drug mass drug administration in Quartier Morin, Haiti.

<table>
<thead>
<tr>
<th>biomarker and drug arm</th>
<th>age</th>
<th>% change from baseline to follow up</th>
<th>Odds Ratio and Confidence Intervals</th>
<th>biomarker and drug arm</th>
<th>age</th>
<th>% change from baseline to follow up</th>
<th>Odds Ratio and Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Double Drug</td>
<td>&lt;10</td>
<td>-3.6</td>
<td>1.24 (0.77-1.99)</td>
<td>Antigen Double Drug</td>
<td>&lt;10</td>
<td>-4.6</td>
<td>1.51 (0.85-2.68)</td>
</tr>
<tr>
<td></td>
<td>10-19</td>
<td>-9.1</td>
<td>1.52 (1.11-2.11)*</td>
<td></td>
<td>10-19</td>
<td>-3.6</td>
<td>1.38 (0.89-2.15)</td>
</tr>
<tr>
<td></td>
<td>20+</td>
<td>-14.3</td>
<td>1.94 (1.53-2.46)*</td>
<td></td>
<td>20+</td>
<td>-5.0</td>
<td>1.24 (0.99-1.54)</td>
</tr>
<tr>
<td>Overall</td>
<td>-10.6</td>
<td>-10.6</td>
<td>1.66 (1.40-1.98)*</td>
<td></td>
<td>Overall</td>
<td>-3.9</td>
<td>1.22 (1.02-1.45)</td>
</tr>
<tr>
<td>Antibody Triple Drug</td>
<td>&lt;10</td>
<td>3.9</td>
<td>0.78 (0.47-1.29)</td>
<td>Antigen Triple Drug</td>
<td>&lt;10</td>
<td>3.0</td>
<td>0.80 (0.46-1.38)</td>
</tr>
<tr>
<td></td>
<td>10-19</td>
<td>-3.5</td>
<td>1.23 (0.87-1.74)</td>
<td></td>
<td>10-19</td>
<td>0.1</td>
<td>0.99 (0.66-1.49)</td>
</tr>
<tr>
<td></td>
<td>20+</td>
<td>-2.8</td>
<td>1.18 (0.89-1.56)</td>
<td></td>
<td>20+</td>
<td>-3.5</td>
<td>1.20 (0.93-1.55)</td>
</tr>
<tr>
<td>Overall</td>
<td>-1.8</td>
<td>-1.8</td>
<td>1.11 (0.91-1.36)</td>
<td></td>
<td>Overall</td>
<td>-0.6</td>
<td>1.04 (0.85-1.26)</td>
</tr>
</tbody>
</table>

* Statistically Significant
3.6 Discussion

Our study aimed to assess the utility of anti-filarial antibody data collected at the population level as a potential surveillance tool for LF elimination programs, specifically in settings where triple drug therapy is used for MDA. While antibody prevalence was different at a population level across the drug arms at follow up, the results were challenging to interpret because antibody population prevalence was also significantly different at baseline between the two drug arms. The 10 localities in Quartier Morin were randomly assigned to each drug arm; the localities were small in size and geographically interspersed amongst each other. By chance, the communities randomized into the double drug arm had higher levels of LF than the triple drug arm. While our results make it difficult to determine if one treatment regimen was more effective than the other, the overall decrease in both arms after treatment reiterates that both treatments regimens were effective. This finding is important for GPELF because the strategic framework for interrupting transmission is based on treating populations at risk.

Anti-filarial antibody is known to persist for many years but will eventually clear after successful treatment. In program settings where double drug regimens have been used for MDA, significant declines have not often been observed one year after treatment. However, the kinetics of filarial antibody responses in IDA settings are not well understood, and it is unclear how soon a meaningful decline in antibody at the population level can be observed. Our results were surprising in that participants in only one treatment arm, the standard double drug arm, had a significant decrease in antibody levels. One potential factor influencing this outcome may have been MDA compliance rates. Compliance in the double drug arm was 63.8%, whereas compliance in the triple drug treatment arm was only 55.0%. It is possible residents were suspicious of the new drug or deterred by the additional number of pills required. Also, baseline prevalence in the double drug arm was higher than in the triple drug arm (35.1% vs 22.0%). With a higher baseline prevalence, it may have been easier to detect a statistical difference. The antibody prevalence at follow up for the double drug arm was still higher than the baseline antibody prevalence for the triple drug arm.

It is important to account for age when discussing biomarkers such as antigen and anti-filarial antibody. Older individuals have had more opportunities to be infected (repeatedly), and once infected, remain seropositive for many years. Our results supported this as older individuals were more likely to be positive by both biomarkers than children were. While high prevalence of infection-specific antibody in older people at a single time point does not necessarily distinguish between recent or historic infection, observing a meaningful decline in any age group over a period of time may indicate
transmission has been interrupted. When stratifying into three primary age groups in our study, the prevalence patterns were not as clear cut as expected. This may have been influenced by differences in MDA compliance among the age groups, though that was not in the scope of this analysis.

Our results indicated that both overall and across most age groups that antibody prevalence was higher than antigen prevalence in both drug arms. Integration of antibody tools into program monitoring may make it easier to detect meaningful changes without compromising program feasibility. Smaller sample sizes may be sufficient to detect changes in antibody compared to antigen due to the more ubiquitous response at the population level. The decrease in antibody prevalence in both arms further supports the utility of inclusion of antibody tools regardless of treatment regimen, standard double drug or triple drug. Additionally, antigen positivity results from the presence of adult worms; in most healthy individuals the immune system has already begun creating anti-filarial antibodies to fight LF infection before worms reach maturity and begin producing CFA. Anti-filarial antibody is the first detectable biomarker after infection, followed by CFA antigen and lastly Mf.21 Adding antibody markers as a surveillance tool may allow programs to capitalize on early detection and intervene before transmission worsens or recrudescence occurs.

The results of our investigation support efficacy of MDA and the addition of antibody testing as a surveillance tool. Treatment with either regimen was effective. While administering ivermectin, DEC and albendazole as part of triple drug therapy is quite promising, it should be acknowledged DEC and albendazole in tandem are also effective though progress is slower. Poor population compliance with either drug regimen will have a detrimental effect on transmission interruption. In places where continued compliance is a known difficulty - also known as MDA fatigue - triple drug implementation, when accepted amongst most of the community, has the potential to alleviate some of this burden due to the reduced number of years of required treatment intervention. After one year, our results indicated a decline in antibody responses at the population level. This means monitoring progress using antibody may be equally useful throughout the duration of a short triple drug MDA program or a longer double drug MDA program.

It is clear there is still LF transmission in Quartier Morin, Haiti. Seeing a signal of any kind in the youngest age group suggests recent transmission, an indication MDA should continue in Quartier Morin. Continued treatment with either drug regimen would be effective; though previous work in this commune provides a unique opportunity for continued analysis of relative efficacy between the two drug treatment strategies over a longer period of time.
It is important to build on the research presented here to both strengthen the knowledge base around antibody persistence in the population as well as guide its use in diverse and evolving LF elimination programs. This should be done by repeating the community prevalence study in the same Quartier Morin localities as soon as possible. This will provide an additional time point and provide clarity on how both treatment regimens affect trends in biomarker decline. Similar assessments should be carried out in other countries eligible for treatment with triple drug therapy, especially those with other vector species. These additional data will provide a better understanding of whether these results are unique to Haiti or if they can be applied globally.

This analysis focused on one specific anti-filarial antibody, Wb123, a marker of infection against *W. bancrofti*. Future research into other anti-filarial antibodies like Bm14 and Bm33 or new markers not yet identified may help expand the antibody tool portfolio. Finally, it is critical to identify better and more reliable field deployable tests for program implementation. There is not currently a true gold standard LF diagnostic; having one could be what GPELF needs to help the remaining 34 countries who have not completed all necessary MDA as well as all countries conducting ongoing surveillance. In the meantime, measuring Wb123 anti-filarial antibody using the MBA should be considered; the results garnered are worth investing in this laboratory-based assay.

This analysis utilized two cross sectional studies which may be a better representation of the ‘real world’ in terms of population MDA compliance, however a longitudinal study design following up with all the participants from year 0 would allow for a direct assessment of biomarker change over time. When assessing anything over time the analysis becomes increasingly robust the more time points that are available. This is particularly important for biomarkers with long half-lives like Wb123 anti-filarial antibody and CFA antigenemia.

Additional limitations specific to diagnostic testing include the lack of gold standard comparisons. Most LF tests available have some major technical weakness (i.e. Mf smears low sensitivity, FTS proxy for infection and unreliable function, Brugia Rapid™ test design and unreliable function, MBA cutoff determination and highly technical operation) making it difficult not only to implement them in programs but also to interpret their results and make meaningful decisions.

Our results have contributed to the evidence base surrounding population-level antibody data and help to inform programmatic decision making around treatment strategies. They also highlight the importance of high MDA compliance and the impact it has on population level community infection
indicators like anti-filarial antibody and CFA antigenemia. Our results also support the effectiveness of both MDA treatment regimens, the DA double drug therapy and the IDA triple drug therapy.

4.0 REFERENCES


