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Evaluating lymphatic filariasis antigen and antibody results from TAS-2 surveys in American Samoa, Philippines, and Tanzania

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Abstract

Evaluating lymphatic filariasis antigen and antibody results from TAS-2 surveys in American Samoa, Philippines, and Tanzania

By Rachel Hartman

Lymphatic filariasis (LF) is a neglected tropical disease affecting roughly 50 million people worldwide. LF, which is most common in Africa and southeast Asia, is a vector-borne, filarial disease spread through bites from infected mosquitos. LF can cause serious, disfiguring symptoms such as lymphedema and elephantiasis.

The World Health Organization has committed to eliminating LF and has indicated that interrupting disease transmission is possible with Mass Drug Administration (MDA). MDA has been successfully implemented in many countries, and several have achieved elimination. The worldwide prevalence of LF has significantly declined over the years due to the success of these programs. However, some countries are experiencing continued transmission and even disease recrudescence despite years of MDA.

Transmission Assessment Surveys (TAS) are used to determine if there is evidence that transmission has been interrupted and MDA can be stopped. Current diagnostic tools for LF are not perfect because the filarial worms have a complex life cycle that can result in delayed or inaccurate diagnosis. For TAS, filarial test strips (FTS) detect antigen in a finger prick blood sample and are used for rapid diagnoses. These tests are convenient and effective, but may be unable to discriminate between active and historic infection in adults, and do not signify active transmission.

Antibody data has recently become more popular for diagnosing NTDs because it can detect new infections sooner than traditional antigen testing. This thesis examined whether LF antibody data corroborated FTS antigen data, or if cases were possibly being missed by antigen tests alone. Results from TAS-2 data from American Samoa, Philippines, and Tanzania suggested that FTS may be missing new infections. Additionally, using all ages combined masked individual age group effects, so keeping age groups separate may be beneficial. Antibody prevalence is not a good predictor of FTS prevalence at the individual level and the two are not significantly correlated at the cluster level. The datasets were not perfect and the antibody positive/negative cutoffs could be called into question. More analyses should be conducted on other TAS datasets to corroborate and expand on these results.

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Table of Contents

Chapter 1. Introduction and statement of and context for the problem and purpose

I. Introduction and Rationale

Lymphatic Filariasis (LF) is a neglected tropical disease (NTD) transmitted by mosquitos that can cause a myriad of symptoms including swelling in the legs and genitals and elephantiasis (CDC, 2021). Roughly 51 million people are currently infected by LF, and the disease is most common in southeast Asia and Africa (Cromwell et al., 2020; Kamgno & Djeunga, 2020; Lourens & Ferrell, 2019). The World Health Organization (WHO) has enacted mass drug administration (MDA) programs to combat LF, which have been successful, but continued disease transmission is still an issue (Lau et al., 2020; World Health Organization, 2011). Transmission Assessment Surveys (TAS) are conducted after a country completes an MDA program. A cluster within a country, typically a village, is the primary sampling unit within a country, and an evaluation unit for a country is typically larger than a cluster, usually a district. (World Health Organization, 2011)

The most common method for diagnosing LF is the filariasis test strips (FTS), which is an antigen tests that can provide rapid results in the field. A more specific test is the microfilaria blood test, in which blood is tested for juvenile LF worms (microfilariae), although this test must be administered at night in most settings, making it impractical for larger field surveillance (Lammie et al., 2004; Ottesen, 2006; Weil et al., 2013). Antibody data has increasingly been used as a more sensitive diagnostic tool for other diseases including malaria, Giardia, and schistosomiasis, and it can detect asymptomatic cases (Arnold et al., 2019; Cai et al., 2019; Helb et al., 2015).

II. Problem Statement

Current surveillance methods of LF monitoring in countries that have completed MDA are not always sensitive enough to detect low levels or hotspots of ongoing transmission that could signify disease recrudescence.

III. Purpose Statement

This thesis will address these concerns by investigation the relationship between commonly-used LF antigen diagnostic test results and antibody results over different spatial scales. The current age ranges used to determine LF recrudescence will also be evaluated by comparing the same diagnostic tools to determine if expanding or changing the age range would have value for future surveillance programs.

IV. Research Questions Addressed

The thesis will consist of three main questions:

1. Is cluster-level antigen prevalence correlated to cluster-level level antibody seroprevalence?
2. Do cluster-level antigen and antibody prevalences change over spatial scales? Are the *W. bancrofti* antibody prevalence results consistent when compared to *Brugia Sp.* antibody prevalence results?
3. Can indicators such as individual seropositivity, years lived in a village, or frequent travel be used to predict whether an individual will have a positive antigen test?

V. Significance

Antigen testing is a powerful diagnostic tool for many diseases, including LF, but the complex life cycle of LF can result in cases being missed by antigen testing alone.

Including antigen and antibody results in further LF surveillance could improve diagnostic power and identify areas of ongoing transmission before the case numbers become high enough to be considered disease recrudescence. MDA could be easily and rapidly administered in these hotspots.

The long-implemented age range for LF surveillance has been in children (World Health Organization, 2011), however this thesis will also examine the utility of this and the possibility that the age range should be expanded to include older subjects. A model will also be constructed for each age range to assess whether an individual positive antigen result can be predicted by a number of other factors. This model could be significant if it could create an algorithm to identify attributes that could identify at-risk individuals.

VI. Definition of Terms

- LF – Lymphatic filariasis
- MDA – Mass Drug Administration
- TAS – Transmission Assessment Survey. Used to determine whether MDA can be stopped and post-MDA surveillance can begin.
- TAS-2 – Second Transmission Assessment Survey. Used to ensure there has not been ongoing disease transmission or recrudescence in a region that has stopped MDA.
- Cluster – primary sampling unit for a TAS, usually a village.
- Evaluation Unit – administrative area across which a mass treatment decision is made, based on the results of a TAS; typically corresponds to a district.

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Chapter 2. Literature Review

I. Introduction

Lymphatic filariasis (LF) is a neglected tropical disease caused by certain species of parasitic helminths. LF infection can often be asymptomatic, but some individuals will develop symptoms such as lymphedema and elephantiasis, which can cause permanent disfigurement and be subject to stigma within the community (CDC, 2021). LF is the most common cause of permanent disfigurement worldwide, affecting 40 million people (Lourens & Ferrell, 2019). The disease has the highest prevalence in southeast Asia and Africa, where mass drug administration (MDA) programs are still needed despite overall global reductions in prevalence (Cromwell et al., 2020). As of 2018, 51 million people were estimated to be currently infected with LF (Cromwell et al., 2020; Kamgno & Djeunga, 2020), a significant, continued decrease from previous years (Ramaiah & Ottesen, 2014).

MDA programs are currently being employed with the goal of LF elimination worldwide. Figure 1 shows the global distribution of LF and the projected progression of MDA programs (Institute for Health Metrics and Evaluation, 2022), and many countries with endemic LF have been verified to have disrupted transmission. While programs have been successful and the global prevalence of LF has been steadily decreasing, some regions are experiencing continued transmission and recrudescence. Current diagnostic tools, particularly Filariasis Test Strip (FTS), can miss new infections due to the complex mechanisms of disease progression and detectability. The goals of this thesis are to

1. Investigate disparities between antibody and FTS data.

2. Determine whether antibody data can be used in tandem with FTS to identify hotspots of ongoing disease transmission.

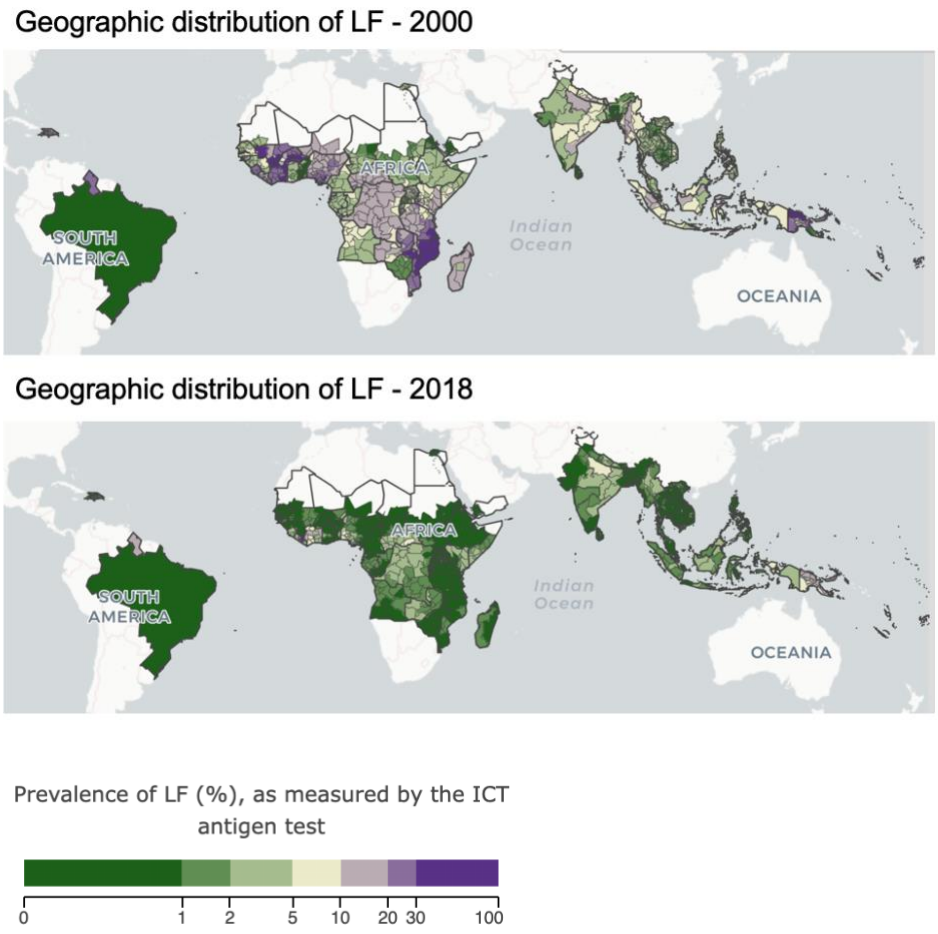

Figure 1 – Global distribution of LF and progress towards elimination by MDA programs from 2000 to 2018 (Institute for Health Metrics and Evaluation, 2022).

II. Transmission

The three parasitic helminths that cause LF are *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*; *W. bancrofti* causes 90% of LF infections. The vector of transmission of helminths to humans is the mosquito, usually *Aedes, Anopheles*, *Culex*, or *Mansonia* (Ottesen, 2006). Figure 2 shows the life cycle of *W. bancrofti* (CDC, 2018), which is similar to the life cycles of *B. malayi* and *B. timori*.

First, a mosquito infected with helminth larvae takes a blood meal. The larvae are introduced into the human bloodstream, where the worms grow to the adult stage. The adult worms will then grow and reproduce, producing microfilariae that circulate in the blood during specific hours at night. In some regions, such as the Philippines, there is also daytime periodicity in microfilariae circulation. It is only possible to test for microfilariae by taking a blood sample during these specific day/night hours, since they are undetectable during other hours. This has resulted in significant difficulty in accurately diagnosing the disease. In the last stage of the life cycle, an uninfected mosquito takes a blood meal from the infected human and acquires the microfilariae, which can then be transmitted to another human (CDC, 2018, 2021).

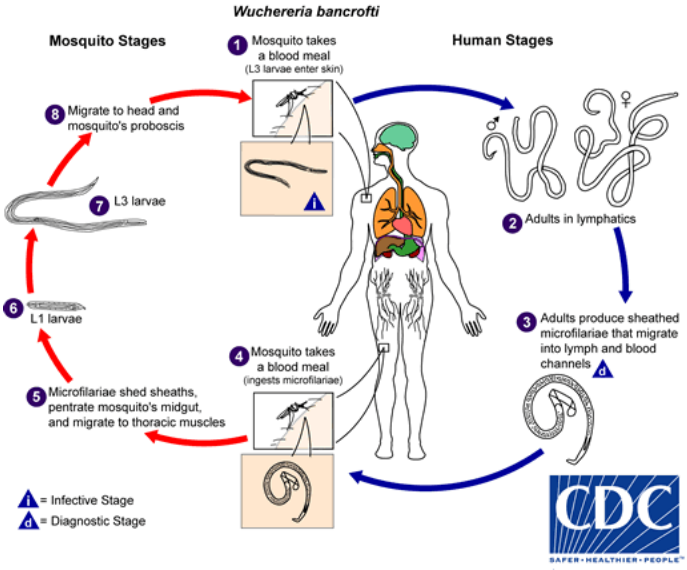

Figure 2 – Life cycle of *W. bancrofti*, which is similar for other LF-causing species (CDC, 2018).

III. Symptoms, Treatment, and Prognosis

Many cases of LF do not present any symptoms at all, or begin to show symptoms after a delay of many years. The most striking symptoms of LF are hydrocele, elephantiasis, and lymphedema, which can be disfiguring and difficult to treat (Gyapong et al., 2005).

In many regions, infection with LF results in stigma from the community. Oftentimes individuals do not know how the disease is spread or what causes it, so affected individuals are looked down upon or seen as unclean (Abdulmalik et al., 2018). Factors that correlated to higher community stigma against LF patients were advanced disease, young age, female gender, and poverty level (Hofstraat & van Brakel, 2016).

The World Health Organization (WHO) recommends use of diethylcarbamazine (DEC) and albendazole to treat LF, though complications from coinfections with other parasitic helminths, such as onchoceriasis, can necessitate the use of ivermectin in place of DEC (World Health Organization, 2011). Combinations of DEC/albendazole or ivermectin/albendazole are administered one time orally, and have been shown to be safe with minimal side effects (Gyapong et al., 2005). Recent studies have also shown that combination ivermectin/albendazole treatments may be more effective and have longer-lasting effects than DEC treatment alone (World Health Organization, 2017).

Disease Diagnosis

LF is notoriously difficult to diagnose due to several factors. The parasite itself can produce new microfilariae for up to 4-8 years, but once a new person is infected, the parasite can take 6-12 months to develop to this adult stage, at which point it finally becomes detectable via an antigen test (Ottesen, 2006). Diagnosing a heavily infected individual can also be difficult because microfilariae only circulate in the bloodstream for

a few hours each day, often occurring in the middle of the night (Ottesen, 2006). This makes conducting prevalence surveys and making accurate diagnoses difficult for personnel conducting the testing and for the individuals being tested as it requires being woken up in the night for a blood test.

Filariasis test strips (FTS, Alere Inc., Abbott) are antigen tests that are currently used to detect LF antigen in the field and have the advantage that they can be used at any time of the day (Weil et al., 2013). Due to the aforementioned length of parasite development, new infections are not immediately detected by FTS antigen tests, making these tests inadequate for forming a rapid LF surveillance response (Lammie et al., 2004; Ottesen, 2006). Current protocols for the TAS-2 recommend FTS testing for children as an indicator of continued transmission, but this method often misses new infections. A recent study in Samoa showed that FTS prevalence was significantly higher in adults than in children, and that antigen prevalence in children was not correlated with antigen prevalence in adults, so sampling adults may provide a more accurate picture of the overall prevalence (Lau et al., 2020).

Antibody detection provides a more sensitive alternative to antigen detection tests. Antibody quantification by using recombinant filarial antigens have been successfully used as a diagnostic tool with several caveats. A positive antibody test does not always indicate an active infection, since antibodies accumulate after an exposure or may linger following a prior treated infection. There can also be cross-reactivity with other filarial diseases (Lammie et al., 2004). Antibody diagnostics have been widely accepted for other diseases such as malaria and schistosomiasis (Cai et al., 2019; Helb et al., 2015), though issues remain concerning the costs of antibody

diagnostics (Cai et al., 2019). Currently, ELISA immunoassays have been developed for LF antibody detection with acceptable sensitivity and specificity, particularly for *W. bancrofti* (Steel et al., 2013). Luminex multiplex bead assays have also been developed for antibody detection as cheaper and more efficient alternatives to ELISA that enable the detection of multiple antibodies from a single assay (Moss et al., 2011).

Evaluation of diagnostic tests is a crucial component of reducing or eliminating a disease because false positives or negatives could result in a distorted view of disease transmission. Comparing antibody results to the current standard diagnostic tests could provide validation or illuminate missed cases, particularly in children where antibody response has not had time to aggregate over time. For example, Arnold et al. (2019) found that children with *Giardia* infection often presented as asymptomatic and were not shedding the parasite, causing a false negative diagnosis from stool sample analysis.

IV. Current LF Surveillance and MDA Strategies

The goal of MDA is to disrupt transmission of LF and eventually achieve disease elimination. Figure 2 shows the steps for LF elimination, as outline by the World Health Organization in 2011 ((Ichimori et al., 2014; World Health Organization, 2011).

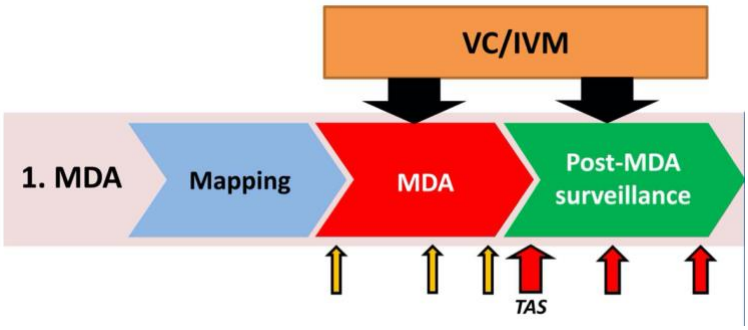

Figure 2 – Schematic plan for LF MDA (Ichimori et al., 2014).

The first step in addressing LF is to conduct mapping surveillance to determine if there is active disease and transmission (World Health Organization, 2011). During this phase, implementation units (IUs) are defined by each individual country and usually consist of districts. The actual mapping surveys consist of antigen and/or microfilaria testing to determine the baseline prevalence in school-aged children or adults, depending on which test the nation decides to implement. If the baseline prevalence of the antigen test is >2% (1% if the mosquito vector is *Aedes*) or the microfilaria test is >1%, the country will require proceeding to the second step, MDA administration (World Health Organization, 2011).

For this disease, one "round" of MDA is defined as one yearly treatment with the appropriate medication (World Health Organization, 2011). LF MDA programs require 4-6 rounds of MDA before completion and several steps are taken throughout the intervention to ensure coverage and efficacy. Coverage is reported in the form of a survey and "spot checks" are conducted to determine if the prevalence of LF is decreasing as expected. At the end of the 6th round of MDA, a transmission assessment survey (TAS) is conducted to determine if the microfilaremia/antigen prevalence is below 1% or 2% respectively. TAS are conducted on evaluation units (EUs) which can correspond to an implementation unit (IU), be a subset of an IU, or be composed of several IUs. Generally, an EU should have <2 million people and have a relatively homogenous risk of LF. Usually, a school-aged children survey is conducted along with a community-wide survey (World Health Organization, 2011).

After an IU prevalence falls below the critical threshold for TAS decision-making, MDA stops and post-MDA surveillance begins. Transmission is determined to be

disrupted at prevalence less than 1%, which is defined by critical cutoff numbers of antigen-positive children (World Health Organization, 2011), however it is possible for recrudescence to occur. The critical cutoff points are determined using the upper end of the 95% confidence interval for 2% antigen prevalence (or 1% in *Aedes* regions). At least 2 years post-MDA, a second TAS survey (TAS-2) should be conducted. If the prevalence is above 2%, MDA will resume, otherwise a third TAS will occur 2-3 years after the TAS-2. If, after this survey, the prevalence is still less than 1%, the country can report that LF transmission has been disrupted (World Health Organization, 2011).

V. Overview of Thesis Topic and Objectives

Disrupting disease transmission is a vital step towards elimination of LF. Identifying regions with continued transmission or those at high risk of transmission will allow for fast and effective administration of MDA. Previous research indicates that LF transmission is highly clustered into hotspots (Boyd et al., 2010). The purpose of this study is to investigate the relationship between the current LF antigen detection methods and the corresponding antibody response at different spatial scales, accounting for risk factors such as age and travel history. Additionally, it will be determined whether antibody data can be used to identify high-risk areas for ongoing transmission or recrudescence.

First, correlations will be conducted to determine the relationship between quantitative antibody values and seroprevalence among children, adults, and the whole population. Theoretically, these should be close to a 1:1 relationship, or at least linear since the seroprevalence should come directly from the quantitative antibody values.

Next, correlations will be conducted between FTS prevalence (i.e. current diagnostic tool) and seroprevalence for the three age groups. If FTS alone is a good indicator of disease transmission within a region, FTS prevalence and seroprevalence should be similar among children. Seroprevalence will likely be higher than FTS prevalence in adults due to the accumulation of antibody response over time.

Next, mapping will be conducted in QGIS to visualize the comparisons between FTS prevalence and seroprevalence. Hotspots can be identified and compared easily within each country and maps will be created for each age group. Lastly, a linear regression will be conducted to determine whether seroprevalence can be used to predict FTS prevalence in the three age groups.

This project will include three datasets from the TAS-2 from three separate countries: American Samoa, Tanzania, and Philippines. Tanzania passed this TAS-2, while American Samoa and Philippines failed. The study population sampled included younger children 5-7 years old, which are the standard indicator group for LF transmission, and also adults and older children >7 years old. The datasets contain serological data including individual LF filariasis test strip results and individual antibody results (ELISA or Luminex). Xenomonitoring mosquito PCR data is included at the subdistrict level. Mosquitos were collected in traps, pooled, and PCR conducted on the pool. Demographic information such as age and sex were also provided in the dataset. This project will be carried out under the supervision of members of the NTD Support Center.

VI. Overview of Current LF Status by Country

American Samoa

The data used in this thesis from American Samoa are from a 2017 TAS-2 survey in which the country failed and was recommended to restart MDA. Recent studies show evidence of recrudescence in the country, which may be overlooked by the TAS-2 survey as it currently operates (Lau et al., 2016, 2020). It has also been suggested that using the children age group is not an accurate depiction of the true prevalence in a given evaluation unit (Lau et al., 2020), so examining antibody data for more than one age group could be useful for further TAS efforts.

Philippines

The Philippines in general has responded well to LF MDA programs. Treatment began in 2001, at which point LF was endemic in 46 provinces (delos Trinos et al., 2021). As of 2018, LF was declared to no longer be a public health problem in 31 of the 46 provinces originally identified (*Filariasis Elimination Program*, 2018). In 2020, the number of provinces that ceased MDA increased to 43 out of 46 (Yajima & Ichimori, 2020). The dataset from 2017 collected in the Philippines, which will be analyzed for this thesis, demonstrates that the Philippines did not pass the TAS-2 and there is ongoing transmission of LF. The inability to identify LF hotspots through the TAS-2 and the delay in accurate diagnosis are likely contributing factors to the TAS-2 failure.

Tanzania

Tanzania began a LF elimination program in 2000 as part of a national program to end neglected tropical diseases, with a goal to eliminate LF by 2020 (Parker & Allen,

2013). There have been issues with MDA coverage and compliance of Tanzania citizens, with some refusing treatment (Parker & Allen, 2013). A study from 2019 found that antigen prevalence in one district was 5.2% indicating that the goal of elimination, which requires antigen prevalence <2%, would likely be unattainable by 2020 (Fimbo et al., 2020). Regardless, the dataset from Tanzania used in this analysis is from a TAS-2 that was passed, i.e. MDA did not need to restart. This is the only country in this analysis with data from a passed TAS-2, so comparisons with countries that failed will likely be useful.

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Chapter 3. Methodology

I. Introduction

This thesis will address the issue of ongoing LF transmission in American Samoa, Philippines, and Tanzania by comparing antigen and antibody diagnostic test results at the village (aka cluster) level. The correlation between mean antigen prevalence (FTS) and mean antibody seroprevalence (Luminex Multiplex Assay or Enzyme-Linked Immunosorbent Assay (ELISA), Wb123 marker) results will be assessed at the cluster level to determine if the antigen tests are missing recent exposures, particularly in children who have not had extended antibody responses built up over time. An antibody response in children could also signal that there are more active infections in adults. If the antigen and antibody tests produce similar prevalences, the two measures should be significantly correlated.

Mean cluster-level antigen and Wb123 antibody test results will then be mapped for each country to identify LF hotspots and visually compare prevalence over geographical location. Using a different marker for comparison can call into question the meaning of the antibody results and cutoff points from the Wb123 marker data. Theoretically, both tests (Wb123 seroprevalence and FTS) should exhibit very similar spatial patterns, and the seroprevalence hotspots should mirror the FTS hotspots.

Lastly, a model will be constructed to determine if a positive antigen test can be predicted from factors such as Wb123 seroprevalence, presence of infected mosquitos within the cluster, age, years lived in the village, and frequent travel outside the village. This model will be evaluated for individual-level data and will be stratified by the three age groups, i.e. three models per country.

II. Population and Sample

Datasets from combined school-based (only children) and community-level (all ages) TAS-2 surveys were analyzed from three countries- American Samoa, Philippines, and Tanzania. These surveys were conducted in 2016-2017. The TAS-2 involved obtaining consent, a survey of questions for each individual to answer, and finger prick blood collection for antibody and antigen testing. Each dataset included the latitude and longitude of the village, village name, and a de-identified barcode for each individual. The village represented the primary sampling unit for the survey, which will be referred to as the "cluster" from now on.

Each dataset also included analysis variables such as FTS diagnostic results, ELISA or Luminex Multiplex Assay quantitative antibody results, and seroprevalence results using predetermined positive/negative cutoff antibody values. Serological results were included for the three different LF species markers: *Wuchereria bancrofti* (Wb123)*, Brugia malayi* (Bm14 and Bm33).

The Emory University Institutional Review Board was consulted on this project and was granted a non-human subjects determination.

III. Research Design

This project was a secondary data analysis using TAS-2 data collected in 2016-2017. The TAS-2 was conducted by field research teams in which individuals were asked survey questions and blood samples were collected. All individuals provided informed consent and the data was de-identifed. Clusters were pre-determined by the

LF programs in place by each country. More detail about TAS-2 sampling in general can be found in the WHO document (World Health Organization, 2011). This thesis was written 5-6 years after the data was collected.

IV. Procedures

Data were cleaned in SAS prior to analysis. Briefly, duplicates were eliminated based on de-identified barcode. The village names in the form provided had inconsistent character lengths and formats, therefore the names were stripped and assigned uniform character length to facilitate later analyses. Variables were then checked for feasibility; for example, one individual in the Philippines dataset had a reported age of 405, which could be attributed to data entry error. These types of observations were eliminated from the datasets.

The Wb123 seropositivity cutoffs for American Samoa and Tanzania were calculated using finite mixture modeling by the CDC prior to obtaining the datasets for this thesis. The Bm14 and Bm33 cutoffs for American Samoa were also predetermined by the CDC, while we determined the cutoffs for Tanzania and Philippines by separating the quantitative serology results into deciles. The upper decile was defined as seropositive and the lower deciles as seronegative; i.e. the upper 10% of antibody data was defined as seropositive for the sake of the correlation analyses in this thesis.

V. Instruments

All data were analyzed in SAS version 9.4 (SAS Institute Inc, Cary, NC, USA) and all maps were constructed in QGIS version 3.16.

VI. Data sources

All data were obtained from the Neglected Tropical Disease Support Center (NTDSC) at the Task Force for Global Health located in Decatur, GA.

VII. Data Analysis

Pearson Correlations

To examine the relationship between cluster-level mean fluorescence intensity (MFI) and Wb123 seroprevalence, the means were calculated for each cluster in each dataset, using the most common marker for disease, Wb123. This was done for children 10 years and younger (i.e. most likely to indicate active LF transmission), adults 20 years and older, and for all ages together. Mean cluster-level MFI and Wb123 seroprevalence were then analyzed using non-parametric Pearson correlations, since the data was not normally distributed. Next, the relationship between Wb123 seroprevalence and FTS prevalence was evaluated by again calculating the cluster-level means for children, adults, and all ages. The Pearson correlations were calculated and mean cluster-level Wb123 seroprevalence was plotted against mean FTS prevalence. All correlations were reported as the correlation coefficient and p-value with alpha=0.05 as the benchmark for significance. A strong correlation was defined as a correlation coefficient >0.59, a moderate correlation as 0.4-0.59, and a weak correlation as 0-0.39 (Akoglu, 2018).

Bm14 and Bm33 markers were included in the analysis by determining if Wb123 seroprevalence correlated with Bm14 and Bm33 seroprevalence. This analysis was

used to explore whether requiring more than one positive antibody marker resulted in a more specific signal.

Mapping

Cluster-level mean Wb123 seroprevalence and mean FTS prevalence were mapped using QGIS version 3.16. The OpenStreetMap base layer was used as the background for all maps. Latitude and longitude coordinates were provided for each individual observation in the dataset, so the cluster-level mean coordinates were used to plot each cluster on the map. The SAS datasets for each country were converted to CSV format and imported into QGIS using the default coordinate reference system WGS 84 (EPSG:4326).

The cluster-level data points were converted to graduated color schemes to represent mean Wb123 seroprevalence (red gradient) or mean FTS prevalence (blue gradient). The size of the points was altered to reflect the sample size of each cluster. The prevalence/seroprevalence scales were kept consistent between all maps for all countries. Maps were generated for three age groups: children 10 years and younger, adults 20 years and older, and the whole population sampled.

Regression Analysis

Logistic regression analyses were conducted for each country for children 10 and younger, adults 20 and older, and the entire sampled population. The regressions were completed at the individual level.

Model

The full model to be considered for each country/age group was:

Log [p(FTS)/1-p(FTS)] = β_0 + β_1 (Wb123 sero) + β_2 (mosq_ind) + β_3 (years_lived) + β_4(travel) + β_5 (Wb123 sero*mosq-ind) + β_6 (Wb123 sero*years_lived) + β_7 (Wb123 sero*travel) + ε

Forward stepwise selection was used within the LOGISTIC procedure for each country/age group to select the final model. Interaction terms were selected using -2Log Likelihood "chunk" tests. Overall model fit and significance of individual predictors was analyzed. Notably, mosquito data was not available for the Philippines

Variables

1. FTS: Outcome variable. FTS antigen diagnostic result coded as 0 for negative and 1 for positive.
2. Wb123 Sero: Main predictor variable. Wb123 antibody diagnostic result coded as 0 for negative and 1 for positive.
3. Mosq_ind: Secondary predictor variable. Indicator coded as 1 if >=1 positive mosquito was identified in a cluster, 0 if no positive mosquitos were identified. NOTE: Philippines did not have mosquito data
4. Years_lived: Control variable. Years lived in the current village; continuous variable between 1 and 4 years.
5. Travel: Control variable. Whether individual frequently travels outside the village; coded as 0 for no, 1 for yes.

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Chapter 4. Results

I. Key Findings – American Samoa

Table 1 summarizes the demographic data for American Samoa, which was stratified by FTS diagnostic result and Wb123 antibody result. In general, Wb123 seroprevalence was much higher than FTS prevalence in American Samoa (20% and 3%, respectively). Both FTS and Wb123 seroprevalence increased with age. For the other two LF antibody markers, Bm14 and Bm33, most positive FTS cases were also seropositive. Comparing Wb123 to the Bm14 and Bm33 antibody markers, some Wb123 seropositive cases were not positive for Bm14 or Bm33.

American Samoa (Total N = 3769)

		FTS Positive (%)	*FTS Negative (%)*		*Wb123 Seropositive (%)*	*Wb123 Seronegative (%)*	
		n=129 (3)	n=3640 (97)	Chi-square p-value	n=744 (20)	n=3025 (80)	Chi-square p-value
Age Category (years)	5-6	2 (0.3)	581 (99.7)		35 (6)	548 (94)	
	7-8	8 (1)	620 (99)		67 (11)	561 (89)	
	9-13	4 (1)	414 (99)		39 (9)	375 (91)	
	14-24	10 (2)	635 (98)		154 (24)	491 (76)	
	25-34	12 (4)	300 (96)		74 (24)	238 (76)	
	35-49	36 (7)	468 (93)		148 (29)	356 (71)	
	50+	51 (8)	554 (92)	**<0.0001**	197 (33)	408 (67)	**<0.0001**
Frequent Travel Coded as travel)	Yes	33 (4)	757 (96)		176 (22)	614 (78)	
	No	87 (5)	1753 (95)	0.58	474 (26)	1366 (74)	0.058
Years Lived in Village (Coded as years_lived)	1 – <1 year	13 (5)	253 (95)		66 (25)	200 (75)	
	2 – 1-2 years	11 (5)	202 (95)		42 (20)	171 (80)	
	3 – 3-5 years	4 (1)	298 (99)		48 (16)	254 (84)	
	4 - >5 years	100 (3)	2888 (97)	**0.05**	588 (20)	2400 (80)	0.067
Positive Mosquito (Pooled sample) (Coded as mxpoolpos)	Yes	108 (85)	2738 (88)		597 (85)	2249 (88)	
	No	19 (15)	390 (12)	0.41	105 (15)	304 (12)	**0.031**
Bm14 Seroprevalence	Positive	98 (77)	243 (7)		245 (33)	96 (3)	
	Negative	30 (23)	3398 (93)	**<0.0001**	499 (67)	2929 (97)	**<0.0001**
Bm33 Seroprevalence	Positive	120 (94)	1297 (36)		560 (75)	857 (28)	
	Negative	8 (6)	2344 (64)	**<0.0001**	184 (25)	2168 (72)	**<0.0001**
Wb123 Mean Fluorescence Intensity	*Mean*	*Median*	*Min*	*Max*			
	749	166	1	29993			

Summary of Missing Data - American Samoa	
Variable	**Number missing**
FTS	0
Wb123 Seroprevalence	0
Age	0

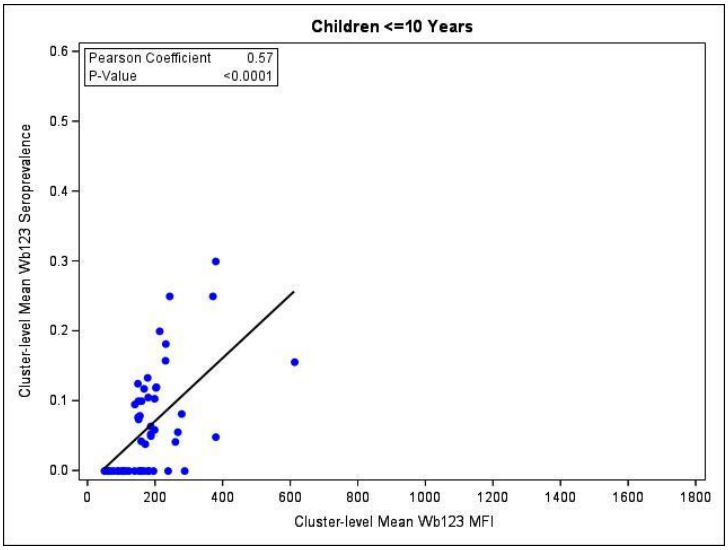
Frequent Travel	1139
Years lived in village	0
Positive Mosquito (pooled sample)	514
Bm14 Seroprevalence	0
Bm33 Seroprevalence	0
Wb123 Mean Fluorescence Intensity	0

Table 1 – Data summary for American Samoa. Demographic predictors are stratified by FTS diagnostic result and Wb123 antibody result. Bolded p-values denote significant (alpha=0.05). A summary of missing data is also included.

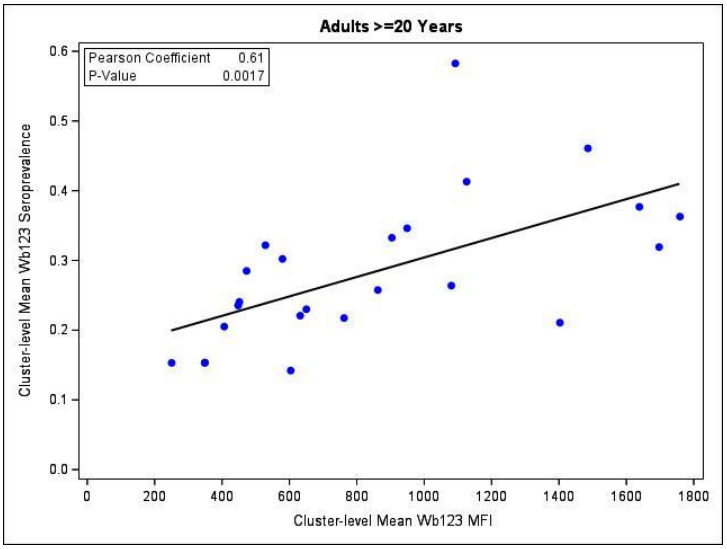
Figure 1 displays non-parametric Pearson correlations between cluster-level mean Wb123 seroprevalence and quantitative MFI for American Samoa. Cluster-level mean Wb123 seroprevalence and Wb123 MFI were significantly correlated for all three age groups. Figure 2 displays non-parametric Pearson correlations between cluster-level Wb123 seroprevalence and FTS prevalence. For children and adults, cluster-level Wb123 seroprevalence and FTS prevalence were not significantly correlated, while the correlation was significant for all ages together. Wb123 seroprevalence tended to be higher while FTS prevalence remained relatively low throughout most clusters.

Figure 1 – Pearson correlation plots for American Samoa. Cluster-level mean Wb123 seroprevalence was correlated with cluster-level mean Wb123 MFI results for the three age groups, A) Children <=10 years, B) Adults >=20 years, and C) All ages. The insets in the top left corner of the plots indicate the Pearson correlation coefficients and p-values.

A)



B)



C)

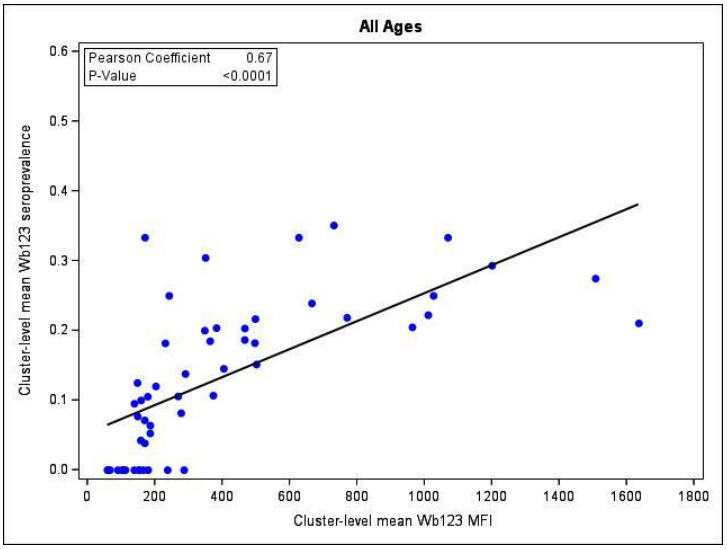
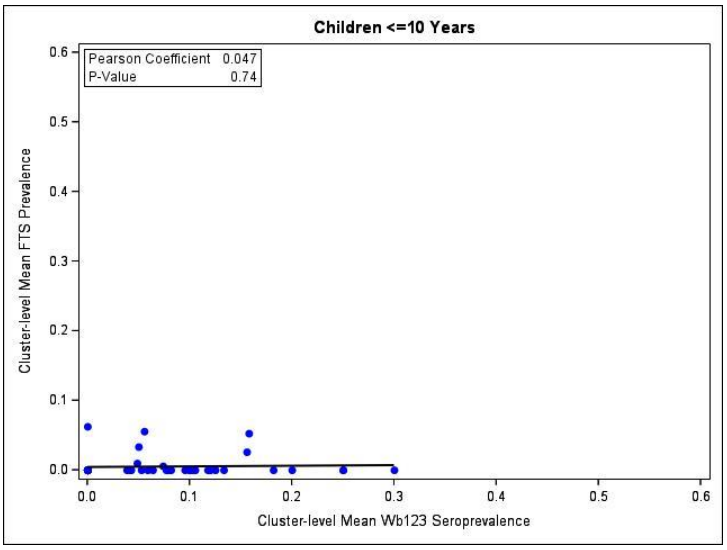
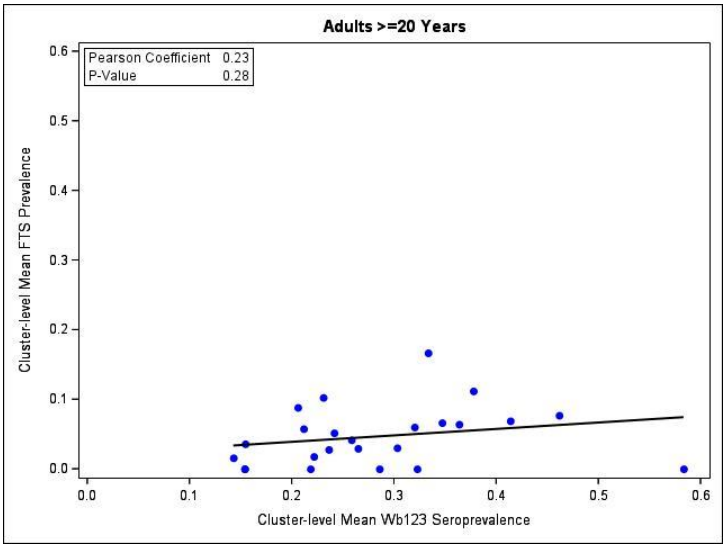


Figure 2 – Pearson correlation plots for American Samoa. Cluster-level mean Wb123 seroprevalence was correlated with cluster-level mean FTS prevalence for the three age groups, A) Children <=10 years, B) Adults >=20 years, and C) All ages. The insets in the top left corner of the plots indicate the Pearson correlation coefficients and p-values.

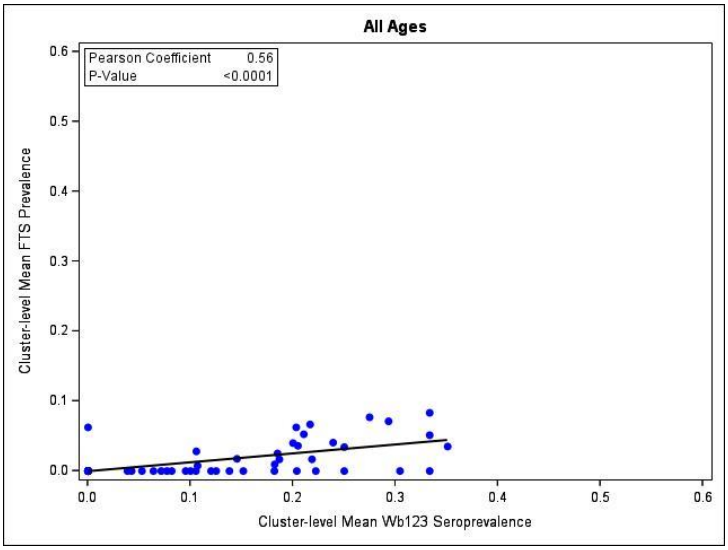
A)


Children <=10 Years
Pearson Coefficient 0.047
P-Value 0.74
Cluster-level Mean FTS Prevalence
Cluster-level Mean Wb123 Seroprevalence
0.0
0.1
0.2
0.3
0.4
0.5
0.6

B)


Adults >=20 Years
Pearson Coefficient 0.23
P-Value 0.28
Cluster-level Mean FTS Prevalence
Cluster-level Mean Wb123 Seroprevalence
0.0
0.1
0.2
0.3
0.4
0.5
0.6

C)



Cluster-level non-parametric Pearson correlation results are displayed in table 2. For each cluster, the sample size, Pearson correlation coefficients, and p-values were given for each comparison at the top of each column. Only clusters that had cases were shown because the comparisons could not be conducted without at least one case for at least FTS and Wb123. In general, cluster-level FTS antigen results were positively correlated with cluster-level Wb123 prevalence alone, cluster-level prevalence of individuals with both Wb123 and Bm14 markers, and cluster-level prevalence of individuals with all three antibody markers together, though some exceptions existed. Additionally, Wb123 alone was generally significantly positively correlated with Bm14 and Bm33, with few exceptions. More comparisons were able to be done for the adults and entire population, compared to children.

A.1 Children <=10 Years
Correlations between FTS and Wb123 marker only (Ab1), Wb123 and Bm14 combined (Ab2), and Wb123, Bm14, and Bm33 combined

Cluster	*N*	*Correlation Coefficient – FTS, Ab1*	*P-value*	*Correlation Coefficient – FTS, Ab2*	*P-value*	*Correlation Coefficient – FTS, Ab3*	*P-value*
Fagali'i	7	0.55	0.2	0.73	0.062	0.73	0.062
Fagatogo	60	0.38	**0.003**	0.7	**<0.0001**	0.7	**<0.0001**

Malaeloa/ Aitulagi	19	0.54	**0.016**	1	**<0.0001**	1	**<0.0001**
Pago Pago	103	0.44	**<0.0001**	1	**<0.0001**	1	**<0.0001**
Satala-Anua-Atuu	18	1	**<0.0001**	1	**<0.0001**	1	**<0.0001**
Tula	12	0.52	0.082	0.52	0.82	0.52	0.082
Vaitogi	77	0.38	**0.0007**	0.7	**<0.0001**	0.7	**<0.0001**

A.2 Children <=10 Years

Correlations between Wb123 and Bm14 and between Wb123 and Bm33.

Cluster	*N*	*Correlation Coefficient - Wb123 with Bm14*	*P-value*	*Correlation Coefficient - Wb123 with Bm33*	*P-value*
Afono	15	-	-	-0.15	0.58
Amouli	17	-	-	-0.063	0.81
Aoa	20	-	-	0.10	0.66
Aoloau	25	-	-	0.12	0.56
Asili	8	-0.22	0.60	-0.45	0.27
Aua	52	-	-	0.19	0.173
Auto	4	-	-	0.58	0.42
Fagaitua-Utusia	8	-	-	-0.14	0.74
Fagalii	7	0.75	0.052	0.73	0.062
Fagasa	19	-	-	0.39	0.095
Fagatogo	60	0.57	**<.0001**	0.13	0.30
Faleniu	47	-	-	-0.0154	0.92
Futiga	24	-	-	-0.0629	0.77
Iliili	87	0.20	0.064	0.28	**0.0093**
Laulii	39	-	-	0.22	0.18
Leloaloa	10	0.51	0.13	0.76	**0.010**
Leone	39	-	-	0.070	0.67
Malaeimi	34	-	-	0.53	**0.0013**
Malaeloa-Aitulagi	19	0.54	**0.016**	0.60	**0.0062**
Mapusagafou	48	-	-	0.11	0.47
Mesepa	19	0.69	**0.0012**	0.14	0.58
Nuuuli	98	0.22	**0.029**	0.22	**0.027**
Pago Pago	103	0.44	**<.0001**	0.31	**0.0013**
Pavaiai	84	-	-	0.20	0.074
Poloa	9	-	-	0.60	0.089
Satala-Anua-Atuu	18	0.69	**0.0017**	0.34	0.16
Seetaga	10	-	-	0.67	**0.035**
Tafuna	176	-0.021	0.78	0.11	0.15
Tula	12	0.68	**0.014**	0.41	0.19
Vailoatai	23	-	-	-0.14	0.52
Vaitogi	77	0.15	0.18	0.40	**0.0003**
Vatia	20	-	-	0.69	**0.0008**

B.1 Adults >=20 Years

Correlations between FTS and Wb123 marker only (Ab1), Wb123 and Bm14 combined (Ab2), and Wb123, Bm14, and Bm33 combined (Ab3)

Cluster	*N*	*Correlation Coefficient – FTS with Ab1*	*P-value*	*Correlation Coefficient – FTS with Ab2*	*P-value*	*Correlation Coefficient – FTS with Ab3*	*P-value*
Afono	52	0.48	**0.0003**	0.55	**<.0001**	0.65	**<.0001**
Amaua	13	0.43	0.14	1.00	**<.0001**	1.00	**<.0001**
Amouli	68	0.29	**0.016**	0.28	**0.019**	0.28	**0.019**
Asili	23	0.44	**0.036**	0.59	**0.0029**	0.59	**0.0029**
Auma	26	0.31	0.12	0.48	**0.014**	0.53	**0.0057**
Fagalii	36	0.27	0.11	0.44	**0.0075**	0.44	**0.0075**
Fagamalo	8	0.58	0.13	0.77	**0.024**	0.77	**0.024**
Fagatogo	137	0.29	**0.0007**	0.51	**<.0001**	0.51	**<.0001**
Fatumafuti	4	0.33	0.67	-	-	-	-
Futiga	72	0.30	**0.0094**	0.29	**0.015**	0.29	**0.015**
Iliili	196	0.28	**<.0001**	0.38	**<.0001**	0.38	**<.0001**
Laulii	63	0.31	**0.013**	0.70	**<.0001**	0.70	**<.0001**
Leloaloa	29	0.63	**0.0003**	0.67	**<.0001**	0.67	**<.0001**
Malaeimi	78	0.35	**0.0017**	0.52	**<.0001**	0.52	**<.0001**
Malaeloa-Aitulagi	58	0.23	0.080	0.65	**<.0001**	0.38	**0.003**
Pago Pago	132	0.17	**0.049**	0.27	**0.002**	0.27	**0.002**
Pavaiai	169	0.25	**0.0009**	0.37	**<.0001**	0.37	**<.0001**
Satala-Anua-Atuu	50	0.37	**0.0085**	0.76	**<.0001**	0.76	**<.0001**
Seetaga	29	0.32	0.087	0.68	**<.0001**	0.68	**<.0001**
Tafuna	120	0.16	0.076	0.31	**0.0005**	0.33	**0.0002**

Tula	34	0.61	**0.0001**	0.80	**<.0001**	0.80	**<.0001**
Utumea West	6	0.63	0.18	0.63	0.18	0.63	0.18
Vaitogi	143	0.41	**<.0001**	0.55	**<.0001**	0.55	**<.0001**
Vatia	39	0.02	0.93	0.09	0.59	0.09	0.59

B.2 Adults >=20 Years

Correlations between Wb123 and Bm14 and between Wb123 and Bm33.

Cluster	N	Correlation Coefficient – Wb123 with Bm14	P-value	Correlation Coefficient – Wb123 with Bm33	P-value
Afono	52	0.23	0.11	0.35	**0.011**
Alao	31	0.10	0.59	0.32	**0.081**
Amaua	13	0.43	0.14	0.53	0.064
Amouli	68	0.30	**0.014**	0.42	**0.0004**
Asili	23	0.37	0.082	0.48	**0.020**
Auma	26	0.55	**0.0034**	0.35	0.080
Aumi	13	0.68	**0.011**	0.28	0.35
Fagalii	36	0.64	**<.0001**	0.49	**0.0022**
Fagamalo	8	0.75	**0.034**	0.65	0.078
Faganeanea	13			0.18	0.56
Fagatogo	137	0.35	**<.0001**	0.27	**0.0012**
Fatumafuti	4			-0.33	0.67
Futiga	72	0.37	**0.0014**	0.38	**0.0012**
Iliili	196	0.33	**<.0001**	0.29	**<.0001**
Laulii	63	0.27	**0.035**	0.39	**0.0014**
Leloaloa	29	0.74	**<.0001**	0.53	**0.0029**
Malaeimi	78	0.51	**<.0001**	0.55	**<.0001**
Malaeloa-Aitulagi	58	0.16	0.22	0.32	**0.014**
Masausi	14			0.4	0.16
Nua	12	0.49	0.11	0.51	0.092
Pago Pago	132	0.31	**0.0003**	0.28	**0.0013**
Pavaiai	169	0.31	**<.0001**	0.33	**<.0001**
Satala-Anua-Atuu	50	0.19	0.18	0.47	**0.0005**
Seetaga	29	0.26	0.17	0.31	0.10
Tafuna	120	0.47	**<.0001**	0.27	**0.0032**
Taputimu	55	0.19	0.16	0.43	**0.001**
Tula	34	0.27	0.13	0.52	**0.0017**
Utumea West	6	0.71	0.12	0.5	0.31
Vaitogi	143	0.55	**<.0001**	0.48	**<.0001**
Vatia	39	0.63	**<.0001**	0.44	**0.005**

C.1 All Ages

Correlations between FTS and Wb123 marker only (Ab1), Wb123 and Bm14 combined (Ab2), and Wb123, Bm14, and Bm33 combined (Ab3)

Cluster	*N*	*Correlation Coefficient – FTS, Ab1*	*P-value*	*Correlation Coefficient – FTS, Ab2*	*P-value*	*Correlation Coefficient – FTS, Ab3*	*P-value*
Afono	83	0.38	**0.0004**	0.56	**<.0001**	0.65	**<.0001**
Amaua	19	0.46	**0.050**	1.00	**<.0001**	1.00	**<.0001**
Amouli	119	0.25	**0.0067**	0.27	**0.0031**	0.27	**0.0031**
Asili	33	0.43	**0.012**	0.62	**0.0001**	0.62	**0.0001**
Auma	39	0.33	**0.041**	0.50	**0.0013**	0.55	**0.0003**
Fagalii	47	0.33	**0.023**	0.47	**0.0008**	0.47	**0.0008**
Fagamalo	15	0.64	**0.0095**	0.85	**<.0001**	0.85	**<.0001**
Fagatogo	249	0.29	**<.0001**	0.53	**<.0001**	0.53	**<.0001**
Fatumafuti	7	0.47	0.29				
Futiga	119	0.20	**0.03**	0.23	**0.011**	0.23	**0.011**
Iliili	369	0.27	**<.0001**	0.41	**<.0001**	0.41	**<.0001**
Laulii	132	0.25	**0.0033**	0.70	**<.0001**	0.70	**<.0001**
Leloaloa	45	0.55	**<.0001**	0.66	**<.0001**	0.66	**<.0001**
Malaeimi	146	0.33	**<.0001**	0.50	**<.0001**	0.50	**<.0001**
Malaeloa-Aitulagi	100	0.28	**0.0047**	0.74	**<.0001**	0.56	**<.0001**
Pago Pago	300	0.20	**0.0004**	0.32	**<.0001**	0.32	**<.0001**
Pavaiai	315	0.21	**0.0002**	0.32	**<.0001**	0.32	**<.0001**
Satala-Anua-Atuu	91	0.38	**0.0002**	0.73	**<.0001**	0.73	**<.0001**
Seetaga	57	0.26	0.051	0.44	**0.0006**	0.44	**0.0006**
Tafuna	344	0.13	0.013	0.31	<.0001	0.32	<.0001
Tula	60	0.51	<.0001	0.64	<.0001	0.64	<.0001
Utumea West	12	0.43	0.17	0.67	0.016	0.67	0.016
Vaitogi	267	0.37	<.0001	0.51	<.0001	0.51	<.0001
Vatia	64	0.03	0.81	0.14	0.28	0.14	0.28

C.2 All ages

Correlations between Wb123 and Bm14 and between Wb123 and Bm33.

Cluster	N	Correlation Coefficient - Wb123 with Bm14	P-value	Correlation Coefficient - Wb123 with Bm33	P-value
Afono	83	0.18	0.11	0.23	**0.041**
Alao	54	0.14	0.30	0.25	0.067
Amaua	19	0.13	0.59	0.49	**0.033**
Amouli	119	0.31	**0.0006**	0.29	**0.0014**
Aoa	20	-	-	0.10	0.66
Aoloau	25	-	-	0.12	0.56
Asili	20	0.30	0.085	0.24	0.18
Aua	52	-	-	0.19	0.17
Auma	39	0.58	**<.0001**	0.55	**0.0003**
Aumi	23	0.32	0.13	0.03	0.90
Auto	4	-	-	0.58	0.42
Fagaitua-Utusia	8	-	-	-0.14	0.74
Fagalii	47	0.63	**<.0001**	0.55	**<.0001**
Fagamalo	15	0.76	**0.0011**	0.66	**0.0072**
Faganeanea	28	-	**-**	0.16	0.42
Fagasa	19	-	**-**	0.39	0.095
Fagatogo	247	0.38	**<.0001**	0.27	**<.0001**
Faleniu	47	-	**-**	-0.02	0.92
Fatumafuti	7	-	**-**	-0.09	0.85
Futiga	119	0.34	**0.0001**	0.31	**0.0007**
Iliili	369	0.32	**<.0001**	0.33	**<.0001**
Laulii	132	0.23	**0.0091**	0.42	**<.0001**
Leloaloa	45	0.69	**<.0001**	0.59	**<.0001**
Leone	39	-	-	0.07	0.67
Malaeimi	146	0.54	**<.0001**	0.55	**<.0001**
Malaeloa-Aitulagi	100	0.19	0.055	0.35	**0.0003**
Mapusagafou	48	-	-	0.11	0.47
Masausi	29	-	-	0.55	**0.0019**
Mesepa	19	0.69	**0.0012**	0.14	0.58
Nua	23	0.40	0.056	0.48	**0.021**
Nuuuli	98	0.22	**0.029**	0.22	**0.027**
Pago Pago	300	0.39	**<.0001**	0.34	**<.0001**
Pavaiai	315	0.30	**<.0001**	0.36	**<.0001**
Poloa	9	-	-	0.60	0.089
Satala-Anua-Atuu	91	0.32	**0.0017**	0.50	**<.0001**
Seetaga	57	0.48	**0.0002**	0.52	**<.0001**
Tafuna	344	0.36	**<.0001**	0.27	**<.0001**
Taputimu	99	0.20	**0.048**	0.44	**<.0001**
Tula	60	0.35	**0.0068**	0.34	**0.0083**
Utumea West	12	0.41	0.19	0.60	**0.040**
Vailoatai	23	-	-	-0.14	0.52
Vaitogi	267	0.50	**<.0001**	0.51	**<.0001**
Vatia	64	0.51	**<.0001**	0.47	**<.0001**

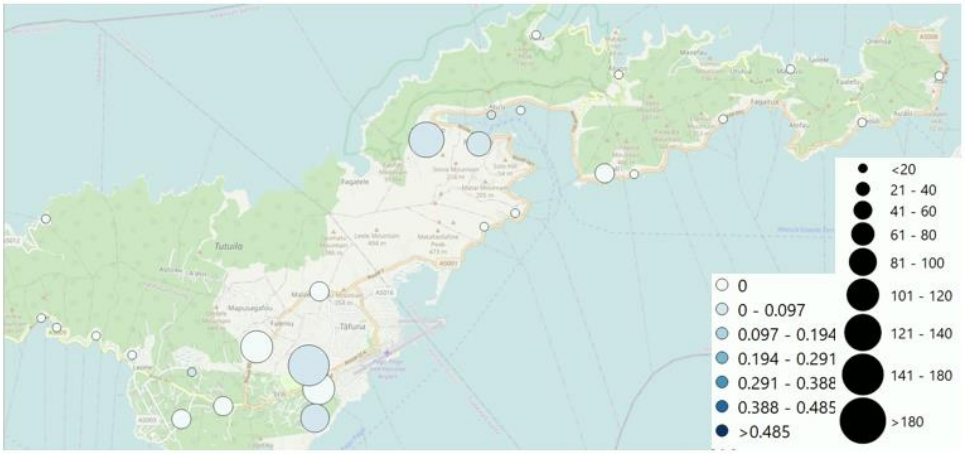
Table 2 – Non-parametric Pearson correlations. Bold p-values indicate significant result.
A.1 – Children <=10 years; comparing cluster-level FTS prevalence with Wb123 marker seroprevalence alone (Ab1), with Wb123 and Bm14 marker combined prevalence (Ab2), and with Wb123, Bm14, and Bm33 marker combined prevalence (Ab3).
A.2 – Children <=10 years; comparing Wb123 marker with Bm14 marker, and Wb123 marker with Bm33 marker.
B.1 - Adults >=20 years; comparing cluster-level FTS prevalence with Wb123 marker seroprevalence alone (Ab1), with Wb123 and Bm14 marker combined prevalence (Ab2), and with Wb123, Bm14, and Bm33 marker combined prevalence (Ab3).
B.2 – Adults >=20 years; comparing Wb123 marker with Bm14 marker, and Wb123 marker with Bm33 marker.
C.1 – All ages; comparing cluster-level FTS prevalence with Wb123 marker seroprevalence alone (Ab1), with Wb123 and Bm14 marker combined prevalence (Ab2), and with Wb123, Bm14, and Bm33 marker combined prevalence (Ab3).
C.2 – All ages; comparing Wb123 marker with Bm14 marker, and Wb123 marker with Bm33 marker.

The mapping results for cluster-level mean FTS prevalence and cluster-level mean Wb123 seroprevalence are displayed in figure 3. For children, the mean seroprevalence was generally much higher than the FTS prevalence, but sample sizes were low. Similar prevalence hotspots existed in both maps near the center of American Samoa. For adults, similar trends were observed. The mean Wb123 seroprevalence was very high compared to FTS prevalence, and both were higher than were observed in children. Almost all clusters had a high Wb123 seroprevalence for adults. For all ages combined, results mirrored the adults' maps. Sample sizes were large and almost all clusters had a very high Wb123 seroprevalence. For both adults and all ages, Wb123 seroprevalence hotspots could not be readily identified since seroprevalence values were consistently high throughout the country. The same can be said for FTS prevalence, to a lesser degree due to generally lower FTS prevalence.

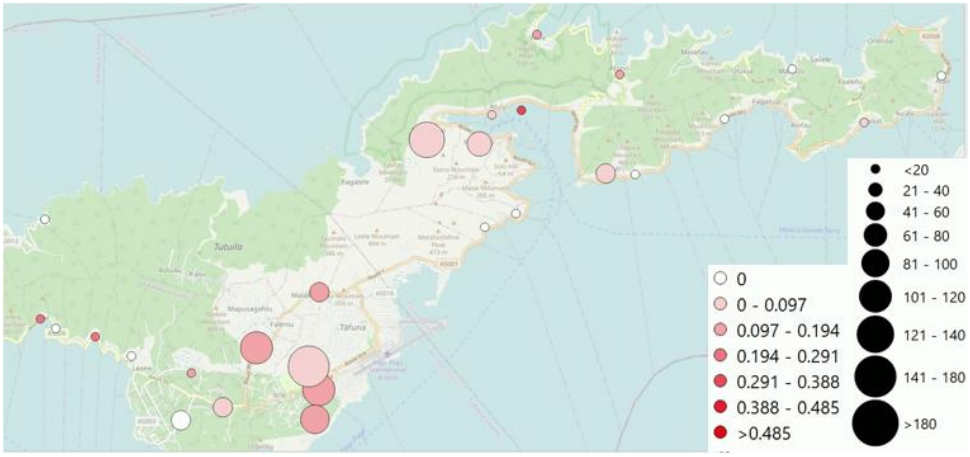
Figure 3 – Mapping results from American Samoa for the three age groups, A) Children <=10 years, B) Adults >=20 years, and C) All ages. Each point on the map represents a cluster and the size of the point corresponds to the cluster-level sample size. Maps on the top, in blue, display cluster-level mean FTS prevalence. Maps on the bottom, in red, display cluster-level mean Wb123 seroprevalence.

A) Children <=10 years

Cluster-level Mean FTS Prevalence

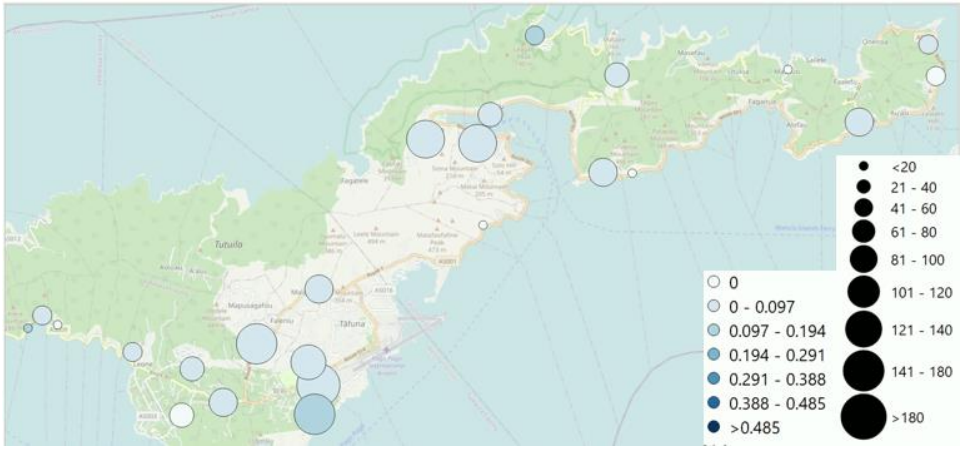


Cluster-level Mean Wb123 Seroprevalence

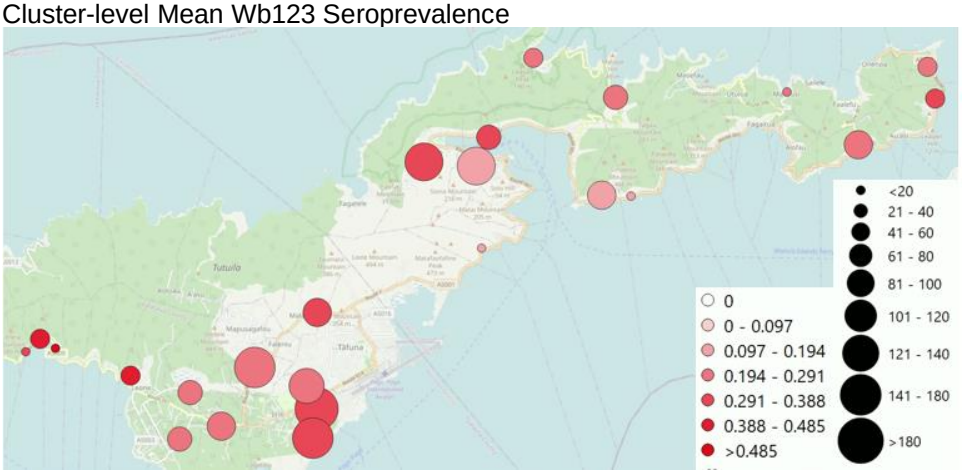


B) Adults >=20 years

Cluster-level Mean FTS Prevalence

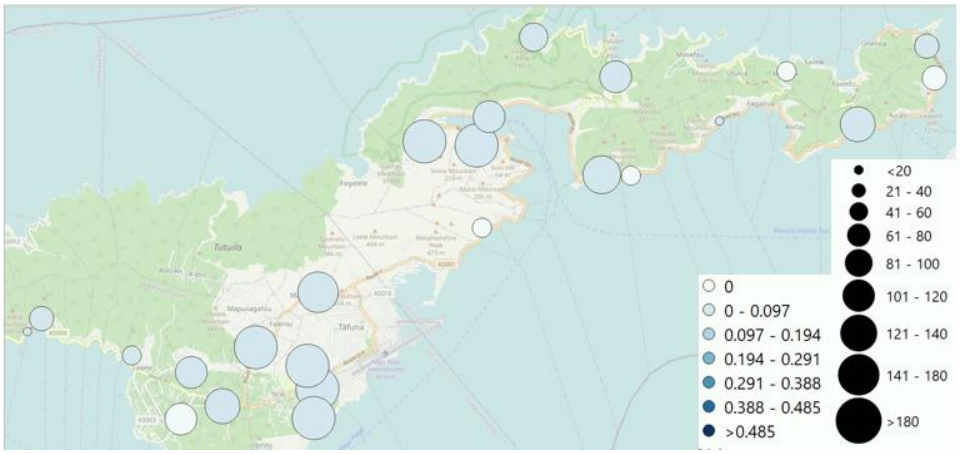


Cluster-level Mean Wb123 Seroprevalence

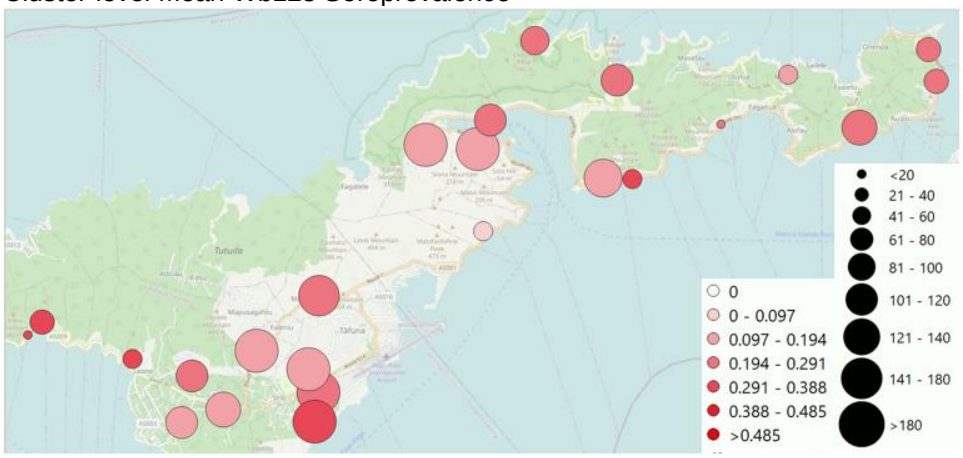


C) All ages

Cluster-level Mean FTS Prevalence



Cluster-level Mean Wb123 Seroprevalence



The results of the logistic regressions for American Samoa are presented in table 3. For children, the model selection indicated quasi-separation of data points when including the indicator for positive mosquito pool, and/or travel, so these variables were excluded. Quasi-separation of data is an error produced by SAS when a variable is almost perfectly split, for example, if all clusters with positive mosquito pools had positive FTS cases, while all clusters with no positive pools had no FTS cases. This results in a “perfect” model, for which parameter estimates can reach infinity. Years lived in the village was not a significant predictor of FTS ($p>0.05$), but was included in the model as a control. The interaction between Wb123 seroprevalence and years lived

in the village was examined, but was determined to be insignificant using -2LogLikelihood chunk test. The global likelihood ratio test showed that Wb123 and years lived in the village were overall reasonable predictors of FTS ($p<0.0001$). The odds of being FTS positive were 32.9 times higher for those that were Wb123 seropositive than seronegative.

For adults, the only variable that did not cause quasi-separation of the data was Wb123 seroprevalence, which was a significant predictor of FTS. The overall model was significant, and the odds ratio for Wb123 was also significant. The odds of being FTS positive were 23.13 times higher for those that were Wb123 positive than Wb123 negative. For the whole sampled population, again Wb123 was the only predictor variable able to be used in the model. The model was significant and the odds of being FTS positive were 27.16 times higher for those that were Wb123 positive than Wb123 negative.

Exact logistic regression can be used to force odds ratio estimates of variables when the number of cases is small, or some cells may have values of 0; i.e. quasi or complete data separation. These were run to attempt to use positive mosquito pool and travel in the models, but the upper limit on the odds ratio confidence interval was always infinity. This made interpreting these results questionable, and the results are not presented here.

A) Children <=10 years

$Ln(p/(1-p) = Intercept + \beta_1(Wb123) + \beta_2(years\_lived) + Error$

Global Likelihood Ratio Test	
Chi-square	32.5
P - value	<0.0001

Parameter	**Level**	**Estimate**	**P-value**	**Odds Ratio Estimate**	**95% CI for Odds Ratio**

Intercept		-4.12	<0.0001		
Wb123		1.75	<0.0001	32.9	8.67, 124.6
Years_lived	1 (ref)				
	2	0.018	0.98	0.67	0.055, 8.27
	3	-0.19	0.82	0.55	0.045, 6.66
	4	-0.24	0.63	0.52	0.102, 2.69

B) Adults >= 20 years

$\text{Ln}(p/(1-p) = \text{Intercept} + \beta_1(\text{Wb123}) + \text{Error}$

Global Likelihood Ratio Test	
Chi-square	178.91
P - value	<0.0001

Parameter	**Estimate**	**P-value**	**Odds Ratio Estimate**	**95% CI for Odds Ratio**
Intercept	-3.032	<0.0001		
Wb123	1.57	<0.0001	23.13	12.56, 42.59

C) All ages

$\text{Ln}(p/(1-p) = \text{Intercept} + \beta_1(\text{Wb123}) + \text{Error}$

Global Likelihood Ratio Test	
Chi-square	267.083
P - value	<0.0001

Parameter	**Estimate**	**P-value**	**Odds Ratio Estimate**	**95% CI for Odds Ratio**
Intercept	-3.41	<0.0001		
Wb123	1.65	<0.0001	27.16	16.56, 44.54

Table 3 – Logistic regression model selection and parameter results from children (A), adults (B), and all ages (C).

II. Key Findings – Philippines

Table 4 summarizes the demographics from the Philippines dataset. All xenomonitoring mosquito data is missing as it was not collected during the TAS-2 for this country. All other variables were either entirely present or had very low numbers of missing data. In general, FTS and Wb123 antibody prevalences were lower compared to American Samoa, but were comparably low to Tanzania. Most FTS cases were also seropositive for Bm14 and Bm33, though the majority of Wb123 seropositive cases were not seropositive for Bm14 or Bm33.

B) Philippines (Total N= 4905)

Variable	*FTS Positive (%)*	*FTS Negative (%)*	*Wb123 Seropositive (%)*	*Wb123 Seronegative (%)*

		n=54 (1)	n=4851 (99)	Chi-square p-value	n=490 (10)	n=4415 (90)	Chi-square p-value
Age Category (years)	5-6	10 (0.8)	1257 (99.2)		148 (12)	1119 (88)	
	7-8	9 (0.7)	1284 (99.3)		161 (12)	1132 (88)	
	9-13	1 (0.2)	437 (99.8)		26 (6)	412 (94)	
	14-24	5 (1)	496 (99)		30 (6)	471 (94)	
	25-34	11 (3)	414 (97)		50 (12)	375 (88)	
	35-49	9 (2)	505 (98)		37 (7)	477 (93)	
	50+	9 (2)	458 (98)	**0.003**	38 (8)	429 (92)	**<0.0001**
Frequent Travel	Yes	0 (0)	474 (100)		27 (6)	447 (94)	
(Coded as travel)	No	54 (1)	4361 (99)	**0.016**	463 (10)	3952 (90)	**0.01**
Years Lived in Village	1 - <1 year	0 (0)	45 (100)		4 (9)	41 (91)	
(Coded as	2 – 1-2 years	2 (7)	26 (93)		5 (18)	23 (82)	
years_lived)	3 – 3-5 years	3 (6)	49 (94)		6 (12)	46 (88)	
	4 - >5 years	49 (1)	4732 (99)	**0.0001**	475 (10)	4305 (90)	0.54
Positive Mosquito (Pooled sample) (Coded as mxpoolpos)	Yes	-	-		-	-	
	No	-	-	-	-	-	-
Bm14 Seroprevalence	Positive	49 (91)	442 (9)		181 (37)	310 (7)	
	Negative	5 (9)	4409 (91)	**<0.0001**	309 (63)	4105 (93)	**<0.0001**
Bm33 Seroprevalence	Positive	48 (89)	442 (9)		158 (32)	332 (8)	
	Negative	6 (11	4409 (91)	**<0.0001**	332 (68)	4083 (92)	**<0.0001**
Wb123 Mean Fluorescence Intensity	*Mean*	*Median*	*Min*	*Max*			
	244	89	1	28401			

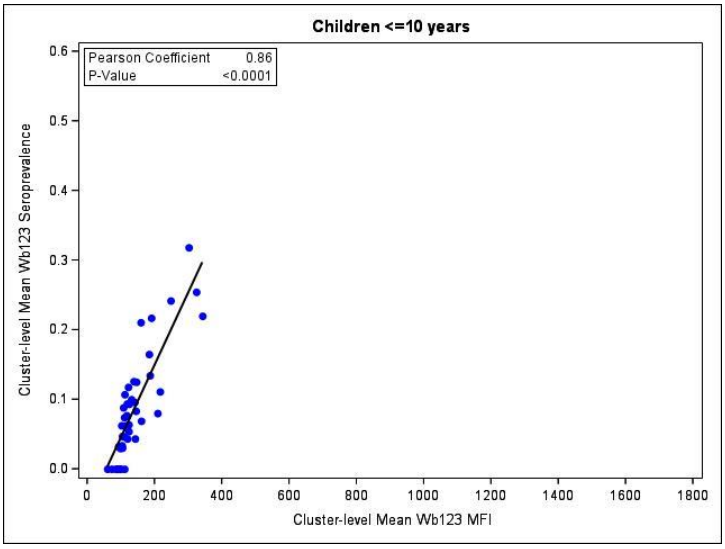
Summary of Missing Data - Philippines	
Variable	**Number missing**
FTS	16
Wb123 Seroprevalence	0
Age	1
Frequent Travel	16
Years lived in village	0
Positive Mosquito (pooled sample)	4905 (all)
Bm14 Seroprevalence	0
Bm33 Seroprevalence	0
Wb123 Mean Fluorescence Intensity	0

Table 4 – Data summary for Philippines. Demographic predictors are stratified by FTS diagnostic result and Wb123 antibody result. Bolded p-values denote significant (alpha=0.05). A summary of missing data is also included.

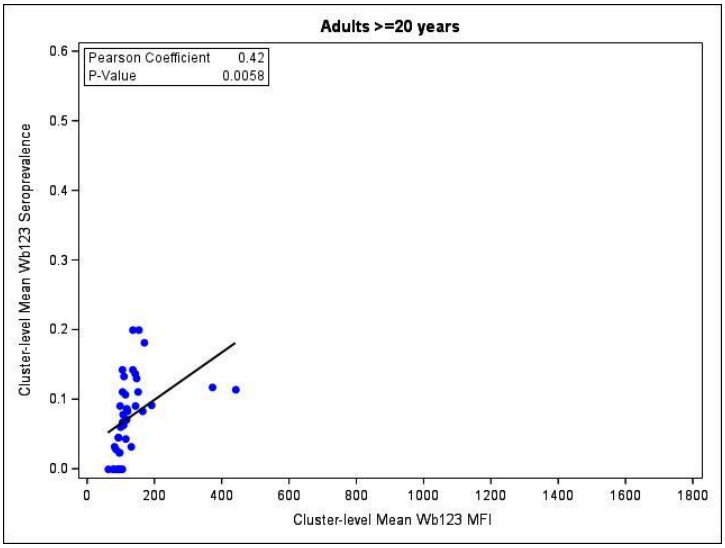
Figure 4 contains the Pearson correlation plots for the Philippines, comparing Wb123 seroprevalence and Wb123 MFI at the cluster level. For all age groups, these two factors were highly correlated, particularly for adults. Figure 5 contains the Pearson correlation plots comparing cluster-level mean Wb123 seroprevalence and FTS prevalence. For all three age groups, Wb123 seroprevalence and FTS prevalence were not significantly correlated. For high values of antibody seroprevalence, FTS prevalences remained very low and were zero for many of the clusters.

Figure 4 –Pearson correlation plots for Philippines. Cluster-level mean Wb123 seroprevalence was correlated with cluster-level mean Wb123 MFI results for the three age groups, A) Children <=10 years, B) Adults >=20 years, and C) All ages. The insets in the top left corner of the plots indicate the Pearson correlation coefficients and p-values.

A)


Children <=10 years
Pearson Coefficient 0.86
P-Value <0.0001
Cluster-level Mean Wb123 Seroprevalence
Cluster-level Mean Wb123 MFI
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
200
400
600
800
1000
1200
1400
1600
1800

B)


Adults >=20 years
Pearson Coefficient 0.42
P-Value 0.0058
Cluster-level Mean Wb123 Seroprevalence
Cluster-level Mean Wb123 MFI
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
200
400
600
800
1000
1200
1400
1600
1800

C)

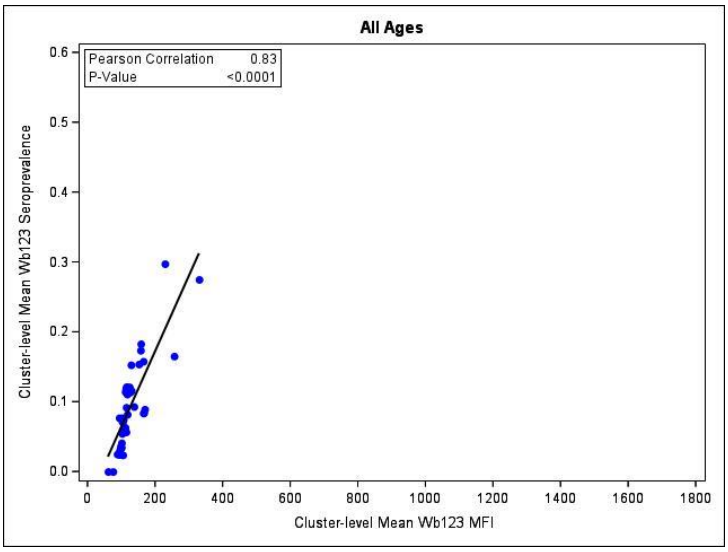
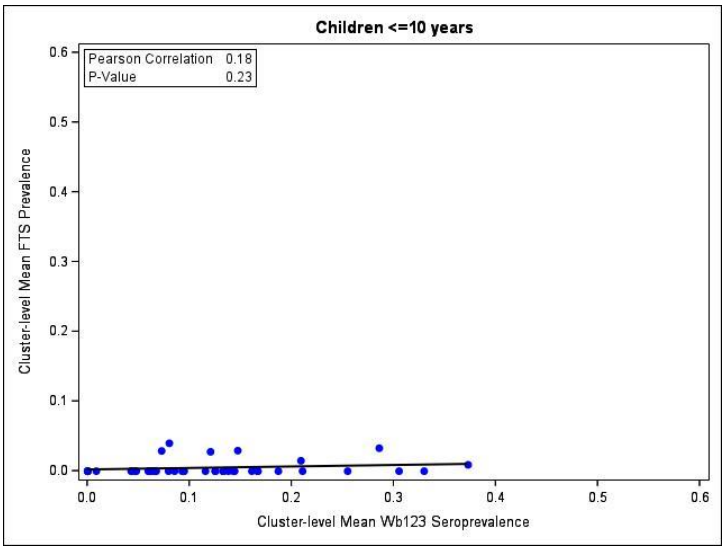
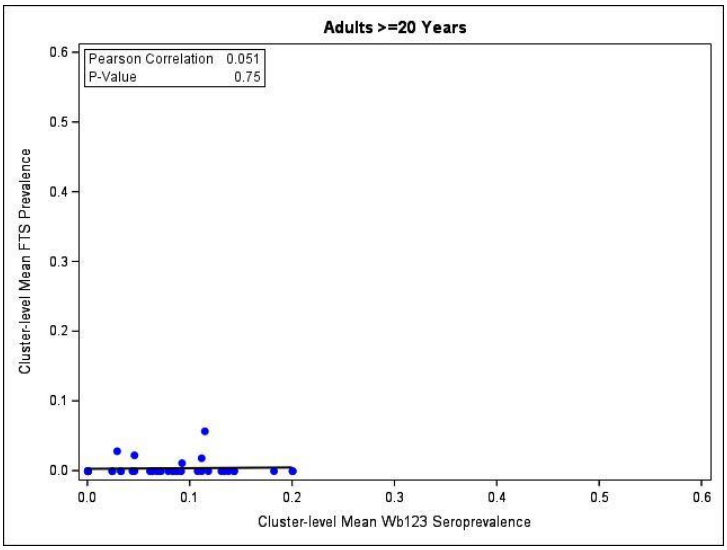


Figure 5 – Pearson correlation plots for Philippines. Cluster-level mean Wb123 seroprevalence was correlated with cluster-level mean FTS prevalence for the three age groups, A) Children <=10 years, B) Adults >=20 years, and C) All ages. The insets in the top left corner of the plots indicate the Pearson correlation coefficients and p-values.

A)



B)



C)

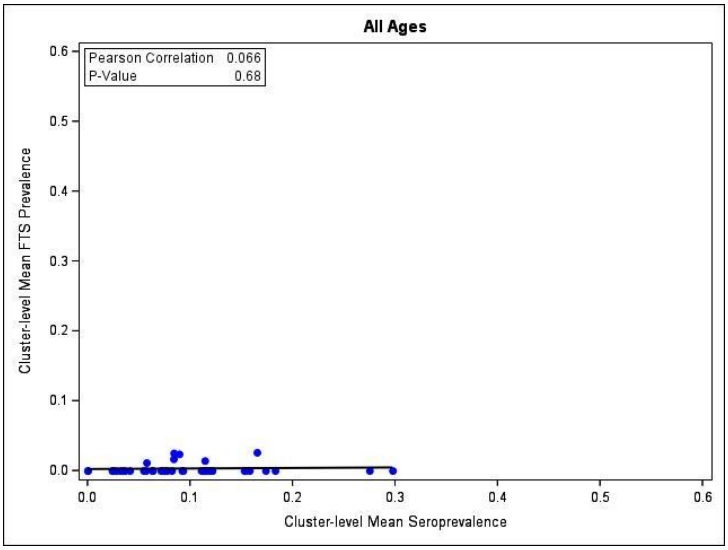


Table 5 shows the results from the cluster-level Pearson correlations for the Philippines. Tables 5A.1, 5B.1, and 5C.1 show the correlations between FTS and the Ab marker combinations for each age group. Tables 5A.2, 5B.2, and 5C.2 show the correlations between Wb123, Bm14, and Bm33. Within the child age group, only one cluster was able to be analyzed for FTS/Ab, and the correlations were not significant. While many clusters were able to be analyzed for the Wb123-Bm14/Bm33 comparisons, only one cluster had a significant correlation. For adults, several clusters had significant

correlations between FTS and Wb123 alone, but no adults had positive results for the Bm14 and Bm33 markers and FTS, so those were unable to be analyzed since positive cases are needed for both variables being correlated. There was a total of twenty-four adults positive for both Wb123 and FTS, zero adults positive for both Bm14 and FTS, and zero adults positive for both Bm33 and FTS, out of 1,610 total adults sampled. For the adult age group comparisons between the three markers, there were no significant correlations between Wb123 and the other two markers for any cluster. Considering the entire population, FTS correlated well with Wb123 alone, but not well with Wb123 combined with Bm14 and/or Bm33. There were two clusters with significant correlations between Wb123 and the other two markers.

A.1 Children <=10 Years
Correlations between FTS and Wb123 marker only (Ab1), Wb123 and Bm14 combined (Ab2), and Wb123, Bm14, and Bm33 combined

Cluster	*N*	*Correlation Coefficient – FTS with Ab1*	*P-value*	*Correlation Coefficient – FTS with Ab2*	*P-value*	*Correlation Coefficient – FTS with Ab3*	*P-value*
4	69	-0.065	0.50	-0.00092	0.92	-	-

A.2 Children <=10 Years
Correlations between Wb123 and Bm14 and between Wb123 and Bm33.

Cluster	*N*	*Correlation Coefficient - Wb123 with Bm14*	*P-value*	*Correlation Coefficient - Wb123 with Bm33*	*P-value*
1	38	0.32	0.051	-0.085	0.61
10	29	-0.051	0.79	-	
117	28	-0.096	0.63	-0.096	0.63
12	134	0.045	0.61	-0.027	0.76
13	110	-0.028	0.77	-0.15	0.12
15	106	0.11	0.28	-0.062	0.53
18	101	-0.042	0.68	-0.083	0.41
19	108	-0.015	0.88	-0.14	0.16
2	91	-0.098	0.36	-0.098	0.36
20	118	-0.12	0.21	0.022	0.82
21	91	-0.16	0.14	-0.16	0.12
22	68	-0.078	0.52	-	
23	31	-0.11	0.56	-0.089	0.63
24	24	-0.063	0.77	-0.091	0.67
26	32	-0.15	0.40	-0.15	0.40
30	54	-0.14	0.30	0.15	0.27
31	125	-0.067	0.46	-0.029	0.75
32	91	-0.13	0.23	-0.16	0.14
33	106	-0.18	0.069	-0.16	0.10
34	75	-0.13	0.26	0.055	0.64
36	34	0.098	0.58	-0.031	0.86
37	52	-0.22	0.12	-0.13	0.35
38	33	-0.16	0.37	-0.14	0.43
39	48	-0.16	0.29	-0.098	0.51
4	69	-0.060	0.63	-0.066	0.59
40	72	-0.16	0.18	-0.10	0.39
41	67	-0.21	0.086	-0.15	0.24

42	26	-0.21	0.30	-0.10	0.61
43	74	0.13	0.28	-0.057	0.63
44	60	-0.13	0.33	-0.11	0.40
45	16	-0.067	0.81	-0.067	0.81
5	143	-0.11	0.19	-0.13	0.14
6	50	-0.075	0.61	-0.075	0.61
7	43	-0.049	0.76	0.082	0.60
8	23	-0.066	0.77	-0.083	0.71
9	47	0.47	**0.0008**	-0.055	0.71

B.1 Adults >=20 Years
Correlations between FTS and Wb123 marker only (Ab1), Wb123 and Bm14 combined (Ab2), and Wb123, Bm14, and Bm33 combined (Ab3)

Cluster	*N*	*Correlation Coefficient – FTS with Ab1*	*P-value*	*Correlation Coefficient – FTS with Ab2*	*P-value*	*Correlation Coefficient – FTS with Ab2*	*P-value*
13	57	0.21	0.13	-	-	-	-
19	44	-0.02	0.88	-	-	-	-
2	48	0.48	**0.0006**	-	-	-	-
37	21	0.62	**0.0028**	-	-	-	-
4	87	0.40	**0.0001**	-	-	-	-
40	56	0.48	**0.0002**	-	-	-	-
41	35	0.80	**<.0001**	-	-	-	-
6	56	-0.03	0.85	-	-	-	-

B.2 Adults >=20 Years
Correlations between Wb123 and Bm14 and Wb123 and Bm33

Cluster	N	*Correlation Coefficient - Wb123 with Bm14*	P-value	*Correlation Coefficient - Wb123 with Bm33*	P-value
1	22	-0.048	0.83	-	-
10	23	-0.095	0.67	-	-
117	31	-0.033	0.86	-0.048	0.80
12	69	-0.053	0.67	-0.026	0.83
13	57	-0.11	0.43	-0.19	0.16
15	32	-	-	-0.068	0.71
17	74	-0.028	0.81	-0.020	0.87
18	51	-0.12	0.40	-0.13	0.35
19	44	-0.023	0.88	-0.099	0.52
2	48	-	-	-0.089	0.55
20	69	-0.062	0.62	-0.096	0.43
21	22	-0.10	0.65	-0.15	0.51
22	27	-0.1	0.62	-0.069	0.73
23	28	-	-	-0.037	0.85
24	12	-	-	-0.13	0.68
25	11	-0.15	0.66	-0.1	0.77
30	22	-0.087	0.70	-0.069	0.76
31	43	-0.024	0.88	-0.024	0.88
32	30	-0.17	0.38	-0.17	0.38
33	42	-0.051	0.75	-0.087	0.58
34	79	-0.039	0.73	-0.039	0.73
36	24	-0.063	0.77	-0.093	0.66
38	14	-0.21	0.46	-0.17	0.57
39	33	-0.045	0.80	-	-
4	87	-0.029	0.79	-0.029	0.79
40	56	-0.16	0.23	-0.093	0.50
41	35	-0.094	0.59	-0.075	0.67
42	15	-0.16	0.57	-0.10	0.71
45	14	-0.11	0.70	-0.17	0.57
46	15	-	-	-0.15	0.58
5	140	-0.030	0.73	-0.061	0.48
6	54	-0.063	0.65	-0.038	0.78
7	31	-0.060	0.75	-0.12	0.53

C.1 All ages
Correlations between FTS and Wb123 marker only (Ab1), Wb123 and Bm14 combined (Ab2), and Wb123, Bm14, and Bm33 combined (Ab3)

Cluster	*N*	*Correlation Coefficient – FTS with Ab1*	*P-value*	*Correlation Coefficient – FTS with Ab2*	*P-value*	*Correlation Coefficient – FTS with Ab3*	*P-value*
13	170	0.13	0.095	-0.015	0.85		

19	168	0.39	**<.0001**	-0.021	0.79	-0.017	0.83
2	147	0.33	**<.0001**	**-**			
36	70	0.43	**0.0002**	-0.014	0.91	-0.014	0.91
37	87	0.47	**<.0001**	**-**	-	-	-
4	179	0.35	**<.0001**	**-**	-	-	-
40	145	0.28	**0.0007**	-0.030	0.72	-	-
41	115	0.29	**0.0019**	**-**	-	-	-
6	119	0.21	**0.023**	**-**	-	-	-
9	87	-0.02	0.85	-0.017	0.88	-	-

C.2 All ages
Correlations between Wb123 and Bm14 and Wb123 and Bm33

Cluster	N	*Correlation Coefficient - Wb123 with Bm14*	P-value	*Correlation Coefficient - Wb123 with Bm33*	P-value
1	65	0.19	0.14	-0.050	0.69
10	57	-0.081	0.55	-	-
117	65	-0.066	0.60	-0.074	0.56
12	220	0.011	0.87	-0.028	0.68
13	170	-0.054	0.49	-0.16	**0.035**
15	154	0.088	0.28	-0.056	0.49
16	29	-	-	-0.036	0.85
17	215	-0.020	0.77	-0.013	0.85
18	183	-0.070	0.35	-0.088	0.24
19	168	0.035	0.66	-0.12	0.13
2	147	-0.079	0.34	-0.092	0.27
20	205	-0.099	0.16	-0.024	0.73
21	121	-0.14	0.13	-0.16	0.089
22	107	-0.11	0.27	-0.035	0.72
23	65	-0.067	0.60	-0.062	0.62
24	43	-0.042	0.79	-0.099	0.53
25	40	-0.046	0.78	-0.037	0.82
26	73	-0.073	0.54	-0.067	0.57
27	42	-0.024	0.88	-	-
30	82	-0.13	0.26	0.088	0.43
31	190	-0.053	0.47	-0.028	0.70
32	144	-0.14	0.086	-0.17	**0.047**
33	164	-0.13	0.090	-0.13	0.10
34	174	-0.083	0.28	0.037	0.62
36	70	0.021	0.86	-0.053	0.67
37	87	-0.17	0.13	-0.10	0.34
38	53	-0.20	0.15	-0.17	0.23
39	97	-0.096	0.35	-0.062	0.55
4	179	-0.045	0.55	-0.053	0.48
40	145	-0.15	0.075	-0.095	0.25
41	115	-0.17	0.064	-0.12	0.19
42	42	-0.19	0.24	-0.10	0.52
43	111	0.11	0.26	-0.042	0.66
44	72	-0.12	0.30	-0.11	0.37
45	33	-0.080	0.66	-0.1	0.58
46	44	-0.059	0.70	0.086	0.58
5	352	-0.074	0.17	-0.088	0.099
6	119	-0.062	0.50	-0.048	0.60
7	88	-0.059	0.58	-0.003	0.98
8	82	-0.037	0.74	-0.046	0.68
9	87	0.24	**0.028**	-0.036	0.74

Table 5 – Non-parametric Pearson correlations. Bold p-values indicate significant result.
A.1 – Children <=10 years; comparing cluster-level FTS prevalence with Wb123 marker seroprevalence alone (Ab1), with Wb123 and Bm14 marker combined prevalence (Ab2), and with Wb123, Bm14, and Bm33 marker combined prevalence (Ab3).
A.2 – Children <=10 years; comparing Wb123 marker with Bm14 marker, and Wb123 marker with Bm33 marker.
B.1 - Adults >=20 years; comparing cluster-level FTS prevalence with Wb123 marker seroprevalence alone (Ab1), with Wb123 and Bm14 marker combined prevalence (Ab2), and with Wb123, Bm14, and Bm33 marker combined prevalence (Ab3).
B.2 – Adults >=20 years; comparing Wb123 marker with Bm14 marker, and Wb123 marker with Bm33 marker.
C.1 – All ages; comparing cluster-level FTS prevalence with Wb123 marker seroprevalence alone (Ab1), with Wb123 and Bm14 marker combined prevalence (Ab2), and with Wb123, Bm14, and Bm33 marker combined prevalence (Ab3).

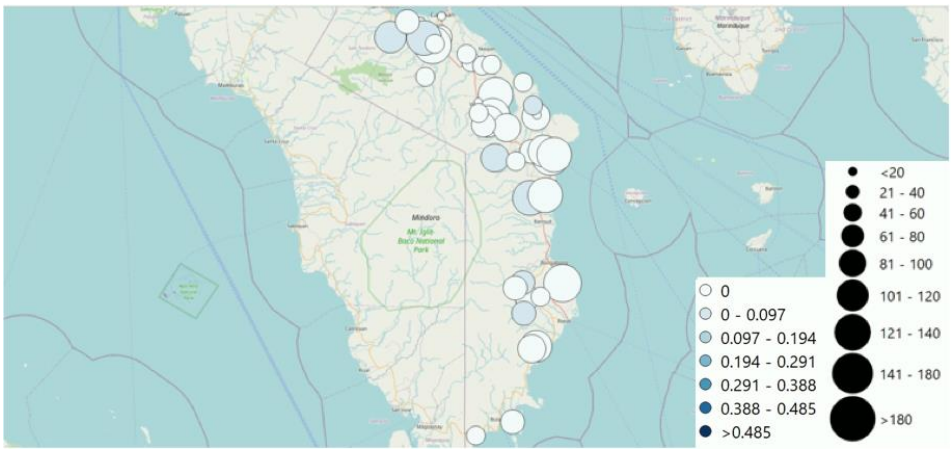
C.2 – All ages; comparing Wb123 marker with Bm14 marker, and Wb123 marker with Bm33 marker.

Figure 6 contains the maps for the Philippines. For children, it can be seen that the FTS and Wb123 hotspots were in similar locations, but they did not directly correspond in adults, indicating discrepancies between antibody and FTS data. For all ages, the entire country had a high Wb123 seroprevalence, but the FTS prevalence was low. In comparison to American Samoa, the sample sizes of the clusters in the Philippines are generally lower.

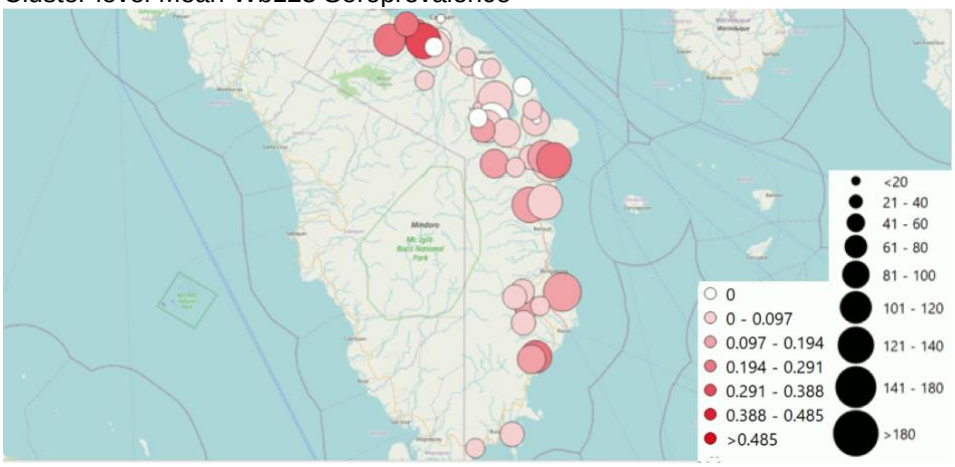
Figure 6 – Mapping results from Philippines for the three age groups, A) Children <=10 years, B) Adults >=20 years, and C) All ages. Each point on the map represents a cluster and the size of the point corresponds to the cluster-level sample size. Maps on the top, in blue, display cluster-level mean FTS prevalence. Maps on the bottom, in red, display cluster-level mean Wb123 seroprevalence.

A) Children <=10 years

Cluster-level Mean FTS Prevalence

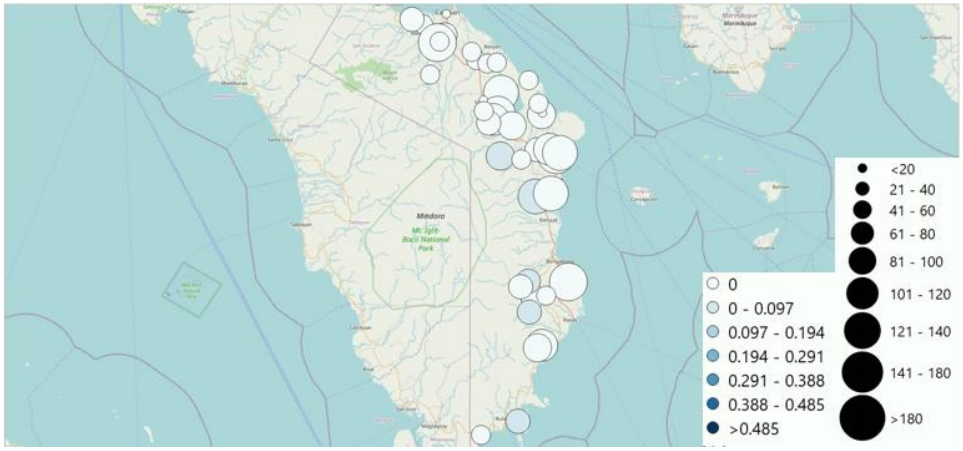


Cluster-level Mean Wb123 Seroprevalence

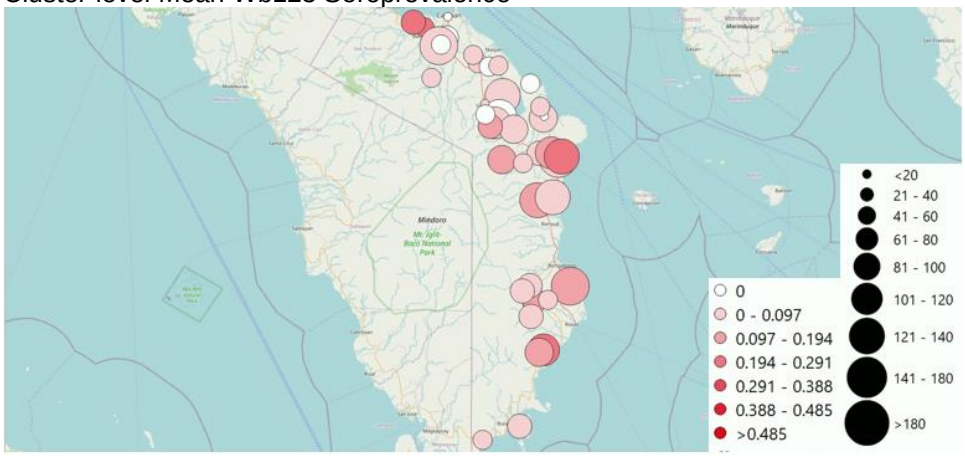


B) Adults >=20 years

Cluster-level Mean FTS Prevalence

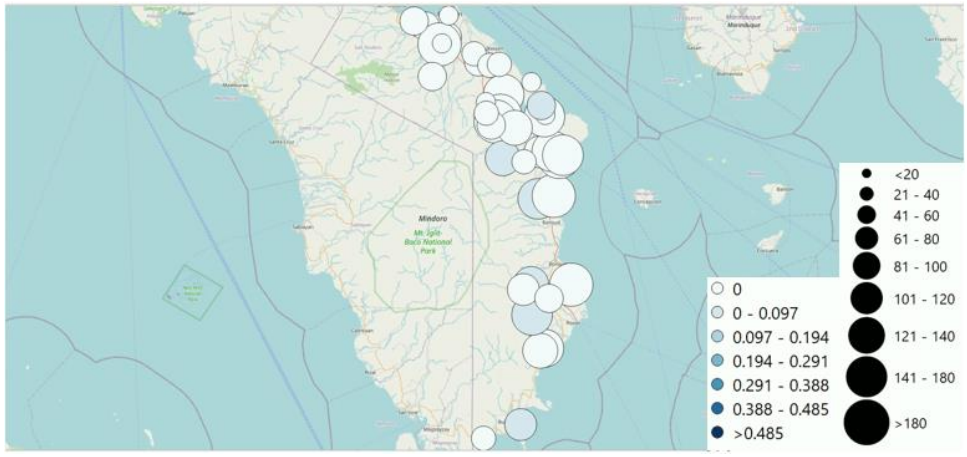


Cluster-level Mean Wb123 Seroprevalence



C) All ages

Cluster-level Mean FTS Prevalence



Cluster-level Mean Wb123 Seroprevalence

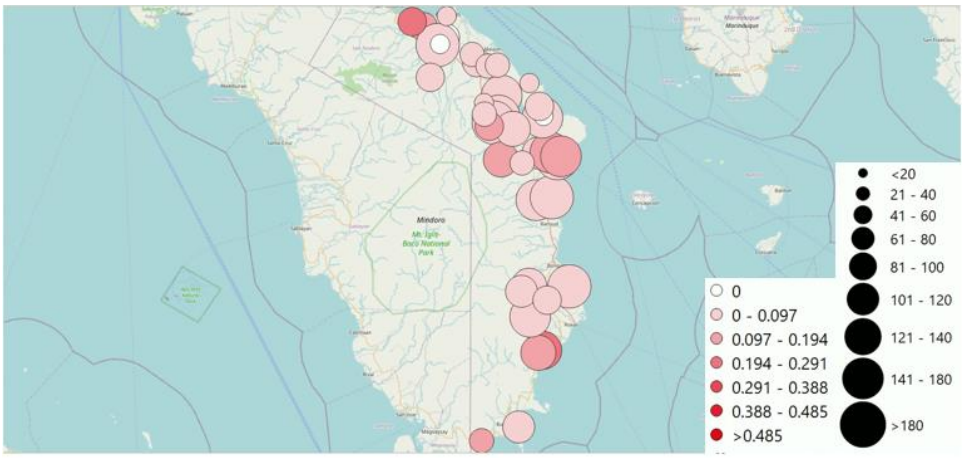


Table 6 shows the logistic regression results for the Philippines. For each of the three age groups, the basic model containing FTS as the outcome and Wb123 as the predictor was the only possible model because of quasi-separation of the data. The global likelihood ratio tests for all three age groups were significant, indicating that Wb123 is a good predictor of FTS. The model selection did indicate that years lived in the village was a significant predictor in some cases, but quasi-separation of the data occurred both for this predictor and travel in all age groups.

The odds ratios were also highly significant for all three age groups. For children, the odds of being FTS positive were 18.61 times greater than the odds for being Wb123 seropositive. For adults, the odds of being FTS positive were 87.96 times greater than the odds for being Wb123 seropositive. For all ages, the odds of being FTS positive were 133.41 times greater than the odds for being Wb123 seropositive. The confidence intervals were very wide, making the actual odds ratio estimate of questionable certainty, but the relationship is highly significant.

As with American Samoa, exact logistic regressions were attempted, but the results were not reported due to questionable significance and validity of odds ratio estimates.

A) Children <=10 Years

$Ln(p/(1-p) = Intercept + \beta_1(Wb123) + Error$

Global Likelihood Ratio Test	
Chi-square	37.93
P - value	<0.0001

Parameter	Estimate	P-value	Odds Ratio Estimate	95% CI for Odds Ratio
Intercept	-4.56	<0.0001		
Wb123	1.46	<0.0001	18.61	7.1, 48.78

B) Adults >=20 Years

$Ln(p/(1-p) = Intercept + \beta_1(Wb123) + Error$

Global Likelihood Ratio Test	
Chi-square	87.96
P - value	<0.0001

Parameter	Estimate	P-value	Odds Ratio Estimate	95% CI for Odds Ratio
Intercept	-3.38	<0.0001		
Wb123	1.83	0.0063	39.26	17.24, 89.4

C) All ages

$Ln(p/(1-p) = Intercept + \beta_1(Wb123) + Error$

Global Likelihood Ratio Test	
Chi-square	133.41
P - value	<0.0001

Parameter	Estimate	P-value	Odds Ratio Estimate	95% CI for Odds Ratio
Intercept	-4.079	<0.0001		
Wb123	1.69	<0.0001	29.11	15.75, 53.82

Table 6 – Logistic regression model selection and parameter results from children (A), adults (B), and all ages (C).

III. Key Findings – Tanzania

Table 7 shows the demographic data summary for Tanzania. Notably, there is no data for the Bm14 or Bm33 antibody markers. Significant proportions of data are missing for xenomonitoring, years lived in the village, and travel outside the village. Like the Philippines, Tanzania had a very low FTS antigen prevalence (2%) and also a relatively low Wb123 seroprevalence (8%). Additionally, the Luminex antibody data is recorded as optical density, an analogous but different metric than MFI measured with ELISA.

C) Tanzania (Total N = 4434)

Variable		*FTS Positive (%)*	*FTS Negative (%)*		*Wb123 Seropositive (%)*	*Wb123 Seronegative (%)*	
		n=77 (2)	n=4360 (98)	Chi-square p-value	n=368 (8)	n=4079 (92)	Chi-square p-value
Age Category (years)	5-6	2 (0.25)	816 (99.5)		61 (7)	757 (93)	
	7-8	5 (0.5)	1088 (99.5)		69 (6)	1024 (94)	
	9-13	3 (0.3)	1128 (99.5)		87 (8)	1044 (92)	
	14-24	11 (3)	379 (97)		35 (9)	355 (91)	
	25-34	16 (6)	264 (94)		35 (12)	245 (88)	
	35-49	18 (5)	312 (94)		35 (11)	295 (89)	
	50+	22 (6)	370 (94)	**<0.0001**	44 (11)	348 (89)	**0.0022**
Frequent Travel (Coded as travel)	Yes	3 (2)	177 (98)		13 (7)	167 (93)	
	No	65 (4)	1414 (95)	0.081	159 (11)	1320 (89)	0.14
Years Lived in Village (Coded as years_lived)	1 - <1 year	0 (0)	3 (100)		0 (0)	3 (100)	
	2 – 1-2 years	1 (2)	45 (98)		1 (2)	45 (98)	
	3 – 3-5 years	2 (3)	63 (97)		3 (5)	62 (95)	
	4 - >5 years	65 (4)	1480 (96)	0.85	168 (11)	1377 (89)	0.093
Positive Mosquito (Pooled sample) (Coded as mxpoolpos)	Yes	7 (1)	476 (99)		32 (7)	452 (93)	
	No	4 (0.7)	612 (99.3)	0.19	36 (6)	584 (94)	0.58
Bm14 Seroprevalence	Positive	-	-		-	-	
	Negative	-	-	-	-	-	-
Bm33 Seroprevalence	Positive	-	-		-	-	
	Negative	-	-	-	-	-	-
Wb123 Luminex	*Mean*	*Median*	*Min*	*Max*			
	0.0634	0.053	-0.001	1.535			

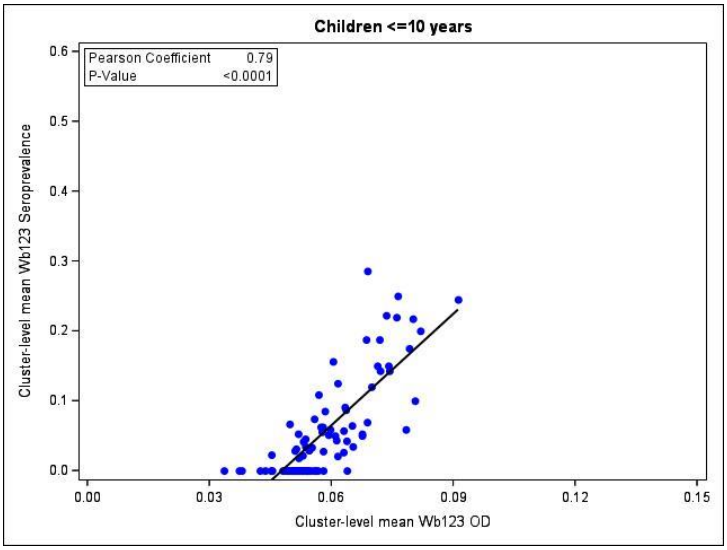
Summary of Missing Data - Tanzania	
Variable	**Number missing**
FTS	0
Wb123 Seroprevalence	0
Age	0
Frequent Travel	2775
Years lived in village	2775
Positive Mosquito (Pooled sample)	3335

Table 7– Data summary for Tanzania. Demographic predictors are stratified by FTS diagnostic result and Wb123 antibody result. Bolded p-values denote significant (alpha=0.05). A summary of missing data is also included.

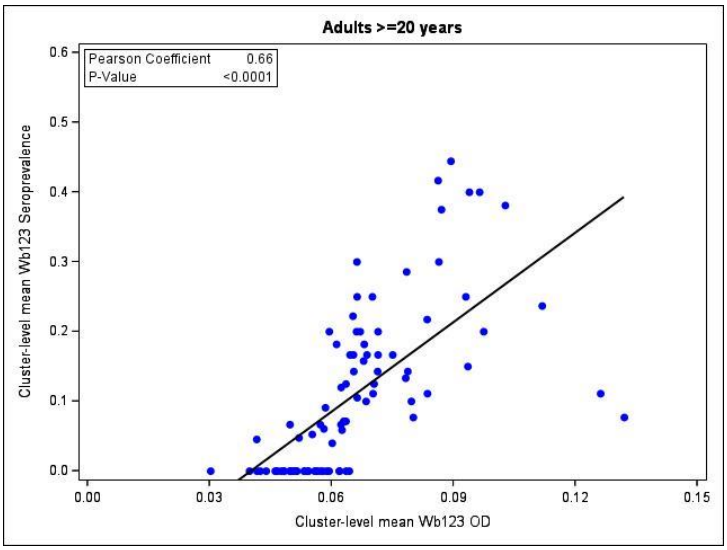
Figure 7 shows the Pearson correlation plots for Tanzania comparing cluster-level mean Wb123 seroprevalence and antibody data. It should be noted that the quantitative antibody data for Tanzania comes from Luminex Multiplex Bead Assays, and is therefore represented as optical density (OD). Cluster-level mean quantitative Wb123 seroprevalence was significantly correlated with mean OD in all age groups. Figure 8 shows the Pearson correlation plots for Wb123 seroprevalence and FTS prevalence at the cluster level. For all three age groups, there was no significant correlation between seroprevalence and FTS prevalence. In fact, almost all clusters had FTS prevalence of zero while the seroprevalence was variable.

Figure 7 – Pearson correlation plots for Tanzania. Cluster-level mean Wb123 seroprevalence was correlated with cluster-level mean Wb123 MFI results for the three age groups, A) Children <=10 years, B) Adults >=20 years, and C) All ages. The insets in the top left corner of the plots indicate the Pearson correlation coefficients and p-values.

A)



B)



C)

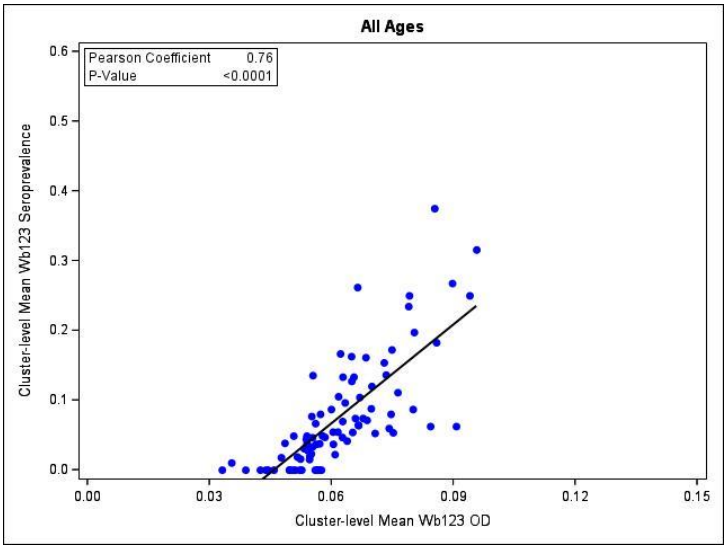
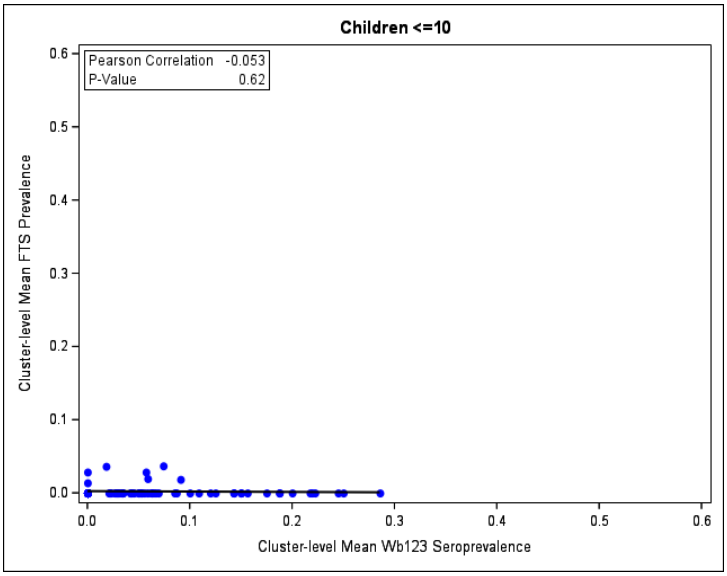
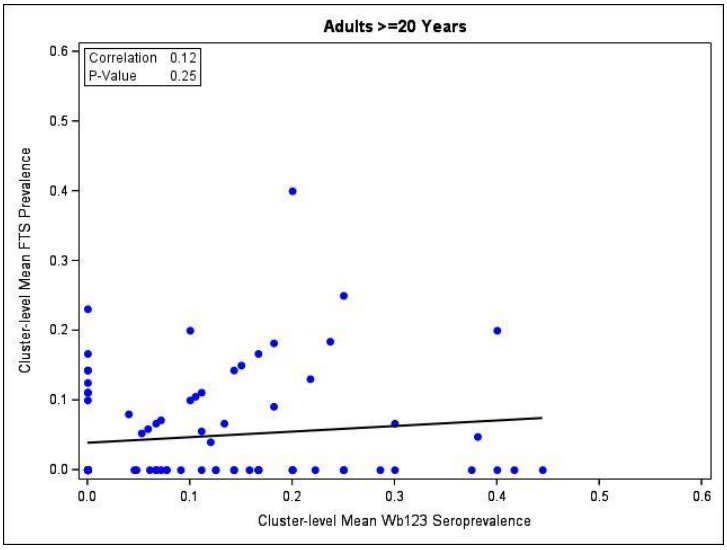


Figure 8 – Pearson correlation plots for Tanzania. Cluster-level mean Wb123 seroprevalence was correlated with cluster-level mean FTS prevalence for the three age groups, A) Children <=10 years, B) Adults >=20 years, and C) All ages. The insets in the top left corner of the plots indicate the Pearson correlation coefficients and p-values.

A)


Children <=10
Pearson Correlation -0.053
P-Value 0.62
Cluster-level Mean FTS Prevalence
Cluster-level Mean Wb123 Seroprevalence
0.0
0.1
0.2
0.3
0.4
0.5
0.6

B)


Adults >=20 Years
Correlation 0.12
P-Value 0.25
Cluster-level Mean FTS Prevalence
Cluster-level Mean Wb123 Seroprevalence
0.0
0.1
0.2
0.3
0.4
0.5
0.6

C)

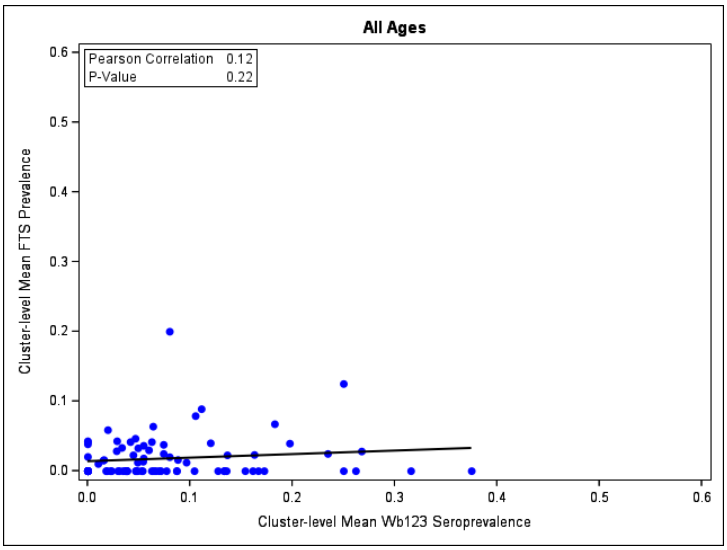


Table 8 contains the cluster-level Pearson correlations for Tanzania. There are no correlations involving Bm14 or Bm33 because there was no data for these markers for this country. The correlations between FTS and Ab1 were less significant than those calculated in American Samoa or Philippines. The adults age group had fewer individuals sampled. Only one cluster had a significant correlation for both children and adults. There were several significant correlations when considering the entire population sampled. FTS and Ab1 do not appear to correlate well and Wb123 seroprevalence may not be a good predictor of FTS status in Tanzania.

***Correlations involving Bm14 and Bm33 markers were not possible because there is no Bm14 or Bm33 data for Tanzania.

A) Children <=10 Years

Correlations between FTS and Wb123 marker only (Ab1).

Cluster	*N*	*Correlation Coefficient – FTS with Ab1*	*P-value*
43	41	-0.15	0.34
153	27	-0.055	0.78
342	51	-0.035	0.81
372	54	-0.044	0.75
425	55	-0.026	0.85
448	35	0.70	**<.0001**

B) Adults >=20 Years

Cluster	*N*	*Correlation Coefficient – FTS with Ab1*	*P-value*
123	10	0.5	0.14
130	11	-0.15	0.66
183	30	0.12	0.54
191	5	-0.41	0.50
198	12	0.4	0.20

213	18	0.69	**0.0017**
217	17	-0.063	0.81
229	19	-0.12	0.63
232	9	-0.13	0.75
241	38	0.055	0.74
259	21	0.29	0.21
266	11	0.39	0.24
274	15	-0.10	0.71
293	15	-0.10	0.71
304	20	0.22	0.36
327	5	0.61	0.27
342	14	-0.077	0.79
345	9	0.5	0.17
360	25	-0.06	0.78
365	23	0.11	0.62
407	25	-0.075	0.72
425	15	-0.071	0.80
440	4	-0.33	0.67
448	10	-0.17	0.65
456	14	0.42	0.14
463	19	-0.056	0.82
501	20	-0.11	0.64

C) All Ages

Cluster	*N*	*Correlation Coefficient – FTS with Ab1*	*P-value*
43	53	-0.14	0.33
123	25	0.59	**0.0019**
130	44	-0.03	0.83
145	25	-0.08	0.72
153	43	-0.07	0.66
160	43	-0.05	0.76
183	81	0.10	0.38
191	11	-0.24	0.48
198	53	0.32	**0.020**
213	74	0.49	**<.0001**
217	63	-0.016	0.90
229	61	-0.042	0.75
232	24	-0.043	0.84
241	104	0.072	0.47
259	71	0.28	**0.017**
266	38	0.22	0.19
274	44	-0.061	0.70
293	48	-0.054	0.72
304	45	0.14	0.37
319	66	-0.015	0.90
327	16	0.22	0.42
328	97	-0.010	0.92
342	67	-0.044	0.72
343	51	-0.035	0.81
345	50	0.48	**0.0004**
360	70	-0.029	0.81
365	76	0.069	0.55
372	82	-0.037	0.74
387	55	-0.033	0.81
407	82	-0.025	0.82
425	70	-0.036	0.77
440	30	-0.034	0.86
448	47	0.29	**0.050**
456	81	0.26	**0.020**
463	55	0.38	**0.0041**
501	125	-0.040	0.66

Table 8 – Non-parametric Pearson correlations for Tanzania for A) Children <=10 years B) Adults >=20 years C) All ages. Bold p-values indicate statistical significance. FTS antigen prevalence was correlated with Wb123 marker seroprevalence alone (Ab1).

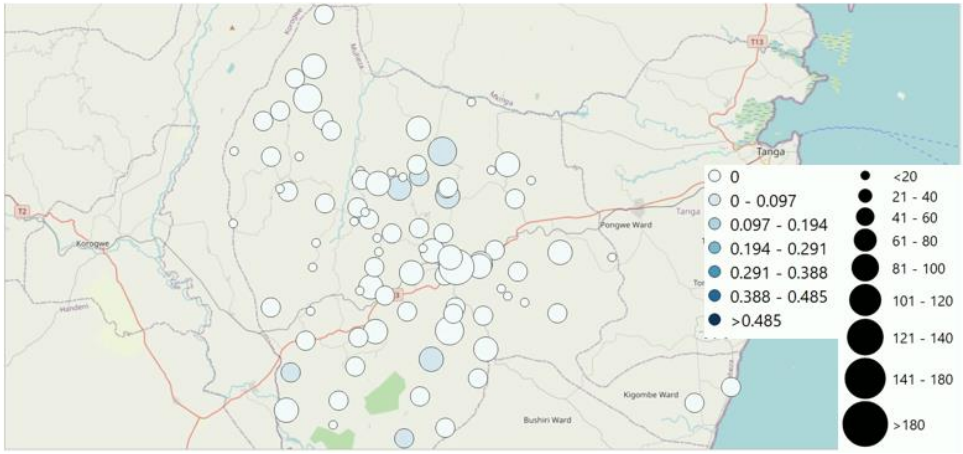
Figure 9 contains the FTS antigen prevalence vs Wb123 seroprevalence mapping for Tanzania. For children, the FTS hotspots do not correspond to the Wb123

hotspots – some clusters with FTS cases had zero seropositive cases, and vice versa. For adults, there was a low sample size for almost every cluster, which could have ramifications for data interpretation and utility in this age group. The FTS and Wb123 hotspots were correlated, unlike the data for the children. For all ages together, the hotspots were not in the same locations and differed significantly.

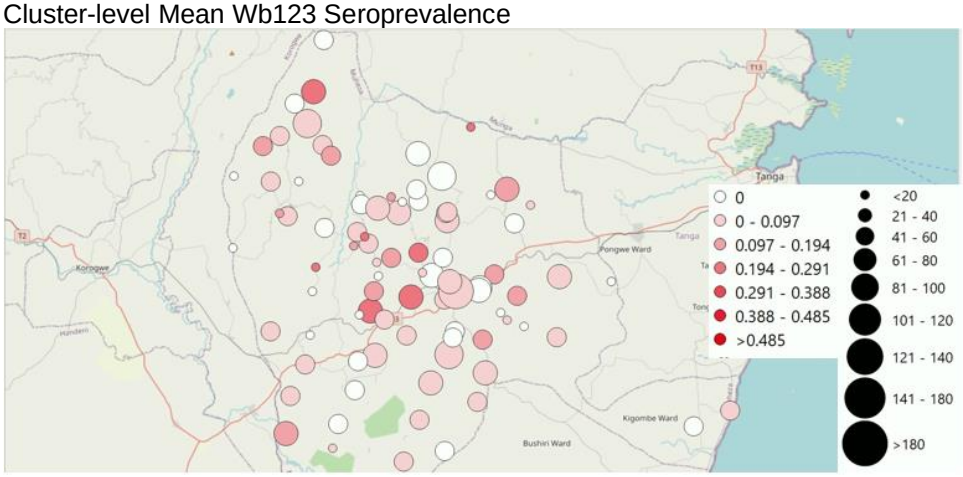
Figure 9 – Mapping results from Tanzania for the three age groups, A) Children <=10 years, B) Adults >=20 years, and C) All ages. Each point on the map represents a cluster and the size of the point corresponds to the cluster-level sample size. Maps on the top, in blue, display cluster-level mean FTS prevalence. Maps on the bottom, in red, display cluster-level mean Wb123 seroprevalence.

A) Children <=10 years

Cluster-level Mean FTS Prevalence

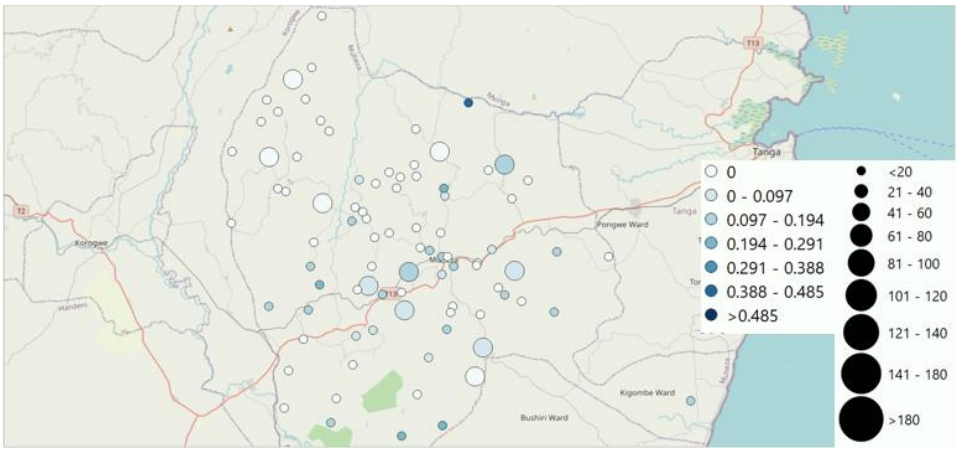


Cluster-level Mean Wb123 Seroprevalence

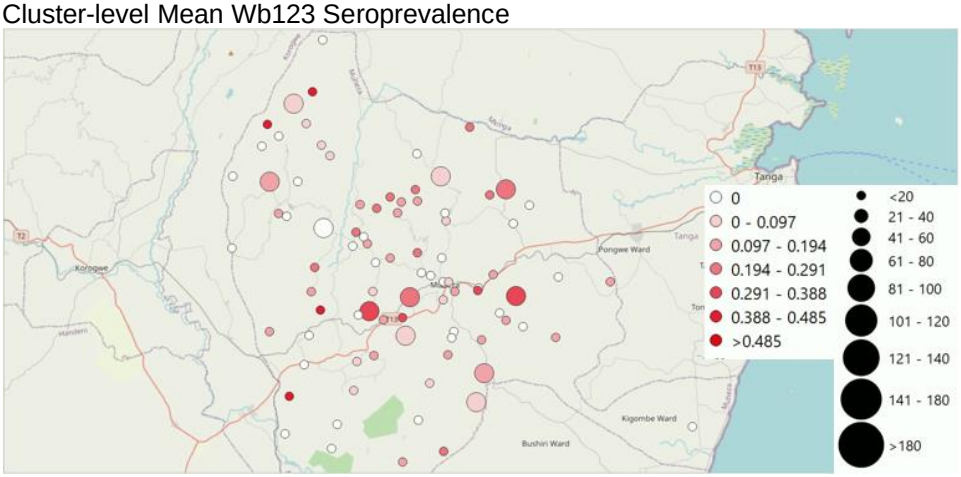


B) Adults >=20 years

Cluster-level Mean FTS Prevalence

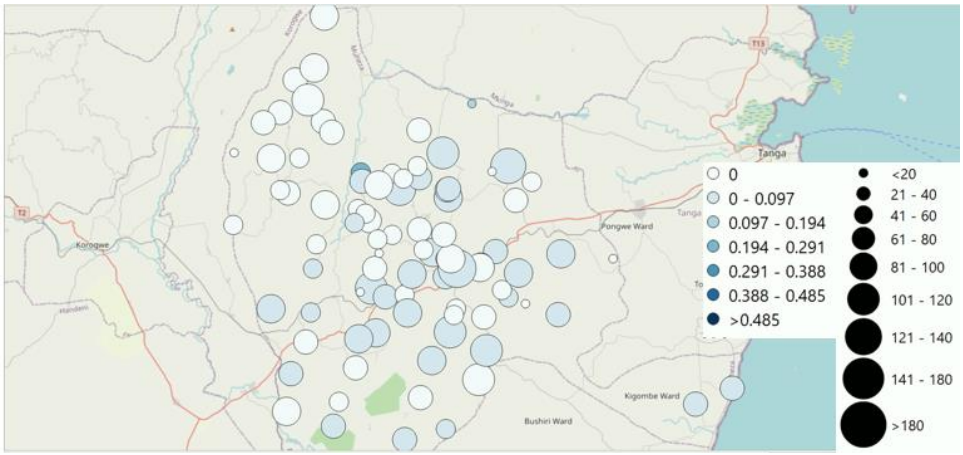


Cluster-level Mean Wb123 Seroprevalence



C) All ages

Cluster-level Mean FTS Prevalence



Cluster-level Mean Wb123 Seroprevalence

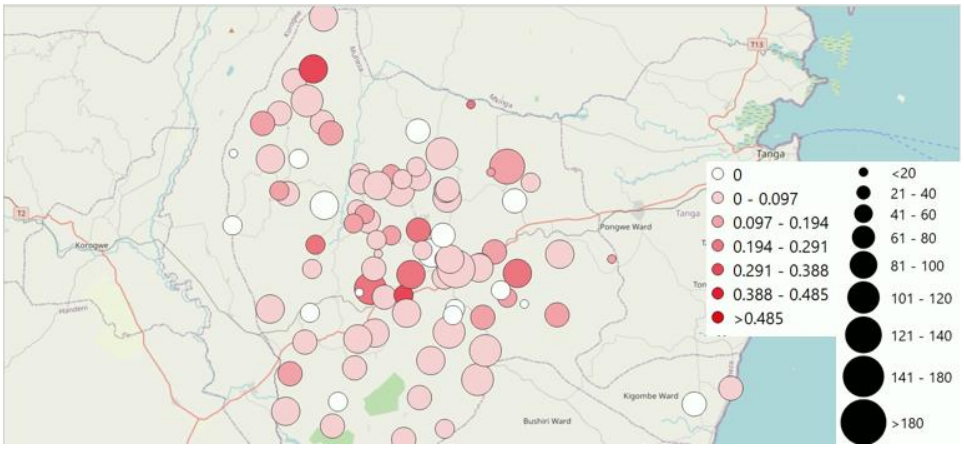


Table 9 shows the model selection and individual-level logistic regressions for the three age groups in Tanzania. For children, the model selected included Wb123 and positive mosquito pool, but when actually running the regression, there was quasi-separation of the data points, indicating one or more predictors were nearly completely separated by the outcome variable. Wb123 was kept in the model while positive mosquito pool was eliminated due to the high degree of missingness in that variable. The global LR test was not significant, indicating the model is not a good fit for the data. Also supporting this conclusion, the Wb123 parameter and odds ratio estimates were not significant. There is a significant amount of unexplained error in the model and Wb123 alone cannot be used to predict FTS results in children.

For adults, the model selected included Wb123, years lived in the village, and positive mosquito pool. As occurred in the children age group, quasi-separation resulted in lived in the village, travel, and positive mosquito pool being removed from the model. For Wb123, the parameter and odds ratio estimates were significant. The odds ratio indicated that adults in Tanzania that were Wb123 seropositive had 2.39 times higher odds to be FTS positive. The global LR test was significant, indicating Wb123 is a

reasonable predictor of FTS. However, there are still unexplained sources of error and there are likely more predictors capable of improving the model fit.

When considering all ages, model selection indicated the same model as the children; Wb123 and positive mosquito pool. Again, similar to the children, positive mosquito pool had to be removed from the model due to quasi-separation. For Wb123, the parameter and odds ratio estimates were significant. Individuals that were Wb123 seropositive had 3.25 times higher odds to be FTS positive. The global LR test was significant, indicating Wb123 is generally a good indicator of FTS when not considering age groups. Again, there were likely missing predictors in these models and many factors that had not been considered simply because the data does not exist.

As with American Samoa and Philippines, exact logistic regressions were attempted, but the results were not reported due to questionable significance and validity of odds ratio estimates.

A) Children <=10 Years

$Ln(p/(1-p) = Intercept + \beta_1(Wb123) + Error$

Global Likelihood Ratio Test	
Chi-square	0.19
P - value	0.66

Parameter	**Estimate**	**P-value**	**Odds Ratio Estimate**	**95% CI for Odds Ratio**
Intercept	-5.55	<0.0001		
Wb123	0.247	0.64	1.64	0.20, 13.17

B) Adults >=20 Years

$Ln(p/(1-p) = Intercept + \beta_1(Wb123) + Error$

Global Likelihood Ratio Test	
Chi-square	6.47
P - value	0.011

Parameter	**Estimate**	**P-value**	**Odds Ratio Estimate**	**95% CI for Odds Ratio**
Intercept	-2.56	<0.0001		
Wb123	0.439	0.0063	2.39	1.28, 4.47

C) All ages

$Ln(p/(1-p) = Intercept + \beta_1(Wb123) + Error$

Global Likelihood Ratio Test	
Chi-square	14.2
P - value	0.0002

Parameter	Estimate	P-value	Odds Ratio Estimate	95% CI for Odds Ratio
Intercept	-3.61	<0.0001		
Wb123	0.589	<0.0001	3.25	1.87, 5.62

Table 9 – Logistic regression model selection and parameter results from children (A), adults (B), and all ages (C).

Chapter 5. Conclusions, Implications, and Recommendations

I. Summary of Study

The purpose of this thesis was to evaluate lymphatic filariasis diagnostic methods, specifically FTS antigen tests and antibody results. The data came from TAS-2 surveys from American Samoa, Philippines, and Tanzania. The diagnostic test results were evaluated using various statistical and visualization methods including Pearson correlations, geospatial mapping of prevalence data, and logistic regression.

Results showed that antibody data generally identified more potential LF cases than antigen data alone, which could be due to the delay in filarial worm maturation. Antigen and antibody data were often not significantly correlated, most likely due to generally low or zero FTS prevalence in many clusters, or due to historical infections that have already cleared. Antigen and antibody prevalence hotspots usually corresponded to the same general geographic location, but not always. These discordant sites could signify active LF cases that have not matured enough to be detected by FTS antigen testing. In countries with more FTS cases, i.e. failed a TAS, Wb123 seropositivity can be a good predictor of FTS positivity, but other significant predictors are not known at this time from the data provided. Previous studies have identified factors such as the species of mosquito vector, elevation, and population density that may be predictive of whether a country will fail the pre-TAS, i.e. the survey that decides whether an area is ready for a TAS (Burgert-Brucker et al., 2020). Incorporating some of these variables into the within-country analyses may prove useful in the future.

There were many issues present within each dataset that call into question the validity of these results; however, this study showed that it may be possible to use antibody data to detect early signs of recrudescent transmission or as a more sensitive signal of ongoing transmission.

II. Discussion of Key Results

Pearson Correlations – Wb123 seroprevalence with Wb123 quantitative antibody

For all age groups in all countries, cluster-level mean Wb123 MFI was significantly correlated with cluster-level mean Wb123 seroprevalence, which was expected since seroprevalence was directly derived from the quantitative antibody results.

Pearson Correlations – Wb123 seroprevalence with FTS antigen

For children and adults in all three countries, Wb123 seroprevalence and FTS prevalence were not significantly correlated. In fact, FTS prevalence was zero for almost all of the clusters. This could indicate that exposures were still occurring, particularly among children since they had been living in MDA/post-MDA times. For all ages in Philippines and Tanzania, the antigen prevalence and seroprevalence were not significantly correlated.

Antigen prevalence and Wb123 seroprevalence were significantly correlated when considering all ages data in American Samoa, only. A problem this thesis was initially investigating was that TAS-2 surveys of a large geographic location could be

missing smaller focal points of exposure, therefore in this case the all ages analyses masked the effects seen in the children and adults age groups.

Previous studies with malaria have shown that antibody responses can be used as a method to predict areas of high infection intensity (Corran et al., 2007). While Wb123 seroprevalence is not necessarily indicative of a corresponding FTS hotspot, seroprevalence could be used to identify regions of high exposure potential.

Pearson Correlations – FTS correlation with Wb123 alone (Ab1), Wb123+Bm14 (Ab2), and Wb123+Bm14+Bm33 (Ab3)

In American Samoa, correlating FTS prevalence with Ab2 and Ab3 prevalence was enough to improve the significance of cluster-level correlations, particularly in adults. In some cases, this changed a previously-insignificant correlation to a significant correlation. This could mean that the Bm14 and Bm33 markers are important enough to include in analyses even though Wb123 is the most common marker. Additionally, the Wb123 marker alone may be a better indicator of exposure, but the combined markers may be better indicators of antigenemia. In general, however, there were far fewer positive cases for Bm14 and even fewer for Bm33, so the increased correlation could be due to the decreasing sample size.

In the Philippines, there were very few individuals, especially in the adult age groups, that were positive for FTS, Wb123, and/or Bm14 and Bm33. Only 18 individuals total were positive for both Wb123 and Bm14, and 4 were positive for all three markers. Many clusters were excluded from the analyses. One reason for this could be, again, the questionability of the antibody cutoffs. Recently in Togo, Wb123 seroprevalence

was examined in children 5-6 years old in previously-endemic subdistricts. The seroprevalence was so similar between subdistricts that the data could not be used to establish cutoffs useful for LF surveillance programs (Dorkenoo et al., 2021).

Pearson Correlations – Wb123 with Bm14, Wb123 with Bm33

In American Samoa, 11 clusters had significant correlations for antibody marker comparisons for children. The majority of clusters significant correlations for Wb123-Bm14 and Wb123-Bm33, for adults and all ages. It can be concluded that, in some clusters, Wb123 could be better correlated with Bm14 than Bm33, and vice versa. More information would be needed to determine which is the better marker to use based on the geographic location of the cluster.

In the Philippines, for children the correlations between antibody markers were non-significant for every cluster for children and adults, but all ages had one cluster significant for the correlation between Wb123 and Bm33. The number of individuals positive for the three markers simultaneously was very low. A higher sample size and further analyses would be needed to draw conclusions about these correlations in the Philippines.

There was no data for the Bm14 or Bm33 markers in Tanzania, so these analyses were not completed. These indicators were not collected due to the expected geographic location of *Brugia malayi* (CDC, 2021).

Cluster-level mean FTS prevalence and Wb123 seroprevalence mapping

For American Samoa, transmission/exposure hotspots mostly corresponded for child data, but not for adult data. A similar trend was observed in all ages data. We concluded from this data that the antibody signal in the adult data in American Samoa made it difficult to identify areas of high transmission because adult transmission has been occurring for years. Identifying high exposure areas did seem possible considering child data because the antigen and seroprevalences were lower and any signal likely denotes recent exposure/transmission.

For the Philippines, the trend was mostly the same as in American Samoa. There was some agreement between antigen and antibody hotspots in child data, however, particularly in the middle-south of the study area, there were many hotspots for Wb123 that had a corresponding zero FTS prevalence. For adult data, this relationship was similar, For all ages data, this relationship is slightly more difficult to ascertain since the antibody signal is high throughout the country. In conclusion, in the Philippines, antibody data could be a useful indicator of ongoing transmission, especially for children, that may not be observable yet from the antigen data. Furthermore, I would recommend keeping the datasets separate to look at ongoing transmission because hotspots and areas of high transmission may be missed with combining the data into one dataset.

In Tanzania, it should be noted that most clusters had a very low sample size. For children, there were many clusters with a strong Wb123 response, but very few clusters with any FTS cases. This could mean that exposure has been successfully interrupted in these clusters, since Tanzania did pass the TAS-2, however it is very difficult to determine if an antibody response comes from historic exposure or new exposure. In adults, there were higher FTS prevalence and correspondingly high

Wb123 seroprevalences, but the sample sizes were smaller compared to the child data. These numbers are plausible because when transmission is interrupted in children, those results may not be seen in adults due to persistent antibody response and positive antigen response from previously-cleared infections. For all ages data, FTS prevalence is low, Wb123 seroprevalence is high, and the sample sizes are larger. In conclusion, the relationship between antibody exposure data and antigen infection data is not as clear in a true case of LF elimination. Trends are more difficult to discern, in contrast to the other two countries.

Next, we addressed the question of predicting antigen hotspots from antibody hotpots using mapping. We identified a hotspot as a cluster with >2% antigen prevalence. For American Samoa in children, four clusters were antigen hotspots. The highest seroprevalence clusters were not hotspots for antigen prevalence in children. For adults, there were 16 hotspot clusters and all of these had seroprevalences ranging from 15% to over 40%. For all ages, 14 hotspot clusters were identified and the seroprevalences were between those of children and adults.

For Philippines child data, five clusters were defined as hotspots. The FTS prevalences lined up fairly well with the antibody seroprevalences; while seroprevalence was always higher, this effect was less than was seen in American Samoa. Only three clusters were identified as hotspots for the adult data. Again, the seroprevalence was higher than FTS, but not by much. For all ages, three clusters were identified as hotspots, and followed the same trend as the other two age groups.

For Tanzania child data, four hotspots were identified. Interestingly, one cluster was a hotspot for FTS but had 0% seroprevalence. Another cluster had a lower

seroprevalence than antigen prevalence. The same trend can be seen in adult data, where out of 32 identified hotspots, eight had 0% seroprevalence. Five clusters had a seroprevalence less than the FTS prevalence. This could be more evidence that transmission has been significantly decreased and/or disrupted in Tanzania. The FTS prevalences may be due to old infections that have since been treated. The same trend can be seen in the 30 hotspots identified with all ages together. This is further evidence that Tanzania is an outlier, and that age categories should remain separate.

Individual-level logistic regression

For American Samoa child data, Wb123 seropositivity and years lived in the village were selected model predictors while Wb123 was the only significant predictor. For adults and all ages, only Wb123 was a selected and significant model predictor, but the overall model fit was better than that of the children's model. This could be because there were generally more FTS cases in the adult age group, so the relationship was more defined. This can also be seen in the odds ratio estimates and confidence intervals for Wb123; the estimate was less precise in children.

A similar trend was seen in the models for the Philippines. All model fits were significant, while the children's model fit was the worst of the three. Model fit improved for adults and was the best for all ages. Interestingly, adults had the highest odds ratio, but the widest confidence intervals, likely due to the smaller sample size of adults for this country.

Tanzania was again an outlier for this analysis. Similar to the other two countries, Wb123 seropositivity was the only variable selected for the models. The children's

overall model was not significant, but the adults and all ages models were significant. The odds ratio and Wb123 parameter were not significant in the children's model, but were significant in the adults and all ages models, with narrow confidence intervals compared to the other two countries. Tanzania is likely an outlier in model fit due to the fact that it passed the TAS-2 and had very few FTS and Wb123 seropositive individuals compared to the other two countries. Logistic regression models do not run well when any of the crosstab cell sizes are too small. Bias is introduced that distorts the values of odds ratios away from the null (OR=1), so these estimates may seem significant when they are not (Nemes et al., 2009). The same, to a lesser degree, can be said for the other two countries; odds ratio estimates for small sample sizes are difficult to interpret. Logistic regression has proven to be a powerful tool for LF surveillance in the past, as it has been previously used to generate population at risk maps (Cromwell et al., 2020; Lindsay & Thomas, 2000). Incorporating antibody data into risk analyses and maps could improve accuracy since it measures exposure risk instead of actual cases.

III. Limitations and Strengths

The analysis of epidemiologic datasets can come with a long list of limitations and caveats, as opposed to randomized controlled trials with fewer limitations. The three datasets used in this thesis were from American Samoa, Philippines, and Tanzania, but there was an additional dataset from Haiti that was not used in the analysis. The CDC and analysts at the NTD Support Center were unable to establish antibody cutoffs for Haiti due to the quality of the data, so the dataset was put aside. The three other

datasets also had many problems that are worth considering when interpreting any results from these analyses.

American Samoa had the most robust dataset of the three. The Wb123, Bm14, and Bm33 antibody cutoffs were already established and implemented into the dataset, so there were fewer questions of validity. Additionally, while there was still a high degree of missingness, this dataset had the highest amount of usable xenomonitoring mosquito data. The analyses using this dataset were more robust and encountered fewer errors than the analyses from the other two countries. This could be partially due to the fact that American Samoa had many more FTS-positive cases than the other countries, so more analyses were possible without the case number limitations.

The Philippines dataset most notably did not have xenomonitoring mosquito data. This variable was unable to be used in the regressions, so those potential effects were not examined. This dataset also only had 54 FTS-positive cases, whereas American Samoa had 129. This is likely due to the fact that the Philippines was closer to passing this TAS-2 than American Samoa was. Another issue was with the Wb123, Bm14, and Bm33 seropositive cutoffs. The CDC and the NTD support center were unable to establish the cutoffs for Wb123 definitively, while no cutoffs were attempted for Bm14 or Bm33. The decision to sort the quantitative antibody results into deciles was the most reasonable, but likely does not represent the true cutoffs for these antibody markers. Recent work with malaria has called into question the assumptions accepted while establishing serology cutoffs and methods are being developed to analyze this data without the need for cutoffs (Kyomuhangi & Giorgi, 2022).

The Tanzania dataset also had issues with missing data. Almost all of the values for years lived in the village and travel were missing, which can be attributed to the fact that these questions were not asked during the school-aged children survey. They were asked during the community-level survey, but the sample size was much lower. The sample size for the adults age group was too small to do many robust analyses, particularly at the cluster level since most clusters had less than 20 individuals.

Lastly, there was an inherent omission in the individual-level logistic regression analyses. Some individuals sampled came from the same household and were not truly independent of each other. Future analyses would need to include hierarchical logistic regression to account for different levels of correlation within the data. The results obtained from these analyses likely had narrower confidence intervals than the hierarchical analyses.

IV. Implications

This thesis demonstrated that antibody data can be a valuable tool in LF surveillance and in identifying regions of ongoing transmission. While most of the analysis techniques came with a fair number of caveats related to biases and issues with the data, they were an important next step in improving the utility of the TAS for later surveillance efforts. This thesis also highlighted the need for consistent data collection techniques in the field, though the logistics of this are difficult to implement due to potential unforeseen circumstances, available resources, and human error.

V. Recommendations

This project examined three real-world datasets from an LF TAS-2 from 2016-2017. Many of these analyses were exploratory, meaning we were determining what was possible with the data we had. In future TAS surveillance, more variables could be incorporated into these datasets, possibly distance to water, more mosquito data, or more detailed demographic information. This would increase the robustness of our current analyses and the variety of possible analyses.

VI. Conclusion

The main problem addressed in this thesis was that current LF surveillance methods (i.e. transmission assessment surveys) are not capable of detecting hotspots low levels of transmission or exposure. We used TAS-2 datasets from three countries (American Samoa, Philippines, and Tanzania) to show through data analysis that antibody data may be a useful tool to detect hotspots of ongoing LF exposure, when used in combination with antigen test results that pick up active cases. LF antibody data did not always correlate with areas of high antigen prevalence, indicating areas of high exposure with low actual case prevalence. In Tanzania, this relationship was not as clear because there were generally low numbers of cases due to this country passing the TAS-2.

Previous studies have shown the value of mapping the population at risk of this disease, and this study has shown the potential value of antibody data in evaluating risk of exposure. In the future, these methods could be validated and expanded upon as more data from TAS becomes available.

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