Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world-wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Justin Willingham Date

Serological Assessment of Malaria and Lymphatic Filariasis in the Dominican Republic

By

Justin Willingham

MPH

Epidemiology

_____

Lauren Christiansen-Lindquist, PhD, MPH

Committee Chair

_____

Gregory S. Noland, PhD, MPH

Committee Member

Serological Assessment of Malaria and Lymphatic Filariasis in the Dominican Republic

By

Justin Willingham

B.S.

Appalachian State University

2010

Faculty Thesis Advisor: Lauren Christiansen-Lindquist, PhD, MPH

An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology

2019

Abstract

Serological Assessment of Malaria and Lymphatic Filariasis in the Dominican Republic

By Justin Willingham

Background:
The island of Hispaniola, comprised of the Dominican Republic and Haiti, is the only island in the Caribbean with active malaria transmission and accounts for 95% of the lymphatic filariasis (LF) burden in the Americas.

Methods:
A cross-sectional, household cluster survey was conducted in *bateyes*—agricultural settlement villages home to Haitian migrant workers, their descendants, and ethnic Dominicans—in the Dominican Republic from March to April 2016 to estimate the prevalence of malaria and LF by rapid antigen test and microscopy. The study also included collection of dried blood spots from one adult and one other randomly selected household resident of any age to estimate the seroprevalence of malaria and LF antibodies using a multiplex bead assay.

Results:
This study reports serology results from 1,331 samples with matched diagnostic results (median age: 34 years; range: 2—96). Although no (0%) person was *Plasmodium*-positive by RDT or microscopy, overall seroprevalence to *P. falciparum* antigens MSP-1, AMA-1, CSP, and LSA-1 was 16.9%, 10.0%, 1.5%, and 1.2%, respectively. Seroprevalence of long-lived antibodies MSP-1 and AMA-1 increased with age from 2.5% and 1.3%, respectively, in those <10 years of age to 23.8% and 19.1% in those ≥60 years of age. Seroprevalence of short-lived antibodies CSP and LSA-1 was uniformly low across age groups. For LF, six individuals were FTS-positive (0.5%), but none (0%) were microfilariae-positive. Overall seroprevalence to Wb123, Bm14, and Bm33 antibodies was 1.3%, 16.2%, and 7.7%, respectively. Seroprevalence to Bm14, but not Bm33 or Wb123, increased with age. Univariate analysis for malaria seropositivity (MSP-1) revealed significantly higher odds of infection in the Southwest region and among those without access to a bed net, but no significant difference by ethnicity, when considered by itself. Multivariable analysis for malaria seropositivity (MSP-1) revealed that ethnicity was significantly associated with infection when considered alongside other factors.

Conclusion:
These results indicate very low recent exposure to malaria and LF in the Dominican Republic and provide important data to prospectively monitor transmission elimination in Hispaniola.

Serological Assessment of Malaria and Lymphatic Filariasis in the Dominican Republic

By

Justin Willingham

B.S.

Appalachian State University

2010

Faculty Thesis Advisor: Lauren Christiansen-Lindquist, PhD, MPH

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology

2019

Acknowledgements

I would first like to thank my field advisor, Dr. Noland, for the opportunity to work on this project and his insight and guidance throughout. I would also like to express my gratitude for my faculty advisor, Dr. Christiansen-Lindquist, for her willingness to take on this project and coach me through the process. I sincerely appreciate all the hard work and assistance of those at the Carter Center and CDC who conducted the survey and serology testing that made my thesis possible, particularly Dr. Eric Rogier, Hunter Keys, and Manuel Gonzales. I lastly want to thank my girlfriend, Carrie Ripkey, for the endless string of "does this sound right?" and "does this make sense?".

Table of Contents

Abbreviations

EDC	1-ethyl-3-[3'-dimethylaminopropyl] carbodiimide hydrochloride
AMA	Apical Membrane Antigen
BSA	Bovine Serum Albumin
CDC	Centers for Disease Control and Prevention
CENCET	Centro Nacional Para El Control De Las Enfermedades Tropicales
CSP	Circumsporozoite Protein
CI	Confidence Interval
DDT	Dichlorodiphenyltrichloroethane
DEC	Diethylcarbamazine
DALY	Disease-Adjusted Life Years
CONABIOS	Dominican Consejo Nacional de Bioetica en Salud
DR	Dominican Republic
FTS	Filariasis Test Strip
GPELF	Global Programme to Eliminate Lymphatic Filariasis
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated bed Net
LDH	Lactate Dehydrogenase
LSA-1	Liver Stage Antigen-1
LLIN	Long-Lasting Insecticidal Nets
LF	Lymphatic Filariasis
MDA	Mass Drug Administration
MFI	Median Fluorescence Intensity
MSP1	Merozoite Surface Protein 1
OBMICA	Observatorio Migrantes del Caribe
OR	Odds Ratio
PBS	Phosphate-Buffered Saline
PPS	Proportional to Population Size
RDT	Rapid Diagnostic Test
RBC	Red Blood Cells
RT	Room Temperature
NaP	sodium phosphate
sulfo-NHS	sulfo N-hydroxysulfosuccinimide
TAS	Transmission Assessment Surveys
TPE	Tropical Pulmonary Eosinophilia
WHO	World Health Organization

Chapter I: Background

Malaria

Malaria is an intracellular parasitic disease transmitted through the bite of female *Anopheles* mosquitoes. In 2017, there were an estimated 219 million cases worldwide, spread over 90 countries, resulting in 435,000 deaths. This is compared with an estimated 217 million cases in 2016 and 239 million cases in 2010 (1). Five species of the genus *Plasmodium* are known to cause malarial infection in humans: *P. falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax*. Infections can be caused by any one of these species individually, but mixed species infections have been found to be frequent in endemic areas (2, 3). *P. falciparum* and *P. vivax* infections are the most common worldwide, with *P. falciparum* infections the most serious threat to public health due to its potential for severe and fatal disease (3).

Malaria has been described in documents as early as 2700 BCE in China and has been featured in the writings of notorious authors such as Homer, Plato, Chaucer, and Shakespeare (4). The parasite was first discovered in a patient's blood in 1880 by Alphonse Laveran, but the sexual stages were not discovered for another seventeen years (5). Ronald Ross would later discover that *Plasmodium* is transmitted through the bite of an infected "brown" mosquito and began to develop a transmission cycle for the parasite, eventually earning him the Nobel Peace Prize in 1902 (6). Following Ronald Ross' discovery in 1897, a group of Italian malariologists were the first to demonstrate that human malaria was transferred through the anopheline mosquitos (5).

Once prevalent throughout the globe, Malaria has been eliminated in Canada, Europe, Russia, and the United States (2). The World Health Organization (WHO)

reports the greatest burden of malaria in sub-Saharan Africa, followed by South-East Asia, Eastern Mediterranean, Western Pacific region, and the Americas (7). Failed efforts to eliminate malaria worldwide in the 1950s through the 1980s, have resulted in mosquitoes resistant to Dichlorodiphenyltrichloroethane (DDT) and malathion as well as *Plasmodium* resistant to chloroquine and pyrimethamine (8).

Biology:

Of the five species of Plasmodium known to cause disease in humans, *P. falciparum*, *P. ovale*, and *P. vivax* are known to have the greatest reliance on humans as their primary reservoir. *P. malariae* is common to man, African apes, and probably some South American monkeys (3). *P. knowlesi* has been documented to infect humans in parts of Southeast Asia, but its natural host is the monkey *Macaca fascicularis* (4). The malaria parasite has a life cycle well-adapted to survive in both the mosquito vector as well as the vertebrate host. The cycle begins with the sporozoite form of the parasite being transmitted through the blood meal of an infected female Anopheles mosquito (Figure 1). The sporozoites enter the capillary bed of the host and subsequently travel through the venous blood stream to invade hepatocytes. Once in the liver, the sporozoites' nuclei replicate and develop into a hepatic schizont, taking anywhere from 5 to 15 days to form (4). The continuing division and ingestion of cell contents leads to the eventual rupture of the cell, spilling thousands of merozoites into the bloodstream. Once in the bloodstream, merozoites attach to erythrocyte surface receptors and infiltrate the red blood cells (RBCs). The enveloped merozoite differentiates into a trophozoite that multiplies and causes mechanical strain on the cell, leading to hemolysis (4).

The release of a new generation of merozoites into the blood stream starts the cycle over, during which a parasite population can increase 6 to 20-fold each cycle. The length of this secondary cycle varies amongst *Plasmodium* species, with *P. malariae* taking 72 hours, *P. falciparum*, *P. ovale*, and *P. vivax* taking approximately 48 hours, and *P. knowlesi* only 24 hours (2, 4). The characteristic clinical presentation of undulating fever with chills seen in malaria patients is a result of this periodic release of merozoites and pyrogens from ruptured red blood cells.

Some of the merozoites produced in this cycle differentiate into male and female forms called gametocytes. When another mosquito feeds on this infected host, the gametocytes are ingested in the blood meal and travel to the mosquito's stomach. After emerging from the red blood cell, the male and female gametes fuse, producing a zygote. The newly formed zygotes will then elongate and form ookinetes which will penetrate through the stomach wall. Once through the wall, the ookinete develops into an oocyst, forming more than 10,000 sporozoites (4). The oocyst then ruptures and releases the sporozoites into the coelomic cavity, which will migrate to the salivary glands, ready to start another life cycle. Once the female anopheline mosquito is infected with malaria, it will remain so for the rest of its life, spreading the sporozoites with each additional blood feed (4).

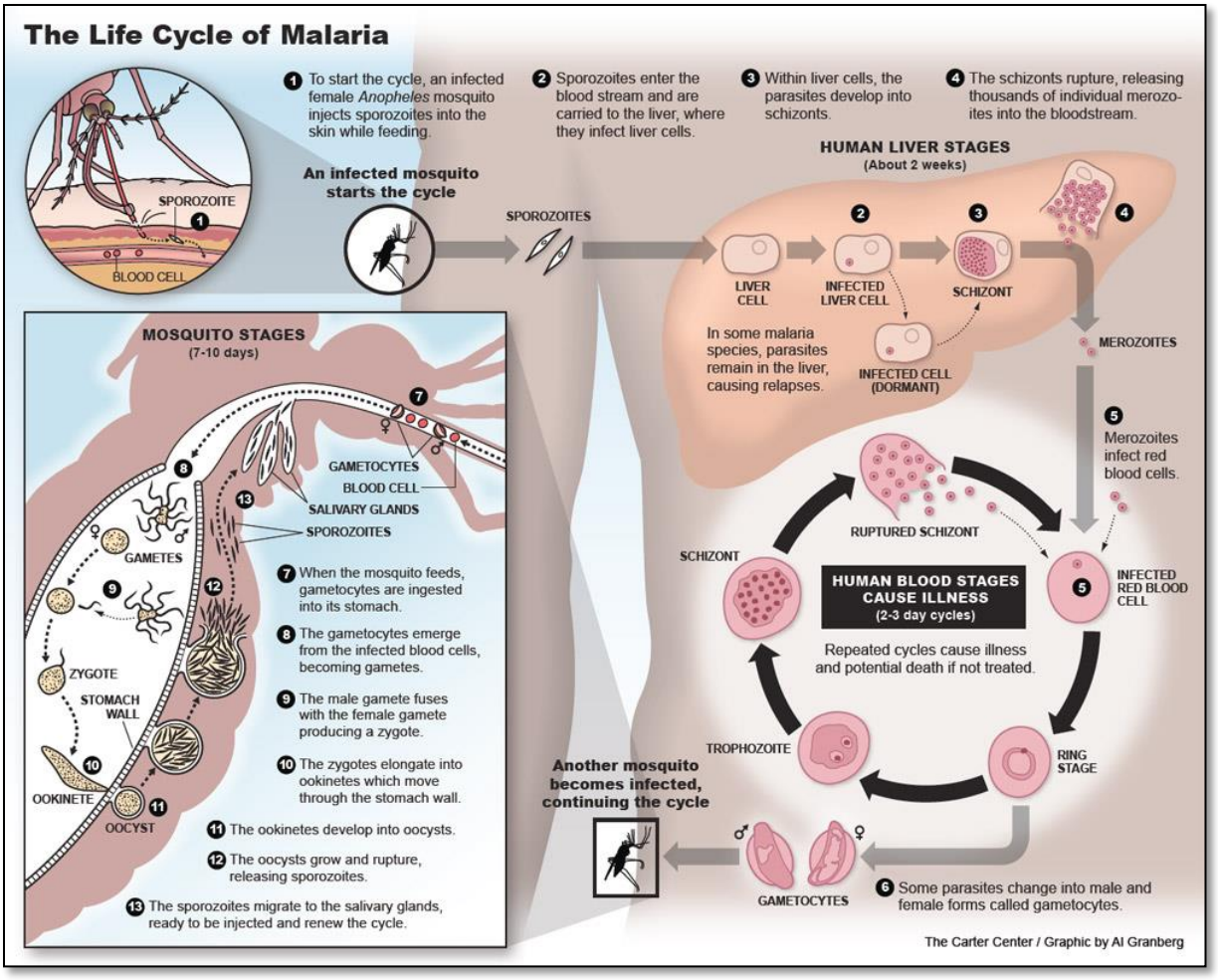


Figure 1. Life Cycle of Malaria (The Carter Center, graphic by Al Granberg)

Serology:

Multiplex serology is a newly developed method that detects antibodies to multiple antigens simultaneously, allowing for the evaluation and monitoring of several diseases (9). When using this method to test for malaria, different antigens are targeted at different stages of the parasite's lifecycle (Figure 1). Some antigens that have been used are: merozoite surface protein 1 (MSP1), circumsporozoite protein (CSP), liver stage antigen-1 (LSA-1), and apical membrane antigen (AMA).

To make the transition from the end of one cycle to the start of another, one antigen, a multifunctional surface protein called circumsporozoite protein, plays an integral role in allowing the *Plasmodium* sporozoite to travel from the mosquito's midgut to the human liver. The protein has been shown to have two different conformational states, allowing it to have two main functionalities. One conformation allows for a migratory state, in which the sporozoite can travel to the target organ of the host, while the other conformation assists in sporozoite development and hepatocyte invasion (10).

After hepatocyte invasion, *P. falciparum* infections also lead to the production of another targeted antigen, Liver Stage Antigen-1. This antigen accumulates in the parasitophorous vacuole within the infected hepatocyte, surrounding thousands of replicating merozoites (11). Although its role in parasite development has not been determined, it is believed to be involved in liver schizogony and merozoite release. LSA-1 has been well documented to evoke specific humoral, cellular, and cytokine immune responses (12).

Due to its presence in all species of *Plasmodium*, merozoite surface protein-1 has been used as a target antigen to identify *P. falciparum*, *P. malariae*, and *P. vivax* infections. MSP1 is a major protein component on the surface of the merozoite, the parasitic form involved in erythrocyte invasion. The surface protein undergoes two proteolytic maturation steps, which are essential for its ability to invade and infect erythrocytes (step 5) after its release from hepatocytes that rupture in the host (13).

The last antigen of note is the Apical Membrane Antigen 1, a micronemal protein that is found on the surface of both merozoites and sporozoites. Experimental evidence has suggested that this protein plays a role, when interacting with another parasitic protein, in

merozoite invasion of RBCs and sporozoite invasion of hepatocytes (14). Because all of these antigens are believed to play critical roles in initiating and mediating important stages in the parasite's lifecycle, they are also of great interest and the subjects of current studies to identify potential vaccine candidates (14).

Clinical Manifestation:

The symptomatic stage of infection begins 6 to 8 days after the parasite escapes from the hepatocyte and into the blood stream, reaching a parasite density of approximately 50/μL (2). The release of the parasites and erythrocytic components from the ruptured hepatocyte and the activation of the cytokine cascade are largely responsible for many of the signs and symptoms of an uncomplicated malaria infection (15). Although the pathogenesis and severity of disease can vary among *Plasmodium* species, the presentation of symptoms are not sufficient on their own to make species identification and need additional laboratory studies to confirm diagnosis (3).

An initial untreated attack can last anywhere from a week to a month, potentially longer, but varies drastically depending on the host's immune status and the *Plasmodium* species involved (3,15). Fever is the cardinal feature of malaria but is most commonly accompanied with a range of non-specific symptoms such as headache, fatigue, muscle aches, and abdominal discomfort (15). These symptoms are frequently accompanied by nausea, vomiting, orthostatic hypotension, and profuse sweating. Some cases will result in physical manifestations such as hepatomegaly and splenomegaly which in rare cases can lead to rupture (15). Human malarial infection, excluding those by *P. falciparum*, are usually not life-threatening and parasitaemias are typically less than 1%. However, there have been increasing reports of more severe cases of *P. Vivax* and *P. knowlesi* (2, 15).

Following an afebrile period, the flu-like syndrome can recur daily, every other day, or every third day depending on the synchronized schizogony (3, 15). Between these febrile periods, infected individuals are typically afebrile and feel well. If the condition goes untreated, the attacks can range from weeks to months and then spontaneously resolve over time (15).

After initial Infections with *P. vivax* and *P. ovale*, relapses can occur from weeks to years after a latent period with no parasitemia, depending on geographical ethnicity (2, 3). Reinfection of the blood stream by merozoites occurs when hypnozoites awake from dormancy, resulting in renewal of clinical symptoms and parasitemia. The clinical manifestation mimics the initial attack but is typically milder, shorter in duration, and has a more abrupt onset with absence of irregular fever (15). Infections with *P. malariae* may persist for life with or without recurrent febrile episodes (3).

Although other species of *Plasmodium* can lead to complications, almost all severe cases and deaths resulting from malaria are caused by *P. falciparum* (16). The manifestation and degree of severity within these cases are largely dependent upon the individual's immune status and age (2). *P. falciparum* has some variations in its life cycle compared to other *Plasmodium* species, which can result in major complications such as: cerebral malaria, pulmonary edema, acute renal failure, severe anemia, parasitemia, acidosis, and hypoglycemia (8, 16). Some complications such as coma and acidosis occur indiscriminately of age but some manifestations occur more commonly in certain age groups. Children are more prone to severe anemia and hypoglycemia, whereas acute pulmonary edema, acute kidney injury, and jaundice are more common among adults (2). The case fatality rate of children and non-immune individuals with

uncomplicated falciparum malaria is approximately 0.3%, increasing to 15-20% once complications appear. If left untreated, severe *falciparum* malaria cases are almost always fatal (3).

Lymphatic Filariasis

Lymphatic Filariasis (LF) is a neglected tropical parasitic disease transmitted by the bite of an infected mosquito. The mosquito genera responsible for transmission varies based on geographical location, the 4 genera include: *Aedes*, *Anopheles*, *Culex,* and *Mansonia* (17). The disease is caused by three closely related nematodes from the family Filariodidea: *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* (18). *W. bancrofti* is the most widespread of the three parasites accounting for approximately 90% of LF cases and is endemic to Africa, Asia, the Caribbean, parts of Latin America, and the Pacific Islands (19). The remaining cases are caused by Brugian parasites, most common to Southeast Asia and several western Pacific Island groups (3, 4).

Currently, 856 million people remain at risk for LF in 52 countries worldwide (17). The most recent data suggest that there are around 67.88 million people currently infected with LF in 73 countries. Of the 67.88 million individuals currently infected, 19 million suffer with genital hydrocele and 16 million report varying degrees of lymphoedema (20). Although LF is not typically associated with mortality, the WHO states that LF accounts for 2 million disease-adjusted life years (DALYs) as determined in 2016 (21).

Biology:

The lifecycle of all three parasites have five distinct morphological stages (Figure 2). The cycle begins with the disposition of L3 larvae in the skin of a host following the bite of an infected mosquito (18). The larvae then travel to the site of puncture and

migrate to the vessels and nodes of the lymphatic system. After reaching the lymphatic system, the process of molting results in the development of L4 larvae. These larvae migrate centrally in the lymphatic system and eventually develop into sexually-mature adults worms, taking around 6-9 months (19). The adult worms appear thread-like and vary in size between sexes, with males approximately 40mm in length and females around 100mm (4).

After fertilization, adult female worms release up to 10,000 microfilariae per day into the host's blood stream. Microfilariae are approximately 250 μm in length and 10 μm in width and are most abundant in circulation at night, referred to as nocturnal periodicity. During the day they remain in deep vascular beds, which is suspected to increase the survival period for the microfilariae to around 9 months (4). Once in the host blood stream, the microfilariae are able to be consumed by a secondary mosquito during a blood meal and taken to the mosquito's gut where it will penetrate the gut wall and migrate to the thoracic muscle (18). Once in the muscle, the microfilariae are able to undergo two molts, transitioning to L2 and finally L3 to return to its infectious form in around 10 – 14 days (19).

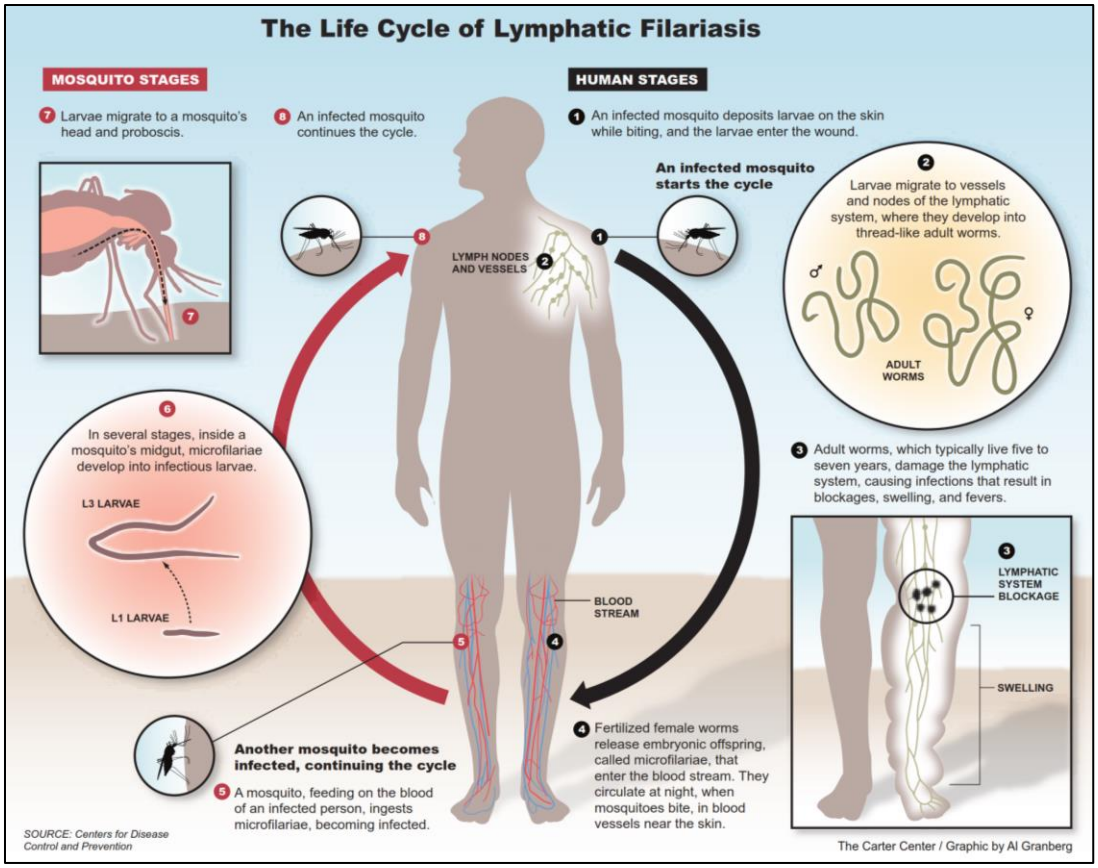


Figure 2. Life cycle of Lymphatic Filariasis (The Carter Center, graphic by Al Granberg)

Serology:

As with malaria, multiplex serology can detect several different antibodies to targeted antigens, serving as a reliable surveillance tool. Two such target antigens used to detect Lymphatic Filariasis are Bm14 and Bm33. Bm14, also known as SXP-1, belongs to a family of related genes that encode proteins that are strong immunogens in many parasitic infections (22, 23). Studies have shown that Bm33, also known as Bm-AP-1, is thought to be present throughout the adult and larval portions of the parasite's lifecycle, suggesting it is not a stage-specific antigen. Although its particular role is unknown, evidence suggests it may play protective role for the parasite and cause tissue damage in the host (24).

Although both Bm14 and Bm33 have been proven to be effective markers of infection, they also have been shown to have cross-reactivity with other related filarial parasites (25). The cross activity seen with the antigens has both advantages and disadvantages. The cross reactivity can allow for detection of both *W. bancrofti* and *B. malayi*, but also has also been shown to be reactive with other filarial parasites such as *Onchocerca volvulus*, *Loa loa*, and *Mansonella perstans* limiting its usefulness in areas where such parasites are prevalent (23). Notably, Bm33 appears earlier than other antigens, allowing the detection of filarial parasites before other targeted antigens are detectable (26).

Another target antigen used to detect LF is wb123, which contains 372 amino acids and is thought to belong to the serpin family of proteins. This family of proteins are serine protease inhibitors that have been found to be highly immunogenic in humans (27). The advantage of testing for this antigen over the other two is that it has a high sensitivity and specificity for Wb infections, resulting in no cross reactivity with other filarial parasites (27). Because of this attribute, Wb123 works as an effective target in both diagnosing individual Wb cases as well as an early serologic marker to confirm LF in surveillance operations in areas of geographical overlap with other filarial infections (25, 27).

Clinical Manifestation:

Lymphatic filariasis can present with both clinical and subclinical manifestations, the most common and well-known being elephantiasis, hydrocele, and lymphoedema (18). The three different species that are responsible for LF present with very similar signs and symptoms except for urogenital disease and chyluria which are not present with *Brugia*

infections (19). In regions where LF is endemic, clinical manifestation can be broken down into five categories: 1) Not infected, but exposed; 2) Infected and asymptomatic; 3) Acute filarial disease (with or without microfilaremia); 4) Chronic infection with lymphostasis; and 5) Tropical pulmonary eosinophilic syndrome (3, 18).

Most individuals infected with LF are asymptomatic even though they have been found to have microfilariae or filarial antigen in their peripheral blood (19). These individuals are carriers of the parasites and play a role in the continual transmission in the population (28). Although they may not present with signs or symptoms, better imaging techniques have shown that almost all individuals with active infection have some level of lymphatic abnormality (29). Although the majority of those infected are asymptomatic, approximately 30-40% of those infected will present with clinically apparent cases (29).

In cases of acute filarial disease, individuals present with recurrent fever accompanied by inflammation of lymph nodes, most commonly the inguinal, axillary, and epitrochlear nodes (28, 29). Additionally, infections of W. bancrofti tend to spread to males' genitalia, resulting in funiculitis, epididymitis, and/or orchitis (28). Two mechanisms thought to cause these clinical features are an inflammatory immune response to the dead or dying adult worm and the development of a plaque-like lesion of cutaneous or sub-cutaneous inflammation that is accompanied by ascending lymphangitis and regional lymphadenitis (28).

Chronic forms of LF often take years to develop, causing debilitating damage that is detrimental to a person's health as well as having social and economic consequences (20). The main clinical sequelae of the chronic form of disease are: hydrocele,

lymphoedema, chyluria, and elephantiasis of the limbs, breasts, and genitalia (3). The pathologic cause of these symptoms is thought to be a granulomatous reaction upon death of the adult worm in which the hosts' inflammatory response to the parasites and its secreted antigens induce dilation and thickening of the lymphatic vessels (28, 29). To prevent these possible sequelae, recent evidence suggests a combination of three medications (albendazole, ivermectin, and diethylcarbamazine citrate) can safely clear microfilariae from infected individuals within weeks of drug initiation (30).

The last clinical category of LF is tropical pulmonary eosinophilia (TPE). The syndrome presents with paroxysmal cough, nocturnal wheezing, weight loss, adenopathy, low-grade fever, and eosinophilia (18). The syndrome has a higher prevalence in males, with a male to female ratio of 4:1, and is most common among men ages 20-40 (19). Although the pathogenesis is uncertain, it is theorized to be an allergic type hypersensitivity reaction to the microfilariae in the pulmonary blood vessels. If left untreated, infected individuals can progress to chronic restrictive lung disease with diffuse interstitial fibrosis (19).

Vector Distribution

Although both diseases are transmitted by mosquitos, malaria is transmitted by the *Anopheles* genus in all regions, whereas LF varies between geographical locations. The *Anopheles* mosquitos is a shared vector for both diseases in much of West Africa, eastern Mediterranean, Southeast Asia, and the western Pacific regions. Elsewhere, members of the *Culex pipiens* complex and *Aedes* genera are the principle vectors of LF, with *Culex quinquefasciatus* playing an important role in the Americas and urban areas of east Africa (31). The similarities that exist between malaria and LF transmission vectors,

being of the same genus or related genera, can be used to help control the diseases in areas of overlapping distribution. In these areas, vector control interventions such as long-lasting insecticidal nets (LLINs) or indoor residual spraying (IRS) can aid in the attempts to reduce and eradicate both diseases simultaneously (31).

Dominican Republic

The island of Hispaniola, divided into the Dominican Republic (DR) and Haiti, is the only remaining island in the Caribbean with active malaria transmission and also accounts for 95% of the LF burden in the Americas (32). In the DR, Malaria is primarily caused by *P. falciparum* and its principal vector is the mosquito *Anopheles albimanus*. The DR has experienced a decline in malaria since 2005, with the exception of surges seen after an earthquake that struck neighboring Haiti in 2010. As a result, the DR is currently classified as being in the pre-elimination phase for malaria (33). Since the surge in 2010, the total number of confirmed cases in the country has fallen from 1,616 cases in 2011 to 398 cases in 2017 (7). Haiti has also seen a decline in malaria in the last decade, with a 90% decrease in reported cases between 2010 and 2018 (33).

The Global Programme to Eliminate Lymphatic Filariasis (GPELF), which was launched in 2000, set a goal of eliminating LF as a public health problem by 2020. This is defined as reducing infection prevalence in an area to below target thresholds and providing the appropriate care to patients with lymphoedema and/or hydrocele (34). To reach these goals the WHO has recommended annual Mass Drug Administration (MDA) of albendazole in combination with ivermectin or diethylcarbamazine (DEC) to all populations living in endemic areas. In the DR, baseline mapping revealed that LF transmission was restricted to 19 municipalities in three regions: The East, La Ciénaga in

Santo Domingo, and the Southwest (32). By 2018, all three regions had passed at least one transmission assessment survey (TAS), meaning that all previously endemic areas qualified to stop MDA. Haiti has seen similar progress, with 118 of the 140 communes (84%) meeting the criteria to stop MDA (32).

In 2009, both Haiti and the DR committed to bi-national elimination of malaria and LF by 2020. The decrease in prevalence of both malaria and LF highlights the need for additional tools that can be used to monitor low levels of disease prevalence as transmission declines and programs near elimination endpoints. Since antibody detection has a higher sensitivity in comparison to microfilaria (mf) and antigen detection, there is potential for antibody response to be used as an earlier signal of recrudescence or decreased transmission over time (35). Although more information is needed to understand how to appropriately interpret responses to different antibody markers, antibody response has the potential to be a useful tool to complement current surveillance methods (35).

Chapter II: Manuscript

Introduction

Malaria and lymphatic filariasis (LF) are mosquito-transmitted parasitic diseases that cause the highest global health burden of all vector-borne diseases (31). In 2017, there were an estimated 219 million cases of malaria spread over 90 countries worldwide that resulted in 435,000 deaths (7). The most recent data on LF suggest there are around 67.88 million people currently infected in 73 countries. Of these, 19 million suffer with genital hydrocele and 16 million report varying degrees of lymphoedema (20). Five species of the genus *Plasmodium* are responsible for malarial infections in humans, while LF is caused by three different nematodes: *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. The two diseases overlap in their distribution in most of Africa, Southeast Asia, and some parts of Latin America (31).

Although both diseases are transmitted by mosquitos, malaria is transmitted by the *Anopheles* genus in all regions, whereas the LF vector varies by geographical location. The *Anopheles* mosquitos is a shared vector for both diseases in much of West Africa, eastern Mediterranean, Southeast Asia, and the western Pacific regions. Elsewhere, members of the *Culex pipiens* complex and *Aedes* genera are the principle vectors of LF, with *Culex quinquefasciatus* playing an important role in the Americas and urban areas of east Africa (31). The fact that malaria and LF share mosquitos as a common transmission vector can be used to help control the diseases in areas of overlapping distribution (36, 37). In these areas, vector control interventions such as long-lasting insecticidal nets (LLINs) or indoor residual spraying (IRS) can aid in the attempts to reduce and eradicate both diseases simultaneously (31).

The island of Hispaniola, divided into the Dominican Republic (DR) and Haiti, is the only remaining island in the Caribbean with active malaria transmission and also accounts for 95% of the LF burden in the Americas (32). In the DR, Malaria is primarily caused by *P. falciparum* and its principal vector is the mosquito *Anopheles albimanus*. The DR has experienced a decline in malaria since 2005, with the exception of surges seen after an earthquake that struck neighboring Haiti in 2010. As a result, the DR is currently classified as being in the pre-elimination phase for malaria (33). Since the surge in 2010, the total number of confirmed cases in the country has fallen from 1,616 cases in 2011 to 398 cases in 2017 (7). Haiti has also seen a decline in malaria in the last decade, with a 90% decrease in reported cases between 2010 and 2018 (32).

The Global Programme to Eliminate Lymphatic Filariasis (GPELF), which was launched in 2000, set a goal of eliminating LF as a public health problem by 2020. This is defined as reducing infection prevalence in an area to below target thresholds and providing the appropriate care to patients with lymphoedema and/or hydrocele (17). To reach these goals the WHO has recommended annual Mass Drug Administration (MDA) of albendazole in combination with ivermectin or diethylcarbamazine (DEC) to all populations living in endemic areas, with the goal of stopping transmission. In the DR, baseline mapping revealed that LF transmission was restricted to 19 municipalities in three regions: The East, La Ciénaga in Santo Domingo, and the Southwest (32). By 2018, all three regions had passed at least one transmission assessment survey (TAS), meaning that all previously endemic areas qualified to stop MDA. Haiti has seen similar progress, with 118 of the 140 communes (84%) meeting the criteria to stop MDA (32).

In 2009, both Haiti and the DR committed to bi-national elimination of malaria and LF by 2020. The decrease in prevalence of both malaria and LF highlights the need for additional tools that can be used to monitor low levels of disease prevalence as transmission declines and programs near elimination endpoints. Since antibody detection has a higher sensitivity in comparison to microfilaria (mf) and antigen detection, there is potential for antibody response to be used as an earlier signal of recrudescence or decreased transmission over time (35). Although more information is needed to understand how to appropriately interpret responses to different antibody markers, Antibody response has the potential to be a useful tool to complement current surveillance methods (35).

The primary goal of this study is to investigate the sero-prevalence of antibodies to multiple malaria and LF antigens in three main agricultural areas of the Dominican Republic. In the DR, malaria and LF are historically associated with agricultural regions on the border with Haiti, which rely heavily on migrant farm workers from the neighboring country. To capture this target population, the survey was conducted in *bateyes*, agricultural settlement villages adjacent to vast sugar cane and agricultural plantations (38). The secondary objective of this study is to identify risk factors associated with malaria seropositivity.

Methods

Study design:

Samples for this study were obtained in the context of a study to determine the prevalence of malaria and LF in *bateyes* of the DR (38). In brief, a cross-sectional multi-stage cluster survey was conducted in the Dominican Republic between March 19th and

April 24th, 2016. The target population included all residents of *bateyes* in the Dominican Republic. The sampling frame for the study was based on a nationwide census of *bateyes* conducted by the Observatorio Migrantes del Caribe (OBMICA) in 2012. After excluding the capital region, which was thought to be less reliant on migrant labor due to its waning importance in agricultural production, the survey designated the Southwest, East, and North regions as individual strata, with the goal of obtaining representative results within each region. Across the three regions, a total of 51 clusters (*bateyes*), or 17 *bateyes* within each region, were selected from a random start using probability of selection proportional to population size (PPS). Within each *batey*, 15 households were then systematically selected from a random start using a map of the households created by field staff before surveying. The sample population was defined as consenting individuals present in selected households, within selected *bateyes*, at the time of survey collection. The survey was sponsored by the Carter Center and conducted in collaboration with the Dominican Ministry of Public Health (*Ministerio de Salud Pública*) / Centro Nacional Para El Control De Las Enfermedades Tropicales (CENCET).

Data Collection:

The study comprised two components: a household-level questionnaire and blood sampling. Survey participants were required to be at least 18 years of age and self-identify as either head-of-household or spouse of head-of-household. Participants that met these criteria and gave verbal informed consent were then asked to complete the survey in their preferred language, Haitian Kreyòl or Spanish. The questionnaire included questions on: demographics, travel, fever and illness, treatment-seeking behavior, knowledge and practices regarding malaria and LF. Data were recorded by the

interviewers in the field using Eagle Survey software (The Carter Center, mobile v.1.3.3) on Samsung GalaxyTab3 tablets.

Blood samples were collected from the questionnaire participant and one other randomly selected individual of any age within the household. Laboratory technicians collected finger-prick samples to diagnose malaria (by rapid diagnostic test (RDT) and microscopy) and LF (by filariasis test strip (FTS)) infection as well as filter paper dried blood spot samples for serological analysis. Filter paper samples were labeled with participant study ID, stored in zip-lock bags and stored at the CENCET laboratory in the DR. Samples were then sent to the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA for analysis.

Ethical approval:

The study was approved by the ethical review board of the Dominican Consejo Nacional de Bioetica en Salud (CONABIOS) and declared a non-research public health activity by Emory University.

Serology assays for anti-malaria antibodies and malaria antigens:

To test for IgG antibodies against *Plasmodium* spp., six *Plasmodium* antigens were used for this study: the species-specific merozoite surface protein 19kD (MSP1) fragments for *P. falciparum*, *P. vivax*, and *P. malariae*, and three other *P. falciparum* antigens: liver stage antigen 1 (LSA1), circumsporozoite protein (CSP), and apical membrane antigen 1 (AMA1). The three *Plasmodium* MSP1 antigens were recombinantly produced and purified as described previously (39). The LSA1 and CSP *P. falciparum* antigens were produced as peptides, and AMA1 produced as described previously (40). All antigens were coupled to magnetic beads (Luminex Corporation, Austin, TX) in the same manner as previously (39). Briefly, beads were pulse vortexed,

transferred to a microcentrifuge tube and centrifuged for 1.5 minutes at 13,000g. Supernatant was removed and beads were washed with 0.1M sodium phosphate, pH 6.2 (NaP). Beads were activated by suspending in NaP with 5 mg/mL of EDC (1-ethyl-3-[3'-dimethylaminopropyl] carbodiimide hydrochloride) and 5 mg/mL sulfo-NHS (sulfo N-hydroxysulfosuccinimide) and incubating with rotation for 20 minutes at room temperature (RT) protected from light. After a wash with coupling buffer (50 mM 2-(4-morpholino)-ethane sulfonic acid, 0.85% NaCl at pH 5.0), antigens were coupled to beads in presence of coupling buffer for 2h at an antigen concentration of 20 ug/mL for all antigens except for CSP at 30 ug/mL, LSA1 at 60 ug/mL and GST at 15 ug/mL. Beads were washed once with PBS, and suspended in PBS with 1% bovine serum albumin (BSA) with incubation for 30 minutes at RT by rotation. Beads were then resuspended in storage buffer (PBS, 1% BSA, 0.02% sodium azide and 0.05% Tween-20) and stored at 4°C.

For the IgG assay, all incubation steps were performed at RT in 50 µl reaction volumes protected from light. Incubation steps were followed by washes with 200 µl PBS pH 7.2 containing 0.05% Tween-20 (PBST) performed by attaching plates to a handheld magnet (Luminex Corp.) for one minute then inverting to evacuate supernatant. A master mix of beads was made by combining 125,000 beads per region in 5.5 mL of Buffer A (PBS pH 7.2 plus 0.5% BSA, 0.05% Tween-20, 0.02% sodium azide). Bead master mix was aliquoted into a black flat-bottom 96-well plate (Bio-Rad, Hercules, CA) for a total of 1250 beads per well per region. Beads were then incubated with samples for 90 min, followed by 45 min with secondary antibodies diluted in Buffer A (50 ng/well biotinylated mouse anti-human IgG and 20 ng/well biotinylated mouse anti-human IgG4;

Southern Biotech, Birmingham, AL). Incubations with 250 ng/well of streptavidin-R phycoerythrin (Invitrogen) for 30 min and Buffer A alone were carried out as previously described. Beads were resuspended in 100 µL PBS and then stored overnight at 4°C protected from light and read the following day. Before reading, plates were shaken at room temperature for at least 20 min, and the old PBS was removed. Fresh 100 uL PBS was added and plates were read on a BioPlex-200 machine (Luminex Corporation, Austin, TX) with BioPlex Manager 6.1 (BioRad). Median fluorescence intensity (MFI) signal was generated for a minimum of 50 beads/region, and background MFI from wells incubated with Buffer B was subtracted from each sample to give a final value of MFI-bg.

The presence and quantification of antigens was performed with similar methodology as described previously using the bead-based Luminex® platform (Luminex Corp., Austin, TX) (9). Three unique bead regions (Bio-Plex COOH bead, BioRad, Hercules, CA; 171506XXX) were individually coated by the EDC/Sulfo-NHS intermediate reaction with separate antibodies specific for each antigen to be captured: *Plasmodium* aldolase (12.5µg/12.5x10^6 beads, rabbit IgG anti-aldolase, Abcam, Cambridge, UK; ab207494), *Plasmodium* LDH (12.5µg/12.5x10^6 beads, mouse IgG anti-LDH, BBI Solutions, Cardiff, UK; BM355-Z8F7), and *P. falciparum* PfHRP2 (20µg/12.5x10^6 beads, mouse IgG anti-HRP2, Abcam; ab9206). For the assay, a mix of the three coupled bead regions was made in 5mL Buffer A (PBS, 0.5% BSA, 0.05% Tween20, 0.02% NaN_3) so that 1,500 of each bead region would be added per well in the assay plate. Samples were incubated with 50µL of the bead mix in filter bottom plates (Millipore; MABVN1250) for 90min under gentle shaking and subsequently washed

three times with 100µL wash buffer (PBS, 0.05% Tween20). Beads were incubated for 45min with a 50µL mix of detection antibodies: anti-pAldo (1:1000x, rabbit anti-aldolase, Abcam; ab207494), anti-pLDH (1:500x of 2:1:1 mixture (BBI Solutions BM355-P4A2: BioRad Pv-pLDH HCA156: BioRad Pf-pLDH HCA158)), and anti-HRP2 (1:500x, mouse IgG anti-HRP2, Abcam, ab9203). All detection antibodies were previously biotinylated by Thermo Scientific EZ-Link Micro Sulfo-NHS-Biotinylation Kit (ThermoFisher Scientific, Waltham, MA) according to the manufacturer's protocol. Plates were washed three times, and wells subsequently incubated with 50µL streptavidin-phycoerythrin (1:200x, Invitrogen, Carlsbad, CA) for 30min. Plates were washed three times, and after a final 30min wash step with reagent diluent, beads were washed once and re-suspended in 100µL PBS and read on a Bio-Plex 200 instrument (BioRad, Hercules, CA**)** by generating the median fluorescence intensity (MFI) signal for 50 beads in each unique region, and then the mean fluorescence intensity of the MFIs among duplicates. The final measure, denoted as MFI-bg, was reported by subtracting MFI values from beads on each plate only exposed to sample diluent during the sample incubation step.

To extrapolate an assay signal to an antigen concentration, standard curves of known recombinant antigens were run in order to generate equations to derive a concentration from a signal intensity of the bead assay (9). Recombinant pLDH and HRP2 antigens were provided by Microcoat Biotechnologie GmbH (Bernried, Germany), and lyophilized preparations were rehydrated according to the manufacturer's instructions. The *Plasmodium vivax*-specific isoform of aldolase was produced at the CDC as described previously (9).

Data Analysis:

A total of 1,439 individuals from 780 different households participated in the study. Electronic data recorded using Eagle Survey software were exported to Excel file format and merged with serological results from the CDC. Of the 1,439 observations, 108 were excluded due to unreconcilable data, resulting in a total of 1,331 observations with linked household information and serology results. All statistical analyses were conducted using Stata v15.1. Estimates and 95% confidence intervals (CIs) were calculated using Stata's *svy* survey commands that incorporated stratification, selection probabilities, and study design. Sampling weights were derived as the inverse of the product of sampling probabilities.

Age groups were constructed using 10-year intervals, with the oldest age group containing all individuals 60 years of age and older. Maps were constructed using the ArcMap desktop application of ArcGIS v10.6.1. Shapefiles and administrative borders were downloaded from DIVA-GIS using Transverse Mercator projection and GCS_WGS_1984 geographic coordinate system.

To evaluate risk factors for seropositivity to malaria, a univariate logistic regression analysis was conducted for each potential risk factor, using seropositivity to *Plasmodium spp.* MSP-1 antigens as the outcome of interest. MSP-1 exhibits moderate immunogenicity and elicits a long-lived immune response, making it useful for estimating malaria prevalence across a range of transmissions levels (41). A univariate analysis was first conducted for each variable to investigate the strength of the association with malaria seropositivity when considered alone. A multivariable logistic regression model that accounted for the survey weights was then developed using stepwise backwards elimination. All non-collinear variables were included in the

stepwise analysis and variables were sequentially removed when found to have a significance level of greater than 15% ($P > 0.15$). Variables determined significant by stepwise analysis were included in a logistic regression analysis with adjustments for survey design.

Results

Survey Demographics:

This study includes results from 1,331 individuals from 747 unique households from three geographic regions in the DR (Table 1). Females made up 59.2% of the sample population, and the mean age was 36 years (range 2 – 96). The survey contained 470 individuals from the Eastern region, 459 individuals from the Northern region, and 402 individuals from the Southwest region. Participants' ethnicity was defined by the individuals' nation of birth and reported descent; this sample population was composed of individuals that could be described as Dominican born with no Haitian descent (n=485, 36.6%), Dominican born with Haitian descent (n=384, 29.0%), and Haitian born (n=455, 34.4%) (Table 7).

Survey questions were also used to assess the coverage of traditional mosquito control measures within the *bateyes*. In the survey, 778 individuals (58.5% of respondents) reported having a mosquito net in the area for their use. Of those who reported having a mosquito net, 761 (97.8%) reported owning the net and 631 (81.1%) reported sleeping under the net the night before completing the survey. In the 12 months preceding the questionnaire, 475 participants (35.7%) reported having indoor residual spraying (IRS) in their household and 548 participants (41.2%) reported having insecticide sprayed outdoors.

Malaria Descriptive Analysis:

Seroprevalence for antibodies to the MSP-1 antigen for *P. falciparum*, *P. vivax*, and *P. malariae* was plotted by 10-year age categories to examine the distribution of malaria seropositivity by age within the study population (Figure 1). *P. falciparum* MSP-1 seroprevalence steadily increased across age categories from 2.5% in those aged 0-9 years to 23.8% in those aged 60 years or older. Seroprevalence to *P. malariae* and *P. vivax* MSP-1 was uniformly low across all age groups, with *P. malariae* ranging from 1.0 and 4.8% and *P. vivax* ranging from 2.3 and 4.2%.

In addition to MSP-1, the seroprevalence of *P. falciparum* AMA-1, CSP, and LSA-1 was also estimated (Figure 2). AMA-1 followed a similar increase with age as seen with MSP-1, though at reduced levels, increasing from 1.3% in those aged 0-9 years to 19.1% in those aged 60 years or older. CSP and LSA-1 were both found to maintain a low seroprevalence across all age categories, ranging from 0.0% to 2.8% across all age groups.

Seropositivity to multiple antigens within individuals was also examined. The level of test concordance between *Plasmodium* antibody seropositivity varied by both inter- and intra- species comparisons (Table 3). Test concordance between different *Plasmodium* species appeared to be weak across all index antigens with the exception of the 30% concordance found between the anti-MSP-1 antibodies of *P. falciparum* and *P. malariae*. All other inter-species tests had a considerably lower concordance index (range 0.0 – 6.7%). Intra-species antibody testing for *P. falciparum* had a wide range of test concordance (0.0-68.8%). All index antigens, except PvMSP-1, were found to have a high test concordance with PfMSP-1 (30.0 – 46.3%). The highest intra-species test

concordance was found between the *P. falciparum* index antigen LSA-1 and AMA-1 (68.8%) and the lowest was found between the index antigen PfAMA and CSP (1.5%).

Seroprevalence of *Plasmodium* anti-MSP-1 antibodies were also stratified by sex and ethnicity. No statistically significant difference was observed between males and females, though prevalence appeared slightly higher among females aged 20-60 years (Figure 3). Regarding ethnicity, seroprevalence was generally highest among Haitian born individuals, followed by individuals born in the DR of Haitian descent, and lowest among those born in the DR without Haitian descent, though differences were not statistically significant (Figure 4).

Geo-spatial distribution of seroprevalence to MSP-1 was plotted for each survey cluster, including for all age groups (Figure 5), and for 20-year age groupings (Figures 6-8). The regional distribution of seropositivity was found to vary spatially. For all age groups, the North region had the lowest seroprevalence to *Plasmodium spp.* MSP-1 antigens (Mean 14.5%, range 3.5%-39.1%) followed closely by the East region (Mean 16.1%, range 0.0%-40.0%). The Southwest region had highest seroprevalence (Mean 34.3%, range 2.4%-63.0%) as well as the highest number of *bateyes* with >30% seroprevalence (n=8, 53.3%). Similar trends were observed when stratified by 20-year age groups (Figures 6-8).

Lymphatic Filariasis Descriptive Analysis:

Seroprevalence of antibodies to the filarial antigens Bm14, Bm33, and Wb123 were also examined (Figure 10). Seroprevalence of anti-Bm14 antibody was found to be the highest of the three antibodies in each age category and increased steadily over each age group (range 11.1%-24.9%). Anti-Bm33 antibody was found to have the second

highest seroprevalence in each age group but varied minimally across the age groups (range 6.3-9.3%). Anti-Wb123 antibody was found to have the lowest seroprevalence of the three antibodies and, like anti-Bm33, varied minimally across all age groups (range 0.0-2.3%).

The level of test concordance between filarial antibody seropositivity was examined using each antibody as the index (Table 5). Test concordance was found to be the highest with Wb123 as the index, which resulted in Bm14 and Bm33 having 82.4% and 58.8% concordance respectively. When anti-Bm33 was used as the index antibody, test concordances of 9.8% for Wb123 and 31.4% for Bm14 were observed. The lowest concordance occurred when Bm14 was used as the index antibody, with Wb123 and Bm33 having 6.5% and 14.9% concordance respectively.

GIS maps and seroprevalence graphs stratified by regions were created to explore the geographical distribution of LF. Seroprevalence to Bm14 tended to be highest across each survey region, followed by Bm33, and Wb123, though differences generally were not statistically significant (Figures 11-13). Anti-Bm14 antibody was the only LF antibody that tested positive in all *bateyes* sampled during the study and had the highest mean prevalence in all three regions (Figures 16). Anti-Bm33 was found to have the second highest mean prevalence across all age groups and was found in all but 8 *bateyes* sampled in the study (Figure 15). The lowest mean prevalence across all age groups was found to be anti-Wb123 antibody, which was only found in one out of twelve *bateyes* in the North region (8.3%), four out of seventeen in the East region (23.5%), and six out of fifteen *bateyes* in the Southwest region (40.0%) (Figure 14).

Household Risk Factor Analysis:

The following risk factors were strongly associated with seropositivity to *Plasmodium spp*. MSP-1 antigens in univariate analyses: age (as continuous variable), age (twenty year age categories), Southwest region (categorical variable), not having access to a mosquito net (binary variable), and not sleeping under a mosquito net the night before the survey was administered (binary variable) (Table 7). Other variables were moderately associated with malaria seropositivity, such as Haitian birth (ethnicity categorical variable), construction (occupation categorical variable), and female (gender binary variable). Indoor residual spraying and outdoor insecticide spraying in last 12 months were weakly associated malaria seropositivity. Neither recent travel to Haiti in last 12 months or working at night were associated with malaria seropositivity.

Stepwise elimination resulted in a final model containing age, region, ethnicity, and access to a mosquito net. Malaria seropositivity was significantly associated with older age [OR = 1.02, 95% CI 1.01-1.04], living in the Southwest (vs. North) region [OR = 4.27, 95% CI 2.16-8.43], being Haitian born (vs. Dominican born with no Haitian descent) [OR = 1.87, 95% CI 1.15-3.06], and lacking access to a mosquito net [OR = 1.81, 95% CI 1.25-2.62] (Table 8).

Discussion

This study is the first to describe antibody serological response to malaria and lymphatic filariasis in the Dominican Republic. Cost, feasibility, and test sensitivity of existing diagnostic methods for disease surveillance such as hospital-based, entomological, and parasitological measures have led researchers to explore antibody response as a possible addition to current surveillance strategies (41). Antibody response

analysis offers a theoretical advantage over other methods, allowing a single measurement to provide transmission trends over an extended period of time, overcoming sampling biases that can occur with seasonal variation and changes in peak transmission (42).

Recent publications have focused on malaria antibody testing, believing it could provide a tool for estimating both long-term and recent malaria transmission in a population using cost effective cross-sectional studies (41, 43). This study measured levels of species-specific antibodies to MSP-1 antigens for *P. falciparum*, *P. malariae*, and *P. vivax* as well as antibodies to three other *P. falciparum* antigens: AMA-1, CSP, and LSA-1. Although a number of factors including antibody acquisition and seroreversion rate play a role in serum levels of antibodies, antibody half-life has been a large focus of many studies due to its importance in interpretation of test results. Antibodies to AMA-1 and MSP-1 have both been reported to have a long, potentially lifetime, half-life for individuals over 10 years of age (42, 43). Antibodies to LSA-1 have been found to have a short half-life for young children but increase to an intermediate length, defined as > 10 years, for individuals over 50 years of age (43). Literature suggests that of the four *P. falciparum* antibodies measured, only CSP has a short half-life across all ages (43). In addition to having a shorter half-life, CSP and LSA-1 have also been thought to be less immunogenic than MSP-1 and AMA-1, suggesting that repeated infections would be needed to show a high level of either antigen (9, 41).

Malaria in the Dominican Republic:

Malaria rapid diagnostic tests (RDT) and microscopy performed in the Dominican Republic as part of the larger *batey* survey showed 0% prevalence in the study population

(38). Blood samples collected from the same individuals and analyzed using multiplex antibody testing suggested very low to minimal transmission within the study population at the time of sampling. The increase in seroprevalence of *P. falciparum* MSP-1 and AMA antibodies in older generations, in contrast to low reactivity in younger age groups, are suggestive of historic exposure. Although no previous studies have reported malaria antibody response in the DR, a cross-sectional study in Haiti conducted in 1998 reported age-specific seroprevalence estimates of MSP-1 antibody. The study reported the same trend, increasing in seroprevalence across age groups, ranging from 6% in the lowest age group (0-5 years of age) to 54% in the highest age group (>40 years of age) (44). With *P. vivax* and *P. malariae* known to be non-endemic in the DR, the extremely low and uniform seroprevalence seen in the study population is suggestive of false positives or cross-reactivity. Therefore, a higher analytic threshold should be considered for *P. vivax* and *P. malariae* in future studies.

Given the lack of long half-life antibody markers seen in the younger cohorts and assuming seroconversion to AMA-1 and MSP-1 after only a single or few malarial infections, it appears that over time a smaller proportion of younger individuals are being infected compared to preceding generations. If the observed increases in AMA-1 and MSP-1 were due to more recent infections, a proportional increase in CSP and LSA-1 would be expected. The increase of both MSP-1 and AMA-1 across age categories, combined with the low, stable prevalence of CSP and LSA-1 suggest two important findings: 1) *P. falciparum* transmission within the *bateyes* is currently very low and 2) the majority of seropositivity for AMA-1 and MSP-1 is the result of past exposure. These

conclusions agree with the antibody predictive profiles across age groups for AMA-1 and CSP in an extremely low transmission area of Kapsisiywa and Kipsamoite, Kenya (43).

Using this interpretation of the serological data, in which the youngest cohort is representative of current transmission while the oldest cohort is better representative of previous exposure, seropositivity to *P. falciparum* MSP-1 by 20-year age groups can be used to assess malaria transmission by region. The Northern region of the Dominican Republic had the lowest prevalence of *Plasmodium* anti-MSP-1 antibodies among the youngest cohort, as well as the fewest number of *bateyes* with seropositive cases within this age group. In the same region, seroprevalence among the oldest cohort was much higher and seropositive individuals were found in every *batey*, sharply contrasting the youngest cohort. These findings, in combination with low seroprevalence of shorter half-life antibodies (CSP & LSA-1), suggest a very low level of transmission in the Northern region.

A similar trend is seen in the Eastern region, with a higher number of malaria free *bateyes* and lower prevalence in the lowest age cohort in comparison to the oldest age cohort. The Southwest region had the highest prevalence in each of the three age cohorts but generally followed the same temporal trend. Although the region has a higher prevalence, age stratification shows improvements over time with a decrease in overall prevalence and the emergence of three *bateyes* with no seropositive cases. Although the degree of success varies by region, results indicate that the Dominican Republic has made substantial progress in the reduction of malaria across the country.

This survey was also designed to assess risk factors for seropositivity to develop more targeted interventions. Age was found to have an association with seropositivity

both by itself and when considered in a multivariable model. This relationship was thought to be largely influenced by the extended half-life of the MSP-1 antibody discussed previously. There were some differences in seroprevalence when considering gender and occupation, but neither was found to be significant by univariate analysis. This lack of statistically significant association between malaria and gender is consistent with previous studies (45). Haitian ethnicity was not found to be strongly associated with the outcome by univariate analysis but became so when considered in the full multivariable model. Given that Haiti typically accounts for >95% of the malaria cases reported in Hispaniola, individuals from Haiti are at greater risk for past malaria infection (46). Although an association was found with Haitian ethnicity, it is important to note the lack of a meaningful association between those who visited Haiti in the last 12 months. When considering region as a risk factor, living in *bateyes* of the Southwest region was found to be strongly associated with seropositivity in both univariate and multivariable models. The self-reported low bed net usage and low IRS seen in the southwest region is thought to be contributing or compounding factors leading to this regional discrepancy (38).

All of the traditional malaria control measures were found to have at least a weak protective effect. Having access to a mosquito net and sleeping under a mosquito net the previous night were found to have the strongest protective associations. Over half of respondents (58.5%) reported having a mosquito net in the household, with 81.2% of bed net owners reporting use the night before. This is an improvement from the 2014 estimates on insecticide-treated bed net (ITN) usage within the DR (33). While these measures may not be directly comparable as this survey did not specify insecticide

treatment of bed nets, it does demonstrate the continued use of protective measures in areas of reduced transmission, a key concern of many elimination programs. Indoor residual spraying in last 12 months and outdoor insecticide spraying in last 12 months were both found to be protective, but neither were found to have a significant association. Previous studies examining the impacts of IRS on malaria transmission have shown a protective association but vary on whether these association are significant (45, 47).

Lymphatic Filariasis in the Dominican Republic:

Like malaria, lymphatic filariasis is a neglected tropical disease that has been the target of many national and international elimination programs. Successful MDA programs have created the need for the standardization of a diagnostic tool for monitoring transmission of LF in both endemic and non-endemic areas (35). After effective MDA, blood levels of microfilariae and antigens decline in the population, making it difficult to use these as markers of transmission (48). This leaves antibody response as a promising measure of transmission in post-MDA low intensity settings, but research is needed to better understand the limitations and interpretations of this method.

Results from LF antibody testing were compared to results of rapid antigen testing (FTS) in the same population to examine diagnostic test concordance as well as explore transmission levels within the Dominican Republic. Of the 1418 individuals included in FTS testing, six participants tested positive for LF antigens, resulting in a prevalence of 0.5% across all three regions (38). The six individuals who tested FTS positive were found to be microfilariae-negative in confirmatory night blood testing. In contrast, overall seroprevalence of antibodies to Wb123, Bm33, and Bm14 were found to be 1.3%, 7.7%, and 16.2% respectively. Although no previous studies have reported LF antibody

response in the DR, a longitudinal study of children in Haiti, conducted between 1990 and 1999, reported the seroprevalence of antibodies to Wb123, Bm33, and Bm14 to be 92%, 100%, and 95% respectively, in children that tested antigen and microfilaria negative (22).

It is still unclear how single markers of LF exposure or infection should be interpreted in individuals who tested negative for LF antigens (35). In previous studies, concordance of antibody seropositivity has been found to be "good" in antigen positive individuals but "poor" amongst those who tested antigen negative (35). The differences seen between Wb123, Bm14, and Bm33 antibody response could be contributed to different life stages or levels of exposure, but more research is needed to understand how to interpret LF antibody results in a low transmission environment where the majority of individuals test negative to both antigens and microfilariae (22, 35).

The previous analysis by Keys et al. reported the regional distribution of the 6 antigen positive cases to be as follows: two in the Southwest (0.4% prevalence), one in the East (0.3% prevalence), and three in non-endemic provinces believed to have been imported cases (38). Antibody seroprevalence to Wb123, Bm33, and Bm14 was found to be low across all regions. In the Southwest they were - 0.8%, 3.5%, and 7.3% respectively, in the East - 0.3%, 4.4%, and 1.7%, and in the North - 0.2%, 2.4%, and 4.5%. Although no previous studies in the DR provide LF antibody prevalence measurements for direct comparison, the seroprevalence was found to be greater than or equal to all antigen prevalences captured in the same survey. One potential explanation is cross-reactivity that occurs between Bm14/Bm33 and related filarial parasites such as *Loa loa* and *Onchocerca volvulus* (49, 50). In summary, this study's LF data provide

conflicting results depending on the antigen biomarker: Wb123 seroprevalence suggests minimal LF transmission is present in the *batey* populations, whereas Bm14 and Bm33 suggest low to moderate levels of past exposure and/or transmission.

There are several limitations of the current analysis. First, our sampling frame, containing only agricultural areas of the DR, biases the results towards risk factors of rural areas versus potential risk factors of urban and peri-urban areas. Secondly, although survey activities were designed to capture a representative sample of the population, females completed the household survey more often than males. Males were potentially under-represented due to working at the time or having migrated prior to the survey being conducted. Finally, the introduction of antibody response as a proxy measurement for disease transmission in low intensity environments introduces its own limitations. Studies have suggested that altitude and seroreversion rate of children can alter seropositivity for P. falciparum MSP-1 and should be considered in future studies (42, 43).

The study results presented here for malaria and lymphatic filariasis contribute to the ongoing evaluation of antibody response as a means of low transmission surveillance. These results represent the current antibody seroprevalence in the Dominican Republic and provide important data to prospectively monitor transmission elimination in Hispaniola. This method could provide a standardized tool for efficient, cost effective disease monitoring for elimination programs in the future.

Table 1. Sample demographics by region for serology samples from *batey* survey, Dominican Republic, 2016

	Total (n = 1,331)[a]	Region		
		East (n = 470)	North (n = 459)	Southwest (n = 402)
	n (%)	n (%)	n (%)	n (%)
Sex				
Female	787 (59.2)	236 (50.2)	295 (64.4)	256 (63.68)
Male	543 (40.8)	234 (49.8)	163 (35.6)	146 (36.3)
Age (years)				
0-9	80 (6.0)	21 (4.5)	26 (5.7)	33 (8.2)
10-19	226 (17.0)	67 (14.3)	86 (18.8)	73 (18.2)
20-29	257 (19.3)	105 (22.3)	82 (17.9)	70 (17.4)
30-39	206 (15.5)	79 (16.8)	64 (14.0)	63 (15.7)
40-49	194 (14.6)	63 (13.4)	80 (17.5)	51 (12.7)
50-59	177 (13.3)	58 (12.3)	67 (14.7)	52 (12.9)
≥60	189 (14.2)	77 (16.4)	52 (11.4)	60 (14.9)
Place of Birth				
Dominican Republic	874 (65.8)	221 (47.0)	329 (71.7)	324 (81.0)
Haiti	455 (34.2)	249 (53.0)	130 (28.3)	76 (19.0)
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Descent, if Dominican Republic Birth				
Haitian Descent	384 (29.0)	113 (24.2)	77 (16.8)	194 (48.7)
Non-Haitian Descent	940 (71.0)	355 (75.9)	381 (83.2)	204 (51.3)
Access to Mosquito net?				
Yes	778 (58.5)	258 (54.9)	291 (63.4)	229 (57.3)
No	551 (41.5)	212 (45.1)	168 (36.6)	171 (42.8)
Slept Under Mosquito Net Last Night?[b]				
Yes	631 (47.4)	202 (43.0)	240 (52.3)	189 (47.0)
No	700 (52.6)	268 (57.0)	219 (47.7)	213 (53.0)
Indoor Residual Spray in last 12 months?				
Yes	475 (35.7)	285 (60.6)	49 (10.7)	141 (35.3)
No	838 (63.1)	175 (37.2)	405 (88.2)	258 (64.5)
Don't know	16 (1.2)	10 (2.13	5 (1.1)	1 (0.3)
Outdoor Insecticide Spray in last 12 months?				
Yes	548 (41.2)	251 (53.4)	144 (31.4)	153 (38.3)
No	662 (49.8)	160 (34.0)	292 (63.6)	210 (52.5)
Don't know	119 (9.0)	59 (12.6)	23 (5.0)	37 (9.3)
Traveled to Haiti in last 12 months?				
Yes	132 (10.0)	36 (7.7)	63 (13.8)	33 (8.3)
No	1,192 (89.9)	434 (92.3)	394 (86.2)	364 (91.2)
Don't know	2 (0.2)	0 (0.0)	0 (0.0)	2 (0.5)
Do You Work at Night?				
Yes	125 (15.8)	45 (14.8)	39 (16.5)	41 (16.3)
No	668 (84.2)	259 (85.2)	198 (83.5)	211 (83.7)

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

[b] Among those with access to a bed net

Table 2. Seropositivity (%) to Malarial antigens by age groups, Dominican Republic, 2016

	P. falciparum				P. malariae	P. vivax
Age Group	**PfMSP-1**	**PfAMA-1**	**PfCSP**	**PfLSA-1**	**PmMSP-1**	**PvMSP-1**
0-9 (n=80)	2.5	1.3	0.0	0.0	1.3	3.8
10-19 (n=226)	9.3	4.0	1.8	0.4	2.2	3.1
20-29 (n=257)	16.7	9.7	0.8	0.8	1.6	2.3
30-39 (n=206)	16.0	11.7	1.9	1.5	1.0	3.4
40-49 (n=194)	20.1	11.9	2.1	2.6	3.1	3.1
50-59 (n=177)	23.7	8.5	2.8	0.6	1.7	4.0
>60 (n=189)	23.8	19.1	0.5	2.1	4.8	4.2
Total (n=1,329)	16.9	10	1.5	1.2	2.3	3.3

Table 3. Antibody test concordance for *Plasmodium* species antigens, Dominican Republic, 2016

		Concordance with positive Index*					
		P. falciparum				P. malariae	P. vivax
Index	**Total # positive/N (%)**	**PfMSP1**	**PfAMA**	**CSP**	**LSA1**	**PmMSP**	**PvMSP**
PfMSP1	225/1,331 (16.9)		62/225 (27.6)	6/225 (2.7)	6/225 (2.7)	9/225 (4.0)	6/225 (2.7)
PfAMA	134/1,331 (10.1)	62/134 (46.3)		2/134 (1.5)	11/134 (8.2)	6/134 (4.5)	5/134 (3.7)
CSP	20/1,331 (1.5)	6/20 (30.0)	2/20 (10.0)		1/20 (5.0)	0 (0.0)	1/20 (5.0)
LSA1	16/1,331 (1.2)	6/16 (37.5)	11/16 (68.8)	1/16 (6.3)		1/16 (6.3)	0 (0.0)
PmMSP	30/1,331 (2.3)	9/30 (30.0)	6/30 (20.0)	0 (0.0)	1/30 (3.3)		2/30 (6.7)
PvMSP	44/1,331 (3.3)	6/44 (13.6)	5/44 (11.4)	1/44 (2.3)	0 (0.0)	2/44 (4.6)	

*Number of samples that tested positive for ab to specified antigen / Number of samples that tested positive for index antigen (%)

Table 4. Seropositivity (%) to LF antigens by age groups, Dominican Republic, 2016

	Lymphatic Filariasis		
Age Group	**Wb123**	**Bm14**	**Bm33**
0-9 (n=80)	1.3	11.3	6.3
10-19 (n=226)	0.0	11.1	7.5
20-29 (n=257)	2.3	14.8	9.3
30-39 (n=206)	1.5	16.0	9.2
40-49 (n=194)	1.6	18.0	6.7
50-59 (n=177)	0.6	15.8	6.8
≥60 (n=189)	1.6	24.9	6.4
Total (n=1,329)	1.3	16.2	7.7

Table 5. Antibody test concordance for filarial antigens, Dominican Republic, 2016

		Concordance with positive Index		
Index	**Total # positive/N (%)**	**Wb123**	**Bm14**	**Bm33**
Wb123	17/1,331 (1.3)		14/17 (82.4)	10/17 (58.8)
Bm14	215/1,331 (16.2)	14/215 (6.5)		32/215 (14.9)
Bm33	102/1,331 (7.7)	10/102 (9.8)	32/102 (31.4)	

Table 6. Filarial antigen seropositivity by region, Dominican Republic, 2016

Age Group	**Wb123 (%)**	**Bm14 (%)**	**Bm33 (%)**
Southwest	0.75	7.29	3.53
North	0.23	4.51	2.40
East	0.30	4.36	1.73
Total (n=1,329)	1.28	16.16	7.66

Table 7. Univariate logistic regression analysis of household risk factors for Malaria Seropositivity (P. falciparum, P. malariae, and P. vivax MSP1 antigen), Dominican Republic, 2016

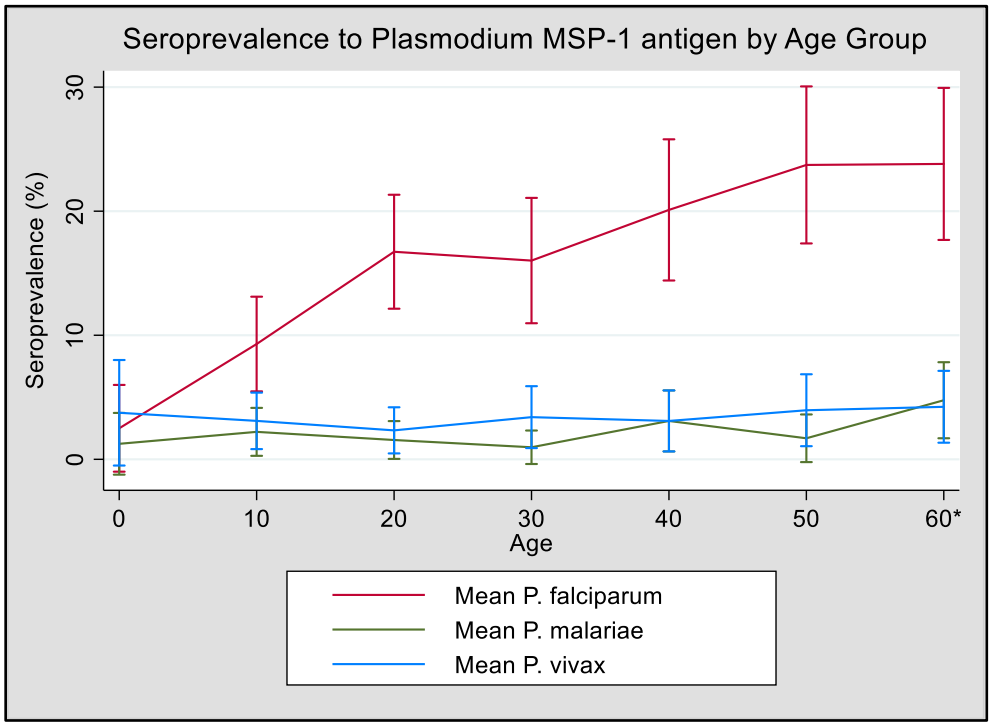
Risk factor	n	% Seropositive	Malaria Seropositivity	
			Odds ratio (95% CI)	P-value
Age (per year)	1,329		1.02 (1.01-1.03)	<0.00
Age (per 20 year age group)				
0-19	306	11.8	1.0	
20-39	463	20.3	1.83 (1.18-2.84)	0.01
40-59	371	25.3	2.37 (1.40-4.01)	<0.00
≥60	189	31.2	3.96 (1.88-8.35)	<0.00
Sex				
Male	538	23.4	1.0	
Female	791	19.6	0.74 (0.50-1.10)	0.14
Birth				
Dominican Republic	874	19.7	1.0	
Haiti	455	24.4	1.45 (0.92-2.26)	0.11
Ethnicity				
Dominican born with no Haitian descent	485	16.1	1.0	
Dominican born with Haitian descent	384	24.2	1.28 (0.84-1.96)	0.24
Haitian born	455	24.4	1.65 (0.99-2.76)	0.06
Region				
North	459	14.4	1.0	
East	470	16.2	1.17 (0.66-2.08)	0.59
Southwest	402	35.1	3.46 (1.96-6.12)	<0.00
Occupation				
Farmer	257	27.2	1.0	
Domestic Work	158	25.3	1.47 (0.75-2.90)	0.26
Construction	47	10.6	0.28 (0.06-1.25)	0.09
Market Vendor / Shop	142	23.9	1.41 (0.63-3.11)	0.39
Tourism	2	0.0	-	-
Unemployed	536	19.2	0.54 (0.24-1.18)	0.12
Other	187	16.6	0.66 (0.35-1.26)	0.20
Access to mosquito net?				
Yes	778	18.0	1.0	
No	551	26.0	1.81 (1.25-2.62)	< 0.00
Slept Under Mosquito Net Last Night? [a]				
Yes	631	18.1	1.0	
No	700	24.1	1.71 (1.14-2.55)	0.01
Indoor Residual Spray in last 12 months?				
Yes	475	18.3	1.0	
No	838	23.0	1.09 (0.68-1.75)	0.72
Don't know	16	18.8	1.42 (0.33-6.04)	0.63
Outdoor Insecticide Spray in last 12 months?				
Yes	548	19.0	1.0	
No	662	23.1	1.16 (0.81-1.67)	0.41
Don't know	119	21.9	0.74 (0.28-1.96)	0.54
Traveled to Haiti in last 12 months?				
Yes	132	22.0	1.0	
No	1,192	21.3	0.77 (0.34-1.77)	0.53
Do You Work at Night?				
Yes	125	21.6	1.0	
No	668	22.9	1.10 (0.15-0.68)	0.78

[a] Among those with access to a bed net

Table 8. Multivariable analysis of household risk factors for Malaria Seropositivity (P. falciparum, P. malariae, and P. vivax MSP antigen), Dominican Republic, 2016

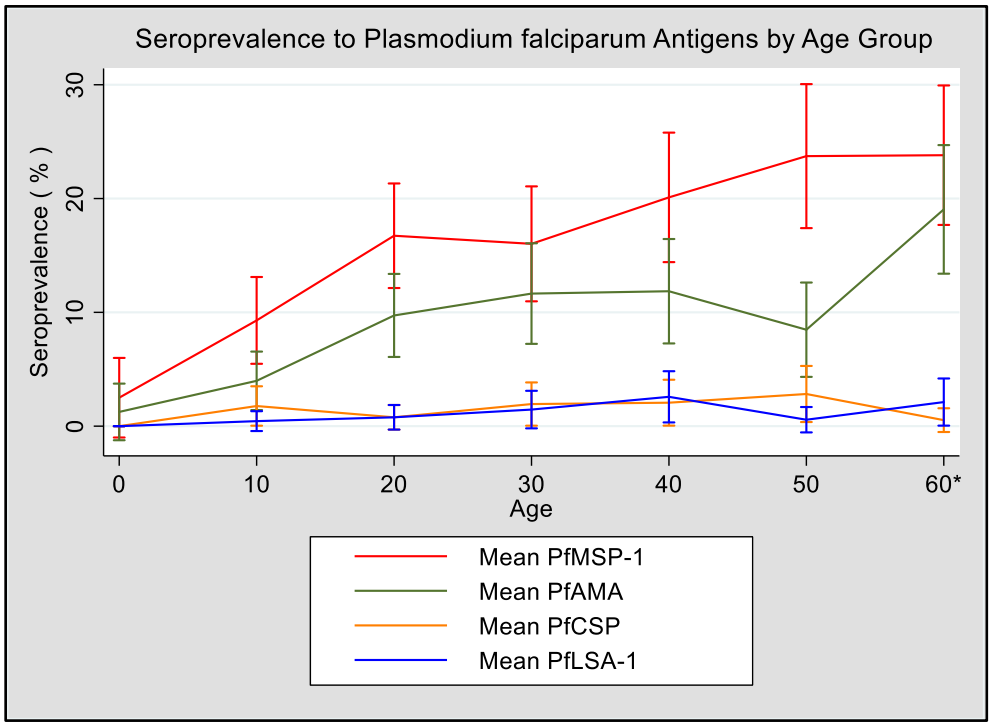
Risk factor	Odds ratio (95% CI)	P-value
Age (per year)	1.02 (1.01-1.04)	<0.00
In this area, is there a mosquito net for you?		
No	1.0	
Yes	0.54 (0.33-0.87)	0.01
Region		
Northern Region	1.0	
Southern Region	4.27 (2.16-8.43)	<0.00
Origin (Haitian born)		
Dominican born with no Haitian descent	1.0	
Haitian born	1.87 (1.15-3.06)	0.01

Figure 1. Mean seroprevalence with 95% confidence intervals to *P. falciparum*, *P. malariae*, and *P. vivax* MSP-1 antigen by 10-year age categories among *batey* residents in the Dominican Republic, 2016



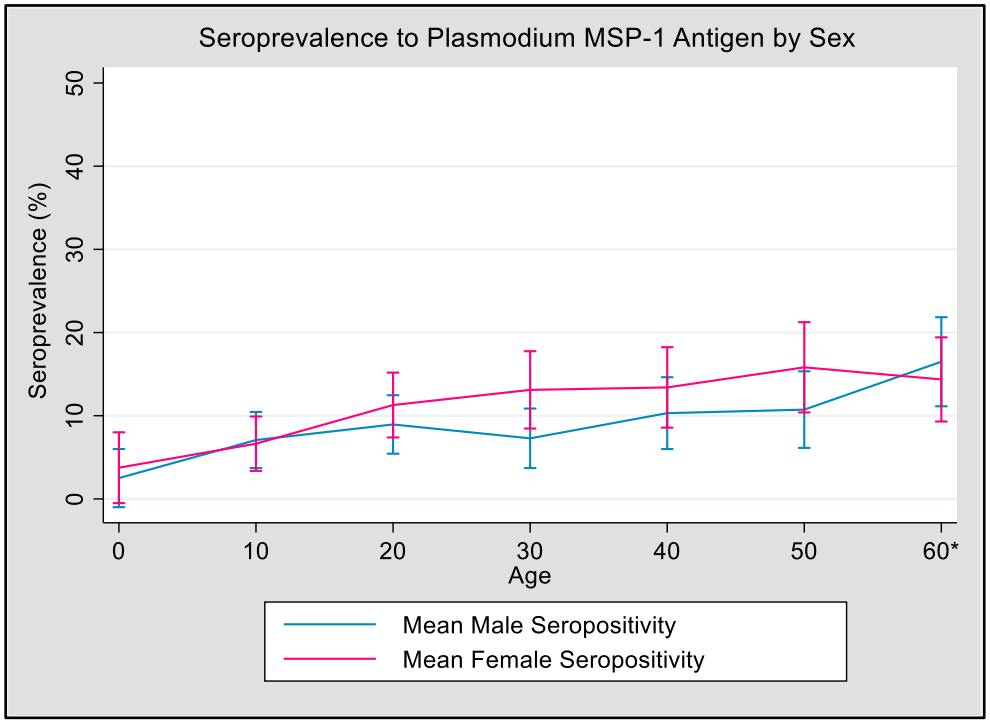
*Last category includes all individuals aged 60 years or older.

Figure 2. Mean seroprevalence with 95% confidence intervals to *Plasmodium falciparum* MSP-1, AMA, CSP, and LSA-1 by 10-year age categories among *batey* residents in the Dominican Republic, 2016



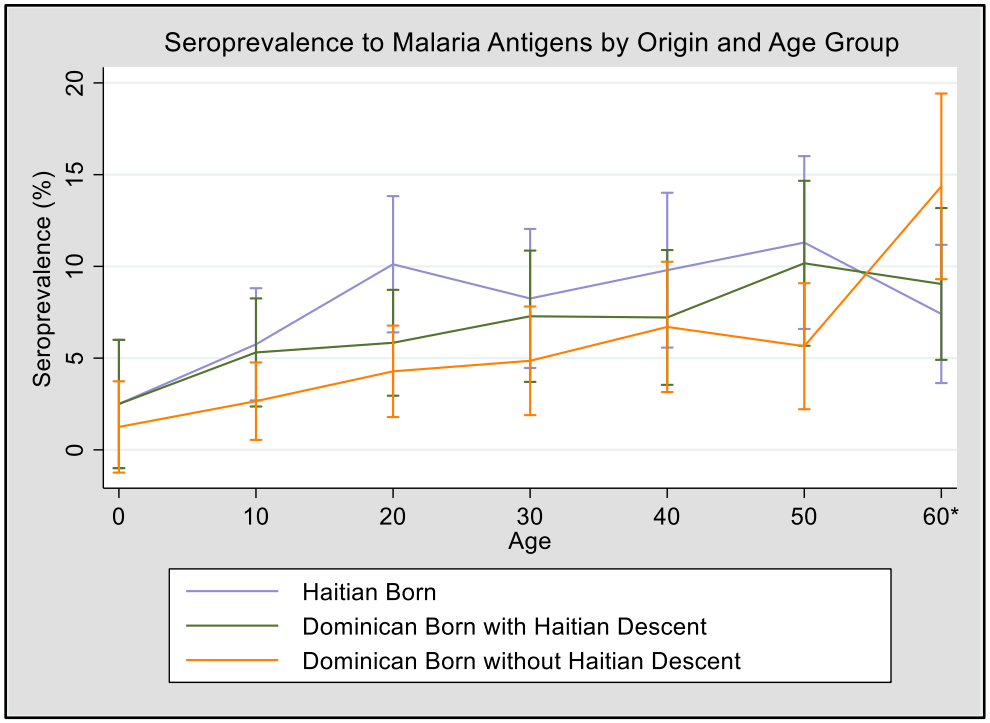
*Last category includes all individuals aged 60 years or older.

Figure 3. Mean seroprevalence with 95% confidence intervals to *Plasmodium spp.* MSP-1 antigens by gender over 10-year age categories among *batey* residents in the Dominican Republic, 2016



*Last category includes all individuals aged 60 years or older.

Figure 4. Mean seroprevalence with 95% confidence intervals to *Plasmodium spp.* MSP-1 antigens by ethnicity over 10-year age categories among *batey* residents in the Dominican Republic, 2016



*Last category includes all individuals aged 60 years or older.

Figure 5. Cluster-specific seroprevalence to *Plasmodium spp.* MSP-1 antigens among *batey* residents (all ages) in the Dominican Republic.

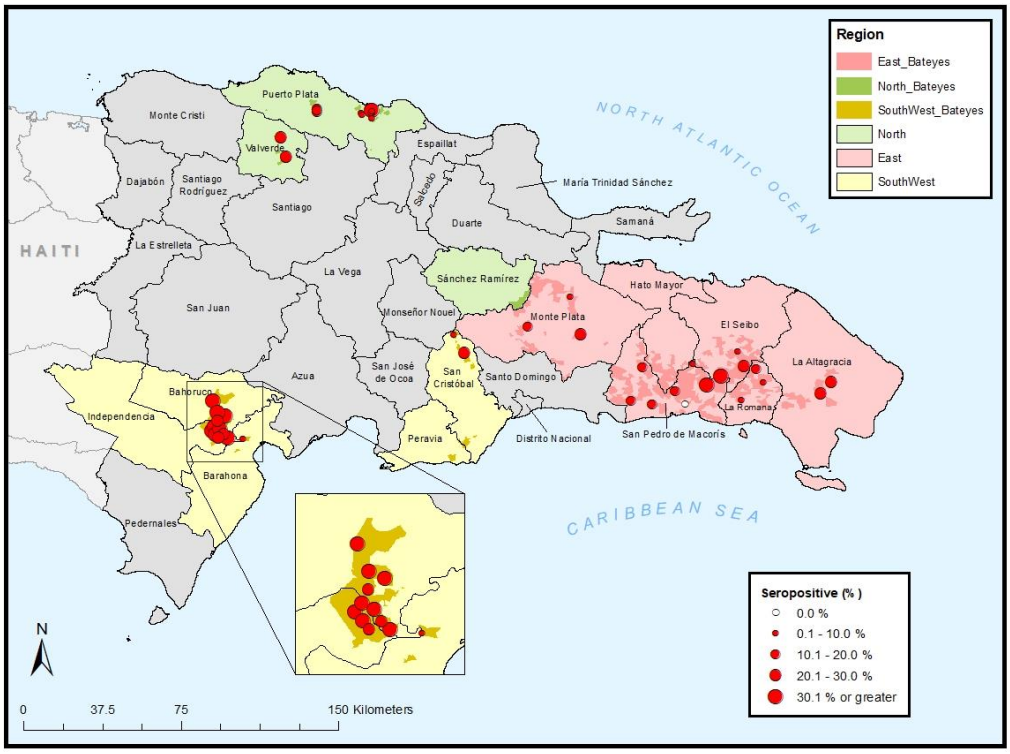


Figure 6. Cluster-specific seroprevalence to *Plasmodium spp.* MSP-1 antigens among *batey* residents (0-19 years old) in the Dominican Republic.

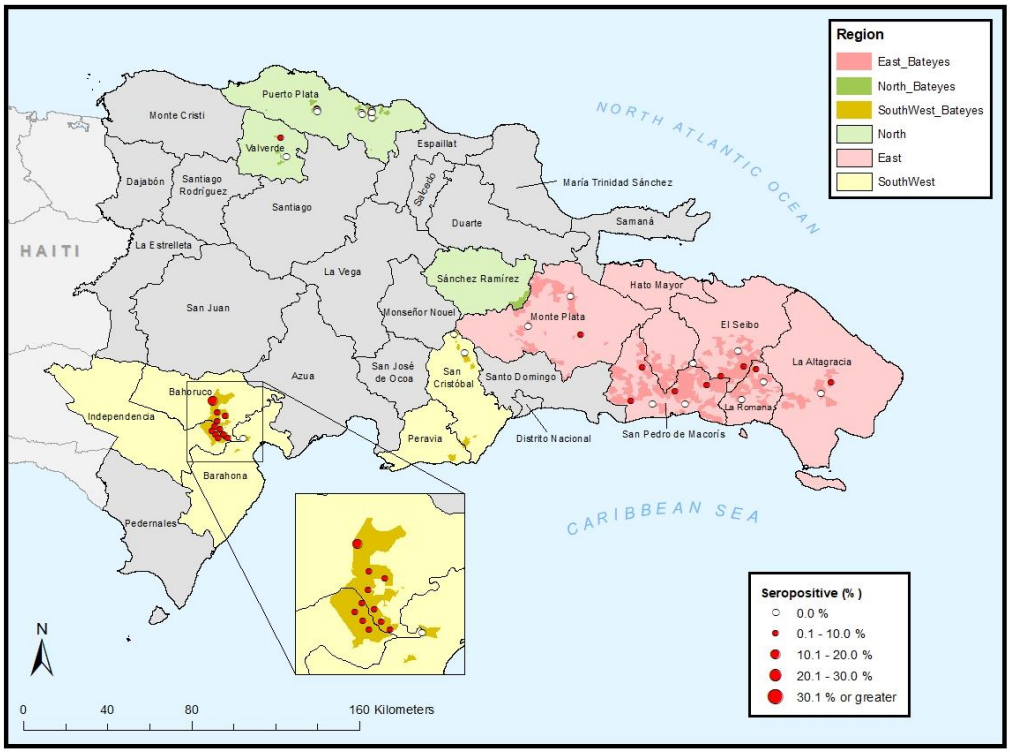


Figure 7. Cluster-specific seroprevalence to *Plasmodium spp.* MSP-1 antigens among *batey* residents (20-39 years old) in the Dominican Republic.

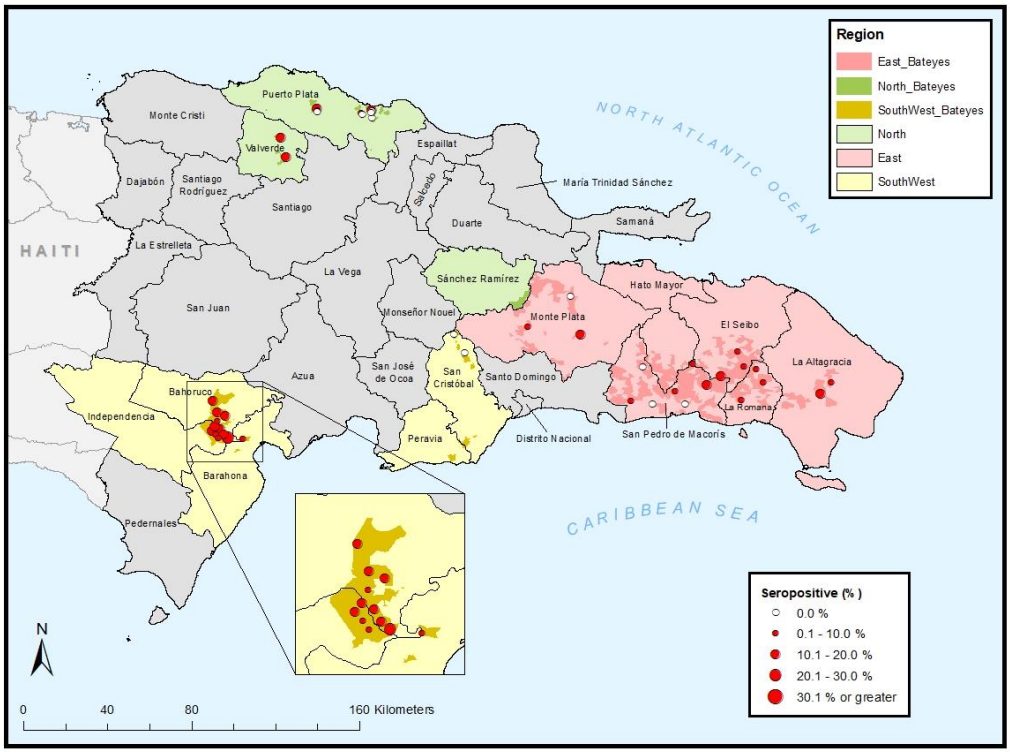


Figure 8. Cluster-specific seroprevalence to *Plasmodium spp.* MSP-1 antigens among *batey* residents (40 years and older) in the Dominican Republic.

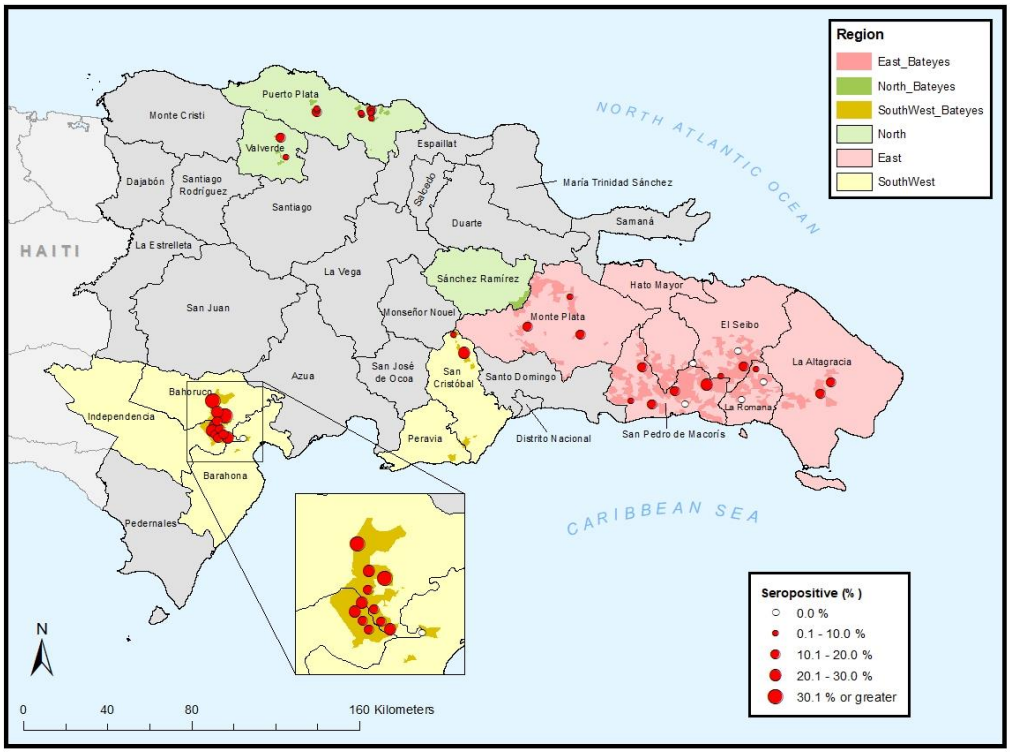


Figure 9. Cluster-specific seroprevalence to *P. falciparum* CSP and LSA-1 antigens among *batey* residents (all ages) in the Dominican Republic.

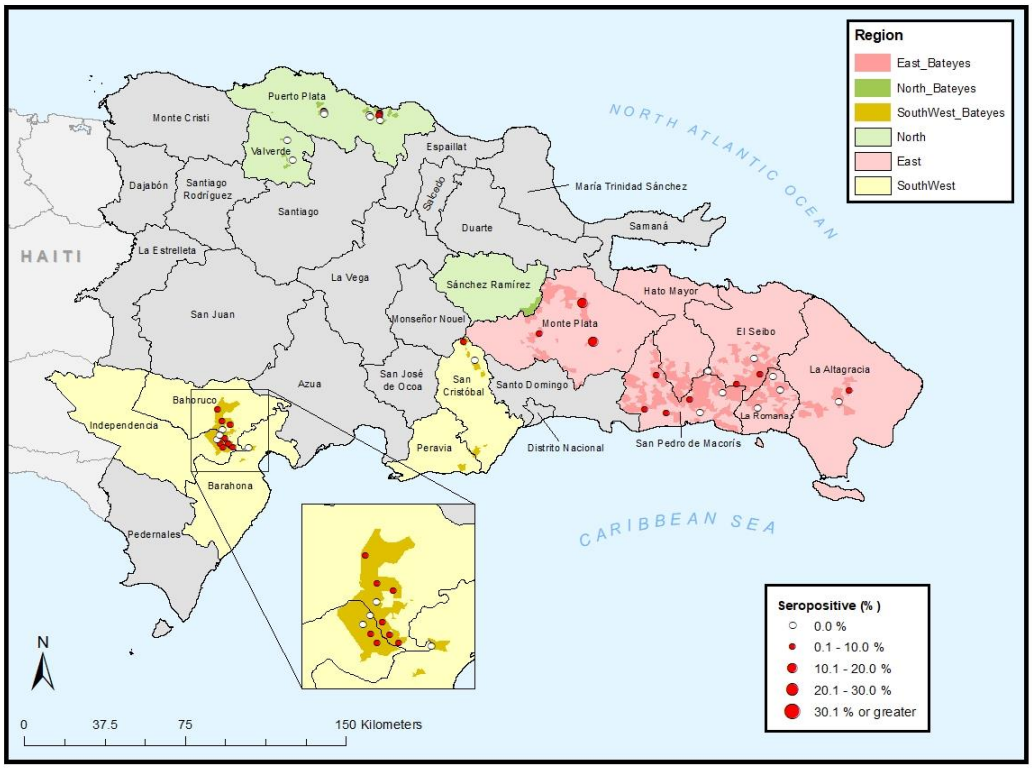
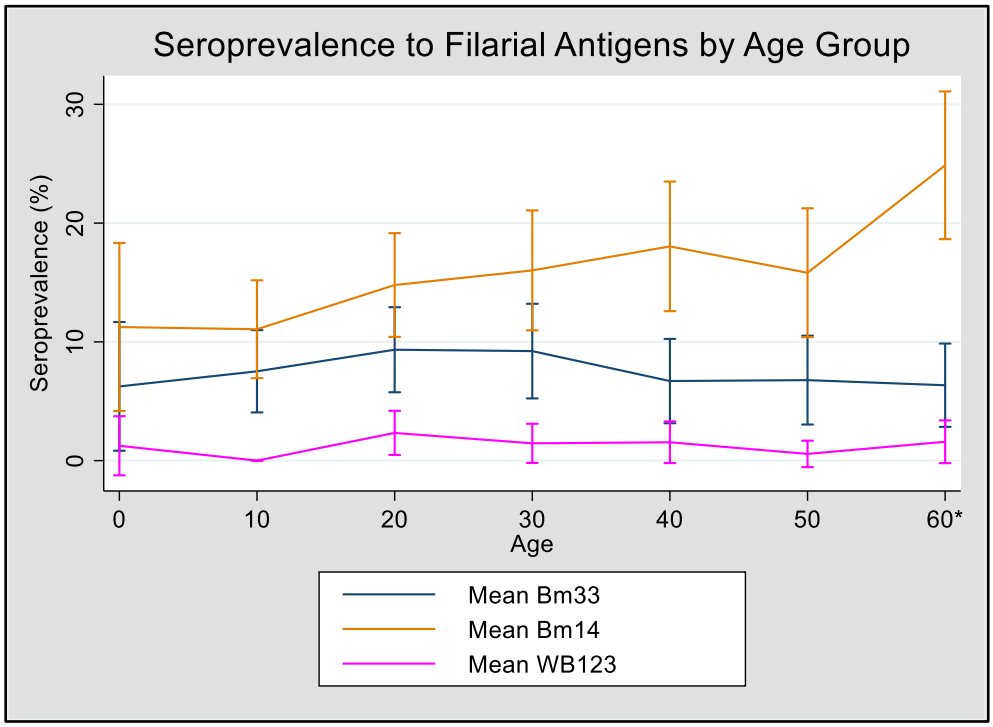
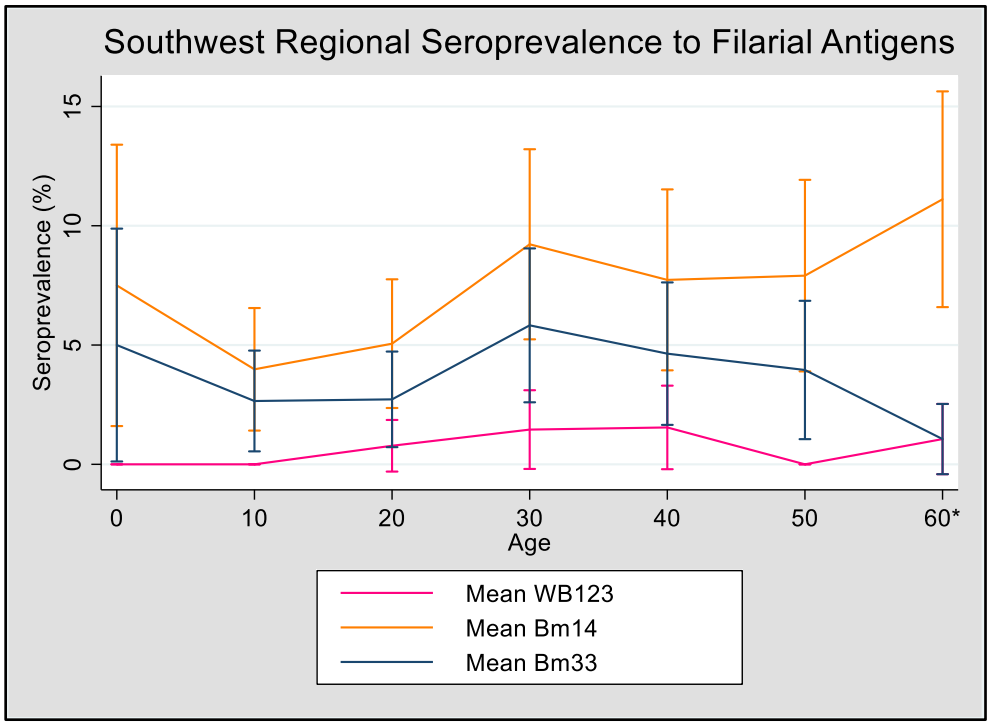


Figure 10. Mean seroprevalence with 95% confidence intervals to filarial antigens Bm14, Bm33, and Wb123 over 10-year age categories among *batey* residents in the Dominican Republic, 2016



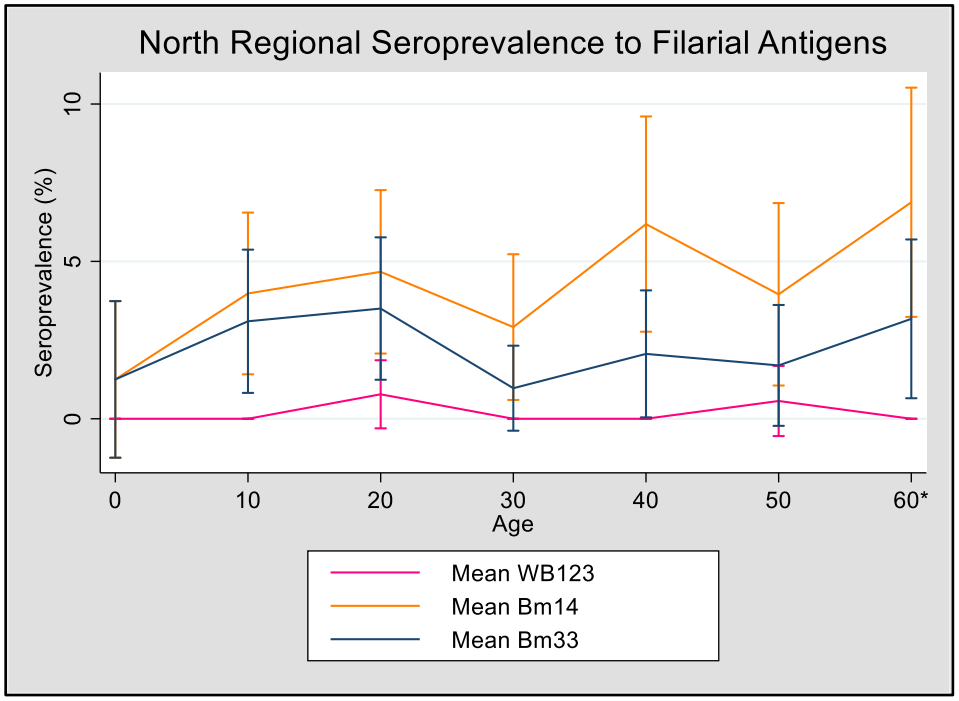
*Last category includes all individuals aged 60 years or older.

Figure 11. Mean seroprevalence with 95% confidence intervals to filarial antigens Bm14, Bm33, and Wb123 in Southwest region over 10-year age categories among *batey* residents in the Dominican Republic, 2016



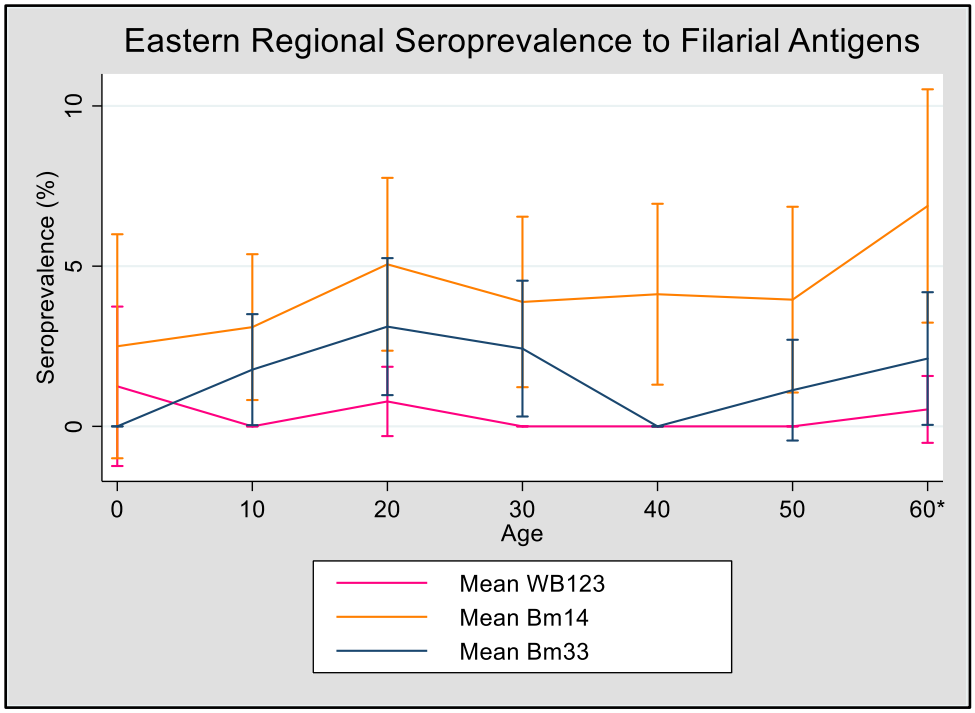
*Last category includes all individuals aged 60 years or older.

Figure 12. Mean seroprevalence with 95% confidence intervals to filarial antigens Bm14, Bm33, and Wb123 in Northern region over 10-year age categories among *batey* residents in the Dominican Republic, 2016



*Last category includes all individuals aged 60 years or older.

Figure 13. Mean seroprevalence with 95% confidence intervals to filarial antigens Bm14, Bm33, and Wb123 in Eastern region over 10-year age categories among *batey* residents in the Dominican Republic, 2016



*Last category includes all individuals aged 60 years or older.

Figure 14. Cluster-specific seroprevalence to lymphatic filariasis Wb123 antigen for all age groups, among *batey* residents in the Dominican Republic.

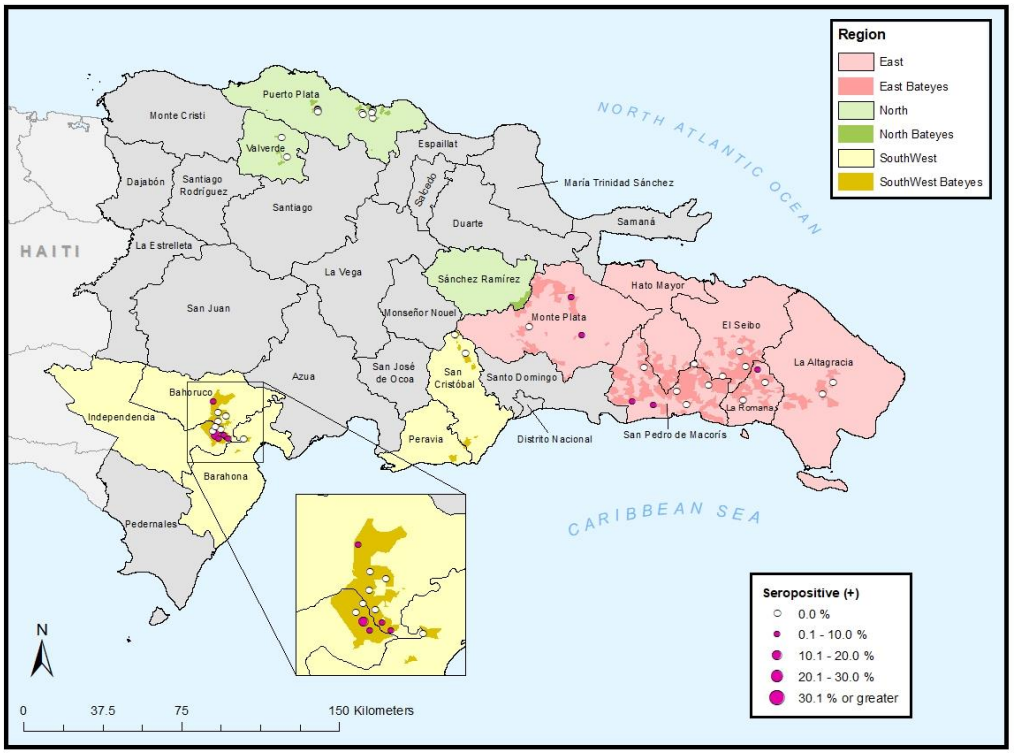


Figure 15. Cluster-specific seroprevalence to lymphatic filariasis Bm33 antigen for all age groups, among *batey* residents in the Dominican Republic.

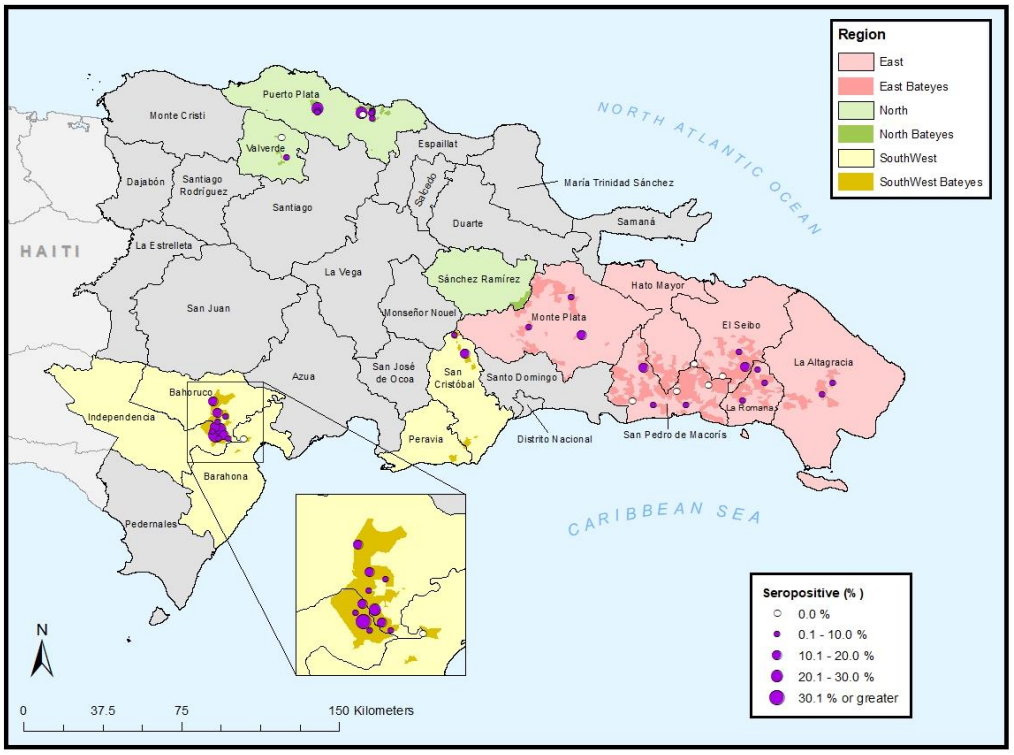
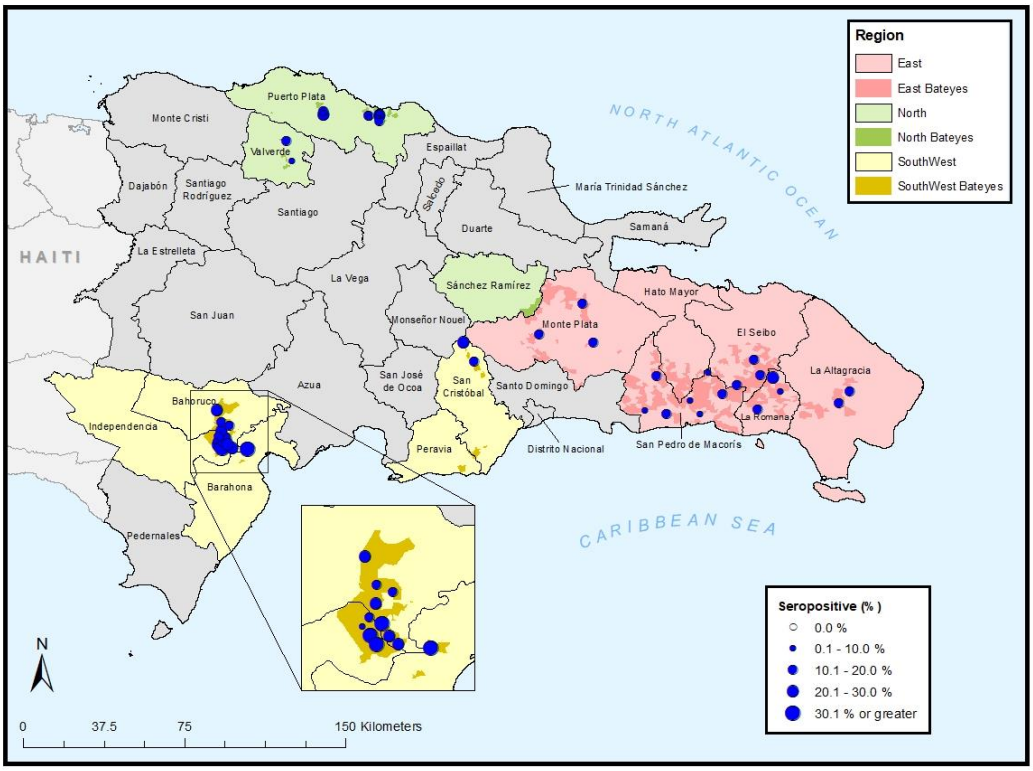


Figure 16. Cluster-specific seroprevalence to lymphatic filariasis Bm14 antigen for all age groups, among *batey* residents in the Dominican Republic.



Chapter III: Study Summary & Future Public Health Implications

Summary

This study is the first to describe antibody serological responses to malaria and lymphatic filariasis in the Dominican Republic. The study used individuals' antibody seropositivity to estimate malaria and lymphatic filariasis prevalence in agricultural regions of the Dominican Republic. Results showed an overall seroprevalence to *P. falciparum* antigens MSP-1, AMA-1, CSP, and LSA-1 of 16.9%, 10.0%, 1.5%, and 1.2%, respectively. Seroprevalence of long-lived antibodies MSP-1 and AMA-1 increased with age from 2.5% and 1.3%, respectively, in those <10 years of age to 23.8% and 19.1% in those ≥60 years of age. Seroprevalence of short-lived antibodies CSP and LSA-1 was uniformly low across age groups. For LF, overall seroprevalence to Wb123, Bm14, and Bm33 antibodies was 1.3%, 16.2%, and 7.7%, respectively. Univariate analysis for malaria seropositivity (MSP-1) revealed significantly higher odds of infection in the Southwest region and among those without access to a bed net, but no significant difference by ethnicity when considered by itself. Multivariable analysis for malaria seropositivity (MSP-1) revealed that ethnicity had significantly higher odds of infection when considered in the full model. These results indicate very low recent exposure to malaria and LF in the Dominican Republic and provide important data to prospectively monitor transmission elimination in Hispaniola.

Future Public Health Implications

Malaria and lymphatic filariasis are diseases currently targeted for elimination by many nations. The WHO has suggested that after interruption of transmission of malaria or lymphatic filariasis, national programmes should implement surveillance systems to confirm the interruption of transmission and detect new foci (7, 51). This study hopes to

contribute to the ongoing evaluation of antibody response as a means of transmission surveillance in these nations post-interruption. If current elimination programs would include this method as part of their disease surveillance early in the process, it would allow for more accurate interpretations of post-interruption results as well as aid our understanding of different antibody responses. If results from similar studies continue to correlate with previous research, this method could provide a more sensitive measure of disease exposure and transmission trends over an extended period of time (35, 42).

Looking forward, these results provide measures of antibody prevalence in the Dominican Republic that can be used for direct comparisons for future surveillance. Further research is needed to substantiate this method as an efficient and effective surveillance tool. Continued validation could lead antibody response to be included as a standardized diagnostic tool in WHO's monitoring and epidemiological assessment strategy for disease surveillance.

References

1. WHO. World malaria report 2018. World Health Organization, 2018:166.
2. White NJ, Pukrittayakamee S, Hien TT, et al. Malaria. *Lancet (London, England)* 2014;383(9918):723-35.
3. Rietveld AEC, Newman RD. Malaria. *Control of Communicable Diseases Manual*.
4. Kenrad E. Nelson CMW. *Infectious disease epidemiology : theory and practice*. Third edition. Burlington, MA : Jones & Bartlett Learning, [2014] ©2014; 2014.
5. Cox FE. History of the discovery of the malaria parasites and their vectors. *Parasites & vectors* 2010;3(1):5.
6. Sinden RE. Malaria, mosquitoes and the legacy of Ronald Ross [electronic article].
7. WHO. World Malaria Report 2018. Geneva, Switzerland, 2018.
8. Kumar V, Abbas AK, Aster JC, et al. *Robbins basic pathology*. 2018.
9. Plucinski MM, Candrinho B, Chambe G, et al. Multiplex serology for impact evaluation of bed net distribution on burden of lymphatic filariasis and four species of human malaria in northern Mozambique. *PLoS neglected tropical diseases* 2018;12(2):e0006278.
10. Coppi A, Natarajan R, Pradel G, et al. The malaria circumsporozoite protein has two functional domains, each with distinct roles as sporozoites journey from mosquito to mammalian host. *The Journal of experimental medicine* 2011;208(2):341-56.

11. Nicoll WS, Sacci JB, Rodolfo C, et al. Plasmodium falciparum liver stage antigen-1 is cross-linked by tissue transglutaminase. *Malaria journal* 2011;10:14.

12. Hillier CJ, Ware LA, Barbosa A, et al. Process development and analysis of liver-stage antigen 1, a preerythrocyte-stage protein-based vaccine for Plasmodium falciparum. *Infection and immunity* 2005;73(4):2109-15.

13. Pizarro JC, Chitarra V, Verger D, et al. Crystal structure of a Fab complex formed with PfMSP1-19, the C-terminal fragment of merozoite surface protein 1 from Plasmodium falciparum: a malaria vaccine candidate. *Journal of molecular biology* 2003;328(5):1091-103.

14. Draper SJ, Angov E, Horii T, et al. Recent advances in recombinant protein-based malaria vaccines. *Vaccine* 2015;33(52):7433-43.

15. Bartoloni A, Zammarchi L. Clinical aspects of uncomplicated and severe malaria. *Mediterranean journal of hematology and infectious diseases* 2012;4(1):e2012026.

16. Trampuz A, Jereb M, Muzlovic I, et al. Clinical review: Severe malaria. *Critical care (London, England)* 2003;7(4):315-23.

17. Organization WH. Global programme to eliminate lymphatic filariasis: progress report, 2017. *Weekly Epidemiological Record*: World Health Organization, 2018:589-604.

18. Babu S, Nutman TB. Chapter 16 - Vascular Responses in Human Lymphatic Filariasis. In: Gavins FNE, Stokes KY, eds. *Vascular Responses to Pathogens*. Boston: Academic Press, 2016:209-20.

19. Fox LM, King CL. 110 - Lymphatic Filariasis. In: Magill AJ, Hill DR, Solomon T, et al., eds. *Hunter's Tropical Medicine and Emerging Infectious Disease (Ninth Edition)*. London: W.B. Saunders, 2013:815-22.

20. Ramaiah KD, Ottesen EA. Progress and impact of 13 years of the global programme to eliminate lymphatic filariasis on reducing the burden of filarial disease. *PLoS neglected tropical diseases* 2014;8(11):e3319.

21. Famakinde DO. Mosquitoes and the Lymphatic Filarial Parasites: Research Trends and Budding Roadmaps to Future Disease Eradication. *Tropical medicine and infectious disease* 2018;3(1).

22. Hamlin KL, Moss DM, Priest JW, et al. Longitudinal Monitoring of the Development of Antifilarial Antibodies and Acquisition of Wuchereria bancrofti in a Highly Endemic Area of Haiti. *PLoS neglected tropical diseases* 2012;6(12):e1941.

23. Lammie PJ, Weil G, Noordin R, et al. Recombinant antigen-based antibody assays for the diagnosis and surveillance of lymphatic filariasis - a multicenter trial. *Filaria journal* 2004;3(1):9.

24. Krushna NS, Shiny C, Dharanya S, et al. Immunolocalization and serum antibody responses to Brugia malayi pepsin inhibitor homolog (Bm-33). *Microbiology and immunology* 2009;53(3):173-83.

25. Won KY, Sambou S, Barry A, et al. Use of Antibody Tools to Provide Serologic Evidence of Elimination of Lymphatic Filariasis in The Gambia. *The American journal of tropical medicine and hygiene* 2018;98(1):15-20.

26. Steel C, Golden A, Kubofcik J, et al. Rapid Wuchereria bancrofti-specific antigen Wb123-based IgG4 immunoassays as tools for surveillance following mass drug administration programs on lymphatic filariasis. *Clinical and vaccine immunology : CVI* 2013;20(8):1155-61.

27. Kubofcik J, Fink DL, Nutman TB. Identification of Wb123 as an Early and Specific Marker of Wuchereria bancrofti Infection. *PLoS neglected tropical diseases* 2012;6(12):e1930.

28. Babu S, Nutman TB. Immunopathogenesis of lymphatic filarial disease. *Seminars in immunopathology* 2012;34(6):847-61.

29. Nutman TB. Insights into the pathogenesis of disease in human lymphatic filariasis. *Lymphatic research and biology* 2013;11(3):144-8.

30. WHO. WHO Lymphatic Filariasis Factsheet, 2018. World Health Organization, 2018.

31. van den Berg H, Kelly-Hope LA, Lindsay SW. Malaria and lymphatic filariasis: the case for integrated vector management. *The Lancet Infectious diseases* 2013;13(1):89-94.

32. Carter Center. Summary of the Twenty-Ninth Meeting of the International Task Force for Disease Eradication (ITFDE). The Carter Center, 2019.

33. PAHO. Report on the Situation of Malaria in the Americas. Pan American Health Organization, World Health Organization, 2014:1-114.

34. Organization WH. Lymphatic filariasis: A handbook of practical entomology for national lymphatic filariasis elimination programmes. *World Health Organization*, 2013:1-92.

35. Won KY, Robinson K, Hamlin KL, et al. Comparison of antigen and antibody responses in repeat lymphatic filariasis transmission assessment surveys in American Samoa. *PLoS neglected tropical diseases* 2018;12(3):e0006347.

36. Eigege A, Kal A, Miri E, et al. Long-Lasting Insecticidal Nets Are Synergistic with Mass Drug Administration for Interruption of Lymphatic Filariasis Transmission in Nigeria. *PLoS neglected tropical diseases* 2013;7(10):e2508.

37. Reimer LJ, Thomsen EK, Tisch DJ, et al. Insecticidal Bed Nets and Filariasis Transmission in Papua New Guinea. *New England Journal of Medicine* 2013;369(8):745-53.

38. Keys H. Prevalence of malaria and lymphatic filariasis (LF) in bateyes of the Dominican Republic. *In review* 2019.

39. Priest JW, Plucinski MM, Huber CS, et al. Specificity of the IgG antibody response to Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale MSP119 subunit proteins in multiplexed serologic assays. *Malaria journal* 2018;17(1):417.

40. Rogier E, Wiegand R, Moss D, et al. Multiple comparisons analysis of serological data from an area of low Plasmodium falciparum transmission. *Malaria journal* 2015;14:436.

41. Wong J, Hamel MJ, Drakeley CJ, et al. Serological markers for monitoring historical changes in malaria transmission intensity in a highly endemic region of Western Kenya, 1994-2009. *Malaria journal* 2014;13:451.

42. Drakeley CJ, Corran PH, Coleman PG, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure.

Proceedings of the National Academy of Sciences of the United States of America 2005;102(14):5108-13.

43. Ondigo BN, Hodges JS, Ireland KF, et al. Estimation of recent and long-term malaria transmission in a population by antibody testing to multiple Plasmodium falciparum antigens. *The Journal of infectious diseases* 2014;210(7):1123-32.

44. Arnold BF, Priest JW, Hamlin KL, et al. Serological measures of malaria transmission in Haiti: comparison of longitudinal and cross-sectional methods. *PloS one* 2014;9(4):e93684.

45. Graves PM, Richards FO, Ngondi J, et al. Individual, household and environmental risk factors for malaria infection in Amhara, Oromia and SNNP regions of Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2009;103(12):1211-20.

46. WHO. World Malaria Report, 2017. Geneva, Switzerland: World Health Organization, 2017.

47. Deressa W, Ali A, Berhane Y. Household and socioeconomic factors associated with childhood febrile illnesses and treatment seeking behaviour in an area of epidemic malaria in rural Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2007;101(9):939-47.

48. Gass K, Beau de Rochars MV, Boakye D, et al. A multicenter evaluation of diagnostic tools to define endpoints for programs to eliminate bancroftian filariasis. *PLoS neglected tropical diseases* 2012;6(1):e1479.

49. Weil GJ, Curtis KC, Fischer PU, et al. A multicenter evaluation of a new antibody test kit for lymphatic filariasis employing recombinant Brugia malayi antigen Bm-14. *Acta tropica* 2011;120 Suppl 1:S19-22.

50. Schmaedick MA, Koppel AL, Pilotte N, et al. Molecular xenomonitoring using mosquitoes to map lymphatic filariasis after mass drug administration in American Samoa. *PLoS neglected tropical diseases* 2014;8(8):e3087.

51. Organization WH. Monitoring and epidemiological assessment of mass drug administration in the global programme to eliminate lymphatic filariasis : a manual for national elimination programmes. *World Health Organization* 2011.