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Application of Freedom From Infection Analysis to Nigerian Cross-Sectional Lymphatic Filariasis Survey Data

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Faculty Thesis Advisor: Benjamin Lopman, Ph.D.

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Abstract

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By Gregory S. Noland

Lymphatic filariasis (LF), or "elephantiasis", is a mosquito-transmitted parasitic disease affecting 67.9 million people in 73 countries. Infection leads to lymphatic dysfunction that results in swelling of limbs (lymphedema) and genitals (male hydrocele), and painful recurrent inflammation. The World Health Organization (WHO) targets "elimination of LF as a public health problem" through 1) mass drug administration (MDA) to interrupt parasite transmission and 2) morbidity management and disability prevention (MMDP) to care for those already affected by LF. WHO guidelines currently exist for validation of LF elimination as a public health problem through a series of transmission assessment surveys (TAS) with a critical threshold of 2% (1% in areas where LF is transmitted by *Aedes* mosquitoes)—the levels below which transmission is presumed unsustainable. WHO also recently indicated that countries may request verification of elimination of LF transmission (elimination *sensu stricto*), but acknowledged that specific requirements for such verification have not yet been agreed. This study assessed Freedom From Infection (FFI) analysis to fill this gap. FFI employs probability theory to estimate the probability that disease prevalence in a population is below a pre-determined threshold. This novel application of FFI was conducted using data from a series of cross-sectional LF antigen surveys in eight districts of Plateau and Nasarawa states, Nigeria encompassing 31,714 individuals tested over an eleven-year period (2007—2017) through school-based TAS surveys of children aged 6—7 years old and community-based cluster surveys of individuals > 2 years. Results indicate a high probability (>0.90) at all examined thresholds (2%, 1%, 0.1%, 1 case) that areas with lower baseline LF transmission ($\leq$20% antigenemia) were free from LF infection by 2007, five to six years after the start of MDA, while high FFI probability (>0.99) was observed between 2012—2015, around 11—13 years after the start of MDA, for three areas of higher baseline transmission. These results correspond well with cross-sectional survey conclusions. In summary, initial application of FFI analysis to LF shows good concordance with cross-sectional survey data, thereby offering a tool, that with refinement, could serve as a viable analytic framework to verify elimination of LF transmission.

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Last, but most importantly, thank you to my family for giving me the time, space, and grace to finish this goal. To my children, my greatest desire is that you find a vocation as fulfilling and rewarding as the one I have in public health.

Table of Contents

Abbreviations

ADLA	Acute dermatolymphangioadenitis
CFA	Circulating filarial antigen
DP	Design prevalence
EU	Evaluation Unit
FFI	Freedom from infection
FMOH	Federal Ministry of Health
FTS	Filariasis test strip
GPELF	Global Programme to Eliminate LF
ICT	Immunochromatographic test
IRB	Institutional Review Board
LF	Lymphatic filariasis
LGA	Local Government Area
LQAS	Lot quality assurance sampling
MDA	Mass drug administration
MMDP	Morbidity management and disability prevention
NHREC	Nigerian National Health Research Ethics Committee
NPV	Negative predictive value
NTD	Neglected tropical disease
PacELF	Pacific regional program to eliminate LF
PTS	Post treatment surveillance
RB	River blindness
TAS	Transmission assessment survey
TCC	The Carter Center
WHO	World Health Organization

Chapter I: Background

Biology

Lymphatic filariasis (LF), also known as "elephantiasis", is a mosquito-transmitted parasitic disease currently affecting an estimated 67.9 million people in 73 tropical and subtropical countries (1). It is caused by infection with one of three filarial nematodes (roundworms): *Wuchereria brancrofti*, *Brugia malayi*, and *Brugia timori*. Approximately 90% of cases globally are caused by *W. bancrofti*, with *Brugia* transmission limited to areas of eastern and southern Asia (2). The principal vectors of LF include a wide variety of *Culex*, *Anopheles*, *Aedes*, and *Mansonia* mosquitoes (3).

LF was the first disease determined to be transmitted by mosquitoes: in 1878 in China, Sir Patrick Manson discovered larval parasites in the blood meal of a recently fed *Culex* mosquito (3). Though the full transmission cycle between man—mosquito—man was not demonstrated until 1900 by Low (Manson initially believed that mosquitoes only fed on humans once, that infective filariae were released into water pools by dying mosquitoes, and that humans became infected by ingesting contaminated water) (4), Manson's observations led to identification of the mosquito's role in the transmission of many other diseases including malaria, by Manson and Sir Ronald Ross in the late 1890's, and yellow fever, by Walter Reed and colleagues in 1900.

As shown in Figure 1, the life cycle of filarial parasites begins when infective third-stage larvae (L3) escape through a mosquito's proboscis into a bite wound on human skin (3). The larvae migrate through tissue and enter the lymphatic system, where they mature and develop for 6-12 months. In afferent lymphatic vessels and lymph nodes of the definitive human host, adult male (2.5—4 cm in length) and female (5—10 cm in

length) worms mate, producing thousands of microfilariae (mf) over the life span of the female worm, which is estimated to be five to seven years. Mf (245—300 μm in length) flow through the circulatory system exhibiting nocturnal periodicity in most endemic areas: mf are mainly absent from peripheral circulation during the day. However, between the hours of 10 pm and 4 am, corresponding to the nocturnal biting habits of mosquitoes that transmit LF, mf emerge into peripheral circulation, from where they can be taken up in a mosquito blood meal. Mf seem to reside in the pulmonary capillaries and vessels when absent from peripheral circulation and have a lifespan of approximately 12 months (5). Once ingested by a female mosquito, mf penetrate the mosquito midgut, migrate to the thoracic musculature, and molt twice over a 10 day period into infective L3 larvae. The larvae escape into the mosquito's body cavity and will continue the infection cycle when the mosquito bites another human host.

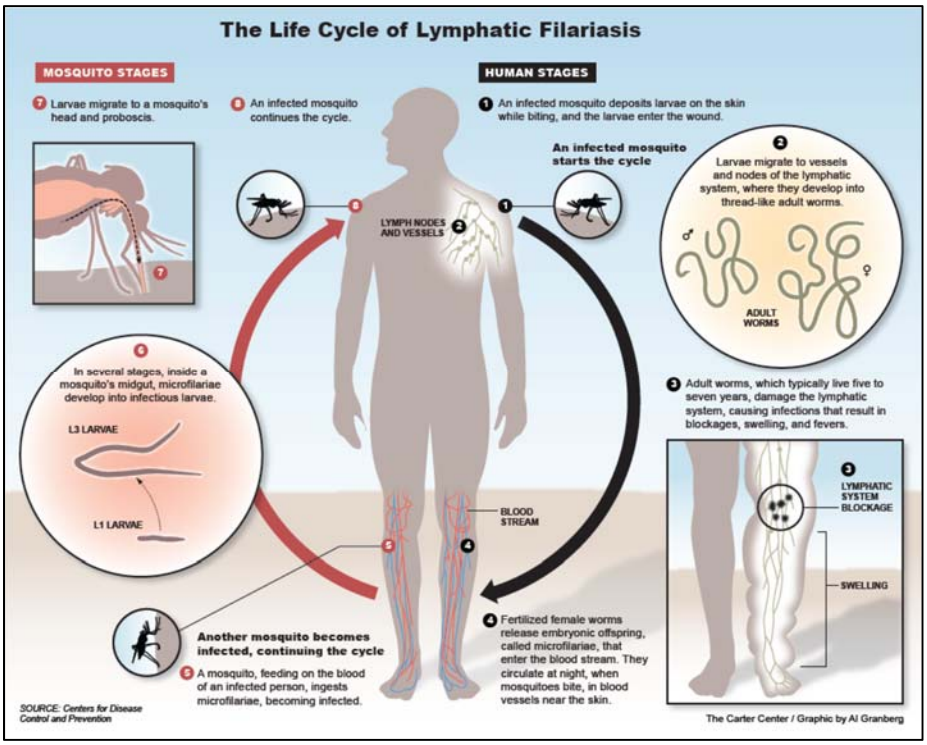


Figure 1. Life cycle of the parasites causing lymphatic filariasis.

Pathology

LF is a leading cause of global disability and accounts for 5.8 million disability-adjusted life years (6). LF is not a fatal condition, but 30-40% of individuals develop overt pathology that can include lymphedema and genital swelling in males (hydrocele) (7). These conditions are the result of chronic physiological dysfunction caused by adult worms, as well as inflammatory reaction to worms, particularly at worm death. The granulomatous inflammatory reaction associated with worm death may be caused by, or exacerbated by, antigen release from *Wolbachia* bacterium that is an endosymbiont of filarial parasites (7). In highly endemic areas, exposure to infected mosquitoes results in parasite infection in children as young as 3 years old (8), though the associated sequelae of lymphedema and/or hydrocele typically do not appear until adolescence or later (9). Pathology includes swelling of the legs, arms, scrotum, chyluria, and tropical pulmonary eosinophilia (10). Lymphedema is typically categorized using a seven-stage grading scheme (11). Advanced lymphedema leaves patients susceptible to secondary bacterial infections that cause acute dermatolymphangioadenitis (ADLA), painful 'acute attacks' that are a common symptom of LF. Though recent studies indicate modest improvement in pathology following drug treatment (12, 13), lymphedema is generally considered irreversible, and morbidity management focuses on secondary and tertiary prevention. Morbidity management and disability prevention (MMDP) includes basic hygiene and skin care to prevent secondary infections and ADLA, motor exercises to promote lymphatic circulation, and psychological and socioeconomic care to maintain active, productive lives (10). Hydrocele can be corrected surgically (10), though surgeons trained in this procedure are rare in LF-endemic areas, and patients likely do not have the

financial resources to afford surgery. These conditions lead to reduced mobility, impairment of daily activities, and social isolation for affected individuals (14, 15). A recent study found that 20% of patients with overt LF pathology met criteria for clinical depression (16), highlighting the need for physical and psycho-social morbidity care.

Diagnostics

Ascribing infected/non-infected status—and the related, but distinct, diseased/non-diseased—is a fundamental task for epidemiological analysis. Until the advent of modern diagnostic tools, filarial infection was diagnosed by microscopic demonstration of mf in stained thick blood smears collected in finger prick samples from suspected patients. Microscopic diagnosis poses several significant limitations apart from the necessity for reagents and trained laboratory personnel. First, there is a 6-12 month "diagnostic lag" between infection and the start of mf release by fecund worms (5). Secondly, night-time blood sampling is required due to the nocturnal periodicity of most filarial parasites. While feasible for research studies, this is challenging to operationalize in a large-scale program context. Thirdly, not all infections result in patent mf production (17, 18)—perhaps the result of aberrant adult worm development, transient mf production, or successful immune response by the human host (19).

Antigen-based diagnostics for *W. bancrofti* were developed beginning with an enzyme-linked immunosorbent assay (ELISA) in the late 1980's using the Og4C3 monoclonal antibody that detects circulating filarial antigen (CFA) (20). CFA is a 200-kilodalton protein released in large amounts by adult female worms and is present in the

serum of *W. bancrofti*-infected individuals (21). Lateral flow rapid tests to detect CFA in whole blood or serum followed: first the immunochromatographic card test (ICT) introduced in 1997 (22), then more recently, the filariasis test strip (FTS) in 2013 (23). CFA-based tests exhibit good correlation with intensity of adult worm burden, and high sensitivity and specificity (24), though recent evidence suggests a previously unrecognized cross-reactivity with *Loa loa*, a closely related filarial parasite endemic in parts of central Africa (25). Antigen persists for years after treatment, however, complicating interpretation of a positive antigen test result—particularly in post-treatment surveillance (PTS) settings (24). Antigen tests are not currently available for *Brugia* infections.

Antibody-based tests detecting IgG4 are available for *Brugia* infections in both ELISA and rapid test formats (Brugia Rapid) (26). Commercial antibody tests for *W. bancrofti* are not yet available, though several candidates are under consideration including Bm14 and Wb123 (27, 28). However, sero-conversion and sero-reversion rates are not fully defined for these markers, and antibody assays pose additional challenges to interpreting exposure versus infection.

Global LF Elimination Program

Prior to global LF elimination efforts, nearly 1.1 billion people in 80 countries—nearly 18% of the world's population—were at risk of LF, with 120 million infected (2). In 1993, the International Task Force for Disease Eradication declared LF one of six eradicable diseases (29). In 1997, the World Health Assembly called for the "elimination

of LF as a public health problem" (30), followed by the launching in 2000 of the Global Programme to Eliminate Filariasis (GPELF) by the World Health Organization (WHO). The global strategy consists of 1) annual mass drug administration (MDA) to interrupt transmission; and 2) morbidity management and disability prevention (MMDP) to alleviate disability for those with LF sequelae (Figure 2).

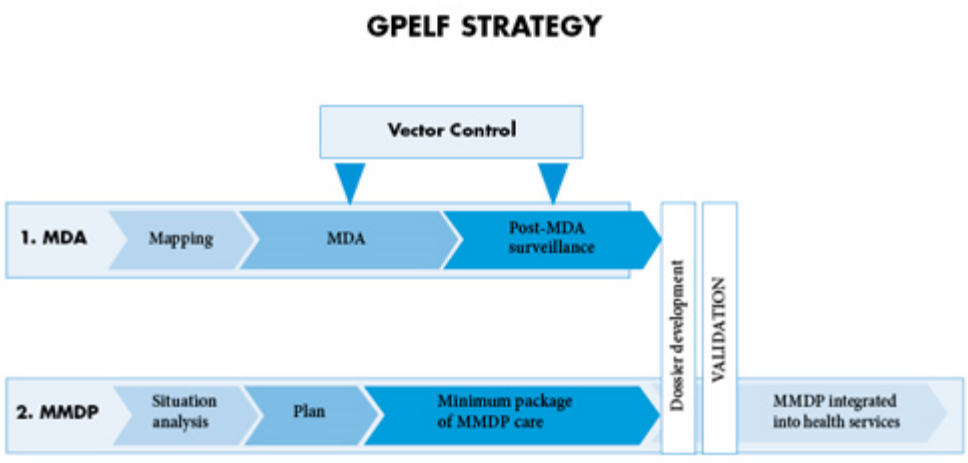


Figure 2. Twin pillars of the Global Programme to Eliminate Lymphatic Filariasis (GPELF). ***Source: (31).***

The drugs used for MDA—albendazole (donated by GlaxoSmithKline) co-administered with either diethylcarbamazine (DEC, donated by Eisai Co.) or ivermectin (Mectizan®, donated by Merck)—reduce the number of viable infectious stage mf found in circulation of the human host (5), thereby preventing transmission to mosquitoes. Annual MDA for 4—6 years at effective coverage (> 65%) in at-risk populations is thought to reduce infection prevalence to levels below which transmission is no longer sustainable (32).

There are four phases of LF elimination programs (Figure 2): mapping, MDA, post-treatment surveillance (PTS), and validation (formerly called verification). To progress from MDA to PTS and from PTS to validation, WHO recommends a series of transmission assessment surveys (TAS) (32). TAS-1 is recommended after a minimum of five years of MDA to determine whether parasite antigen prevalence has been reduced to less than 2% (1% in areas where *Aedes* mosquitoes are the primary vector)—levels below which LF transmission is presumed unsustainable (32)—and MDA can stop. Once MDA has stopped, two additional TAS surveys (TAS-2 and TAS-3) are recommended at two- to three-year intervals as part of PTS activities (32). If antigen prevalence throughout a country remains less than 2% for at least four years of PTS, countries may apply to WHO for validation of elimination of LF as a public health problem.

LF Survey Designs

The current WHO-recommended methodology for assessing whether LF prevalence has been reduced below sustainable transmission thresholds is the Transmission Assessment Survey (TAS) (32). TAS is a lot quality assurance sampling (LQAS)-type survey measuring LF antigen prevalence among children 6-7 years old through school- or community-based (household) sampling. This population is targeted because they are born after commencement of MDA, and therefore should be LF-free if MDA successfully interrupts local transmission. The LQAS design was favored because it provides a “pass” or “fail” outcome based on the number of positive samples above or

below a critical threshold level—an outcome that does not require sophisticated statistical software and one that is easy for program managers to interpret.

Introduced in 2011, TAS replaced the previous recommended suite of transmission assessment surveys that involved multiple LQAS surveys of approximately 500 individuals in sentinel and spot-check villages (areas suspected of having high transmission) followed by a larger LQAS survey of 3000 children aged 6-7 years old (33). The larger school-based survey was designed to determine whether LF antigen prevalence was above or below 0.1%. For this expected prevalence, the sample size of 3000 had alpha (α) of 0.05, but beta (β) of 0.95, meaning that there was high likelihood of failing areas that had met the 0.1% target (34). For this reason, the 2011 TAS increased the threshold antigen prevalence to 2.0%. The corresponding TAS sample sizes are calibrated such that α remains 0.05, but β is reduced to 0.75 if the true antigen prevalence is 1.0% (half of the threshold level).

An alternative survey design that pre-dated TAS but did not gain widespread adoption is the WHO Pacific regional program to eliminate LF (PacELF) "C-survey" (35). PacELF C-surveys encompass several design approaches, including a health-facility-based LQAS survey and a community-based cluster survey design, with the latter being synonymous with a PacELF C-survey. Like TAS, the threshold for PacELF surveys is 2% at the 95% confidence level. However, unlike TAS, which tests primary school children (6-7 years old), PacELF C-survey includes testing of all aged individuals older than 2 years.

Disease Control Definitions

There has been much debate regarding the interpretation of "elimination as a public health problem" for LF since the WHA Resolution was passed in 1997 (36). National programs and implementing agencies have tended to interpret the goal to mean elimination of transmission—i.e. zero incident infections nationally—consistent with traditional disease control spectrum definitions: **extinction** (pathogen extermination), **eradication** (zero incident infections globally), **elimination** (zero incident infections in a defined geographic area), and **control** (reduction in incidence or prevalence) (29). However, WHO recently defined "elimination as a public health problem" as a distinct phase between elimination and control that would apply to LF, blinding trachoma, and human African trypanosomiasis (37). The corresponding country-level review processes undertaken by WHO and reviewing authority for each classification are shown in Table 1.

Table 1. WHO Disease control classifications and corresponding review processes. Information from (37).

Classification	WHO Review Process	Reviewing Authority
Extinction	n/a	
Eradication	Certification	International Commission established by World Health Assembly Resolution
Elimination of transmission	Verification	Ad-hoc international Reviewing Authority
Elimination as a public health problem	Validation	Ad-hoc international Reviewing Authority
Control	n/a	n/a

The quantitative threshold for attaining elimination of LF as a public health problem is defined as maintaining less than 2% antigen prevalence (<1% in areas where LF is transmitted by *Aedes* mosquitoes) for at least 4 years after MDA has stopped (38). This threshold is set based on a combination of theoretical modeling of LF breakpoints (39, 40) and programmatic feasibility and survey power/sample sizes (41). Because reduction to a non-zero number requires programs to maintain surveillance activities indefinitely (38), there is benefit to develop and define methodologies demonstrating elimination of transmission.

The procedures for confirming and acknowledging endpoints for disease eradication/elimination require countries to complete a WHO disease-specific dossier. In the case of LF, the dossier contains the following main sections: i) endemicity description and classification; ii) description of MDA and other interventions; iii) monitoring data; iv) results from TAS surveys; v) existing patient burden; vi) MMDP service availability; vii) capacity to sustain post-validation surveillance (38). A dossier should be completed by the national LF program manager in consultation with relevant partners and submitted to WHO any time after the minimum four-year PTS period has elapsed. The WHO Regional Office then convenes an ad-hoc Review Authority composed of at least three international LF experts to review the dossier and make a recommendation validating the claim of elimination as a public health problem or postponing the validation where additional evidence is deemed necessary (38).

Even after validation, countries must continue post-validation surveillance and support for MMDP services. WHO recently indicated that countries may additionally request *verification of elimination of transmission*, despite the fact that "specific

requirements for such verification have not yet been agreed" (38). This work seeks to contribute to this current gap by describing an analytic framework designed to estimate the probability of freedom from infection and applying this approach to analysis of sequential TAS and PacELF C-surveys in the central Nigerian states of Plateau and Nasarawa.

Chapter II: Manuscript

Title: Freedom From Infection: Evaluation of Lymphatic Filariasis Survey Data in Plateau and Nasarawa States, Nigeria

Authors: Gregory S. Noland, Gillian Stresman, Morgan Smith, Edwin Michael, Chris Drakeley, Abel Eigege, Solomon Adelamo, Bulus Mancha, Benjamin Lopman, Frank O. Richards, Jr.

Abstract: Lymphatic filariasis (LF), or "elephantiasis", is a mosquito-transmitted parasitic disease affecting 67.9 million people in 73 countries. Infection leads to lymphatic dysfunction that results in swelling of limbs (lymphedema) and genitals (male hydrocele), and painful recurrent inflammation. The World Health Organization (WHO) targets "elimination of LF as a public health problem" through 1) mass drug administration (MDA) to interrupt parasite transmission and 2) morbidity management and disability prevention (MMDP) to care for those already affected by LF. WHO guidelines currently exist for validation of LF elimination as a public health problem through a series of transmission assessment surveys (TAS) with a critical threshold of 2% (1% in areas where LF is transmitted by *Aedes* mosquitoes)—the levels below which transmission is presumed unsustainable. WHO also recently indicated that countries may request verification of elimination of LF transmission (elimination *sensu stricto*), but acknowledged that specific requirements for such verification have not yet been agreed. This study assessed Freedom From Infection (FFI) analysis to fill this gap. FFI employs

probability theory to estimate the probability that disease prevalence in a population is below a pre-determined threshold. This novel application of FFI was conducted using data from a series of cross-sectional LF antigen surveys in eight districts of Plateau and Nasarawa states, Nigeria encompassing 31,714 individuals tested over an eleven-year period (2007—2017) through school-based TAS surveys of children aged 6—7 years old and community-based cluster surveys of individuals > 2 years. Results indicate a high probability (>0.90) at all examined thresholds (2%, 1%, 0.1%, 1 case) that areas with lower baseline LF transmission (≤20% antigenemia) were free from LF infection by 2007, five to six years after the start of MDA, while high FFI probability (>0.99) was observed between 2012—2015, around 11—13 years after the start of MDA, for three areas of higher baseline transmission. These results correspond well with cross-sectional survey conclusions. In summary, initial application of FFI analysis to LF shows good concordance with cross-sectional survey data, thereby offering a tool, that with refinement, could serve as a viable analytic framework to verify elimination of LF transmission.

Introduction

Lymphatic filariasis (LF), or "elephantiasis", is a mosquito-transmitted parasitic disease caused primarily by *Wuchereria bancrofti*. Infection leads to lymphatic system dysfunction that results in swelling of limbs (lymphedema) and genitals (hydrocele in males), and painful recurrent inflammation. The World Health Organization (WHO) targets "elimination of LF as a public health problem" through 1) mass drug administration (MDA) to interrupt parasite transmission and 2) morbidity management and disability prevention (MMDP) to care for those already affected by chronic LF (30).

The quantitative endpoint for attaining elimination of LF as a public health problem is defined as maintaining less than 2% antigen prevalence (<1% in areas where LF is transmitted by *Aedes* mosquitoes) at the 95% confidence level for at least 4 years after MDA has stopped (37, 38). This threshold is set based on a combination of theoretical modeling of LF breakpoints (39, 40) and programmatic feasibility and survey power/sample sizes (41).

Recent WHO guidance indicates that after achieving elimination as a public health problem—i.e. reduction below 2%—countries could request verification of elimination of transmission (elimination *sensu stricto*). However, specific criteria do not exist to substantiate this claim. The lack of accepted protocols to measure transmission elimination for LF is due in part to several reasons. First, available diagnostic tools are imperfect: the presence of microfilariae (the transmissible larval form of the parasite generally found only in the blood at night) is poorly correlated with infection, and parasite antigen and antibody persist for years after infection is cleared (24). Secondly,

proving a negative is a challenge statistically and philosophically, unless the entire population is tested with a perfect diagnostic device. Basic sampling theory can be used to generate prevalence estimates and corresponding confidence limits. However, even large-scale population surveys push the limits of programmatic feasibility for costs and logistics. For example, WHO elimination guidelines for onchocerciasis (river blindness, RB) require a sample of 2000 children per survey domain to detect a sero-prevalence of 0.1% or less at the 95% upper confidence limit (42). A similar threshold level of <0.1% antigen prevalence in children was recommended by the regional Pacific Program to Eliminate LF (PacELF) "D survey" to confirm interruption of transmission (43). At one time, WHO recommended a survey of 3000 5 year-old children could be completed at the end of the five-year post-treatment surveillance (PTS) period to satisfy claims for LF elimination (33). However, this methodology was not widely adopted, and WHO's current position is that "specific requirements for such verification [of elimination of transmission] have not yet been agreed" (38).

Freedom From Infection (FFI) is an analytic framework originally developed in veterinary epidemiology to estimate the probability of a freedom from disease in animal livestock herds (44). The method uses probability theory to estimate the probability that disease prevalence is below a pre-determined threshold, which can then be extended to estimate the probability of freedom from infection in a population—equivalent to a negative predictive value (45). In the few applications of FFI to date for human disease, including poliovirus (46), and malaria (45), investigators have focused on evaluating the sensitivity of passive surveillance systems to detect an infected individual using scenario tree modeling. Incorporation of active surveillance or cross-sectional survey data into

FFI analysis has been proposed (45), but not has not yet been evaluated. This project proposes to apply FFI analysis to cross-sectional LF survey data from eight districts—called local government areas (LGAs)—in Plateau and Nasarawa states, Nigeria, and to assess whether these areas are likely free from LF transmission.

Methods

Survey area

Nigeria has the largest population at risk for LF in sub-Saharan Africa—120 million people—and the second largest globally behind India. In order to demonstrate the feasibility of eliminating LF in a highly-endemic area, the Nigerian Federal Ministry of Health (FMOH), state and local ministries, and with implementing assistance by The Carter Center, established an LF elimination program in the central Nigerian states of Plateau (2015 population estimate 4.2 million) and Nasarawa (2.7 million) in 1997. This initiative built upon the FMOH/Carter Center onchocerciasis elimination program that annually distributed ivermectin in 12 RB hyper-endemic districts of Plateau and Nasarawa since 1992 (47). Around 80% of inhabitants in Plateau and Nasarawa practice subsistence farming, and the area is a mixture of Christian and Islamic permanent residents and itinerate Fulani herdsmen.

Baseline LF mapping in Plateau and Nasarawa was conducted from 1999-2000 and consisting of convenience sample testing of 50—100 individuals 15 years or older in each of 1 to 4 villages suspected of having LF transmission per LGA in Nigeria. LGA-level mean antigen prevalence was 23% (range 4%—58%) in the 30 districts of the two-

state area (48). Annual mass drug administration (MDA) for LF with albendazole and ivermectin was launched in two LGAs in 2000, with all 30 LGAs under annual MDA by 2003. In 2007-2008, after a minimum of five years of LF MDA, a stop-MDA survey using the PacELF C-survey found that ten of the 30 LGAs met the criterion for stopping MDA (49). The remaining LGAs qualified to stop MDA through transmission assessment surveys (TAS-1) conducted in 2012. In total, 36.1 million treatments for LF were provided, with median of MDA duration of 11 years (range 7—13 years). After the halt of MDA, TAS-2 and TAS-3 surveys as well as PacELF C-surveys were conducted to meet WHO requirements for post-treatment surveillance and for operational research purposes.

This analysis includes survey data from eight LGAs—six located in Plateau and two in Nasarawa (Figure 1). Population data, baseline LF antigenemia and MDA history in the eight LGAs are summarized in Table 1. Baseline antigenemia ranged from 11%-58%, except in urban Jos North where baseline prevalence was only 4%. LF MDA was conducted between 7 and 12 years, with five LGAs stopping MDA in 2009 and three stopping in 2012. Kanke LGA in Plateau state was also considered hyper-endemic for onchocerciasis, meaning that eight rounds of annual MDA with ivermectin mono-therapy were provided prior to initiation of albendazole-ivermectin MDA for LF in 2001. The remaining LGAs in this analysis were considered hypo-/meso- endemic for onchocerciasis and were not treated prior to initiation of LF MDA.

Survey Designs

Data were obtained between 2007 and 2017 in the context of cross-sectional surveys following either the transmission assessment survey (TAS) or Pac-ELF C-survey study designs. TAS is the current WHO-recommended methodology for assessing whether LF prevalence has been reduced below sustainable transmission thresholds (32). TAS is a lot quality assurance sampling (LQAS) survey measuring LF antigen prevalence among children 6-7 years old through school- or community-based (household) sampling. Programmatically, TAS-1 is recommended after a minimum of five years MDA that exceed the minimum effective coverage level (65%) to determine whether MDA can be halted. Two additional surveys (TAS-2 and TAS-3) are recommended during the five-year post-treatment surveillance (PTS) to assess whether transmission has recrudesced or has been imported.

PacELF C-survey is an alternative survey design developed and used by the WHO Pacific regional program to eliminate LF ("PacELF") prior to formalization of TAS methodology. Though encompassing a suite of survey options, PacELF is most commonly understood to mean a community-based cluster (household) survey of individuals older than 2 years of age with a prevalence threshold of 2% at the 95% confidence level similar to TAS (35).

Survey-specific details are as follows:

2007 PacELF C-survey. The 2007 PacELF C-survey that was conducted as the first stop-MDA assessment in Plateau and Nasarawa states. It was implemented as a household-based cluster survey. Full survey details are summarized by King et

al. (49). Briefly, each LGA was considered its own survey domain, and cluster surveys of at least 13 households in 20 clusters per LGA were conducted in all 30 LGAs across Plateau and Nasarawa states. LF antigen testing for circulating filarial antigen was performed from finger prick blood samples with the BinaxNOW Filariasis immunochromatographic test (ICT) according to manufacturer's instructions (Alere Inc., Scarborough, ME).

2012 TAS. The 2012 TAS was conducted as a school-based cluster survey to determine whether MDA could stop in areas that continued to treat after failing to meet stop-MDA criteria in the 2007 PacELF C-survey. Details are summarized by Eigege et al. (50). Briefly, 21 LGAs were grouped into four survey domains called evaluation units (EUs). EUs contained between 4 and 7 LGAs. TAS guidelines specify that the total population of an EU be no more than 2 million people (32). The target sample size in each EU was around 1,700 children aged 6-7 years old. TAS sample sizes and critical cutoff values are powered so that an EU has at least a 75% chance of passing if the true antigen prevalence is 1.0% and no more than about a 5% chance of passing (incorrectly) if the true antigen prevalence is ≥2.0% (32). Antigen testing was performed from finger prick blood samples using ICT according to manufacturer's instructions. Four of the LGAs considered in this analysis were included in the survey.

2014 TAS and PacELF C-survey. Post-treatment surveillance TAS-2 and PacELF C-survey surveys were conducted as operational research to compare LF antigen prevalence between the two study designs in the same geographic areas [Noland et al., in preparation]. Four LGAs included in this analysis (Jos North, Keanna,

Keffi, and Langtang South were considered a single TAS EU survey domain for school-based testing of children 6-7 years old. Each of the four LGAs along with Barkin Ladi LGA were also evaluated as independent survey domains for household-based PacELF C-surveys of individuals older than 2 years old. Antigen testing was performed using either ICT or filariasis test strip (FTS) (Alere Inc., Scarborough, ME).

2015 TAS. Post-treatment surveillance TAS-2 was conducted in 26 LGAs, including four LGAs considered for this analysis. School-based testing of children 6-7 years old using ICT or FTS was conducted according manufacturer's instructions.

2016 TAS and PacELF C-survey. Post-treatment surveillance TAS-3 was conducted in the four LGAs assessed in 2014 TAS surveys. In 2016, the two Plateau state LGAs were considered an EU survey domain, while the two Nasarawa state LGAs were considered a separate EU. In addition, epidemiological and diagnostic operational research studies for LF and RB were conducted in Kanke, Mikang, and Kanam [Noland et al., in preparation]. Each LGA was considered a separate survey domain for PacELF C-survey design. For TAS study design, LGAs were grouped into two EUs based on RB endemicity: Kanke (RB meso-/hyper-endemic); and Mikang, Kanam (RB hypo-endemic). School-based testing of children 6—7 years old for TAS and household testing of individuals older than 2 years was performed using ICT or FTS testing according to manufacturer's instructions.

2017 TAS. Post-treatment surveillance TAS-3 was conducted in 26 LGAs, including four LGAs considered for this analysis. School-based testing of children 6-7 years old using ICT or FTS was conducted according manufacturer's instructions.

Ethical approval and consent procedures

All surveys were considered non-research public health evaluations by Emory University Institutional Review Board (IRB) and were approved by the Nigerian National Health Research Ethics Committee (NHREC) under the following approval numbers: NHREC/01/01/2007- 20/04/2015; NHREC/01/01/2007-03/02/2016; NHREC/01/01/2007-10/02/2016; NHREC/01/01/2007-18/04/2017.

Participation in the surveys was voluntary. Individual oral consent was obtained for participants 18 years or older, while oral assent was collected from children less than 18 along with consent from their parent or care taker.

Descriptive statistics

LGA-specific antigen prevalence point estimates and two-sided 95% confidence intervals were calculated from individual-entry survey primary data sets. Calculations took into account the selection probabilities of the cluster survey designs and were performed using the SVY routine in Stata v14 (StataCorp, College Station, TX).

Freedom from Infection Analysis

To calculate FFI probability estimates at individual survey time points for each LGA, the on-line FreeCalc tool was used (AusVet) (51). The tool calculates the exact probability of observing a given number or proportion of diseased individuals, given a specified finite population, test sensitivity and specificity, and type I and type II error. Statistical details of the tool are provided in Cameron and Baldock (44). The hypothetical disease prevalence is termed 'design prevalence' (DP); it can be specified as a proportion or fixed number of diseased elements in the population. Input values are summarized in Table 2.

Results

Descriptive Results of Cross Sectional Surveys

The results from cross-sectional TAS and PacELF C-surveys reported in this analysis encompass a total of 31,714 individuals tested for LF antigen over an eleven-year period (2007—2017) in eight LGAs of Plateau and Nasarawa states, Nigeria (Figure 2). The LGAs can be grouped into high and low burden according to prevalence estimates from population-wide (individuals older than 2 years old) 2007 PacELF C-survey (Table 3a): three LGAs with 14% antigenemia (Kanke, Kanam, and Mikang); the other five LGAs (Langtang South, Barkin Ladi, Keffi, Keanna, and Jos North) had antigen prevalence point estimates less than 2% at this time point.

Five years later, in the context of stop-MDA TAS-1 surveys conducted among primary school children in the three high-burden LGAs and in Barkin Ladi in 2012, positive samples were detected in three of the four areas tested (Table 3a), while no positive samples found in Kanke. The antigen prevalence point estimate was 2% or less in all areas; however, only in Kanam and Kanke was the 95% upper confidence limit less than 2%.

In 2014, PacELF C-surveys and TAS-2 post-treatment surveillance surveys were conducted in lower burden LGAs (according to 2007 antigen prevalence). Two of five areas assessed by PacELF C-survey (Jos North and Keffi) had no antigen positive samples, while two of four LGAs assessing primary school children in TAS surveys (Keanna and Keffi) did not detect antigen positive samples. Among the LGAs with positive samples in TAS or PacELF C-survey, antigen prevalence ranged from 0.15% to 0.66%, with all confidence intervals less than 2%.

From 2015 onwards, all samples from TAS surveys (n=9,317) were antigen negative (Table 3b). Only in the context of community-wide PacELF C-survey testing were positive samples identified. In 2016 PacELF C-surveys, nine antigen positive individuals were found in two of the three included LGAs (all samples in Mikang were negative). Median age of positive individuals was 44.5 (range 22—75). Antigen prevalence estimates and corresponding 95% confidence intervals were less than 2% in all three areas.

In summary, cross sectional survey data indicate that LF antigen prevalence was reduced to significantly less than 2%—the hypothetical LF transmission breakpoint—by

2014 across all eight LGAs included in this analysis. Furthermore, considering antigen persistence in previously infected individuals and the absence of infection in children sampled in TAS and PacELF C-surveys suggests that these areas were like free of LF infection by 2015-2016.

Freedom From Infection Analysis

Before calculating LGA-specific FFI probabilities, the impact of test specificity was examined over a range of values from 95% to 99.9%, keeping other parameters constant (95% test sensitivity, 5% type I and type II error, using Barkin Ladi LGA as an example. Observed survey antigen prevalence in Barkin Ladi was low, but non-zero from 2007-2014, after which point, observed prevalence decreased to 0%. As shown in Figure 3, FFI probability at 2% DP increased from 0.90 in 2007 to high (>0.98) probability for test specificity of 99.9% beginning in 2014. Other test specificity values (95%, 98% and 99%) yielded certainty of FFI (probability=1.0) or near-certainty for all time points. In the case of 2007 PacELF C-survey data where observed prevalence was 1.74%, the high or certain probability of FFI is initially surprising. However, the imperfect test specificity assumes that the relative low number of positive samples are false positives. For all subsequent analysis, a test specificity of 98% was selected.

Next, LGA-specific FFI probabilities were calculated for each survey time point at varying DPs (2%, 1%, 0.1% and 1 case). As shown in Figure 4, two general patterns emerged that follow the categorization of transmission burden in 2007. In Kanke, Kanam, and Mikang, LGAs where 2007 antigen prevalence was 14%, the corresponding

FFI probability at 2007 was 0.0001 for all DPs—meaning the population is considered diseased. However, by the next time point, 2012, and all following time points, probability of FFI had reached near certainty (>0.98), except where an intermediate range of values (0.38—0.92) was observed at 2012 in Mikang due to border-line observed TAS survey prevalence (2.01%) and low sample size (n=249) not being able to distinguish the population with specified DP from a disease-free population. In the five low-burden LGAs, FFI probability across DP of 2%, 1%, 0.1% and 1 case started high (>0.90 in Barkin Ladi; ≥0.97 in Jos North, Keana, Keffi, and Langtang South) and reached certainty or near-certainty (>0.98) by 2014, providing strong evidence that the population is free from disease.

Discussion

This study conducted a novel application of freedom from infection (FFI) analysis, a framework originally developed in veterinary epidemiology to estimate the probability that disease prevalence is below a pre-determined design prevalence (DP) threshold, to assess whether areas of central Nigeria were free from LF infection utilizing repeated cross-sectional LF survey data. Results indicate a high probability (>0.90) at all examined DP thresholds (2%, 1%, 0.1% and 1 case) that areas with lower baseline LF transmission (≤20% antigenemia) were free from LF infection as early as 2007, five to six years after the start of MDA, while high FFI probability (>0.99) was achieved between 2012 and 2015, around 11-13 years after the start of MDA, for three areas of higher baseline transmission. These results correspond well with observed prevalence

estimates from cross-sectional PTS surveys that from 2007 onwards for the lower transmission areas and from 2012 onwards for the higher transmission areas were less than or equal to 2%—the level at which LF transmission is believed to be no longer sustainable (32). Prior to 2012, the three areas of higher transmission intensity had extremely low (<0.0001) probability of freedom consistent with cross-sectional survey results.

In general, the high FFI probabilities obtained in this analysis are not surprising given the very low or negative antigen prevalence observed in the cross-sectional data from 2014 onwards. On the other hand, high FFI probabilities (>95%) even at DP of 0.1% and 1 case, were somewhat surprising in instances like Langtang South and Keana, where 10 and 15, respectively, antigen positive samples were detected in 2007 PacELF C-surveys. However, as illustrated in Figure 3, FFI probabilities are strongly influenced by the specificity of the diagnostic test. For example, at 95% specificity, 50 positive reactors in a sample of 1000 would be assumed to be false-positives. Values of 10 and 15 positive reactors in samples of around 1200 individuals tested are within the acceptable range of false-positives at 95% or even 98% specificity. While early evaluations claimed 100% sensitivity and 100% specificity of the ICT rapid antigen test compared to parasitological diagnosis of blood samples from endemic and non-endemic areas, an additional 25% of the endemic parasite-negative samples were antigen positive (52). This is attributable to antigen persistence after parasite clearance, which has been estimated at 22.5—65 months (53). Classification of formerly infected individuals as antigen-positive reactors leads to a conservative estimate of the true parasite infection prevalence in communities; however, it complicates survey-based evaluations seeking to determine

parasite elimination (24), as well as accurate valuation of the test specificity for FFI analysis. The FFI analysis conducted here assumed a test specificity of 98% to balance a low false positive rate with the reality that a proportion of antigen positive individuals are no longer parasite-infected. The current generation FTS diagnostic has similar performance profile with improved storage and shelf-life characteristics (23). For this analysis, which included surveys conducted with both ICT and FTS, sensitivity and specificity parameters were assumed equal for both tests.

There are several limitations of the current analysis. First, the FFI analysis conducted here only considered each time point as an independent sampling event. To harness the full power of FFI framework, additional analysis should be conducted to account for the repeated measures in the same geographical population. While adaptations of scenario-tree modeling framework normally applied to passive surveillance data has been proposed for this purpose (45), FFI analysis of repeated cross-sectional survey data does not appear to have been performed to date. Secondly, the FFI estimates derive from observational survey data that may be prone to biases. Selection bias in particular is a risk in this area of Nigeria, where ethnic conflicts mean that a proportion (usually <5%) of clusters are inaccessible to survey teams. It is conceivable that such areas also may have been inaccessible to MDA campaigns, and therefore may serve as undetected reservoirs of infection. FFI results therefore may over-estimate the freedom from disease. Thirdly, while PacELF C-surveys benefited from large samples sizes in each LGA (typically >1,000 individuals tested per survey), TAS samples sizes tended to be much smaller (median 432), as TAS surveys usually included multiple LGAs in a single TAS survey domain, called an evaluation unit (EU). This resulted in

low power to exclude the null hypothesis that a given number or fewer test positive samples would be observed if the population was diseased at a level equal to or greater than the specified design prevalence. For example, the intermediate FFI results in Mikang (n=249) and in Barkin Ladi (n=208) in 2012, particularly at DPs of 0.1% and 1 case, likely result from the small sample size coupled with a non-zero number of test positive samples. Slightly larger samples sizes and a less conservative DP can be applied. For example, a study by Cruz et al. calculated a minimum sample size of 297 was sufficient to exclude a seroprevalence greater than 1% at 95% confidence using a similar FFI approach applied to cross-sectional serological evaluation of equine infectious anemia virus in Spanish horses (54). A final limitation is that the FreeCalc tool employed in the present analysis assumes simple random sampling, whereas the Nigerian TAS and PacELF surveys were implemented as cluster surveys. Additional tools in the AusVet toolkit would need to be modified and applied to the present data in order to obtain more accurate FFI probabilities that account for the cluster nature of the survey.

In conclusion, this initial application of FFI analysis to LF shows good concordance with cross-sectional survey data conclusions, thereby offering a tool, that with refinement, could serve as a viable analytic framework to verify elimination of LF transmission.

Tables

Table 1. Information for the eight local government areas (LGAs) of Plateau and Nasarawa states, Nigeria, included in analysis, ranked in order of baseline antigen prevalence.

State	LGA	Population (2014 Est.)	1999-2000 Baseline LF Antigen prevalence (%)	Years LF MDA
Plateau	Kanke	156,222	58	12 (2001-2012)
Plateau	Kanam	213,441	44	11 (2002-2012)
Plateau	Mikang	125,327	25	10 (2003-2012)
Plateau	Langtang South	136,770	20	7 (2003-2009)
Plateau	Barkin Ladi	225,495	18	8 (2002-2009)
Nasarawa	Keana	88,765	14	8 (2002-2009)
Nasarawa	Keffi	103,660	11	8 (2002-2009)
Plateau	Jos North	552,330	4	7 (2003-2009)

Table 2. Input parameters for FreeCalc freedom from infection calculations.

Population Size *Size of the total population (herd) being evaluated*	*Pac-ELF C-Surveys.* Estimated total 2014 population size for each LGA was used. 2014 was selected, as it represents the mid-point year for the range of years in which surveys were conducted. Calculated based on 2006 census figures and assuming a 3.2% annual growth rate. *TAS Surveys*. Estimated 2014 population size of 6-7-year olds within each LGA was used. Calculated as 8% of the total population.
Sample size	Number of people tested in each LGA at each survey time point.
Number positive	Number tested positive
Test Sensitivity	95%
Test Specificity	Various: 95%, 98%, 99%, 99.9%
Design Prevalence *The hypothetical prevalence to be detected. Can be specified as either a fixed number of elements from the population or a proportion of the population;*	Various: 2%, 1%, 0.1%, 1 case
Desired type I error	0.05
Desired type II error	0.05
Calculation method *hypergeometric (for small populations); or simple binomial (for large populations*	Hypergeometric
Population threshold for binomial method	The population size threshold, above which the simple binomial method is used regardless of which calculation method has been selected;
Precision (significant digits)	4

Table 3a. Transmission assessment survey (TAS) and PacELF C-survey lymphatic filariasis antigen test results in eight local government areas (LGAs) of Plateau and Nasarawa states, Nigeria (2007-2014), ranked in order of baseline antigen prevalence. Prevalence point estimates and 95% confidence intervals (CI) account for complex, clustered survey designs.

State	LGA	2007 PacELF			2012 TAS			2014 PacELF			2014 TAS		
		Tested	Pos.	Prevalence (95% CI)	Tested	Pos.	Prevalence (95% CI)	Tested	Pos.	Prevalence (95% CI)	Tested	Pos.	Prevalence (95% CI)
Plateau	Kanke	1,306	161	14.14 (9.55—20.44)	242	0	0						
Plateau	Kanam	1,088	176	14.76 (10.00—21.26)	537	4	0.74 (0.32—1.71)						
Plateau	Mikang	1,086	160	14.73 (10.85—19.71)	249	5	2.01 (0.53—7.37)						
Plateau	Langtang South	1,235	10	0.62 (0.27—1.42)				1,093	1	0.17 (0.03—1.08)	619	1	0.16 (0.03—0.93)
Plateau	Barkin Ladi	947	13	1.74 (0.73—4.13)	208	1	0.48 (0.07—3.36)	1,301	3	0.15 (0.03—0.71)			
Nasarawa	Keana	1,207	15	1.29 (0.76—2.19)				1,211	7	0.66 (0.26—1.64)	512	0	0
Nasarawa	Keffi	1,485	6	0.42 (0.16—1.10)				1,208	0	0	229	0	0
Plateau	Jos North	1,056	2	0.19 (0.03—1.36)				1,323	0	0	399	1	0.25 (0.05—1.38)

Table 3b. Transmission assessment survey (TAS) and PacELF C-survey lymphatic filariasis antigen test results in eight local government areas (LGAs) of Plateau and Nasarawa states, Nigeria (2015-2017), ranked in order of baseline antigen prevalence. Prevalence point estimates and 95% confidence intervals (CI) account for complex, clustered survey designs.

State	LGA	2015 TAS			2016 TAS			2016 PacELF			2017 TAS		
		Tested	Pos.	Prevalence (95% CI)	Tested	Pos.	Prevalence (95% CI)	Tested	Pos.	Prevalence (95% CI)	Tested	Pos.	Prevalence (95% CI)
Plateau	Kanke	341	0	0	1724	0	0	1,309	4	0.31 (0.06—0.83)	256	0	0
Plateau	Kanam	488	0	0	987	0	0	1,272	5	0.39 (0.10—1.23)	498	0	0
Plateau	Mikang	239	0	0	574	0	0	1,275	0	0	432	0	0
Plateau	Langtang South				1141	0	0						
Plateau	Barkin Ladi	213	0	0							224	0	0
Nasarawa	Keana				1130	0	0						
Nasarawa	Keffi				415	0	0						
Plateau	Jos North				655	0	0						

Figures

Figure 1. Map of Plateau and Nasarawa states, Nigeria, showing locations of the eight local government areas (LGAs) selected for analysis.

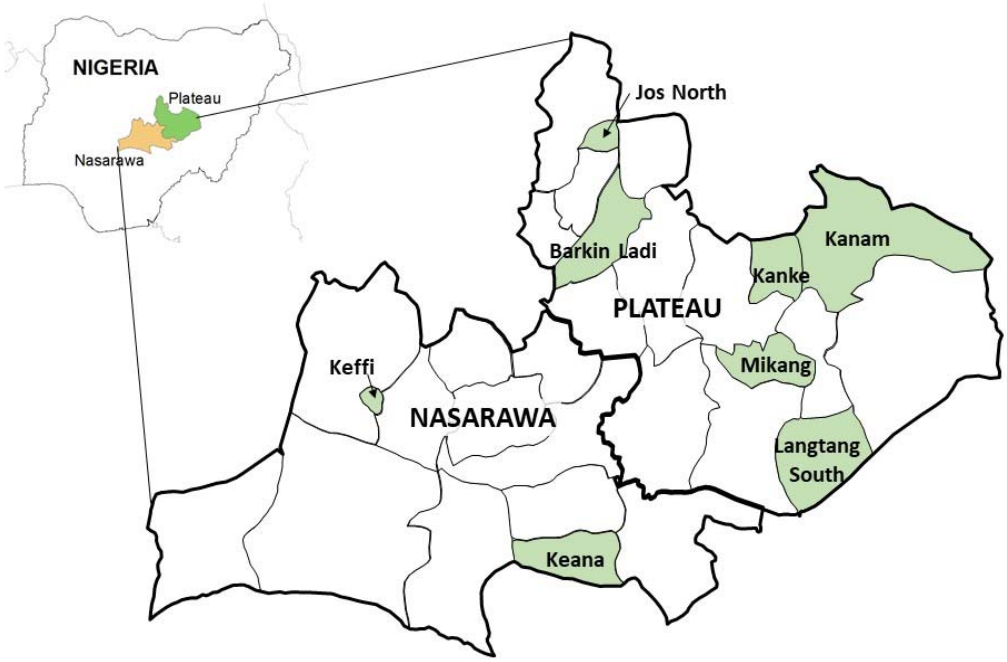


Figure 2. LF Antigen prevalence from transmission assessment survey (TAS) or Pac-ELF C-surveys in eight local government areas (LGAs) of Plateau and Nasarawa states, Nigeria, by year.

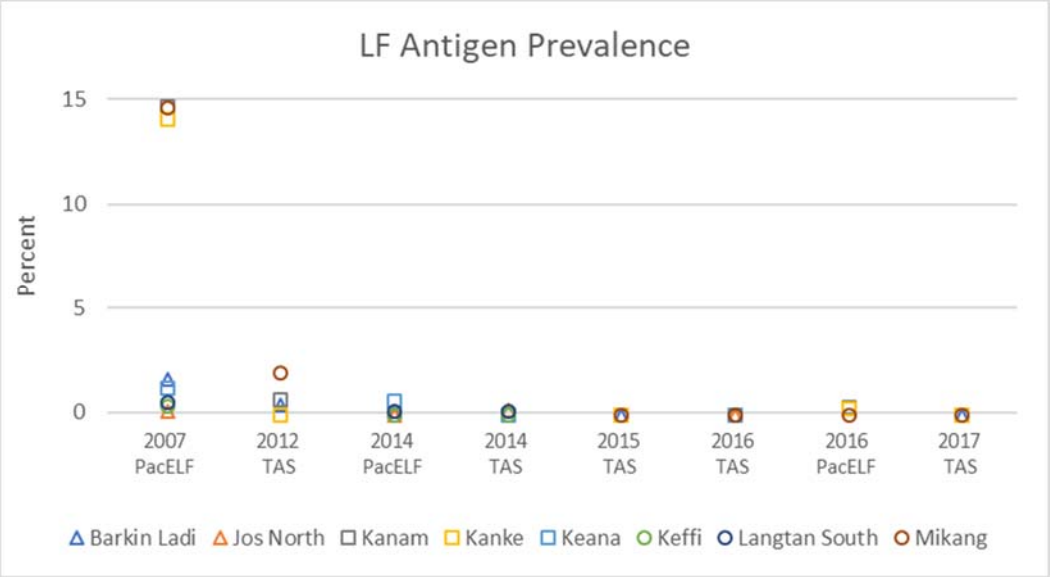


Figure 3. Probability of freedom from infection over time at various test specificity values (95%, 98%, 99%, and 99.9%) in Barkin Ladi LGA at 95% test sensitivity, 5% type I and type II error, and 2% design prevalence.

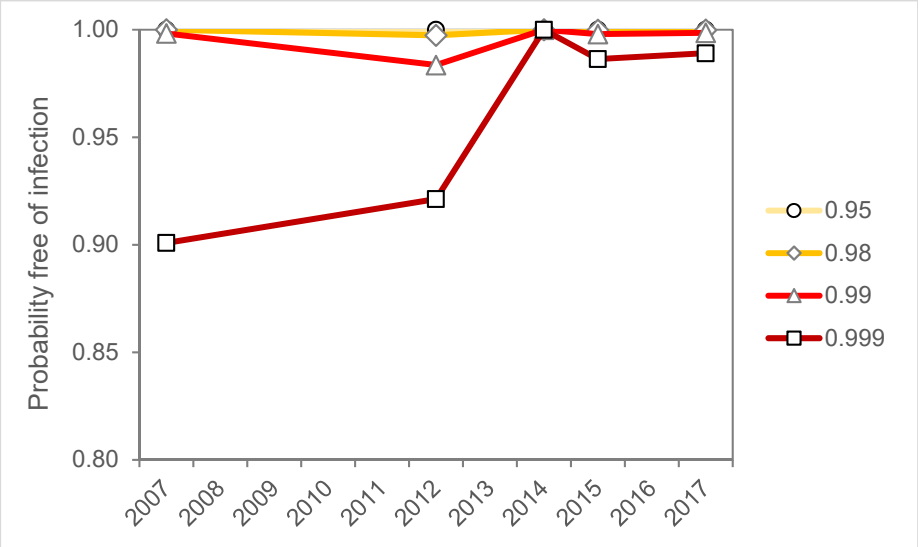
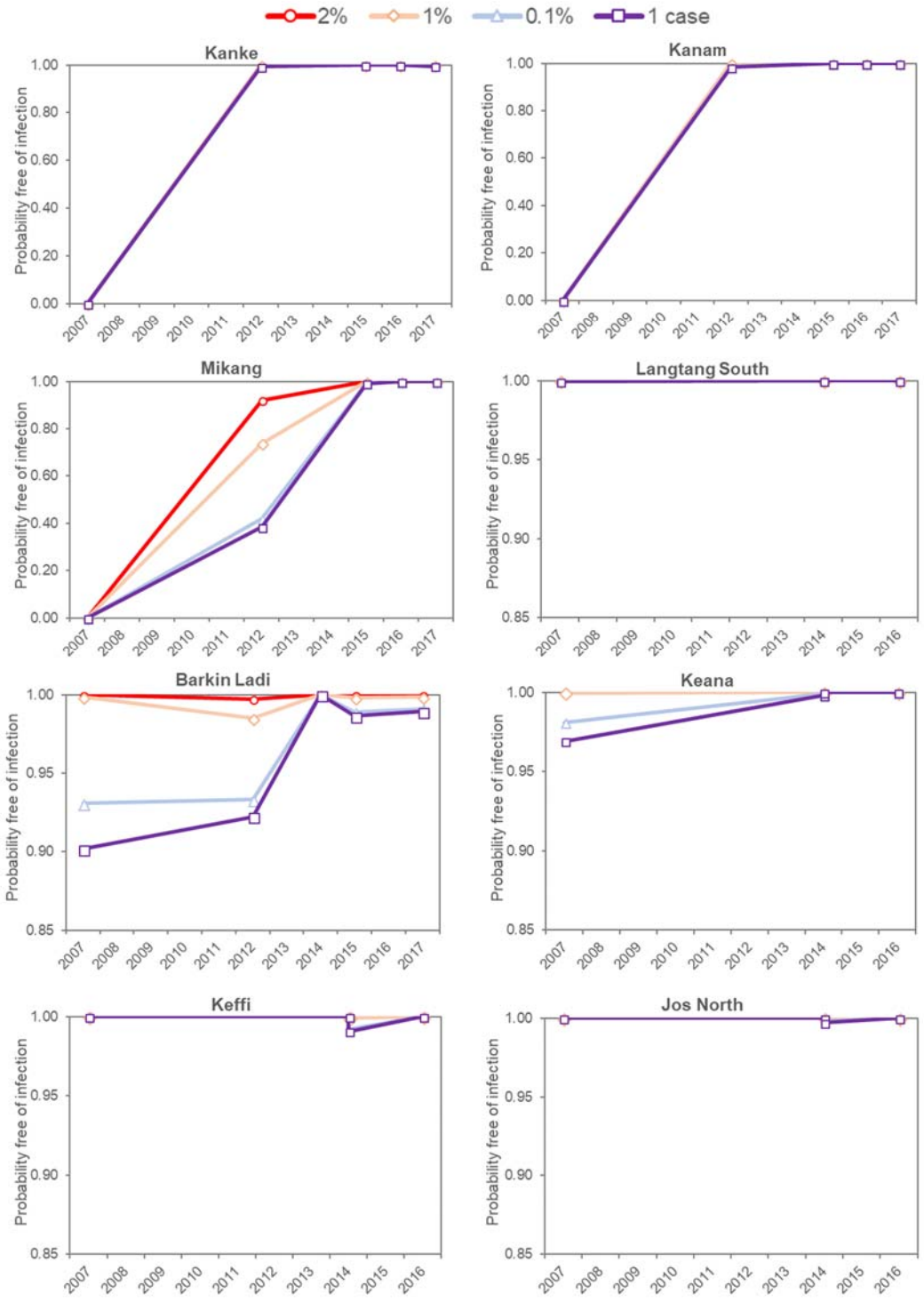


Figure 4. Probability of freedom from infection over time in each LGA at various design prevalence values (2%, 1%, 0.1%, and 1 case) at 95% test sensitivity, 98% test specificity, and 5% type I and type II error. Note different y-axis scale for Kanke, Kanam, and Mikang (0.00-1.00) versus other LGAs (0.85-1.00).



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

Summary

This study conducted a novel application of freedom from infection (FFI) analysis, a framework originally developed in veterinary epidemiology to estimate the probability that disease prevalence is below a pre-determined design prevalence (DP) threshold, to assess whether areas of central Nigeria were free from LF infection utilizing repeated cross-sectional LF survey data. Results indicate a high probability (>0.90) at all examined DP thresholds (2%, 1%, 0.1% and 1 case) that areas with lower baseline LF transmission (≤20% antigenemia) were free from LF infection as early as 2007, five to six years after the start of MDA, while high FFI probability (>0.99) was achieved between 2012 and 2015, around 11-13 years after the start of MDA, for three areas of higher baseline transmission. In summary, this initial application of FFI analysis to LF shows good concordance with cross-sectional survey data conclusions, thereby offering a tool, that with refinement, could serve as a viable analytic framework to verify elimination of LF transmission.

Public Health Implications

LF is one of eleven neglected tropical diseases (NTDs) currently targeted for elimination at the global (eradication), regional, or national levels (37). WHO guidelines currently exist to validate LF elimination as a public health problem in a country through a series of stop-MDA (TAS-1) and post-treatment surveillance (TAS-2, TAS-3) surveys with a critical threshold for passing TAS set at 2% (1% in areas of *Aedes* transmission) (32). WHO also recently indicated that countries may additionally request verification of

elimination of LF transmission, but acknowledged that "specific requirements for such verification have not yet been agreed" (38).

This study sought to assess the potential utility of FFI analysis to fill this methodological gap. FFI framework is based on the recognition that surveys designed to substantiate freedom from disease area fundamentally different from surveys designed to estimate a non-zero disease prevalence with associated confidence interval (44). FFI employs probability theory to estimate the probability that disease prevalence is below a pre-determined threshold, which can then be extended to estimate the probability of freedom from infection in a population—equivalent to a negative predictive value (45). Results from this study showed that FFI could be a viable approach to verify claims of LF elimination (*sensu stricto*). In principle, it could therefore have relevance for all elimination-targeted NTDs.

If implemented programmatically, FFI would likely rely on the existing sequence of TAS surveys from formerly endemic areas as the primary data source of human infectivity data. To gain programmatic acceptance, FFI would need to show benefit beyond competing 'elimination' survey designs such as the outdated WHO survey of 3,000 five year old children per survey domain (33). An alternative approach would be to extend the 'sentinel site' monitoring scheme currently recommended during MDA phase (32). In such a scenario, more frequent, but smaller sized surveys would be conducted and analyzed by FFI framework. Further work is needed then to compare the statistical, logistical, and financial value of these various elimination survey approaches. This is an area of active research that offers opportunity to directly inform policy gaps in the LF and broader NTD community.

Possible Future Directions

Several additional aspects should be considered in order to improve the viability of FFI analytic framework for programmatic use. First, to capture the repeated measures nature of the cross-sectional surveys contained in this dataset, the full FFI scenario tree modeling framework should be applied. This framework is available in the RSurveillance package for R statistical software (45). However, work will be required to adapt the scenario tree modeling framework to accommodate repeated cross-sectional survey data, since most FFI analyses currently focus on passive surveillance data analysis. Given that GPELF programmatic guidelines rely on a series of TAS-1, TAS-2, and TAS-3 surveys, this component will be the most important to address.

Secondly, simulations are needed to determine a minimum acceptable sample size for cross sectional surveys that will permit valid FFI determinations across a range of DPs, taking into account cluster survey design, and other LF-specific input parameters. Simulations should also consider the utility of smaller, but more frequent 'sentinel site'-type sampling strategies.

Thirdly, mosquito infectivity data could be incorporated into FFI analysis. Xenomonitoring has been useful in corroborating trends in human infection prevalence (55, 56), and recently has been used as part of post-validation surveillance in Togo (57). Xenomonitoring data would provide direct evidence of LF transmission potential without the complications associated with antigen testing and antigen persistence.

An age-based component could also be incorporated into FFI scenario tree models, as has already been done for poliovirus FFI analysis to account for differential risk and surveillance targeting likelihoods (46). For LF, age-based stratification seems relevant given the specific targeting of primary school children (aged 6—7 years old) in TAS surveys versus the all age-group sampling of PacELF C-surveys and other community-based testing such as sentinel site surveys. The TAS survey approach is likely to continue as the WHO recommended methodology for stop-MDA and post-treatment surveillance surveys. Results from the current dataset indicate similar results and programmatic interpretations (<2% at 95% confidence level) between TAS and PacELF C-surveys of individuals older than 2 years of age when conducted in the same area at the same time point. However, a recent study from American Samoa found that TAS was inferior to community-based all age-group surveys in detecting areas of ongoing LF transmission (58), meaning that all age-group sampling surveys like PacELF C-survey may be favored for comprehensive post-treatment surveillance. Interpretation of population-wide antigen prevalence is more difficult to interpret, though, due to antigen persistence. Estimates of antigen persistence (53) could be used to account for age-based exposure and antigen clearance allowing flexibility in FFI to account for TAS or PacELF C-survey-type survey designs, or a combination of designs as represented by this dataset.

References

1. 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.

2. World Health Organization. Lymphatic filariasis. *Wkly Epidemiol Rec* 2001;76(20):149-54.

3. Markell EK, John DT, Krotoski WA. *Markell and Voge's Medical Parasitology*. 8th ed. Philadelphia: W.B. Saunders; 1999.

4. Eldridge BF. Patrick Manson and the discovery age of vector biology. *J Am Mosq Control Assoc* 1992;8(3):215-20.

5. Ottesen EA. Lymphatic filariasis: Treatment, control and elimination. *Adv Parasitol* 2006;61:395-441.

6. Fenwick A. The global burden of neglected tropical diseases. *Public Health* 2012;126(3):233-6.

7. Nutman TB. Insights into the pathogenesis of disease in human lymphatic filariasis. *Lymphat Res Biol* 2013;11(3):144-8.

8. Bal MS, Beuria MK, Mandal NN, et al. Antigenemia in young children living in Wuchereria bancrofti-endemic areas of Orissa, India. *Trans R Soc Trop Med Hyg* 2009;103(3):262-5.

9. Dreyer G, Noroes J, Figueredo-Silva J, et al. Pathogenesis of lymphatic disease in bancroftian filariasis: a clinical perspective. *Parasitol Today* 2000;16(12):544-8.

10. World Health Organization. Lymphatic filariasis: managing morbidity and preventing disability. Geneva: WHO, 2013.

11. Dreyer G. *Basic Lymphedea Management: Treatment and Prevention of Problems Associated with Lymphatic Filariasis*. Hollis, New Hampshire: Hillis Publishing; 2002.

12. Kar SK, Dwibedi B, Das BK, et al. Lymphatic pathology in asymptomatic and symptomatic children with Wuchereria bancrofti infection in children from Odisha, India and its reversal with DEC and albendazole treatment. *PLoS Negl Trop Dis* 2017;11(10):e0005631.

13. Mand S, Debrah AY, Klarmann U, et al. Doxycycline improves filarial lymphedema independent of active filarial infection: a randomized controlled trial. *Clin Infect Dis* 2012;55(5):621-30.

14. Person B, Bartholomew LK, Addiss D, et al. Disrupted social connectedness among Dominican women with chronic filarial lymphedema. *Patient Educ Couns* 2007;68(3):279-86.

15. Krishna Kumari A, Harichandrakumar KT, Das LK, et al. Physical and psychosocial burden due to lymphatic filariasis as perceived by patients and medical experts. *Trop Med Int Health* 2005;10(6):567-73.

16. Obindo J, Abdulmalik J, Nwefoh E, 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.

17. Beaver PC. Filariasis without microfilaremia. *Am J Trop Med Hyg* 1970;19(2):181-9.

18. Weil GJ, Ramzy RM, Chandrashekar R, et al. Parasite antigenemia without microfilaremia in bancroftian filariasis. *Am J Trop Med Hyg* 1996;55(3):333-7.

19. McGreevy PB, Ratiwayanto S, Tuti S, et al. Brugia malayi: relationship between anti-sheath antibodies and amicrofilaremia in natives living in an endemic area of South Kalimantan, Borneo. *Am J Trop Med Hyg* 1980;29(4):553-62.

20. Weil GJ, Jain DC, Santhanam S, et al. A monoclonal antibody-based enzyme immunoassay for detecting parasite antigenemia in bancroftian filariasis. *J Infect Dis* 1987;156(2):350-5.

21. Weil GJ, Liftis F. Identification and partial characterization of a parasite antigen in sera from humans infected with Wuchereria bancrofti. *J Immunol* 1987;138(9):3035-41.

22. Weil GJ, Lammie PJ, Weiss N. The ICT Filariasis Test: A rapid-format antigen test for diagnosis of bancroftian filariasis. *Parasitol Today* 1997;13(10):401-4.

23. Weil GJ, Curtis KC, Fakoli L, 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.

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

25. Bakajika DK, Nigo MM, Lotsima JP, et al. Filarial Antigenemia and Loa loa Night Blood Microfilaremia in an Area Without Bancroftian Filariasis in the Democratic Republic of Congo. *The American Journal of Tropical Medicine and Hygiene* 2014.

26. Jamail M, Andrew K, Junaidi D, et al. Field validation of sensitivity and specificity of rapid test for detection of Brugia malayi infection. *Trop Med Int Health* 2005;10(1):99-104.

27. Steel C, Golden A, Kubofcik J, 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.

28. Hamlin KL, Moss DM, Priest JW, et al. Longitudinal monitoring of the development of antifilarial antibodies and acquisition of Wuchereria bancrofti in a highly endemic area of Haiti. *PLoS Negl Trop Dis* 2012;6(12):e1941.

29. Centers for Disease Control and Prevention. Recommendations of the International Task Force for Disease Eradication. *Morbidity and Mortality Weekly Report* 1993;42(RR-16):1-38.

30. World Health Assembly. Resolution 50.29. Elimination of lymphatic filariasis as a public health problem. 1997,

31. World Health Organization. Global Programme to Eliminate Lymphatic Filariasis. 2018. (http://www.who.int/lymphatic_filariasis/elimination-programme/en/). (Accessed 7/14/2018).

32. 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: WHO, 2011.

33. World Health Organization. Monitoring and epidemiological assessment of the programme to eliminate lymphatic filariasis at implementation unit level. Geneva: WHO, 2005.

34. Deming M, Lee H. Background and technical notes for Filarial antigenemia surveys to decide if mass drug administrations to eliminate lymphatic filariasis can be stopped: a manual for survey planners. unpublished, 2009.

35. Pacific Programme to Eliminate Lymphatic Filariasis (PacELF). PacMAN (PacELF Monitoring and Analysis Network) Book: Country-Specific Monitoring and Evaluation, and Mosquito Control Plans. Suva, 2004.

36. Addiss DG. Global elimination of lymphatic filariasis: addressing the public health problem. *PLoS Negl Trop Dis* 2010;4(6):e741.

37. World Health Organization. Generic Framework for Control, Elimination and Eradication of Neglected Tropical Diseases. Geneva: World Health Organization,, 2016.

38. World Health Organization. Validation of elimination of lymphatic filariasis as a public health problem. Geneva: World Health Organization,, 2017.

39. Michael E, Malecela-Lazaro MN, Maegga BT, et al. Mathematical models and lymphatic filariasis control: monitoring and evaluating interventions. *Trends Parasitol* 2006;22(11):529-35.

40. Stolk WA, Swaminathan S, van Oortmarssen GJ, et al. Prospects for elimination of bancroftian filariasis by mass drug treatment in Pondicherry, India: a simulation study. *J Infect Dis* 2003;188(9):1371-81.

41. Chu BK, Deming M, Biritwum NK, 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.

42. World Health Organization. Guidelines for stopping mass drug administration and verifying elimination of human onchocerciasis: criteria and procedures. Geneva, 2016.

43. World Health Organization, Pacific ROftW. *The PacELF way : towards the elimination of lymphatic filariasis from the Pacific, 1999-2005*. Manila : WHO Regional Office for the Western Pacific; 2006.

44. Cameron AR, Baldock FC. A new probability formula for surveys to substantiate freedom from disease. *Prev Vet Med* 1998;34(1):1-17.

45. Stresman G, Cameron A, Drakeley C. Freedom from Infection: Confirming Interruption of Malaria Transmission. *Trends Parasitol* 2017;33(5):345-52.

46. Watkins RE, Martin PA, Kelly H, et al. An evaluation of the sensitivity of acute flaccid paralysis surveillance for poliovirus infection in Australia. *BMC Infect Dis* 2009;9:162.

47. Hopkins DR, Eigege A, Miri ES, et al. Lymphatic filariasis elimination and schistosomiasis control in combination with onchocerciasis control in Nigeria. *Am J Trop Med Hyg* 2002;67(3):266-72.

48. Richards FO, Eigege A, Miri ES, 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.

49. King JD, Eigege A, Umaru 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, 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. Sergeant E. Epitools epidemiological calculators. Ausvet Pty Ltd; 2018. (http://epitools.ausvet.com.au/content.php?page=FreeCalc1). (Accessed 5/30/2018).

52. Njenga SM, Wamae CN. Evaluation of ICT filariasis card test using whole capillary blood: comparison with Knott's concentration and counting chamber methods. *J Parasitol* 2001;87(5):1140-3.

53. Singh BK, Michael E. Bayesian calibration of simulation models for supporting management of the elimination of the macroparasitic disease, Lymphatic Filariasis. *Parasites & vectors* 2015;8:522.

54. Cruz F, Fores P, Ireland J, et al. Freedom from equine infectious anaemia virus infection in Spanish Purebred horses. *Vet Rec Open* 2015;2(1):e000074.

55. Eigege A, Kal A, Miri E, et al. Long-lasting insecticidal nets are synergistic with mass drug administration for interruption of lymphatic filariasis transmission in Nigeria. *PLoS Negl Trop Dis* 2013;7(10):e2508.

56. Pilotte N, Unnasch TR, Williams SA. The Current Status of Molecular Xenomonitoring for Lymphatic Filariasis and Onchocerciasis. *Trends Parasitol* 2017;33(10):788-98.

57. Dorkenoo MA, de Souza DK, Apetogbo Y, et al. Molecular xenomonitoring for post-validation surveillance of lymphatic filariasis in Togo: no evidence for active transmission. *Parasites & vectors* 2018;11(1):52.

58. Sheel M, Sheridan S, Gass K, 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.