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Post-Mass Drug Administration Serological Assessment of Lymphatic Filariasis and Onchocerciasis in Plateau State, Nigeria

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By

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B.S.
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An abstract of
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Abstract

Post- Mass Drug Administration Serological Assessment of Lymphatic Filariasis and Onchocerciasis in Plateau State, Nigeria

By Rebecca Castor

Background:
Nigeria bears the highest burden in Africa for lymphatic filariasis (LF) and onchocerciasis, two vector-transmitted filarial diseases caused by *Wuchereria bancrofti* and *Onchocerca volvulus*, respectively. This study compared results of a novel Ov16/Wb123 biplex rapid antibody test with laboratory-based ELISA for anti-Ov16 and anti-Wb123 antibodies and LF circulating filarial antigen (CFA) by immunochromatographic card test (ICT) during post-treatment surveillance for LF in 3 local government areas (LGAs) of Plateau State, Nigeria.

Methods:
From April to May 2016, finger-prick blood samples were collected from consenting individuals in school-based Transmission Assessment Surveys (TAS) of first and second-year primary school children (approximately 6-7 years old) and community-wide household surveys of individuals >2 years of age in each LGA. Rapid tests (Ov16/Wb123 biplex and ICT) were conducted in the field; dried blood spots (DBS) were prepared for laboratory-based ELISA testing.

Results:
A total of 6,854 individuals (median age: 7 range: 2-95) had matched demographic data and valid results from all laboratory tests. Overall prevalence estimates for LF CFA, and Wb123 by ELISA and biplex test were 0.13% (95% upper confidence limit [uCL]: 0.23), 1.75% (95% uCL: 2.03), and 0.13% (95% uCL: 0.23), respectively. CFA prevalence generally increased with age, whereas Wb123 prevalence by both ELISA and Biplex tended to be highest in children 5-9 and in the oldest age groups. Of the 9 samples positive for CFA by ICT, only one was positive for Wb123 antibody biplex positive and none were Wb123 ELISA positive. None of the 120 individuals positive by Wb123 ELISA were positive by Wb123 biplex or for CFA by ICT. Overall prevalence estimates for Ov16 by ELISA and biplex were 0.01% (95% uCL: 0.07) and 0.03% (95% uCL: 0.09), respectively. All Ov16-positive individuals were at least 50 years old.

Conclusion:
Compared to ELISA, the biplex rapid antibody test demonstrated good performance for Ov16, but poorer performance for Wb123. This study strongly suggests that transmission interruption of both diseases has been achieved in the study areas and provides important data on the age distribution of Wb123 and Ov16 in low transmission settings.

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List of Abbreviations

ADL	Adenolymphangitis
APOC	African Programme for Onchocerciasis Control
CDC	Centers for Disease Control and Prevention
CDTI	Community-Directed Treatment with Ivermectin
CFA	Circulating Filarial Antigen
DALYs	Disability-adjusted life years
DBS	Dried blood spots
ELISA	Enzyme-linked immunosorbent assay
EA	Enumeration Area
ESPEN	Expanded Special Project for the Elimination of Neglected Tropical Disease
EU	Evaluation Unit
FMOH	Federal Ministry of Health (Nigeria)
FTS	Filariasis Test Strip
GPELF	Global Programme for the Elimination of Lymphatic Filariasis
ICT	Immunochromatographic Card Test
IDA	Ivermectin, Diethylcarbamazine, and Albendazole
IRS	Indoor Residual Spraying
IU	Implementation Unit
LF	Lymphatic filariasis
LGA	Local Government Area
MDA	Mass drug administration
MF	Microfilariae
MMDP	Morbidity Management and Disability Prevention
NTD	Neglected tropical disease
OEPA	Onchocerciasis Elimination Program for the Americas
OCP	Onchocerciasis Control Programme in West Africa
PacELF	Pacific program to Eliminate Lymphatic Filariasis
PES	Post-Elimination Surveillance
PTS	Post-Treatment Surveillance
RB	River Blindness
TAS	Transmission Assessment Survey
TCC	The Carter Center
WHO	World Health Organization

Chapter I: Background

Lymphatic filariasis

Lymphatic filariasis (LF), also known as elephantiasis, is a vector-borne neglected tropical disease (NTD) transmitted by mosquitos. The disease is caused by any of three species of filarial parasites, with *Wuchereria bancrofti* being the most common and contributing to 90% of global LF cases, and *Brugia malayi* and *B. timori* accounting for the remaining 10% of the cases, especially in Asian countries. These parasites are microscopic, thread-like nematode worms that can lead to disruption of the body's lymphatic system (1). Through inhabiting the lymphatic system, these worms cause severe morbidity and can lead to lymphedema, elephantiasis, swelling of the scrotum in men (hydrocele), and swelling of the breasts and vulva in women (2, 3). Adenolymphangitis (ADL) is often the first clinical sign of LF and is characterized by an area of plaque-like cutaneous inflammation that may be with or without ascending lymphangitis or satellite adenitis (4).

LF is endemic in 72 subtropical and tropical countries throughout Africa, the Caribbean, Asia, the Western Pacific, and South America, and affects more than 120 million people (1). Although most infected people are asymptomatic, nearly all have some level of subclinical lymphatic damage (5). Globally, an estimated 15 million people suffer from lymphedema and 25 million men suffer from hydrocele with still 880 million at risk for the disease (6, 7). Although not typically associated with mortality, LF is the second leading cause of permanent and long-term disability with over 5.5 million disability-adjusted life years (DALYs) (8, 9). In countries where LF is endemic, there is a total estimated annual loss of $1 billion and economic impairment of up to 88% (5). Studies have shown that lymphedema reduces one's ability to carry out daily tasks

independently, impacting their ability to work and increasing their need for treatment, leading to financial strain for the individual and their families. Along with the aforementioned economic impact and impaired health consequences, the psychosocial aspects of LF can contribute to the negative mental health of LF patients as well (6, 10).

Mosquitos belonging to the *Anopheles, Aedes, Culex,* and *Mansonia* genera are responsible for transmission of filaria. The *Anopheles* mosquito is the most common vector in Africa while in the Americas, it is the *Culex* vector. In the Western Pacific and Asia, the *Aedes* and *Mansonia* are the most common vector (1).

History

Although there are statue depictions of people suffering from elephantiasis as early as 2000 BC, the earliest known documentation of lymphatic filariasis symptoms was by Jan Huygen Linschoten between 1588 and 1592 during an exploration of Goa, India. He wrote that the descendants of those who killed St. Thomas were "all born with one of their legs and one foot from the knee downwards as thick as an elephants' leg" (11). Soon after, additional documentation of LF appeared in other parts of Asia and Africa.

In 1863, Jean-Nicolas Demarquay, a French surgeon, was the first to see and document microfilariae in hydrocele fluid. Three years later, microfilariae were discovered in urine by Otto Henry Wucherer, but it was a Scottish physician named Timothy Lewis who confirmed finding microfilariae in urine and blood, making the connection to elephantiasis. In 1876, the adult worm was identified by Joseph Bancroft which led to the name *Filaria bancrofti.* Transmission by mosquitoes was then discovered by Patrick Manson in 1877 and viewed as the most important discovery not only for lymphatic filariasis, but also for tropical medicine in general. He was the first to

identify the microfilariae in mosquitos, which was later applied to other diseases such as malaria, but incorrectly hypothesized the mode of transmission of *Wuchereria bancrof*ti to humans. In 1900, George Carmichael Low identified that the true mechanism of transmission of the parasite was through the bite of an infected mosquito (11).

Biology and Pathology

Human infection begins when L3 larvae (infectious-stage larvae) are deposited into the skin when bitten by an infected mosquito (Figure 1). Larvae then enter the lymphatics and lymph nodes and begin a 6-9 month process where they molt into L4 larvae and finally develop into adult worms. Adult male and female worms live in the lymphatics of the lower extremities, scrotum, inguinal canal, upper limb and thorax (breasts for females) for an average of 5-7 years and are resistant to the host's immune attacks (12, 13). Worms mate and can produce millions of microfilariae during their lifetime. Lymphatic dilation and thickening of the vessel wall occur due to host immuno-pathological reaction to adult worms and their antigens. Histologically, the worm elicits little reaction while alive. After the worm dies, a granulomatous reaction occurs, resulting in lymphatic dilation and later lymphatic dysfunction and eventually lymphedema (8). Microfilariae produced by female worms are passed into the bloodstream where they can be taken up by a mosquito during a blood feeding (12). The microfilariae develop inside the mosquito for about a week to form L2 and L3 larvae and are (14).

Microfilariae are most numerous in the blood circulation at night, sequestering in the deep vascular beds during the day. This nocturnal periodicity is thought to prolong microfilariae survival, resulting in high levels of microfilaria in some individuals (e.g. >10,000/ml) (15).

The pathological changes in filariasis depend on factors such as the number of infected mosquito bites, the number of L3 larvae, time between infected bites, the site of the bite and localization of the adult worm, the age of the patient at initial infection, severity of immunologic reaction, secondary infections, and other complicating diseases such as varicose veins (12).

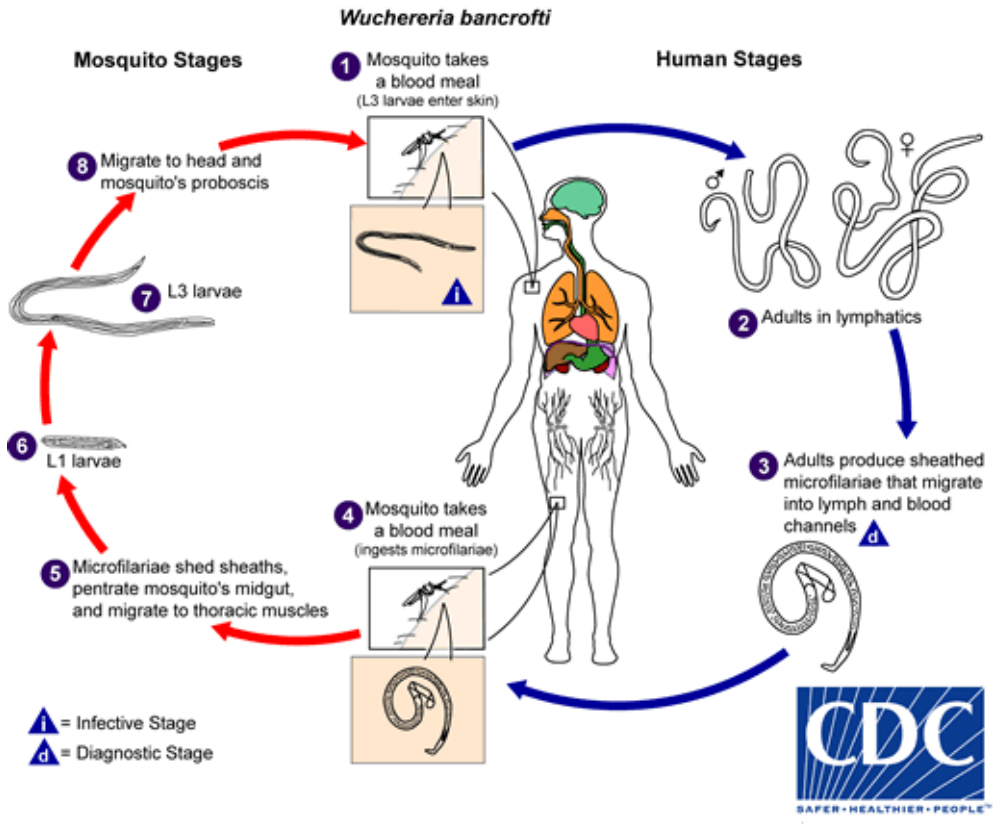


Figure 1. Life Cycle of *Wuchereria Bancrofti* (1)

Diagnostic Tests

Microscopy

Microscopic examination of thick blood film for microfilariae is the standard method of diagnosing active infection. This diagnostic technique is performed in the middle of the night between midnight and 2:00 a.m., due to the nocturnal periodicity of microfilariae (16).

Antigen Tests

Aside from microscopy, there are a variety of diagnostic tests for lymphatic filariasis that may test for the presence of either the LF antigen or antibody in the blood. These tests are mainly used in order to determine whether or not to stop large-scale treatment in the effort to eliminate lymphatic filariasis. Antigen tests measure the presence of circulating filarial antigen (CFA) in the blood. However, LF antigen may remain in the blood for years after adult worms die meaning that a positive test does not necessarily indicate an active infection, making it difficult to monitor the progress of LF elimination programs. Furthermore, antigen testing is costly and is not yet developed for *Brugia* spp (16).

Og4C3 ELISA

The first method available to detect lymphatic filariasis antigen in *Wuchereria bancrofti* endemic areas was an enzyme-linked immunosorbent-assay (ELISA) that detects the Og4C3 antigen. This parasite antigen test is a lab-based test that provides a quantitative measurement of parasite antigen in a sample. Qualitative positive/negative determinations can be assigned by using reference samples from endemic and non-endemic areas. Serum, hydrocele fluid, or plasma can be used to carry out the test, but dried blood spots (DBS) are also commonly collected on filter paper and subsequently used to carry out the Og4C3 ELISA (17).

Immunochromatographic Card Test

The immunochromatographic card test (ICT) is a point-of-care test used for detecting 200 kDa *Wuchereria bancrofti* CFA. The development of a point-of-care rapid test revolutionized LF programs as they do not limit testing to nighttime hours in order to

align with microfilariae periodicity. Testing procedures require 100 microliters of a plasma sample to be added to the sample pad of the card, which contains a polyclonal anti-filarial antibody that binds to the CFA. The pad then comes into contact with a nitrocellulose strip once the card is closed. The antibody-antigen complex continues to move along the nitrocellulose strip until it is trapped by an anti-filarial monoclonal antibody (AD12.1) in the strip's coating. The test should be read at 10 minutes; false positive results can occur if read after 10 minutes. Although recent evidence indicates that the sensitivity of ICT might vary depending on sex, age, presence of living adult worms, and microfilarial density, the test is useful in that it can be administered easily with minimal resources necessary. This was the recommended diagnostic tool for TAS surveys (18). Limitations of the ICT include an inability to detect infection prior to the development of adult parasites, which can take up to 18 months after exposure to infective stage larvae. The ICT also needs a cold-chain, has limited shelf-life, and lacks positive controls in test kits (19, 20).

Filariasis Test Strip

The Filariasis Test Strip (FTS) is an updated point-of-care test with a higher sensitivity and result stability that is used to detect *W. bancrofti* CFA. The FTS also has a longer shelf life at ambient temperatures (15-37°C) compared to the ICT test. Testing procedures for the FTS require 75 µl of blood to be collected from a finger prick. Blood samples are placed onto a nitrocellulose strip and read after 10 minutes (21, 22).

Antibody Tests

Due to antibody persistence, antibody diagnostic tests cannot definitively identify an active LF infection nor distinguish between past and current infection. They may be

useful, however, for surveillance purposes to estimate prevalence trends across age groups and to provide an early marker of infection (23). Antibody test are available for all species in either ELISA (*Wb*123, *Bm*14, *Bm*33) or point-of-care rapid test format (Wb123 monoplex, Ov16/Wb123 biplex).

Wb123, Bm14, Bm33 ELISA.

As with antigens, ELISA testing can also be used to detect different antibodies to targeted antigens. There are three target antigens that can be used to detect anti-filarial IgG4 antibodies: Wb123, Bm14, and Bm33 (24). As with the ELISA testing for LF antigen, this lab-based test provides a quantitative and qualitative measure of antibody in whole blood, plasma, or serum.

Wb123 monoplex

The Wb123 monoplex test is a point-of-care rapid test used in order to detect anti-filarial IgG4 antibodies that are produced in response to the presence of Wb123 filarial antigen in the blood (22).

Ov16/Wb123 biplex

Some areas, mainly in sub-Sarahan Africa, that are endemic for LF are also co-endemic for onchocerciasis, similar parasitic filarial disease. For this reason, a Ov16/Wb123 biplex test was created in order to test for the presence of antibodies to the Wb123 and Ov16 proteins at the same time (25).

Because different tests provide different information about LF infection and can be carried out in different settings, there is no gold standard diagnostic test for LF.

Global Elimination Program

In 1993, the International Task Force for Disease Eradication classified LF as one of six potentially eradicable infectious diseases, with the other five being dracunculiasis (Guinea worm disease), poliomyelitis, mumps, rubella, and taeniasis/cysticercosis (pork tapeworm) (26). Subsequently, in 1997, the World Health Assembly passed Resolution 50.29 calling for the elimination of LF as a public health problem. In 2000, the World Health Organization (WHO) launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF) with the goal of eliminating LF by the year 2020. The GPELF is a partnership between the WHO and local ministries of health, donors, non-governmental organizations, and pharmaceutical companies. Drugs, such as Diethylcarbamazine (DEC), Albendazole, Ivermectin, and Doxycycline have been effectively used to kill microfilariae. However, people with lymphedema and elephantiasis are unlikely to benefit from treatment because most are no longer infected with the parasite. Thus, LF elimination programs focus on two program components: 1. stopping the spread of infection through mass drug administration (MDA); and 2. alleviating suffering caused by LF through morbidity management and disability prevention (MMDP) (2).

Mass Drug Administration

Mass drug administration (MDA) is a method of preventive chemotherapy in which drugs are administered to an entire at-risk population of people. WHO guidelines for LF elimination recommend annual administration of two drugs given together for at least a five-year period. In countries where onchocerciasis ("River Blindness") is prevalent, DEC is contraindicated because it can worsen onchocercal eye disease. Thus, the recommended drugs include either a combination of DEC and albendazole (for

countries not co-endemic for onchocerciasis), ivermectin and albendazole (for countries co-endemic for onchocerciasis), or semi-annual doses of albendazole (in areas co-endemic for Loiasis, a fly transmitted skin and eye disease caused by the nematode worm *Loa loa* (27). The goals of MDA are to reduce the density of circulating LF microfilaria (mf) in the blood of infected individuals, and to reduce the infection prevalence in the community to below a threshold (<2% antigen prevalence at the 95% confidence limit) at which transmission can no longer be sustained in the absence of drug intervention(2). MDA is typically implemented at the district or equivalent administrative unit, which is called the MDA implementation unit (IU) for LF programs.

Within the first 17 years of the GPELF, over 890 million people living in endemic areas received at least one LF treatment for a total of more than 7.1 billion LF treatments administered (2). An estimated 554 million people no longer require MDA which is a 38% reduction of those living in endemic areas in 2000. An estimated 18.73 million hydrocele and 5.49 million lymphedema cases were prevented (28). Furthermore, microfilaremia prevalence dropped by 68%, hydrocele prevalence dropped by 49%, and hydrocele prevalence dropped by 25% for a reduction in the global prevalence of LF by 59% from (3.55% to 1.47%).

Stop-MDA decisions are based on a Transmission Assessment Survey (TAS) that determine if LF antigen or mf prevalence in endemic areas have reached the critical threshold of transmission sustainability. During TAS, blood samples from 6-7-year-old children are taken as this population was born during the MDA implementation years and should be free from LF infection if MDA succeeded in transmission interruption. TAS are carried out at the evaluation unit (EU) level. An EU may be made up of part of an IU,

an entire IU, or a combination of two or more IUs that have a common border or are similar epidemiologically (29). TAS is a lot quality assurance survey (LQAS) design. If the total number of positive cases in an EU is at or below the critical threshold of infection, MDA can be stopped. However, if the number of positive cases is above the critical threshold of infection, MDA should be implemented for at least two additional rounds (29).

TAS surveys design may vary between countries and EUs. These survey designs are dependent upon primary school enrollment rates in the EU, number of schools, target population, target population size, sample size, survey type, critical cutoff, and diagnostic tool (29). In order to qualify for the TAS, implementation units must have completed at least 5 rounds of MDA, have an MDA coverage of greater than 65% of the implementation unit, and sentinel and spot-check sites must have an mf prevalence of less than 1% or CFA prevalence of less than 2% (for *W bancrofti* transmitted by *Anopheles* or *Culex* mosquitoes) among convenience samples of at least 300 people over the age of 5 years at all sites after the last round of MDA. Sentinel sites are established at baseline, prior to MDA implementation, to monitor infection levels and the impact of MDA, while spot check sites are selected based on local knowledge of suspected higher transmission areas in order to provide a conservative bias for progressing to TAS (2).

Morbidity Management and Disability Prevention

In order to achieve the second goal of preventing disease progression and alleviating suffering due to LF, a minimum package of care for the management of lymphedema and hydrocele must be available in primary health care systems and accessible to LF patients. The goal is to provide the minimum package in all known areas

where there are LF patients. Surgery for patients with hydrocele, treatment for ADL episodes, management of lymphoedema in the prevention of ADL and disease progressions, are all part of the basic care package for MMDP (2). Morbidity management should begin as soon as resources are available and are required as part of the requirements for validating a country's elimination of LF as a public health problem.

The implementation of both MDA and MMDP together is found to have a more significant impact on decreasing the burden of LF than the implementation of either one of these programs alone. A study in India showed that areas with programs to assist with morbidity management tended to lead to better adherence to MDA (6, 30) .

Post Treatment Surveillance

Once MDA has been stopped in an area, post-treatment surveillance (PTS) can begin. WHO recommends approximately 4 years of post-treatment surveillance in order to confirm the absence of sustained LF transmission (31). The most common option for PTS includes repeated Transmission Assessment Surveys (TAS-2) and (TAS-3) conducted at two year intervals following stop MDA TAS-1.

Transmission Assessment Surveys

Similar to stop-MDA TAS surveys, PTS TAS surveys are LQAS surveys that help to determine if infection prevalence remains below a level where recrudescence is unlikely to occur in the absence of MDA. Surveys should be performed at least twice after MDA has stopped and be implemented 2-3 years apart. Transmission is then assumed to be interrupted, though this is difficult to ascertain in reality.

TAS Surveys target first- and second-year primary school children as they are expected to be in the target age range of 6-7 years old. Children in this age range should

be protected from LF infection given MDA was successful in transmission interruption, and antigenaemia in these children is used as a marker for recent transmission (31).

The methodology for TAS-2 and TAS-3 surveys is the same as for TAS-1. Ideally EU configuration within TAS areas are maintained across TAS surveys for consistency of monitoring. Areas that 'pass' TAS-2 or TAS-3 maintain stop-MDA status, while areas that 'fail' TAS-2 or TAS-3 must resume MDA for at least two years before restarting TAS-1 and PTS assessments.

Pacific Programme for the Elimination of Lymphatic Filariasis

The Pacific Programme for the Elimination of Lymphatic Filariasis (PacELF) was established in 1999 to coordinate LF elimination in 22 Pacific countries and territories. It was the first regional LF control program in the pacific with the goal of eliminating LF through MDA and clinical management to minimize disease progression in those already with the disease (32). The PacELF guidelines for stopping MDA were based on a 'C-survey' conducted after 5 rounds of MDA and which determined the antigen prevalence in the general population by testing individuals at least 2 years old. If the percentage of antigenemia in the sample population was <1% with an upper CI of <2%, MDA could be stopped. The PacELF 'C-survey' methodology has been superseded by the GPELF TAS methodology, however there are concerns that surveys of children may not be a valid indicator of community wide LF transmission due to differential risk factors between children and adults (33, 34).

Onchocerciasis

Onchocerciasis, often referred to as river blindness (RB), is a parasitic disease caused by the filarial nematode *Onchocerca volvulus.* RB is transmitted through repeated bites from blackflies of the *Simulium* genus (35). The disease is termed river blindness because the blackflies that cause the disease breed near fast-flowing rivers and streams. Infection causes severe itching, nodules under the skin, dermatitis, depigmentation, and lesions in the eye that can lead to blindness (36).

RB is endemic in 30 countries across sub-Saharan Africa, six countries in Latin America, and also in Yemen. It is the second leading infectious cause of blindness worldwide, after trachoma, and the fourth leading cause of preventable blindness after cataract, glaucoma, and trachoma. The WHO estimates 25 million people are infected with *O. volvulus* with 99% of the disease burden being in Africa (37). Of those infected, 800,000 are visually impaired in some capacity, and 300,000 have become blind (35). About 200 million people remain at risk for contracting the disease-causing parasite (37). The distribution of RB is connected to transmission zones formed by the interaction of infection maintained between blackflies and humans (38). There is no vaccine currently available for river blindness, nor medication that can prevent *O. volvulus* infection, however, transmission can be eliminated from communities with 12-15 years of MDA with ivermectin, corresponding to the lifespan of the adult female worm whose death leads to the permanent interruption of transmission (39, 40). For those already infected with *O. volvulus*, ivermectin also serves as the recommended treatment and is given annually, semi-annually, or quarterly, depending on the intensity of infection, for a minimum of 15 years. Ivermectin primarily kills the *Onchocerca* larvae and prevents them from causing damage, such as skin rash and blindness, but does not kill the adult parasites (35).

Onchocerciasis was target for elimination in the Americas by 2015 and has already been eliminated in Colombia, Ecuador, Mexico, and Guatemala, with Brazil and

Venezuela still yet to request for verification of elimination. Select African countries have been targeted for elimination by 2020 with parts of Uganda and Sudan in the post-treatment surveillance phase (40).

History

Microfilaria were first recognized in 1875 in symptomatic individuals by a British naval surgeon named John O'Neill in present day Ghana. Rudolf Leuckart then identified the adult worm in 1890 which was later documented in a book by Patrick Manson. Because the adult worms have the appearance of a curved hook-tail, the name Onchocerca comes from the Greek words *onchos* and *cercos*, which mean hook and tail, respectively. Onchocerciasis was first mentioned in 1915 after consultations with symptomatic patients in Guatemala and later confirmed to be caused by the same parasite seen in Africa. In 1923, Donald Breadalbane Blacklock discovered that the blackfly is the vector for *O. volvulus* transmission. Jean Hissette was the first to link eye troubles and blindness to onchocerciasis in a study of persons along the Sankuru and Uéle rivers in the Belgian Congo in 1930 (41).

Biology and Pathology

Onchocerciasis is spread to humans through an infectious blackfly. A blackfly bites an *O. volvulus*-infected person and microfilariae from the subcutaneous tissue of the infected person develops into third-stage filarial larvae inside of the blackfly over a two-week period, after which time the larvae are infectious to humans. The infected blackfly bites only during the day and deposits larvae that penetrate the skin during the process. The worm larvae can only reproduce in humans and can take up to one year to develop into an adult and about 10 to 20 months before they are found in nodules in subcutaneous

connective tissues. Adult worms can live in nodules for about 15 years. Adult female worms measure 33 to 50 cm in length and 270 to 400 μm in diameter and can produce millions of new larvae within their reproductive lifespan of about 10 years. Male worms measure 19 to 42 mm in length and 130 to 210 μm in diameter. Because larvae complete some of their development in the blackfly, the number of worms inside an individual directly correlates to the number of infectious bites sustained by an individual. Symptoms usually begin after adult worms begin producing mf. Individuals experiencing many infectious bites over a long period of time are more likely to suffer from blindness (35). As onchocerciasis is neither vaccine- nor drug-preventable, the best prevention methods are to avoid bites by blackflies by using insecticides and wearing long sleeve shirts and pants.

In some areas, infective vectors can travel distances of up to 500 km, spreading the disease between different communities and maintaining transmission despite local control efforts (37).

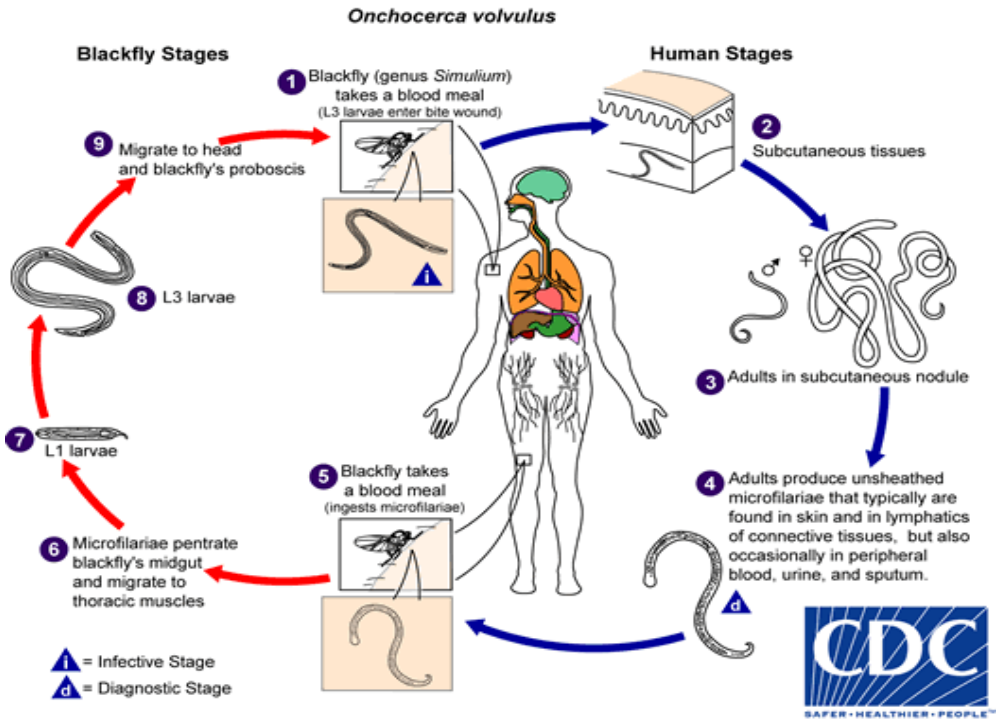


Figure 2. Life Cycle of *Onchocerca volvulus* (35)

Diagnostic Tests

There are no antigen tests available to test for onchocerciasis, but skin snip biopsies to test for mf and two antibody tests used to detect IgG4 antibodies to the recombinant antigen Ov-16 are available. Antibody tests cannot distinguish between past and current infection but the presence of Ov-16 antibodies in children can be evidence for recent transmission (42). Since 2001, the WHO has been utilizing the Ov-16 antibody testing to verify transmission interruption of onchocerciasis in humans (43).

Skin Biopsies

Superficial skin biopsies, or "skin snips", weighing 1-3 mg can be taken from the iliac crests using field-sterilizable corneoscleral punches. Skin snips are incubated in saline for 24 hours and then examined by microscopy for mf to determine *O. Volvulus* infection (44).

Ov-16 ELISA

ELISA testing is used to detect IgG4 antibodies in the blood to the Ov-16 recombinant antigen. It is a lab-based test that provides quantitative and qualitative measurement of Ov-16 using a standard curve created by dilutions of a positive control as a reference. Cut-offs are determined using arbitrary units after analyzing positive and negative samples (43).

Ov-16 monoplex

The *Ov-16* monoplex test is a point-of-care rapid test used to detect *Ov-16* antibodies in the blood. It requires a drop of blood for testing (43).

Ov16/Wb123 biplex

A combined Ov16/Wb123 biplex test was developed to enable simultaneous testing for LF and RB because of the co-endemicity of the two diseases in many areas-particularly in Africa (25).

Onchocerciasis Control Programs

In 1974, the Onchocerciasis Control Programme in West Africa (OCP) began large scale onchocerciasis control through vector control by aerial spraying and used skin snip procedures for epidemiologic evaluation of the disease. Taking into account the ten-year reproductive lifespan of an adult female onchocercal worm, 14 years of vector control is needed in order to achieve onchocerciasis elimination (37). Before OCP closed in 2002, it relieved 40 million people from infection and prevented blindness in 600,000 people (40).

The African Programme for Onchocerciasis Control (APOC) was launched in 1995 with its main strategy being community-directed treatment with ivermectin (CDTI).

It operated in onchocerciasis-endemic countries throughout Africa until 2015 when the transition to disease elimination began. It took into account the epidemiologic and ecologic settings for onchocerciasis in Africa that vary widely and range from areas with low levels of infection, to large areas with efficient vectors that maintain a high level of infection (37). More than 119 million people were treated with ivermectin in APOC's final year with many countries significantly decreasing onchocerciasis associated morbidity.

Post-Treatment Surveillance

According to WHO guidelines, the decision to stop MDA is based on results of entomological (O-150 PCR Poolscreen) testing of black flies. After a PTS period of between 3 and 5 years, transmission interruption is confirmed by entomological testing and, if necessary, serological testing. This 3-5 years of post-treatment surveillance may be extended in areas co-endemic for LF where treatment may continue after the stop MDA decision for onchocerciasis is justified (39). If there is an insufficient number or absence of flies, MDA can be stopped when serological results reveal an Ov-16 antibody prevalence of less than 0.1% at the 95% confidence limit (CL) among children less than 10 years of age. However, the current diagnostic tools available are not able to reasonably detect <0.1% prevalence of Ov-16 due to imperfect specificity. The lack of an adequate diagnostic tool makes it difficult to make stop-MDA decisions and conduct post-treatment surveillance for onchocerciasis elimination (45). During PTS for RB, programs must seek to identify people harboring the parasite, not just those who exhibit symptoms of onchocerciasis. Entomological evaluation confirms transmission interruption at the end of post-treatment surveillance and programs can then enter the last phase of elimination,

post-elimination surveillance (PES). PES's goal is to detect disease recrudescence or reintroduction. As part of elimination, the WHO 2016 guideline requires an oversight committee to be in place by each country's Ministry of Health that is independent of the national onchocerciasis program (46). The WHO Regional Office for Africa supervises the Expanded Special Project for the Elimination of Neglected Tropical Disease in Africa (ESPEN) whose goal is to eliminate the five preventive chemotherapy NTDs: lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminths (ascariasis, trichuriasis, and hookworm infection), and trachoma, and which assumed regional programmatic oversight in the post-APOC era (40).

Chapter II: Manuscript

Introduction

In Nigeria, Lymphatic Filariasis (LF) is caused by the parasite *Wuchereria bancrofti.* It is the second most endemic country in the world for LF, behind India, and the most endemic in all of Africa (47). With over 190 million inhabitants, approximately 120 million Nigerians are at risk for LF and an estimated 25 million have the disease, making it the fifth most prevalent neglected tropical disease in Nigeria (48). In 1997, The Carter Center assisted the Nigerian Federal Ministry of Health (FMOH) in establishing an LF elimination program in Plateau and Nasarawa States. Baseline LF mapping in Plateau and Nasarawa States occurred from 1998-2000 and found all 30 local government areas (LGAs) to be endemic with a mean antigen prevalence of 23% (range: 4-62%) among adults (Eigege et al., 2017). Annual albendazole-ivermectin mass drug administration (MDA) occurred in all 30 LGAs by 2003 and by 2007/2008, PacELF 'C-surveys' in each LGA showed that 10 LGAs met the stop-MDA criteria with an LF antigen prevalence less than 2% at the 95% confidence limit among individuals at least 2 years old. Among the 20 LGAs that did not meet stop-MDA criteria, the LGAs with the highest antigen prevalence (14.1% - 14.8%) were Kanam, Mikang, and Kanam of Plateau State (49). After five additional rounds of MDA, these and the remaining LGAs successfully passed transmission assessment surveys (TAS) surveys in 2012 and thus entered post-treatment surveillance (50).

Nigeria holds the largest burden of onchocerciasis, often referred to as river blindness (RB), in the world with 40% of the global at-risk population. There are an estimated 50 million people at risk for contracting RB throughout 40,000 communities in

Nigeria. During the African Programme for Onchocerciasis Control (APOC), 30 million people in Nigeria were treated with annual ivermectin MDA (51).

Of the *Simulium* black flies, it is specifically the *Simulium damnosum* complex that transmits the nematode in Africa (52). *Simulium damnosum* s.l. is a complex of closely related sibling species and in Nigeria, there are 9 known sibling species in the *S. damnosum* complex which all transmit onchocerciasis (53).

The Onchocerciasis Program began in 1991 in Plateau State with a disease mapping exercise based on superficial skin biopsies of adults (44). Nasarawa State was originally the eastern part of Plateau State before splitting off in 1996. Villages that had a microfilaria (mf) prevalence of at least 5% were considered to be mesoendemic. Any LGA that had at least one mesoendemic village was considered in need of MDA in its entirety. LGAs with at least one village with an mf prevalence of at least 80% were considered hyperendemic. Villages with an mf prevalence of less than 5% were considered hypo-endemic and not in need of MDA. Of the original 23 LGAs in Plateau State, 10 were classified as meso- or hyperendemic and in need of MDA, which corresponded to 12 LGAs in the two-state configuration following the formation of Nasarawa state. Eighteen LGAs were considered either non-endemic or hypo-endemic and therefore were not in need of MDA. Of the LGAs chosen for this study, Kanke was classified as meso-endemic and Mikang and Kanam were classified as non/hypo-endemic (44).

From 1992 to 1993, MDA with ivermectin began in the 12 meso/hyper-endemic LGAs with a treatment coverage goal of at least 80%. By 1994, all MDA eligible communities were under treatment and in 1995, greater than 80% coverage was achieved.

In 1996, the APOC community-directed treatment with ivermectin (CDTI) strategy was adopted, with at least 80% coverage continuing to be reported. Kanke received CDTI from 1993-2017 with annual albendazole added for LF elimination from 2001-2012 (Table 1) (44). Although Mikang and Kanam were non/hypo-endemic and did not qualify for CDTI, they received annual ivermectin-albendazole MDA from 2002 and 2003 respectively to 2012 as part of LF MDA.

In order to verify the absence of LF transmission, the World Health Organization (WHO) guidelines recommends two cross-sectional TAS surveys of 6-7 year-old children be conducted 2-3 years apart at 2-3 years after the last round of MDA. However, there is concern that the TAS target population may not accurately reflect the risk of LF transmission in the general population. Therefore, this study aims to compare LF antigen prevalence between TAS sample populations (school-age children aged 6-7 years old) versus the PacELF 'C-survey' community-wide population (>2 years old) in low- and high-recrudescence risk LGAs of Plateau State. This study also aims to compare results of the Ov16/Wb123 biplex rapid antibody test with laboratory-based ELISA for anti-Ov16 and anti-Wb123 antibodies in 3 LGAs (Kanke, Mikang, and Kanam) in Plateau State, Nigeria. These LGAs had the highest LF antigen prevalence in 2007-2008 surveys and were predicted to be at greatest risk of LF transmission recrudescence after stopping MDA for LF in 2013. This study also takes advantage of the differing RB baseline endemicity between LGAs to compare the age distribution of Ov16 in RB meso-endemic LGA Kanke versus non-/hypo-endemic LGAs Mikang and Kanam.

Methods

Survey Design:

Two different surveys were conducted as part of this Post-MDA Operational Research study from April 11 – May 5, 2016 in Kanke, Mikang, and Kanam LGAs of Plateau State. The first survey was a school-based transmission assessment (TAS) survey of first- and second-year primary school children. Two evaluation units (EUs) were formed based on RB endemicity: Kanke was considered one EU; Mikang-Kanam was considered a second EU. Based on the estimated number of children in each EU being 8% of the total population, target sample sizes for the Kanke and Mikang-Kanam EUs were 1,540 and 1,556 respectively, with critical cutoff values for "passing" the TAS of 18 antigen-positive children for both EUs. TAS sample sizes and critical cutoff values are powered so each EU has at least a 75% chance of passing if the true antigen prevalence is 1.0% and no more than a 5% chance of passing (incorrectly) if the true prevalence is greater than or equal to the 2% threshold level.

The TAS design employed two stages of selection. In the first stage, 45 schools per EU were selected by interval (systematic) selection following a random start from an ordered list of all schools within each EU. In the second stage of selection, class 1 and 2 pupils were recruited from each school, as these grades were expected to contain the target age-group of 6 and 7-year-old children. All children in these classes were eligible for selection regardless of age. Approximately 45 children were selected for inclusion at each school with a maximum of 55 children selected from any single school.

The PacELF 'C-survey' was the second survey conducted in this study. This survey was a two-stage household cluster survey that was conducted in each LGA to

evaluate the sensitivity in detecting LF recrudescence versus the TAS survey design. Each LGA was considered as an individual survey domain, with the target population being individuals older than two. Assuming a non-response rate of 20% and an average of 6 residents per household, 20 clusters with an average of 15 households per cluster was needed. One sentinel site village from each LGA was also purposively sampled. Within each LGA, 20 census enumeration areas (EAs) were randomly selected from a list of EAs. In order to determine if LGA infection levels were less than 2% at the upper 95% confidence limit (uCL) a total of 1,180 individuals in each LGA was needed. When necessary, maps of each selected EA were used to divide EAs into segments with approximately 15 households per segment. A village chief then randomly selected one segment in which all households were eligible for inclusion. Households were then randomly selected within each EA and all individuals over 2 years of age within the household were eligible to be included in the survey.

Data Collection:

Data for these surveys were collected by six teams, each team consisting of a data recorder, a lab scientist, and a lab technician. For the TAS survey, summary information from each school (including school/cluster name, location, GPS coordinates, and total number of students present) was collected. Using a standard collection TAS survey paper form, trained data collectors recorded the sex, age, and consent/assent to participate from assenting children in classes 1 and 2 of the selected schools. Participants were also asked if they had lived in the respective LGA their whole life (for the purpose of determining whether any identified LF infections represented endemic transmission or were the result of importation). Blood samples were taken from assenting participants and used for

point-of-care Binax Now® Filariasis Immunochromatographic Card Test (ICT) (Alere Inc., Scarborough, ME) and Ov16/Wb123 biplex (PATH, Seattle, WA) testing. Dried blood spots (DBS) from each participant were prepared, sorted into airtight zip-lock bags by school or cluster, and sent to The Carter Center lab in Jos, Plateau State and frozen for subsequent testing of LF and RB antibodies by laboratory-based ELISA. DBS were processed for both IgG4 antibodies against Ov16 recombinant antigens using the Onchocerciasis Elimination Program for the Americas (OEPA) Ov16 ELISA methodology (42), and for IgG4 antibodies against the recombinant Wb123 antigen using the Filaria *Detect*™ IgG4 ELISA kit (24). Household level information was obtained for the PacELF 'C-survey'. Information was collected simultaneously on hand-held tablet computers using Eagle version 1.3.3 survey software and on paper forms. For each household, the household number, cluster number, and LGA were recorded along with the sex and age of the head of household, the number of people, sleeping spaces, and nets in the household, if indoor residual spraying (IRS) for mosquitoes was done in the past 12 months, and if there was someone in the household with lymphoedema or hydrocele. Demographic data was collected for each assenting participant in the household over the age of two, including their sex, age, whether they slept under a bed net the previous night, and if they lived in the respective LGA their whole life. Every participant was assigned a unique study ID number. Blood samples were taken from assenting participants and used for point-of care ICT and Ov16/Wb123 biplex testing. DBS from each participant were taken and sent to The Carter Center lab in Jos, Plateau State and frozen for subsequent testing of LF and RB antibodies by laboratory-based ELISA using the same ELISA methodologies as the TAS Survey.

Ethical Approval:

The survey protocol was approved by the Emory University Institutional Review Board (IRB00086795) as well as The Nigeria National Health Research Ethics Committee (NHREC/01/01/2007). Informed verbal consent was received from each participant before interviewing and before blood testing. Participation in the survey was voluntary. Children 6-17 years old were able to give assent to participate in the study and those under six required parental consent to participate in the study.

All those who tested CFA positive by ICT were offered ivermectin and albendazole treatment for LF and advised to sleep under their mosquito bed nets.

Data Analysis:

A total of 7,668 individuals across the two surveys participated in the study. Paper forms were transcribed onto excel spreadsheets and merged with serological results from The Carter Center lab in Jos, Nigeria. Of the 7,668 observations, 814 were excluded due to unreconcilable data, resulting in a total of 6,854 observations with linked demographic information and serological results. All statistical analyses were conducted using Stata v16.1. Point estimates and upper 95% CLs were calculated using the Stata *svy* survey command to account for the complex survey design. Sampling weights were calculated as the inverse of the product of the sampling probabilities.

Age groups for the PacELF 'C-survey' were created using 10-year intervals, with the oldest age group consisting of all participants 80 years old and above. Maps were created using the ArcMap desktop application of ArcGIS v10.7.1.

To evaluate risk factors for seropositivity to lymphatic filariasis and onchocerciasis, univariate logistic regression analyses were conducted for each potential

risk factor, using seropositivity to Wb123 and Ov16 antibodies and *Wuchereria bancrofti* CFA. Seropositivity estimates and upper 95% CLs for each EU was determined for each survey type in order to determine LF antigen and LF and RB antibody prevalence within each EU.

Results

Survey Demographics:

The total number of households selected for the PacELF 'C-survey' was 855 throughout 63 clusters, and overall 4,386 individuals were surveyed. Due to missing demographic data, 14 households were excluded from the final dataset. There were 337 individuals who did not consent to blood testing and household level data was missing for an additional 43 individuals. There were 277 Ov16 and Wb123 ELISA results that did not have corresponding demographic data and 423 observations with demographic data that did not have corresponding ELISA results. A further 14 observations were dropped due to duplicate entry errors and all observations from one cluster were dropped due to missing demographic data. Due to these factors, the PacELF dataset includes 833 households and 3,569 individuals: 1,175 from Kanam, 1,143 from Kanke, and 1,251 from Mikang (Table 2). The mean household size for the PacELF survey was 8.7 people and the mean age of individuals tested was 23.7 years (range. 1-95). 43.2% of survey participants were male and 96.8% of individuals surveyed lived in the LGA in which they were surveyed their whole lives.

A total of 83 schools were selected for inclusion in the TAS survey throughout the two EUs. All 41 schools selected in Kanke were surveyed whereas the target sample size had been met in Mikang-Kanam after sampling only 36 of the 42 selected schools for a

total of 77 schools surveyed across both EUs. Of the 3,291 students selected for testing, 6 refused, resulting in a total of 3,285 (99.8%) students tested. Of the students tested, 1,724 students were from the Kanke EU and 1,561 students were from the Mikang-Kanam EU. The mean age of students tested was 6.6 years with a standard deviation of 0.9. 50.4% of the students were male and 99.9% of students surveyed lived in the LGA in which they were surveyed their whole lives (Table 3).

The final dataset includes results for both surveys and contains 6,854 (89.3% of those selected for testing) individuals, 77 schools, and 833 households.

Coverage of Lymphatic Filariasis Control Measures:

Survey questions were used in the PacELF Survey design to assess the coverage of traditional mosquito control measures (Table 2). The mean number of sleeping spaces in the house was 4.4 and 2,800 individuals (78.5%) reported having access to a mosquito net, with a greater proportion in Kanke (93.9%) versus the other two LGAs. Of people with access to a mosquito net, 29.0% reported sleeping under a net the previous night, with a lower proportion in Mikang (9.3%) compared to the other two LGAs. In the 12 months preceding the survey, no respondents report having indoor residual spraying (IRS) in their household. Additionally, no respondent report having had lymphedema in the past 3 months and no male respondent report having hydrocele.

Lymphatic filariasis and Onchocerciasis Descriptive Analysis:

Antigen and antibody prevalence and corresponding upper 95% CLs by LGA for the PacELF survey are shown in Table 4. The overall antigen prevalence was 0.25% (95% uCL 0.44). Overall seroprevalence of antibodies to the Wb123 filarial antigen by biplex and ELISA was 0.14% (95% uCL 0.29) and 1.01% (95% uCL 1.33) respectively.

Overall seroprevalence of antibodies to the Ov16 filarial antigen by biplex and ELISA was 0.06% (95% uCL 0.18) and 0.03 (95% uCL 0.13) respectively.

Antigen and antibody prevalence and corresponding upper 95% CLs by RB co-endemicity for the TAS survey are shown in Table 5. The TAS survey did not find any participant positive for LF CFA by ICT or for RB Ov16 antibodies by both ELISA and biplex tests. Seroprevalence of antibodies to the Wb123 filarial antigen by biplex was similar for both RB meso/hyper-endemic and hypo-endemic areas, 0.12% (95% uCL 0.36) and 0.13% (95% uCL 0.40) respectively while seroprevalence by ELISA was significantly higher at 3.71% (95% uCL 4.55) and 1.28% (95% uCL 1.86) for RB meso/hyper and hypo-endemic areas respectively.

Age distribution of LF antigen and LF and RB antibody prevalence and corresponding upper 95% CLs for the combined PacELF and TAS samples populations are shown in Table 6. *Wuchereria bancrofti* CFA prevalence was 0.13% (95% uCL 0.23) overall, with an apparent increasing trend with age: prevalence was highest among the 40-49-year age category at 0.98% (95% uCL 2.51). Seroprevalence of antibodies to the Wb123 filarial antigen by ELISA was greater than 0 for all age categories with the highest seroprevalence among 5-9 year olds and lowest among those less than 5 years old (Figure 1). However, seroprevalence point estimates by Wb123 biplex were greater than zero in only the 5-9 year and ≥ 50 year age categories. Seroprevalence of antibodies to the Ov16 filarial antigen was greater than zero only for the ≥ 50 age category for both the ELISA and biplex tests at 0.26% (95% uCL 1.21) and 0.51% (95% uCL 1.61) respectively.

Geo-spatial distribution of *Wuchereria bancrofti* CFA positive cases was plotted for each survey by cluster (school or EA) throughout the Kanam, Mikang, and Kanke LGAs (Figure 2). There were no cases positive by *Wuchereria bancrofti* CFA among the students in the TAS school survey. The greatest number of *Wuchereria bancrofti* CFA positive cases within a cluster for the PacELF 'C-survey' was 3 within the Kanam LGA while there were no cases positive by *Wuchereria bancrofti* CFA in Mikang. Geo-spatial distributions of the number of positive cases by Ov16/Wb123 biplex, Wb123 ELISA, and Ov16 ELISA were also plotted for each survey by cluster (Figures 3-6). There was a maximum of one case per cluster positive by Wb123 biplex in both the TAS and PacELF surveys. These cases were present throughout the 3 LGAs. The number of positive cases by Wb123 ELISA was scattered throughout the 3 LGAs with as many as 4 positive cases in PacELF 'C-survey' clusters and as many as at least 5 positive cases in the TAS survey schools. The TAS survey shows a high concentration of cases positive by Wb123 ELISA in Kanke. There was a maximum of one case per cluster positive by Ov16 biplex in the PacELF 'C-survey' with no positive cases for the TAS survey and no positive cases for either survey in Mikang. There was only one positive case by Ov16 ELISA which was located in Kanke and identified through the PacELF 'C-survey'.

Test concordance between the ICT for *Wuchereria bancrofti* CFA and filarial antibody seroprevalence to the Wb123 filarial antigen by ELISA and biplex tests was examined using each test as the index (Table 7). Test concordance was fairly low when using either of the three tests and the index. When using ICT as the index test, only one of nine (11.1%) ICT positive samples was also Wb123 biplex positive, while none of the nine were positive for Wb123 by ELISA. Similarly, of the nine Wb123 biplex positives,

only one (11.1%) was ICT positive, and none were Wb123 ELISA positive. Of the 120 Wb123 ELISA positive samples, none were positive for CFA by ICT or for Wb123 by biplex rapid test.

Test concordance between filarial antibody seroprevalence to the Ov16 filarial antigen by ELISA and biplex tests was also examined using each test as the index (Table 8). The one Ov16 ELISA sample was one of the two Ov16 biplex test positive samples,

Household Risk Factor Analysis:

Univariate logistic regression analyses for *Wuchereria bancrofti* CFA and Wb123 and Ov16 biplex and ELISA test positivity were performed in order to determine their association with household risk factor variables. Data from TAS and PacELF surveys were combined for variables collected in both surveys. The following risk factors were strongly associated with an increased odds of *Wuchereria bancrofti* CFA positivity in univariate analyses: female (gender binary variable) [odds ratio (OR)= 3.21, 95% CI 1.10-9.37, *P*=0.03] and not having access to a mosquito net (binary variable) (OR= 5.36, 95% CI 1.75-16.39, *P*=0.004), while the number of sleeping spaces in household per additional sleeping space (discrete integer variable) was significantly associated with a decreased odds in *Wuchereria bancrofti* CFA prevalence (OR= 0.82, 95% CI 0.72-0.94, *P*=0.004), (Table 9). Univariate analyses was not possible for the 0-9 and 10-19 year age categories, nor for the variables of lived in respective LGA whole life, slept under a mosquito net the previous night, interior walls sprayed for mosquitos in the last 12 months, had lymphedema for the last 3 months, and has hydrocele, due to cells with a prevalence of 0%. Odds of *W. bancrofti* CFA positivity was lower in the 20-39 and 40-59-year age categories compared to the 60 years and older age category, but this

association was not statistically significant (*P*=0.21, 0.65 respectively). There was no association between the number of people in the household per additional person (discrete integer variable) and odds of CFA positivity (OR= 1.02, 95% CI 0.97-1.08, *P*=0.36).

Table 10 shows univariate logistic regression analysis of risk factors for seropositivity to Wb123 biplex. The number of sleeping spaces in household per additional sleeping space (discrete integer variable) was significantly associated with a decrease in odds of seropositivity to the Wb123 filarial antigen by biplex test (OR= 0.77, 95% CI 0.60-0.99, *P*=0.04) and not living in the respective LGA whole life (binary variable) was significantly associated with an increase in odds of seropositivity (OR= 16.00, 95% CI 3.09-82.78, *P*<0.001). Sex, age, number of people in household, and mosquito net access were not significantly associated with odds of seropositivity to Wb123 filarial antigen by biplex test.

A decrease in odds of seropositivity to the Wb123 filarial antigen by ELISA test was significantly associated with female (OR= 0.51, 95% CI 0.27-0.99, *P*=0.05) while not sleeping under a mosquito net the night before the survey was significantly associated with an increased risk of odds of seropositivity (OR= 18.48, 95% CI 2.36-144.80, *P*=0.01) (Table 11). There was no significant association found with odds of seropositivity to Wb123 filarial antigens and any of the other variables in the analyses.

The only variable that saw a significant association with seropositivity to Ov16 antigens by biplex test in univariate analyses was not living in the respective LGA whole life, which was significantly positively associated with seropositivity (OR= 21.14, 95% CI 1.06-422.58, *P*=0.05) (Table 12). Seropositivity by Ov16 ELISA test saw a significant

positive association with people in household per additional person (OR= 1.04, 95% CI 1.00-1.07, *P*=0.03) and sleeping spaces in household per additional sleeping space (OR= 1.22, 95% CI 1.13-1.32, *P*<0.001) (Table 13).

Discussion

This survey was performed as part of the post-treatment surveillance phase of the GPELF framework following the decision to stop-MDA for LF in Plateau and Nasarawa States, Nigeria. A community-based household survey was simultaneously conducted in order to verify the use of the TAS survey to accurately represent LF transmission risk in the broader population. This study measured *Wuchereria bancrofti* CFA prevalence in a TAS survey design and compared it to the antigen prevalence in the community-based household survey design. It also compared prevalence of LF and RB serological biomarkers across diagnostic test types. Because TAS surveys allow for faster, more convenient, and cheaper testing than community-based surveys of LF prevalence in the population compared to previous methodologies, their adoption has allowed for more efficient LF stop-MDA surveys and post-treatment surveillance surveys to be conducted. Recent studies comparing school-based TAS surveys and community-based surveys suggest that community-based surveys can provide a good indication of overall CFA prevalence in older age groups and identify foci of ongoing transmission while school-based TAS surveys do not (33). The results of this study indicate that the PacELF survey design was not superior to the school-based TAS design in detecting evidence of LF transmission in the survey areas, as prevalence was extremely low by both methodologies. However, some studies found that testing adults would be more efficient in detecting transmission in low prevalence settings compared to testing children 6-7

years old (54). One study conducted during LF PTS in Sri Lanka found that community-based adult-TAS combined with molecular xenomonitoring (molecular detection of filarial DNA in systematically sampled mosquitoes) provided an alternative surveillance approach to verify absence of LF transmission and identify areas requiring additional intervention (55).

Despite scale-up of MDA throughout LF endemic areas of Nigeria, transmission interruption has only occurred in Plateau and Nasarawa states (56). In 2017, 14.3% of all people needing treatment for LF were living in Nigeria with people living in 583 of 774 Local Government Areas (LGAs) in Nigeria requiring preventive chemotherapy for LF (57). The WHO is currently working with the Nigerian Government to distribute medications through MDA in 520 LGAs. In 2018 over 79 million people were reached with preventive chemotherapy.

There are, however, several limitations to the analysis of the current study. This study was conducted in 2016 and ivermectin was distributed in the Kanke LGA until 2017. Ivermectin monotherapy exerts microfilarial activity against *W. bancrofti* and ivermectin MDA can suppress microfilaremia over time among infected persons and prevent recrudescence (50). Taking this into consideration, we must question whether or not repeated TAS or PacELF surveys in areas with ongoing ivermectin MDA can truly be considered as part of LF PTS. Secondly, because children were enrolled by class and not age, children's ages were outside the target 6-7 year old age range, with children up to 12 included in the survey. Although this TAS study saw an antigen prevalence of 0.00%, future studies that find an antigen prevalence above zero may therefore be overestimating, provide greater certainty of stop-MDA threshold achievement. Thirdly,

TAS methodology results in equal selection probabilities for individuals by selecting participants with probability proportional to school size. The method of selection of participants in the TAS survey for this study did not result in equal selection probabilities for each individual, but given there were no antigen positives in the survey, the results are still believed to not alter the decision to pass the EUs.

Recent studies suggest the use of serologic tools for LF antibody testing to guide LF elimination program decision making and support surveillance (24). Because blood levels of mf and antigens decline in the population after effective MDA, it is difficult to use these as transmission markers, but more research is needed in order to understand the use of antibody testing in LF elimination and establish LF antibody thresholds (58). According to the GPELF, MDA implementation in Nigeria is projected to cease in 2020 and shift focus towards morbidity management, however, 33 of the 583 LGAs endemic for LF in Nigeria have yet to begin MDA (57).

Nigeria has seen a decreased level of infection and notable reduction in the burden of onchocerciasis. In certain communities, epidemiological assessments conducted in the past few years have shown low or zero prevalence. The goal is to interrupt onchocerciasis transmission by the year 2020, eliminating disease transmission for good by the year 2025 (51). This study provides evidence of no recent transmission of onchocerciasis in Plateau State. Because the evolution of mf in skin and Ov16 incident infection response is slow, continued monitoring for reintroduction of *O. volvulus* transmission is suggested to occur using positive PCR pools from vector blackflies (44). Reintroduction of onchocerciasis from bordering states is also possible through the

movement of infected blackflies and/or humans, necessitating the need for continued surveillance.

Tables and Figures

Table 1. Years of Mass Drug Administration of Ivermectin and Albendazole for 3 LGAs in Plateau State, Nigeria

LGA	Onchocerciasis endemicity	Ivermectin	Albendazole
Kanke	Meso -endemic	1993-2017	2001-2012
Kanam	Non-/Hypo-endemic	2002-2012	2002-2012
Mikang	Non-/Hypo-endemic	2003-2012	2003-2012

Table 2. PacELF 'C Survey' demographics and household-level risk factors by LGA, Plateau State, Nigeria, 2016

	Total (n=3, 569)	Local Government Area		
		Kanam (n= 1,175)	Kanke (n= 1,143)	Mikang (n= 1,251)
	n (%)	n (%)	n (%)	n (%)
Sex				
Female	2,026 (56.8)	686 (58.4)	656 (57.4)	684 (54.7)
Male	1,543 (43.2)	489 (41.6)	487 (42.6)	567 (45.3)
Age(years)				
0-9	1,037 (29.1)	298 (25.4)	329 (28.8)	410 (32.8)
10-19	799 (22.4)	245 (20.9)	301 (26.3)	253 (20.2)
20-29	576 (16.1)	237 (20.2)	134 (11.7)	205 (16.4)
30-39	461 (12.9)	186 (15.8)	123 (10.8)	152 (12.2)
40-49	307 (8.6)	102 (8.7)	104 (9.1)	101 (8.1)
50-59	155 (4.3)	49 (4.2)	51 (4.5)	55 (4.4)
60-69	113 (3.2)	30 (2.6)	49 (4.3)	34 (2.7)
70-79	63 (1.8)	12 (1.0)	33 (2.9)	18 (1.4)
> 79	58 (1.6)	16 (1.4)	19 (1.7)	23 (1.8)
People in household*	8.7 (5.1)	8.5 (4.7)	8.4 (5.7)	9.1 (5.0)
Sleeping spaces in household*	4.4 (2.4)	4.3 (2.6)	4.3 (2.1)	4.4 (2.4)
Lived in respective LGA whole life?				
Yes	3,456 (96.8)	1,118 (95.2)	1,108 (96.9)	1,230 (98.3
No	113 (3.2)	57 (4.9)	35 (3.1)	21 (1.7)
Has access to a mosquito net?				
Yes	2,800 (78.5)	815 (69.4)	1,073 (93.9)	912 (72.9)
No	769 (21.6)	360 (30.6)	70 (6.1)	339 (27.1)
Slept under a mosquito net the previous night?[a]				
Yes	812 (29.0)	299 (36.7)	428 (39.9)	85 (9.3)
No	1,988 (71.0)	516 (63.3)	645(60.1)	827 (90.7)
Interior walls sprayed for mosquitos in the last 12 months?				
Yes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No	3,569 (100.0)	1,175 (100.0)	1,143 (100.0)	1,251 (100.0)
Had lyphedema for the last 3 months?				
Yes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No	3,569 (100.0)	1,175 (100.0)	1,143 (100.0)	1,251 (100.0)
Has hydrocele?[b]				
Yes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No	1,543 (100.0)	489 (100.0)	487 (100.0)	567 (100.0)

*Value reported as mean (standard deviation)

[a] Among those with access to a bed net

[b] Among males

Table 3. TAS Survey demographics by RB-co-endemicity, Plateau State, Nigeria 2016

	Total (n=3,285)	Local Government Area	
		Kanke (n=1,724) Meso-Endemic	Mikang-Kanam (n=1,561) Hypo-Endemic
	n (%)	n (%)	n (%)
Sex			
Female	1631 (49.7)	866 (50.2)	765 (49.0)
Male	1654 (50.4)	858 (49.8)	796 (51.0)
Age(years)*	6.61 (0.9)	6.6 (0.9)	6.6 (0.9)
Lived in respective LGA whole life?			
Yes	3281 (99.9)	1720 (99.8)	1561 (100.0)
No	4 (0.1)	4 (0.2)	0 (0.0)

*Age is reported as mean (standard deviation)

Table 4. Antigen and antibody crude prevalence for PacELF Survey by LGA, Plateau State, Nigeria 2016

LGA	Number of Clusters	ICT		Wb123				Ov16			
		CFA Positive Total # positive/ N (%)	95% uCL [a] (One-sided)	Positive by Biplex Total # positive/ N (%)	95% uCL (One-sided)	Positive by ELISA Total # positive/ N (%)	95% uCL (One-sided)	Positive by Biplex Total # positive/ N (%)	95% uCL (One-sided)	Positive by ELISA Total # positive/ N (%)	95% uCL (One-sided)
Kanam	20	5/1,175 (0.43)	0.89	2/1,175 (0.17)	0.53	9/1,175 (0.77)	1.33	1/1,175 (0.09)	0.40	0/1,175 (0.00)	0.25
Mikang	21	0/1,251 (0.00)	0.24	0/1,251 (0.00)	0.24	23/1,251 (1.84)	2.59	0/1,251 (0.00)	0.24	0/1,251 (0.00)	0.24
Kanke	21	4/1,143 (0.35)	0.79	3/1,143 (0.26)	0.68	4/1,143 (0.35)	0.80	1/1,143 (0.09)	0.41	1/1,143 (0.09)	0.41
	Total	9/3,569 (0.25)	0.44	5/3,569 (0.14)	0.29	36/3,569 (1.01)	1.33	2/3,569 (0.06)	0.18	1/3,569 (0.03)	0.13

[a]Upper confidence limit

Table 5. Antigen and antibody prevalence for TAS Survey by RB co-endemicity, Plateau State, Nigeria 2016

LGA	Number of Schools	ICT		Wb123				Ov16			
		CFA Positive Total # positive/ N (%)	95% uCL[a] (One-sided)	Positive by Biplex Total # positive/ N (%)	95% uCL (One-sided)	Positive by ELISA Total # positive/ N (%)	95% uCL (One-sided)	Positive by Biplex Total # positive/ N (%)	95% uCL (One-sided)	Positive by ELISA Total # positive/ N (%)	95% uCL (One-sided)
Kanke (Meso/Hyper-Endemic)	41	0/1,724 (0.00)	0.17	2/1,724 (0.12)	0.36	64/1,724 (3.71)	4.55	0/1,724 (0.00)	0.17	0/1,724 (0.00)	0.17
Mikang/ Kanam (Hypo-Endemic)	36	0/1,561 (0.00)	0.19	2/1,561 (0.13)	0.40	20/1,561 (1.28)	1.86	0/1,561 (0.00)	0.19	0/1,561 (0.00)	0.19
	Total	0/3,285 (0.00)	0.09	4/ 3,285 (0.12)	0.28	84/ 3,285 (2.56)	3.06	0/3,285 (0.00)	0.09	0/3,285 (0.00)	0.09

[a]Upper confidence limit

Table 6. Age distribution of LF and RB antigen and antibody prevalence for TAS and PacELF surveys combined, Plateau State, Nigeria 2016

Age Group	ICT		Wb123				Ov16			
	CFA Positive Total # positive/ N (%)	95% uCL[a] (One-sided)	Positive by Biplex Total # positive/ N (%)	95% uCL (One-sided)	Positive by ELISA Total # positive/ N (%)	95% uCL (One-sided)	Positive by Biplex Total # positive/ N (%)	95% uCL (One-sided)	Positive by ELISA Total # positive/ N (%)	95% uCL (One-sided)
< 5	0/336 (0.0)	0.88	0/336 (0.0)	0.89	1/336 (0.30)	1.40	0/336 (0.0)	0.89	0/336 (0.0)	0.89
5-9	0/3,962 (0.0)	0.08	5/3,962 (0.13)	0.27	96/3,962 (2.42)	2.86	0/3,962 (0.0)	0.08	0/3,962 (0.0)	0.08
10-14	0/454 (0.0)	0.66	0/454 (0.0)	0.66	3/454 (0.66)	1.70	0/454 (0.0)	0.66	0/454 (0.0)	0.66
15-19	0/369 (0.0)	0.81	0/369 (0.0)	0.81	4/369 (1.08)	2.46	0/369 (0.0)	0.81	0/369 (0.0)	0.81
20-29	1/576 (0.17)	0.82	0/576 (0.0)	0.52	5/576 (0.87)	1.82	0/576 (0.0)	0.52	0/576 (0.0)	0.52
30-39	3/461 (0.65)	1.67	0/461 (0.0)	0.65	4/461 (0.87)	1.97	0/461 (0.0)	0.65	0/461 (0.0)	0.65
40-49	3/307 (0.98)	2.51	0/307 (0.0)	0.97	4/307 (1.30)	2.96	0/307 (0.0)	0.97	0/307 (0.0)	0.97
≥50	2/389 (0.51)	1.61	4/389 (1.03)	2.34	3/389 (0.77)	1.98	2/389 (0.51)	1.61	1/389 (0.26)	1.21
Total	9/6,854 (0.13)	0.23	9/6,854 (0.13)	0.23	120/6,854 (1.75)	2.03	2/6,854 (0.03)	0.09	1/6,854 (0.01)	0.07

[a]Upper confidence limit

Table 7. Lymphatic Filariasis Diagnostic Test Concordance for TAS and PacELF Surveys, Plateau State, Nigeria, 2016

		Concordance with Index		
Index	**Total # positive/N**	**CFA ICT**	**Wb123 biplex**	**Wb123 ELISA**
CFA ICT	9/6,854		1/ 9 (11.1)	0/ 9 (0.0)
Wb123 biplex	9/6,854	1/ 9 (11.1)		0/ 9 (0.0)
Wb123 ELISA	120/6,854	0/ 120 (0.0)	0 /120 (0.0)	

Table 8. Onchocerciasis Test Concordance for TAS and PacELF Surveys, Plateau State, Nigeria, 2016

		Concordance with Index	
Index	**Total # positive/N**	**Ov16 biplex**	**Ov16 ELISA**
Ov16 biplex	2/6,854		1/2 (50.0)
Ov16 ELISA	1/6,854	1/1 (100.0)	

Table 9. Univariate logistic regression analysis of risk factors for *W. bancrofti* CFA positivity by ICT from TAS and PacELF surveys in 3 LGAs of Plateau State, Nigeria, 2016

Risk factor	Total(n=6,854)[a]		CFA Prevalence	
			Odds ratio (95% CI)	P-value
	n	% Prevalence[d]		
Sex				
Male	3,197	0.06	ref	
Female	3,657	0.19	3.21 (1.10-9.37)	0.03*
Age (years)				
0-9	4,298	0.00	-	-
10-19	823	0.00	-	-
20-39	1,037	0.39	0.27 (0.03- 2.15)	0.21
40-59	462	0.65	0.83 (0.37- 1.86)	0.65
≥ 60	234	0.85	ref	
People in household (per additional person)[b]	8.67	5.14	1.02 (0.97-1.08)	0.36
Sleeping spaces in household (per additional sleeping space)[b]	4.35	2.37	0.82 (0.72-0.94)	0.004*
Lived in respective LGA whole life?				
Yes	6,737	0.13	-	-
No	117	0.00	-	-
Has access to a mosquito net?				
Yes	2,800	0.14	ref	
No	769	0.65	5.36 (1.75-16..39)	0.004*
Slept under a mosquito net the previous night?[c]				
Yes	812	0.49	-	-
No	1,988	0.00	-	-
Interior walls sprayed for mosquitos in the last 12 months?				
Yes	0	0.00	-	-
No	3,569	0.25	-	-
Had lymphedema for the last 3 months?				
No	3,569	0.25	-	-
Yes	0	0.00	-	-
Has hydrocele?[e]				
No	1,543	0.13	-	-
Yes	0	0.00	-	-

[a]All variables do not total to study population due to missing values

[b]Value reported as mean (st. dev)

[c]Among those with access to a bed net

[d]Crude Prevalence

[e]Among males

** $p < 0.05$ was considered statistically significant*

Table 10. Univariate logistic regression analysis of risk factors for *W. bancrofti* seropositivity by Wb123 biplex from TAS and PacELF surveys in 3 LGAs of Plateau State, Nigeria, 2016

Risk factor	Total(n=6,854)[a]		Wb123 Biplex Seropositivity	
			Odds ratio (95% CI)	P-value
	n	% Seropositivity[d]		
Sex				
Male	3,197	0.13	ref	
Female	3,657	0.14	0.80 (0.10 - 6.62)	0.84
Age (years)				
0-9	4,298	0.12	0.17 (0.02-1.62)	0.12
10-19	823	0.00	-	-
20-39	1,037	0.00	-	-
40-59	462	0.43	0.85 (0.10-7.34)	0.88
≥ 60	234	0.85	ref	
People in household (per additional person)[b]	8.67	5.14	0.95 (0.81-1.12)	0.56
Sleeping spaces in household (per additional sleeping space)[b]	4.35	2.37	0.77 (0.60-0.99)	0.04*
Lived in respective LGA whole life?				
Yes	6,737	0.10	ref	
No	117	1.71	16.00 (3.09-82.78)	<0.001*
Has access to a mosquito net?				
Yes	2,800	0.11	ref	
No	769	0.26	1.46 (0.16-13.00)	0.73
Slept under a mosquito net the previous night?[c]				
Yes	812	0.37	-	-
No	1,988	0.00	-	-
Interior walls sprayed for mosquitos in the last 12 months?				
Yes	0	0.00	-	-
No	3,569	0.14	-	-
Had lymphedema for the last 3 months?				
No	3,569	0.14	-	-
Yes	0	0.00	-	-
Has hydrocele?[e]				
No	1,543	0.19	-	-
Yes	0	0.00	-	-

[a]All variables do not total to study population due to missing values

[b]Value reported as mean (st. dev)

[c]Among those with access to a bed net

[d]Crude seropositivity

[e]Among males

** $p < 0.05$ was considered statistically significant*

Table 11. Univariate logistic regression analysis of risk factors for *W. bancrofti* seropositivity by Wb123 ELISA from TAS and PacELF surveys in 3 LGAs of Plateau State, Nigeria, 2016

Risk factor	Total(n=6,854)[a]		Wb123 ELISA Seropositivity	
			Odds ratio (95% CI)	P-value
	n	% Seropositivity[d]		
Sex				
Male	3,197	2.35	ref	
Female	3,657	1.23	0.51 (0.27 - 0.99)	0.05*
Age(years)				
0-9	4,298	2.26	1.23 (0.21-7.32)	0.82
10-19	823	0.85	0.53 (0.08-3.72)	0.52
20-39	1,037	0.87	0.55 (0.11-2.71)	0.46
40-59	462	0.87	0.66 (0.09-5.15)	0.69
≥ 60	234	1.28	ref	
People in household (per additional person)[b]	8.67	5.13	1.01 (0.95- 1.08)	0.72
Sleeping spaces in household (per additional sleeping space)[b]	4.35	2.37	1.07 (0.87-1.31)	0.53
Lived in respective LGA whole life?				
Yes	6,737	1.77	ref	
No	117	0.85	1.63 (0.19-14.12)	0.66
Has access to a mosquito net?				
Yes	2,800	1.07	ref	
No	769	0.78	1.11 (0.35-3.57)	0.86
Slept under a mosquito net the previous night?[c]				
Yes	812	0.12	ref	
No	1,988	1.46	18.48 (2.36-144.80)	0.01*
Interior walls sprayed for mosquitos in the last 12 months?				
Yes	0	0.00	-	-
No	3,569	1.01	-	-
Had lymphedema for the last 3 months?				
No	3,569	1.01	-	-
Yes	0	0.00	-	-
Has hydrocele?[e]				
No	1,543	1.36	-	-
Yes	0	0.00	-	-

[a]All variables do not total to study population due to missing values

[b]Value reported as mean (st. dev)

[c]Among those with access to a bed net

[d]Crude seropositivity

[e]Among males

** $p < 0.05$ was considered statistically significant*

Table 12. Univariate logistic regression analysis of risk factors for *O. volvulus* seropositivity by Ov16 biplex from TAS and PacELF surveys in 3 LGAs of Plateau State, Nigeria, 2016

Risk factor	Total(n=6,854)[a]		Ov16 Biplex Seropositivity	
			Odds ratio (95% CI)	P-value
	n	% Seropositivity[d]		
Sex				
Male	3,197	0.03	ref	
Female	3,657	0.03	1.24 (0.07-20.90)	0.88
Age(years)				
0-9	4,298	0.00	-	-
10-19	823	0.00	-	-
20-39	1,037	0.00	-	-
40-59	462	0.22	0.78 (0.04-13.67)	0.86
≥ 60	234	0.43	ref	
People in household (per additional person)[b]	8.67	5.14	0.95 (0.77-1.18)	0.66
Sleeping spaces in household (per additional sleeping space)[b]	4.35	2.37	1.13 (0.95-1.34)	0.17
Lived in respective LGA whole life?				
Yes	6,737	0.01	ref	-
No	117	0.85	21.14 (1.06-422.58)	0.05*
Has access to a mosquito net?				
Yes	2,800	0.04	ref	
No	769	0.13	2.30 (0.13-39.71)	0.56
Yes	812	0.00	-	-
No	1,988	0.05	-	-
Interior walls sprayed for mosquitos in the last 12 months?				
Yes	0	0.00	-	-
No	3,569	0.06	-	-

[a]All variables do not total to study population due to missing values

[b]Value reported as mean (st. dev)

[c]Among those with access to a bed net

[d]Crude seropositivity

** $p < 0.05$ was considered statistically significant*

Table 13. Univariate logistic regression analysis of risk factors for *O. volvulus* seropositivity by Ov16 ELISA from TAS and PacELF surveys in 3 LGAs of Plateau State, Nigeria, 2016

Risk factor	Total(n=6,854)[a]		Ov16 ELISA Seropositivity	
			Odds ratio (95% CI)	P-value
	n	% Seropositivity[d]		
Sex[b]				
Male	3,197	0.00	-	-
Female	3,657	0.03	-	-
Age(years)				
0-9	4,298	0.00	-	-
10-19	823	0.00	-	-
20-39	1,037	0.00	-	-
40-59	462	0.22	-	-
≥ 60	234	0.00	-	-
People in household (per additional person)[b]	8.67	5.14	1.04 (1.00-1.07)	0.03*
Sleeping spaces in household (per additional sleeping space)[b]	4.35	2.37	1.22 (1.13-1.32)	<0.001*
Lived in respective LGA whole life?				
Yes	6,737	0.01	-	-
No	117	0.00	-	-
Has access to a mosquito net?				
Yes	2,800	0.04	-	-
No	769	0.00	-	-
Slept under a mosquito net the previous night?[c]				
Yes	812	0.00	-	-
No	1,988	0.05	-	-
Interior walls sprayed for mosquitos in the last 12 months?				
Yes	0	0.00	-	-
No	3,569	0.03	-	-

[a]All variables do not total to study population due to missing values

[b]Value reported as mean (st. dev)

[c]Among those with access to a bed net

[d]Crude seropositivity

** $p < 0.05$ was considered statistically significant*

Figure 1. LF prevalence with upper 95% confidence limits for TAS and PacELF 'C' surveys by age categories among residents of 3 LGAs in Plateau State, Nigeria, 2016.

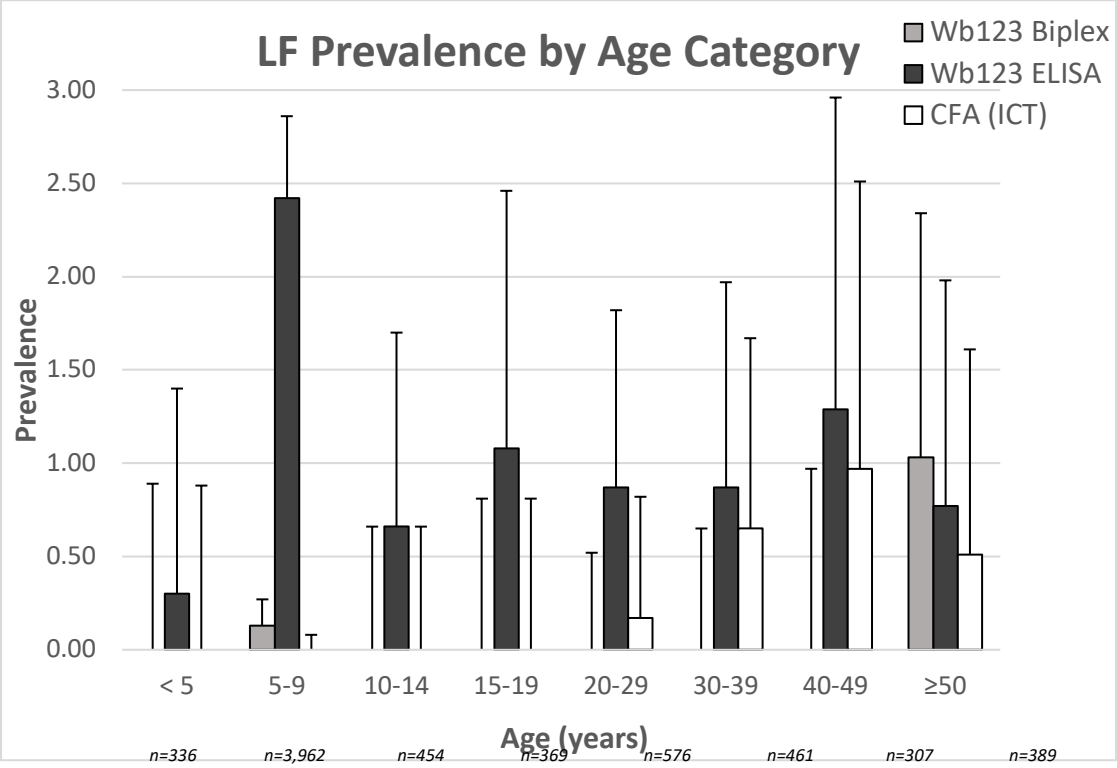


Figure 2. Cluster and school-specific CFA-positive cases by ICT among Kanam, Mikang, and Kanam LGA residents in Plateau State, Nigeria, 2016

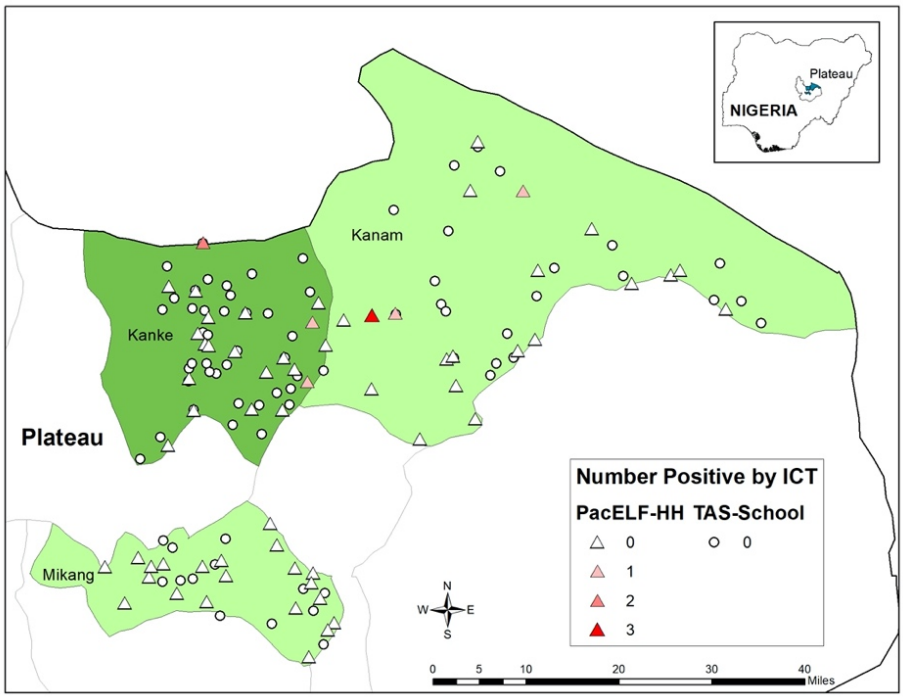


Figure 3. Cluster and school-specific positive cases by Wb123 biplex among Kanam, Mikang, and Kanam LGA residents in Plateau State, Nigeria, 2016

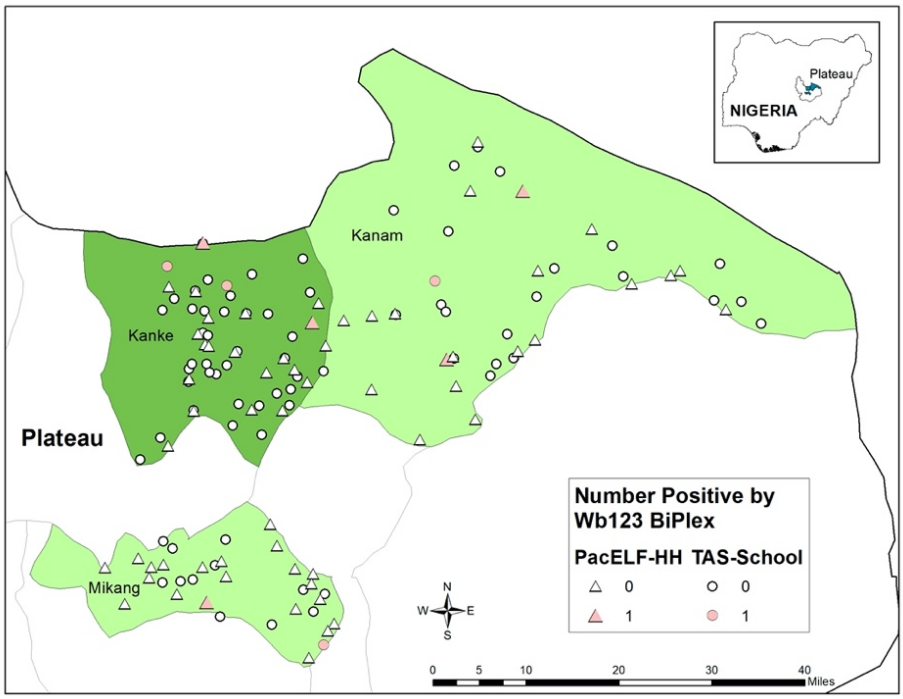


Figure 4. Cluster and school-specific positive cases by Wb123 ELISA among Kanam, Mikang, and Kanam LGA residents in Plateau State, Nigeria, 2016

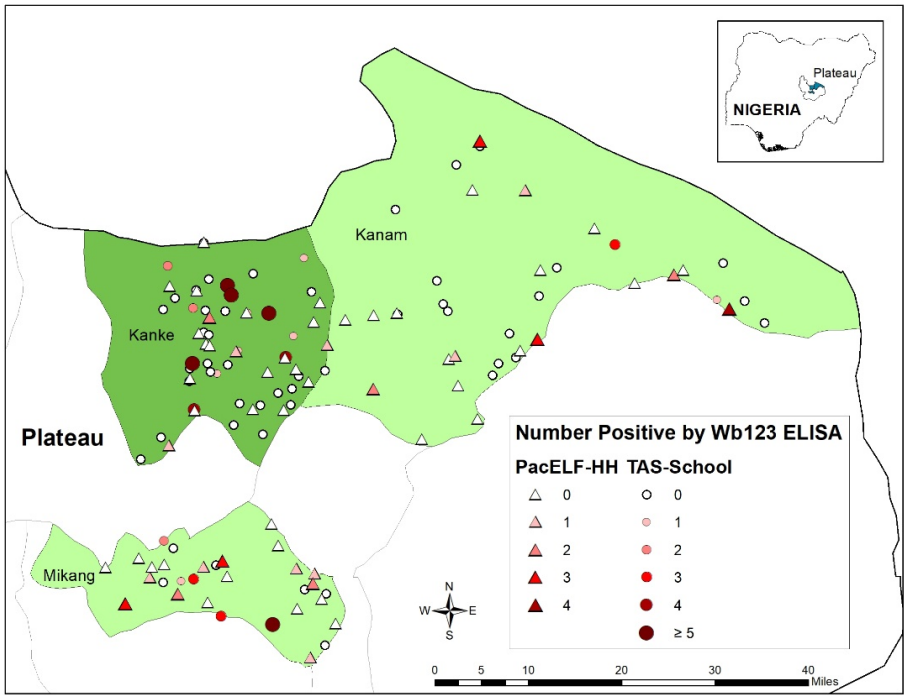


Figure 5. Cluster and school-specific positive cases by Ov16 biplex among Kanam, Mikang, and Kanam LGA residents in Plateau State, Nigeria, 2016

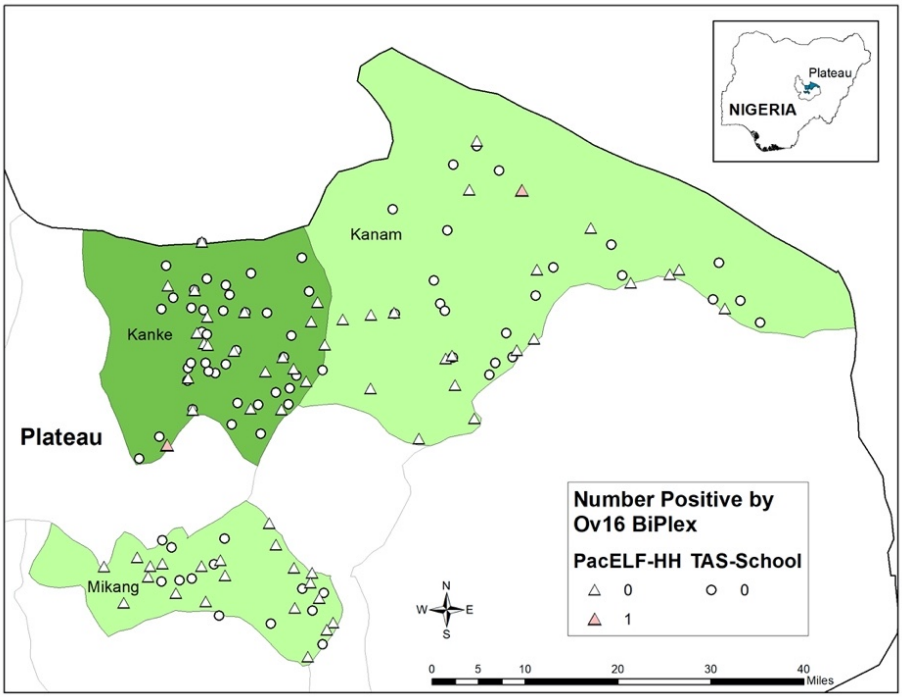
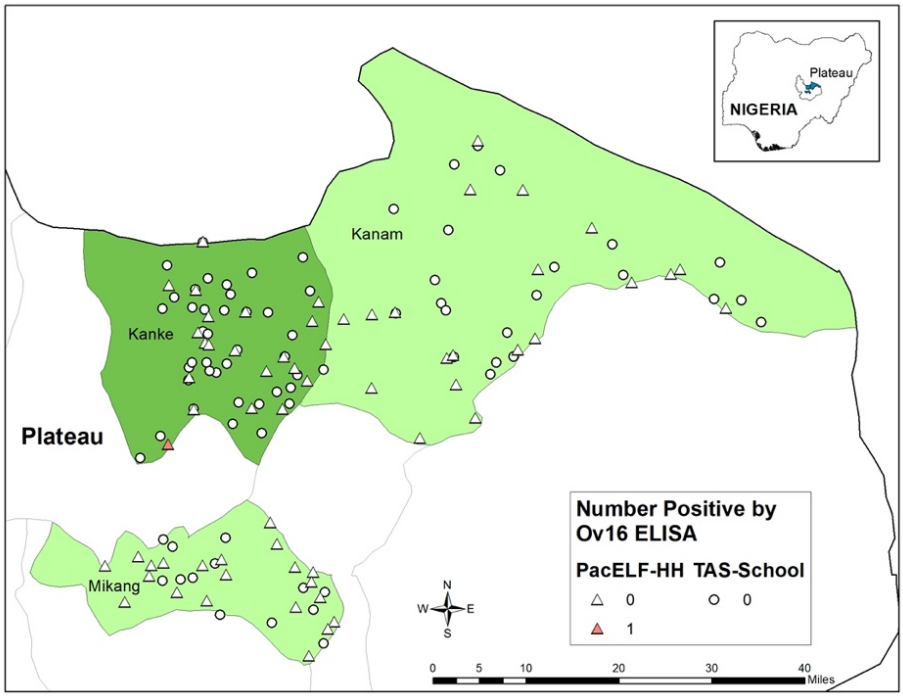


Figure 4. Cluster and school-specific positive cases by Ov16 ELISA among Kanam, Mikang, and Kanam LGA residents in Plateau State, Nigeria, 2016



Chapter III: Summary, Public Health Implications, and Future Directions

Summary

These studies were the last surveys in the LF post-treatment surveillance phase in Plateau State, Nigeria. The surveys were conducted in order to compare LF antigen prevalence from school-based TAS of 6-7 year olds to a community-wide household survey of individuals over 2 years old ("PacELF" survey design). A secondary aim of these studies was to determine the age distribution of LF and RB seroprevalence using a point-of-care rapid diagnostic test and laboratory-based ELISA. Results showed no (0.00%) person was *Wuchereria bancrofti* CFA positive by ICT for the TAS Survey and 0.25% for the PacELF survey with an overall prevalence of 0.13%. RB seroprevalence was greater than 0.00% only among the ≥ 50 age category at 0.51% and 0.26% for the biplex and ELISA tests respectively. LF seroprevalence by biplex test was 0.00% in all age categories except for the 5-9 year age category and ≥ 50 age category at 0.13% and 1.03% respectively. Wb123 ELISA testing showed a seroprevalence above 0.00% in all age categories with the highest prevalence among the 5-9 year age category at 2.42% and an overall seroprevalence of 1.75%. Univariate analysis reveals access to a mosquito net is a significant contributor in the prevention of LF transmission and that not having lived in the respective LGA your whole life is a significant contributor to an increase in LF and RB seropositivity. LF test concordance was low between LF antigen and antibody tests and high among RB antibody tests.

These results indicate no recent exposure to onchocerciasis and very low recent exposure to lymphatic filariasis in Plateau State, Nigeria with an LF antigen prevalence below the 2% threshold. Low levels of overall LF antigen prevalence and zero evidence of recent LF antigen transmission strongly suggest that sustainable LF transmission is not

possible in Plateau State, Nigeria and the shift from post-treatment surveillance into the next phase of verifying the absence of sustainable LF transmission can be initiated.

Future Public Health Implications

Onchocerciasis and lymphatic filariasis are two neglected tropical diseases targeted for elimination in many nations by the WHO. After passing a series of post-treatment surveillance surveys, the next step of verifying transmission absence can begin. This study hopes to contribute to the evaluation of school-based TAS surveys to accurately represent community-based surveys in the determination of community LF antigen prevalence and the verification of little to no recent LF or RB transmission. Elimination programs using the TAS survey methodology in lieu of the community-based survey experience faster, more convenient, and cheaper testing in the determination of community LF and RB prevalence. Furthermore, this study suggests that Ov16/Wb123 point-of-care biplex test showed minimal correlation with Wb123 ELISA testing, but good correlation with Ov16 ELISA testing.

Future Directions

These results provide continued evidence of RB transmission interruption as well as evidence to suggest that school-based TAS surveys may continue to be used in lieu of more costly, and time consuming community based surveys to represent community LF antigen prevalence. Further research is needed in order to improve the performance of Wb123 rapid antibody tests and to develop antibody prevalence thresholds to enable the use of antibody tests as a compliment or replacement of antigen tests for LF stop-MDA and post-treatment surveillance surveys.

References

1. CDC. Parasites - Lymphatic Filariasis 2013 [Available from: http://www.cdc.gov/parasites/lymphaticfilariasis/index.html.

2. World Health Organization. Global programme to eliminate lymphatic filariasis: progress report, 2017. 2018 Nov 3. Contract No.: 44.

3. Famakinde DO. Mosquitoes and the Lymphatic Filarial Parasites: Research Trends and Budding Roadmaps to Future Disease Eradication. Trop Med Infect Dis. 2018;3(1).

4. Mues KE, Lammie PJ, Klein M, Kleinbaum DG, Addiss D, Fox LM. Changes in Antibody Levels during and following an Episode of Acute Adenolymphangitis (ADL) among Lymphedema Patients in Leogane, Haiti. PLoS One. 2015;10(10):e0141047.

5. World Health Organization. What is lymphatic filariasis 2019 [Available from: https://www.who.int/lymphatic_filariasis/disease/en/.

6. Eneanya OA, Garske T, Donnelly CA. The social, physical and economic impact of lymphedema and hydrocele: a matched cross-sectional study in rural Nigeria. BMC Infect Dis. 2019;19(1):332.

7. Kalyanasundaram R, Khatri V, Chauhan N. Advances in Vaccine Development for Human Lymphatic Filariasis. Trends Parasitol. 2020;36(2):195-205.

8. Babu S, Nutman TB. Immunopathogenesis of lymphatic filarial disease. Semin Immunopathol. 2012;34(6):847-61.

9. Gavins FNE, Stokes KY. Vascular responses to pathogens. Amsterdam ; Boston: Elsevier/AP, Academic Press is an imprint of Elsevier; 2016. xi, 239 pages p.

10. Obindo J, Abdulmalik J, Nwefoh E, Agbir M, Nwoga C, Armiya'u A, et al. Prevalence of depression and associated clinical and socio-demographic factors in people living with lymphatic filariasis in Plateau State, Nigeria. PLoS Negl Trop Dis. 2017;11(6):e0005567.

11. Cox FEG. History of Human Parasitology. Clinical Microbiology Reviews. 2002;15(4):595-612.

12. Lymphatic filariasis. New York, NY: Springer Berlin Heidelberg; 2016. pages cm p.

13. Tyagi BK. Lymphatic filariasis : epidemiology, treatment and prevention - the indian perspective. New York, NY: Springer Berlin Heidelberg; 2018. pages cm p.

14. Goel TC, Goel A. Lymphatic Filariasis. Singapore: Springer Verlag, Singapore; 2016.

15. Magill AJ. Hunter's tropical medicine and emerging infectious diseases. Ninth edition / ed. London: Saunders/Elsevier; 2013. xxiv, 1190 pages p.

16. Weil GJ, Ramzy RM. Diagnostic tools for filariasis elimination programs. Trends Parasitol. 2007;23(2):78-82.

17. Masson J, Douglass J, Roineau M, Aye KS, Htwe KM, Warner J, et al. Relative Performance and Predictive Values of Plasma and Dried Blood Spots with Filter Paper Sampling Techniques and Dilutions of the Lymphatic Filariasis Og4C3 Antigen ELISA for Samples from Myanmar. Trop Med Infect Dis. 2017;2(2).

18. Gounoue-Kamkumo R, Nana-Djeunga HC, Bopda J, Akame J, Tarini A, Kamgno J. Loss of sensitivity of immunochromatographic test (ICT) for lymphatic filariasis

diagnosis in low prevalence settings: consequence in the monitoring and evaluation procedures. BMC Infect Dis. 2015;15:579.

19. Rebollo MP, Bockarie MJ. Shrinking the lymphatic filariasis map: update on diagnostic tools for mapping and transmission monitoring. Parasitology. 2014;141(14):1912-7.

20. Steel C, Golden A, Kubofcik J, LaRue N, de Los Santos T, Domingo GJ, et al. Rapid Wuchereria bancrofti-specific antigen Wb123-based IgG4 immunoassays as tools for surveillance following mass drug administration programs on lymphatic filariasis. Clin Vaccine Immunol. 2013;20(8):1155-61.

21. Weil GJ, Curtis KC, Fakoli L, Fischer K, Gankpala L, Lammie PJ, et al. Laboratory and field evaluation of a new rapid test for detecting Wuchereria bancrofti antigen in human blood. Am J Trop Med Hyg. 2013;89(1):11-5.

22. Wanji S, Esum ME, Njouendou AJ, Mbeng AA, Chounna Ndongmo PW, Abong RA, et al. Mapping of lymphatic filariasis in loiasis areas: A new strategy shows no evidence for Wuchereria bancrofti endemicity in Cameroon. PLoS Negl Trop Dis. 2019;13(3):e0007192.

23. Kubofcik J, Fink DL, Nutman TB. Identification of Wb123 as an early and specific marker of Wuchereria bancrofti infection. PLoS Negl Trop Dis. 2012;6(12):e1930.

24. Won KY, Sambou S, Barry A, Robinson K, Jaye M, Sanneh B, et al. Use of Antibody Tools to Provide Serologic Evidence of Elimination of Lymphatic Filariasis in The Gambia. Am J Trop Med Hyg. 2018;98(1):15-20.

25. Steel C, Golden A, Stevens E, Yokobe L, Domingo GJ, de los Santos T, et al. Rapid Point-of-Contact Tool for Mapping and Integrated Surveillance of Wuchereria bancrofti and Onchocerca volvulus Infection. Clin Vaccine Immunol. 2015;22(8):896-901.

26. CDC. Recommendations of the International Task Force for Disease Eradication. MMWR Recomm Rep. 1993;42(RR-16):1-38.

27. CDC. Parasites-Loiasis. 2015.

28. Ramaiah KD, Ottesen EA. Progress and impact of 13 years of the global programme to eliminate lymphatic filariasis on reducing the burden of filarial disease. PLoS Negl Trop Dis. 2014;8(11):e3319.

29. Chu BK, Deming M, Biritwum NK, Bougma WR, Dorkenoo AM, El-Setouhy M, et al. Transmission assessment surveys (TAS) to define endpoints for lymphatic filariasis mass drug administration: a multicenter evaluation. PLoS Negl Trop Dis. 2013;7(12):e2584.

30. Cantey PT, Rout J, Rao G, Williamson J, Fox LM. Increasing compliance with mass drug administration programs for lymphatic filariasis in India through education and lymphedema management programs. PLoS Negl Trop Dis. 2010;4(6):e728.

31. World Health Organization. Monitoring and Epidemiological Assessment of Mass Drug Administration in the Global Programme to Eliminate Lymphatic Filariasis: A Manual for National Elimination Programmes. Geneva, Switzerland: World Health Organization,; 2011. Available from: https://apps.who.int/iris/bitstream/handle/10665/97377/9789241505444_eng.pdf?sequence=1.

32. Ichimori K, Graves PM. Overview of PacELF—the Pacific Programme for the Elimination of Lymphatic Filariasis. Tropical Medicine and Health. 2017;45(1):34.

33. Sheel M, Sheridan S, Gass K, Won K, Fuimaono S, Kirk M, et al. Identifying residual transmission of lymphatic filariasis after mass drug administration: Comparing school-based versus community-based surveillance - American Samoa, 2016. PLoS Negl Trop Dis. 2018;12(7):e0006583.

34. Rao RU, Nagodavithana KC, Samarasekera SD, Wijegunawardana AD, Premakumara WD, Perera SN, et al. A comprehensive assessment of lymphatic filariasis in Sri Lanka six years after cessation of mass drug administration. PLoS Negl Trop Dis. 2014;8(11):e3281.

35. CDC. Parasites- Onchocerciasis (also known as River Blindness) 2015 [Available from: https://www.cdc.gov/parasites/onchocerciasis/index.html.

36. Makenga Bof JC, Mansiangi P, Nsangi H, Mafuta E, Aujoulat I, Coppieters Y. Community Participation in and Perception of Community-Directed Treatment with Ivermectin in Kinshasa, DRC. Trop Med Infect Dis. 2019;4(3).

37. Dadzie Y, Amazigo UV, Boatin BA, Seketeli A. Is onchocerciasis elimination in Africa feasible by 2025: a perspective based on lessons learnt from the African control programmes. Infect Dis Poverty. 2018;7(1):63.

38. Gebrezgabiher G, Mekonnen Z, Yewhalaw D, Hailu A. Reaching the last mile: main challenges relating to and recommendations to accelerate onchocerciasis elimination in Africa. Infect Dis Poverty. 2019;8(1):60.

39. World Health Organization. Guidelines for Stopping Mass Drug Administration and Verifying Elimination of Human Onchocerciasis: Criteria and Procedures.

Guidelines for Stopping Mass Drug Administration and Verifying Elimination of Human Onchocerciasis: Criteria and Procedures. WHO Guidelines Approved by the Guidelines Review Committee. Geneva2016.

40. World Health Organization. Onchocerciasis 2019 [Available from: https://www.who.int/news-room/fact-sheets/detail/onchocerciasis.

41. Kluxen G. A short History of Onchocerciasis.

42. Richards FO, Nwoke BEB, Zarroug I, Tukahebwa E, Negussu N, Higazi TB, et al. The positive influence the Onchocerciasis Elimination Program for the Americas has had on Africa programs. Infect Dis Poverty. 2019;8(1):52.

43. Hernandez-Gonzalez A, Moya L, Perteguer MJ, Herrador Z, Nguema R, Nguema J, et al. Evaluation of onchocerciasis seroprevalence in Bioko Island (Equatorial Guinea) after years of disease control programmes. Parasit Vectors. 2016;9(1):509.

44. Richards FO, Eigege A, Umaru J, Kahansim B, Adelamo S, Kadimbo J, et al. The Interruption of Transmission of Human Onchocerciasis by an Annual Mass Drug Administration Program in Plateau and Nasarawa States, Nigeria. Am J Trop Med Hyg. 2020;102(3):582-92.

45. Gass KM. Rethinking the serological threshold for onchocerciasis elimination. PLoS Negl Trop Dis. 2018;12(3):e0006249.

46. Cantey PT, Roy SL, Boakye D, Mwingira U, Ottesen EA, Hopkins AD, et al. Transitioning from river blindness control to elimination: steps toward stopping treatment. Int Health. 2018;10(suppl_1):i7-i13.

47. Richards FO, Eigege A, Miri ES, Kal A, Umaru J, Pam D, et al. Epidemiological and entomological evaluations after six years or more of mass drug administration for lymphatic filariasis elimination in Nigeria. PLoS Negl Trop Dis. 2011;5(10):e1346.

48. Hotez PJ, Asojo OA, Adesina AM. Nigeria: "Ground Zero" for the high prevalence neglected tropical diseases. PLoS Negl Trop Dis. 2012;6(7):e1600.

49. King JD, Eigege A, Umaru J, Jip N, Miri E, Jiya J, et al. Evidence for stopping mass drug administration for lymphatic filariasis in some, but not all local government areas of Plateau and Nasarawa States, Nigeria. Am J Trop Med Hyg. 2012;87(2):272-80.

50. Eigege A, Evans DS, Noland GS, Davies E, Umaru J, Adelamo SE, et al. Criteria to Stop Mass Drug Administration for Lymphatic Filariasis Have Been Achieved Throughout Plateau and Nasarawa States, Nigeria. Am J Trop Med Hyg. 2017;97(3):677-80.

51. Federal Ministry of Health A. Nigeria Onchocerciasis Elimination Plan. Abuja; 2017.

52. Mackenzie CD, Homeida MM, Hopkins AD, Lawrence JC. Elimination of onchocerciasis from Africa: possible? Trends Parasitol. 2012;28(1):16-22.

53. Post RJ, Onyenwe E, Somiari SA, Mafuyai HB, Crainey JL, Ubachukwu PO. A guide to the Simulium damnosum complex (Diptera: Simuliidae) in Nigeria, with a cytotaxonomic key for the identification of the sibling species. Ann Trop Med Parasitol. 2011;105(4):277-97.

54. Harris JR, Wiegand RE. Detecting infection hotspots: Modeling the surveillance challenge for elimination of lymphatic filariasis. PLoS Negl Trop Dis. 2017;11(5):e0005610.

55. Rao RU, Samarasekera SD, Nagodavithana KC, Punchihewa MW, Ranasinghe USB, Weil GJ. Systematic sampling of adults as a sensitive means of detecting persistence of lymphatic filariasis following mass drug administration in Sri Lanka. PLoS Negl Trop Dis. 2019;13(4):e0007365.

56. Eigege A, Noland GS, Adelamo SE, Nwodu K, Sallau A, Umaru J, et al. Post-Treatment Surveillance for Lymphatic Filariasis in Plateau and Nasarawa States, Nigeria: Results of Transmission Assessment Surveys. Am J Trop Med Hyg. 2020.

57. World Health Organization. WHO sustains Lymphatic Filariasis treatments in over 500 local government areas across Nigeria: World Health Organization 2019 [Available from: https://www.afro.who.int/news/who-sustains-lymphatic-filariasis-treatments-over-500-local-government-areas-across-nigeria.

58. Gass K, Beau de Rochars MV, Boakye D, Bradley M, Fischer PU, Gyapong J, et al. A multicenter evaluation of diagnostic tools to define endpoints for programs to eliminate bancroftian filariasis. PLoS Negl Trop Dis. 2012;6(1):e1479.