UNIVERSITY OF CAPE COAST

IMPACT OF MALARIA MASS-TEST TREAT EXERCISE ON ANTI-*Pf*EBA-175 ANTIBODY LEVELS IN A MALARIA ENDEMIC COMMUNITY

PROSPER KOFI TEY

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BY

PROSPER KOFI TEY

Thesis submitted to the Department of Microbiology and Immunology, of the School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Infection and Immunity

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature…… Date ……..

Name: Prosper Kofi Tey

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Supervisor's Signature……Date…..…….

Name: Prof. Dorcas Obiri-Yeboah

Co-Supervisor's Signature……Date…..……

Name: Prof. Linda Eva Amoah

ABSTRACT

One key factor influencing the manifestation of malaria is naturally acquired immunity (NAI) against the parasite, which is developed after repeated exposure by people living in endemic areas. Efforts to further decrease the incidence of malaria have resulted in the implementation of a wide variety of malaria control and elimination measures, each with the potential to alter the development of NAI. We monitored total IgG against *Pf*EBA-175 antigen quarterly over a 13-month period in individuals living in a high malaria transmission setting (Obom) where a mass test, treat and track (MTTT) exercise was rolled out. 1200 individuals without any symptoms of malaria aged between 7 months and 90 years were enrolled on the study and donated finger-pricked blood that was used to prepare dried blood spots (DBS). Out of this number, 314 individuals who were present for all four sampling time points were selected for the study. Genomic DNA was extracted from the DBS from these individuals for PCR estimate of *P. falciparum.* Antibodies were also eluted from the DBS and used for *Pf*EBA-175-specific ELISA. The results were stratified by age into 0-4 years, 5-9 years, 10-15 years and 16+ years groups. Across visits, there was a gradual rise in antibody levels at each time point, however, this was not statistically significant. Individuals aged 16 and above had higher antibody titres than individuals aged 15 and below. The study revealed that all the age categories had higher antibody titres with mean significant differences at the end of the period except those in the age group 10-15 years whose differences in antibody titres at the end of the study were not statistically significant. Generally, higher antibody levels were associated with those infected compared to the uninfected. This study established that MTTT intervention does not significantly reduce antibody titres against the *Pf*EBA-175 antigen after a year of its rollout in an endemic community.

KEYWORDS

Naturally acquired immunity

Antibodies

Immunity

Erythrocyte binding antigen

Sub-patent parasitaemia

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DEDICATION

This work is dedicated to my parents, Mr. Franklin Tey and Mrs Leticia Setuagbe Tey, and also in memory of my late sisters, Jennifer Tey and Irene Yayra Tey.

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LIST OF ACRONYMS

NAI	naturally acquired immunity
MTTT	mass test treat and tracking
WHO	world health organisation
SSA	sub-Saharan Africa
RBCs	red blood cells
HB	haemoglobin
IRS	Indoor residual spraying
ITNs	insecticide-treated nets
IPTp	intermittent preventive treatment in pregnancy
IPTi/IPTc	intermittent preventive treatment in infants/children
SMC	seasonal malaria chemoprevention
MDA	mass drug administration
MSPs	merozoites surface proteins
EBP	erythrocyte binding-like proteins
RBL/RfRh	reticulocyte binding-like or reticulocyte homologue proteins
EBAs	erythrocyte binding antigens
*Pf*EBA-175	*Plasmodium falciparum* erythrocyte binding antigen-175
RDTs	rapid diagnostic tests
*Pf*EMP1	*Plasmodium falciparum* erythrocyte membrane protein-1
IEs	infected erythrocytes
DDT	dichlorodiphenyltrichloroethane
GMAP	Global Malaria Action Plan
NMCP	National Malaria Control Programme
NMEP	National Malaria Elimination Programme

CDC	Center for Disease Control and Prevention
ANC	antenatal care
SP	sulfadoxine-pyrimethamine
FSaT	focal screening and treatment
MFT	mass fever treatment
PRRs	pathogen-recognition receptors
IS	immune system
DCs	dendritic cells
CSP	circumsporozoite protein
IgG	immunoglobulin G
TNF	tumour necrosis factor
DBP	duffy-binding proteins
DNA	deoxyribonucleic acid
ELISA	enzyme link immunosorbent assay
PCR	polymerase chain reaction
PBS	phosphate buffered saline
PET-PCR	photo-induced electron transfer- polymerase chain reaction
DBS	dried blood spot
OD	optical density
CT	cycle thresholds
AU	arbitrary units
ADAMSEL	Auditable Data Analysis and Management System for ELISA

CHAPTER ONE

INTRODUCTION

Millions of individuals are affected every year across the globe with malaria and sub-Saharan Africa (SSA) bears the greatest burden of the disease. Ghana, like many other countries on the continent, continues to roll out several malaria interventions to mitigate the effects of the disease. These interventions in one way or another may alter the naturally acquired immunity (NAI) developed by people in endemic areas after repeated exposure to the parasite. Gaining an understanding of these interventions on NAI is critical to the implementation of these policies. This current research sought to assess the dynamism associated with antibody titres/response after the implementation of a mass-test, treat and track (MTTT) intervention in an endemic area.

Background

As a disease of major public health concern, malaria is a vector-borne infectious disease caused by the *Plasmodium* species. Five (5) of these species have been identified to infect humans; *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax, and Plasmodium knowlesi*. *Plasmodium falciparum* is regarded as the most dangerous among the *Plasmodium* species (Rout & Mahapatra, 2019) which accounts for over 90% of all malaria cases (Hassen & Dinka, 2020) with the most of them happening among children below the ages of five and pregnant women. The devastating effect of malaria - about 247 million reported cases and 619,000 deaths (World Health Organization, 2022), continues to hit the world despite several efforts to curb it. Sub-Sahara Africa (SSA) is the continent most burdened

with the disease; accounting for about 95% of all cases and death related to malaria. Five (5) countries in sub-Saharan Africa for instance accounted for 55% of the malaria cases and over half the malaria deaths. Ghana, a malaria mesoendemic country accounts for 2.2% and 2.0% of world malaria cases and death respectively, making it part of the 15 highest-burden malaria countries in the world (Amoah et al., 2022; WHO, 2020).

Tremendous milestone has been chalked in significantly decreasing the burden caused by this disease, especially from the early 2000s when the disease received massive attention. The World Health Organization reports that about 1.5 billion cases and 7.6 million deaths due to malaria have been prevented between the periods of 2000 – 2019 (World Health Organization, 2020). This massive success has been achieved due to various control measures put in place over the years. These control measures are broadly classified under preventive and treatment modules (Desai et al., 2020). The preventive module consists mainly of controlling the vector; insecticide-treated nets (ITNs), and indoor residual spraying (IRS). Chemoprophylaxis, intermittent preventive treatment in pregnancy (IPTp) and or in infants/children (IPTi/IPTc), seasonal malaria chemoprevention (SMC), mass drug administration (MDA) and mass test, treat and track (MTTT) forms the treatment module. However, the gains made over the years appear to be under threat. The number of incidents and fatalities has fluctuated over the past few years, with no appreciable drops observed as compared to those made from the 2000s to 2015. Some studies attribute the stagnation observed to factors such as vector resistance, and parasite diversity; which consequently results in drug

resistance, ecological changes and host and vector variables (Haile et al., 2020).

Though millions of individuals are infected, a few proportions, mostly children show clinical symptoms of the disease. This is seen by the disparity between the number of cases and deaths reported globally. Longitudinal studies which focus on the disease incidence among people over a period of time have also produced similar outcomes (Akpogheneta et al., 2010; Coulibaly et al., 2021; Julien & Wardemann, 2019; Yman et al., 2019). The reduction in the number of people who develop clinical malaria is attributed to the natural acquisition of immunity by people in endemic areas over time (Doolan et al., 2009).

The complex nature of malarial immunity makes its full understanding a daunting task. Like any other immune response, it is influenced by host and environmental factors. Antibodies against merozoites antigens of the parasite are crucial step in preventing the manifestation of the severe forms of the disease (Ismail et al., 2014). Several families of merozoites proteins/antigens such as the merozoites surface proteins (MSPs), the erythrocyte binding-like proteins (EBP) and the reticulocyte binding-like or reticulocyte homologue proteins (RBL or RfRh) help parasite to invade the erythrocytes. The erythrocyte binding antigens (EBAs); EBA-175, EBA-181, and EBA-140, antigens located in the micronemes of the merozoites of which the *Pf*EBA-175 have been studied with these studies pointing to the fact that higher titres of antibodies produced against this antigen have protected children from severe forms of the diseases (Akpogheneta et al., 2010; Julien & Wardemann, 2019). Another form of protection that has been linked to immunity is protection

against anaemia. Anaemia in malarial endemic areas is multifactorial and hence cannot be entirely said to be caused by *Plasmodium falciparum*. Though nutritional deficiencies, infectious diseases and haemoglobinopathies are factors contributing to anaemia in malaria endemic settings (Crookston et al., 2010; Maketa et al., 2015; R. E. Phillips & Pasvol, 1992; Sumbele et al., 2016), it does not negate the fact that *Plasmodium falciparum* carriage is associated with anaemia, especially in young children (Kimbi et al., 2013; Teh et al., 2018).

All malaria interventions aim to drastically reduce the morbidity and mortality associated with the disease. These interventions are usually determined by the overall objectives the implementers seek to achieve. Mass, test, treat and track (MTTT) is said to be a modified form of MDA. In MDA which involves the treatment of the whole population in a defined area with anti-malarial drugs irrespective of the presence of infection or symptoms, MTTT establishes the presence of the infection usually through testing with rapid diagnostic tests (RDTs) before administering anti-malarial drugs. The intention of MTTT is to tackle the main concerns raised with regards to MDA- the issue of drug pressure resulting in drug resistance and other factors such as coverage and sustainability (Newby et al., 2015). Also, according to Ndong *et al*, MTTT is generally accepted by the population since it is believed to be helpful in reducing the disease's incidence (Ndong et al., 2019).

While malaria control interventions have proven to contribute substantially in decreasing malarial morbidity and mortality, there are some challenges associated with these control interventions (Barnes et al., 2009; Hershey et al., 2017). Resistance to several pesticides used in vector control,

as well as parasites developing drug resistance (Oladipo et al., 2022). Another concern raised is the issue of immunity among the population considering the fact that malarial immunity is mostly associated with exposure over time. Though some studies have ruled out this "fear", exploring the dynamism and impact associated with antibody titres against a blood stage antigen, in this case, *Pf*EBA-175 after an MTTT control intervention is worth studying. Using samples from a 13-month MTTT survey, where participants present during the entire sampling points were selected, this study aims to assess antibody responses against the *Pf*EBA-175 antigen in the midst of an intervention.

Problem Statement

Malaria continues to cause significant havoc on humans despite tremendous efforts to decrease disease's burden. The stagnation observed in the cases and death recorded due to malaria for the past few years is evident that new measures or modifications to some existing measures are required to further decrease morbidity and mortality to the barest minimum and subsequently ultimate elimination. With the birth of the elimination agenda, attention has been shifted to finding effective ways to build and sustain immunity towards the disease. The acquisition of protective immunity against malaria builds over time with repeated exposure (Ismail et al., 2014; Nielsen et al., 2022). This acquired immunity is what prevents the development of the clinical symptoms associated with the disease. Since the fight against malaria is geared towards the production of effective vaccines, with one of them being accepted by WHO and others at various stages of clinical trials, it is apparent that more researches are needed to fully comprehend the dynamism behind the acquisition of natural immunity in the midst of several control interventions.

Studies (Julien & Wardemann, 2019; White et al., 2014; Yman et al., 2019) suggest that antibodies produced against antigens of the parasite are short-lived and thus immunity is lost over time when exposure is removed (Beeson, 2015).

In wanting to understand the dynamics of natural immunity, questions such as; what effects do control interventions (in this case MTTT) have on antibody levels against *Pf*EBA-175? Do other factors like changes in transmission seasons, the persistence of infection and parasite diversity also influences natural immunity? Currently, limited studies assess antibody response to *Pf*EBA-175 antigen after an MTTT malarial control intervention. Also, very few studies have been conducted in the country assessing *Pf*EBA -175 antibody titres across all age groups.

Aim and Objectives

Aim

To assess the impact of a 13-month mass testing, treatment, and tracking (MTTT) intervention on antibody responses to *Plasmodium falciparum* erythrocyte binding antigen (*Pf*EBA-175) and sub-patent parasitaemia

Specific Objectives

In line with the main aim, this study is set;

1. To measure the *Pf*EBA-175 antibody titres in individuals from Obom at baseline and at the end of a 13-month MTTT intervention
2. To evaluate the prevalence of *Plasmodium falciparum* infection among individuals living in Obom before and during the course of a 13-month MTTT intervention study participants in an endemic area

3. To determine the relationship between the *Pf*EBA-175 antibody titres and *P. falciparum* infectivity among study participants

Justification

The fight against one of the world's deadliest illnesses (malaria) continues unabatedly with the employment of several control methods from the past till current time. Whilst these methods in the past have proven to be of substantial benefits, the fight still continues especially in SSA. In our quest to achieve this incredible objective of decreasing the burden of malaria on population, several interventions are being rolled out without us critically assessing the effects of these interventions on the population. The conflicting study reports with regards to the malarial immunity of the population coupled with the stall in malaria related cases and its associated deaths for the past few years are enough for researchers to critically assess and review, where necessary, all existing malaria interventions. Aside from the limited knowledge available with regards to antibody response against a blood-stage antigen (*Pf*EBA-175), and a leading blood-stage vaccine candidate after MTTT intervention in an endemic population, investigation of malarial immunity after a rollout of such an intervention will provide malaria-related data which would be used in planning and implementation of other interventions. Lastly, the knowledge from this study would add up to other findings which together will help policymakers in identifying threats to malaria control and elimination and suitably adjust their policies when required.

Organisation of the Study

This study is structured into five (5) chapters. The first chapter, chapter one provides the entire background of the study. The literature review forms chapter two of this study. This chapter mainly discusses malaria disease and its pathogenesis. It also discusses malarial immunity. The chapter three (3) presents the research methods employed in undertaking the study. The study design, study site, sample collection and analysis have all been explained in this section. The results of the study obtained and the discussion of major findings forms the core of the fourth chapter. The concluding chapter- chapter five (5) puts together the conclusion made from the study and also provides recommendations for further studies.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This section, reviewed a brief history of malaria, the *Plasmodium* parasite biology and the pathogenesis of the disease. The chapter further evaluates the various control measures or interventions that have been implemented to curb the devasting effects of the disease. The antigen of interest in this study was also reviewed as well as its role in malarial immunity. "PubMed", "Google Scholar", "PLOS" "Web of Science", etc. were the various databases used in conducting the literature review.

Brief History

Malaria, an ancient vector-borne protozoan infectious disease continues to torment human life from time past till now. The first identification of this parasite in the blood of infected patients by Laveran in 1880 marks the beginning of a new era for the parasite. Despite this great discovery, the incrimination of the mosquitoes as vectors for transmission of the parasites was not known until 1897 when it was revealed by Ronald Ross through the study of avian malaria. Human malaria was subsequently also discovered by Italian malariologists as reviews by (Francis EG Cox, 2010; Neghina et al., 2010). The stories leading to the understanding of the disease we have today juxtaposed with the strikes made over the years tell us how far we have come in dealing with this deadly disease. Despite the tremendous progress made in battling this debilitating disease, there seems to be an

unending fight against the disease, especially in tropical countries with stakeholders having more to do.

The Disease: Malaria's Burden

Globally, malaria is endemic in 84 countries and as reported by the WHO, this is a reduction in the 108 countries that were formerly endemic in the 2000s. It is projected that approximately half the world's population is at risk of malaria with most of such individuals situated in the tropical and subtropical regions (Choutos et al., 2023). Infants, children below 5 years, pregnant women, immuno-compromised individuals, non-immune immigrants as well as tourists are at a higher chance of developing the disease (del Prado et al., 2014).There were estimated 247 million cases of malaria globally in 2021 with its estimated accompanying death of 619, 000. Since 2016, malaria cases in the world have been steadily increasing. This situation was evidently seen between 2019 and 2020 which recorded a rise of 13 million in malaria cases as a result of disruption encountered due to the COVID-19 pandemic. Children below 5 years accounted for most malaria-associated death recorded (World Health Organization, 2022).

The global trends in malaria differ significantly according to WHO's malarious regions. The world is classified into six (6) malaria regions; Africa, Eastern Mediterranean, South East Asia, Western Pacific, European, and Americas region. The Africa region alone accounts for between 95-96% of malaria cases and deaths followed by South East Asia region which accounts for about 2% of world cases and deaths. The European region, since 2015 has recorded no malaria cases and no malaria-related death since 2000. Unlike the WHO Africa region where almost every country in the region bore the burden

of the disease, just a few countries in the other regions bear the burden of the region. For instance, in South East Asia region (which records approximately 5.4 million cases and 9000 deaths), India bears about 83% and 82% of the malaria cases and deaths respectively in South East Asia (Rahi & Sharma, 2022). Also, Papua New Guinea bears 87% of cases and 94% of death in Western Pacific region, whilst the Republic of Venezuela, Brazil and Columbia together bores the burden (79% of cases) of that of the WHO region of Americas (World Health Organization, 2022).

Though the amount spent in 2021 in controlling and elimination of malaria is about half (US$3.5 billion) the estimated amount required (US$7.3 billion) by the WHO, this amount still poses a significant financial burden. Of the amount spent which is mostly donations, 36% of this amount was donated by the United States of America, 33% from governments of endemic countries, with other countries and donors donating between 1% - 8% of the amount. The US$3.5 billion spent in 2021 is higher than the amount spent in 2019 (US$3.0 billion) and 2020 (US$3.3 billion) (Rannan-Eliya, 2022).

Globally, there have been some countries that have succeeded in recording zero malaria cases after several years of investment and commitment toward malaria. China, being the first country in the WHO Western Pacific region to eliminate malaria did so after 70 years of investment. This feat was achieved after several years of well-thought-out and executed plans (Feng et al., 2022; Zhang et al., 2018). The World Health Organization report about some countries submitting a request to be officially declared malaria-free which shows that countries across the world are striving so hard to achieve a similar feat (World Health Organization, 2019).

Out of the five (5) existing species known to cause infection in humans, more attention is paid to two of them; *P. falciparum* and *P. vivax*. The global distribution of human-pathogenic *Plasmodium* species shows how widely distributed *Plasmodium falciparum* is in the African region. Most cases of *P. vivax* infections are recorded outside the WHO Africa region. Of all the cases recorded outside the African region, approximately 72% were caused by *P. vivax* (World Health Organization, 2022).

In the African sub-region, the burden of malaria is devastating and it is known to account for about 9% of all diseases on the continent (Korzeniewski et al., 2021). The African region accounted for about 95-96% of global cases and deaths which primarily makes it the disease's hub. The continent has most of its countries been endemic to malaria. About 27 countries in Africa account for all the disease's cases and deaths. The most alarming situation for the continent is that more than half (55%) of the illnesses and fatalities are attributed to five (5) nations in the region with Nigeria leading the chart with 26.6% and 31.3% for cases and deaths respectively. Aside from Nigeria leading the chart for cases and death, the Democratic Republic of Congo, the United Republic of Tanzania and Niger also have a higher burden of the disease. The same story can be said for other countries in Central and Eastern Africa (Economist Intelligence Unit Limited, 2020).

For example, countries such as the Central African Republic and Chad record as high as about 75% and 61% malaria prevalence respectively in some communities (Nzoumbou-Boko et al., 2022). The alarming part of this situation is that most of the cases and deaths reported in the country according to WHO are mostly not the true representative of the situation on the ground.

Access to proper healthcare is one of the challenges faced by most countries in that part of Africa with some communities having to travel more than 24 hours to access the closest health facility (Korzeniewski et al., 2021). It will not be out of place for one to project that the situation is not so different from other African countries hence making the continent's contribution of about 95% of global cases and deaths not surprising.

Plasmodium falciparum is the predominant malaria species in SSA accounting for as much as 99.7% of infections in the region (Kojom & Singh, 2020). Cases of infection associated with *Plasmodium ovale, and Plasmodium malariae* mostly exist as a mixed infection with *Plasmodium falciparum* (Akala et al., 2021).

The African story of the malaria burden is not all doom and gloom as countries in the northern part of the continent are relatively doing well concerning malaria control and elimination. Algeria, Morocco and Mauritius have been declared malaria-free (Health Organization, 2016). These countries have consistently invested in the fight against diseases. Strategies such as free healthcare, effective and efficient workforce, early treatment and diagnosis, and effective preventive measures adopted by some of these countries have contributed significantly to their success story. Algeria for example has domestically funded its malaria intervention (World Health Organization, 2019). Some Southern African countries are also on track to the elimination of the disease (Moonasar et al., 2016).

Ghana is considered one of the endemic countries to malaria. Differences in ecological zones with varying seasons allow the disease to persist throughout the year. In other words, Ghana is considered a

mesoendemic country (Amoah et al., 2016). In 2021, the WHO indicates that Ghana contributed about 2.2% of the global cases representing approximately 5.4 million cases and about 12,000 deaths (World Health Organization, 2022). These numbers demonstrate the extent of the disease's burden in the country. It is not surprising Ghana falls under the top 15 most burdened countries in the world. Locally, the burden of the disease varies from one ecological zone to another due to seasonal variations in the country. Most of the burden of the disease in Ghana is borne by rural communities in the country (Savi et al., 2022).

With regards to the population at risk, the entire population in the country (about 33 million) are at risk of the disease with the greatest burden being on children below five (5) and pregnant women. The dominant malaria parasite is *P. falciparum* (96.3%), *P. ovale,* and *P. malariae* (1% - 4%) (Amoah et al., 2019).

The *Plasmodium* Parasite

Plasmodium, the causative organism for malaria belongs to the genus, *Plasmodium*. The *Plasmodium* parasite is considered one of the most successful groups of eukaryotic protozoan parasites considering its ability to infect a wide group of animals such as mammals, lizards and birds. As a member of the rank phylum *Apicomplexa*, these groups of parasites fall in the same group as some eukaryotic parasites of medical importance - *Toxoplasma, Theileria, Eimeria, Babesia, and Cryptosporidium*. Another characteristic feature of these groups is the ability to go through a complex life cycle which has contributed to their survival (Cowman & Crabb, 2006).

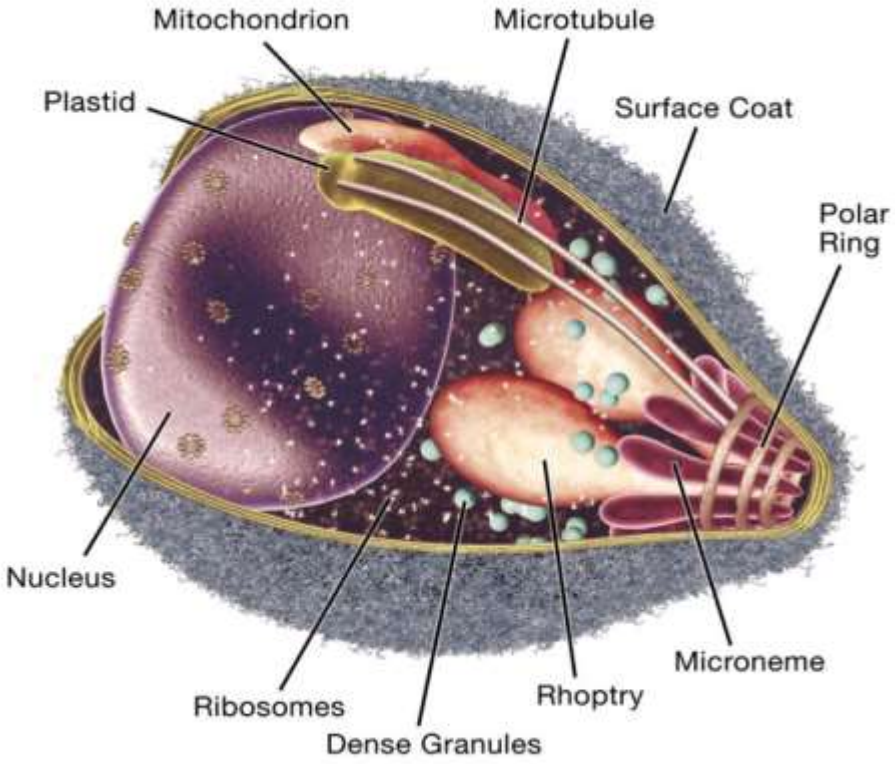

Figure 1: A representative structure of the *Plasmodium* parasite (taken from (Cowman & Crabb, 2006).

The *Plasmodium* parasite has an elliptical shape with a broader base at the posterior end and an apex region at the anterior end that forms the apical complex **(Figure 1)** which is used by the parasite to attach and penetrate the host cells (Guerra & Carruthers, 2017; Harding & Frischknecht, 2020). Approximately 156 species have been identified as of now. (*CDC - DPDx - Malaria*, n.d.).

Species such as *P. relictum, P. juxtanucleare* and *P. elongatum* infect birds (avian malaria) and are known to infect birds such as falcons, ducks, pigeons and penguins. Other species like *P. falciparum, P. gaboni and P. reichenowi* have been isolated in chimpanzees and gorillas and *P. floridense* and *P. mexicanum* have also been found in reptiles (*Avian Malaria | Bird Disease | Britannica*, n.d.). The three most relevant species that have been

widely utilized as model organisms for studying malaria extensively in mice are *P. berghei, P. chabaudi,* and *P. yoelii* (Stephens et al., 2012).

However, out of this number, only five (5) species of the *Plasmodium* parasites have been formally described to cause and establish infection in humans; *P. falciparum, P. malariae, P. ovale, P. vivax,* and *P. knowlesi* (Sato, 2021). *Plasmodium ovale* is widely distributed in West Africa with some being found in Asia. In contrast, *P. malariae is* widely distributed across the globe. While infection caused by *P. ovale* (*P. ovale wallikeri* and *P. ovale curtisi)* and *P. malariae* are asymptomatic, mild, and usually associated with low parasitaemia (Fuehrer et al., 2022; Goerdeler et al., 2021), *P. falciparum* and *P. vivax* infection are severe and pose the greatest threat - estimated to cause about 95% of infections (Garcia, 2010) in humans with the former accounting for more of the world's cases and death. The latter is predominantly notorious outside WHO African region accounting for most malaria cases recorded outside of Africa (Battle et al., 2019; Weiss et al., 2019).

The Life Cycle of the Parasite

General Overview

The *Plasmodium* parasite has a complicated life cycle that alternates between the anopheline mosquito vector and the vertebrate host **(Figure 2)** (e.g., human) (Tuteja, 2007). The female *Anopheles* mosquito delivers the infective form of the parasite-sporozoites into the dermis of the human host while taking bloodmeal. The sporozoites subsequently make their way into the liver via the circulation where they multiply asexually to form schizonts. Upon reaching maturation, the liver releases the merozoites into circulation which

go to infect RBCs (Prudêncio et al., 2006). In the RBCs, the parasite further undergoes maturation to release new merozoites which go to infect other uninfected RBCs. A limited proportion of these new merozoites differentiates into male and female gametocytes, generating the parasite's sexual forms (Talman et al., 2004) which is then picked up by the female *Anopheles* vector during a bloodmeal. The entire life cycle of the *Plasmodium* can be classified into 3 main stages; the pre-erythrocytic stage, the asexual/erythrocytic stage and the sexual/ post-erythrocytic stage (Antinori et al., 2012). The above stages are further described as follows.

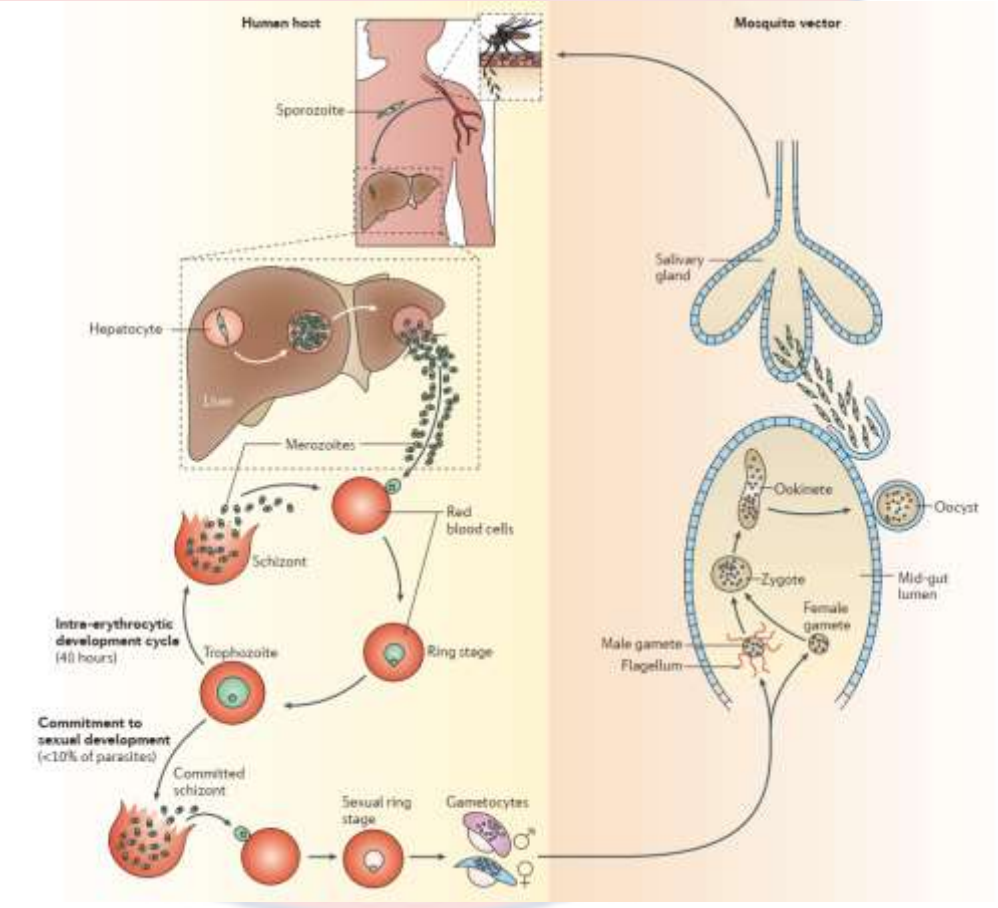

Figure 2: Life cycle of *Plasmodium falciparum* (taken from (Josling & Llinás, 2015)

Erythrocytic/exoerythrocytic stage

This stage, also known as hepatic invasion lasts for 2-7 days depending on the *Plasmodium* species and is termed the clinically silent stage of the malaria infection (Clemence et al., 2011). In other words, this stage is associated with no disease symptoms. This stage starts when an infected *Anopheline* vector inoculates the sporozoites (infective stage) from its salivary gland into the dermis of its host during a blood meal **(Figure 2)**. As reviewed by Graumanns *et al*, several studies suggest that only a small fraction (8 to 39 sporozoites i.e., approximately 1%) of the sporozoites are inoculated into the dermis. It is worth noting however that very few *Anopheles* mosquitoes, termed as high-injectors can inject more sporozoites, >100 sporozoites i.e., about 7 to 36% (Graumans et al., 2020). Once in the dermis, the sporozoites; an elongated actively motile cells by gliding motility move through the bloodstream and get into the liver infecting the hepatocytes which results in the formation of the parasitophorous vacuole **(Figure 2)**. According to some reviews, this process lasts between 30-60 minutes (Garcia, 2010; Prudêncio et al., 2006; van der Watt et al., 2022).

Once the final invasion is completed, each sporozoite develops and undergoes pre-erythrocytic multiplication (schizogony) in the hepatocyte resulting in the generation of several new uninucleate merozoites; a sporozoite is capable of releasing approximately 10^4 merozoites into circulation (van der Watt et al., 2022).

Asexual Erythrocytic Stage

This stage of the parasite's life cycle is usually associated with the clinical symptoms of the disease. Upon release of the merozoites from the

hepatocytes, the merozoites flood out into the blood and invade the erythrocytes.

Merozoite invasion of erythrocytes is based on interactions between particular receptors on the erythrocyte membrane and ligands on the merozoite surface. The entire invasion process occurs rapidly and happens in about 30s–60s (Cowman et al., 2016a; Fujioka & Aikawa, 2002). In a review by (Bannister & Mitchell, 2003a), the invasion process involves;

1) Adhesion and apical orientation; this involves primary attachment of the apical portion of the parasite to the RBCs.

2) Parasitophorous vacuole formation; after attachment, there is the formation of tight-junction leading to the gradual invagination of the parasite into the RBC. As this continues there is full engulfing of the parasite by the RBC resulting in the formation of a suitable environment for parasite growth (Cowman et al., 2016a).

3) Merozoite interiorization which involves the beginning of the formation of the invasion pits which is constant contact with the infected erythrocytes (Bannister & Mitchell, 2003b)

4) Dense granule release and merozoite transition to the ring stage. At this stage, they develop into trophozoites and subsequently into schizonts which burst to release merozoites upon maturation to initiate another replication cycle (Venugopal et al., 2020).

Sexual/ Post-erythrocytic Stage (Gametocytogenesis)

The mechanism that triggers this phase of the parasite's development is not clearly understood though it has been thought to be connected with the host and environmental stressors. The question remains why would some

portion of the parasite decide to commit to this stage of development? The epidemiological answer to this question is to ensure the constant availability of the parasites' progeny for propagation. At this stage, a few proportions (approximately 0.1 -5%) of the numerous asexual parasites released into circulation take the path to form non-replicating sexual gametes. The development into micro and macro gametes only occur in infected erythrocyte located in the spleen and bone marrow (Fujioka & Aikawa, 2002). In the case of *P. falciparum,* gametocyte development takes about approximately 8-12 days after which the microgamete and macrogamete are picked up by the *Anopheles* mosquito which then undergoes the sexual phase of the life cycle **(Figure 2)**. After fertilization, a motile zygote known as the ookinete is formed within the lumen of the mosquito gut where it undergoes development and maturation (Whitten et al., 2006) . The ookinete then travels through the midgut from the apical side to the basal lamina which then later transforms into oocysts. Upon maturation, the oocyst results in the development of sporozoites which then travel to the salivary glands of a mosquito (Matuschewski, 2006) and are injected into a new host when the mosquito feeds.

Pathogenesis of the Disease

The severity of malaria infection depends on several parasite and host factors. For instance, despite the very high number of recorded cases, relatively low numbers result in diseases which are attributed to the partial immunity acquired by people in endemic areas. Parasite inoculation into the unsuspecting human host by an infected anopheline mosquito marks the onset of malaria (Silvie et al., 2008). However, the clinical manifestation of the

disease is not shown until the parasite enters the blood stage of its life cycle which is mostly characterized by the invasion and distraction of the host's RBCs. The huge periodic amplification of the parasite population at this stage also enhances the severity of the disease (Miller et al., 1994). For every replication cycle, there is the release of new merozoites to reinfect new erythrocytes and this is accompanied by haemolysis of *Plasmodium* spp.-infected erythrocytes and malarial endotoxins thus resulting in its associated chills and fevers. Though fever seems to be a symptom of most illnesses or diseases, malarial fever has somewhat distinctive (i.e., cyclical and predictable) characteristics (Oakley et al., 2011). Fever associated with *P. falciparum* infection is usually experienced every 24 hours (quotidian) (King K. Holmes, Stefano Bertozzi, Bary R. Bloom, 2010), *P. ovale* and *P. vivax* fevers are usually experienced every 48 hours (tertian) and that of *P. malariae* every 72 hours (quartan). Some patients may sometimes complain of headaches and abdominal discomforts and can suffer from diarrhoea. Children may also experience some form of vomiting (Mawson, 2013).

The initial symptoms of malaria are mostly not life-threatening and thus usually classified as uncomplicated malaria. The progressive ability of this disease especially infection with *P. falciparum* makes it more complicated and deadly due to the parasite's capacity to evade the immune system of the host. Upon invasion of the RBCs, the parasites alter the infected RBCs by the insertion of some parasite-derived proteins, e.g., *P. falciparum* erythrocyte membrane protein-1 (*Pf*EMP1) into and unto the membrane of the infected erythrocytes (IEs) (Emile et al., 2012). *Plasmodium falciparum* erythrocyte membrane protein-1 (*Pf*EMP1), a highly diversified protein once on the

surface of the IEs, the parasite switches to antigenically distinct isoforms resulting in immune evasion by the parasite (as reviewed by (Cowman et al., 2016a). The expression of a specific variant of the *Pf*EMP1 mediates cytoadhesion which leads to the sequestration of the IEs in the microvasculature thus enhancing the parasites' ability to avoid clearance by the spleen. The obstruction of blood circulation at the capillary level disturbed tissue perfusion, and hypoxia results in the various complications associated with the disease (Helms et al., 2016; Smith et al., 2013).

Malaria Control; Successes, Challenges and the Way Forward

The benefits of malaria control especially in the 19th and 20th centuries can never be overlooked. Over a billion cases and millions of deaths (WHO, 2020) averted due to the various malaria control measures implemented. It is evident that these control measures are effective. For this reason, these measures must be continued with few and or necessary modifications that is capable of ensuring the malaria elimination and eradication agenda. These control measures have been of great benefits to the people leaving in Europe and North America in the mid-nineties in eradicating malaria from the region. The use of dichlorodiphenyltrichloroethane (DDT) in the mid-nineties, to control the vectors' breeding grounds was very effective and was later abandoned after cost and environmental concerns were raised in the 1970s (Hammami, 2014; M. A. Phillips et al., 2017). Various control measures have been employed since the launch of the Global Malaria Action Plan (GMAP) in partnership with Roll Back Malaria and Millennium Development Goals (as reviewed (King K. Holmes, Stefano Bertozzi, Bary R. Bloom, 2010) which could be classified into two main groups-preventive and treatment modules

(Desai et al., 2020). The preventive module consists mainly of control of vectors; insecticide-treated nets (ITNs) and indoor residual spraying (IRS). Chemoprophylaxis, intermittent preventive treatment in pregnancy (IPTp) and or in infants (IPTi), seasonal malaria chemoprevention (SMC) can also be classified under preventive. Mass drug administration (MDA), mass testing and treating (MTaT) and mass testing, treatment and tracking (MTTT) forms the treatment module.

Indoor Residual Spraying (IRS)

Indoor residual spraying (IRS) encompasses spraying of a lasting insecticide (the use of DDT at the initial stages) on the walls and other surfaces in a house to kill mosquitoes and other insects that settles on the sprayed wall after feeding making it more effective against endophilic mosquitoes due to their indoor biting and resting behaviours thereby preventing transmission of infection to other persons. Depending on the kind of chemical formulation and the malaria transmission period, spraying is usually done between once and three times in a year. About 16 formulations grouped into five main classes (carbamates, organochlorines, organophosphates, pyrethroids and neonicotinoids) are used in IRS. Dichlorodiphenyltrichloroethane (DDT) wettable powder (WP), fenitrothion WP, malathion WP, pirimiphos-methyl WP, bendiocarb WP, propoxur WP, WP in sealed water-soluble (WP-SB) and emulsifiable concentrate (EC), suspension concentrate (SC) are some examples of the WHO recommended insecticides or formulation used in IRS (Suuron et al., 2020; Tangena et al., 2020).

This malaria control tool was very effective in the early stages of malaria control and elimination until concern about the use of DDT was raised. The use of DDT in the early days of IRS led to the complete elimination of malaria in 37 countries in the 1970s, especially in Europe and the Americas (Tangena et al., 2020).

The progress made in the early days of IRS was evident and thus recommended by WHO to other countries, especially in SSA. There have been significant improvements in IRS coverage in many countries over the years. For instance, IRS coverage in SSA was 26% in 1997 but has increased to about 63% by 2017 (Tangena et al., 2020). The increase in IRS coverage has significantly resulted in reducing disease's incidence in some SSA countries. Indoor residual spraying (IRS) played a major role in the decline of cases in Southern Africa. A study in Uganda cited by Nankabirwa *et al* reports more than 80% reduction in malaria incidence in 5 districts after 4-5 years of implementation of this method (Nankabirwa et al., 2022). A study in Western Kenya also reports a reduction in OPD cases when IRS was used together with ITNs (Dulacha et al., 2022).

As part of the strategic plan for malaria control (2008–2015), The Ghana NMCP in 2009 employed IRS method as part of its vector control strategy to lessen the burden of the disease, more so in regions with high *P. falciparum* transmission (MOH/GHS/NMCP, 2008). A decrease in the transmission of malaria is the aim of IRS. A study in some districts in Northern Ghana has reported a decrease in transmission when IRS was employed and saw an increase when it was withdrawn (Coleman et al., 2017).

Another also reports a decrease in self-reporting malaria related incidence among women within the reproductive age bracket (Alhassan et al., 2022).

The use of chemicals against biological agents comes with some associated challenges. The reported cases of resistance to some of the insecticides used in IRS have been the main challenge. To curb this issue, other insecticides were introduced. However, these chemicals were expensive hence the inability to sustain this programme (CDC – Centers for Disease Control and Prevention, 2019a). With recent reports on the effectiveness of the IRS (Wagman et al., 2021; Zhou et al., 2022), many countries especially the endemic once are revisiting this control method.

Insecticide Treated Nets (ITNs)

Insecticide treated nets (ITNs) usage is one of the approaches employed as a means of reducing transmission. These nets are made of fine fibres woven together and impregnated with the insecticide pyrethroid thus serving two main purposes a physical barrier and a toxic substance to the vectors. As cited by (Lindsay et al., 1989), results from studies conducted in different areas have shown a significant reduction in the biting rate of the Anopheles mosquitoes. Another major benefit of using ITNs is the reduction in clinical malaria cases. A study also showed that in areas (i.e., rooms having ITNs) where ITNs were used, there was a significant reduction in mosquito numbers as compared to areas without them. This study also demonstrated that there was about 83-92% reduction in biting on men by mosquitoes (Lindsay et al., 1989). Countries in SSA have adopted the use of ITNs as part of their malaria control interventions. Several countries have reported mass distribution of ITNs to their inhabitants (Fru et al., 2021; Masaninga et al.,

2018; Scates et al., 2020; Singh et al., 2013; van Bortel et al., 2022). Reports indicate that the use of ITNs is a contributory factor for the 68% of the 600 million case reductions between 2000 and 2015 (Van Bortel et al., 2022). Consequently, ITNs usage has been highly recommended for pregnant women and children below five. This has resulted in preventing infection during pregnancy and decreasing its associated complications. For instance, there is a strong correlation between the use of ITNs and a decline in the prevalence gestational malaria and anaemia. According to the data, using ITNs is strongly associated with a decrease in stillbirths, better baby birth weights, and a decline in the prevalence of parasitaemia and anemia in expectant mothers (Singh et al., 2013).

Ghana as a country has also rolled out several mass distributions of ITNs since 2000. The Ghana National Malaria Control Programme (NMCP) now National Malaria Elimination Programme (NMEP) has distributed 12.5 million ITNs within two years. The NMCP further made a distribution of 15.5 million ITNs in 2018. The ultimate goal of this massive distribution is to ensure over 90% of the total targeted population get access to treated nets as it is believed that a large population usage of ITNs has the propensity to decrease the burden of malaria in the general population (Afagbedzi et al., 2022; National Malaria Control Programme, 2013).

Despite the success of this method of controlling the vector, there are still some challenges -the major challenge being vectors becoming resistive to the chemicals used in treating these nets. Another challenge is mainly associated with human behaviour. Some have reported the discomfort (i.e., heat) associated with the use of the ITNs (Watanabe et al., 2014). Some have

reported some reactions they experience after sleeping in the net and many other reasons (Koenker et al., 2023). These reasons have contributed to the general usage of ITNs. According to Ricotta *et al* more than 50% of homes in SSA own at least a treated net and not less than 30 % of such homes have reported sleeping in it the night before the survey (Ricotta et al., 2019). Though these values may be in the past, the disparity in net usage and ownership is alarming and maybe a true reflection of the current situation. To circumvent the challenge of resistant vectors, new insecticides such as alpha cypermethrin, bifentrin, clothianidin 50%, bendiocarb, deltamethrin (The Global Fund, 2023) are being used in treating the nets by making some additions to the existing ones. Also, with the behavioural part, more public education is been rolled out.

Chemoprophylaxis

Chemoprophylaxis is still one of the most important therapeutic strategies for reducing the severity and mortality associated with malaria. This mode of malaria prevention is mainly employed by travellers moving from non-endemic areas to endemic areas. Though personal protection may serve as an important tool, it is not sufficient. There are two (2) types of malaria chemoprophylaxis- blood-stage prophylaxis and Liver stage prophylaxis (Schwartz, 2012).

The use of chemoprophylaxis especially in travellers has always been a challenge. The benefits of developing severe malaria have to be weighed against the effects of using the drug. This situation becomes dicier when travellers are moving to low transmission settings. Also, the issue of

continuing with drug use for some weeks even after returning from such trips further complicates the issue (Chaves et al., 2017).

Chemoprophylaxis usage in an endemic population in the form of MDA comes with its challenges (especially drug resistance) hence various modifications have been adopted.

Intermittent Preventive Treatment in Pregnancy (IPTp)

It is a fact that pregnant women are one of the groups that bear the brunt of malaria diseases. Aside from the risk of death, the adverse effects on both mother and child are a great burden. Maternal anaemia, premature delivery and delivery of low-birth infants, just to mention a few are all associated with malaria in pregnancy (CDC - Centers for Disease Control and Prevention, 2019b). This strategy includes the administration of a full treatment dose of an antimalarial drug (currently sulfadoxine-pyrimethamine) to pregnant women when they come for antenatal care (ANC) at the clinic, regardless of whether the expecting woman is infected with malaria or not.

The WHO recommends the intake of IPTp-SP at every ANC visit starting as early as the second trimester. As cited by Anto *et al*, doses should be at least one month apart to receive a minimum of four doses before delivery (Anto et al., 2019).

Studies have shown that women who receive a minimum of 3 doses before delivery have better delivery outcomes than women with less than 3 doses (Anto et al., 2019; Dosoo et al., 2021). The major challenge with this method has got to do with the willingness of pregnant women to attend ANC as it has been found that people with high education backgrounds and those in

healthy marriages are more likely to follow through till delivery (Anto et al., 2019).

Intermittent Preventive Treatment in Infants (IPTi)

Intermittent preventive treatments in infants (IPTi) is the provision of preventive antimalarial drugs of sulfadoxine-pyrimethamine (SP) to infants at intervals corresponding to routine vaccination visits. This is given to infants in moderate to high transmission areas regardless of their malaria infection status (CDC - Centers for Disease Control and Prevention, 2019b). Like the IPTp, the ultimate goal is to protect the child from clinical malaria. According to a systematic review, the general impact of IPTi is a close to 30% reduction in the incidents of malaria in infants (Esu et al., 2019). Though IPTi is an effective way of reducing morbidity and mortality related to malaria, there have been concerns raised about drug resistance and naturally acquired immunity (O'Meara et al., 2005).

Seasonal Malaria Chemoprevention (SMC)

Formerly referred to as Intermittent Preventive Treatment in children (IPTc), SMC is defined as the intermittent administration of full treatment doses of an antimalarial drug during malaria transmission periods to prevent illness due to malaria and to maintain effective therapeutic concentrations in the blood throughout the transmission period where there is a higher risk of infection. Children aged five (5) years and below are the primary focus of this mode of malaria prevention. This control method is safe, effective, cost-effective, and generally feasible in communities with high seasonal malaria transmission (World Health Organization, 2012).

Despite SMC's success, in reducing the burden of malaria in areas of high transmission, it still comes with challenges which include; logistical burden, the use of a course of medication lasting 3 days and limited inclusion of areas that are equally qualified for SMC (Coldiron et al., 2017).

Mass Testing, Treatment, and Tracking (MTTT)

Mass testing, treatment, and tracking (MTTT) or Mass screening and treatment (MSAT) is a variation or a modified form of MDA. Other forms of MDA include Focal screening and treatment (FSaT), Mass fever treatment (MFT). These methods are employed based on the geographic area such as smaller villages, households or hot spots (CDC - Centers for Disease Control and Prevention, 2019b). Unlike MDA where the entire population is treated irrespective of one's malaria infection status, MTTT always tests the entire population using RDTs before treating those confirmed as positive for malaria.

While some studies have shown a decrease in the prevalence and or incidence of malaria cases (Larsen et al., 2015), other studies show otherwise (Cook et al., 2015; Halliday et al., 2014; Tiono et al., 2013). With MTTT, the challenges associated with it are; the use of RDTs for screening which may not be able to detect very low-level parasitemia, missing possible asymptomatic reservoirs. Also, study participants' compliance to the medications given since these people are not actively monitored in taking the medications (Cook et al., 2015).

Immunity to the Parasite

Over the years, the complexity surrounding malarial immunity has been unfolding gradually. Though there is a broad understanding of immunity

to malaria, especially concerning those in the endemic area. There is still more to do to ensure a complete understanding of malarial immunity.

One key contributor to this complexity is the *Plasmodium* parasite. The long list of various proteins expressed by the parasite at each specified phase throughout its entire life cycle is evidenced enough of how the body's immune system (IS) is going to react to most if not all of these antigenic proteins expressed by the parasite. Coupled with the production of these numerous proteins are the cunning ways adopted by the parasite to evade the IS of its host.

Innate Immunity and the *Plasmodium*

The innate immune response as the primary line of defence against any invading pathogen places a critical role in protecting the body. In the case of the *Plasmodium*, this part of the immune system influences the disease's progression and immunity building. The clinically silent nature of the parasite at the liver stage created the impression that the parasite goes undetected by the IS, however recent studies in mice have shown that the developing parasites are recognized by the pathogen-recognition receptors (PRRs) of the liver cells thus inciting antiparasitic type 1 interferon response (Gowda & Wu, 2018).

Some reviews have also implicated the skin as playing a role in immunity towards the *Plasmodium* sporozoites. The barrier function performed by the skin thus requiring the sporozoites to traverse cells before exiting the skin is evidence of its importance. In so doing this also limit the number of sporozoites that gets to the liver. These immobilized sporozoites in the skin are later eliminated by the phagocytes. Additionally, a large number

of dendritic cells on the skin is likely to interact with the parasite thus presenting it to the T cells (Osii et al., 2020; Sinnis & Zavala, 2012). It should also be noted that the evasive ability of the *Plasmodium* parasite to the IS has somewhat rendered the dendritic cells (DCs) ineffective towards the parasite. This also contributes to the limited understanding of the IS with regard to dealing with *Plasmodium* infection. Aside from the skin, the lymphatic system also clears some sporozoites to the lymph nodes which serve as a primer for the activation of T- cell responses (Clemence et al., 2011).

Adaptive Immunity – Antibody-mediated

Antibody production towards *Plasmodium* parasite antigen has been established to contribute significantly to malaria immunity. Several early studies in both human and animal models show that passive serum transfer from immune individuals to malaria-naïve recipients leads to a reduction in parasitemia and disease (Cohen1961, n.d.) (Narum et al., 2000; Valero et al., 1998). Whilst most malaria antibodies produced are towards the erythrocytic stage of the parasite, high titres of antibodies produced against the sporozoite antigen, circumsporozoite protein (CSP) have also shown to be of neutralizing effect hence impeding the sporozoites' ability to infect the hepatocytes (Clemence et al., 2011).

Though different isotypes of antibodies are produced, immunoglobulin G (IgG) is the most important isotype when it comes to malaria infection. These immunoglobulins may either be polyclonal or specific (Perlmann et al., 2002). Of the IgG isotype, IgG1 and IgG3 subtypes are the most dominant in malaria-infected individuals (Hviid et al., 2022; Villasis et al., 2012). Additionally, in vitro experiments have identified antibodies from

subjects with a higher quantity of IgG1 to IgG3 ratio have the most efficient neutralizing effects on parasites. Antibodies can provide protection against malaria through one or two of these mechanisms; inhibition of erythrocyte invasion by merozoites, inhibition of intra-erythrocytic growth and enhanced clearance of infected erythrocyte through binding to their surfaces hence promoting elimination by the spleen and also preventing the parasites' ability to sequester in small vessels (Perlmann et al., 2002). Opsonization of infected RBCs also increases their predisposition to phagocytosis and cytotoxicity. These opsonized infected erythrocytes also interact with effector cells (neutrophils, monocytes/macrophages) which causes the release of TNF which are toxic to the parasite thus ensuring parasite clearance (Chua et al., 2021).

Despite the effectiveness of antibodies protecting infected persons, antigenic variation of the parasite greatly affects this ability. In cases where an infected individual with a particular strain of the parasite is exposed to a different strain, the individual may come down with the disease (Cowman et al., 2016b).

Erythrocyte Binding Antigen (EBA) Family

Erythrocyte Binding Antigen (EBA), an invasion ligand is a member of the erythrocyte binding-like (EBL) family of proteins that has members in both *Plasmodium falciparum* and *Plasmodium vivax* (Salinas et al., 2019). Erythrocyte binding-like (EBL) homologues includes the Duffy-Binding Proteins (DBP) found in *P. vivax* and *P. knowlesi.* At least six (6) members of the EBAs found in *P. falciparum* have been identified; namely EBA-175, EBA-140, EBA-181, EBA-165, MAEBL AND EBL-1. These proteins generally have been involved in erythrocyte receptor recognition or

contributing alternatively in the invasion process (Iyer et al., 2007; John H. Adams, 2001; Vera-Bravo et al., 2005).

Erythrocyte Binding Antigen-175 (EBA-175)

Plasmodium falciparum Erythrocyte Binding Antigen-175 (*Pf*EBA-175) is a 175 kDa erythrocyte-binding protein situated in the apical micronemes of merozoites. The gene for this protein is found on chromosome 7 and it is encoded by 1575 amino acids (John H. Adams, 2001). Its primary protein structure is divided into 7 major domains **(Figure 3)**, labelled as regions I to VII (Badiane et al., 2013).

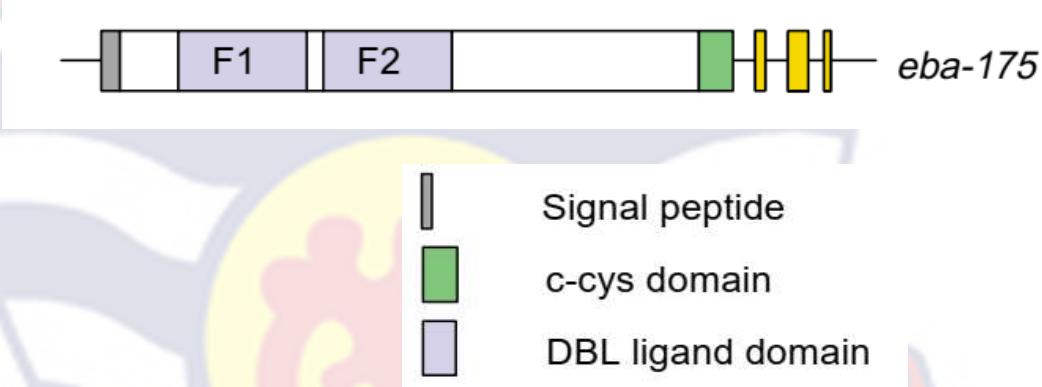

Figure 3: Protein structure of EBA-175 of *Plasmodium falciparum* (taken from (John H. Adams, 2001)

They consist of N-terminal, a long extracellular hydrophilic cysteine-rich domain (Region II), a C-terminal, a short cytoplasmic cysteine-rich domain (Region VI), highly conserved domain (Region III-V), and transmembrane and cytoplasmic domains (Carrillo et al., 1997).

Plasmodium falciparum Erythrocyte Binding Antigen-175 (*Pf*EBA-175) facilitates parasite invasion of host erythrocytes in a sialic acid-dependent manner through attachment of the Duffy-binding-like (DBL) domains in its extracellular domain on the *O*-linked glycans of glycophorin-A (Wanaguru *et*

al., 2013). This pathway has been thought to be the main ligand facilitating invasion however other studies have demonstrated otherwise (Duraisingh et al., 2003; Malpede et al., 2013).

Chapter Summary

This chapter reviewed existing literature about the progressed made from the discovery of malaria to the current understanding of the disease. The parasite's biology was also described in this section. The burden posed by malaria on the African sub region was expounded lying bear the associated efforts been made by the continent. It also further explored the various control measured that have been used in curbing the threat posed by malaria to the population at risk of the disease. Additionally, the chapter explained some challenges associated with the malaria control and how these challenges were somewhat contained. The antigen of interest and the role it plays on immunity was discussed.

CHAPTER THREE

METHODOLOGY

Introduction

This longitudinal study was carried out from September 2020 to September 2021 at Obom, a suburban area in the Ga South Municipal Assembly of the Greater Accra Region, Ghana. This was a random sampling of participants between the ages of 6 months and 90 years. Samples were taken at quarterly intervals during the 13-month period. Genomic DNA was extracted for parasite density estimation using PCR and antibody titres against *Pf* EBA-175 was measured using ELISA. Graph pad Prism (version 9.0.0) was used to analyze the data obtained. This current chapter provides details of the research design employed, study site, study population, sampling procedure, processing, and data processing and analysis. Additionally, the criteria for exclusion and inclusion for the study are well-defined and explained. The ethical issues concerning this study are also outlined in this chapter and a chapter summary.

Research Design

This is a 13-month longitudinal study conducted from September 2020 to September 2021 where an average of 1200 participants were sampled at four time points (quarterly intervals) after providing written informed consent. Out of this number, 314 of the participants present at all four visits were selected for this study.

Ethical Considerations

This study forms part of a broader study approved by the Institutional Review Board of the Noguchi Memorial Institute for Medical Research

(Reference number 077/19-20). Meetings were also held with the community leaders to seek their approval and explain the study's objectives to them. Individual written informed consent was obtained from the adults and the parents/guardians of children before enrolment. Assent was also administered to minors between the ages of 12 years and 17 years.

Study area and Population

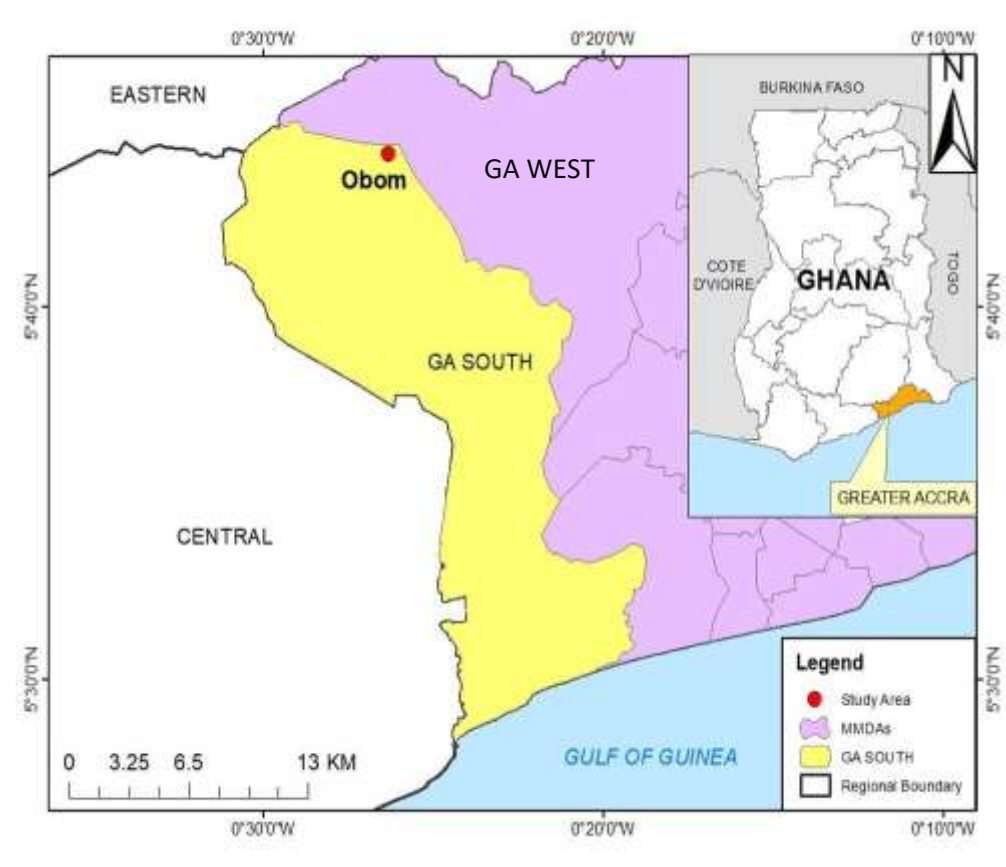

Figure 4: Map of the study site in the Ga South Municipality

Obom (05˚34' N, 0˚ 20' W), a suburban community in the Ga South Municipal of the Greater Accra Region, Ghana **(Figure 4)**, and also situated in the Coastal Savannah belt with annual average rainfall between 790 mm to 1270 mm. It is about 37 km away from the nation's capital, Accra. It has a health centre that serves as the primary health care provider to the about 2500 inhabitants and some surrounding communities. The community is considered

endemic to malaria where malaria transmission is perennial but peaks during the rainy season between June and August. Parasite prevalence by microscopy is estimated to be about 35% (Amoah et al., 2016). Over the years, some malaria control interventions have been carried out in this community and a decline in parasite prevalence has been reported (Ayanful-Torgby et al., 2018). The major economic activity of the inhabitants is mostly farming with a few engaging in petty trading.

Sampling Procedure

As a community survey, all individuals residing in the study area who were present and willing to partake in the study were enrolled before the sample collection date. On the day of enrolment, participants were requested to provide basic demographic information (i.e., name, age, sex,.) after consent has been obtained. Study participants were assigned unique identification codes after registration. Sampling was done at four-time points; September 2020, January 2021, May 2021, and September 2021. An average total of 1200 participants were sampled during the study period. However, this particular study selected participants that were present at all four sampling time points, a total of 314 participants.

Inclusion Criteria

Individuals residing in the community at the time of sampling and were above the age of 6 months were recruited and were present for all four sample collection time points.

Exclusion Criteria

Individuals who were absent for at least one sampling time point.

Sample Collection

Sampling was in September 2020, January 2021, May 2021, and September 2021. Samples were obtained in the morning or late afternoon by trained community volunteers.

First, the body temperature of the study participant was measured using an infrared thermometer gun and recorded. Finger-prick peripheral blood was subsequently collected from each individual. From this, about 5 µL was used for RDT testing for *P. falciparum*. The result of the RDT test was read after 15 minutes.

Disclaimer: The RDT results for this study were undisclosed and thus there is no access to the data. The parasite prevalence data used in this study is based on PCR results.

In addition, 10 µL of the finger-pricked blood of children aged 15 and below was used to determine haemoglobin levels using the Urit 12 haemoglobin meter (Guangzhou Medsinglong Medical Equipment Co., Ltd., China). About 150 µL was also spotted on the Whatman grade 3 filter paper (Whatman, Maidstone, UK). Filter paper blood spots were allowed to air dry for about 2–3 minutes and were placed in each bag with a desiccant which was later stored at 4 ^{o}C before processing.

LABORATORY PROCEDURES

Extraction Parasite's DNA

Genomic DNA was extracted from two 3 mm punches of DBS using the Tween-Chelex extraction protocol with slight modifications (Amoah et al., 2016; Obboh et al., 2020). Briefly described; For each sample, two (2) punches of about 3 mm in diameter of blood-stained filter paper were punched

into a sterile pre-labelled 1.5 mL centrifuge tube. A 1000 µL of 0.5% Tween-20/PBS solution was aliquoted unto the samples and incubated overnight at room temperature on a shaker (Thermo Scientific, USA). After the overnight incubation, the samples were vortexed and spun at 14,000 rpm for 2 minutes and the reddish supernatant was discarded. The samples were then washed with 1000 µL of 1X PBS, vortexed to aid a uniform mixture, and incubated for 30 minutes at 4^{0} C. After the 30-minute incubation, the samples were spun at 14,000 rpm for 2 minutes. Finally, 50 µL of 20% Chelex (Sigma-Aldrich, USA) in DNA/RNA free water and 100 µL of DNA/RNA free water were aliquoted unto the samples and incubated at 95 °C for 10 minutes, with vortexing at 2 minutes intervals. After the heating stage, final high-speed centrifugation at 14,000 rpm for 8 minutes was done and the DNA-containing supernatant was picked into clean sterile well-labelled tubes (excluding the Chelex) and stored at −20 °C until used for PCR.

***Plasmodium falciparum* Identification by PET-PCR**

The presence of the parasite was detected using the real-time PET-PCR method as described by (Lucchi et al., 2014) with few modifications. The amplification of 18s rRNA *Plasmodium* gene and r364 target *Plasmodium* falciparum multiplex reaction was performed using QuantStudio™ 3 and 7500 Fast thermocyclers (Applied BioSystems) in 15 µL reaction volume containing a 2X TaqMan environmental master mix 2.0 (Applied BioSystems), nucleus free water, 250 nM of each of the genus forward and reverse primers, the falciparum forward primer, and 125 nM of the *P. falciparum* reverse primer, 2 µL of template DNA. Both reverse primers were labelled with FAM and HEX for the *Plasmodium* genus and *Plasmodium*

falciparum respectively. The PCR cycling conditions used for these reactions were; 95 ^{o}C for both initial and cyclic denaturation for 15 minutes and 30 seconds respectively, 63 ^{o}C annealing for 40 seconds, and 72 ^{o}C extension for 30 seconds, with a total of 45 cycles per run. A 1:10 serial dilution of *P. falciparum* (NF54) DNA with a known concentration, which also serves as the positive control was added to each reaction plate to generate a standard curve which was used to quantify the *P. falciparum* in each sample DNA.

Elution of Antibodies from DBS

Antibodies were eluded from a single 3 mm punch of dried blood blots using 0.05% Sodium Azide /PBS solution as described by Baidjoe *et al*, with few modifications (Baidjoe et al., 2013). Briefly, a single punch of each DBS sample was transferred into 2.0 mL 96-wells deep well plates (Greiner Bio-One GmbH, Germany), 336 µL of 0.05% sodium azide/PBS solution was added to each sample in the well and incubated overnight on a plate shaker (Thermo Scientific, USA). The reconstituted eluate, containing antibodies with an equivalent final serum dilution of 1:100 (Corran et al., 2008) was used for ELISA the following day.

Enzyme-linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) for total IgG against *Pf*EBA-175 was performed as described (Mccarra et al., 2012; Sennang et al., 2014) with few modifications (Abagna et al., 2018a). Briefly, the antibody activity against total IgG was measured as follows: A, 96-well flat bottom plates (F96 MAXISORP NUNC-immuno plate, Denmark) were coated with *P. falciparum* recombinant P*f*EBA-175 antigen in coating buffer (PBS; pH 7.4). The plates were sealed and an overnight incubation was performed at 4 ^{o}C.

The following day, plates were washed 3 times with PBST (PBS with 0.05% v/v Tween 20) and blocked with 3% skimmed milk (MARVEL, UK). The setup was then incubated at 37 ^{0}C for 1 hour. After an hour, the plates were washed and samples containing antibodies eluded from BDS with an estimated final serum dilution of 1:100 were pipetted into wells in duplicate. Each plate also had empty wells as the blank wells (contain just the diluent) and a positive control serial dilutions (sera from hyperimmune individuals) and negative control (sera from naïve individuals). The setup was incubated for an hour at 37 ^{0}C. The plate was then washed 3 times after an hour and goat anti-human IgG diluted (Invitrogen, USA) in 1% skimmed milk with a final dilution factor of 1:3000 was added and the setup was further incubated for an hour at 37 ^{0}C. 3-3'-tetramethylbenzidine (TMB) (Kem-En-Tek Diagnostics) was added to the plate after washing 3 times. The setup was incubated for 10 minutes based on optimization. The reaction was stopped using sulphuric acid (H_2SO_4). Optical densities (OD) for each well were read immediately at 450 nm using a plate reader (Thermo Scientific MULTISKAN FC, USA).

Data Management

The primary data collected from participants which included their demographics and results generated on the field and in the laboratory were stored and organized in Microsoft Excel.

Data Analysis

For the PET-PCR, each sample was scored positive for *P. falciparum* if the DNA concentration (quantity) is greater than 0.0002 ng/µL (usually above the concentration of the last standard) and cycle thresholds (CT) below forty (40) with all those with CT forty (40) and above were scored as negative.

All analyses were statistically performed using the Mann-Whitney unpaired two-tailed t-test and Kruskal-Wallis one-way analysis of variance (GraphPad Prism v9.0) for comparing various age groups and rounds of MTTT. The results were expressed at means and SDs. Categorical variables were summarized as percentages. Optical Densities (ODs) were converted to concentrations in arbitrary units (AU) using ©ADAMSEL. Tests results obtained were considered statistically significant when *P*-values were < 0.05.

Chapter Summary

As explained above in details, the study- a longitudinal was carried out in Obom in the Ga south district of the greater Accra Region of Ghana **(Figure 4)** between a 13-months period that spans from September 2020 to September 2021. The inclusion and exclusion criteria for this study was clearly stated and duly followed. Genomic DNA was extracted and DBS elution was performed on the samples collected from these willing participants after consent. The data was collated and analysed using Graph pad Prism (version 9.0.0). All results with p-values less than 0.005 were considered statistically significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

This study sought to examine the antibody response to a blood-stage antigen; *Pf*EBA-175 after a one-year MTTT intervention in an endemic area. We studied the antibody levels to *Pf*EBA-175 antigen and *Plasmodium falciparum* infection from blood samples of people living in the endemic areas after a malaria control intervention was rolled out. The data from these parameters were compared with one another to see if there is an association. Furthermore, data from the different time points in the course of the study was also presented. All associations were considered statistically significant if *P*-value was less than 0.05 for all analyses performed. The chapter further provides possible explanations for the observations made in the study.

RESULTS

Demographics of the study population

The study used a total of 314 samples from participants between the ages of 7 months and 90 years that were available for all four sampling time points. This number consists of 123 (39.2%) males and 191 (60.8%) females. The age group 16+ years makes up the majority of the study participants representing 135 (43.0%), followed by 5 – 9 years (22.0%) and then 10 – 15 years (19.1%) with 0 – 4 years (15.9%) representing the least group. The mean age of the entire study population was 22.1±20.9 years **(Table 1).**

Table 1: Demographic characteristics of Study Participants

Age Group	**Age**		**Males (N)**	**Females (N)**	**Total (%)**
(years)	**Mean**	**SD**			
0 – 4	2.7	1.1	29	21	50 (15.9)
5 – 9	6.6	1.5	34	35	69 (22.0)
10 – 15	12.4	1.6	28	32	60 (19.1)
16+ y	41.5	18.3	32	103	135 (43.0)
General (%)	22.1	20.9	123	191	314

SD = Standard Deviation, N = number

Clinical characteristics of the study population

Table 2 represents some clinical parameters (temperature and haemoglobin) of study participants across the entire sampling time points. The mean temperature of the study participants was found to be 36.3^0C, 35.9^0C, 36.4^0C and 36.3^0C respectively for visit 1 (September 2020), visit 2 (January 2021), visit 3 (May 2021) and visit 4 (September 2021). The mean haemoglobin (Hb) concentration for participants of the various age category has been shown in **Table 2**.

Table 2 Clinical characteristics of the study population

Parameter		September 2020	January 2021	May 2021	September 2021
Temperature (^{0}C)					
	Mean	36.3	36.1	36.4	36.3
	SD	0.4	0.8	0.4	0.4
	Range	34.9 – 37.7	34.0 – 38.1	35.0 – 37.8	35.0 – 38.0
Haemoglobin (g/dL)					
0 – 4 years		**Normal range (10.0g/dL – 13.0g/dL)**			
	Mean	9.8	9.8	9.4	9.6
	SD	1.8	1.6	2.2	1.4
	Range	5.1 – 14.0	6.0 – 13.6	4.2 – 12.5	4.7 – 12.3
5 – 9 years		**Normal range (11.5g/dL – 13.0g/dL)**			
	Mean	10.6	10.3	10.3	10.2
	SD	1.7	1.9	1.2	1.7
	Range	7.6 – 16.0	6.0 – 14.5	7.4 – 12.6	6.4 – 13.8
10 – 15 years		**Normal range (11.5g/dL – 14.7g/dL)**			
	Mean	11.0	10.9	10.4	10.5
	SD	1.6	1.6	1.5	1.7
	Range	6.7 – 14.9	7.1 – 14.2	6.1 – 13.7	6.6 – 13.4
16+ years		n/a	n/a	n/a	n/a

SD = Standard Deviation, n/a = not available

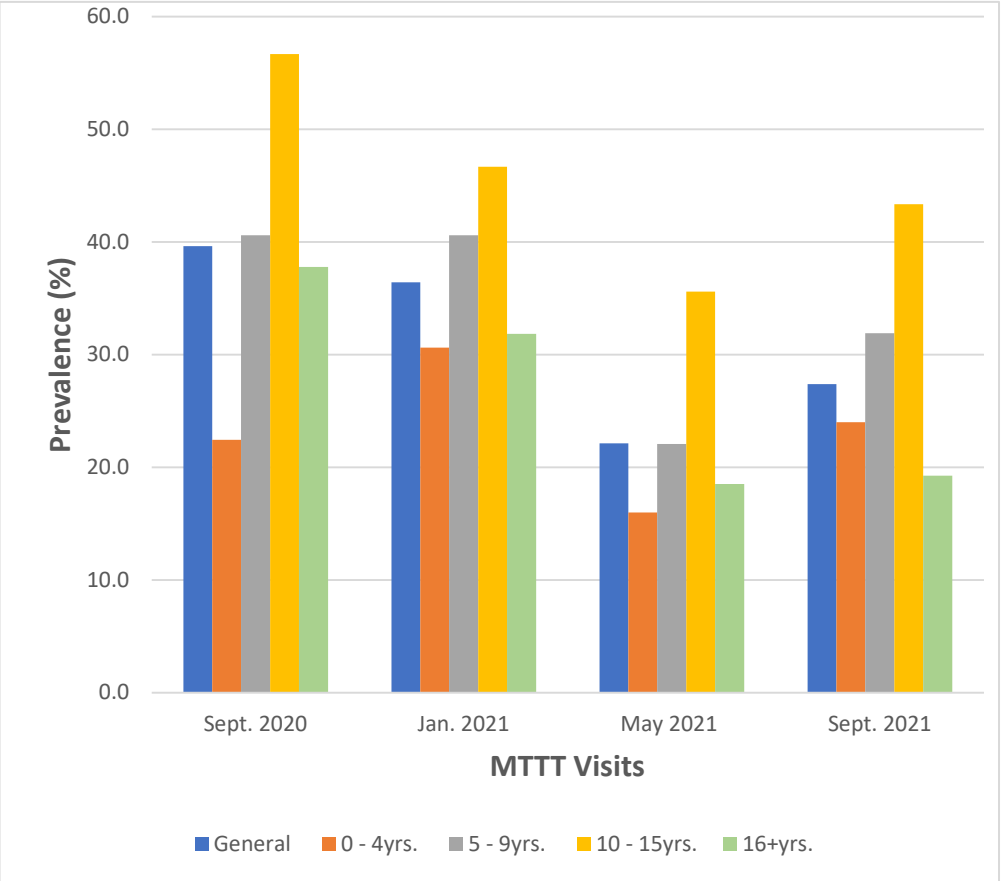

Figure 5: *Plasmodium falciparum* prevalence (PCR) among the various age categories.

The figure above **(Figure 5)** gives the general overview of the *Plasmodium* infection prevalence in the study population. The general study population had a prevalence of 39.6% at baseline (September 2020), which decreased to 27.4% at the end of the study (September 2021). Further stratification of the study population into various age categories showed that the age group 10–15 years had the highest prevalence at baseline (56.7%) with the age group 0 – 5 years having the least of 22.4% at the beginning of the study **(Figure 5)**. We also observed that parasite prevalence declines gradually within all the age groups as the study progressed except for the final visit (September 2021) where an increase in parasite prevalence was recorded. One

key observation made is the drastic decline in parasite prevalence among the 10 – 15 years age category during the first 3 sampling time points **(Figure 5)**.

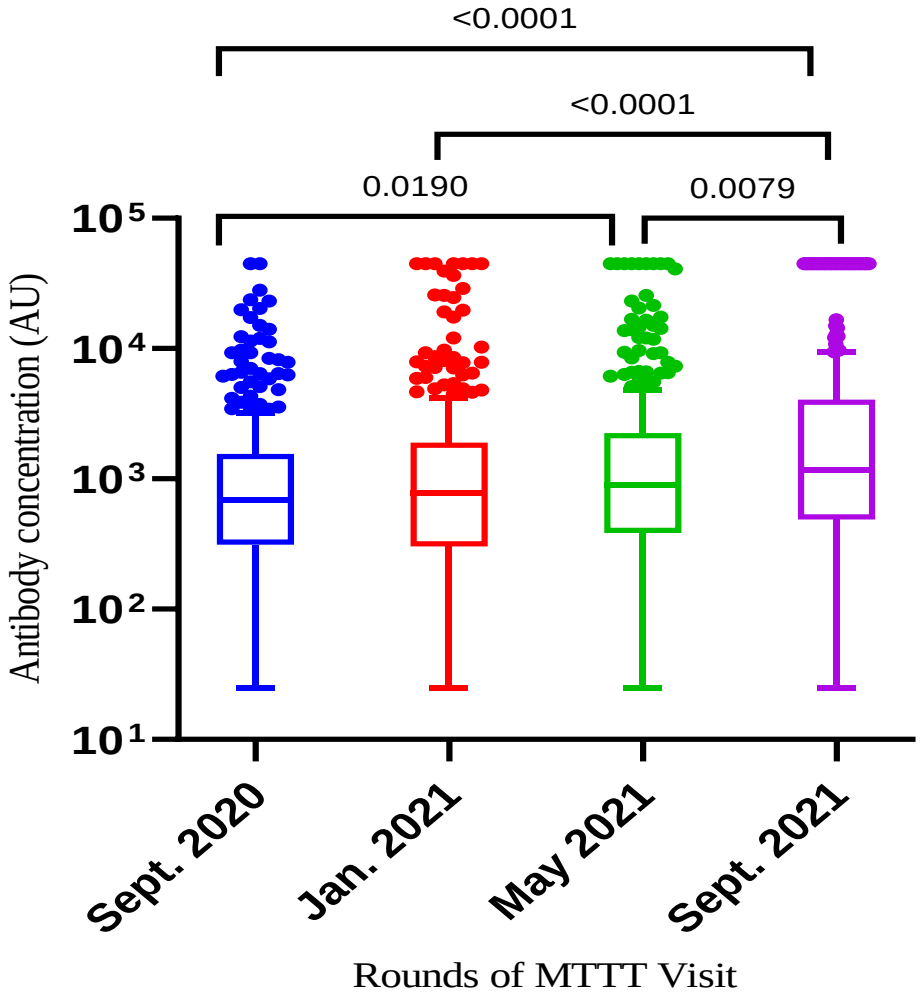

Figure 6: General antibody response of study participants against the *Pf*EBA-175 antigen across the four MTTT rounds.

In measuring the antibody response of the entire study population**,** there was a mean significant difference between antibody titres of the first visit (September 2020) and the final visit (September 2021) (*p*-value = <0.0001), first visit (September 2020) and third visit (May 2021) (*p*-value = 0.0190), second visit (January 2021) and final (September 2021) (*p*-value = <0.0001) and third visit (May 2021) and final visit (September 2021) (*p*-value

= 0.0079). However, there was no mean significant difference between the first visit (September 2020) and the second visit (January 2021), and that between the second visit (January 2021) and third visit (May 2021); p = >0.9999 and p = 0.9928 respectively **(Figure 6)**.

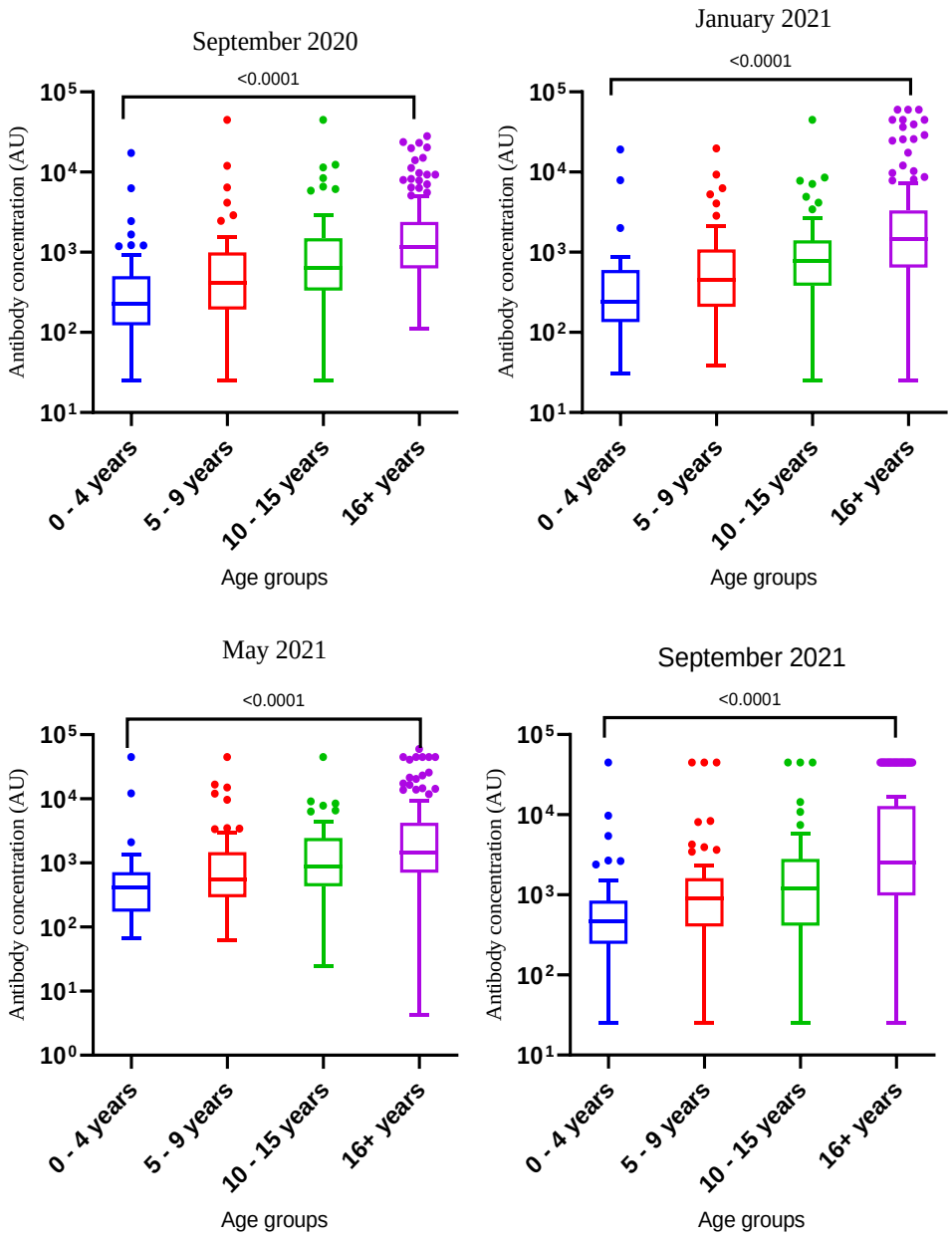

Figure 7: Antibody titres against *Pf*EBA-175 at each visit among the age categories.

We observed that there was a mean significant difference in antibody response at each visit among the age categories **(Figure 7).**

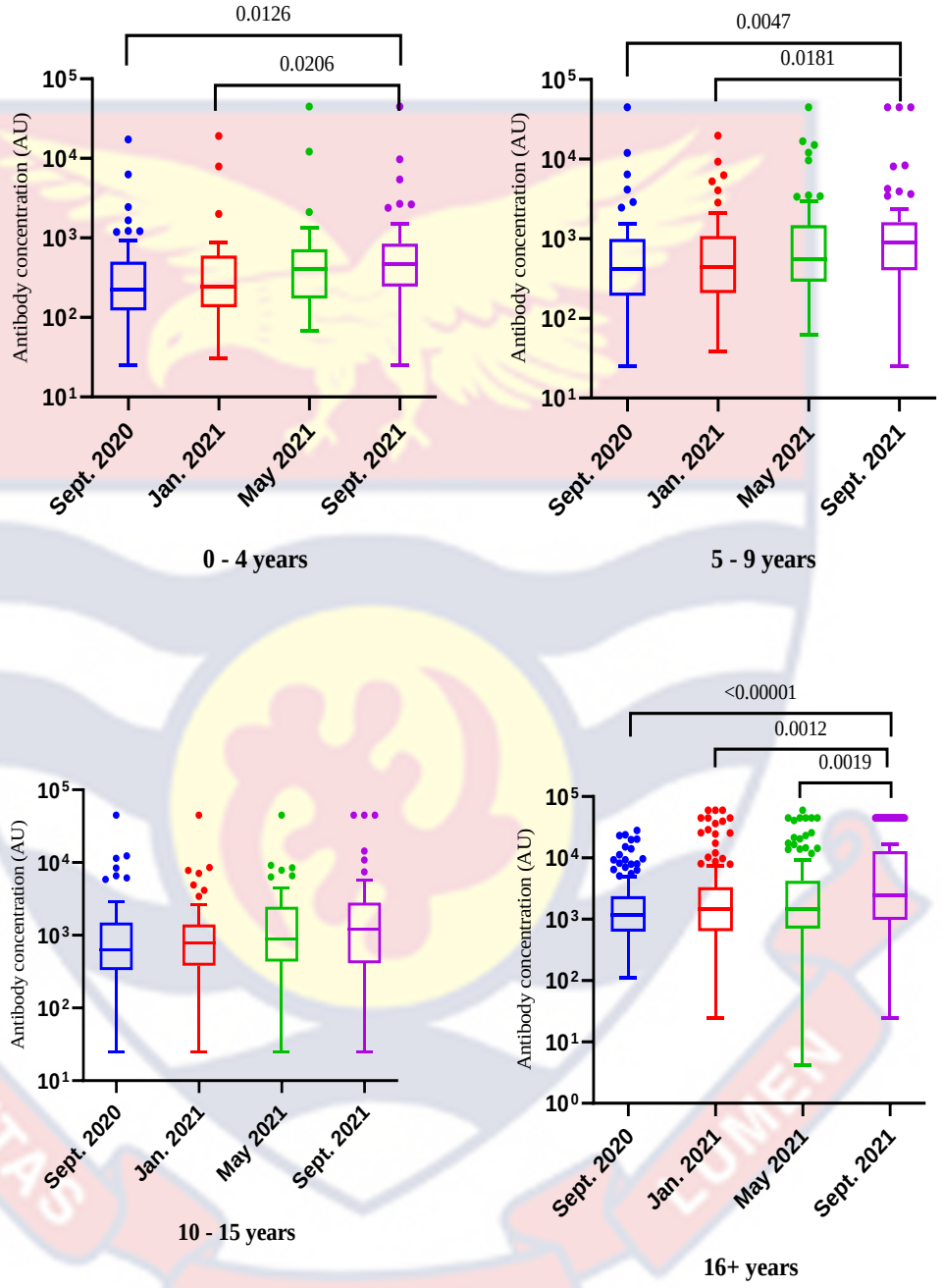

Figure 8: Antibody titres against *Pf*EBA-175 across all visits within each age category.

Comparing antibody response within each age group across all four visits, there were statistically significant differences (*p*-value of 0.0372,

0.0194, and <0.0001 for 0-4 years, 5-9 years and 16+ years respectively) except that of the age group 10-15 years where though there was a rise, it was not statistically significant with an overall *p*-value of 0.3229 **(Figure 8)**.

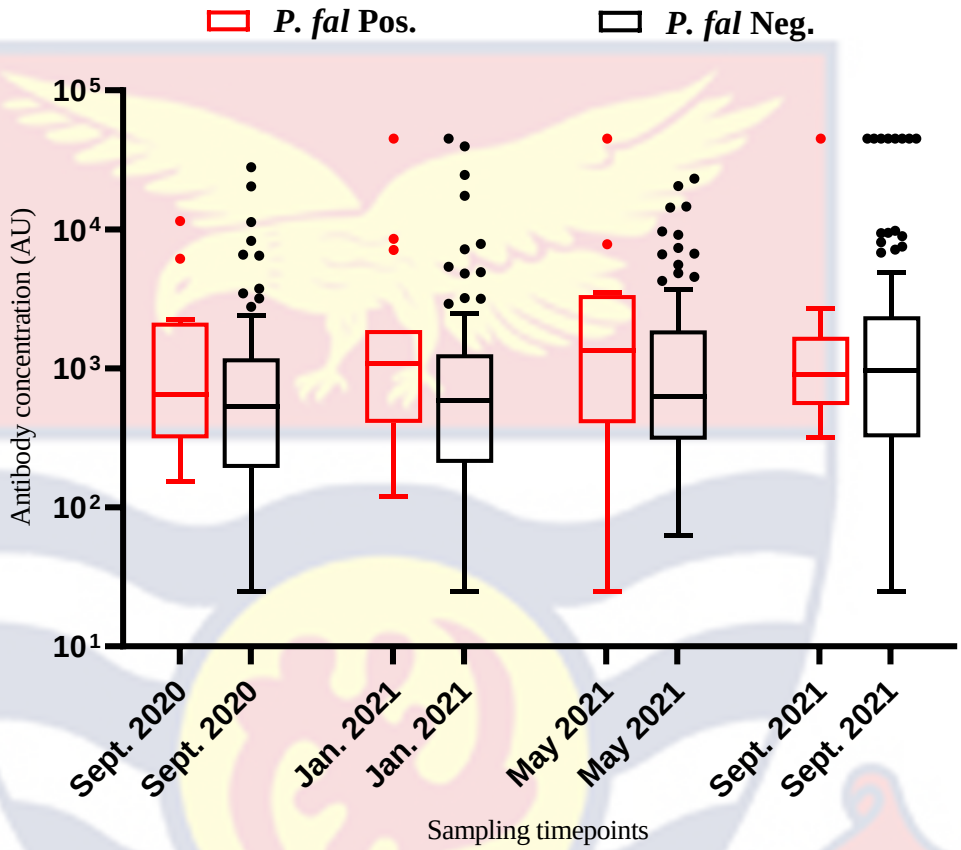

Figure 9: Antibody titres against *Pf*EBA-175 of individuals who were *Plasmodium falciparum-infected* and non-infected throughout the study period.

This figure **(Figure 9)** represents participants that were infected with *Plasmodium falciparum* throughout and those that were non-infected throughout as well. Mean antibody titres for those infected with *Plasmodium falciparum* (indicated in red colour) during the entire study period were higher than the non-infected (indicated in black colour). However, there was statistically no mean significant difference in their antibody titres.

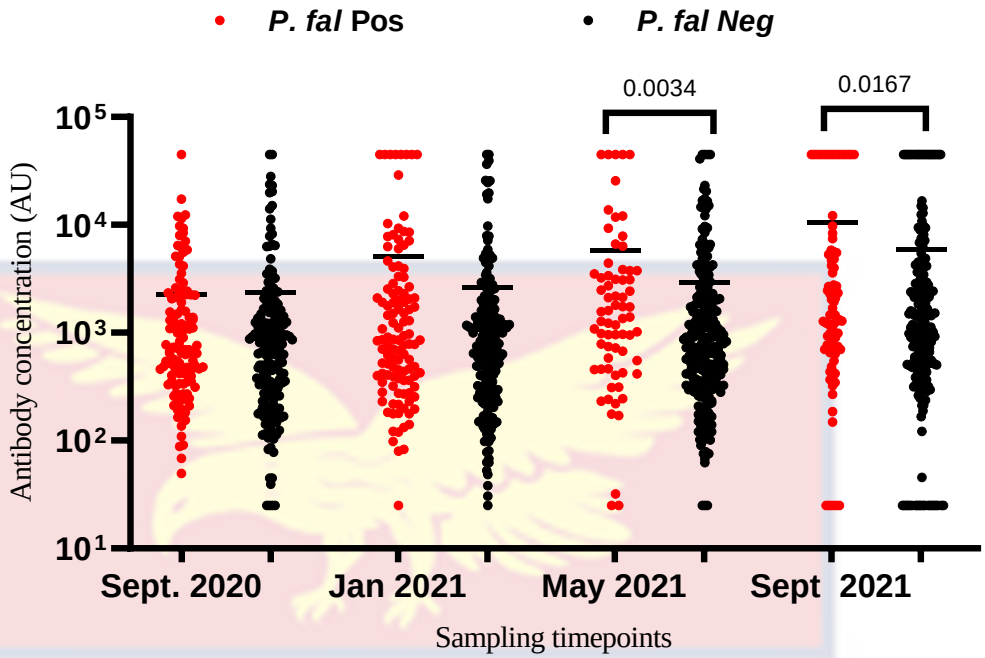

Figure 10: Antibody titres against *Pf*EBA-175 of *P falciparum-infected* individuals and non-infected individuals each time sampling point.

Comparing *Plasmodium falciparum*-infected individuals (indicated in red colour) and non-infected individuals (indicated in black colour) at each visit **(Figure 10)**, there was no statistically significant difference between the infected and non-infected for September 2020 visit (p=0.1262) and January 2021 visit (p=0.0511) despite higher mean antibody titres for infected. Conversely, mean antibody titres were statistically significant **(Figure 10)** between the infected and non-infected for May 2021 visit (p=0.0034) and September 2021 visit (0.0167)

Discussion

The severity of malaria is a known fact across the globe, especially infections caused by *Plasmodium falciparum*. Despite the high number of cases recorded every year, the number of deaths is relatively low - though these numbers remain unacceptable. For people in endemic areas, it is a fact that naturally acquired immunity plays a significant role in the disease's

manifestation and severity (Reeder & Brown, 1996). An era of renewed strength to combat this deadly disease has led to the employment of various control and elimination methods. How does a control strategy (in our case, MTTT) introduced in an area interfere with the natural immunity mainly influenced by repetitive exposure? In this study, we examined changes in antibody levels against a blood stage antigen- *Pf*EBA-175 after a year of MTTT rollout in an endemic area.

Characteristics of the study population

The results from the demographic data suggest that females' involvement and willingness to partake in malaria-related interventions and/or studies in sub-Saharan Africa (SSA) is very common compared to males. This outcome is not surprising because, during the sampling time, the male family heads were willing to allow the wives and children to participate in the study but not them. Also, the adult males were usually not at home during sampling. This observation confirms the already existing belief concerning men's attitude towards seeking healthcare which is mostly influenced by societal constructs especially cultural and patriarchal norms (Latunji & Akinyemi, 2018; Olanrewaju et al., 2019).

As expected, the mean body temperature across the visit was within the normal range. Mean haemoglobin levels among children within the ages of 6 months and 15 years were found to be about 10 g/dL across visits. This value is similar to mean haemoglobin levels found in a review of other studies among children leaving in endemic areas.

Prevalence among the general study population.

P. falciparum prevalence for this study by PCR was found to be 39.6% at baseline; the first-time visit (September 2020) of the study, qualifying this area as a moderate to high transmission zone. These zones are classified as areas where malaria infection and disease present a significant burden on the local population (Idro et al., 2006). The prevalence obtained in this study is similar to a previous study conducted in the same area and areas with similar ecological zones (Acquah et al., 2020). This prevalence is however lower than those from studies conducted earlier in the same area which detected a relatively higher outcome both by microscopy and PCR (Adjah et al., 2018; Amoah et al., 2018). The varying outcome may be due to the differences in sampling time points. September serves as the transition month between the major rainy season (June to August) and the minor rainy season (usually October – November) (Nyasa et al., 2021). Another probable factor for the decline in prevalence could be because of the malaria control interventions rolled out in the study area over the years. At the second visit (January 2021), a 3% decline in prevalence among the study population is expected since this time is not so far off from the minor peak seasons. An important finding is a decline in prevalence to almost half of the baseline prevalence in the third visit (May 2021) during the study period. Two reasons may account for this observation; firstly, the period falls after the long off-peak seasons (usually from December to March) which are usually associated with a decrease in vector population resulting in a subsequent decrease in transmission. Secondly, the treatment of asymptomatic parasite carriers through the MTTT may also influence the decline in prevalence. The

effectiveness of MTTT interventions can be seen in the outcome (27.4%) observed after the last intervention (September 2021). This result, though higher than the preceding visit (May 2021) is lower than the prevalence recorded from the previous year around the same period (September 2020) even after the return of the rains which is known to influence parasite transmission. Another factor that could be attributed to this decline may be the results of the malaria education given to the study participants in the course of the study period.

Prevalence among the various age groups in the study population.

In grouping the study populations into the different age groups, it was observed that, aside from the final visit (September 2021), those within the age group of 0 – 4 years have the least prevalence across the study period. This result corroborates with a study that reported the least prevalence among children under five (Workineh et al., 2021). Other studies within the sub-region have however reported a high prevalence among this age category (Dao et al., 2021; Nwaorgu & Orajaka, 2011). The reason for the former observation is due to the extra attention given to this age group when it comes to malaria intervention. Children within this age bracket usually sleep in treated nets with their mothers (Afoakwah et al., 2018). They are also likely to spend limited time outside and thus have little exposure to mosquitoes. Additionally, most interventions are mainly geared towards them. Another important finding is the high prevalence observed in the age groups 5–9 years and 10–15 years (Haji et al., 2016). This situation could also be because these age groups tend to be on their own as they age and hence lost their "mother's protection" when it comes to ensuring that they are protected from mosquito

bites. Also, this age group mostly falls out of the intervention geared towards children under five (5) and hence are left on their "own" (Touré et al., 2016; Workineh et al., 2021). The group that tends to manifest the effect of the intervention is those in groups 10–15 years who have consistently had a significant decline in parasite prevalence except that of the last visit. This is because the last visit falls just after the peak seasons where malaria transmission is usually high.

Antibody titres of the general population.

The result of our study showed a general rise in mean antibody titres of the study participants against the *Pf*EBA-175 antigen as the study progresses from the baseline sampling point (September 2020) to the final sampling point (September 2021). This rise was strongly significant ($P<0.0001$). The probable explanation for this observation is the presence of parasite at the submicroscopic level in the blood which could not be detected hence thus was not cleared which further contributed to the increase in antibody titres despite the intervention. Statistically there were no mean significant differences in antibody titres between the first and second visits, which coincided with parasite prevalence between the baseline and the second visits, which was about 3%. One would expect that for a reduction in parasite prevalence, there should somewhat be a reduction in antibody titres as well, as it is one key characteristic in malaria immunity where the absence of exposure is mostly associated with a decrease in immunity (Crompton et al., 2014; Doolan et al., 2009). The fact that parasite prevalence was not statistically significant could probably explain this observation.

Conversely, the assertion made for the insignificant differences in antibody titres between the baseline visit and the second visit cannot be made for the third and fourth visits where there was a substantial increase in antibody levels despite the decrease in parasite prevalence. This observation is close to a study in the same area where there was no statistically significant difference in antibody titres against the *Pf*EBA-175 antigen among children despite the varying seasons in sampling (Abagna et al., 2018b).

The results from this study proved an age-long knowledge that antibodies to malaria antigen in an endemic population increases with age (Doolan et al., 2009; Ondigo et al., 2014). The results for each sampling time point showed that antibody titres against the *Pf*EBA-175 were higher for people in the age bracket of 16 years and above as compared to those below this age bracket (15 years and below) across visits. These outcomes could possibly be the reason why adults hardly come down with the diseases in an endemic community despite harbouring the parasite. Though this study did not assess the disease's incidence in the population, other studies have established an association between antibody titres and disease incidence (Dodoo et al., 2011; Hamre et al., 2020; Offeddu et al., 2017).

Also, varying seasons did not affect the antibody levels among the various age group. The 16+ years age category had higher antibody titres than those below 16 years irrespective of the time of sampling, showing that antibody acquisition in an endemic area is always influenced by age. We also observed that there were significant differences in antibody response between the baseline and final visits across all the age categories except for the age group 10–15 years. Though there was some increase in mean antibody titres

against the *Pf*EBA-175 antigen as the study progresses, the difference was not significant. This observation could be attributed to the drastic decline in parasite prevalence among this age category after the baseline survey especially that of the third visit. It must, however, be stated that this group like the other age category showed a persistent decline in parasite prevalence except for the final visit where there was an increase in prevalence for all the age groups. The high clearance of the parasite from the blood possibly might have contributed to this outcome.

The rise in *Plasmodium* prevalence at the final visit further proved the returns of the rains hence the possibility of an increase in infection due to its corresponding increase in mosquitoes' breeding sites as a result of the rain. This observation contradicts those made in the other groups where though there was a decline in parasite carriage; mean antibody levels were still high and significant.

***Plasmodium falciparum* Infection and Antibody Response.**

Generally, *P. falciparum-infected* individuals have higher antibody titres against the antigen. Those who were infected throughout (i.e., had the infection for all four visits), had higher antibody titres but the mean difference between this group and those who were not *P. falciparum-infected* was not significant. The gradual rise in antibody levels against the antigen especially for those who were not infected at all during the entire sampling period could be a result of two reasons. Firstly, due to the period difference in the sampling times (about three months), the parasites were cleared after infection and thus were not captured during the time of sampling. This previous infection might have caused an increase in antibody titres. Secondly, the parasites present in

the individual may be below the limit of detection for PCR which ranges from 1–10 parasite/μL (Aschar et al., 2022) of blood and thus was missed. The fact that the *P. falciparum*-infected group had higher antibody titres than the non-infected group is evident that the presence of the parasite boosts antibody levels which in effect provide partial immunity to people living in such areas. Though our current study did not assess the number of people who were symptomatic during the study periods, records from other studies proved that higher antibody titres were mostly synonymous to been asymptomatic to the disease with some individuals in the asymptomatic group having antibody titres of about 8 times more than the symptomatic group (Addy et al., 2021; Richards et al., 2010).

Additionally, comparing the infected with non-infected at each sampling time point also showed that the former had high antibody tires at all sampling time points. There were no mean significant differences between the two groups for the first two visits (September 2020 and January 2021). This however cannot be said about the last two visits (May 2021 and September 2021) where there was a mean significant difference (P=0.0034 and P=0.0167 respectively). The period might have accounted for the differences. The month of May falls within the major peak seasons (usually between April and July) and that of September with the minor peak seasons (usually between September and October) that is mostly associated with higher transmission thus resulting in a significant increase in infection which further influences immunity.

Chapter Summary

This chapter put together all the results obtained after the analysis was done. The trends observed in parasite prevalence among the four (4) sampling time points and that observed among the various age categories was compared to see the presence of an association. Antibody response to *Pf*EBA-175 was also compared with parasite prevalence and infection at each visit. The chapter also provided some plausible explanations to the trends observed.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

Introduction

This chapter brings finality to the thesis. It summarizes the theoretical and empirical underpinnings of the study, what we sought to do and the scientific rationale for doing it that way. It also summarizes the scientific methods used in this study. The present chapter summarizes the major determinations of our research and makes conclusions from the results of the study. Lastly, this chapter presents cogent recommendations (directed at policy and practice) based on our research findings. These recommendations also include suggestions for future research in the present area of research.

Summary of the study

Our study sought to assess the immunological dynamics associated with employing a control intervention in an endemic area. Several interventions have been employed in many endemic areas in SSA and these interventions have helped decrease the burden of the disease. The immense benefits of these interventions cannot be disregarded and thus very timely in the control of the disease. Just like a double-edged sword, these benefits come with some downsides; the issue of the loss of partial immunity.

The intervention used in our case was MTTT where individuals in an endemic area were tested and treated which results in the clearing of blood-stage parasites. These parasites serve as an immunogen which stimulates the immune system to build immunity towards them thus providing such individuals with what is termed anti-disease immunity.

To explore the dynamism associated with partial immunity, antibody titres were measured against a blood-stage antigen-*Pf*EBA-175. Studies have shown that this antigen is very promising, especially its established role in erythrocyte invasion. Antibodies against this antigen have also proven to be effective in disease prevention.

To achieve this aim, 314 participants in an endemic area who were present during the four-time sampling points were selected. Blood samples collected were processed to extract genomic DNA and antibody elution from DBS. PET-PCR was run for these samples and an Enzyme-linked immunosorbent assay (ELISA) was run from the DBS elution against the *Pf*EBA-175 antigen to measure the antibody titres. Data were analyzed descriptively and the associations that existed between variables were identified.

Summary of Findings

Three main objectives were examined by this study. Of the 314 participants recruited; between the ages of 7 months and 90 years, 191 of them were females and 123 were males. The average age of the study participants was 22.1±20.9 years.

For objective one, we found out that *Plasmodium falciparum* prevalence among the general study population at the baseline (September 2020) was 39.6% and at the end of the intervention, it reduced to 27.4% in September 2021. When the study participants were categorized according to age, the 10–15-year group had the highest *P. falciparum* prevalence across all visits, whilst the 0-4 years age group had the least across visits except for the final visits (September 2021).

For objective two, significant difference in the mean antibody titres against the *Pf*EBA-175 antigen were observed in the population across the different time points. There was also a significant difference in the mean antibody titres among the various age categories, except for participants within the 10-15 years age group. Also, antibody response at each visit among the various age categories showed a similar trend where the visit did not affect the differences that existed among the group.

Lastly, the study found an association between those infected with *Plasmodium falciparum* and their antibody levels. Despite the insignificant difference in antibody titres among those who were infected at all visits and those who never got infected during the study period, the former had higher antibody titres than the latter. Also, comparing those infected at each visit and those not infected, there was no mean significant difference between the September 2020 visit and the January 2021 visit. This however was not the case for May 2021 and September 2021 visits which recorded mean significant differences between the infected and non-infected.

Conclusion

This study revealed a consistent increase in antibody titres against *Pf*EBA-175 among study participants throughout the entire 13-month study period despite the intervention. We found out that all the age categories showed higher antibody titres with mean significant differences at the end of the period except those in the age group 10-15 years whose differences in antibody titres at the end of the study were not significant. The probable explanation for this deviation could be a result of the drastic decline in parasite prevalence after the first visit. We also found a strong mean significant

difference in antibody titres between the *Plasmodium falciparum*-infected and non-infected groups during the third (May 2021) and final (September 2021) visits but not that of the first (September 2020) and second (January 2022) visits. This study reveals that MTTT intervention does not significantly reduce antibody titres against the *Pf*EBA-175 antigen after a year of its rollout in an endemic community. However, some deviations observed in some groups could give a clue of a possible reduction or loss of antibody titres against this antigen if the intervention is carried out for a longer period of time as suggested by some studies.

Recommendations

1. For a direct community benefit, we recommend regular education of community members on the adherence to some education given to them with regard to malaria prevention. The leadership of the community should also encourage their people to observe these protocols.
2. The government in collaboration with the National Malaria Elimination Programme (NMEP) should also strengthen the malaria prevention protocols such as indoor residual spraying and the use of long-lasting insecticide-treated nets, chemoprevention, etc. to help further reduce the burden of the disease in the area.
3. Ghanaian government should sponsor the Universities and Research Centres to conduct serological surveillance to monitor malaria interventions that are rolled out.
4. We recommend that all interventions employed by the various malaria control organisations in SSA should include the measurement of

immunological parameters as a tool for monitoring malaria disease interventions and their outcomes given that these studies are limited.

5. Globally, more funds should be pumped into Research Institutions to put in much effort to develop more potent malaria vaccines in addition to the current ones to help curb the disease.

Suggestion for Further Research

1. Future research may consider tracking the participants and taking into account those that become symptomatic in the course of the study so as to help us understand the changing dynamics of parameters across different study periods. Also, we recommend that the sampling period should be shorter so to help us understand the dynamics associated with immunological response to these antigens. It will be worthwhile if future researchers can explore this gap.
2. We recommend serological surveillance during the course of other malaria interventions. Our current intervention is one of the many interventions employed and thus larger studies will help us to understand a much bigger picture of the disease dynamics across the population.
3. Future studies may also consider the effects of other blood-stage antigens not captured in this study and establish if the combinations of antibodies from these antigens significantly affect disease outcomes.

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