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Estimating *Plasmodium falciparum* Malaria Prevalence in Haiti Using Serologic Testing

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An abstract of
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Hispaniola is the only island in the Caribbean where endemic malaria transmission still occurs. Elimination of malaria in Hispaniola has been viewed as an attainable goal when malaria control and elimination efforts are focused in Haiti, where the majority of cases are. We estimated the prevalence of *P. falciparum* malaria in Haiti using serological assays, which are more useful at detecting exposure and transmission of the disease in low-endemic countries. We used Luminex® assay to assess Immunoglobulin G (IgG) antibody prevalence to three *P. falciparum* antigens (AMA-1, MSP-1-19 & LSA-1) measured through nationwide surveys in 2012 and 2015. Persons with antibodies to all three antigens were found throughout the country, with MSP-1-19 being the most prevalent in both surveys, followed by AMA-1 and LSA-1. Higher antibody prevalence was found in pregnant women, older age groups (>50-years old), rural areas and in the administrative region (department) of Centre. A poor agreement between microscopy and Rapid Diagnostic Tests (RDTs) to Luminex® assay indicated serologic testing, rather than diagnostic testing, is more suitable for identification of transmission in areas with low prevalence of malaria. This study suggests that ongoing monitoring of *P. falciparum* antibody prevalence should continue under the country's malaria prevention and control efforts, and that interventions should be intensified in rural areas, especially in the geographic region of Centre for the attainment a malaria-free Haiti.

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Estimating *Plasmodium falciparum* Malaria Prevalence in Haiti Using Serologic Testing

Definition of terms

Agent – a pathogen (e.g., virus, bacteria, or parasite) that causes an infection (Nelson & Williams, 2014, p.45).

Antibody – "a specialized serum protein (e.g., immunoglobulin) produced by B lymphocytes in the blood in response to an antigen exposure. The antibodies specifically bind to the antigens that induce the immune response and help defend the body against infectious agents such as parasites" (CDC, 2012).

Antigen – "foreign substances, such as parts of an invading parasite, that stimulates the immune system to produce antibodies" (CDC, 2012).

AMA-1 – apical membrane antigen from *Plasmodium* (Sylla et al., 2015).

Chloroquine – "A drug used for both prevention and treatment of malaria" (CDC, 2012). Chloroquine works by targeting the blood stages of the *Plasmodium* parasite's life cycle, not the liver stages (YG, 2016).

CSP – circumsporozoite protein *Plasmodium* antigen (Sylla et al., 2015).

Elimination – "reducing all local transmission of a disease (e.g., malaria) down to zero cases within a defined geographic location (e.g., Haiti)" (CDC, 2012).

Endemic – "the habitual presence of a disease or infectious agent within a given geographic area or a population group; may also refer to the usual prevalence of a given disease within an area" (Heymann, 2015 p. 695).

Endemicity – "endemicity or disease intensity is a measure of disease prevalence in a particular region" (MAP, n.d. a).

Endophagic – "a mosquito that feeds indoors" (CDC, 2012).

Exophagic – "a mosquito that feeds outdoors" (CDC, 2012).

Gametocytes – "The sexual stage of malaria parasites. Male gametocytes (microgametocytes) and female gametocytes (macrogametocytes) are inside red blood cells in the circulation. If they are ingested by a female *Anopheles* mosquito, they undergo sexual reproduction which starts the extrinsic (sporogonic) cycle of the parasite in the mosquito. Gametocytes of *Plasmodium falciparum* are typically banana or crescent-shaped" (CDC, 2012).

Host – "a person or another living animal that affords subsistence or lodgment to an infectious agent under natural conditions" (Heymann, 2015 p. 696).

Hotspot – Mosha et. al. (2014b) defines it as "a cluster of higher than expected prevalence of a disease as compared to surrounding areas" (Mosha, Sturrock, Greenwood, et al., 2014).

Immunity – "protection generated by the body's immune system, in response to previous malaria attacks, resulting in ability to control or lessen a malaria attack" (CDC, 2012).

Immunization – "the process by which a subject (e.g., person) is rendered immune, or resistant to a specific disease (e.g., malaria)" (CDC, 2012).

Incidence – the number of infections by diagnostic tests (RDT, PCR, microscopy) or the number of IgG antibody positives by serology during a given period in a specified population (Heymann, 2015 p.697).

Infection – "the invasion of an organism by a pathogen such as parasites (e.g. *Plasmodium*). Not all infections lead to disease" (CDC, 2012).

MSP-1-19 – merozoite surface protein antigen from *Plasmodium* (Wilson et al., 2011).

MSP-1-42-(D)-(F) – merozoite surface protein antigen from *Plasmodium* (Sylla et al., 2015).

Parasite – "Any organism that lives in or on another organism without benefiting the host organism" (CDC, 2012).

Parasitaemia- "the presence of parasites in the blood or the quantity of parasites in the blood" (CDC, 2012).

Plasmodium – "the genus of the parasite that causes malaria" (CDC, 2012).

Prevalence – the total number of infections by diagnostic tests (RDT, PCR, microscopy) or the total number of IgG antibody positives by serology in a specified population at a particular time, without distinction between old and new cases (Heymann, 2015 p.702).

Resistance – "the ability of an organism to develop ways to be impervious to specific threats to their existence". Some malaria parasites have developed resistance to chloroquine drugs while some mosquitoes have developed resistance to insecticides (CDC, 2012).

Serology – "the branch of science dealing with the measurement and characterization of antibodies and other immunological substances in body fluids, particularly serum" (CDC, 2012).

Sequestration – "the adherence of infected erythrocytes (red blood cells) containing late developmental stages of the parasite (trophozoites and schizonts) to the endothelium of capillaries and venules; it is characteristic of Plasmodium falciparum infections" (David et. al., 1983).

Sporozoite – "A stage in the life cycle of the malaria parasite. Sporozoites are produced in the mosquito and migrate to the mosquito's salivary glands. They can be inoculated into a human host when the mosquito takes a blood meal on the human. In the human, the sporozoites enter liver cells where they develop into the next stage of the malaria parasite life cycle (the liver stage or exo-erythrocytic stage)" (CDC, 2012).

Titration – measuring antibodies present in a blood sample dilutions (Draper, 1971).

Transmission – a category of risk at which malaria is spread in a population (MAP, n.d. b).

Vector – "an organism (e.g., *Anopheles* mosquitoes) that transmits an infectious agent (e.g. malaria parasites) from one host to the other (e.g., humans)" (CDC, 2012).

Chapter I. Introduction

Malaria is a life-threatening disease caused by parasites from the genus *Plasmodium*. People acquire malaria from the bite of an infected female *Anopheles* mosquito. When the *Anopheles* mosquito takes a blood meal from a malaria infected human, it ingests the gametocytes which have to multiply inside the mosquito's stomach and midgut before making its way to the salivary glands where it can inoculate a new human host by injecting the sporozoites and continuing the malaria lifecycle (CDC, 2016a). There are five species of *Plasmodium* parasites that cause malaria in humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*, the first one being the most dangerous (WHO, 2016a). *P. falciparum* is found in the tropics and subtropical regions, and predominates in Africa. *P. falciparum* is the most lethal species because it multiplies quickly in the blood causing severe malaria and anemia due to serious blood loss (CDC, 2016b). Additionally, an infection with *P. falciparum* can lead to cerebral malaria, a fatal complication that occurs when the parasites clog small blood vessels in the brain (CDC, 2016b).

The vector of malaria, *Anopheles sp.*, are crepuscular or nocturnal mosquitoes, biting between dusk and dawn (CDC, 2015a). Their biting behavior differs since some are endophagic (prefer to bite indoors) and others are exophagic (prefer to bite outdoors) (CDC, 2015a). The *Plasmodium* parasite, the *Anopheles* vector, the human host, and the environment are all factors that determine the intensity of malaria transmission in a given place (WHO, 2016a). In order to transmit the parasite, the mosquito needs to have a long lifespan so that the parasite can mature and develop inside the vector to successfully infect others while taking a blood meal. The environmental and climate conditions also play a big role in malaria transmission since rainfall, temperature, and humidity influences the mosquitoes' survival (WHO, 2016a). In many countries,

malaria transmission occurs seasonally, peaking after the rainy seasons (WHO, 2016a). In areas where the population has little to no immunity, malaria epidemics can occur unexpectedly when the environment and climate conditions favor the mosquito population (e.g., earthquakes and floods). In moderate to high malaria endemic areas, partial immunity is developed as people are exposed throughout life. Partial immunity does not provide complete clinical protection, but can reduce the risk of severe malaria from occurring (WHO, 2016a). In endemic settings, when there is low transmission of malaria, all age groups are at risk of severe disease since they have low or no immunity to the parasite, whereas in high transmission settings, young children are at a greater risk since they do not have the acquired immunity that develops with age (WHO, 2016a).

In order to accurately determine the prevalence and distribution of malaria, sensitive and specific tests are needed to detect the disease. Microscopy is the "gold standard" for malaria laboratory confirmation, while Rapid Diagnostic Tests (RDTs) are useful for specific parasitic antigen detection during epidemiological surveillance studies since they are easy to use, do not require electricity, and provide fast results (Lucchi et al., 2014; Makler, Palmer, & Ager, 1998; Moody, 2002). A shortcoming of microscopy and RDTs is that both can underestimate the prevalence of the parasite, especially in in low transmission areas, where the majority of the infected population is asymptomatic (Ghinai et al., 2017; Lucchi et al., 2014). Polymerase Chain Reaction (PCR) is a more sensitive test when compared with microscopy and RDTs, however, it is also only useful for detecting current active infections (Ghinai et al., 2017).

Serological assays such as the enzyme-linked immunosorbent assay (ELISA) and Luminex® bead-based assay are simple and cheap to perform (Pothin, Ferguson, Drakeley, & Ghani, 2016). Serological data is used to measure an individual's antibody responses to specific antigens produced by pathogens. Serology can provide an estimate of past exposure to a disease

since it determines the levels of antibodies to a specific agent (Pothin et al., 2016). Since antibodies can persist for months or years after infection, the effect of seasonality in malaria transmission is not as large of a concern (Pothin et al., 2016). A meaningful estimate of malaria antibody prevalence may be determined in low malaria transmission areas when examining the longevity of the antibodies since a higher proportion of antibody positive persons indicates higher prevalence of disease (Pothin et al., 2016). Considering that serology data provides useful information on the prevalence of exposure to malaria and helps demonstrate the parasites transmission dynamics in a specific area, serology becomes a more useful method in low-transmission malaria settings where active cases are rare and difficult to find (Rogier et al., 2016).

Despite the ongoing efforts to reduce malaria worldwide, the disease continues to be a public health concern in 91 countries, primarily in Africa, South-East Asia and the Americas (WHO, 2016b). According to the World Health Organization (WHO), in 2015 there were an estimated 212 million cases of malaria and 429,000 deaths worldwide for which *P. falciparum* accounted for 99% of the malaria deaths (WHO, 2016b). WHO reported that between 2010 and 2015, there has been a 21% decrease in malaria incidence and a 29% decrease in malaria mortality rates worldwide and a 37% decrease in the malaria mortality rate in the American region (WHO, 2016c).

Currently, there is only one island in the Caribbean with endemic malaria transmission: Hispaniola (Boncy et al., 2015; Carter et al., 2015; Frederick et al., 2016; Lucchi et al., 2014). Hispaniola is comprised of Haiti and Dominican Republic. Malaria is endemic in both countries, but the majority of malaria cases occur in Haiti (Elbadry et al., 2015). The *P. falciparum* parasite is the causative agent for malaria in this region and the *Anopheles albimanus* mosquito is the principal vector that transmits the disease (Elbadry et al., 2015; Frederick et al., 2016).

Transmission of *P. falciparum* occurs mostly after Haiti's rainy seasons, from March through May and October through November (CDC, 2015b).

Today, Haiti continues to be the poorest country in the Western Hemisphere (CIA, 2017). With a population size of 10,485,800, Haiti suffers excessive mortality due to AIDS and other infectious diseases which has led to lower life expectancy, higher death rates, and lower population growths (CIA, 2017). Haiti has a tropical semiarid climate, its terrain is mostly uneven and mountainous, and has a mean elevation of 470 m, with the largest concentrations of people located near the coast (CIA, 2017). Many of the low-elevation areas of Haiti have natural springs and irrigation canals, which can create ideal habitats for mosquito breeding sites (Frederick et al., 2016). The island has been known to suffer from various natural disasters such as hurricanes, storms, flooding, earthquakes, and periodic droughts (CIA, 2017).

Natural disasters negatively impact the health and livelihood of Haitians. Earthquakes and hurricanes have been associated with increased malaria transmission in the island (Frederick et al., 2016). Additionally, movement of individuals from Haiti to Dominican Republic has contributed to the spread of malaria and an uncertain geographic distribution of disease (Frederick et al., 2016). Unfortunately, on January 12, 2010, an earthquake of a 7.0 magnitude hit Haiti and left approximately 500,000 people homeless and approximately 200,000 people were killed (Mung et al., 2010). The earthquake devastated the lives of many Haitians, especially those who lived in densely populated areas (Boncy et al., 2015). Haiti's public health system was weakened since many hospitals and laboratory facilities were destroyed (Boncy et al., 2015; Carter et al., 2015). Consequently, many Haitians were exposed to increased mosquito populations and increases in *P. falciparum* transmission (Carter et al., 2015). After the earthquake, travel to Haiti was increased as people from around the world who came to provide health services and relief. Although this

was helpful and necessary at the time, it offered the potential spread of malaria to other countries and the possible introduction of a drug-resistant *Plasmodium* parasite in the population (Boncy et al., 2015). Since *A. albimanus* tends to have an exophagic pattern, displaced individuals and emergency responders living outdoors or in shelters were at a greater risk of acquiring malaria (CDC, 2015a; Mung et al., 2010). After the earthquake, malaria cases increased and approximately 86,633 cases of *P. falciparum* malaria cases were reported, and in 2012, 25,423 confirmed malaria cases were reported (Herrera et al., 2015; Lucchi et al., 2014). There is uncertainty in whether these numbers are correct since there continues to be a lack of malaria surveillance and shortages in the information systems in Haiti (Herrera et al., 2015; Lucchi et al., 2014).

Haiti's weakened infrastructure, triggered by the 2010 earthquake, caused there to be successful reservoirs and uncontrolled transmission of infectious diseases, endangering the population and putting them at a higher risk of infections (Boncy et al., 2015). Since then, there has been a renewed international interest in eliminating malaria from Hispaniola by 2020 (Boncy et al., 2015). The island of Hispaniola is known to have a low transmission of malaria with *P. falciparum* being the primary agent of disease. Despite the extensive usage of chloroquine treatment for many years, there is little evidence of resistance to this antimalarial in Haiti (Carter et al., 2015). Additionally, since malaria is not endemic in nearby countries, the island is at a low risk of importing malaria cases (Carter et al., 2015). All of these factors contribute to Haiti's potential for malaria elimination.

A national population-based survey conducted in 2011 estimated that malaria prevalence in Haiti was less than 1% (Frederick et al., 2016; Lucchi et al., 2014). Given the low prevalence and transmission of disease, elimination of malaria in Hispaniola has been viewed as an attainable goal when malaria control and elimination efforts are focused in Haiti (Boncy et al., 2015; Elbadry

et al., 2015). Although current programs to eliminate malaria in Haiti are limited by deficiencies in the surveillance system, current interventions are being implemented in the country, such as the distribution of insecticide-treated nets (ITNs) for vector control (Boncy et al., 2015). Other interventions practiced are the use of RDTs for diagnosis, treatment with a combination of chloroquine and primaquine, and entomological interventions such as larvaciding (Boncy et al., 2015). Even though these interventions are necessary for the control and elimination of malaria in Haiti, there continue to be some weaknesses that affect such interventions. For example, there continues to be a lack of training and dissemination of recent diagnostic guidelines, inaccuracy in the communication and reporting of suspected cases as confirmed, and an absence of devices to send case information to the central level and a lack of feedback once received (Boncy et al., 2015). All of these impediments contribute to the difficulty of eliminating malaria in Haiti. Currently, with the support of the Centers for Disease Control and Prevention (CDC), local Non-profit organizations (NGOs), and the Programme National de Controle de la Malaria (PNCM), the Haitian government has developed the Plan Stratégique National d'Elimination de la Malaria (PSNEM), a national strategic plan for 2016-2022, with the goal of improving case management and the surveillance system for malaria elimination by 2020 (Boncy et al., 2015).

Ongoing monitoring of malaria prevalence is key to achieve malaria elimination by 2020. Since serological methods are more useful in detecting exposure and transmission of malaria in low-endemic countries, gathering serology data to estimate the prevalence of malaria becomes a more useful technique in Haiti. Additionally, serology data becomes very useful when determining where resources should be allocated and which interventions should be targeted to control and eliminate the disease (Rogier et al., 2016). Given the importance of serologic testing, this study

will use serology data to estimate the prevalence of *P. falciparum* malaria in Haiti with the overall goal of contributing to the elimination of malaria in the Caribbean.

Chapter II. Literature Review, Problem, Purpose & Significance Statements

A. Literature Review

a. Epidemiology of malaria

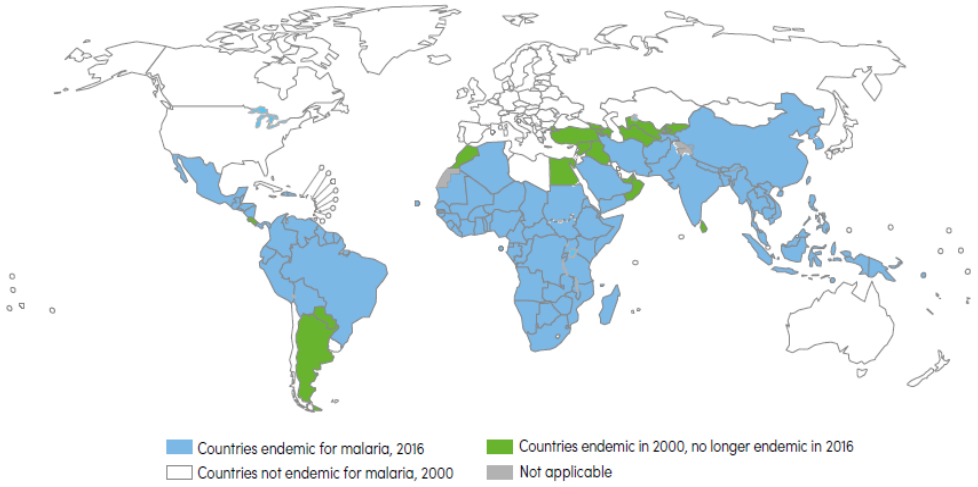
Despite the ongoing efforts to reduce malaria worldwide, the disease continues to be a public health concern in 91 countries, primarily in Africa, South-East Asia and the Americas (Figure 1) (WHO, 2016b). According to the World Health Organization (WHO), in 2015 there were an estimated 212 million cases of malaria and 429,000 deaths worldwide for which *Plasmodium falciparum* accounted for 99% of the malaria deaths (WHO, 2016b). WHO reported that between 2010 and 2015, there has been a 21% decrease in malaria incidence and a 29% decrease in malaria mortality rates worldwide and a 37% decrease in malaria mortality rate in the American region (WHO, 2016c).

According to the WHO's 2016 World Malaria Report, there were an estimated 191 million malaria cases in the African region, 14 million in the South-East Asia region, 3.8 million in the Eastern Mediterranean region, 1.2 million in the Western Pacific region and 0.8 million malaria cases in the American region (WHO, 2016b). Regarding malaria mortality, there were an estimated 394 thousand deaths in the African region, 26 thousand deaths in the South-East Asia region, 7.3 thousand deaths in the Eastern Mediterranean region, 1.5 thousand deaths in the Western Pacific region and 0.5 thousand deaths in the American region (WHO, 2016b). These estimates indicate Africa as the region with the highest transmission of malaria and the Americas with the lowest.

WHO's Global Technical Strategy (GTS) for Malaria 2016-2013 provides a technical framework for all malaria endemic countries and its targets include: reducing malaria incidence

and mortality rates by at least 90% as well as eliminating malaria in at least 35 countries by 2030 and preventing resurgence in malaria-free countries (WHO, 2015). Each country's progress toward malaria elimination depends on their level of investment, biological determinants (e.g. vector and parasite), environmental factors, the strength of their health system, social, demographic, political and economic realities (WHO, 2015). Once malaria programs reduce transmission to a low rate, programs should focus on the prevention, detection, and treatment of every malaria infection (WHO, 2015). In order to achieve this, the epidemiological and entomological surveillance systems need to be strengthened. This improvement can be accomplished through long-term financial and political commitment as well as structural and organizational changes in malaria programs (WHO, 2015). Given Haiti's low estimated malaria prevalence of less than 1%, elimination of malaria in Hispaniola is viewed as an attainable goal when malaria control and elimination efforts are focused in Haiti (Boncy et al., 2015; Elbadry et al., 2015; Frederick et al., 2016; Lucchi et al., 2014). Haiti is currently on the target towards malaria elimination by 2020 through PSNEM, with the help of CDC, NGOs, and the Haitian Ministry of Health.

Figure 1. Malaria endemic countries for 2000 and 2016 (WHO, 2016b).



b. Diagnosis and serologic testing for malaria

Multiple diagnostic tests for malaria have been developed throughout the years and have been published in the literature extensively. A comprehensive review of practical techniques for malaria diagnosis was published by Makler *et. al*., (1998) where traditional and contemporary diagnostic methods were evaluated. The authors discussed the reality that in many developing countries, resources for diagnosis of malaria are unavailable and there is a lack of trained professionals (Makler, Palmer, & Ager, 1998). The "gold standard" laboratory procedure for malaria diagnosis is the preparation of thick and thin blood smears for observation under light microscopy, a technique that was developed in the early 1900s. Investigators discussed that the thick blood smear was more sensitive since it concentrated the blood on a small surface and detected the parasite infection more easily than with the thin smear (Makler et al., 1998). On the other hand, the thin smear was more specific, given that it allowed for *Plasmodium* species identification and the detection of the intensity of parasitemia (Makler et al., 1998). Even though microscopy is still considered the "gold standard", researchers argued that the diagnosis of malaria using this technique was very time consuming (~60 minutes in slide preparation), labor intensive, and the interpretation of results required an expert, especially in low transmission areas (Makler et al., 1998). Another shortcoming of microscopy was that, in those infected with *P. falciparum,* diagnosis of the parasites by microscopy might not be useful since the parasites are not always present in the peripheral blood due to sequestration. Subsequently, this could lead the technologist to miss the infection, since no parasites will be found in the blood smears (Makler et al., 1998).

An additional laboratory diagnosis procedure discussed in Makler *et. al*., (1998) was the detection of the *Plasmodium* parasites nucleic acid sequence. The polymerase chain reaction (PCR) technique amplifies a targeted sequence of the parasites DNA and makes billions of copies

to be analyzed and detected afterwards by gel electrophoresis. An advantage of PCR is its usefulness in detecting infections in low parasitaemic patients; however, PCRs are expensive, labor intensive, and require considerable technical experience (Makler et al., 1998). Even though the researchers argued PCR methods were the most sensitive, they acknowledge its drawbacks such as the expensiveness, specialized equipment, and advanced training it requires. Therefore, they suggested the PCR technique was not appropriate for malaria diagnosis in developing countries (Makler et al., 1998).

Moody et. al., (2002), discussed non-microscopic diagnostic tests available for malaria. Rapid diagnostic tests (RDTs) are immunochromatographic antigen capture tests which means they are capable of detecting the parasites antigen in the blood. This indicates whether the person has active malaria infection. RDTs are fast, generate results in 15-20 minutes, and are commercially available in kits (Moody, 2002). RDTs have been described as equally accurate in providing results as microscopy and are better for remote areas where there is a lack of microscopy-trained technicians (Moody, 2002). RDTs are easy to perform and require little training making them useful in the field and in limited resource settings; however, the authors argue that the sensitivity of the test is a problem in non-immune populations since RDTs might miss malaria infections when parasite densities are low (Moody, 2002). The researcher indicated microscopy with thick and thin blood smears was still necessary for *Plasmodium* identification (Moody, 2002).

The use of serologic tests and their significance in determining the epidemiology of malaria in humans has been a topic of discussion in the literature since the 1970's. Draper (1971) discussed the serology diagnostic tests available for malaria during that time and explained that the measurement of the parasite antibodies was useful for individual diagnosis, especially in areas were malaria was endemic. He discussed that comparing the immunological responses of the

population helped measure the amount of malaria they had been exposed to and helped assess the progress of malaria eradication in the country (Draper, 1971). Draper discussed the two serologic diagnostic tests available for malaria. The indirect haemagglutination test (IHT) for malaria antibodies was quick and convenient to do, allowed for measurement of serum antibodies (titration) at different concentrations and had previously been used for survey and clinical testing purposes (Draper, 1971). Furthermore, Draper referred to the indirect fluorescent antibody (IFA) test as the most widely used serological test for malaria, even though *P. falciparum* cross-reacted with other human parasites. Draper mentioned that IFA tests were useful in clinical practice when evaluating malaria diagnosis retrospectively (person has already been exposed to the parasite) and for identifying malaria in blood transfusion since parasitaemia may be low or intermittent. However, for those living in high endemic areas who had been exposed to malaria frequently, individual interpretation was more difficult by IFA, but the test had proven valuable in epidemiological studies conducted in such settings (Draper, 1971).

Four year later, Voller *et. al.* (1975), described a new serologic test for malaria: the enzyme-linked immunosorbent assay (ELISA). The ELISA test was able to detect malaria antibodies to *P. vivax* and *P. falciparum* by using antigens prepared from *P. knowlesi,* the simian malaria parasite (Voller, Huldt, Thors, & Engvall, 1975). Voller commented on the usefulness and appropriateness of ELISA tests for comprehensive epidemiological programs since blood samples could be easily collected with a finger-prick. The ELISA studies conducted by Voller *et al*., were simple and done with inexpensive materials, which proved they could be easily executed in laboratories worldwide (Voller et al., 1975). Shortcomings of ELISA include: the labor intensive process, requires specific laboratory equipment with well-trained technicians, require a large amount of samples and can

only examine one analyte at a time, whereas Multiplex assays, such as the Luminex® x-MAP, can examine up to 500 analytes simultaneously (Elshal & McCoy, 2006; Gupta, n.d.).

Multiplex assays are proven to be a useful serology technique for measuring malaria transmission intensity in low-endemic areas. In addition to their ability to examine multiple malaria antibodies simultaneously, multiplex assays can be used to evaluate the efficacy of malaria control strategies (Sarr et al., 2011). Sarr et. al. (2011), used the sensitive multiplex based assay (MBA) to examine children's IgG antibody responses to 13 *P. falciparum* antigens simultaneously. Investigators found that antibody positivity increased with age and the increase was significantly correlated with the parasites density (Sarr et al., 2011).

MBAs can be used to detect IgG responses against multiple *Plasmodium* species. *Plasmodium* antigens produce IgG antibody responses that can last for a long time; therefore, serology can detect them long after infection (Rogier et al., 2016). Since serological analysis in children can provide an estimate of lifetime exposure, an MBA assay was conducted to estimate IgG responses to *P. falciparum* (AMA-1 & MSP-1-42) and *P. vivax* (MSP-1-19) antigens in schoolchildren (Rogier et al., 2016). Interestingly, 96% of the children were positive for either or both *P. falciparum* antibodies, whereas only 17% had antibodies to *P. vivax* (Rogier et al., 2016). Investigators found that as age increased, antibody responses to *P. falciparum* only, increased. This discovery can provide insight on the transmission pattern of malaria, likely indicating a sporadic spread of parasite species (Rogier et al., 2016). MBA remains an effective serological analysis tool, especially when wanting to evaluate multiple *Plasmodium* species to determine malaria transmission (Rogier et al., 2016).

Challenges of malaria diagnosis in endemic and non-endemic settings were discussed by Bronzan et. al., (2008). Since malaria is rare in low transmission settings, a very sensitive test is

needed to identify the parasitic infection at very low densities. Meanwhile, in countries of higher malaria endemicity, diagnosis is difficult since individuals may have partial immunity, due to years of exposure, and may be parasitized, but not ill (Bronzan, McMorrow, & Kachur, 2008). In terms of serologic diagnosis of malaria, IFA and ELISA tests are useful in determining prior exposure to the parasite, given they measure antibodies to malaria, which require time to develop. Therefore, investigators stated that neither tests were appropriate for diagnosis of acute malaria infections (Bronzan et al., 2008). Bronzan et. al. (2008) mentioned that, in areas where malaria is endemic, antibody detection is of little value since the majority of the population have had significant exposure to the disease throughout their lifetime. Hence, serology is most useful in non-endemic areas.

Bronzan et. al., (2008) explained that in malaria endemic countries, transmission intensity and appropriate diagnostic tests vary depending on whether the area has high, low or intermediate transmission. In low transmission areas, the local population has less acquired immunity to the disease, the average age at first infection is higher, the infection is more frequently associated with illness, and severe malaria becomes more common in adults than in children (Bronzan et al., 2008). They argued that in low-transmission areas, the application and interpretation of malaria diagnosis approaches that of a non-endemic country (Bronzan et. al., 2008). In a non-endemic malaria country, the detection of malaria antibodies through serology becomes the most useful diagnostic technique, however, microscopy still remains the standard diagnostic test. Given that microscopy skills weaken when technicians are not conducting malaria diagnosis regularly, such happens in low-transmission settings, microscopy becomes less useful. Moreover, RDTs become more convenient in low-transmission settings, especially in poor resource and remote areas (Bronzan et. al., 2008). Even though RDTs are simple and easy to interpret, they are sensitive to heat and

humidity, making it more challenging in malaria endemic countries where it is mostly a tropical climate. Researchers conclude that serology is most useful in non-endemic or low-transmission malaria countries, particularly when conducting epidemiologic investigations (Bronzan et al., 2008).

There are multiple diagnostic techniques and serological methods that have been developed for the detection of malaria. Microscopy continues to be the "gold standard" diagnostic test, however, it becomes a challenging method to employ in low-transmission malaria settings. PCR is useful when detecting infections in low parasitaemic patients; however, it is expensive, labor intensive, and requires specialized laboratory equipment which makes it a difficult technique to conduct in poor resource settings. RDTs are simple and effective in remote areas, while serology methods, such as MBA, are most useful in low-transmission malaria countries since they identify history of exposure to the disease and can be used to estimate the prevalence of the parasite in epidemiologic surveys.

c. Malaria prevalence and transmission studies in low-endemic countries

In low-endemic malaria countries, sensitive tools are needed to monitor the transmission intensity of the parasite and to prioritize health strategies (Rosas-Aguirre et al., 2013). Rosas-Aguirre et. al. (2013) assessed the transmission intensity of malaria using molecular and serological tools by conducting a cross-sectional survey in north-western Peru, an area with low malaria endemicity. Molecular tools included the use of microscopy and PCR to detect parasitic infections. Serology data was obtained using ELISA to detect *P. falciparum* antibodies. The researchers found that serology with ELISA identified a higher overall prevalence rate (9.8% for *P. falciparum*) whereas active malaria infection with microscopy (0%) or PCR (0.04%) was very limited (Rosas-Aguirre et al., 2013). Even though serology identified a higher prevalence, the

combination of molecular diagnostic and serologic tools was useful since it enhanced the detection of current and past exposure to malaria (Rosas-Aguirre et al., 2013).

A six-month cross-sectional study conducted by Bretscher et al. (2013), used ELISA to measure *P. falciparum* antibodies in Indonesian schoolchildren. Antibodies against AMA-1 and MSP-1-19 (long term antigens of *P. falciparum*) were measured in exposed and unexposed children (Bretscher et al., 2013). Measurement of malaria transmission becomes a challenge in low-endemic areas due to difficulties in detecting parasite positive people as malaria approaches zero (Bretscher et al., 2013). Since the antibody responses that develop after infection last longer than the parasitic infection, serology becomes more useful to assess malaria transmission intensity in low endemic areas (Bretscher et al., 2013).

Traditionally, estimating malaria transmission has been based on vector or parasite procedures, but current methods that use serological markers are more convenient to use in low-endemic areas (Cunha et al., 2014). Serology procedures are used in estimating malaria transmission since they can detect long lasting antibody responses (e.g., IgG) and, thus, the antibody prevalence can help determine malaria transmission changes in endemic areas (Cunha et al., 2014). A cross-sectional study, conducted in northern Brazil, determined the IgG antibody prevalence to *P. vivax* and *P. falciparum* antigens (AMA-1 and MSP-1) and successfully proved that serology can be used to measure and monitor malaria transmission (Cunha et al., 2014).

P. falciparum malaria surveillance was studied in Senegal, where there has been a decline of malaria transmission (Sylla et al., 2015). As parasitic infections became lower every year, serological tools have replaced microscopy. Sylla et al. (2015) conducted cross-sectional surveys with children under 10 years old living in central and south Senegal. Although infection was diagnosed with microscopy, *P. falciparum* antibodies (AMA-1, MSP-1-42, & CSP) were

measured through ELISA (Sylla et al., 2015). Logistic regression analyses were conducted to determine risk factors associated with antibody carriage and they found the presence of *P. falciparum* antibodies increased with age, active infection and area of residence (Sylla et al., 2015). Investigators emphasized that serology methods are useful for studying the epidemiology of malaria in low-transmission areas and contribute to the identification of hotspots, useful when directing target interventions (Sylla et al., 2015).

In Thailand's Tak province, a comprehensive cross-sectional study was conducted in the Myanmar border, which once had been known to have the highest burden of malaria, but now, due to control efforts, it is a low-endemic area (Baum et al., 2016). In settings where prevalence of malaria has declined, but transmission is still occurring, traditional detection methods (e.g., microscopy) should not be used since asymptomatic infections will not be detected or treated (Baum et al., 2016). In this study, microscopy, PCR, and serology were used to detect presence of *Plasmodium sp*. Researchers found that 90.2% of the infections were submicroscopic and asymptomatic, 68% of febrile patients had multiple *Plasmodium sp*. infections, and all were misdiagnosed and undertreated when tested by molecular diagnosis, proving once again that microscopy and PCR are not practical diagnostic tools in low-transmission settings (Baum et al., 2016). When serologically tested, all persons had IgG reactive to the parasite antigens, indicating everyone enrolled in the study has been exposed to malaria (Baum et al., 2016). Another advantage of serology is the ability to detect present and past exposure as well as estimating prevalence rates to determine malaria endemicity in a particular place. Additionally, the success of malaria control efforts can be ascertained by serology when blood samples react to a small number of antigens since low antibodies are correlated with reduced parasite exposure (Baum et al., 2016). Nevertheless, low levels of antibodies against *Plasmodium sp*. make the population susceptible to

malaria, children especially. In the Thailand study, people below 15 years old were more vulnerable to the disease (Baum et al., 2016). Baum et al. (2016) mentions that by stratifying malaria prevalence by age, hotspots and changes in transmission can be detected. Once more, serology is recognized as a valuable tool for monitoring the epidemiology of malaria in low-transmission settings (Baum et al., 2016).

As soon as the transmission of malaria starts diminishing, prevention and control interventions require more reliable and accurate identification of high risk areas and high risk population groups before targeting interventions (Mosha, Sturrock, Brown, et al., 2014). Mosha et al., discussed that identification of malaria transmission hotspots are imperative before targeting interventions for control and elimination of the disease. The study, conducted in Misungwi, Tanzania, demonstrated that households located in high-risk malaria hotspots had a strong association with malaria infections the following year (Mosha et al., 2014a). Researchers suggest that, in order to achieve control and elimination of the disease, hotspots with better coverage and control strategies should be targeted first given that it will be a practical use of resources towards achieving elimination (Mosha et al., 2014a).

d. Malaria prevalence and transmission studies in Haiti

Transmission of malaria continues to occur in Haiti, albeit at low levels. Since passive surveillance becomes less appropriate for capturing incidence of malaria, cross-sectional surveys have been done to assess malaria transmission and prevalence rates in Haiti. A study conducted by von Fricken et al. (2014) evaluated age-specific prevalence rates in Haiti's Ouest (West) and Sud-Est (South East) departments using ELISA (von Fricken et al., 2014). Samples were collected from rural communities, schools and clinics, and were screened for malaria antibodies to *P. falciparum's* AMA-1 and MSP-1-19 antigens. Results indicated that infections ranged from 21.1% to 30.3%

(von Fricken et al., 2014). Age was associated with the likelihood of earlier infection and, after stratifying by age, the results indicated the annual transmission of malaria in the departments was approximately 2.5% (von Fricken et al., 2014). The research showed transmission of malaria in Haiti is low, regardless the lack of control efforts, and suggested elimination of malaria is feasible when surveillance is strengthened (von Fricken et al., 2014).

Given the low *P. falciparum* burden in Haiti, accurate serological assays that measure exposure and human IgG responses to the parasite antigens have been developed. A study conducted by Rogier and colleagues (2015) examined samples from a 2012 Haitian nationwide malaria survey and compared them to a true seronegative reference population (US residents) to determine which immunoassay was best for analyzing malaria in a low-transmission setting like Haiti (Rogier et al., 2015). ELISA and MBA assays were used to obtain IgG antibody data to 4 *P. falciparum* antigens: MSP-1-19, MSP-1-42(D), MSP-1-42(F), and AMA-1 (Rogier et al., 2015). When exposure to the parasite is reduced in the population, levels of IgG are also expected to decrease, making identification of seropositive individuals much more challenging (Rogier et al., 2015). Results from the study found MBA to be a better serological assay for both the Haitian and "malaria-naive" US samples, making these (naïve US samples) an effective reference group for malaria MBA serological assays (Rogier et al., 2015).

There is evidence that malaria can be eliminated from Haiti; however, challenges in epidemiology and elimination efforts are keeping Haiti from achieving this goal. More information and research on the transmission and surveillance of malaria in Haiti are needed as well as proper training for healthcare and laboratory technicians to properly identify malaria cases and improve reporting (Boncy et al., 2015). Even though *P. falciparum* parasites have not shown drug resistance in Haiti, studies should continue evaluating the parasite strains throughout the country and

continue monitoring for drug resistance development (Boncy et al., 2015). Elimination of malaria is possible and will require multidisciplinary approaches with national comprehensive strategic planning, currently being executed through the PSNEM, to strengthen Haiti's health system and achieve malaria elimination by 2020 (Boncy et al., 2015).

B. Problem Statement

The current prevalence of *P. falciparum* malaria in Haiti is unknown. In a low-endemic country like Haiti, prevalence of the parasite and geographic patterns of transmission are hard to determine given the low numbers of infections. Studies suggest molecular methods are not effective at detecting prevalence of disease and recommend serology methods for estimating prevalence rates in low-endemic countries and assessing history of disease. Even though very few studies have been conducted using serologic assays in Haiti, they have successfully estimated malaria prevalence in the past. Newer estimates of *P. falciparum* prevalence are not available, and to our knowledge, no characterization of transmission sites and population groups has been done to identify high-risk areas and groups. This makes malaria elimination efforts more challenging to the Haitian government.

C. Purpose Statement

The purpose of this study is to estimate the prevalence of *P. falciparum* malaria in Haiti, using the serologic Luminex® assay to assess IgG antibody responses to three *P. falciparum* antigens (AMA-1, MSP-1-19 & LSA-1) measured through two nationwide surveys conducted in in 2012 and 2015. The prevalence estimates, as well as the geographic and population distribution of *P. falciparum* antibodies throughout the country will determine what areas and population groups have a higher immunity as well as parasitic transmission. This will provide data for decision

making that will help Haiti's Ministry of Health and its partners determine where to allocate resources and which strategies to implement for achieving malaria elimination.

D. Research Questions

1. What is the estimated *Plasmodium falciparum* antibody prevalence in Haiti?

    a) Overall in the years 2012 and 2015
    b) By gender
    c) By pregnancy status
    d) By age groups
    e) By administrative region (department)
    f) By geographic location (urban vs. rural)

2. What is the agreement of diagnostic testing via RDT and microscopy with serologic testing with Luminex® in identifying *P. falciparum* positives?

E. Significance Statement

By using serological testing to estimate the prevalence of *P. falciparum* malaria in Haiti, this study will contribute to determining history of infection and transmission of the parasite and will help the Haitian Ministry of Health and other involved partners synthesize malaria elimination strategies while strengthening Haiti's health system. Additionally, by comparing and assessing the agreement of serologic testing to traditional diagnostic tests, this study will provide evidence on the usefulness of serology methods in estimating the prevalence of malaria in a low-endemic country.

Chapter III: Manuscript

Abstract

Hispaniola is the only island in the Caribbean where endemic malaria transmission still occurs. Elimination of malaria in Hispaniola has been viewed as an attainable goal when malaria control and elimination efforts are focused in Haiti, where the majority of cases are. We estimated the prevalence of *Plasmodium falciparum* malaria in Haiti using serological assays, which are more useful at detecting exposure and transmission of the disease in low-endemic countries. We used Luminex® assay to assess Immunoglobulin G (IgG) antibody prevalence to three *P. falciparum* antigens (AMA-1, MSP-1-19 & LSA-1) measured through nationwide surveys in 2012 and 2015. Persons with antibodies to all three antigens were found throughout the country, with MSP-1-19 being the most prevalent in both surveys, followed by AMA-1 and LSA-1. Higher antibody prevalence was found in pregnant women, older age groups (>50-years old), rural areas and in the administrative region (department) of Centre. A poor agreement between microscopy and Rapid Diagnostic Tests (RDTs) to Luminex® assay indicated serologic testing, rather than diagnostic testing, is more suitable for identification of transmission in areas with low prevalence of malaria. This study suggests that ongoing monitoring of *P. falciparum* antibody prevalence should continue under the country's malaria prevention and control efforts, and that interventions should be intensified in rural areas, especially in the geographic region of Centre for the attainment a malaria-free Haiti.

A. Introduction

Despite the ongoing efforts to reduce malaria worldwide, the disease continues to be a public health concern in 91 countries, primarily in Africa, South-East Asia and the Americas (WHO, 2016b). There are multiple diagnostic techniques and serological methods that have been developed for the detection of malaria. Microscopy continues to be the "gold standard" diagnostic test; however, it becomes challenging to conduct in low-transmission malaria settings. Polymerase Chain Reaction (PCR) is useful when detecting infections in low parasitaemic patients; however, it is expensive, labor intensive, and requires specialized laboratory equipment which makes it a difficult technique to conduct in poor resource settings. RDTs are simple and effective in remote areas, while serology methods, such as multiplex based assay (MBA), are most useful in non-endemic and in low-transmission malaria countries. Serologic tests can identify history of exposure

to the disease and can be used to estimate the prevalence of the parasite in epidemiologic surveys (Bronzan et. al., 2008).

Currently, there is only one island in the Caribbean with endemic malaria transmission: Hispaniola (Boncy et al., 2015; Carter et al., 2015; Frederick et al., 2016; Lucchi et al., 2014). Malaria is endemic in both Haiti and Dominican Republic, but the majority of malaria cases occur in Haiti (Elbadry et al., 2015). *P. falciparum* is the causative agent and *Anopheles albimanus* is the principal vector that transmits the disease in Haiti (Elbadry et al., 2015; Frederick et al., 2016). Transmission of *P. falciparum* occurs mostly after Haiti's rainy seasons, from March through May and October through November (CDC, 2015b).

Natural disasters, such as earthquakes and hurricanes, negatively impact the health and livelihood of a population and have been associated with increased malaria transmission in Haiti (Frederick et al., 2016). An earthquake of a 7.0 magnitude hit Haiti on January 12, 2010 leaving approximately 500,000 people homeless and approximately 200,000 people were killed (Mung et al., 2010). The earthquake devastated the lives of many Haitians, especially those who lived in densely populated areas (Boncy et al., 2015). Haiti's public health system was weakened given that many hospitals and laboratory facilities were destroyed (Boncy et al., 2015; Carter et al., 2015). Displaced individuals and emergency responders living outdoors or in shelters were at a greater risk of acquiring malaria since *A. albimanus* prefers biting outdoors (CDC, 2015a; Mung et al., 2010). Consequently, many Haitians were exposed to increased mosquito populations and increases in *P. falciparum* transmission (Carter et al., 2015).

Haiti's weakened infrastructure caused there to be successful reservoirs and uncontrolled transmission of infectious diseases, endangering the population and putting them at a higher risk of infections (Boncy et al., 2015). Since then, there has been a renewed interest in eliminating

malaria from Hispaniola by 2020 (Boncy et al., 2015). The island of Hispaniola is known to have a low transmission of malaria with *P. falciparum* being the causative agent of disease. Despite the extensive usage of chloroquine treatment for many years, there is little to no resistance of the antimalarial in Haiti (Carter et al., 2015). Additionally, since malaria is not a concern in nearby countries, the island is at a low risk of importing malaria cases (Carter et al., 2015). All of these factors contribute to Haiti's potential for malaria elimination.

A national population-based survey conducted in 2011 estimated that malaria prevalence in Haiti was less than 1% (Frederick et al., 2016; Lucchi et al., 2014). Given the low prevalence and transmission of disease, elimination of malaria in Hispaniola has been viewed as an attainable goal when malaria control and elimination efforts are focused in Haiti (Boncy et al., 2015; Elbadry et al., 2015). Nowadays, with the support of the Centers for Disease Control and Prevention (CDC), local Non-profit organizations (NGOs), and the Programme National de Controle de la Malaria (PNCM), the Haitian government has developed the Plan Stratégique National d'Elimination de la Malaria (PSNEM), a national strategic plan for 2016-2022, with the goal of improving case management and the surveillance system for malaria elimination by 2020 (Boncy et al., 2015).

Given that serological methods are more useful in detecting exposure and transmission of malaria in low-endemic countries, gathering serology data to estimate the prevalence of malaria becomes a more useful technique in Haiti. Additionally, serology data becomes very useful when determining where resources should be allocated and which interventions should be targeted to control and eliminate the disease (Rogier et al., 2016). The purpose of the study is to estimate the prevalence of *P. falciparum* malaria in Haiti using the serologic Luminex® assay to assess IgG antibody responses to three *P. falciparum* antigens (AMA-1, MSP-1-19 & LSA-1) measured through two nationwide surveys conducted in 2012 and 2015. In addition, we will compare

serologic status to RDT and microscopy. The prevalence estimates, as well as the geographic and population distribution *P. falciparum* antibodies throughout the country will determine what areas and population groups have a higher immunity as well as parasitic transmission. The study will contribute to determining history of infection and transmission of the parasite and will provide data for decision making that will help Haiti's Ministry of Health and its partners determine where to allocate resources and which strategies to implement for achieving malaria elimination. Additionally, by comparing serologic status to RDT and microscopy, the study will provide evidence on the usefulness of serology methods in estimating the prevalence of malaria in a low-endemic country.

B. Methods

The survey design, sample collection, and laboratory analysis were planned and conducted by CDC's Division of Parasitic Diseases and Malaria Branch.

a. Survey design

Two Tracking Results Continuously (TRaC) surveys, which are quantitative research studies that collect data on specific populations through multi-round surveys, were conducted in October 2012 to February 2013, and December 2014 to February 2015 in Haiti as part of Global Fund's Round 8 activities that builds on the Strategic Plan against Malaria in Haiti. Global Fund is a partnership organization that provides financial support to countries responding to malaria, HIV/AIDS and tuberculosis with the goal of accelerating the end of these epidemics. The surveys conducted were cross-sectional and implemented by Population Services International (PSI) utilizing a national community-based household design in collaboration with Haiti's Ministry of Health, the Clinton Health Access Initiative (CHAI), and the CDC.

The Haitian Institute of Statistics and Informatics (IHSI) provided information on sampling sites throughout the 10 administrative regions (departments) of Haiti. The sites chosen for sampling were based on population density with a minimum of 2 sites sampled in each department. Since the Ouest department is the most densely populated with a total of 4,029,700 inhabitants, and 65% of its population lives in the metropolitan area (AM) in the district of Port-au-Prince (IHSI, 2015), the Ouest department was divided into two categories: AM and Ouest without AM. At each site sampled, 20 households were randomly selected and all household members were invited to participate in the survey. With an 11% non-response rate for testing, the estimated sample size for 2012 was 5,170 and the estimated sample size for 2015 was 2,691. Age of participants ranged from 0-99 years and all household members were eligible for parasite screening tests, except for infants less than one month old. The head of the household provided information on household residents, assets, mosquito-net ownership and use, while mothers or guardians of children under 5 years old and pregnant females answered questions related to knowledge and attitudes towards malaria. The survey was administered in a questionnaire format and included information related to participant's experience with malaria and mosquito net use as well as questions regarding participant's exposure to interventions from PSI and other partners.

b. Data and sample collection

In the 2012 survey, the total sample size was 5,169, while in 2015, the total sample size was 4,460. Demographic characteristics, such as age and gender, whether or not women of childbearing age were pregnant, the administrative region (department) and whether or not the household was in an urban or rural location was assessed. Symptoms of fever in the 2 weeks prior to the survey was also assessed during interviewer-administered questionnaires along with whether or not participants sought medical treatment, or were on any antimalarial medication (e.g.

chloroquine). The data collected in the surveys varied by year since data on medical treatment was assessed in 2012 only and antimalarial medication data was assessed in 2015 only.

After consent, each enrolled person had a slide produced for later microcopy, and was given an RDT for onsite diagnosis. Dried blood spots (DBS) were obtained for each individual consenting to participate by spotting finger-prick blood onto Whatman 903 Protein Saver Cards or Whatman 1 circular filter paper (GE Healthcare, Piscataqway, NJ), and air drying for at least 2 hours. Samples were individually packaged into plastic bags with desiccant and stored at 4^{o}C prior to shipping to CDC Atlanta for analysis. At the laboratory, samples were analyzed via microscopy, Polymerase Chain Reaction (PCR) and Luminex® assay. Data collection varied for both surveys, since microscopy and PCR testing was conducted in 2012 only, while RDTs and Luminex® assays were conducted in 2012 and 2015.

c. Blood spot elution and immunoassays

A 6mm circular punch was taken from the center of each blood spot, corresponding to 14uL whole blood, for elution. Samples were shaken overnight at room temperature in 140 uL protein elution buffer containing: PBS (pH 7.2), 0.05% Tween-20, 0.05% sodium azide, and stored at 4^{o}C until analysis. Elution from blood spots provided an initial 1:10 dilution of whole blood, and samples were further diluted 1:40 in Luminex sample diluent for a final whole blood dilution of 1:400, corresponding to a serum dilution of approximately 1:800 with the assumption of 50% hematocrit in whole blood. The *P. falciparum* merozoite surface protein 1 (MSP-1) 42kD fragment of the 3D7 allele was employed as well as the external domain of Apical Membrane Antigen- 1 (AMA-1) both of which were produced at Walter Reed Army Institute of Research (WRAIR, Silver Springs, MD) (Dent et al., 2015). The Pl1043 epitope from *P. falciparum* Liver Stage

Antigen 1 (LSA-1) was synthesized and coupled to beads at a concentration of 60ug/mL at pH 5.0 (Yang et al., 1995).

For the Luminex® assay, antigens were coupled to BioPlex® COOH beads (BioRad, Hercules, CA) according to manufacturer's protocol in the presence of 50 mM 2-(4-morpholino)-ethane sulfonic acid, 0.85% sodium chloride (NaCl) at pH 5.0 and an antigen concentration of 20 ug/mL for all antigens. Sulfo-NHS was purchased from ThermoFisher and *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (EDC) from Sigma-Aldrich. As a control to test for any serum IgG against glutathione-S-transferase (GST), a bead was included in the panel which was coupled to GST. Samples were diluted in blocking buffer containing 0.5% Polyvinyl alcohol (Sigma), 0.8% Polyvinylpyrrolidine (Sigma), 0.1% casein (ThermoFisher), 0.5% BSA (Millipore), 0.3% Tween-20, 0.1% sodium azide, and 0.01% *E. coli* extract to prevent non-specific binding. Reagent diluent (Buffer C) consisted of PBS-T plus 0.5% BSA, 0.02% sodium azide. Filter bottom plates (Multiscreen 1.2 μm, Millipore) were pre-wetted with PBS-T and 1,500 beads/analyte incubated with sample in duplicate for 1.5h under gentle shaking. Secondary antibodies tagged with biotin (1:500 anti-human IgG_{1-3}, Southern Biotech, Birmingham, AL; 1:2,500 anti-human $IgG_{4,}$ Sigma) were incubated for 45 minutes, and subsequent incubation with streptavidin-phycoerythrin (1:200, Invitrogen) for 30 minutes. Plates had a final wash incubation with reagent diluent for 30 minutes and were read on a Bio-Plex 200 machine by generating the median fluorescence signal for 50 beads/analyte and then the mean fluorescence intensity (MFI) between the duplicate wells. Final MFI was reported for a sample after subtracting MFI values from blank background beads that were included on each plate, providing MFI-bg values.

d. Control sample collection

Samples from persons never exposed to malaria were gathered from blood donated to a community blood bank in Memphis, TN. No identifying information was given to the blood units which would allow tracing back to the donor. All blood units were from persons that had screened negative for HIV and hepatitis B viruses and had no reported history of international travel in the last 6 months. To determine antibody positivity cutoff values for high confidence in a positive IgG signal, 92 of the US donors were screened by all antigens used in the study, MFI-bg values were log transformed to estimate a mean and standard deviation for a 'nonimmune' population, and the MFI-bg cutoff value was determined by adding three standard deviations to the mean and exponentiating back to a linear scale.

e. Malaria prevalence

Positive diagnostic tests (microscopy, PCR, RDT) and positive IgG antibody responses (Luminex® assay) were recorded for each sample. To determine antibody positive cutoff values, the samples were compared to the 'nonimmune' population (US donors), and the cutoff value for each *P. falciparum* antigen was determined by adding three standard deviations to the mean and exponentiating back to a linear scale. Responses to each antigen were assessed for every blood sample. After blood samples were analyzed, a positive diagnostic tests or a positive IgG antibody response by serology was individually recorded for each sample and assigned as a dichotomous variable (positive versus negative).

Parasite prevalence, which was gathered for microscopy, PCR and RDT test, was determined by dividing the total diagnostic test positives by the total sample x 100% (e.g. (total RDT positives/ total sample) x 100%). Antibody prevalence by Luminex® was determined by

dividing the total antibody positives for each *P. falciparum* antigen by the total population (e.g. (total IgG positives/total population) x 100%). Antibody prevalence was assessed individually by antigen (AMA-1, LSA-1, MSP-1) and as a combined IgG category in each survey. For the combined IgG category, all three antigen responses were combined, where a positive response to any of the three antigens was classified as a positive case. Antibody prevalence was assessed by age groups, by gender, by pregnancy status, by geographic location (urban vs. rural), and by administrative region (department). In the Ouest department, antibody prevalence was calculated separately for AM and Ouest without AM, but the geographic antibody prevalence distribution was calculated and presented as one department.

f. Demographic and clinical variables

Demographic variables such as participant's age, gender, female's pregnancy status, as well as participant's administrative region (department) and whether they lived in an urban or rural area were collected. Age was stratified into five categories according to the participants age: 0-5, 6-15, 16-25, 26-50 and >50. The administrative region category indicated participant's department of residence. Participant's belonged to one of the following ten departments: Artibonite, Centre, Grand-Anse, Nippes, Nord, Nord-Est, Nord-Ouest, Ouest, Sud, and Sud-Est. The metropolitan area (AM) of Haiti, which is in the Ouest department, was assessed separately; therefore, the Ouest department represented in the results tables do not include the metropolitan area, while the Ouest department in the geographic maps does include AM.

Fever was assessed as a symptom as were other clinical variables such as whether participant's sought medical treatment at least two weeks prior to the survey or if they were taking any antimalarial medication. Data on participant's seeking medical treatment was only evaluated in 2012, while antimalarial medication intake was evaluated in 2015.

g. Data analysis

TRaC 2012 and 2015 data entry was performed by CDC's Division of Parasitic Diseases and Malaria Branch. Data cleaning and management were conducted prior to statistical analysis and approximately <5% of data was excluded due to missing values. Data on each survey was analyzed separately. SAS v9.4 (SAS Institute Inc.) was used for statistical analysis. Statements such as PROC FREQ, CHISQ, FISHER, AGREE, and KAPPA TEST were used to conduct the analysis. Differences in individual and combined *P. falciparum* antibody prevalence rates by gender, pregnancy status, age groups, departments and geographic location were analyzed via Chi-square test or Fisher's exact test. In instances where cell size was less than 5, Fisher's exact tests was used which only occurred when evaluating pregnant females and LSA-1 in the 2012 survey. Chi-square test for administrative unit (department) was obtained after re-categorizing into 5 groups [North (Nord, Nord-Ouest, Nord-Est), South (Sud & Sud-Est), Ouest (AM & Ouest without AM), Center (Centre & Artibonite), and GrandNippes (Grand-Anse & Nippes)] because of small counts. The metropolitan area (AM) of Haiti, which is in the Ouest department, was assessed separately from the Ouest department; therefore, the Ouest department (Ouest without AM) represented in the results tables do not include AM. To determine the agreement between diagnostic (microscopy, RDT) and serologic testing (Luminex®), Kappa statistics was used. The kappa test was conducted between RDT and Luminex® positives for both surveys and microscopy and Luminex® positives for 2012 only. The kappa coefficient was used to determine the agreement between the diagnostic tests using the cutoff values discussed in Viera & Garrett (2005) study (Viera & Garrett, 2005). ArcGIS was used to develop the *P. falciparum* antibody prevalence maps by department. In the geographic distribution maps, AM is included in the Ouest department.

h. Ethical considerations

The study received Institutional Review Board (IRB) exempt status approval (IRB00094539) by Emory University's IRB since it was a secondary data analysis and all data was de-identified prior to analysis. The survey protocols were approved by CDC's IRB and the Haiti ethical review committee in 2012 and 2014 at the Ministry of Health in Port-au-Prince, Haiti. All persons participating in the surveys provided written consent. They were assigned a 6-digit identification number that cannot be traced back to the individual. All laboratory data was collected at the CDC under non-engagement research status as approved by the CDC's IRB.

C. Results

a. Demographic characteristics

In the 2012 survey, the age range of the participants was 0-99 years (median= 16 years), with younger individuals (0-15 years old) making up more than half of the population. Females constituted 61% of the population, of whom 10% (N= 229) were pregnant [Table 1]. In 2015, the age range of the population was also 0-99 years (median= 25 years), with older individuals (16-50 years old) consisting the majority of the population (52%). Females still constituted 61% of the population, out of which only 3% (N=77) were pregnant.

Of the administrative regions (departments) sampled across Haiti, Ouest had the highest participation in both surveys. In 2012, 21% of the study participants were from Ouest's metropolitan area (including Port-au-Prince) while 16% were from the remaining areas of the department. In 2015, 19% of participants were from metropolitan area while 14% were from the remaining areas of Ouest. Participants from the Artibonite department, made up 21% and 17% of the study population in 2012 and 2015, respectively. Participants from the Centre department made

up 11% of the study in 2012 and 7% in 2015, whereas the Nord department made up 7% of the study's sample in 2012 and 11% in 2015. In the remaining departments, smaller portions of the population participated ranging from 2% to 8%. Those included Grand-Anse, Nippes, Nord-Ouest, Nord-Est, Sud and South-Est. As for the geographic location of households, 59% of the 2012 survey population lived in a rural zone while in 2015, this proportion was 61% [Table 1].

b. Malaria clinical and diagnostic data

Fever status in the two weeks prior to the survey was the only clinical symptom assessed [Table 2]. Only 7% and 5% of the population had fever in the two weeks prior to the survey in 2012 and 2015, respectively. Data on whether participant's sought medical treatment was only included in the 2012 study and was available on 305 participants; 188 participants indicated they sought medical treatment in the two weeks before the survey. In 2015 only, antimalarial medication was assessed and only 8 participants were found to be taking Chloroquine.

Malaria diagnostic and serologic tests were conducted with each sample to determine the occurrence of disease [Table 2]. Microscopy and PCR were conducted in 2012 only and RDT was conducted in both years. Microscopy, the "gold standard" diagnostic test for malaria, only identified 3 people (0.1%) with malaria, whereas PCR identified 17 (0.3%). In 2012, RDT was able to identify 50 people (1%) with infection and 24 people (0.5%) in 2015. Only eight RDT positive participants were taking Chloroquine. Of the 3 people who were positive by microscopy, no one reported having fever while, of the 17 PCR positives, only 2 reported experiencing fever in the previous two weeks of the survey. Meanwhile, of the 50 RDT positives for 2012, only 1 had fever and sought medical treatment, while, in the 24 RDT positives for 2015, 6 reported experiencing fever and 8 were taking Chloroquine.

c. Antibody prevalence

i. Overall prevalence

The study identified responses to all three antibodies throughout the country, with MSP-1-19 having the highest occurrence in both 2012 and 2015, followed by AMA-1 and LSA-1. The overall antibody prevalence for MSP-1-19 was 17%, with 891 positives, in 2012 and 22%, with 979 positives, in 2015. AMA-1 was the second highest with a total of 695 positives in 2012 and with 825 positives in 2015, making the AMA-1 prevalence 14% and 19% in 2012 and 2015, respectively. The least prevalent antibody response was LSA-1 where only 1% of the population (N= 54) tested positive in 2012 and 2% (N= 85) tested positive in 2015. The prevalence of the combined IgG antibodies was 22% for 2012 and 29% for 2015. The combined IgG showed that 1,156 people in the population of 2012 had an antibody response to any of the three *P. falciparum* antigens and 1,287 in 2015 [Table 2].

ii. Prevalence by gender

In the 2012 survey, when assessing for differences in individual antibodies by gender, no statistically significant difference was found; however, a statistically significant difference in gender was found when evaluating for the combined IgG category (p= 0.04) [Table 3]. In 2015, there was no statistically significant difference found between gender and the combined IgG category nor between gender and individual AMA-1 or MSP-1-19 antibodies; nonetheless, a statistically significant difference was found between gender and LSA-1 (p= 0.01) [Table 3].

a. Males

In the 2012 TRaC survey, MSP-1-19 antibody prevalence was 16%, the highest of all three antigens with 329 males exhibiting the antibody. AMA-1 prevalence was 13% with 262 males

demonstrating the antibody and LSA-1 prevalence was only 1% with 23 men being positive. For 2015, MSP-1-19 also had the highest prevalence of the three with a 23% prevalence. AMA-1 prevalence was 19% and LSA-1 was the lowest with 2% prevalence. When combining the antibodies into one category (combined IgG), where antibody positivity was determined if at least was positive for one antibody, the prevalence was higher in 2015 (29%) than in 2012 (21%).

b. Females

In the 2012 TRaC survey, as observed in males, MSP-1-19 had the highest prevalence of all three antibodies (18%, N= 562 positive) in females. AMA-1 prevalence was 14% with a total of 433 persons exhibiting the antibody, whereas LSA-1 prevalence was only 1% in the female population. For 2015, MSP-1-19 had the highest prevalence of 22%, for a total of 586 individuals. AMA-1 was the second most prevalent with 497 being positive for a total of 19%, and LSA-1 was the lowest with only 41 positives (1%). Once the antibody prevalence was combined into one category overall (Combined IgG), a higher prevalence was observed in 2015 (29%) than in 2012 (23%).

iii. Prevalence by pregnancy status

Statistically significant differences were found between pregnancy status and AMA-1 (p= 0.001), MSP-1-19 (p= 0.02) and the combined IgG category (p=0.001) in 2012 only, with pregnant women having higher rates on all three measures, except LSA-1 [Table 3]. For 2015, no statistically significant differences were found between pregnancy status and individual nor combined IgG antibodies.

When assessing for *P. falciparum* antibody prevalence in pregnant females, the 2012 survey identified a 25% prevalence for MSP-1-19, 23% for AMA-1 and a 0.9% for LSA-1. In 2015, pregnant women had a 26% prevalence of MSP-1-19 antibody, a 12% prevalence of AMA-

1 and a 4% prevalence of LSA-1. The AMA-1 prevalence decreased in 2015 while the LSA-1 prevalence increased, compared to 2012. Comparing the surveys by the combined antibody category, the prevalence of *P. falciparum* antibodies in pregnant women was 35% in 2012 and 29% in 2015. When assessing for antibody prevalence in non-pregnant women, the 2012 identified a 19% prevalence for MSP-1-19, 15% for AMA-1, and 1% for LSA-1. In 2015, non-pregnant women had a 22% prevalence of MSP-1-19 antibody, a 19% prevalence for AMA-1 and a 1% prevalence for LSA-1. AMA-1 and MSP-1-19 prevalence increased in 2015, compared to 2012. Comparing the surveys by the combined antibody category, the prevalence of *P. falciparum* antibodies in non-pregnant women was 25% in 2012 and 29% in 2015.

iv. Prevalence by age groups

Participants were stratified into age groups (0-5, 6-15, 16-25, 26-50, >50) where each antibody prevalence was assessed. In both surveys, statistically significant differences were found between the age groups and all individual and combined IgG antibody categories with p-values <0.0001 [Table 3]. In both surveys, participants age >50 had the highest prevalence compared to the younger age groups, and the 0–5 years age group had the lowest antibody prevalence. For AMA-1, the difference in prevalence by years was only 1%, except for the >50 group, where there was a 4% increase in 2015 (34% in 2012 to 38% in 2015). For LSA-1, the highest antibody prevalence was found in the oldest age group with 5% and 4% in 2012 and 2015. LSA-1 had the lowest prevalence in each age group compared to AMA-1 and MSP-1. In 2012, the lowest LSA-1 prevalence was observed in the 6–15 years age group (0.1%), and in 2015 no one in the 0–5 age group had immunity to LSA-1. For MSP-1-19, the 26–50 age group had the highest antibody prevalence in 2012 (27%), whereas the >50 years age group had a 25% prevalence. In 2015, the antibody prevalence increased by age groups, reaching 33% in the >50 years age group. The

prevalence of the other age group categories also increased in 2015, except for the 0–5-year old's where it stayed at 2% for both years. When combining antibody positivity into the combined IgG category [Table 3], the highest and lowest overall prevalence were observed in the oldest and youngest participants, respectively. In both years, as the age group categories increased, so did the antibody prevalence.

v. Prevalence by geographic location

The geographic location of each household was categorized into urban or rural. Statistically significant differences were found between the geographic location and combined IgG antibody responses in both surveys ($p < 0.0001$) as well as between the geographic location and individual AMA-1 and MSP-1-19 antibodies ($p < 0.0001$) [Table 3]. As with LSA-1, there was a statistically significant difference between geographic location in 2015 ($p=0.04$); however, no statistically significant difference was found in 2012 ($p=0.05$). Whether assessing antibody prevalence individually or in the combined category, prevalence was always greater in the rural area. In both urban and rural areas, and across the two years, MSP-1-19 had the highest prevalence and LSA-1 had the lowest prevalence. The highest antibody prevalence in the combined antibody category was 32% in the 2015 rural area while the lowest was 17% in the 2012 urban area [Table 3].

vi. Prevalence by administrative region (department)

The distribution of *P. falciparum* antibody prevalence was assessed geographically by mapping the combined IgG antibody prevalence in each department [Figure 1; Figure 2]. Of the ten departments in Haiti, Centre had the highest prevalence in both 2012 and 2015 (41% and 51%, respectively). In 2012, the lowest antibody prevalence was found in the Nord-Ouest (9%) department followed by Nord-Est (12%), Grand-Anse (13%), Sud-Est (17%), Nippes (18%), Nord (21%), Artibonite (22%), Sud (29%), Ouest (21%) and Centre (41%) [Figure 1]. During the

following survey in 2015, overall prevalence by department varied with Ouest having the lowest prevalence (21%), followed by Grand-Anse (25%), Sud-Est (25%), Nord-Est (26%), Nord-Ouest (29%), Nord (29%), Artibonite (31%), Sud (37%), Nippes (46%) and Centre (51%) [Figure 2]. Variations in the combined IgG prevalence by department were statistically significant for both 2012 and 2015 [Table 3]. Overall *P. falciparum* antibody prevalence was lower in 2012 than in 2015, except for the Ouest department where it remained the same.

P. falciparum antibody prevalence was assessed for each of the departments in Haiti [Table 3]. AMA-1 and MSP-1-19 immunity were found in all ten departments, however, LSA-1 was not. For 2012, LSA-1 was not found in any individuals residing in Nord-Ouest, Nord-Est, and Sud-Est; however, in the following survey (2015) LSA-1 immunity was identified in the Nord-Ouest department. Statistically significant differences were observed, with p values of <0.0001 (AMA-1, MSP-1-19 and combined IgG) and 0.006 for LSA-1 across both surveys [Table 3]. Prevalence of antibodies in the Ouest department were split into two groups: AM and Ouest without AM. In AM, antibody prevalence increased for all *P. falciparum* antigens from 2012 to 2015. In 2012, prevalence for AMA-1, LSA-1, and MSP-1-19 was 6%, 0.3% and 9%, and in 2015 it increased to 10%, 1% and 12%, respectively. When combining the antibody positives, prevalence in AM was 11% in 2012 and increased to 17% in 2015. In 2012, the Ouest without AM had an antibody prevalence of 23% for AMA-1, 2% for LSA-1 and 28% for MSP-1-19 and in 2015 it decreased to 16% for AMA-1, 1% for LSA-1 and 22% for MSP-1-19. Overall, *P. falciparum* antibody prevalence decreased from 35% to 28% in the Ouest without AM.

Prevalence in the Artibonite department increased from 2012 to 2015 in all three antibodies with MSP-1-19 having the highest prevalence (25%) in 2015. Combined antibody prevalence also increased from 22% to 31% in 2015. The department of Centre had the highest prevalence in each

individual antibody as well as the combined antibody category [Table 3]. In Centre 2012, LSA-1 prevalence was 3%, AMA-1 was 29% and MSP-1-19 was 31%, and in 2015 increased to 5%, 41% and 37%, respectively. The highest overall prevalence was observed in the Centre department with 41% in 2012 and 51% in 2015.

In Grand-Anse, AMA-1 and MSP-1-19 had the same 8% prevalence for 2012, but for 2015, they differed with AMA-1 having a 17% and MSP-1-19 having a 15% prevalence. Overall antibody prevalence was 13% in 2012 and 25% in 2015. In Nippes, overall combined antibody prevalence was 18% in 2012 and 46% in 2015 with MSP-1-19 having the highest prevalence both years. The Nord department combined antibody prevalence increased from 21% in 2012 to 29% in 2015 and again, MSP-1-19 had the highest antibody positivity. Nord-Ouest had the lowest antibody prevalence in 2012 with 7%, 4% and 0% for MSP-1-19, AMA-1 and LSA-1 and overall combined antibody prevalence of 9%. Afterwards, the prevalence increased to 21%, 18%, and 2% in 2015 with a combined antibody prevalence of 29%. Nord-Est also had a lower antibody prevalence that ranged from 12% in 2012 to 26% in 2015. The Sud department's antibody prevalence in 2012 was 20%, 19% and 0.7% and in 2015, the antibody prevalence increased to 32%, 22% and 2% for MSP-1-19, AMA-1, and LSA-1. Finally, in the 2012 survey, the Sud-Est antibody prevalence was 14%, and 7%, while in 2015 the prevalence was 19% and 18% for MSP-1-19 and AMA-1. Given that no participants were positive for LSA-1 antibody, LSA-1 prevalence was 0% in both 2012 and 2015 for the Sud-Est department [Table 3].

d. RDT, Microscopy and Luminex® antibody positive agreement

The agreement between RDT and Luminex® serology test was assessed to determine the degree of concordance between the two tests in identifying malaria positives. Individual antibody positivity as well as the combined antibody category was used to evaluate the agreement in both

years. The kappa coefficient ranged in value from 0.008 to 0.02, indicating poor agreement between the antibodies, individual and combined, and RDT [Table 4].

Given that the study had data available on microscopy for 2012 only, an assessment of the agreement between the "gold standard" diagnostic test and the Luminex® serology test was conducted for 2012 only [Table 6]. The kappa coefficient was assessed with all three antibodies individually as well as the combined antibody category, and the kappa coefficient ranged from –0.001 to 0.002. In every antibody category, a poor agreement was found with microscopy [Table 5].

D. Discussion

This study estimated the prevalence of IgG antibodies to *P. falciparum* in Haiti over two time points, 2012 and 2015, via national community TRaC surveys. Overall, *P. falciparum* antibody prevalence was lower in 2012 than in 2015. The combined IgG showed that 1,156 people in the population of 2012 had an antibody response to any of the three *P. falciparum* antigens, for an antibody prevalence of 22%, and 1,287 people in 2015, for an antibody prevalence of 29%. Of the ten departments in Haiti, Centre had the highest overall antibody prevalence in both 2012 and 2015 (41% and 51%, respectively). When evaluating individual responses to the *P. falciparum* antigens, all three antibody responses were found throughout the country, with MSP-1-19 having the highest occurrence in both years, followed by AMA-1 and LSA-1. Additionally, a poor agreement was found between RDT and Luminex® assay, as well as between microscopy, the "gold standard" diagnostic test for malaria, and Luminex® assay.

Serology assays are a more useful technique for detecting malaria in low endemic settings because they assess the history of exposure to the disease by detecting long lasting antibody responses (e.g. IgG), are useful in estimating historical prevalence of malaria, and, the antibody

prevalence can help determine transmission changes in malaria endemic areas (Bronzan et al., 2008; Cunha et al., 2014). In our two surveys, IgG antibody responses to three *P. falciparum* antigens were assessed with Luminex® bead-based assay for all the participants who provided a blood sample as a way of determining prevalence of antibodies to malaria. The malaria antigens used were AMA-1, LSA-1 and MSP-1-19, all which are *P. falciparum* antigen proteins for which the immune system develops antibodies after infection. AMA-1 and MSP-1-19 are antigens of the blood stage merozoites while LSA-1 is an antigen of the pre-erythrocytic liver stage (Koffi et al., 2015; Wipasa et al., 2010). We found that MSP-1-19 was the most prevalent across the two years, with a rate of 17% in 2012 and 22% in 2015. It was followed by AMA-1 with rates of 14% and 19% and LSA-1 with rates of 1% and 2% for 2012 and 2015, respectively. Given that AMA-1 and MSP-1-19 antigens produce long term antibodies while LSA-1 antigen produces short term antibodies, this might explain the differences in prevalence estimates for the three antibodies found in our study (Bretscher et al., 2013). IgG antibodies to MSP-1-19 have been demonstrated to have greater immunity longevity as well as being able to predict prior malaria transmission in an area, whereas, AMA-1 antibodies have been found to saturate in the population earlier than MSP-1-19 antibodies, indicating a shorter longevity (Ondigo et al., 2014). Nevertheless, there are multiple factors that influence antibody levels such as age, geographic location and transmission intensity (high versus low malaria). Changes and variations in antibody responses in different settings are useful and recommended for malaria monitoring purposes especially when emphasizing ongoing elimination strategies (Koffi et. al., 2015).

In both surveys, no differences in antibody prevalence by gender were observed. Males and females had the same amount of antibody prevalence each year with only 1-2% difference and, in both genders, antibody prevalence was higher for MSP-1-19, followed by AMA-1 and lastly, LSA-

1 antigens. Prevalence in pregnant women was also assessed and the same pattern was observed where a higher prevalence was seen for MSP-1-19 followed by AMA-1 and LSA-1. In 2012, where statistically significant differences were found, overall *P. falciparum* antibody prevalence was higher in pregnant women than non-pregnant women. When there is equal exposure to the parasite, adult males and females are equally vulnerable to malaria infections, except for pregnant women who are more susceptible and at a greater risk of developing severe disease (World Health Organization [WHO], 2007). It is thought that maternal susceptibility to malaria infections during pregnancy is related to physiological immunosuppression during gestation and cytoadherence, the accumulation of *P. falciparum* infected red blood cells in the placenta (Doolan et al., 2009). Other factors that may explain pregnant women's susceptibility to malaria are the impairment of cellular immunity and hormonal immunosuppression due to the increased levels of hormones expressed when pregnant, especially in those pregnant for the first time (Doolan et al., 2009; WHO, 2007). Primigravidae women have higher levels of cortisol which suppress the immune system, likely increasing pregnant women's susceptibility to infection (Doolan et al., 2009). Our study did not collect data on gravidity.

Age also had an impact on *P. falciparum* antibody prevalence. The oldest age group (>50 years) had greater *P. falciparum* antibody prevalence, indicating they have been exposed more to the disease and subsequently developed antibodies against the parasite (Crompton et al., 2014). As expected, people in the youngest age group (0–5 years) had the least antibody prevalence. It is no surprise that older age groups exhibited overall higher antibody prevalence and younger age groups had the lowest, given that antibodies are developed over time and with exposure to the disease, therefore older populations were expected to have higher antibody levels (Doolan et al., 2009; Rosas-Aguirre et al., 2013; von Fricken et al., 2014). Nevertheless, there are two hypotheses that

have been proposed in malaria antibody studies that explain the development of antibody acquisition in malaria endemic populations. The hypothesis most widely accepted is that clinical immunity to the disease is slow and a cumulative product of exposure to multiple parasite infections over time, therefore producing specific immune responses (Doolan et al., 2009; Rosas-Aguirre et al., 2013). The alternate hypothesis attributes the onset of immunity to heavy malaria exposures as well as to the development of cross-reactive immune responses ruled by inherent characteristics that change with age regardless of the parasite exposure throughout the lifetime of the individual (Doolan et al., 2009). Given the low endemic prevalence of malaria in Haiti and the gradual increase of antibody prevalence as the age group category increased, the first hypothesis is favored in our study.

The lack of symptoms and antibody occurrence indicates the presence of asymptomatic malaria infections throughout Haiti which often occurs in malaria endemic countries (Doolan et al., 2009). Crompton et al. (2014) emphasized the significant element of malaria immunity, that resistance develops after years of exposure. People eventually develop resistance to the disease, because of cumulative exposure. However, resistance to the malaria liver infection is rarely achieved; hence adults have frequent asymptomatic infections in endemic areas (Crompton et al., 2014). Although conflicting results have been found, studies conducted in highland areas with low malaria transmission have also found that the development of malaria immunity is developed with age and, in adults, the disease burden is reduced (Rolfes et al., 2012). Further research is still needed; however, experts suggest malaria immunity may be a consequence of repeated intermittent exposure to the parasite, changes in the immune response as people age, or a combination of both (Rolfes et al., 2012).

Prevalence by geographic location was assessed based on whether the households were located in an urban or rural location. The study found higher levels of *P. falciparum* prevalence throughout the rural areas across Haiti. According to The Word Bank (2014), Haiti's biggest disparity is geographical. Almost 70% of rural houses in Haiti are considered chronically poor, meaning they live below the poverty line on less than $2 a day while also lacking access to basic services (The World Bank, 2014). While cities (urban areas) across Haiti are improving, especially in Port-au-Prince, the countryside continues to struggle with only 11% of the rural population having access to energy and 16% to improved sanitation (The World Bank, 2014). Additionally, getting access to clean water can be challenging given that 40% of Haiti's rural population uses water from non-protected sources such as rivers and unprotected wells, increasing their risk to multiple infectious diseases, including malaria (The World Bank, 2014). These factors contribute to the differences in malaria prevalence by geographic region and influence the spread of the disease across Haiti. Since more antibody prevalence of malaria was found in the rural areas, this is indication that the disease is occurring and infecting the population more in rural rather that urban neighborhoods. For this reason, malaria control and elimination interventions should be targeted at both rural and urban communities, but strongly emphasized in rural communities.

All of the three *P. falciparum* antigens were found to have antibody positive persons throughout the country in all ten administrative regions (department), except for LSA-1 which was not identified in the Nord-Ouest, Nord-Est or Sud-Est departments in 2012. In the following survey in 2015, antibodies to LSA-1 were found in Nord-Ouest. Since the presence of antibody responses to all three *P. falciparum* antigens increased from 2012 to 2015, a possible explanation could be that transmission of malaria is spreading more quickly throughout the population infecting more people. Overall prevalence by department was displayed geographically throughout the country,

where the department of Centre had the highest prevalence on both years while the Nord-Ouest department had the lowest prevalence (9%) in 2012 and the Ouest had the lowest prevalence (21%) in 2015. The prevalence increased in 2015 for every department except for Ouest, where it remained the same (21%). According to IHSI, approximately 80% of the population in the Centre department live in a rural area (IHSI, 2015). In agreement with the results from the study, where higher antibody prevalence was found in rural areas, the geographic area of the Centre department, and the amount of population who live in rural neighborhoods could possibly be influencing and supporting malaria transmission throughout the department. Due to the greater amount of antibody prevalence found in the study, it is possible that the intensity of malaria transmission in Centre is higher than in any other department, however, further investigation is needed to determine the relationship and causes of the findings.

Data on fever and whether or not participants sought medical treatment were only collected for 2012. This information was valuable to determine if the population were potentially exposed to malaria, had symptomatic parasitemia, and if they followed the health recommendations of visiting a medical provider when presenting malaria-like symptoms. Out of the 354 persons who reported a fever at time of survey, or in the two weeks prior, 188 sought medical treatment indicating that more than half of those with symptoms visited a medical provider. Regardless of symptoms, if the medical provider suspects the patient was exposed to malaria, they should test for the disease given that asymptomatic malaria is known to occur in malaria endemic areas (Doolan, Dobaño & Baird, 2009).

During the TRaC surveys, blood samples were tested for active malaria infection through diagnostic tests. Since not all participants were tested by all methods, a comparison of active infection by year could not be established. RDT was able to identify 1% malaria active infection,

while PCR identified 0.3% and microscopy 0.1%. Given the serologic method, Luminex® assay, identified more antibody positive people than the diagnostic tests, indicating a higher quantity of persons previously infected with malaria, one can agree with previous researchers that serological testing is more effective in low malaria endemic countries since it can be used to estimate the prevalence and transmission of malaria, by assessing IgG antibody positivity (Rogier et al., 2016; Bronzan et. al., 2008).

Furthermore, a test evaluation was conducted between RDT and Luminex® for both 2012 and 2015, and between microscopy and Luminex® for 2012 only, to determine the antibody positivity agreement between tests. Kappa statistics was used to evaluate the level of agreement between tests. Given that poor or no agreement were found for any *P. falciparum* antigen, the findings suggests neither RDT nor microscopy are useful to conduct in areas where the occurrence of malaria is low. Once again, the findings discussed here and in previous studies provide ample proof of the benefits of serologic testing in regards to malaria prevalence and transmission in areas with low occurrence of disease (Sylla et al., 2015).

Measurement of malaria becomes a challenge in low-endemic countries due to difficulties in detecting parasite positive people as malaria approaches zero (Bretscher et al., 2013). In countries where there is low incidence of malaria, like Haiti, diagnostic tests are not very useful when assessing the prevalence of the disease given that parasite identification might go undiagnosed since *P. falciparum* parasites are not always present in the peripheral blood or because microscopy skills may weaken when technicians are not conducting malaria diagnosis regularly (Bronzan et al., 2008; Ghinai et al., 2017; Makler et al., 1998; Pothin et al., 2016). All of these explanations can lead to undiagnosed malaria cases. These may be reason why so few microscopy, PCR and RDT tests identified malaria infection in the population and why poor agreements were

found. Since the antibody responses that develop after infection last longer than the parasitic infection, serology becomes more useful to assess malaria prevalence and transmission intensity in low endemic areas (Bretscher et al., 2013).

A strength of our study was that, on account of being a nationwide cross-sectional study, sufficient sample sizes were acquired. Sample selection was proportionally distributed among the 10 administrative regions (departments) of Haiti, as well as proportionally distributed between urban and rural areas within each department relative to their population. Additionally, the 20 households surveyed at each sampling site were systematically selected at random in order to avoid sampling bias. Additionally, blood samples were tested by multiple laboratory techniques to identify not only active malaria infection, but history of exposure through antibody detection. A potential limitation of the study was the limited accessibility of areas sampled due to poor road conditions during Haiti's rainy season. Efforts were made to visit all locations sampled, while ensuring the safety of the study staff during field visits. Inconsistencies with age and gender status were found between datasets, possibly due to multiple staff entering data; therefore, those participants were not included in the analysis (<5%). Participation in each survey depended on the person's willingness to provide consent as well a blood sample to conduct the serological analyses. In both surveys, female participation was higher than that of males. Due to the fact that men are typically the household providers and are therefore working during the daytime, this may have influenced study participation and explained why women participated more in both surveys. Additionally, differences in data collection across both surveys was also a limitation. Information on medical treatment, antimalarial medication, and diagnostic tests (microscopy and PCR) were not collected for both surveys, therefore, comparison between years could not be determined for these categories. Even though our study indicated statistically significant differences, further

analyses are needed to determine the association between variables and *P. falciparum* antibody prevalence in order to develop a more comprehensive study.

In conclusion, this study provides *P. falciparum* antibody prevalence as well as knowledge on the transmission of malaria in Haiti based on the prevalence of antibodies found in each administrative region. The results of the study suggest that Haiti's Ministry of Health and its partners should continue to monitor the antibody prevalence of *P. falciparum* throughout the country, as part of the national malaria prevention and control efforts. Our findings indicate that more interventions should be intensified in rural areas, especially in the administrative region of Centre, where antibody prevalence was highest in both surveys, in order to continue the progress towards the goal of malaria elimination in Haiti.

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Tables and figures

Table 1. Participant characteristics, Tracking Results Continuously (TRaC) surveys, Haiti 2012 and 2015

	TRaC 2012	TRaC 2015
	N= 5,169 n (%)	N=4,460 n (%)
Demographic Characteristics		
Gender		
Male	2,030 (39)	1,751 (39)
Female	3,139 (61)	2,709 (61)
Pregnancy Status [1]		
Pregnant	229 (10)	77 (3)
Non-pregnant	2,156 (90)	2,632 (97)
Age Group [2]		
0 – 5	1,189 (23)	459 (10)
6 – 15	1,365 (27)	899 (20)
16 – 25	1,002 (19)	910 (20)
26 – 50	1,232 (24)	1,402 (32)
> 50	367 (7)	782 (18)
Median Age	16	25
Department		
Aire Métropolitaine (AM)	1,093 (21)	848 (19)
Artibonite	1,100 (21)	753 (17)
Centre	557 (11)	315 (7)
Grand-Anse	224 (4)	216 (5)
Nippes	186 (4)	147 (3)
Nord	372 (7)	504 (11)
Nord-Ouest	257 (5)	355 (8)
Nord-Est	101 (2)	152 (3)
Ouest without AM	846 (16)	613 (14)
Sud	151 (3)	336 (8)
Sud-Est	282 (6)	221 (5)
Geographic Location		
Urban	2,115 (41)	1,720 (39)
Rural	3,054 (59)	2,740 (61)

[1] 2012: (n=2,385); 2015: (n=2,709) [2] 2012: (n=5,155); 2015: (n=4,452)

Table 2. Malaria symptoms, diagnostic tests and antibody prevalence, Tracking Results Continuously (TRaC) surveys, Haiti 2012 and 2015

	TRaC 2012	TRaC 2015
	N= 5,169	N=4,460
	n (%)	n (%)
Symptoms		
Fever [1]		
Yes	354 (7)	212 (5)
No	4,564 (93)	4,246 (95)
Sought Medical Treatment [2,3]		
Yes [4]	188 (62)	–
Took Antimalarial Medication [5,6]		
Chloroquine [7]	–	8 (0.2)
Diagnostic Tests		
Microscopy [2,8]		
Positive	3 (0.1)	–
Negative	5,137 (99.9)	–
PCR [2]		
Positive	17 (0.3)	–
Negative	5,152 (99.7)	–
RDT [9,10]		
Positive [11]	50 (1)	24 (0.5)
Negative	4,902 (99)	4,354 (99.5)
Overall IgG Antibody Prevalence to *P. falciparum* Antigens [12]		
AMA – 1		
Positive	695 (14)	825 (19)
Negative	4,445 (86)	3,581 (81)
LSA – 1		
Positive	54 (1)	85 (2)
Negative	5,086 (99)	4,321 (98)
MSP–1–19		
Positive	891 (17)	979 (22)
Negative	4,249 (83)	3,427 (78)
Combined IgG [13,14]		
Positive	1,156 (22)	1,287 (29)
Negative	3,984 (78)	3,119 (71)

[1] 2012 (n=4,920); [2] Data not available for 2015; [3] (n=305); [4] Everyone who sought treatment had fever; [5] Data not available for 2012; [6] (n=4,386) ; [7] Everyone who was taking Chloroquine was RDT+ ; [8] (n=5,140); [9] 10 participants refused RDT in 2015; [10] 2012 (n=4,952), 2015 (n=4,378)

[11] In 2015, all RDT+ took Chloroquine 2 weeks prior; [12] 2012 (n=5,140), 2015 (n=4,406);

[13] Positive if at least 1 antibody was positive; [14] 2012 (n=5,140), 2015 (n=4,406)

Table 3. Malaria antibody prevalence by demographic characteristics, Tracking Results Continuously (TRaC) surveys, Haiti 2012 and 2015

	TRaC 2012			TRaC 2012	TRaC 2015			TRaC 2015
	AMA – 1 n (%)	LSA – 1 n (%)	MSP–1–19 n (%)	Combined IgG [1] n (%)	AMA – 1 n (%)	LSA – 1 n (%)	MSP–1–19 n (%)	Combined IgG [1] n (%)
Demographic Characteristics								
Gender								
Male [2]	262 (13)	23 (1)	329 (16)	424 (21)	328 (19)	44 (2)	393 (23)	507 (29)
Female [3]	433 (14)	31 (1)	562 (18)	732 (23)	497 (19)	41 (1)	586 (22)	780 (29)
p-value [4]	0.38	0.6	0.12	0.04	0.72	0.01	0.5	0.87
Pregnancy Status								
Pregnant [5]	52 (23)	2 (0.9)	58 (25)	80 (35)	9 (12)	3 (4)	20 (26)	22 (29)
Non-pregnant [6]	318 (15)	27 (1)	414 (19)	539 (25)	488 (19)	38 (1)	566 (22)	758 (29)
p-value [7]	0.001	1	0.02	0.001	0.12	0.1	0.34	0.97
Age Group								
0 – 5 [8]	32 (3)	4 (0.3)	64 (5)	77 (7)	8 (2)	0	10 (2)	15 (3)
6 – 15 [9]	112 (8)	2 (0.1)	192 (14)	230 (17)	60 (7)	4 (0.4)	98 (11)	131 (15)
16 – 25 [10]	133 (13)	7 (0.7)	204 (20)	251 (25)	128 (14)	8 (0.9)	173 (19)	225 (25)
26 – 50 [11]	287 (23)	22 (2)	336 (27)	432 (35)	334 (24)	40 (3)	442 (32)	538 (39)
> 50 [12]	124 (34)	19 (5)	93 (25)	159 (43)	293 (38)	33 (4)	252 (33)	374 (48)
p-value [4]	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Department								
Aire Métropolitaine (AM)[13]	62 (6)	4 (0.3)	93 (9)	123 (11)	88 (10)	11 (1)	104 (12)	140 (17)
Artibonite [14]	137 (12)	14 (1)	178 (16)	242 (22)	150 (20)	16 (2)	185 (25)	233 (31)
Centre [15]	159 (29)	15 (3)	172 (31)	227 (41)	128 (41)	17 (5)	117 (37)	162 (51)
Grand-Anse [16]	17 (8)	2 (1)	18 (8)	30 (13)	37 (17)	3 (1)	33 (15)	54 (25)
Nippes [17]	13 (7)	1 (0.5)	29 (16)	34 (18)	45 (31)	7 (5)	53 (36)	67 (46)
Nord [18]	48 (13)	3 (0.8)	64 (17)	78 (21)	86 (17)	9 (2)	102 (20)	144 (29)
Nord-Ouest [19]	11 (4)	0	19 (7)	24 (9)	62 (18)	7 (2)	73 (21)	99 (29)
Nord-Est [20]	6 (6)	0	9 (9)	12 (12)	22 (14)	0	30 (20)	39 (26)
Ouest without AM [21]	193 (23)	14 (2)	239 (28)	294 (35)	96 (16)	8 (1)	133 (22)	168 (28)
Sud [22]	28 (19)	1 (0.7)	30 (20)	43 (29)	72 (22)	7 (2)	106 (32)	125 (37)
Sud-Est [23]	21 (7)	0	40 (14)	49 (17)	39 (18)	0	43 (19)	56 (25)
p-value [24]	< 0.0001	0.006	< 0.0001	< 0.0001	< 0.0001	0.006	< 0.0001	< 0.0001
Geographic Location								
Urban [25]	194 (9)	15 (0.7)	278 (13)	360 (17)	244 (14)	24 (1)	316 (19)	413 (24)
Rural [26]	501 (16)	39 (1)	613 (20)	796 (26)	581 (21)	61 (2)	663 (24)	874 (32)
p-value [4]	< 0.0001	0.05	< 0.0001	< 0.0001	< 0.0001	0.04	< 0.0001	< 0.0001

[1] Positive if at least 1 antibody was positive; [2] 2012 (n=2,014) where (n= denominator); 2015 (n=1,728); [3] 2012 (n=3,126); 2015 (n=2,678); [4] Obtained via Chi-square test; [5] 2012 (n=229); 2015 (n=76); [6] 2012 (n=2,151); 2015 (n=2,602); [7] Obtained via Chi-square test except for LSA-1 which was obtained via Fisher's Exact test; [8] 2012 (n=1,170); 2015 (n=450); [9] 2012 (n=1,360); 2015 (n=893); [10] 2012 (n=1,200); 2015 (n=899); [11] 2012 (n=1,227); 2015 (n=1,384); [12] 2012 (n=367); 2015 (n=772); [13] 2012 (n=1,078); 2015 (n=146); [14] 2012 (n=1,092); 2015 (n=740); [15] 2012 (n=555); 2015 (n=312); [16] 2012 (n=224); 2015 (n=213); [17] 2012 (n=185); 2015 (n=146); [18] 2012 (n=372); 2015 (n=500); 19 2012 (n=257); 2015 (n=345); 20 2012 (n=101); 2015 (n=152); 21 2012 (n=845); 2015 (n=146); 22 2012 (n=150); 2015 (n=334); [23] 2012 (n=281); 2015 (n=221); [24] Obtained via Chi-square after merging departments into 5 categories due to low counts; [25] 2012 (n=2,098); 2015 (n=1,699); [26] 2012 (n=3,042); 2015 (n=2,707)

Table 4. Agreement between *Plasmodium falciparum* rapid diagnostic test and antibody positivity, Tracking Results Continuously (TRaC) surveys, Haiti 2012 and 2015

		Kappa Coeficient	95% CI	Agreement
TRaC 2012	AMA-1	0.0114	[-0.0047 - 0.0276]	Poor
	LSA-1	0.0282	[-0.0239 - 0.0803]	Poor
	MSP-1-19	0.0093	[-0.0043 - 0.0229]	Poor
	Combined IgG	0.0079	[-0.0034 - 0.0192]	Poor
TRaC 2015	AMA-1	0.016	[0.0031 - 0.0289]	Poor
	LSA-1	0.0289	[-0.0209 - 0.0787]	Poor
	MSP-1-19	0.0098	[-0.0004 - 0.0201]	Poor
	Combined IgG	0.0082	[0.0002 - 0.0162]	Poor

Table 5. Agreement between *Plasmodium falciparum* microscopy and antibody positivity, Tracking Results Continuously (TRaC) surveys, Haiti 2012

		Kappa Coeficient	95% CI	Agreement
TRaC 2012	AMA-1	0.0017	[-0.0033 - 0.0067]	Poor
	LSA-1	-0.0011	[-0.0023 - 0.0001]	Poor
	MSP-1-19	0.0011	[-0.0027 - 0.0049]	Poor
	Combined IgG	0.0006	[-0.0023 - 0.0034]	Poor

Figure 1. Prevalence of *Plasmodium falciparum* antibody by department — Haiti 2012

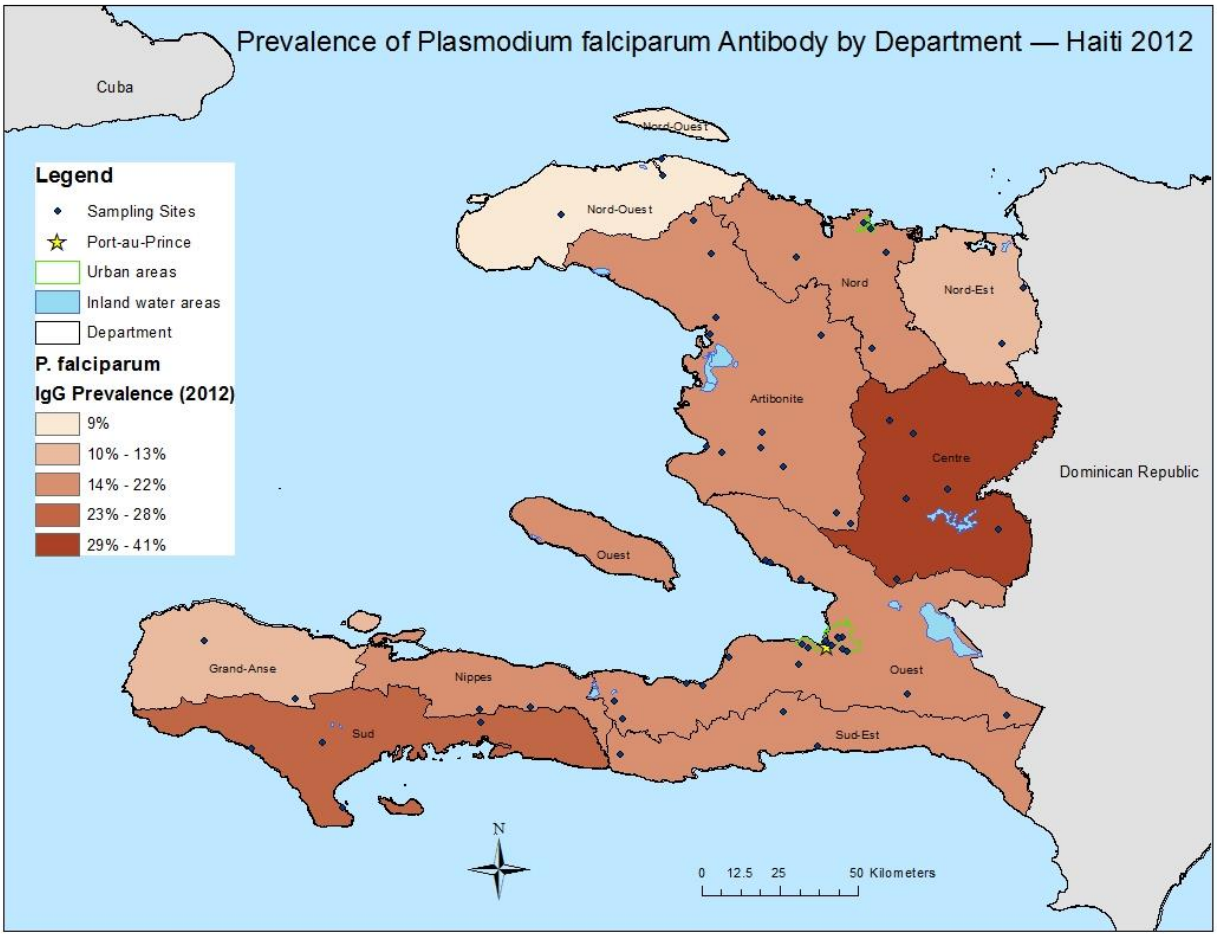
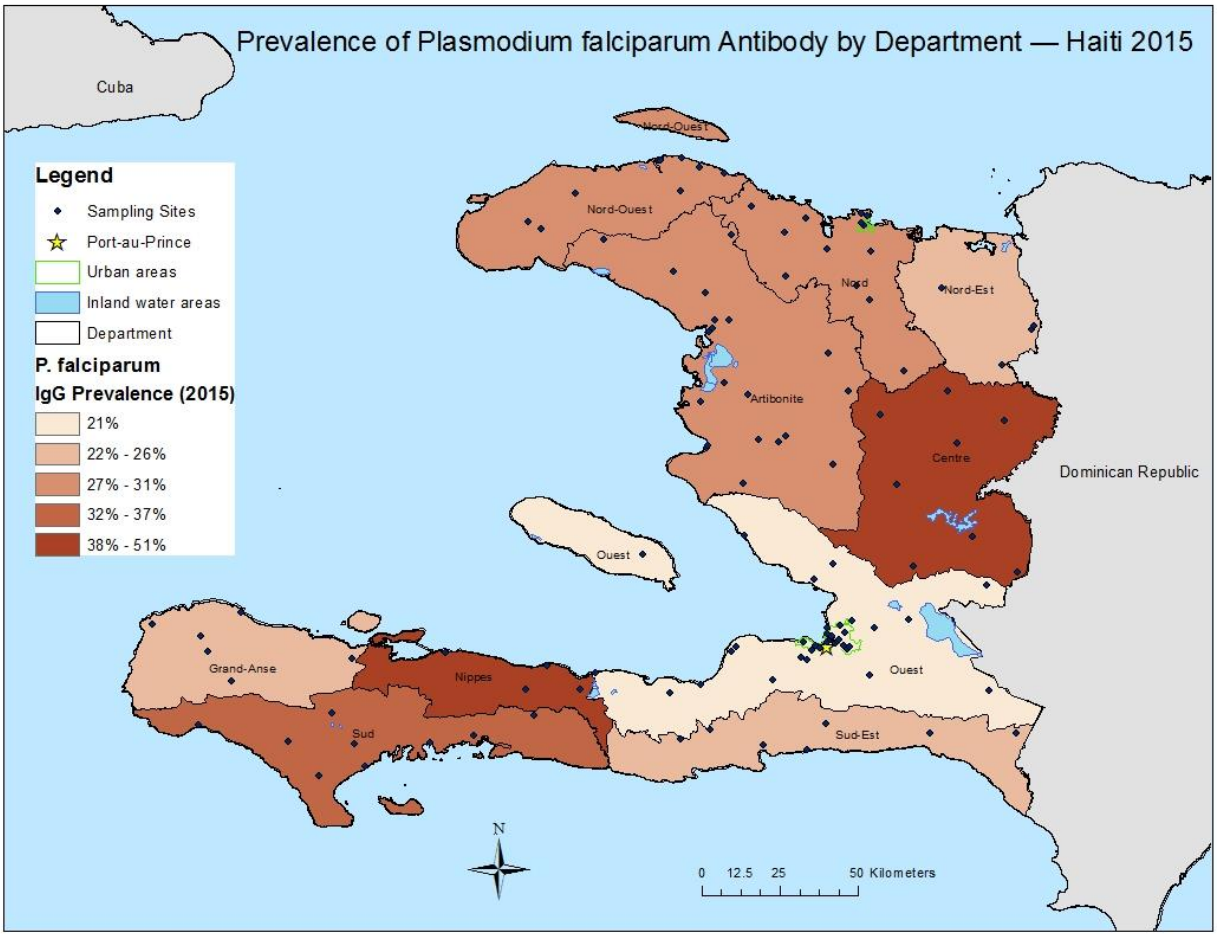


Figure 2. Prevalence of *Plasmodium falciparum* antibody by department — Haiti 2015



Chapter IV: Conclusion and Recommendations

After the 7.0 magnitude earthquake that weakened Haiti's infrastructure and health system in January 2010 there has been a renewed interest to eliminate malaria from the entire island of Hispaniola by 2020. This has been viewed as an attainable goal given malaria's low prevalence on the island and can likely be achieved when malaria control and elimination efforts are focused in Haiti. With support from the CDC, NGOs, and the Programme National de Controle de la Malaria (PNCM), the Haitian government has recently developed the Plan Stratégique National d'Elimination de la Malaria (PSNEM), a national strategic plan for 2016-2022, with the goal of improving case management and the surveillance system for malaria elimination by 2020.

Measuring malaria is a challenge in low-endemic countries since detection of parasitic infection as malaria approaches zero is more difficult. In such settings, malaria diagnostic tests such as microscopy, PCR and RDTs are not very useful when assessing the prevalence of the disease at the population level given that parasite identification might go undiagnosed. Reasons for this occurrence are that *P. falciparum* parasites are not always present in the peripheral blood or because microscopy skills may weaken when technicians are not conducting malaria diagnosis regularly. Given serological methods are more useful in detecting history of malaria exposure in low-endemic countries, serology data was gathered via nation-wide surveys in Haiti to estimate the prevalence of malaria in order to contribute to the elimination of the disease. The findings of this study are useful to the Haitian Ministry of Health since they provide knowledge on the prevalence of *P. falciparum* malaria throughout the country and can contribute to the decision of resource allocation and proper intervention targets in order to successfully achieve elimination of malaria.

The primary purpose of the study, to estimate the prevalence of *P. falciparum* malaria in Haiti by using serologic testing to measure IgG antibody responses to parasitic antigens was accomplished. Antibody prevalence for each *P. falciparum* antigen, as well as the overall antibody prevalence throughout the country was assessed. All throughout Haiti, the highest antibody prevalence was for the MSP-1-19 antigen followed by AMA-1 and LSA-1 antigens. Noteworthy factors that were found to be influencing the prevalence of malaria in Haiti were the geographic location, the age of the population as well as women's pregnancy status. The results of the study indicated that, in rural areas, there was higher transmission of disease. The department of Centre had the highest overall prevalence of malaria for both 2012 and 2015 (41% and 51%). Rural populations of Haiti are at a disadvantage since access to energy, clean water, and improved sanitation are scarce. The findings of the study suggest malaria control and elimination interventions should be targeted at both rural and urban communities, but strongly emphasized in rural communities.

Since a higher prevalence of *P. falciparum* antibodies was found in >50 years age group, the oldest age group category, the findings of the study provide further evidence to the malaria field as to the development of malaria immunity as people age, given that they have been continuously exposed to the parasite throughout their lifetime, and have developed antibody responses to the disease due to re-infection. Moreover, since fever was exhibited in only 5% of the antibody positives, the lack of symptom and antibody occurrence indicated the presence of asymptomatic malaria infections throughout Haiti which often occurs in malaria endemic countries.

The study also provided malaria antibody evidence that concurred with previous research. Serologic methods, such as multiplex based assays (Luminex®), has proven to be a useful

technique to identify the prevalence of malaria in low-transmission areas. PCR, microscopy and RDTs are not efficient at identifying infection of malaria in countries like Haiti and poor agreements (K= <0.02) have been found between diagnostic tests (microscopy and RDT) and Luminex® assay. Serology employs an alternative method of estimating prevalence by examining antibody positivity, therefore indicating historical exposure of disease at the population level.

The study contributes to the field of malaria and public health by providing *P. falciparum* antibody prevalence as well as knowledge on the transmission of malaria in Haiti based on the prevalence of antibodies found in each administrative region (department) of the country. The results of the study also provide knowledge on the characteristics of the population affected, factors likely contributing to the occurrence and spread of disease, as well as the usefulness of serologic methods in low malaria endemic settings. The study suggests that Haiti's Ministry of Health and its partners should continue to monitor the antibody prevalence of *P. falciparum* under the country's malaria prevention and control methods, keeping in mind that most infections are asymptomatic. Our findings also suggest that more resources and interventions should be targeted at rural areas where prevalence is highest, in order to continue progress towards the elimination of malaria in Haiti.

References

Baum, E., Sattabongkot, J., Sirichaisinthop, J., Kiattibutr, K., Jain, A., Taghavian, O., . . . Yan, G. (2016). Common asymptomatic and submicroscopic malaria infections in Western Thailand revealed in longitudinal molecular and serological studies: a challenge to malaria elimination. *Malaria Journal, 15*, 333. doi:10.1186/s12936-016-1393-4

Boncy, P. J., Adrien, P., Lemoine, J. F., Existe, A., Henry, P. J., Raccurt, C., . . . Zervos, M. J. (2015). Malaria elimination in Haiti by the year 2020: an achievable goal? *Malaria Journal, 14*, 237. doi:10.1186/s12936-015-0753-9

Bretscher, M. T., Supargiyono, S., Wijayanti, M. A., Nugraheni, D., Widyastuti, A. N., Lobo, N. F., . . . Drakeley, C. J. (2013). Measurement of Plasmodium falciparum transmission intensity using serological cohort data from Indonesian schoolchildren. *Malaria Journal, 12*. doi:10.1186/1475-2875-12-21

Bronzan, R. N., McMorrow, M. L., & Kachur, S. P. (2008). Diagnosis of malaria: challenges for clinicians in endemic and non-endemic regions. *Molecular Diagnosis & Therapy, 12*(5), 299-306.

Carter, T. E., Malloy, H., Existe, A., Memnon, G., St Victor, Y., Okech, B. A., & Mulligan, C. J. (2015). Genetic Diversity of Plasmodium falciparum in Haiti: Insights from Microsatellite Markers. *PloS One, 10*(10), e0140416. doi:10.1371/journal.pone.0140416

Centers for Disease Control and Prevention [CDC]. (2012). Malaria: Glossary. [Accessed Feb 4, 2017]. Retrieved from https://www.cdc.gov/malaria/glossary.html

Centers for Disease Control and Prevention [CDC]. (2015a). Malaria: Anopheles Mosquitoes. [Accessed Jan 29, 217]. Retrieved from https://www.cdc.gov/malaria/about/biology/mosquitoes/index.html

Centers for Disease Control and Prevention [CDC]. (2015b). Travelers' Health Chapter 4: Haiti. [Accessed Jan 29, 217]. Retrieved from https://wwwnc.cdc.gov/travel/yellowbook/2016/select-destinations/haiti

Centers for Disease Control and Prevention [CDC]. (2016a). Malaria: Biology. [Accessed Apr 4, 2017]. Retrieved from https://www.cdc.gov/malaria/about/biology/index.html

Centers for Disease Control and Prevention [CDC]. (2016b). Malaria: Malaria Parasites. [Accessed Jan 29, 2017]. Retrieved from https://www.cdc.gov/malaria/about/biology/parasites.html

Central Intelligence Agency [CIA]. (2017). The World Factbook Central America and Caribbean: Haiti. [Accessed Jan 30, 2017]. Retrieved from https://www.cia.gov/library/publications/the-world-factbook/geos/ha.html

Crompton, P.D., Moebius, J., Portugal, S., Waisberg, M., Hart, G., Garver, L.S., Miller, L.H., Barillas, C., & Pierce, S.K. (2014). Malaria immunity in man and mosquito: insights into unsolved mysteries of a deadly infectious disease. *Annu Rev Immunol*. 32: 157–187. doi:10.1146/annurev-immunol-032713-120220.

Cunha, M. G., Silva, E. S., Sepulveda, N., Costa, S. P., Saboia, T. C., Guerreiro, J. F., . . . Drakeley, C. J. (2014). Serologically defined variations in malaria endemicity in Para state, Brazil. *PloS One, 9*(11), e113357. doi:10.1371/journal.pone.0113357

Dent, A.E., Malhotra, I., Wang, X., Babineau, D., Yeo. K.T., Anderson, T., … Kazura, J.W. (2015). Contrasting Patterns of Serologic and Functional Antibody Dynamics to *Plasmodium falciparum* Antigens in a Kenyan Birth Cohort. *Clinical and Vaccine Immunology* 23(2):104-16. doi: 10.1128/CVI.00452-15.

Doolan, D.L., Dobaño, C., & Baird, J.K.(2009). Acquired Immunity to Malaria. *Clinical Microbiology Reviews* 22(1), 13-36. doi:10.1128/CMR.00025-08.

Draper, C. C. (1971). Malaria. Laboratory diagnosis. *British Medical Journal, 2*(5753), 93-95. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1795512/pdf/brmedj02255-0045.pdf

Elbadry, M. A., Al-Khedery, B., Tagliamonte, M. S., Yowell, C. A., Raccurt, C. P., Existe, A., . . . Dame, J. B. (2015). High prevalence of asymptomatic malaria infections: a cross-sectional study in rural areas in six departments in Haiti. *Malaria Journal, 14*, 510. doi:10.1186/s12936-015-1051-2

Elshal, M.F., & McCoy, J.P. (2006). Multiplex Bead Array Assays: Performance Evaluation and Comparison of Sensitivity to ELISA. *Methods* vol 38(4):317-323. doi: 10.1016/j.ymeth.2005.11.010

Frederick, J., Saint Jean, Y., Lemoine, J. F., Dotson, E. M., Mace, K. E., Chang, M., . . . Impoinvil, D. E. (2016). Malaria vector research and control in Haiti: a systematic review. *Malaria Journal, 15*(1), 376. doi:10.1186/s12936-016-1436-x

Ghinai, I., Cook, J., Hla, T. T., Htet, H. M., Hall, T., Lubis, I. N., . . . Field, N. (2017). Malaria epidemiology in central Myanmar: identification of a multi-species asymptomatic reservoir of infection. *Malaria Journal, 16*(1), 16. doi:10.1186/s12936-016-1651-5

Gupta, S. (n.d.). Luminex Technology- Innovative Flow-and-Bead Based Technology Measuring Multiple Analytes Simultaneously in a Single Reaction Well. *Cambridge Biomedical*. [Accessed Feb 20, 2017]. Retrieved from http://www.cambridgebiomedical.com/DesktopModules/Bring2mind/DMX/Download.aspx?Command=Core_Download&EntryId=2090&PortalId=0&TabId=155

Haitian Institute of Statistics and Informatics [IHSI]. (2015). Population Totale, Population de 18 Ans Et Plus Ménages Et Densités Estimés En 2015. [Accessed Mar 23, 2017]. Retrieved from http://www.ihsi.ht/pdf/projection/Estimat_PopTotal_18ans_Menag2015.pdf

Herrera, S., Ochoa-Orozco, S. A., Gonzalez, I. J., Peinado, L., Quinones, M. L., & Arevalo-Herrera, M. (2015). Prospects for malaria elimination in Mesoamerica and Hispaniola. *PLoS Neglected Tropical Diseases, 9*(5), e0003700. doi:10.1371/journal.pntd.0003700

Koffi, D., Touré, A.O., Varela, M.L., Vigan-Womas, I., Béourou, S., Brou, S., Ehouman, M.F., … Perraut, R. (2015). Analysis of antibody profiles in symptomatic malaria in three sentinel sites of Ivory Coast by using multiplex, fluorescent, magnetic, bead-based serological assay (MAGPIX™). *Malaria Journal* 14:509. doi**:** 10.1186/s12936-015-1043-2.

Lucchi, N. W., Karell, M. A., Journel, I., Rogier, E., Goldman, I., Ljolje, D., . . . Udhayakumar, V. (2014). PET-PCR method for the molecular detection of malaria parasites in a national malaria surveillance study in Haiti, 2011. *Malaria Journal, 13*, 462. doi:10.1186/1475-2875-13-462

Makler, M. T., Palmer, C. J., & Ager, A. L. (1998). A review of practical techniques for the diagnosis of malaria. *Annals of Tropical Medicine and Parasitology, 92*(4), 419-433.

Malaria Atlas Project [MAP]. (n.d. a). Malaria Endemicity. [Accessed Apr 3, 2017]. Retreived from http://www.map.ox.ac.uk/explore/about-malaria/malaria-endemicity/

Malaria Atlas Project [MAP]. (n.d. b). The Spatial Limits of Malaria Transmission. [Accessed Apr 3, 2017]. Retrieved from http://www.map.ox.ac.uk/explore/about-malaria/spatial-limits-malaria/

Moody, A. (2002). Rapid diagnostic tests for malaria parasites. *Clinical Microbiology Reviews, 15*(1), 66-78. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC118060/pdf/cm0004.pdf

Mosha, J. F., Sturrock, H. J. W., Brown, J. M., Hashim, R., Kibiki, G., Chandramohan, D., & Gosling, R. D. (2014). The independent effect of living in malaria hotspots on future malaria infection: an observational study from Misungwi, Tanzania. *Malaria Journal, 13*. doi:10.1186/1475-2875-13-445

Mosha, J. F., Sturrock, H. J. W., Greenwood, B., Sutherland, C. J., Gadalla, N. B., Atwal, S., . . . Gosling, R. D. (2014). Hot spot or not: a comparison of spatial statistical methods to predict prospective malaria infections. *Malaria Journal, 13*. doi:10.1186/1475-2875-13-53

Mung, K., Renamy, B., Vely, J.F., Magloire, R., Wells, N., Ferguson, J., Townes, D., McMorrow, M., Tan, K., Divine, B., & Slutsker, L. (2010). Malaria Acquired in Haiti --- 2010. *MMWR* 59(08); 217-219.

Ondigo, B.N., Hodges, J.S., Ireland, K.F., Magak, N.G., Lanar, D.E, Dutta, S., Narum, D.L., Park, G.S., Ofulla, A.V, & John, C.C. (2014). Estimation of Recent and Long-Term Malaria Transmission in a Population by Antibody Testing to Multiple *Plasmodium falciparum* Antigens. *The Journal of Infectious Diseases* 210(7): 1123-32. doi: 10.1093/infdis/jiu225.

Pothin, E., Ferguson, N. M., Drakeley, C. J., & Ghani, A. C. (2016). Estimating malaria transmission intensity from Plasmodium falciparum serological data using antibody density models. *Malaria Journal, 15*. doi:10.1186/s12936-016-1121-0

Rogier, E., Moss, D. M., Chard, A. N., Trinies, V., Doumbia, S., Freeman, M. C., & Lammie, P. J. (2016). Evaluation of Immunoglobulin G Responses to Plasmodium falciparum and Plasmodium vivax in Malian School Children Using Multiplex Bead Assay. *American Journal of Tropical Medicine and Hygiene*. doi:10.4269/ajtmh.16-0476

Rogier, E., Wiegand, R., Moss, D., Priest, J., Angov, E., Dutta, S., . . . Barnwell, J. W. (2015). Multiple comparisons analysis of serological data from an area of low Plasmodium falciparum transmission. *Malaria Journal, 14*. doi:10.1186/s12936-015-0955-1

Rolfes, M.A., McCarra, M., Magak, N.G., Ernst, K.C., Dent, A.E, Lindblade, K.A., & John, C.C. (2012). Development of Clinical Immunity to Malaria in Highland Areas of Low and Unstable Transmission. *Am. J. Trop. Med. Hyg*., 87(5), pp. 806–812. doi:10.4269/ajtmh.2012.11-0530.

Rosas-Aguirre, A., Llanos-Cuentas, A., Speybroeck, N., Cook, J., Contreras-Mancilla, J., Soto, V., . . . Erhart, A. (2013). Assessing malaria transmission in a low endemicity area of north-western Peru. *Malaria Journal, 12*. doi:10.1186/1475-2875-12-339

Sarr, J. B., Orlandi-Pradines, E., Fortin, S., Sow, C., Cornelie, S., Rogerie, F., . . . Remoue, F. (2011). Assessment of exposure to Plasmodium falciparum transmission in a low endemicity area by using multiplex fluorescent microsphere-based serological assays. *Parasit Vectors, 4*, 212. doi:10.1186/1756-3305-4-212

Sylla, K., Tine, R. C. K., Ndiaye, M., Sow, D., Sarr, A., Mbuyi, M. L. T., . . . Faye, B. (2015). Sero-epidemiological evaluation of Plasmodium falciparum malaria in Senegal. *Malaria Journal, 14*. doi:10.1186/s12936-015-0789-x

The World Bank (2014). Living Conditions in Haiti's Capital Improve, but Rural Communities Remain Very Poor. [Accessed Mar 23, 2017]. Retrieved from

http://www.worldbank.org/en/news/feature/2014/07/11/while-living-conditions-in-port-au-prince-are-improving-haiti-countryside-remains-very-poor

Viera, A.J. & Garrett, J.M. (2005). Understanding Interobserver Agreement: The Kappa Statistic. *Family Medicine* 37(5):360-3.

Voller, A., Huldt, G., Thors, C., & Engvall, E. (1975). New serological test for malaria antibodies. *British Medical Journal, 1*(5959), 659-661. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1672875/pdf/brmedj01438-0025.pdf

von Fricken, M. E., Weppelmann, T. A., Lam, B., Eaton, W. T., Schick, L., Masse, R., . . . Okech, B. A. (2014). Age-specific malaria seroprevalence rates: a cross-sectional analysis of malaria transmission in the Ouest and Sud-Est departments of Haiti. *Malaria Journal, 13*. doi:10.1186/1475-2875-13-361

Wilson D.W., Fowkes F.J.I., Gilson P.R., Elliott S.R., Tavul L., Michon, P., Dabod, E., Siba, P. M., Mueller, I., Crabb, B. S., & Beeson, J.G. (2011). Quantifying the Importance of MSP1-19 as a Target of Growth-Inhibitory and Protective Antibodies gainst *Plasmodium falciparum* in Humans. *PLOS ONE* 6(11): e27705. doi: 10.1371/journal.pone.0027705

Wipasa, J., Suphavilai, C., Okell, L.C., Cook, J., Corran, P.H., Thaikla, K., Liewsaree, W., Riley, E.M., & Hafalla, J.C.M. (2010). Long-Lived Antibody and B Cell Memory Responses to the Human Malaria Parasites, Plasmodium falciparum and Plasmodium vivax. *PLoS Pathogens* 6(2): e1000770. doi:10.1371/journal.ppat.1000770

World Health Organization [WHO]. (2007). Gender, health and malaria. [Accessed Apr 9, 2017]. Retrieved from http://www.who.int/gender-equity-rights/knowledge/gender_malaria_leaflet/en/

World Health Organization [WHO]. (2015). Global Technical Strategy for Malaria 2016-2030. [Accessed Feb 20, 2017]. Retrieved from http://www.who.int/malaria/publications/atoz/9789241564991/en/

World Health Organization [WHO]. (2016a). Media Center: Malaria fact sheet. [Accessed Jan 30, 2017]. Retrieved from http://www.who.int/mediacentre/factsheets/fs094/en/

World Health Organization [WHO]. (2016b). World Malaria Report 2016. [Accessed Jan 29, 2017]. Retrieved from http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/

World Health Organization [WHO]. (2016c). Fact Sheets: World Malaria Report. [Accessed Jan 29, 2017]. Retrieved from http://www.who.int/malaria/media/world-malaria-report-2016/en/

Yang, C., Shi., Y.P., Udhayakumar, V., Alpers, M.P., Povoa, M.M., Hawley, W.A., Collins, W.E., & Lal, A.A. (1995). Sequence variations in the non-repetitive regions of the liver stage-specific antigen-1 (LSA-1) of Plasmodium falciparum from field isolates. *Mol Biochem Parasitol.* 71(2):291-4.

Your Genome [YG]. (2016). How is Malaria Treated and Prevented? [Accessed Apr 3, 2017]. Retrieved from http://www.yourgenome.org/facts/how-is-malaria-treated-and-prevented