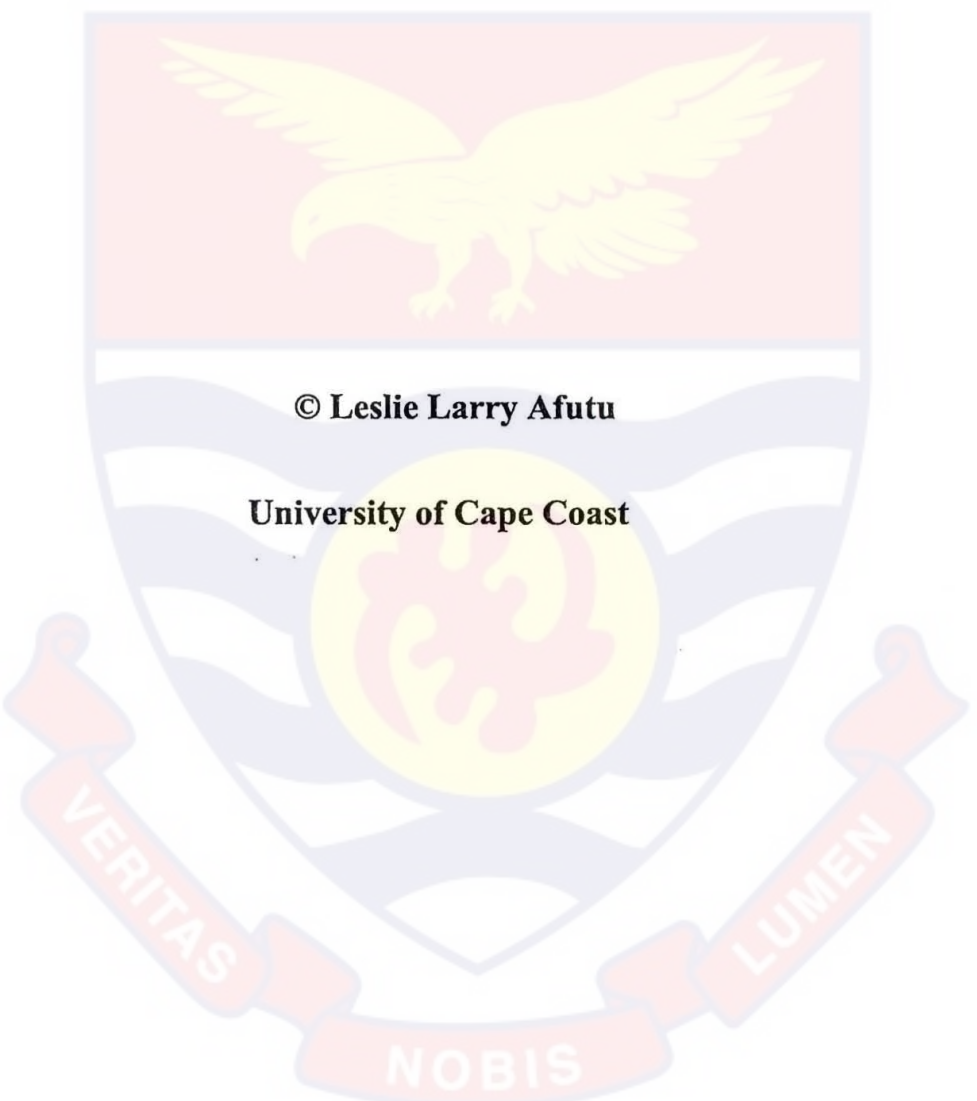

UNIVERSITY OF CAPE COAST

EVALUATION OF THE EFFECTIVENESS OF SULPHADOXINE PYRIMETHAMINE AS INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY IN SELECTED HEALTH FACILITIES IN SEKONDI-TAKORADI, GHANA

BY

LESLIE LARRY AFUTU

Thesis submitted to the Department of Biomedical Sciences of the School of Allied Health Sciences, College of Heath and Allied Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Parasitology

JULY 2018

DECLARATION

Candidate's Declaration

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

Candidate's Signature.....Date.....16/7/18.....

Name: Leslie Larry Afutu

Supervisors' Declaration

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

Principal Supervisor's Signature.....Date.....23/7/2018.....

Name: Professor Johnson Nyarko Boampong

Co-Supervisor's Signature.....Date.....23/7/2018.....

Name: Professor Neils Quashie

ABSTRACT

Malaria in pregnancy still remains a huge public health problem in most parts of Africa and Asia-Pacific. The use of Sulphadoxine-Pyrimethamine as intermittent preventive treatment for malaria in pregnancy (SP-IPTp) in endemic regions of the world has reduced the burden of malaria in pregnancy and minimized the consequences of malaria to both mother and the foetus. However, there have been reports of wide-spread mutations, especially in eastern and some parts of southern Africa, in the *dhfr* and *dhps* genes, which confer to the *Plasmodium* parasite, resistance to pyrimethamine and sulphadoxine respectively. This, coupled with the general preponderance of substandard anti-malaria drugs on the African market, threatens the success of SP-IPTp. The aim of this study was to evaluate the effectiveness of sulphadoxine and pyrimethamine as IPTp in three selected health facilities in Sekondi-Takoradi metropolis of the western region of Ghana. SP was found to be efficacious in clearing parasitaemia amongst parasitaemic yet asymptomatic pregnant women in the face of high prevalence (71.4%) of *dhfr* triple mutations N108, I51 and R59. The quintuple mutation, known to confirm high-grade resistance to the parasites was found in only two (2) isolates in non-pregnant attendants at the general outpatient department. There was no association between number of SP doses taken, the use of insecticide treated nets (ITNs) and maternal anaemia. Higher doses of SP, ITN usage, but not parity, reduced the risk of both placental parasitaemia and low birth weight. The SP tablets in used were found to be of good quality having a USP of 95.3% and 92.8% and dissolution of 95.2% and 83.03% for sulphadoxine and pyrimethamine respectively. This study therefore found the SP tablets in use as IPTp to be of good quality and effective in the face of high prevalence of *dhfr* triple mutations.

ACKNOWLEDGEMENTS

I am sincerely grateful to my supervisors, Professors Johnson Nyarko Boampong and Neils Quashie for their guidance, encouragement and goodwill which guided this work. I am also thankful to the Medical Superintendents, management members and staff of the Obstetrics and Gynaecology Units of Effia Nkwanta Regional Hospital, Takoradi Hospital and Essikado Hospital all in the Sekondi-Takoradi metropolis for not only allowing me to use your facilities, but also assisting me in diverse ways to carry out this work.

I also want to say a very big thank you to Dr. Egya Ndede Yankey and his staff at the Oil Palm Research Institute of the Council for Scientific and Industrial Research in Takoradi for allowing me unlimited access into your laboratory. You also on countless occasions provided me with some laboratory reagents and consumables and gave technical laboratory assistance. I want to also thank the staff of the analytical laboratory of the Food and Drugs Authority in Accra, who helped me with the analysis of the quality of the SP tablets.

I am also grateful to my wife Joana, my children; Lois, Josh and Laurene for their support. To my colleagues, Diane, Gifty, Emmanuel, George and Ishmael, I am most grateful. You guys to a large extent made learning fun and life on campus in general a lot easier. I would also say a big thank you to Dr. Samad Odartey Lamptey, my friend or rather my brother who hosted me at no cost any time I am in Cape Coast for any academic work.

Lastly, but definitely not the least, let me say a big thank you to family members and to friends who in one way or the other have supported me to complete this research work.

DEDICATION

In memory of my late mother

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
AL	Artemether-lumefantrine
AMFm	Affordable Medicine Facility–malaria
ANC	Antenatal care
API	Annual parasite index
AQ	Amodiaquine
AS	Artesunate
AA	Artesunate-Amodiaquine
ASMQ	Artesunate-Mefloquine
ASSP	Artesunate-Sulphadoxine Pyrimethamine
CQ	Chloroquine
CTXp	Co-trimoxazole prophylaxis
CCM	Community case management
CFR	Case fatality rate
CI	Confidence Interval
DDT	Dichloro-diphenyl-trichloroethane
DHA-P	Dihydroartemisinin-piperaquine
DHFR	Dihydrofolate reductase

DHPS	Dihydroptroate synthetase
DOT	Directly Observed Treatment
DVS	Dominant Vector Species
ELISA	Enzyme Linked Immunosorbent Assay
FGR	Foetal Growth Restriction
G6PD	Glucose-6-Phosphate -Dehydrogenase
GDP	Gross domestic product
GHS	Ghana Health Service
H&E	Haematoxylin-Eosin
HPLC	High Performance Liquid Chromatography
IE	Infected Erythrocytes
IPTi	Intermittent Preventive Treatment in infants
IPTp	Intermittent Preventive Treatment in pregnancy
IPTp-SP	Intermittent Preventive Treatment in pregnancy with Sulphadoxine-Pyrimethamine
IQR	Interquartile range
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Mosquito Nets
IUGR	Intra Uterine Growth Restriction

LBW	Low Birth Weight.
LLIN	Long-Lasting Insecticidal Net
LAMP	Loop mediated isothermal Amplification
MDG	Millennium Development Goal
MiP	Malaria in Pregnancy
MOH	Ministry of Health
MTS	Masson's trichrome stain
MPAC	Malaria Policy Advisory Committee
MRDTs	Malaria Rapid Diagnostic tests
MTCT-HIV	Mother to child transmission of HIV
MSP	Merozoites Surface Protein.
MQ	Mefloquine
NMCP	National Malaria Control Programme
OPD	Outpatient department
PABA	Para-aminobenzoic acid
*p*HRP-2	*Plasmodium* Histidine Rich Protein-2
*p*LDH	*Plasmodium* Lactate Dehydrogenase
PCR	Polymerase Chain Reaction
SNPs	Single Nucleotide Polymorphisms

SSA	Sub Saharan Africa
SP	Sulphadoxine-Pyrimethamine
USP	US Pharmacopoeia
WHO	World Health Organization
µl	Microlitre
µm	Micrometre
µM	Micromolar

CHAPTER ONE

INTRODUCTION

Background to the Study

Human malaria, the most important tropical parasitic disease, is caused by five protozoan species belonging to the genus *Plasmodium.* These are; *Plasmodium falciparum, P. vivax, P. Ovale, P. malariae, and P. knowlesi* (White, 2008; Cox-Singh et al., 2008; Sutherland et al., 2010). *P. falciparum* is the most lethal of the species. It causes the severest forms of the disease and mortalities. It also causes the largest burden of the disease (Guerra et al., 2008), especially in South Saharan Africa (SSA).

Malaria occurs throughout most of the tropical regions of the world. The number of malaria cases globally fell from an estimated 262 million in 2000 to 214 million in 2015, a decline of 18%. Fifty-seven (57) of 106 countries that had ongoing transmission in 2000 have reduced malaria incidence (World Health Organization (WHO) 2015). Most cases in 2015 are estimated to have occurred in the World Health Organization Africa Region (88%), followed by South-East Asia Region (10%) and Eastern Mediterranean Region (2%) (WHO).

Apart from children under 5 years, another group that is vulnerable, in terms of risk and outcomes of malaria infection, is pregnant women. This is because pregnant women attract twice the number of *Anopheles gambiae complex*, the predominant African malaria parasite-carrying mosquito

compared to non-pregnant women (Lindsay et al., 2000; Ansell et al., 2002). A woman is also four times as likely to get sick from malaria if she is pregnant and twice as likely to die from the disease as if she is not pregnant (WHO, 2005). The physiological changes of pregnancy and the pathological changes due to malaria have a synergistic effect on the course of each other. The impact of malaria infection on pregnant women is therefore very dire.

There are some 125 million pregnancies at risk of malaria infection every year (Dellicour, Tatem, Guerra, Snow & ter Kuile, 2010; WHO, 2012). Majority of who live in malaria-endemic-region of SSA. For these women, malaria is a threat not only to themselves but to their babies as well. Malaria in pregnancy is known to cause 50% of all low birth weight, which contributes significantly to neonatal and infant morbidity and mortality. The infection is estimated to cause 400,000 cases of severe maternal anaemia and 75,000-200,000 infant deaths annually. Maternal anaemia contributes significantly to maternal mortality and causes an estimated 10,000 deaths per year (Rogerson, Hviid, Duffy, Leke, & Taylor, 2007). Malaria in pregnancy is therefore a huge public health problem.

In sub-Saharan Africa, poor nutrition, micronutrient imbalances (particularly vitamin A, zinc, iron and folate), co-infection with HIV and geo-helminthes, poverty and limited access to effective primary health care and emergency obstetric services usually exacerbate the impact of pregnancy-associated malaria (Sketetee, Nahlen, Parise & Menendez, 2001; Stephenson, Latham & Ottesen, 2000).

To tackle the problem of malaria in pregnancy, the WHO has recommended a set of control interventions in areas with moderate to high

transmission of *P. falciparum*. These include the promotion and use of insecticide-treated nets (ITNs), the administration of intermittent preventive treatment (IPTp) with sulphadoxine-pyrimethamine (IPTp-SP) during pregnancy, and appropriate case management through prompt and effective treatment of malaria and anaemia in pregnant women (Dellicour et al., 2010; WHO, 2014).

ITNs are to be provided to pregnant women as early in pregnancy as possible. Their use should be encouraged for women throughout the pregnancy and during the postpartum period. Appropriate case management involves the prompt treatment of detected cases of malaria during pregnancy using approved efficacious antimalarial drugs according to national guidelines.

The third aspect of the control measures for malaria in pregnancy, which is the IPTp-SP, until recently, consisted of the administration of a curative dose of SP (1500mg sulphadoxine and 75mg pyrimethamine) given intermittently at least two times during the pregnancy. Currently however, the WHO recommends that SP should be administered as early as possible during the second trimester and 1month apart up to the time of delivery (WHO, 2012b). The drug is administered under the direct observation of healthcare giver, a scheme known as Directly Observed Treatment (DOT). This is done during antenatal care (ANC) visits whether or not the pregnant woman is infected with the *Plasmodium* parasite. IPTp-SP has been shown to decrease peripheral and placental parasitaemia, and to increase maternal haemoglobin and infant birth weight, especially in primi and secundigravidae (Rogerson et al, 2007).

Both sulphadoxine and pyrimethamine are anti-folates. They act by inhibiting the enzymes dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*) respectively in the parasite's folate synthesis pathway. Resistance of *Plasmodium* parasite to Sulphadoxine-Pyrimethamine has been attributed mainly to mutations in the genes coding for these enzymes. Mutations at codons **N51I**, **C59R** and **S108N** of the *dhfr* gene are associated with resistance to pyrimethamine, whereas mutations at codons **A437G** and **K540E** of the *dhps* gene are associated with resistance to sulphadoxine.

In Africa, SP resistance appears to be intense in foci of East Africa and is increasing to the West (Mockenhaupt et al., 2005). Though the level of resistance at which IPTp-SP becomes ineffective is unknown, the WHO, in 2005, recommended IPTp-SP for women in countries where the SP failure rate among children is less than 50% in addition to other interventions such as ITN usage (WHO, 2005). In Ghana, SP failure was observed in 28% of children treated (Mockenhaupt et al, 2005). Due to pre-immunity, however, antimalarial treatment is commonly more effective in pregnant women than in children. Findings of efficacy studies in children can therefore not be extrapolated to the preimmuned adult in an endemic region. Currently, the WHO recommends that prior to implementation of IPTp-SP in any region with moderate to high malaria transmission, the prevalence of **K540E** and **A581G** should be determined. IPTp-SP should be used in regions with a prevalence rate **K540E** less than 50% and **A581G** less than 10% (WHO, 2013a).

Triple mutations in the *dhfr* gene (**N51I**, **C59R** and **S108N**) are responsible for SP resistance in Ghana. They confer about 10-fold increase

risk of treatment failure (Mockenhaupt et al., 2008). *Dhps* mutations appear not to be predictive of SP resistance among children in Ghana (Mockenhaupt).

With the increasing report of SP-resistance in children and pregnant women spreading from the eastern parts of Africa to West Africa, there is the need to regularly monitor the efficacy of SP-IPTp to direct policy in Ghana.

Statement of the Problem

Malaria in pregnancy poses significantly huge challenge to most public health managers in sub-Saharan Africa. The infection is estimated to cause 400,000 cases of severe maternal anaemia (which contributes significantly to maternal mortality) and 75,000-200,000 infant deaths annually (Rogerson et al, 2007).

In Ghana, and among pregnant women, malaria accounts for 28.1% of OPD attendance, 13.7% of admissions and 9.0% of maternal deaths (Ghana Health Service (GHS), 2009).

A number of studies in Ghana and SSA have found low levels of efficacy for Sulphadoxine-Pyrimethamine in the general population especially amongst children. SP however appears to be effective amongst pregnant women. SP resistance in IPTp is well documented to be focused in Eastern Africa with spread to the west. Indeed, it has been reported in a study that IPTp does not improve overall pregnancy outcomes in Muheza, Tanzania, where SP-resistant parasites predominate and may increase the odds of foetal anaemia; and as parasite resistance increases in a community, the overall effect of IPTp may transition from net benefit to neutral or net harm (Harrington, Mutabingwa, Kabyemela, Fried & Duffy, 2011). Meanwhile *Plasmodium* parasites in West Africa remain sensitive to SP (Dokomajilar et

al., 2011; Naidoo, & Roper, 2011). In Ghana, there has been a rapid increase in the prevalence of SP resistant *dhfr* gene from 36% to 73% in *P. falciparum* isolated in pregnant women since the introduction of SP (Mockenhaupt et al., 2008).

It is therefore important to conduct the necessary research to monitor the effectiveness of SP as IPTp in Ghana and in West African at large. This would prevent a situation where the whole health system is taken unawares by a complete failure of this all-important intervention. This study therefore comprehensively evaluates the effectiveness of SP as IPTp in Sekondi-Takoradi, a coastal Metropolis in Western Region of Ghana.

Purpose of the Study

The study is in four parts, namely; (i) an SP efficacy study (ii) a maternal and birth outcome study (iii) molecular surveillance for *dhfr* and *dhps* mutations and (iv) a test of the quality of SP tablets in use at the selected health facilities. The study aims at evaluating the effectiveness of SP as IPTp in the control of malaria in pregnancy in selected health facilities in the Western Region of Ghana.

Study objectives

1. To determine the efficacy of SP in clearing parasitaemia in asymptomatic yet parasitaemic pregnant women.
2. To determine the prevalence of *Plasmodium* parasites in peripheral and placental blood amongst delivering mothers.
3. To find out the specific mutation of *Plasmodium falciparum* in peripheral and placental blood isolates.

4. To measure birth weight of babies and correlate it with placental parasitaemia, IPTp-SP usage and maternal anaemia and usage of ITNs.
5. To ascertain the relationship between the number of SP doses and peripheral and placental parasitaemia in delivering mothers.
6. To determine the prevalence of mutations at *dhfr* and *dhps* loci responsible for SP resistance in *Plasmodium* parasite isolates from patients at the study site.
7. To evaluate the quality of the SP tablets in use for IPTp by determining the concentration of the active principle and solubility of the tablets.

Significance of study

Ghana, like many other malaria endemic countries in Africa, has adopted the use of SP as IPT in the control of malaria in pregnancy. There are however very few studies monitoring SP efficacy in pregnant women in our sub-region and for that matter Ghana. This study therefore intends to evaluate the effectiveness of SP as an IPT in pregnant women in the Western region of Ghana. Results of the study could inform policy makers to take appropriate policy action if needed.

Delimitations of the Study

The SP efficacy trial, the birth outcome study and the surveillance for mutation in the *dhfr* and *dhps* genes were limited to health facilities in the Sekondi-Takoradi metropolis of the western region of Ghana. Whilst the efficacy and the birth outcome studies were limited to pregnant women, that of the molecular surveillance was conducted amongst non-pregnant attendants at the OPDs of the study facilities as well. The evaluation of the quality of SP

drug was limited to samples taken from these study facilities and analyzed in the laboratory.

Limitations of the Study

One of the limitations to the study is the passive rather than active recruitment of study subjects. This might have resulted in the inclusion of only a sample which might not precisely reflect the dimension of the parameters being measured in this study. Again, the fact that all studied subjects had subclinical malaria, suggests that the figures herein reported underestimate the actual total prevalence of gestational malaria.

Possible bias due to non-participation may have occurred as this study was based exclusively at the health facilities thereby excluding those pregnant women who did not attend antenatal clinic and who may have a different profile from those that did. The data collected including when the last malaria medication was taken may be subject to recall bias. Using the dissolution analysis of the SP tablets sampled as a surrogate for bioavailability might not be a true reflection of what happens *in vivo*, since in real clinical situations several factors such as presence of co-morbidities, drug-drug interactions and other genetic factors affecting drug metabolism affects the bioavailability and subsequent effectiveness of the drug.

Definition of Terms

Malaria in Pregnancy is malaria infection (symptomatic or asymptomatic) occurring during pregnancy.

Pregnancy-associated malaria is a specific syndrome that occurs only in pregnant women. It is characterized by the accumulation of parasitized red

blood cells in the placenta. Pregnancy-associated malaria causes low birth weight and maternal anaemia.

Efficacy of a drug is the extent to which a drug has the ability to bring about its intended effect under ideal circumstances, such as in a randomized clinical trial.

Effectiveness is defined as the extent to which a drug achieves its intended effect in the usual clinical setting

Drug resistance is the reduction in effectiveness of a drug such as an antimicrobial, anti-helmintic or an anti-neoplastic in curing a disease or condition.

Surveillance is the continuous, systematic collection, analysis and interpretation of health-related data needed for planning, implementation, and evaluation of public health practice.

Asymptomatic or sub-clinical malaria is human *Plasmodium* infections which do not lead to clinical symptoms and therefore remain undetected by fever based surveillance systems.

Prevalence is a statistical concept referring to the number of existing cases that are present in a particular population at a given time.

Parasitaemia is the quantitative content of parasites in the blood. It is used as a measurement of parasite load in the organism and an indication of the degree of an active parasitic infection.

Prematurity is defined as live birth before 37 weeks of pregnancy are completed.

Low birth weight is defined as a birth weight of a liveborn infant of 2,499 g or less, regardless of gestational age.

Recrudescence is a term for recurrence of infection with species of *Plasmodium; P. falciparum, P. malariae* and *P. knowlesi*, which lack hypnozoites. It means that the infection (unless a new infection) has recurred from persistent blood stages of the *Plasmodium* parasite.

Reinfection refers to the infection with a new parasite strain after the past infection has been eliminated.

Genetic mutation is a permanent alteration in the DNA sequence that makes up a gene, such that the sequence differs from what is found in most individuals. Mutations can affect anywhere from a single DNA building block (base pair) to a large segment of a chromosome that includes multiple genes.

Organization of the study

The study is organized into six chapters. Chapter one which is the introduction, deals with background to the study, statement of the problem, purpose of the study, objectives, significance of the study, delimitation, limitations, definition of terms and organization of the study. Chapter two is the review of literature related to the study. Chapter three is the research methodology. It encompasses description of the study sites, study design, sampling procedure, data collection procedure and data analysis. Chapter four covers the results. Chapter five is the discussion of results. Summary of findings, conclusion and recommendations are captured in chapter six.

CHAPTER TWO

LITERATURE REVIEW

Malaria

Malaria is a parasitic disease caused by the infection of red blood cells and other body tissues with a protozoan of the genus *Plasmodium.*

The *Plasmodium* parasite is transmitted mainly by the bites of the female Anopheles mosquito of which the *A. gambiense* complex are the most common and most efficient at transmission. The inoculation of sporozoites of these parasites into human host during a blood meal begins the disease process.

Other comparatively rare mechanisms for transmitting the parasite include congenitally, blood transfusion, contaminated needle stick injuries, and organ transplantation (Filler et al., 2003; Alweis et al., 2004).

Clinical disease

Clinical malaria is usually described as uncomplicated (or simple) or complicated (or severe). Uncomplicated malaria is typically characterized by initial non-specific symptoms such as headaches, lassitude, fatigue, abdominal discomfort and muscle and joint aches, followed by fever, chills, perspiration, anorexia, vomiting and worsening malaise. These symptoms (which are primarily due to schizont rupture and destruction of erythrocytes with its attendant activation of cytokine cascade) are similar to symptoms of any minor systemic viral illness. The similarity of these initial symptoms of malaria with other systemic infections usually leads to an over diagnosis of

malaria especially in endemic regions, if diagnosis is made on the basis of symptoms alone. Symptoms, especially the fever as result of an infection with the various species of the parasite show unique periodicity. At this stage, when malaria is uncomplicated, with no vital organ dysfunction, case fatality is very low. However, with delay in treatment or treatment with drugs that are not efficacious, the parasite burden increases and severe disease may ensue. Severe clinical disease is commoner in children under five years and primigravidae. Severe malaria is now defined by at least one of the following clinical manifestations: unarousable coma (caused by cerebral malaria), convulsions, malarial anaemia, haemoglobinuria, hypoglycaemia, metabolic acidosis (associated with respiratory distress), acute pulmonary oedema, acute renal failure, jaundice, circulatory collapse, hyperparasitaemia, hyperpyrexia, electrolyte disturbance, and or spontaneous bleeding (WHO, 2000). At this stage mortality of those receiving treatment is 15-20% (Miller, Ackerman, Su, & Wellems, 2013). If untreated, severe malaria is almost always fatal.

In regions where malaria transmission is high and all year round, overt clinical disease is rarely seen in the adult population because of acquired partial immunity as a result of repeated parasite inoculation. Overt clinical disease is therefore limited to young children, especially those under five years of age and primigravidae. However, in regions of unstable parasite transmission, prominent clinical manifestations are common across all year groups.

Subclinical malaria

Subclinical malaria (also known as asymptomatic malaria) is said to be human *Plasmodium* infections which do not lead to clinical symptoms and therefore remain undetected by fever based surveillance systems (Galataz, Bassat & Mayor, 2016). Individuals with subclinical malaria are said to be parasitaemic (usually have asexual and sexual blood stage parasites) yet asymptomatic and do contribute to malaria transmission in a population. Being parasitaemic yet asymptomatic, these chronic carriers of the *Plasmodium* parasites evade most passive malaria surveillance systems and therefore remain a threat to malaria elimination programmes. Asymptomatic carriage levels detected by microscopy and or other methods have been reported to be as high as 39%, 8.4%, and 1.36 to 7.7% in African countries, India, and Thailand, respectively (Baliraine et al., 2009; Coleman et al., 2006; Dunyo et al., 2006; Ganguly et al., 2013; Kritsiriwuthinan & Ngrenngarmlert, 2011; Mabunda, Aponte, Tiago & Alonso, 2009; & Vafa, Troye- Blomberg, Anchang, Gracia and Migot-Nabias, 2008).

Though the mechanisms leading to subclinical malaria are not fully understood, interplay of parasite factors, host factors and transmission intensity in a given population is said to account for this phenomenon. Factors affecting virulence of parasites, such as their multiplication rates, cytoadherence and ability to produce toxic substances which elicits deleterious immune response play key roles in determining whether an infection would be clinical or subclinical. The more virulent the parasite strain or subtype, the less likely it would lead to subclinical malaria. Other parasite factors that play a role in determining whether an infection would be clinical or subclinical are a

multiplicity of infecting parasites and drug resistance. When the human host is infected with more than one strain of parasites, the infection is more likely to be subclinical than clinical. Also, parasites that are drug resistant in a given community tend not to lead to clinical malaria.

Host factors such as resistance to parasites, tolerance, genetic background as well as pregnancy and other comorbidities all influence the prevalence of asymptomatic malaria. Asymptomatic human malaria is much common in regions with high transmission intensity where most adults have developed resistance to parasites as result of innate and acquired immunity from repeated exposure to parasites. Tolerance, which is host defence mechanisms that minimizes the damage caused directly by the pathogen or indirectly by the host's immune response irrespective of pathogen burden (Galataz et al., 2016) also, increases the risk of developing asymptomatic malaria.

Comorbidities such as co-infections with Hepatitis B virus (HBV), HIV and soil-helminthes all alter the susceptibility to subclinical malaria in a given population. *Plasmodium* co-infection with hepatitis B virus is more likely to be asymptomatic and to have lower levels of parasitaemia, possibly through an increase in the production of Interferon-γ (IFN-γ) levels induced by the viral infection that can contribute to *Plasmodium* clearance in the liver (Andrade et al., 2011). Meanwhile, co-infection with soil-transmitted helminthes may alter susceptibility to clinical malaria by activating TH2 cytokines and immune-regulatory pathways that downregulate effector functions involved in resistance to malaria (Salgame, Yap & Gause, 2013). HIV can also impair lymphocyte functions against malaria infection

(González, Ataide, Naniche, Menendez, & Mayor, 2012) and thereby increase susceptibility to clinical malaria. Similarly, malnutrition can lead to atrophy of the thymus, leucopenia, and diminished functional T cells that hamper protective immunity to malaria (Schaible & Kaufmann, 2007).

Pregnant women living in endemic regions have an increased risk of asymptomatic malaria compared to those living in non-endemic areas. Indeed, pregnancy tends to increase the risk of asymptomatic malaria especially in these regions with high transmission intensity (Galataz et al., 2016). The prevalence of asymptomatic malaria is therefore higher in pregnant women (especially in primi and secundigravidae women) compared to non-pregnant women and men. Though the malaria infection may remain asymptomatic in these pregnant women, it still can inflict its consequences on both mother and her foetus. Indeed, the risk of maternal anaemia is known to increase with the increasing prevalence of asymptomatic malaria (Bouyou-Akotet et al., 2003; Tagbor et al., 2010; Ayoola et al., 2012 & Huynh et al., 2011).

In areas of low or unstable malaria transmission, women of reproductive age have relatively little or no acquired immunity to malaria. In these areas, all pregnant women, regardless of the number of previous pregnancies, are at similar risk of the adverse consequences of malaria, including severe illness and death as a direct result of severe malaria or malaria-related severe anaemia. Pregnant women with subclinical rather than clinical malaria were used as subjects in the SP efficacy study of this work because those with overt clinical disease would require a different treatment protocol other than the IPTp-SP.

Diagnosis of Malaria

Diagnosis of malaria involves all the procedures, both clinical and laboratory, to establish that one is suffering from the malaria infection. Early and accurate diagnosis of malaria therefore is key, since it allows for prompt and appropriate treatment without which there would be worsening malaria morbidity and increase in mortality (especially in vulnerable groups such as children under five years old and pregnant women). Again, when there is no prompt and appropriate therapeutic intervention, there is an increase in the incidence of non-targets effects of the disease, an increase in the advent of drug resistance and an increase in the overall cost of care.

Not only is effective malaria diagnosis important for the obvious reason of identifying cases to treat effectively, it also limits treatment to only patients who truly have malaria and not other febrile illnesses. This ensures proper use of anti-malaria drugs (Wilson, 2013).

Presently, there are five main ways of diagnosing malaria. These are; clinical/empirical/syndromic diagnosis, microscopy, use of malaria rapid diagnostic tests (MRDTs), histopathology and molecular diagnosis.

Clinical or syndromic diagnosis of malaria is still quite pervasive, especially in resource poor regions of the world. It is essentially the diagnosis of malaria based on the signs and symptoms of a patient. In many endemic areas without adequate diagnostic capacity, patients with febrile illness are presumed to be suffering from malaria. This approach to the diagnosis of malaria has a number of pit falls; (i) there is significant clinical overlap among febrile illnesses; fever alone is too nonspecific to make any particular diagnosis; (ii) co-infections occur, and treatment for one does not treat the

other; (iii) *Plasmodium* parasitaemia occurs that is not the cause of the febrile illness; (iv) dependence on clinical diagnosis alone results in treatment of patients with antimalarial drugs for illnesses other than malaria (Bell, Wongsrichanalai, & Barnwell, 2006). Again, clinical diagnosis as reported by several studies has resulted in an alarming rate of over-diagnosis (Onchiri et al., 2015; Leslie et al., 2012; Ghai, Thurber, Bakry, Chapman & Goldberg, 2016; A-Elgayoum, El-feki, Mahgoub, El-Rayah, & Giha, 2009). The WHO therefore recommends against this practice when and where malaria diagnostic tests are available (WHO, 2011).

In this study, the diagnosis of malaria was not based on symptoms but rather on laboratory methods such as rapid diagnostic test, microscopy and PCR. In the SP efficacy study, the criteria for selection required that only asymptomatic yet parasitaemic pregnant women should be included in the study. For ethical reasons, symptomatic (with temperature > 37.5° C) and parasitaemic pregnant women in this trial were excluded from the study and put on treatment of malaria according to national guidelines.

Light microscopic examination of stained thick and thin blood films is currently the gold standard method for laboratory diagnosis of malaria in clinical settings. The techniques involved, require considerable expertise and adequate quality control. On the other hand, its reliability is questionable, particularly at low levels of parasitaemia and in the interpretation of mixed infection (Hamer et al., 2007; Murray, Gasser, Magill, & Miller, 2008). However, relative to other more sophisticated methods of diagnosis like the molecular methods and histopathology, microscopy involves less equipment; the major equipment being the light microscope. The thick blood film is for

establishing the presence of the parasite and quantifying same whilst the thin film is for parasite speciation. Microscopy can detect parasitaemia as low as 5 parasites/µl (Warrel and Gilles, 2002). A more realistic parasite density threshold of 50-100 parasites/µl is more applicable under field conditions (WHO, 1998; Milne, Chiodini & Warhurst, 1994). Meanwhile, Wongsrichanalai et al (2007) put the threshold at 10-100 parasites/µl.

Sensitivity of microscopic slide evaluation varies in estimation. Given that the expertise of the microscopist is unquestionable, variation in the sensitivity is dependent on the type of infecting species, geographic area, and other factors, but in general diagnostic sensitivity is thought to be no higher than 75% (Wilson, 2011). This figure is based on the rate of detection of parasitaemia in patients with clinical malaria. For patients with non-*falciparum* malaria, low-level parasitaemia, or partial immunity, or those who have been partially treated for malaria, the diagnostic sensitivity is likely to be even lower than 75% (Wilson).

Microscopy however offers significant advantages over other methods. These advantages include; (i) that it allows for definitive species identification as well as mixed infections; (ii) can be used to quantify the parasite density; (iii) can be used for serial examinations to monitor the efficacy of therapy; (iv) requires little laboratory infrastructure; and (v) is comparatively inexpensive. In this study, microscopy was employed as one of tools for malaria diagnosis because apart from being the gold standard for malaria diagnosis in the clinical setting, the microscopes as well as the requisite expertise were readily available. The use of microscopy also helped us to monitor therapy in the SP efficacy trial.

Disadvantages of microscopy include: (i) it does not detect very low parasitaemia; (ii) errors in interpretation are most common with either very low or very high parasitaemia (for which accurate diagnosis is very important); (iii) mixed infections are often missed; and (iv) it is not as useful in areas without endemic malaria because of the inability of persons reading smears to remain sufficiently competent to make accurate and reproducible diagnoses. Also, poor blood film preparation generates artefacts such as bacteria, fungi, stain precipitation, dirt, and cell debris which are commonly mistaken for malaria parasites (Ohrt et al., 2002).

To improve the specificity of microscopy, fluorescent-based staining and fluorescent microscopy are now introduced in clinical laboratories but are expensive. In this study, light microscopy was used as the gold standard for diagnosing malaria and therefore recruiting participants. This was because the microscope and the expertise required were readily available. Other diagnostic tests have been produced to be used in conjunction with or independent of the light microscopy. Examples include the detection of malaria antibodies by indirect immune-fluorescence antibody assay (IFA) and enzyme linked immune-sorbent assay (ELISA) (Sulzer, Wilson & Hall, 1969; Spencer, Collins, Chin & Skinner, 1979).

There are also tests for the detection of malaria antigens by an immune-chromatographic assay. This forms the basis for malaria rapid diagnostic test (MRDT) kits (Moody and Chiodini, 2002; Shiff, Minjas & Premji, 1994). Rapid diagnostic tests kits are currently very much available. They utilize monoclonal antibody to a parasite antigen placed on an immune-

chromatographic strip to detect the presence of parasites in peripheral blood (Bronzan, McMorrow & Kachur, 2008; Wongsrichanalai et al., 2007).

According to Murray et al (2008), an optimal Rapid Diagnostic Test should (i) use simple technology; (ii) should be readily learned by users; (iii) should have results that are easy to interpret (by both users and by providers who ordered the test); (iv) should be reproducible; (v) should not require electricity to run the assay; (vi) should not require refrigerated storage; and, obviously, (vii) should be rapid. These characteristics of the Rapid diagnostic test kits make them useful in resource poor settings, which is the case of most malaria-endemic regions of the world.

Current MRDTs are based on detection of 3 different types of *Plasmodium* antigens (Bell, Wongsgrichanalai & Barnwell, 2006; Murray et al., 2008). The first is *Plasmodium* histidine rich protein (HRP) 2 *(p*HRP-2), which can be specific to *P. falciparum* or *P. vivax*. The second is to *Plasmodium* lactate dehydrogenase *(p*LDH), which can be specific to *P. falciparum* or *P. vivax* or be a variant that is common to all *Plasmodium* species (pan-specific). The last is *Plasmodium* aldolase, which is pan-specific. By combining detection of these 3 antigens on an immune-chromatographic strip (ICS) assay, MRDTs have been developed that can be used to detect any malaria species: *P. falciparum* alone, *P. vivax* alone, or any combination thereof (Bell et al., 2006; WHO. 2011).

Using these 3 antigens however results in some diagnostic limitations. Firstly, none of the 3 antigens is specific for *Plasmodium ovale*, *Plasmodium malariae*, or *Plasmodium knowlesi*; secondly, there are variants of *P. falciparum* in South America that do not produce the 2 most common types of

HRP (*P. falciparum* HRP [*pf*HRP]-2 and *pf*HRP-3), which means that MRDTs based on detection of those antigens would not be useful in that region (Gamboa et al., 2010); and thirdly, cross-reactions with a *pf*HRP-2 assay have been reported from patients with *Schistosoma mekongi* infection (with no cross-reaction with a *p*LDH assay) (Leshem et al., 2011); fourthly, cross-reactions with some assays have been reported for patients with rheumatoid factor or other circulating auto-antibodies (Maltha et al., 2010) ; Again, patients with high levels of *P. falciparum* parasitaemia may give false-positive results with *p*LDH assays designed to detect *P. vivax* (Maltha). Also, unlike microscopy, MRDTs cannot be used to determine the magnitude of parasitaemia; and finally, because *p*HRP-2 is not cleared from blood for up to 30 days after treatment, MRDTs that test for this antigen should not be used to monitor response to therapy (Bell et al., 2006; Murray et al, 2008 & Abba et al, 2011). Although *p*LDH and aldolase are cleared quickly from blood after treatment, gametocytes are not eliminated with standard antimalarial therapy and continue to produce all 3 antigens. As a result, assays testing for these 2 antigens should also not be used to monitor response to therapy (Murray et al., 2008).

In spite of these limitations, MRDTs are more sensitive tests than microscopy. For instance, together, assays that test for HRP-2 showed (meta-analytical) mean sensitivity a of 95.0% and 93.2% for assays that test for LDH (Abba et al., 2011) compared to microscopy that has sensitivity no better than 75-90% even when expertly done (Barat et al., 1999; Reyburn et al., 2004; Reyburn et al., 2006).

Histopathology, in spite of its primary advantage of characterizing the disease process, is not a first-line diagnostic method in malaria control programs. This is because it is insensitive for detecting parasites, unable to identify species and it is too slow and too expensive. Histopathology is however an important diagnostic method for determining the cause of cases of fever of unknown origin in situations where there are multiple causes of febrile illnesses. It is also a very important tool in the diagnosis of placental malaria. It is again useful in autopsies and determining cause of death. Histopathology contributes to public health epidemiology by its usage in the quality control of research in malaria diagnosis and treatment.

In placental malaria, evidence of *Plasmodium* (most commonly mature parasites) or *Plasmodium* products (e.g., hemozoin) in the placenta inter-villous space is a defining feature. There may also be accumulation of maternal immune cells (predominantly monocytes, macrophages and neutrophils) in the placenta and increased levels of inflammatory cytokines in placental malaria.(Abrams et al., 2003; Bayoumi et al., 2009 & Rogerson et al., 2003). Placentas affected by Placental Malaria (PM) can also show other signs of injury including syncytial knotting, thickening of the basement membrane of the trophoblast layer and fibrinoid deposits (Walter, P. R, Garin, Y. & Blot, P. 1982; Ismail et al., 2000).

Tissues for histopathology studies are usually preserved in 10% neutral buffered formalin. Processing of the tissues for analysis involves paraffin embedding and sectioning to about 5μm thickness using standard techniques. The tissue sections are now stained with Haematoxylin-Eosin (H&E) (other stains that can be used are Masson's trichrome stain (MTS) or Geimsa) and

viewed under light microscope usually equipped with Zeiss Axio Cam HRc camera. Some of the tissue parameters canbe evaluated and analyzed using Image J software.

In this study, diagnosis of placental malaria was not made by histopathology, but rather by microscopic examination of placenta villous samples and by polymerase chain reaction (PCR). This was because resources required for histopathological diagnosis were not available.

Diagnosis of malaria using nucleic acid amplification tests is still confined to reference laboratories and for research or epidemiologic purposes. It is expensive to establish nucleic acid amplification laboratories. It also requires highly skilled manpower to undertake such exercises and these are not available in poor regions of the world. The long turnaround time to produce results from these nucleic acid amplification tests make them unattractive for regular clinical diagnosis and for malaria control purposes.

There are two main nucleic acid amplification tests for the diagnosis of malaria; first is the conventional polymerase chain reaction (PCR), which is increasingly becoming the gold standard for malaria diagnosis in research and reference laboratories (Brozan et al., 2008) and a more recent one, the loop mediated isothermal amplification (LAMP). Nucleic acid amplification methods are more sensitive: They are able to detect very low levels of parasitaemia. Some PCR assays can detect less than 1 parasite/μL as the technique validated by Cnops and others in 2011. Not only are nucleic acid amplification tests very sensitive, they also very useful tools for speciation of the *Plasmodium* parasites. For instance, it was a PCR-based test that helped to identify *P. knowlesi* in humans which had been misdiagnosed using

microscopy (Cox-Singh et al., 2008). The procedures for the conventional PCR could be quite laborious and has a longer turn-around time in generating results. It targets mostly genus-or species-specific sequences of the *Plasmodium* 18S subunit rRNA gene. It must be noted that there is also a newly developed single direct PCR for the diagnosis of malaria (Eeheverry et al., 2016). This method amplifies sequences in the cytochrome oxidase III (COX-III) genes. This PCR does not require DNA extraction, it is sensitive, quick, produces clear results, identifies all the *Plasmodium* species infecting humans, it is cost-effective and can be used on when dealing with larger numbers of samples (Echeverry) This method of diagnosis was more sensitive compared to the conventional nested PCR, being as sensitive as detecting 0.6-1 parasite/µl.

The LAMP is a more recent nucleic acid amplification method of diagnosing malaria. It usually gives results in a shorter duration, usually less than 60 minutes and the real-time PCR equipment used is also handy (Lucchi et al., 2010). Detection of positive samples can be by either fluorescence (which can be in-built in the real-time PCR equipment) or by UV light. These characteristics make this method of diagnosis suitable for field studies, relative to the conventional PCR. LAMP has similar sensitivity compared to the conventional PCR method of malaria diagnosis. Lucchi et al (2010) found out that the sensitivity and specificity of Real-Time Fluorescence Loop Mediated Isothermal Amplification (RealAmp) in detecting *P. falciparum* was 96.7% and 91.7% respectively when compared to microscopy and 98.9% and 100% respectively when compared to a standard nested PCR method. In addition, the

LAMP method consistently detected *P. falciparum* directly from boiled blood samples.

Epidemiology of Malaria

Though significant successes have been achieved in the control of malaria, it still accounts for huge medical, social, and economic burdens worldwide. Globally, the number of malaria cases fell from an estimated 262 million in 2000 to 214 million in 2015, a decline of 18% (WHO, 2015). Most cases in 2015 are estimated to have occurred in sub-Saharan African (88%), followed by the South-East Asia (10%) and the Eastern Mediterranean Region (2%)(WHO). The global malaria burden is therefore still concentrated in these three regions of the world (as shown in Figure 1). The incidence of malaria, which takes into account population growth, is estimated to have decreased by 37% between 2000 and 2015. In total, 57 of 106 countries that had ongoing transmission in 2000 have reduced malaria incidence by more than 75%. A further 18 countries are estimated to have reduced malaria incidence by 50–75%. Thus, the target of Millennium Development Goal (MDG) 6 "to have halted and begun to reverse the incidence of malaria" (Target 6C) has been achieved (WHO, 2015).

The number of malaria deaths globally fell from an estimated 839 000 in 2000 to 438 000 in 2015, a decline of 48%. Most deaths in 2015 (90%) occurred in the WHO African Region followed by 7% in the WHO South-East Asia Region and 2% in WHO Eastern Mediterranean Region. The malaria mortality rate, which takes into account population growth, is estimated to have decreased by 60% globally between 2000 and 2015 (WHO, 2015).

In Ghana, about 8.4 million cases of OPD malaria were recorded in 2014. This is a decline in comparison to the 11.4 million cases recorded in 2013, a reduction of approximately 23.6% (Ghana Health Service Annual Report, (GHS) 2014). About thirty percent of all OPD cases were malaria; 27.3% of all admission cases were attributable to malaria and about 7.2% of all deaths on admission were from malaria in the same year (GHS).

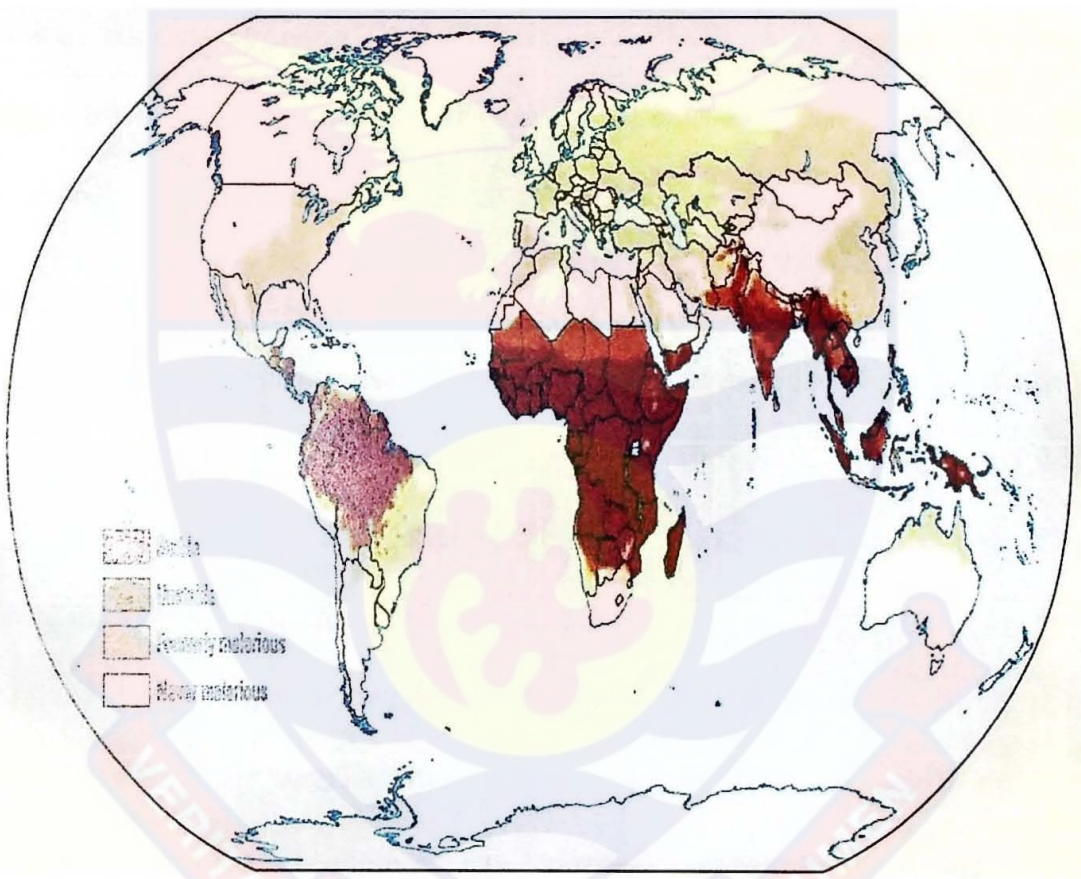

Figure1: Global malaria burden (Source: hptts//ourworldindata/malaria. Retrieved in December 2016)

Malaria Control

Since the year 2000, there has been a World Health Organization led concerted efforts towards malaria control. Although significant successes have been chalked with these malaria control interventions, there still remains a lot more that needs to be done, especially in the endemic regions of the world: Malaria still remains a disease of major public health concern. In the absence

of an effective malaria vaccine to date, for malaria eradication in most endemic regions in the world, these malaria control interventions remain key in our bid to minimize morbidity and mortality due to malaria. The Malaria control interventions include: (i) vector control (which reduces transmission of parasites from humans to mosquitoes and then back to humans), which is achieved largely through the use of insecticide-treated mosquito nets (ITNs) or indoor residual spraying (IRS); chemoprevention (which suppresses blood-stage infection in humans); and case management (which includes prompt diagnosis and treatment of infections (WHO, 2015).

Vector control measures

Historically, successful eradication of malaria in various parts of the world was achieved mainly by vector control (Harrison, 1978). Armed with this knowledge, the WHO and its partners have made vector control one of the focal interventions in its malaria control efforts.

Malaria vector control include all measures that are aimed at minimizing transmission of the parasite from humans to the Anopheles mosquito and back to humans and thereby reducing the incidence and prevalence of the disease and associated morbidity and mortality.

The two main vector control measures being promoted by the WHO in its Global Malaria Programme are the use of Insecticide Treated Nets (ITNs) and the Indoor Residual Spraying (IRS). Less promoted methods of vector control include space spraying, larval control, environmental management, the use mosquito repellents and the wearing of clothing that give adequate covering especially during dusk to night (Raghavendra et al., 2011).

Insecticide Treated Nets (ITNs)

Bed nets act as physical protective barriers against mosquito bites. But untreated bed nets in themselves have not demonstrated significant protective efficacy (White, 2009). When treated with pyrethroid insecticides (introducing a chemical barrier) however, mosquito nets give as twice the protection given by untreated nets and give more than 70% protection compared to a situation where no mosquito bed nets are used (D'Alessandro et al. 1995).The enormous benefit of using of ITNs, that is in reducing the mosquito-human contact thereby reducing malaria morbidity and mortality especially in sub-Saharan Africa is well documented (Curtis and Mnzava, 2000; Lengeler, 2004; Bhattarai et al., 2007; Kleinschmidt et al., 2009; WHO, 2010; Eisele et al., 2012; WHO, 2013). Not only do the ITNs decrease the human–mosquito contact by deterrence or excito-irritability, they also kill the mosquito with their residual insecticidal activity, ITNs by these effects reduce density, feeding frequency, feeding success, and survival of Anopheles mosquito vectors (Giming et al., 2003; Lindblade et al., 2004 & Russell et al., 2010). By reducing vector densities and survival, ITNs do not only decrease malaria exposure for the protected individuals, but also provide protection to the rest of the local human community (Binka, Inkome & Smith, 1998; Howard et al., 2000; Maxwell et al., 2002 & Hawley et al., 2003) when a particular threshold of bed net coverage is reached (Killeen et al 2007). White, in 2009, also found out that, the protection afforded by not sleeping under a net in a community where ITNs are extensively used is greater than that offered by sleeping under ITN in a community where no one uses ITN.

A single impregnation of a mosquito net would provide protection for about a year. (Lindsay & Gibson, 1988; Alonso et al., 1991) after which time the net should be retreated. Currently however, the WHO recommends the use of long-lasting insecticidal nets (LLINs) instead of the ITNs. This is because the LLINs maintain a longer biological activity against the vector even after a number of wash. The biological activity generally lasts as long as the net itself (3–4 years for polyester nets and 4–5 years for polyethylene nets) (WHO 2005). Pyrethroids are the only insecticides that have been used for impregnation of bed-nets due to a very low mammalian toxicity with added rapid knock-down effect and increased residual activity (Raghavendra et al., 2011).

Sustained advocacy and heavy financial investments by WHO and partners as well as governments, have led to a marked increase in the proportion of the population sleeping under an ITN in sub-Saharan Africafrom less than 2% in 2000 to an estimated 46% in 2014and 55% in 2015 (WHO, 2015). The proportion of children aged under-five years in sub-Saharan Africa sleeping under an ITN increased to an estimated 68% in 2015. Although these results represent a substantial increase since 2000, they fall short of universal (100%) coverage of this preventive measure. Ghana's current universal coverage for ITN is said to be 96% and the percentage number of people with access to ITNs is 77% (WHO, 2015).

ITN usage in pregnancy

The WHO together with the Roll Back malaria programme (RBM) advocates for the use of LLINs as one of the main strategies for the control of malaria in pregnancyin addition to IPTp-SP, prompt diagnosis and effective

treatment. LLINs are therefore distributed either for free or heavily subsidized to pregnant women in regions with stable malaria transmission through mainly antenatal clinics or mass nets distribution campaigns. There is a very strong evidence for the efficacy of ITNs in preventing malaria infection and its consequences in pregnancy, as reported by Gamble et al in a Cochrane review in 2009 and in a more recently by Eisele et al in2012 in meta-analysis, which examined malaria prevention in pregnancy data sets from different African nations. Those analyses proved a strong correlation between the use of ITNs and reduction in stillbirths, improvements in birth weights of babies and a reduction in the prevalence of parasitaemia and anaemia in pregnant women.

Indoor Residual Spraying (IRS)

This involves application of long acting chemical insecticides (by spraying) on walls and roofs of houses and domestic animals shelters to kill adult mosquitoes as they land on these treated surfaces (WHO, 2011). Indoor residual spraying has been very effective in interrupting malaria transmission in the 1940s and 1950s (Russell, 1955; MacDonald, 1957). It helped to eradicate malaria from Europe, the former USSR and several countries in Asia and the Caribbean (WHO, 2006). However, the implementation of indoor residual spraying for malaria control and possible eradication of malaria in Africa has not seen much success (Garret-Jones, 1964; De Zuleta et al., 1964; Kouznetsov, 1977).

In spite of several numbers of evidences supporting the efficacy of IRS, its usage has declined considerably. This is due to the lack of commitment on the part of government and concerns about resistance and community acceptance. The WHO is however advocating its reintroduction

and scaling up, especially in areas where there is proven susceptibility of the mosquito to the insecticide.

Effective case canagement of malaria

This arm of malaria control involves the prompt diagnosis of malaria and the use of efficacious drugs for treatment according to national guidelines. Diagnosis of malaria is both clinical and by laboratory methods. The WHO currently emphasizes the need for all clinically suspected cases of malaria to be first confirmed by laboratory methods, mainly RDT and microscopy after Geimsa before treatment is instituted. A T3 (Test, Treat, Track) initiative has therefore replaced the previous presumptive treatment national anti-malaria drug policy in most malaria endemic countries. Drug of choice for treatment is dependent on whether the malaria is uncomplicated or complicated and whether the infection is occurring in a pregnant woman or not, and if pregnant, at which gestational age. These guidelines are all well spelt out in National Anti-Malaria Drug Policies for most countries where malaria is prevalent. They usually based on evidence from research and recommendations from WHO and the Global Malaria Programme (GMP).

Anti-Malaria Drug Policy for Ghana

This document is design by the Ministry of Health in collaboration with the Ghana Health Service, a technical select committee and international health partners. The overall aim of the policy is to provide prompt, safe, effective and appropriate anti-malaria therapy to the entire population (MOH, 2014). Specific policy objectives are:

1. Prevent deaths and complications arising from malaria
2. Shorten the clinical episode of malaria

3. To reduce the consequences of placental malaria infection and maternal associated anaemia through chemoprevention and intermittent preventive treatment during malaria.
4. To delay spread of resistance to anti-malaria medications.

Recommendations for treatment in the revised policy are as follows:

Management of uncomplicated malaria

It is important to note that the T3 initiative must be implemented in all clinical scenarios. If uncomplicated malaria is confirmed in the non-pregnant individual, Artemisinin based combination Therapies (ACTs). The three main ones are Artesunate-Amodiaquine (AA), Arthemeter-Lumefantrim (AL) and Dihydroartemisinin piperaquine (DHAP). Artesunate-Amodiaquine is the drug of choice for children under 5 years. Meanwhile, the treatments of choice for uncomplicated malaria in the pregnant woman are as follows: Oral quinine followed by oral clindamycin in the first trimester. Uncomplicated malaria occurring after the first trimester can be managed with Oral quinine plus oral clindamycin as well as Artesunate-amodiaquine or Arthemeter-Lumefantrim. In the event of a treatment failure, the ACT that has not been used can be used. Oral quinine plus tetracycline or doxycycline or clindamycin can also be used. Treatment Failure occurring in the first trimester can be managed with either the ACT that has not been used or quinine. ACT (yet to be used) or quinine can again be used in infections occurring after the first trimester (MOH, 2014).

Management of complicated malaria

The drug of choice for the treatment of complicated malaria in the non-pregnant is parenteral Artesunate followed by a full course of oral ACT. Parenteral quinine followed by oral quinine is an alternate choice. The

protocol for treating severe malaria in the first trimester of pregnancy is parenteral quinine with a switch to oral quinine as soon as client can tolerate it. Parenteral Artesunate is the drug of choice for severe infections occurring after the first trimester.

Biology of the Malaria Parasite

The *Plasmodium* parasite

The malaria parasite is an obligate intracellular protozoan belonging to the genus *Plasmodium*. There are over 200 species of the *Plasmodium* genus (Igweh, 2012). There are six species known to cause human malaria (White, 2008; Cox-Singh et al., 2008)

Vectors of transmission

Human malaria parasites are transmitted by female mosquitoes belonging to the genus *Anopheles*. Male mosquitoes don't transmit malaria since they don't feed on blood. Only the females do, since they require protein in the blood for the development of their eggs.

There are over 460 named species of the *Anopheles* genus, but only approximately 70 are able to transmit human malaria (Harbach, 2009). Of these 70 species, 52 are said to be Dominant Vector Species (DVS) (Hay et al., 2010). These DVS are species (or species complexes) transmit the majority of human malaria parasites in an area by virtue of their abundance, their propensity for feeding on humans, their mean adult longevity (only old individuals incubate the parasite long enough to transmit the disease), or any combination of these and other factors that increase overall vectorial capacity. Of the DVS, the *A. gambiae* complexes are the most effective and efficient in the transmission of human malaria. This is due to the fact that they are

characteristically anthropophilic and endophilic. The *A. gambiae* complex therefore has the highest entomological inoculation rate in the world (Hay et al., 2010). They are found all over Africa especially in the South of the Sahara and prefer breeding sites represented by sunny and clean water pools devoid of vegetation (Esposito & Habluetzel, 1997). Other important DVS include; *A. arabienses, A. funestus,* complex *A. minimus* complex, *A. nili* and *A. moucheti* (Sinka et al, 2010).

At temperatures below 16°C or above 33°C and at altitudes greater than 200m above sea level, development of the *Plasmodium sp.* in the mosquito is halted (White, 2009). Malaria transmission therefore does not occur under such conditions. Conditions well suited for vector survival and parasite development are those of high humidity, optimal rainfall as well as ambient temperature between 20 and 30°C. These conditions are most prevalent in the tropics and subtropics making malaria endemic in these regions.

Life cycle

The *Plasmodium* parasite exhibits a complex life cycle. It involves a sexual phase (sporogony) in an insect vector (Anopheles mosquito) and an asexual phase (schizogony) in the vertebrate host (humans) as shown in Fig 2.

The infection is initiated when the female Anopheles mosquito, during a blood meal inoculates the human host with sporozoites which are found in its salivary glands. The Sporozoites are carried by the circulatory system to the liver where they invade hepatocytes. In the hepatocytes, the parasite undergoes

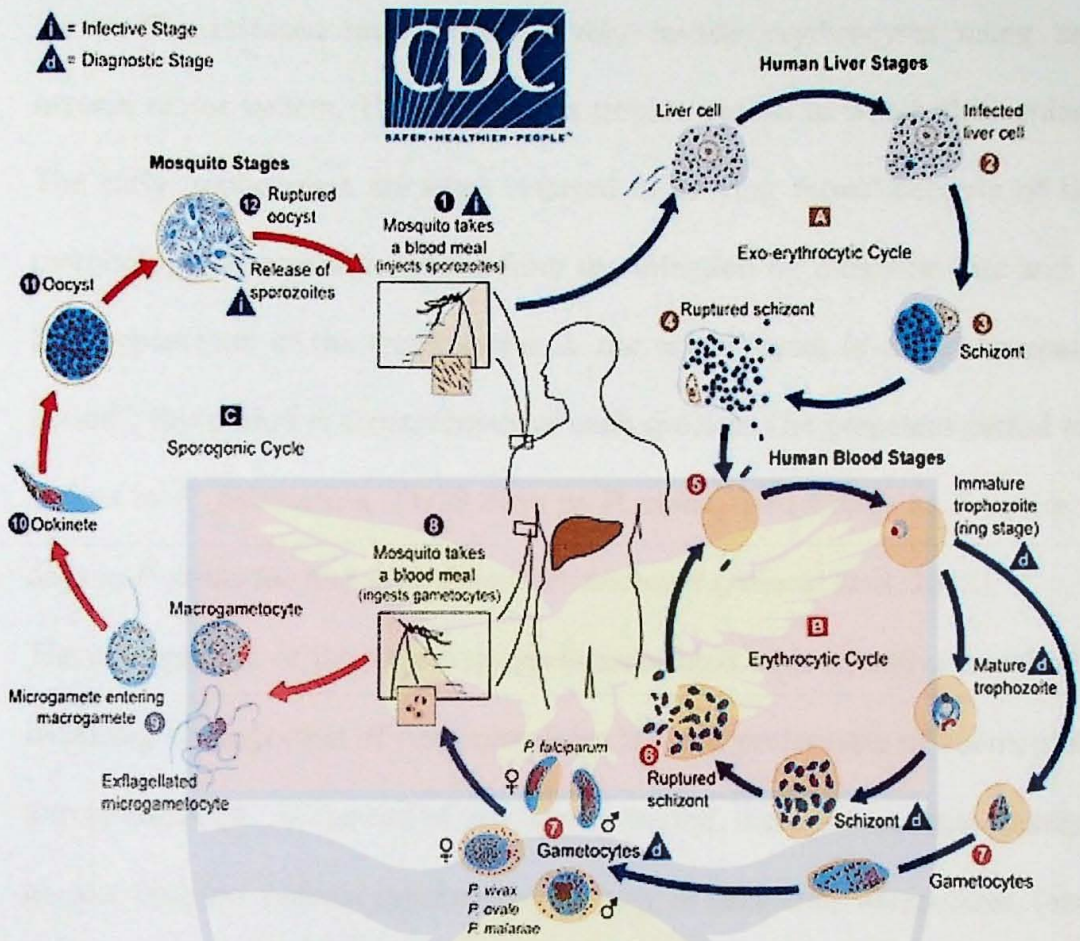

Figure 2: Life cycle of the *Plasmodium sp.* (Source: Centre for Disease Control. Retrieved on 8th December 2016).

an asexual replication known as exoerythrocytic schizogony becoming a tissue schizont that contains thousands of merozoites (about 10,000 in *P. vivax/P.ovale* and up to 30,000 in *P. falciparum*) (Atinori et al, 2012). This exoerythrocytic schizogony is characteristic of all the species of *Plasmodium* that cause human malaria and it lasts for a minimum of 6 days in the case of *P. falciparum* and a maximum of 16 days in the case of *P. malariae*.

A proportion of the liver-stage parasites from *P. vivax* and *P. ovale* go through a dormant period instead of immediately undergoing asexual replication. These are called hypnozoites. These hypnozoites will reactivate several weeks to months (or years) after the primary infection and are responsible for the phenomenon called relapse.

the female Anopheles sp during a blood meal to begin the sexual phase of the parasite's life cycle.

The gametocytes ingested by the mosquito are found within its midgut. Here, the male gametocyte undergoes a rapid nuclear division, producing eight flagellated microgametes. The macrogametocyte also transforms into a macrogamete. Fusion of the micro and macrogametes result in the formation of a motile zygote, the ookinete. The ookinete traverses the mosquito gut wall and encysts on the exterior of the gut wall as an oocyst. In the oocyst, the parasite undergoes nuclear proliferation (sporogony) to form thousands of motile sporozoites (Kayser et al., 2005). Soon, the oocyst ruptures, releasing the sporozoites into the coelomic cavity of the mosquito. They eventually migrate to the salivary glands of the mosquito from where they are inoculated into their human host during a blood meal generally in a number not exceeding 10-100 (Atinori et al., 2012) to begin the asexual cycle in humans. The development of the plasmodium parasite in the mosquito takes 8-35 days depending on the ambient temperature, species of the parasite and species of the vector (White et al., 2009).

Malaria in Pregnancy

Malaria in pregnancy (MiP) can be said to be peripheral and or placental infection with *Plasmodium* parasite during pregnancy. The condition poses significant public health challenges and has substantial risks for the pregnant woman, her foetus, and the new-born child. MiP is associated with mild to severe maternal illness, maternal anaemia, spontaneous miscarriage, stillbirth, preterm delivery and foetal growth retardation (Desai et al., 2007). *Falciparum* malaria is known to be the most important preventable cause of

low birth weight (resulting from maternal anaemia and placental parasitaemia) which is an important contributor to infant morbidity and mortality in Sub-Saharan Africa.

Epidemiological studies estimate 125 million pregnancies are at risk of malaria infection every year (Dellicour et al., 2010; WHO, 2012) within a proposed estimate of 32 million women who become pregnant every year in malaria endemic sub-Saharan Africa countries (WHO, 2012). In highly malaria endemic areas such as sub-Saharan Africa, up to 50% of low birth weight (LBW) deliveries can be attributed to malaria in pregnancy, usually as a consequence of foetal growth restriction (FGR) from placental malaria and maternal anaemia (Desai et al., 2007). Low birth weight is estimated to cause approximately 100 000 infant deaths annually (Desai).

In Ghana, Stephens et al (2014) found the prevalence of *Plasmodium falciparum* peripheral blood parasitaemia in a suburban coastal area to be 5% and placental parasitaemia and low birthweight in that same area were 2.5% and 3% respectively. Another study by Agbozo et al in 2016 found the prevalence of LBW from pregnancy associated malaria to be 9.6%.

Pregnant women have an increased risk of malaria infection, independent of previously acquired immunity (Ataide et al., 2014). Even in areas of high malaria endemicity, where adults are less susceptible to malaria infection, pregnant women are commonly more susceptible to *Plasmodium* infection, because, pregnancy causes a transient depression of cell mediated immunity impairing the pregnant woman's ability to limit parasite replication. Though debatable, increased in susceptibility as a result of hormonal changes has also been described in few studies. For example, increased cortisol levels during

pregnancy have been associated with increased risk of malaria in pregnant women (Boyou-Akotet et al., 2005; Takem & D'Alessandro, 2013). Malaria has also been associated with reduced oestradiol production in late pregnancy (Watkinson et al., 1985). Again, it has been reported that primiparous pregnant women below age 25 years and in their first trimester of pregnancy have even a more heightened risk of developing placental malaria (Uneke, 2008; Falade et al., 2010) compared to their multiparous counterparts. This was evident in a meta-analysis by Adegnika et al in 2011 on four sub-Saharan African countries, which demonstrated a reduction of placental malaria in multi-gravidae women. HIV infection also increases susceptibility to malaria, resulting in more prevalent and higher-density infection, and a relative loss of gravidity-dependent immunity (ter Kuile et al., 2004). In this study, HIV positive pregnant women were excluded. This is because their increase susceptibility to *Plasmodium* infection would have served as a confounding factor in determining the prevalence of malaria in pregnancy (ter Kuile et al., 2004). Also, HIV positive women are not to be put on IPTp-SP because of the possible drug-drug interaction of SP with co-trimoxazole which is given as prophylaxis against opportunistic infections in Persons Living with HIV/AIDS (PLWHA) (WHO, 2012). Multi-gravidae were not excluded in this study because the study was interested in determining how gravidity correlated with *Plasmodium* infection in pregnancy as well as anaemia in pregnancy.

The symptoms and complications of malaria in pregnancy are varied. They are dependent on the malaria transmission intensity in the given geographical area and the individual's level of acquired immunity. In regions of high endemicity, levels of acquired immunity tend to be high, *P. falciparum*

infection is therefore usually asymptomatic in pregnancy. Parasites may however be present in the placenta and contribute to maternal anaemia even in the absence of peripheral parasitaemia (Doolan, Dobano and Baird, 2009; Recker, Bouma, Bamford, Gupta & Dobson, 2009). In malaria-endemic regions of the world, primigravidae suffer the most adverse effects of *P. falciparum* infection in pregnancy because they lack immunity to placental antigens expressed by the *Plasmodium.*

In low-transmission settings however, where women of reproductive age have relatively little acquired immunity to malaria, malaria in pregnancy is associated with anaemia, an increased risk of severe malaria, and it may lead to spontaneous abortion, stillbirth, prematurity and low birth weight. In such settings, malaria affects all pregnant women regardless of their gravidity.

Infection with *P. vivax*, as with *P. falciparum*, leads to chronic anaemia and placental malaria infection, reducing the birth weight and increasing the risk of neonatal death. For women in their first pregnancy, the reduction in birth weight as a result of *P. vivax* infection is approximately two thirds of what is associated with *P. falciparum*. However, the effects of *P. vivax* infection appear to increase with successive pregnancies (ter Kuile & Rogerson, 2008).

The pathology of malaria in pregnancy

The pathology of *P. falciparum* infection during pregnancy may be a non-specific systemic infection which may be similar to those in the non-pregnant. However, *P. falciparum* infection during pregnancy commonly results in the development of placental malaria (PM). This occurs when there is an accumulation of *P. falciparum*-infected erythrocytes, trophozoites and

schizonts forms of the parasite in the placenta with its attendant immuno-pathological effects. *Plasmodium vivax* (which hitherto was thought not to sequester in the placenta) has also been shown to sequester in the placenta, (less commonly though) but it does not appear to induce significant histopathological changes and its clinical consequences are unclear (Rogerson et al., 2007 & Mayor et al., 2012). *P. falciparum* and *P. vivax* mixed-placental infection can occur as well (Carvalho et al., 2011).

The earliest time the placenta is susceptible to malaria infection during pregnancy is unclear. It was previously thought that maternal blood begins to flow into inter-villous spaces around 10–12 weeks of gestation (Gude et al., 2004). However, more recent studies indicate that perfusion of the placenta by maternal blood may occur as early as gestational week (GW) 6 (Merce, 2009). As pregnant women may be most susceptible to malaria infection at 9–20 weeks (Rogerson et al., 2007), this gestational window could represent the first opportunity for *P. falciparum*-infected erythrocytes to sequester in the placenta. Currently, IPTp-SP is administered from the second or third trimester onwards (WHO, 2015), therefore, early pregnancy may be an especially vulnerable time for both mother and foetus.

PM is characterized by pathological changes accompanying the accumulation of parasites, including the presence of hemozoin, infiltration of monocytes and macrophages, and deposition of peri-villous fibrin (Rogerson et al., 2007). More severe inflammation and destruction of the syncytiotrophoblast barrier has also been reported (Crooker, Tanner, Myers & Baker, 2004). Complete loss of villous integrity can also occur (Crooker et al, 2004). The pathology of placental malaria is at the heart of the foetal and

childhood consequences of malaria in pregnancy (Buffet et al., 1999; Scherf, Pouvelle, Buffet & Gysin, 2001).

Changes in the materno-foetal blood exchange form the basis of the pathology of placental malaria (Scherf et al 2001). As mentioned previously, infected erythrocytes (IE) of both *P. falciparum* and *P. vivax* can sequester within the placenta and accumulate in intervillous spaces during malaria infection in pregnancy. The presence of these IE activates mononuclear cells which release chemokines to recruit additional phagocytic cells into the inter-villous spaces. There is also an associated elevation of Tumor Necrosis Factor α (TNF α) and Interleukin 10 (IL-10) levels and have been implicated in poor pregnancy outcomes (Rogerson et al., 2007). Infected Erythrocytes, leukocyte infiltration, fibrin and hemozoin depositions contribute to increase the thickness of the trophoblast basement membrane and to alter the inter-villous and peri-villous spaces, causing reduction of oxygen and nutrient transport to the foetus (Uneke, 2007; Umbers et al., 2011).

P. falciparum IE adhere to chondroitin sulphate A (CSA) expressed in placenta; thus, placenta selects for strains of *P. falciparum* with a CSA-binding phenotype. Primigravidae who have not been exposed to *P. falciparum* CSA-binding phenotype are still susceptible to placental infection. A multigravidae would have however developed antibodies during the previous successful pregnancies and therefore less susceptible to placental malaria (Uneke, 2008). *P. vivax* parasitized erythrocytes can also cytoadhere to the placenta by mechanisms not yet clarified (Carvalho et al., 2010; Costa et al., 2011). Molecular studies have demonstrated that IE binding to chondroitin sulphate A (CSA), expressed on the apical membrane of the placental

syncytiotrophoblast epithelium, is mediated by var2CSA antigen, a variant of the *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) family. There are multiple genes encoding for different var2CSA antigens. A study performed in Cameroon shows that the parasites infecting pregnant women living in high transmission areas have an increased copy number of var2csa genes compared to non-pregnant women (Sander et al., 2011). The multiplicity of var2CSA-type genes confers to *P. falciparum* parasites a greater capacity for antigenic variation and evasion of immune response (Sander et al., 2011). Data from new studies suggest that there could possibly be a new and different pathway of adhesion of the IE apart from that demonstrated by the var2CSA (Bouef et al., 2011; Rovira-Vallbona et al., 2011). This however yet to be fully elucidated.

Complement activation seems to play an important role in the pathogenesis of placental malaria. This is evident by the elevation of the levels of C5a, a factor derived from the complement cascade, in peripheral blood and placental blood of pregnant women with malaria compared to pregnant women without malaria infection (Conroy et al., 2009).

Effects of malaria in pregnancy

As stated previously, malaria in pregnancy poses significant risk and consequences to mother, the foetus and subsequently the child. These consequences are largely influenced by factors such as intensity of malaria transmission in a setting, the species of *Plasmodium* involved in the infection, the age and parity of the infected pregnant woman and the gestational age at which infection occurs.

Maternal effects

In regions of the world where malaria transmission is stable such as in most parts of sub-Saharan Africa, most *Plasmodial* infections are asymptomatic. There is however an increase, substantially, in the risk of maternal anaemia. (Bouyou-Akotet et al., 2003 ; Targbor et al., 2010 ; Ayoola et al., 2011 & Huynh et al., 2011). Both symptomatic and asymptomatic infections can cause anaemia. Severe anaemia is more often observed in stable transmission settings (van Eijk et al., 2007; Tarimo, 2007 & Achidi et al 2005) and more in primigravidae than in multigravidae (Guyatt et al., 2001& Shulman et al., 2001). Malaria infections in the first or second trimester of pregnancy increase the risk of anaemia (Kalilani et al., 2011) though one study reported an increased risk of anaemia also for infections occurring in the third trimester (Huynh et al 2011). Malaria infection prevention by intermittent preventive treatment during pregnancy reduces the risk of anaemia significantly (Kayentao et al 2005; Gies et al., 2009 & Wilson et al., 2011).

Malaria can cause maternal anaemia in areas of unstable transmission as well (Luxemburger et al., 2001; Rodriguez-Morales et al., 2006; Poespoprodjo et al., 2008). It occurs more in primigravidae than in multigravidae and more for *falciparum* infections than for *vivax* infections (Guyatt et al., 2001& Poespoprodjo et al., 2008). Nevertheless, severe anaemia is less common in these settings (O'Donnell et al., 2007; Poespoprodjo et al., 2008).

The pathophysiology of anaemia as a result of malaria infections (though not fully elucidated) in pregnancy is no different from that of anaemia from malaria infection in the non-pregnant. However, the background

haemodilution from increased plasma volume in the state of pregnancy tend to worsen the anaemia from the malaria infection. Again, as mentioned previously, in poor regions of the world, co-morbidities such as geo-helminthes infections and nutritional deficiencies (Iron and folate and vitamin) may also worsen the severity of the anaemia. In this research work, participating pregnant women in both the SP efficacy study and the maternal and birth outcome study were on their routine micronutrient supplementation with iron and vitamins but not folate. This is because, folate at a dose equal or more than 5mg, given concomitantly with SP counteracts its effect as an antimalarial (WHO, 2012). Stool routine examination was also done for these women to rule out the confounding contribution of geo-helminthes to maternal anaemia.

Anaemia from malaria (especially acute *falciparum* infection) infection is due to an in increased removal from the circulation of parasitized and, to a greater extent, non-parasitized erythrocytes through a combination of splenic filtration (Buffet et al., 2011), schizont rupture, macrophage phagocytosis (Wickramasinghe & Abdalla, 2000), complement-mediated haemolysis (Woodruff, Ansdell & Pettitt., 1979) and increased free radical damage (Das & Nanda., 1999; Haldar & Mohandas., 2009). In more chronic infections, decreased marrow production of functional red blood cells due to the direct inhibitory effects of parasites (Abdalla et al., 1980) and cytokines (Wickramasinghe & Abdalla, 2000 & Nussenblatt et al., 2001) along with dysregulation of erythropoietin and iron metabolism (Chang & Stevenson, 2004) adds to the anaemia of ongoing red blood cell loss (Haldar & Mohandas., 2009).

Malaria seems to be known as a common cause of miscarriages in settings of unstable transmission. This is because the most malaria infections in these areas tend to end in a clinical disease with fever, which may by itself increase the risk of miscarriage. Indeed, non-malarial fevers do also increase the risk of miscarriages (Poespoprodjo et al., 2008; McGready et al., 2012). In areas of stable transmission however, little is known about malaria as a cause of miscarriage.

Apart from anaemia and miscarriages, a pregnant woman with malaria infection can also suffer known complications of malaria in the non-pregnant. This is more so commoner in pregnant women living in areas of unstable transmission and may be worse because of the transient reduction of cellular immunity. These complications include; convulsions, haemoglobinuria, hypoglycaemia, metabolic acidosis (associated with respiratory distress), acute pulmonary oedema, acute renal failure, jaundice, circulatory collapse, and electrolyte disturbance and spontaneous bleeding (WHO, 2000).

Malaria was an important cause and contributor to maternal death in some studies (Ramagosa et al 2007; Ali et al., 2012). This was further proven by the substantial reduction in maternal mortality observed in Thailand after the implementation of early detection and treatment of malaria (McGready et al., 2012). When not a direct cause of death, (that is from its complications) malaria in pregnancy is often reported as co-morbidity, in conditions (such as eclampsia) associated with maternal mortality (Adams et al., 2011).

Perinatal effects

Malaria in pregnancy increases the risk of low birth weight (Kassam et al., 2006; Cottrel et al., 2007; Huynh et al., 2011; Ayoola et al., 2012 & Valea

et al., 2012) particularly in primigravidae. This risk of LBW seems to be higher for infections occurring in the first or second trimester (Cotrell et al., 2007; Kalilani et al., 2010; Huynh et al., 2011 & Valea et al., 2012). White et al (2103) also reported an almost doubling of the risk of low birth weight with placental malaria in high transmission intensity areas. The effect being greatest during first pregnancies. Cotrell et al (2007) also found out that low birth weight from malaria infections could also occur with malaria infections in late pregnancy. In Ghana, Asundep et al (2015) reported a prevalence of low birth weight of 11.4% in pregnant women who did not take SP-IPTp compared to 8.4% in those who took SP-IPT in a study carried out in Kumasi. In high malaria transmission settings, LBW is largely due to intrauterine growth restriction (IUGR) rather than pre-term delivery.

The pathophysiology of malaria in pregnancy leading to IUGR and prematurity are poorly understood. IUGR is however thought to be due to immune-pathological consequences following the sequestration of IE and its attendant activation of both syncytiotrophoblasts and monocytes to release chemokines and cytokines. The consequence of this cascade is a reduction in placental growth; reduction of amino acids transport to the foetus; placental hypoxia and a reduction in the activities of Glucose Transporter 1 (GLUT1). These are thought to be the final pathways leading to IUGR (Rogerson et al., 2007). A meta-analysis of 32 cross-sectional data in Africa, showed malaria prevention in pregnancy is associated with 21% reduction in LBW (Eisele et al., 2012).

In areas of unstable malaria transmission, malaria in pregnancy has been associated with preterm deliveries (birth before 37 weeks of gestation),

still births and neonatal deaths (Poespoprodjo et al., 2008). How malaria in pregnancy causes preterm delivery (which has a high risk of death in early life) is not well understood. Preterm delivery is however closely associated with an increase malaria parasite density (Menendez et al., 2000; Tako et al., 2005 & Sullivan et al., 1999), anaemia, and high levels of TNFα and, in particular, interleukin 10 (Suguitan et al 2003). Regardless of parity, and transmission intensity, malaria infections by *P. vivax* are also associated with LBW. However when detected and treated earlier in pregnancy, *P. vivax* (or *P. falciparum*) infection has no significant effect on gestation or birth weight compared to women who never had malaria in their first trimester (McGready et al., 2012).

Effects on new-borns and infants

Cord blood infection with the *Plasmodium* parasite is quite common (Tobian et al., 2000 & Kamwendo et al., 2002). Foetal infection with the *Plasmodium* could be acquired by trans-placental micro transfusion antenatally (Malhrotra et al., 2006). Clinical disease in the newborn baby is however a rarity. This might be due to the fact that trans-placental transfer of variant-specific and other antibodies (e.g. to MSP1) probably protects the infant; although this is debatable (Riley et al., 2001; Branch et al., 1998). Under circumstances of low maternal immunity however, congenital malaria can present as a severe illness 2–6 weeks after birth, and infection in-utero has been associated with stillbirth (Rogerson et al., 2003). Congenital malaria is also known to contribute to infant morbidity and mortality (Bardaji et al., 2011).

In Ghana, Enweronu-Laryea et al, in 2013, reported 2.2% and 12% as the prevalence rates of congenital malaria (malaria in the first week of life) by microscopy and PCR respectively in new-borns in a teaching hospital in Accra.

Relatively, fewer studies have evaluated the impact of pregnancy associated malaria on infant outcomes. Low birthweight due to prematurity or intrauterine growth retardation of full-term babies is however known to be a significant risk factor for perinatal, neonatal and infant death (Sketetee et al., 2001; Singh et al., 2001., & Nosten et al., 1999); and a prominent cause of low birth weight is infection with *Plasmodium falciparum* during pregnancy (Singh et al., 2001; Nosten et al., 1999& Rodriguez-Morales et al., 2006). Malaria in pregnancy resulting in placental malaria has hence, in a number of studies, been shown to increase the risk of infant's death and perinatal mortality, by causing LBW (Luxemburger et al., 2001; Bardaji et al., 2011& Diamond-Smith et al., 2009). This is confirmed by the reduction neonatal mortality, up to 60%, observed after the implementation of preventive interventions targeted to pregnant women, e.g. intermittent preventive treatment (Titaley et al., 2010; Menendez et al., 2010). In another study in primi- and secundi-gravidae, malaria prevention with IPTp or insecticide-treated bed nets was significantly associated with an 18% decreased risk of neonatal mortality (Eisele et al., 2012).

Though the effects of malaria in pregnancy in neonates and infants have been mainly linked to LBW from IUGR and prematurity, other effects on neonates and infants have been reported in other studies independent of LBW. For instance, in 2010, a study in The Gambia has showed that malaria

infection during pregnancy influences infant's growth, independently of LBW (Walther et al., 2010). Placental malaria is also known to decrease trans-placental transfer of maternal IgG antibodies to non-malarial antigens (e.g. measles and Streptococcus pneumonia tetanus) and may make neonates and infants more susceptible to these infections (Okoko et al., 2001; de Moraes-Pinto et al., 1998 & Desai et al. 2007). Much more studies are required to further elucidate this phenomenon.

The fundamental question which is yet to be answered is whether in-utero exposure to malaria alters the long-term development of immunity to malaria. A recent cohort study in Kenya reported that infants who were exposed to malaria in-utero failed to mount malaria-specific immune responses in cord blood and exhibited lower malaria-specific Th1 responses at follow up (Malhotra et al., 2009) as well as higher vulnerability to malaria during early childhood (Malhotra et al.,2009). One possibility is that Tregs (Regulatory T cells) induced in utero may persist long term, and inhibit priming of an effector response to malaria into childhood (LaBeaud et al., 2009). Foetal tolerance therefore, as a result of in utero exposure to parasite antigens appears to be an impediment to protective antimalarial immunity especially in high prevalence settings. The effect of placental malaria on transfer of antimalarial antibodies is less clear. Infection leads to higher antibody titres, increasing antibody transfer to the infant. However, the relative proportion of antibody transferred might be decreased, and transfer of IgG1 and IgG2 subclasses could be particularly affected. How placental malaria influences susceptibility or otherwise needs to be further studied.

Effects on later-childhood, adolescence and adulthood

The long-term impact on health of intrauterine exposure to malaria has not been fully established by concerted research. Malaria in pregnancy causes IUGR and Prematurity leading to LBW. Low birth weight children have an increased incidence of hypertension in later life (Wadsworth etal., 1985; Luyckx & Brenner, 2015; Lewandoski et al., 2015 & Bertagnolli et al., 2016). In a study conducted in Ibadan, Nigeria, infants of mothers who experienced malaria during pregnancy had a higher increase in Blood Pressure levels during the first year of life compared with those who did not (Ayoola et al., 2014). Blood pressure levels track strongly through to adulthood. Such differences in blood pressure could therefore influence theprevalence of adult hypertension significantly (Cruickshank et al., 2005; Juhola et al., 2011; Chen & Wang, 2008). In spite of these strong assertions, a cause and effect relationship between malaria and hypertension is yet to be established by research. For now, it only remains a hypothesis.

Malaria is also associated with hypertensive disorders of pregnancy such as gestational hypertension and preeclampsia especially in young primigravidae (Ndao et al., 2009; Duffy et al., 2007; & Muehlenbachs et al., 2006) and these are also risk factors for low birth weight (Mol et al., 2016).

Other later-life health conditions that placental malaria has been associated with, which are directly or indirectly linked to LBW include; stunting and malnutrition in childhood (Snow et al., 1997 & Kang et al., 2013); neurocognitive and neuropsychiatry disorders (Tran et al., 2016); proteinuria and kidney disease (Luyckx et al., 2013); some cancers and the metabolic syndrome (Christensen et al., 2011).

Public Health Concerns of Malaria in Pregnancy

Malaria in pregnancy still remains a great public health concern. This is so, not only because of the huge burden of the disease and the cost of control but also because the consequences of the disease are of two-fold; it impacts on both mother (on one hand) and foetus, new born, infant, child and even adult (on the other hand). Other areas of growing concern are the resistance of the *Plasmodium* parasites to the sulphadoxine and pyrimethamine being used as IPTp and the resistance of the malaria vector to the insecticides used in treating the bed nets.

Globally, there has been a remarkable achievement in terms of the reduction of the overall number of cases of malaria and reduction in the number of deaths from malaria (WHO, 2015). However, progress in the prevention of malaria in pregnancy during the same time period has been less remarkable (Chico et al., 2015). The burden of malaria in pregnancy is still huge. There are still some 125 million pregnancies are at risk of malaria infection every year (Dellicour et al., 2010; WHO, 2012). In 2012, it was estimated that (based on point prevalence) the peripheral and placental prevalence of malaria parasites in pregnancy in East and Southern Africa as 32% and 26 % respectively; while prevalence of peripheral and placental malaria parasites during pregnancy in West and Central Africa were 38 % and 40 %, respectively (Chico et al., 2012). It can therefore be estimated from these findings that at least one in every four pregnant women in areas of stable *P. falciparum* transmission in Africa will have evidence of malaria infection during her pregnancy at delivery.

In areas of unstable malaria transmission, such as the Asian-Pacific region, prevalence of malaria is lower. It has been estimated to be 15% and 8% for peripheral and placental malaria respectively (Rijken et al. 2012) base on cross-sectional and longitudinal studies conducted over the past 30 years. These figures indicate that the fight against malaria in pregnancy is far from being over and that malaria in pregnancy remains a great public health challenge. This huge malaria burden requires a colossal amount financial and human resource to control the disease.

Malaria infection in pregnancy impacts on both mother and foetus with dire consequences. These rather overwhelming consequences of malaria in pregnancy make it a great public health concern. If we are therefore able to halt the transmission of malaria during pregnancy, we can save both mother and child from morbidities and mortality associated with it.

Other areas of concern are the relatively low coverage of IPTp-SP and LLINs usage in SSA; the growing resistance of the *Plasmodium* parasite to SP and the reported resistance of Anopheles to pyrethroid, the insecticide of choice for malaria vector control.

The proportion of pregnant women receiving at least three doses recommended by WHO in 2012 (WHO, 2012) of intermittent preventive treatment in pregnancy (IPTp) is still at a low of 17%. Increasing this would require educational campaigns involving all stake holders both locally and internationally.

Though ITN usage has increased markedly in sub-Saharan Africa, from less than 2% in 2000 to an estimated 46% in 2014 and 55% in 2015 (WHO, 2015), we still fall short of universal (100%) coverage of this

preventive measure. More aggressive educational campaigns involving members of the community would be required if we are to reach the stipulated target of 100% coverage.

Pyrethroids are the only insecticide recommended for treating bed nets and in indoor residual spraying (Zaim et al., 2000). Pyrethroids are also widely used in the control of agricultural pests worldwide (UN, 2006). There has been increasing reports of pyrethroid resistance in malaria vectors over the past decade (Santolamazza et al., 2008). Pyrethroid resistance in *A. gambiae* was first reported in Cote d'Ivoire in 1993 and is now widespread throughout the western (Corbel et al., 2007; Yawson et al., 2004) and central regions Santolamazza et al., 2008; Awolola et al., 2002) of Africa. In Eastern and Central Africa, *An. gambiae s.s.* and *An. arabiensis* populations are mostly susceptible to pyrethroids in Tanzania (Kulkarni et al., 2007), Mozambique (Coleman et al., 2008) and Madagascar (Ratovonjato et al., 2003) but pyrethroid resistance has been reported in Uganda (Verhaeghen et al., 2010 & Verhaeghen et al., 2006), the Gwave region of Zimbabwe (Muhenga et al., 2008) Sudan (Abdallah et al., 2008 & Himeidan et al 2007) and Ethiopia (Balkew et al., 2003 & Balkew et al 2010). There is therefore the urgent need on mobilizing resources for the development of suitable alternative insecticide(s) for vector control.

Another public health concern regarding malaria in pregnancy is the increasing reports of *P. falciparum resistance* to SP. SP resistance is said to have emerged, originating at the Thailand-Cambodia border and then spreading rapidly to other Asian countries and subsequently to Africa (Barat & Bloland, 1997). This development poses great threats to the whole IPTp-SP

programme. There is an urgent need research into possible alternatives if this trend should continue.

Intermittent Preventive Therapy for Malaria in Pregnancy

IPTp-SP is one of the major interventions in the control of malaria in pregnancy. It involves giving therapeutic doses of SP intermittently (at monthly intervals) to pregnant women whether they have malaria infection or not under the direct observation of a healthcare worker. The aim of the IPTp-SP is to reduce the burden of malaria in pregnancy thereby minimize its effects on both the pregnant mother and the foetus.

History of intermittent preventive therapy

Historically, the first malaria in pregnancy preventive strategies in Africa using chemotherapeutic agents, were implemented in the 1950s (Briand et al., 2007). They consisted of weekly or bi-monthly chemoprophylaxis with chloroquine (CQ) in West African countries and dapsone-pyrimethamine or sulphadoxine-pyrimethamine in East African countries. A large number of trials demonstrated the efficacy of such chemoprophylaxis in preventing low birth weight, maternal anaemia and placental malaria infection (Garner & Gulmezoglu, 2003; Dagne et al., 2002 & Denoeud et al., 2007). Unfortunately, because of the growing resistance of parasites to these drugs and the poor compliance of the women with the treatment, the strategies finally showed a low efficacy (Nahlen et al., 1989 & Simira et al., 2003). Studies that compared prophylaxis with CQ and intermittent administration of treatment doses of SP showed that apart from lower cost and higher compliance SP was significantly superior to CQ at reducing the rate of placental infection, anaemia and low birth weight (Kayentao et al., 2007; Tukur et al., 2007 & Schultz et al., 1994).

Owing to the wide spread resistance of the *Plasmodium* to chloroquine, it was proposed in 1998 and finally implemented in 2004, that chemoprophylaxis should be replaced by intermittent preventive treatment with Sulphadoxine and pyrimethamine for all pregnant women living in areas of stable malaria transmission (WHO, 2004). IPTp-SP is therefore currently used in most parts of Africa where there is stable malaria transmission

Current proposals on IPTp-SP implementation

Prior to these current proposals, IPTp-SP consisted of the administration of a single curative dose of Sulphadoxine-pyrimethamine (SP) (1500mg/75mg) at least twice during pregnancy whether or not the woman is infected. The drug is administered after quickening, under the direct observation of healthcare giver (DOT) during antenatal care (ANC) visits. In 2012 however, the Global Malaria Programme, as part of its new policy making process convened a meeting of an Evidence Review Group (ERG), to review the WHO policy on IPTp with SP. The group, made up of experts in the field of malaria in pregnancy, reviewed evidence from published literature and unpublished studies on the current efficacy and effectiveness of IPTp with SP. They had as their aim to formulate recommendations to the Malaria Policy Advisory Committee (MPAC) for an interim policy statement on IPTp with SP for dissemination to national health authorities of malaria endemic countries where IPTp is implemented. Their specific objectives were to make changes in the current WHO recommendations relating to: (i) The number of treatments with SP that should be given, (ii) The effectiveness of IPTp-SP in areas of high SP resistance (iii) The level of transmission below which SP IPTp is no longer cost effective and (iv) To identify the critical gaps in knowledge and a

priority research agenda for IPTp with SP. (WHO, 2012). Their findings and subsequent recommendations are currently being used as guidelines for the implementation of IPT-SP. Conclusions from the group's deliberations included:

1. Three or more doses are more effective than two.
2. There is some evidence of benefit from SP-IPTp in areas of high prevalence of quintuple mutant *P. falciparum* parasites.
3. There is no evidence of harm from SP-IPTp in areas with a high level of resistance to SP. The findings of increased parasite density and inflammatory changes in women reporting use of IPTp with SP, from an observational study in Tanzanian women need further investigation.
4. There are insufficient data to make a recommendation on the level of malaria transmission below which implementation of SP IPTp is no longer cost effective.

Based on the above conclusions, the following recommendations were made and have subsequently been adopted by the Global Malaria Programme. They are:

- In areas of stable (moderate-to-high) malaria transmission, IPTp with SP is recommended for all pregnant women at each scheduled antenatal care visit.
- The first IPTp-SP dose should be administered as early as possible during the 2nd trimester of gestation.
- Each SP dose should be given at least 1 month apart from the other and up to the time of delivery.

- The last dose of IPTp with SP can be administered late (after 36 weeks) in the 3rd trimester of gestation without safety concerns.
- IPTp should be administered as directly observed therapy (DOT).
- SP can be given on an empty stomach. Folic acid at a daily dose equal or above 5 mg should not be given concomitantly with SP as this counteracts its efficacy as an antimalarial.
- SP is contraindicated in women receiving co-trimoxazole prophylaxis.
- IPTp with SP remains effective in preventing the adverse consequences of malaria on maternal and foetal outcomes in areas where a high proportion of *Plasmodium falciparum* parasites carry quintuple mutations associated with *in vivo* therapeutic failure to SP; therefore, IPTp with SP should still be administered to women in such areas (WHO, 2012).

The group also recommended research into the following areas:

1. Innovative community strategies to increase IPTp coverage that do not detract from ANC services.
2. Operational interventions to improve delivery and use of ITNs to women before they conceive.
3. Effectiveness of IPTp-SP against *P. vivax* infection in pregnancy.
4. The effect of the presence of the *dhps* 581 codon mutation on IPTp-SP effectiveness.
5. The safety of IPTp-SP when given 5 times or more during pregnancy.

6. Interactions between anti-malarials and anti-retrovirals in HIV infected individual.
7. Monitoring protocol for IPTp-SP effectiveness.
8. Methods for using health system information systems for routine monitoring of IPTp-SP implementation and effectiveness.
9. Relationship between malaria transmission intensity level and IPTp-SP effectiveness (risk-benefit and cost-effectiveness analysis based on modeling data).
10. The effect of sustained malaria transmission reduction on IPTp effectiveness.

Challenges to the IPTp-SP programme

In spite of the reasonable successes chalked with IPTp-SP, there are still some challenges if not properly and completely addressed could threaten and even erode the progress made so far with the programme.

The major challenges include issues of emerging resistance to SP, IPT-SP coverage, folate supplementation and HIV co-infection and adverse drug reactions.

Parasite resistance to SP

Treatment with SP is reported to strongly select resistant parasite genotypes (Dzinjalamala et al., 2005). The spread of SP-resistant parasites is a major source of concern as it casts doubts on the issue of its continued usefulness in the intermittent preventive treatment of malaria programme and it's probably the greatest challenge to the IPTp-SP programme. It has been recognized that drug use patterns, pharmacokinetics, and antimalarial

immunity all impact on the spread of drug resistant parasites. These factors converge in a unique synergy in women undergoing intermittent preventive treatment (Hommerich et al., 2007; Masaga et al 2003). Drugs with long half-lives, such as SP, increase the parasite's exposure to sub-therapeutic drug concentrations, and thus selection for resistant mutants (Watkins & Mosobo, 1993; Hyde, 1990). Mutations at specific codons in the dihydrofolate reductase and dihydropteroate synthase genes alter the drug-binding sites of SP. Increasing resistance to SP has been correlated with the stepwise acquisition of specific point mutations (Hyde, 1990; Sibley et al., 2001; Geiger et al., 2014 & Shah et al., 2015). Selection for mutations in *dhfr* gene probably occurrs first and the *dhps* gene mutations following later (Sibley et al., 2001). The presence of quintuple mutation; three mutations in *dhfr* (S108N plus C59R plus N51I) and two mutations in *dhps* (A437G plus K540E) genes have been significantly associated with therapeutic SP resistance (Kublin et al., 2002; Scho¨nfeld et al., 2005). Frequent of mutations at codon 581 on the *dhps* gene and codon 164 on the *dhfr* gene is known to increase the risk of parasite resistance to sulphadoxine (Chico et al., 2015).

Low coverage of IPTp-SP

Achieving coverage of at least 3 doses of IPTp (IPT3) still remains low in most parts of the Africa (WHO, 2015). Though, the proportion of pregnant women receiving three or more doses of IPTp-SP for malaria has increased in recent decades, it is still less than 20% (WHO). Since the WHO revised its recommendation in 2012, the proportion of pregnant women receiving at least three doses ofintermittent IPTp-SP has increased. In 2014, an estimated 52% of eligible pregnant womenreceived at least one dose of IPTp, 40% received

two or more doses, and 17% received three or more doses (WHO). It has been estimated that about 15 million pregnant women still do not receive at least one dose of ITPp-SP (WHO). According to the WHO, Ghana falls into the bracket of countries that had about 60-79% percent of her pregnant women receiving at least one dose of IPTp-SP between 2013 and 2014 (WHO). Meanwhile the IPTp-SP coverage (based on institutional figures from Effia Nkwanta Regional Hospital in Sekondi-Takoradi, the study area for this research work is said to be 57.8% (Orish et al., 2015). Low IPTp-SP coverage in SSA are attributable to (i) Negative perceptions of pregnant women on taking of SP (ii) Negative attitude and practices of health-care workers in the public sector that deter women from going to public hospitals (iii) Poor access and intermittent supplies of medications (iii) Irregular antenatal clinic attendance (iv) Inadequate clinic staff training (Oyibo & Agomo, 2010).

Doku et al (2016) also found out the following to be barriers to achieving high IPT3 coverage in Tamale in the Northern Region of Ghana; (i) shortage of sulphadoxine pyrimethamine (SP) (ii) inadequate supply of portable water for administration of SP (iii) unavailability of IPT during outreach services and (iv) lack of knowledge by ANC staff about the dropout rate in their area of jurisdiction and poor attitude of some health workers. Another study also stressed other challenges with and barriers to accessing ANC such as hidden costs (transportation, time off work, purchase of drugs on the private market when there were no stock in health facilities); cultural factors influencing timing of pregnancy disclosure and healthcare workers' negative attitudes exhibited towards women who present at ANC facilities (Finlayson & Downe, 2013).

These barriers can be easily dealt with when taken case by case. The thrust overcoming these barriers is training and motivation of both healthcare staff and pregnant women. Education of pregnant women and the general population should be by simplified messages. There must also be a concerted effort to mobilise resources for scaling up IPTp-SP coverage by all stakeholders.

Some studies have found that the uptake of preventive treatment of malaria in pregnancy using SP increases with higher levels of formal education (Eijla et al., 2002; Mpungu and Mufubenga, 2008 & Ouma et al., 2007).

Effect of daily folate supplementation

Across SSA, the practice is that pregnant women are placed on 5mg daily dose of folate drugs. This is because folate supplementation in pregnancy is associated with reduction in anaemia and prevention of megaloblastic erythropoiesis (Tamaru et al., 2006). The international guidelines recommend 0.4 or 0.6 mg of folate daily (Stolzfuz and Dreyfuss, 1997). However, the 5mg tablet is more widely available and that is what is used by pregnant women daily. It has been reported that the combined use of SP and daily folate supplementation at adose of 5 mg compromised the efficacy of SP for thetreatment of malaria parasitaemia in pregnant womenwhilethe use of 0.4 mg daily did not. (Ouma et al., 2006). Although the *de novo* biosynthesis of folate for DNA synthesis seems to be the preferred by the *Plasmodium* parasites, they can also salvage exogenous folate especially when that (folate) is in abundance. When biosynthesis of folate is blocked by SP, malaria parasites can switch to the salvage pathway. Enough folate can therefore be

mobilised in the presence of abundant folate for DNA synthesis (Krungkrai et al., 1989 & Wang et al., 2004) and therefore parasite survival. Thus, the abundance of folate may compromise the antifolate effect of SP (Ouma et al., 2006; Mertz, 2007 & Nduati et al., 2008).

IPTp-SP in HIV positive pregnant mothers

In SSA, (where malaria is most endemic) women account for approximately 57% of all people living with HIV (UNAIDS/WHO, 2013). In this same region, approximately 30 million pregnancies occur annually in areas of intense *Plasmodium falciparum* transmission, and HIV-infected women are known to be the most vulnerable to malaria infection (Dellicour et el., 2010 & WHO, 1999). Of the estimated 20 million HIV-infected individuals in SSA living in malaria endemic areas, over 12 million are women of reproductive age (UNAIDS/WHO, 2013). In addition, approximately one million pregnancies each year are complicated by coinfection with malaria and HIV in SSA (UNAIDS/WHO, 2013).

It is also known that the interaction between the two infections is particularly deleterious in pregnancy. HIV increases the severity of malaria infection and disease, and malaria infection increases HIV viral load, which in some studies has been shown to increase the risk of mother-to-child transmission of HIV (MTCT-HIV) (Gozalez et al., 2014).

In spite of all the above, IPTp-SP is contraindicated in HIV infected women. This is to avoid the potentially serious drug interactions with concomitant cotrimoxazole prophylaxis (CTXp), which is currently recommended in all HIV-infected pregnant women to prevent opportunistic infections (WHO, 2013). Thus, even though IPTp-SP is a life-saving and

highly cost-effective intervention, it cannot be used in the most vulnerable group, HIV-infected women. Thus, the problem of malaria–HIV coinfection needs to be revisited to take into account the new context and evolving intervention strategies for both diseases. Otherwise we risk not protecting the most vulnerable group of pregnant women and leaving them and their foetuses, their new born, and their children to the untoward effects of both malaria and HIV infection in pregnancy. HIV positive pregnant women were therefore excluded from participating in both the SP efficacy study and the maternal and birth outcome study.

IPTp-SP in Ghana

Ghana adopted IPT with SP as a policy in 2003 in 20 selected districts, but in 2005, it was scaled up nationwide to all districts to help reduce the severe burden and damaging effects of malaria morbidity and mortality on the pregnant woman and the unborn baby (MOH, 2009 & Roll Back Malaria/WHO, 2000). The drug of choice for IPT in Ghana is SP (1500mg/75mg). This is because of its safety, effectiveness in reproductive-age women, and the feasibility for use in programmes as it can be delivered as a single-dose treatment under direct observation (DOT) by the health worker (MOH, 2009). In addition to the above, SP is inexpensive, and has demonstrated high levels of acceptance by pregnant women (GHS, 2005). Currently Ghana follows recommendations given by the Evidence Review Group through the WHO. IPT-SP is therefore given early in the second trimester or after quickening and at every antenatal visit at least 4 weeks apart. The drug is also delivered as an antenatal package containing haematinics and

anti-helminthic. Screening is also done to rule out G6PD deficiency or any allergies to sulphur containing drugs.

According to the Ghana Health Service Annual Report for 2014, the proportion of pregnant women who were registered and were administered a second dose of Intermittent Preventive Treatment (IPT2) declined, falling from 55.4% in 2013 to 38.8% in 2014. This was a drastic fall back compared to 60.9% coverage achieved in 2011. Currently, Ghana is however categorized amongst countries having IPTp-SP coverage of 60-79% (WHO, 2015).

Properties of Drugs for IPTp-SP

The purpose of IPTp for malaria is to give drugs at doses that are therapeutic and therefore can treat subclinical infection whilst also serving as a prophylactic therapy in preventing malaria infection. The administered drug must also minimize the effects of malaria on the pregnant woman, the foetus, the new-born and later on the child while being safe to both mother and the foetus by reducing maternal anaemia and poor pregnancy outcomes (Shulman & Dorman, 2003, & Greenwood et al., 2004). Drugs for Intermittent Preventive Treatment of malaria in pregnancy must therefore be efficacious, safe to pregnant women and to the foetus, and must be well tolerated. It must also be a long-acting molecule (so as to reduce the frequency of administration), affordable and be easily administered.

Current drugs for IPTp

The current drug of choice for ITPp for malaria in pregnancy is a combination of sulphadoxine and pyrimethamine (SP). It has a good safety profile after the first trimester of pregnancy (Newman et al., 2003 & Peters et al., 2003), it is efficacious, cheap, and is easy to administer in a single dose

regimen. Both molecules inhibit folate biosynthesis, in the *Plasmodium* thereby interfering with its DNA synthesis. A tablet of SP comes in a fixed dose combination of 500mg sulphadoxine and 25mg pyrimethamine.

Sulphadoxine

Sulphadoxine (N1-(5, 6-dimethoxy-4-pyrimidinyl) sulphanilamide), a structural analogue of p-aminobenzoic acid (PABA) (figure 3), is a long acting sulphonamide that is used, usually in combination with other drugs, such as pyrimethamine for treatment of malarial infections. It can also be used in the treatment of respiratory and urinary tract infections. It acts by inhibiting the enzyme Dihydroopteroate synthase *(dhps)* in the folate biosynthesis pathway.

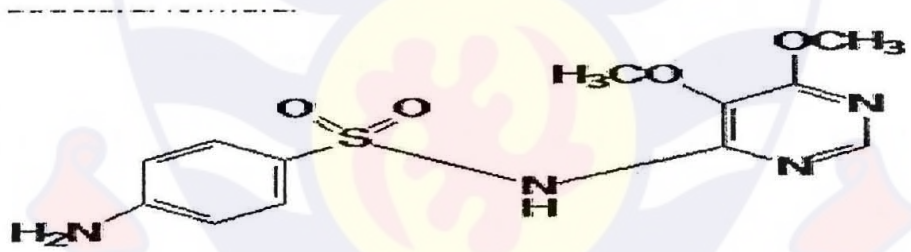

Figure 3: Molecular Structure of Sulphadoxine. (Source: Google Images. Accessed in November, 2016)

Pyrimethamine

Pyrimethamine, (2, 4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine is a synthetic di-amino-pyridine (figure 4), with potent antimalarial properties. It is commonly used as an adjunct in combination with sulphadoxine in *falciparum* malaria treatment and prophylaxis. It acts by competitively

inhibiting the enzyme dihydrofolate reductase *(dhfr)* in the folate biosynthesis pathway. It comes as white scored tablets containing 25 mg pyrimethamine.

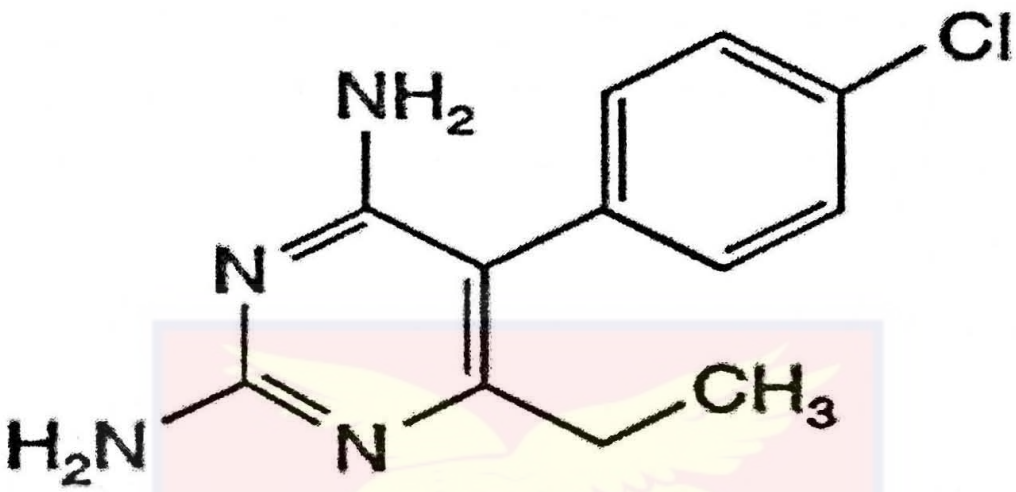

Figure 4: Molecular Structure of Pyrimethamine. (Source: Google Images. Accessed November 2016)

Pharmacokinetics of SP

Both sulphadoxine and pyrimethamine are well absorbed from the gastrointestinal tract. Like other sulphonamides, sulphadoxine is widely distributed in the body. Peak plasma levels are achieved within 2–8 hours. Pyrimethamine has a peak plasma level 2-6 hours and it is distributed mainly in the kidneys, lungs, liver and spleen. Plasma protein binding is about 90% for both pyrimethamine and sulphadoxine. About 5% of sulphadoxine appears in the blood as acetylated metabolite, and 2–3% as glucuronide. Pyrimethamine is transformed to several metabolites in the liver (IPCA, 2006). Sulphadoxine and pyrimethamine are both excreted mainly by the kidneys. The mean elimination half-life of sulphadoxine is 169 h (range: 100–231 hours), while pyrimethamine has a mean half-life of 111 h (range: 54–148 hours) (Drug Information, 2012; IPCA, 2006 & Weidekamm et al., 1982).

Mechanisms of action of SP

Folic acid is needed for the biosynthesis of purines and pyrimidines and hence DNA synthesis and cell multiplication (Bzik et al., 1987 & Selby et al., 2001). Both sulphadoxine and pyrimethamine are antifolates. Pyrimethamine binds to and inhibits the bifunctional enzyme dihydrofolate reductase-thymidylate synthase *(dhfr-ts)* of *Plasmodium* (figure5). Disruption in the catalysis of the NADPH-dependent reduction of 7, 8-dihydrofolate to 5, 6, 7, 8-tetrahydrofolate occurs (Blackley, 1984) leading to the blockage of the biosynthesis of purines and pyrimidines, which are essential for DNA synthesis and cell multiplication. There is a subsequent failure of nuclear division at the time of schizont formation in erythrocytes and liver and subsequent death of the *Plasmodium.* Sulphadoxine on the other hand competitively inhibits dihydropteroate synthase *(dhps)* (Selby et al 2001), leading to the disruption in the coupling of 7,8,-dihydro-6-hydroxymethylpterin pyrophosphate with para-amino benzoic acid (pABA) to yield 7, 8- dihydropteroate (Walter, 1991) (figure 5). It does not destroy gametocytes, but arrests sporogony in the mosquito. The combination of pyrimethamine and sulphadoxine thus offers a two-step synergistic blockade of *Plasmodium* division. SP is schizonticidal and is also active against the other asexual erythrocytic forms of susceptible *Plasmodium*.

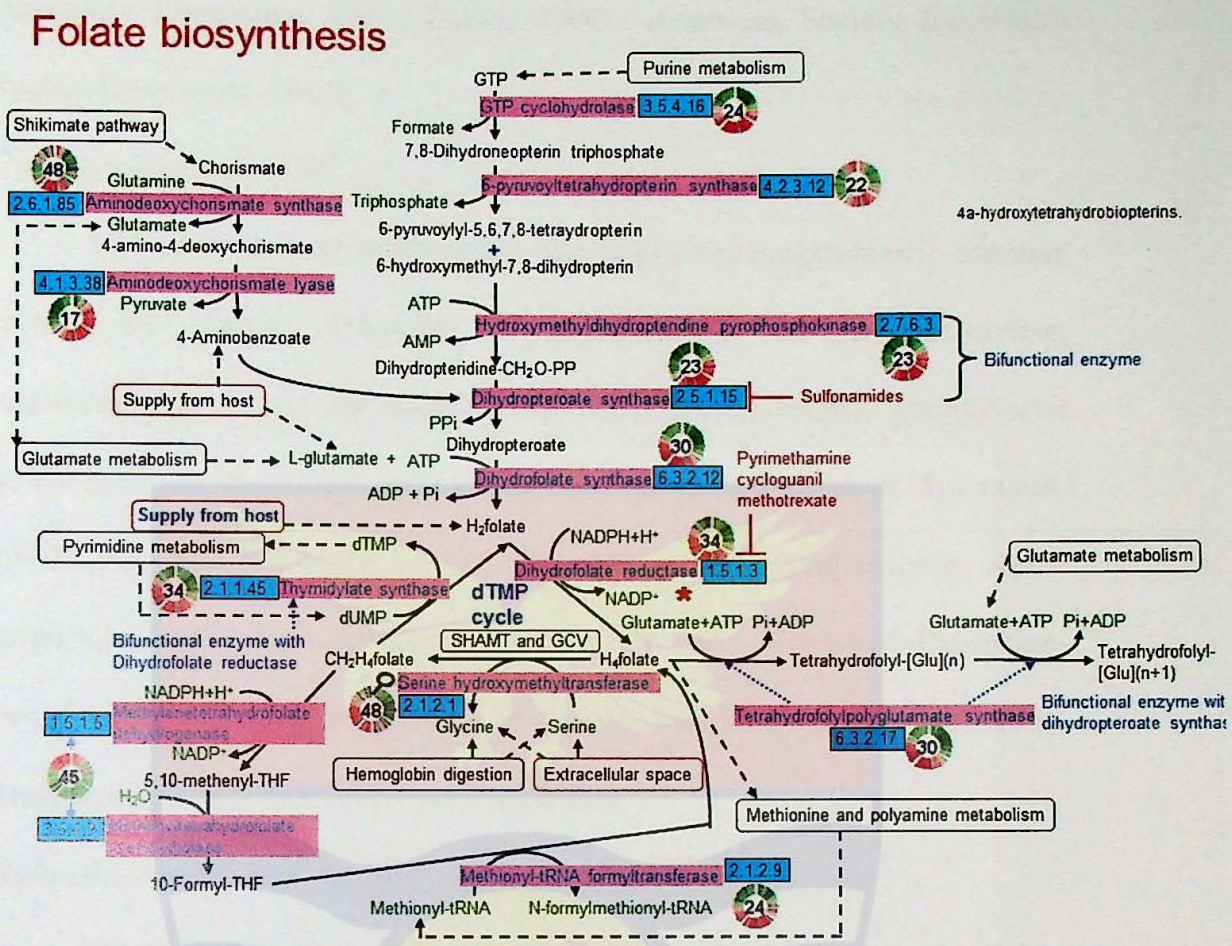

Figure 5: Folate Biosynthesis Pathway and Points of blockade by Sulphadoxine and Pyrimethamine.

(Source: http://mpmp.huji.ac/maps/folatbiopath.html.Accessed November 2016)

Adverse effects of SP

Commonly reported adverse effects of sulphadoxine-pyrimethamine include; hypersensitivity reactions (e.g. itchiness, contact dermatitis, and hives) Gastrointestinal effects (e.g. nausea, vomiting, and diarrhoea) headache and myelo-suppression. Much rarer adverse effects (less than 1% frequency) are Stevens-Johnson syndrome, Toxic epidermal necrolysis, Agranulocytosis, Aplastic anaemia, Disorder of haematopoietic structure, Drug-induced eosinophilia, Thrombocytopaenia, Liver necrosis, Hepatitis, Jaundice, Hepatomegaly, Nephrotoxicity (Drugs.com, 2014; WebMD, 2014; Joint

Formulary Committee, 2013; Roche, 2008 ; American Society for Health System Pharmacist, 2012).

Contraindications of SP

Use of SP is contraindicated in the following: megaloblastic anaemia caused by folate deficiency, hypersensitivity to pyrimethamine, sulphonamides, or any ingredient in the formulation; repeated prophylactic (prolonged) use in patients with kidney or liver failure or blood dyscrasias; infants less than 2 months of age; prophylaxis in nursing women; Acute porphyria and haemoglobinopathies such as Glucose-6-Phosphate-Dehydrogenase defects (Drugs.com, 2014; Joint Formulary Committee, 2013).

Resistance to Sulphadoxine-Pyrimethamine

Molecular structure of the dhfr and dhps enzymes

The two enzymes, *dhfr* and *dhps* are very important in the folate biosynthesis pathway of the *Plasmodium*. Though the parasite can mobilize folate exogenously from host environment, de novo synthesis of folate is preferred and therefore essential for the parasite survival. The *dhfr* enzyme is bifunctional: The dihydrofolate reductase (*dhfr*) and thymidylate synthase (ts) (*dhfr-ts*). The *P. falciparum dhfr-ts*is encoded by a single-copy gene on *P. falciparum* chromosome four, with the two enzymes forming a bifunctional protein (Bzik, Li, Horii, & Inselburg, 1987) similar to other protozoans but distinct from bacteria and higher order eukaryotes. It contains 608 amino acids, the first 231 comprising the *dhfr* domain, the next 89 residues forming the junction region, which joins the remaining 288 residues of the thymidylate synthase domain. Dihydrofolate reductase comprises eight central β-strands between four α-helices, with an additional three short α-helices (Yuvaniyama

et al., 2003). The *dhfr-ts* acts to provide reduced folate for use in the thymidylate cycle, and inhibition of *dhfr-ts* results in arrested DNA synthesis secondary to reduced levels of dTMP (Ferone, 1977).

The *dhps* enzyme is also a bifunctional enzyme. It includes dihydro-6-hydroxymethylpterin pyrophosphokinase (*pppk)* at the N terminus of the *dhps*. The gene encodes a putative protein of 83 kDa that contains two domains that are homologous with the *dhps* and *pppk* enzymes of other organisms. The *pppk-dhps* gene is encoded on chromosome 8 and has two introns (Triglia & Cowman, 1994). SP resistance is linked with substitutions of amino acids of these enzymes which act in the folate biosynthetic pathway (Cowman et al., 1988; Triglia and Cowman, 1994; Brooks et al., 1994). Unlike *dhfr*, there is no known human counterpart to the *dhps* (Gregson and Plowe, 2005).

SP resistance markers

Distinct point mutations in the genes coding for the *dhfr* and *dhps* enzymes are associated with SP resistance. In both genes, each successive mutation has been shown to incrementally increase the parasite's tolerance to the drug in vitro (Triglia et al., 1997, 1998). A point mutation in *dhfr* gene causing a Ser→Asn at codon 108 of the *dhfr* domain has been implicated in resistance to pyrimethamine (Basco et al., 1996; Reeder et al., 1996), while high level in vitro resistance has shown that mutations at residue 51 resulting in (Asn→ Ile) 59 (Cys→Arg), and/or 164 (Ile→Leu) confer some level of resistance (Sirawaraporn et al., 1993; 1997a).

Series of sequence analysis of the coding region of *dhps* has identified a number of point mutations; all associated with in vitro resistance to sulphadoxine.The following mutations in the *Plasmodium dhps* gene are

associated with resistance to sulfadoxine based on in vitro analysis: An Ala→Gly mutation at codon 437 of the *dhps* domain, while high levels of resistance to this drug component are associated with additional mutations at codon 581 (Ala→Gly), 436 (Ser→Phe), and 613 (Ala→Ser) (Triglia, Wang, Sims, Hyde,& Cowman, 1998; Triglia, Menting, Wilson, & Cowman, 1997; Wang, Sims & Hyde, 1997).

Mechanism of SP resistance

Antimalarial drug resistance develops most rapidly when a population of parasites encounters sub-therapeutic concentrations of the drug. These act as a selective pressure filtering out the more resistant parasites within the infecting population. Selection is most efficient when single point mutations confer high level resistance. Mutations that confer resistance may however occur spontaneously independent of drug pressure. Other factors that play significant role in the emergence of resistance include; drug pharmacokinetics, cross-resistance between drugs (from the same family), over usage of drugs, and inadequate treatment through inappropriate prescription or administration, non-compliance, or poor absorption (White, 1999 ; Simpson et al., 2000 ; Iyer et al., 2001 & Hastings et al., 2002). Also, implicated are host immunity and level of transmission (Sibley et al., 2001; Gatton et al., 2001 & White & Pongtavonpinyo, 2003).

SP fall under the categories of drugs with longer terminal elimination phase. *Plasmodium* parasite populations may therefore be exposed to sub-therapeutic concentrations of the drug for weeks after a single therapeutic dose. For pyrimethamine to inhibit the *dhfr* enzyme, the rigid length of the pteridine ring (figure 4) fits between residues 108 and 54 within the active site of the enzyme

(Warhurst, 1998; Yuvaniyama et al., 2003). The primary *dhfr* mutation, S108N, leads to a conformational change in the enzyme. This affects drug accommodation at the active site of the enzyme, rather than affecting entry of the drug into this site. The NH_2 of asparagine 108 interferes with the chlorine atom of pyrimethamine, causing the later to be displaced within the active site (Warhurst, 1998, 2002). Further compromise of the planar aspect of the pyrimethamine pteridine ring is caused by the carboxylate group of asparagine 54 and the pyrimethamine ethyl group. This loss of planarity is said to be responsible for the pyrimethamine resistance caused by the S108N mutation (Delfino, Santos-Filho& Figueroa-Villar, 2002). Though the additional *dhfr* mutations, N51I and C59R are distant from the enzyme active site, they are on the same helix as residue 54, which is critical to substrate binding (Yuvaniyama et al., 2003). Mutations at residues 51 and 59 may therefore impede admission of pyrimethamine to the enzyme binding site (Santos-Filho de Alencastro, & Figueroa-Villar, 2001; Warhurst, 2002). This could be possible throughinteractionsofpolaror charged residues on isoleucine and arginine and the protonated pyrimethamine (Delfino et al., 2002). The effect of the additional mutations at 51 and 59 is essentially silent in the absence of the S108N mutation, but additive when it is present (Sirawaraporn et al., 1997b). Presence of mutation I164L leads to a marked increase in resistance to pyrimethamine in the presence of S108N, N51I, and C59R. This is due to an increase in the active site gap between the α-carbon of C50 and I164L.

In the *dhps* gene, codons 436, 437, and 540 all line the channel to the active site. Meanwhile codons 581 and 613 are only one to three positions away from the channel (Gregson and Plowe, 2005). As in the *dhfr* gene, the

greater the number of point mutations the greater the degree of resistance. It is however unclear that a necessary starting mutation such as A437G must be present, as is S108N in *dhfr*. *Plasmodium* isolates containing S436A, A437G, or K540E singly have been found and all confer some degree of resistance to sulphadoxine.However, higher level sulphadoxine resistance requires the presence of multiple mutations in addition to mutations at codons 436, 437, and/or 540. Mutations at codons 581 and 613 may be compensatory in function, explaining why the two residues are not seen in isolation, but only with mutations at 436, 437, or 540. These mutations lead to conformational changes at the drug binding site hence a limitation of the accommodation of the sulphadoxine molecule and a consequent reduction in parasite susceptibility.

Current status of SP resistance in Africa

In Africa, high grade resistance is of particular concern in eastern and southern Africa (Naidoo et al., 2011), where high frequencies of parasites bearing haplotypes with three mutations in *dhfr* (encoding the N51I, C59R, and S108N) and two in *dhps* (encoding the A437G and K540E substitutions) exist, especially if the additional *dhfr*164L or *dhps* 581G mutations occur (Gesase et al., 2009 & Harrington et al., 2009). The latter has recently been associated with poor birth outcomes in IPTp-SP recipients Harrington et al., 2011), although this association has not yet been confirmed in other studies in eastern and southern Africa (Kalilani et al., 2011; Menendez et al., 2011; Taylor et al., 2012). Again, in East and South Africa, mutations at the 437 and 540 codons are found together while in West and Central Africa the 437 mutation is frequently found on its own (Pearce et al., 2009). Laboratory

studies show that the A437G and K540E substitutions in combination raise sulfadoxine tolerance of sensitive *dhps* by 200-fold, compared to just 10-fold for the A437G substitution alone (Triglia et al., 1997). Hence East African parasites are predicted to withstand higher doses of SP thanWest African parasites. The efficacy of IPTp-SP is being further compromised in east Africa by the additional emergence of *dhps* mutation at codon 581 in northern Tanzania (Gesase et al., 2009) which has been shown to reduce drastically the efficacy of IPTp-SP (Minja et al., 2013) termed super resistance (Naidoo & Roper, 2013).

In contrast, the parasite populations in western Africa seem to be mostly sensitive to SP (Naidoo et al., 2011; Dokomajilar et al., 2006; Diakite et al., 2011& Coulibaly et al., 2006), and IPTp-SP has proven to be highly effective and efficacious in clinical trials and observational studies (Kayentao et al., 2013; Diakite et al., 2011). However, spread of SP resistance from eastern and southern Africa, or the de novo development of high-level SP resistance may occur and monitoring of the effectiveness of SP when employed as IPTp is essential.

Potential Alternative Drugs for IPTp

With the rising concern of resistance to SP, the medical research world is strongly considering alternatives to SP should there be a need for a switch to another anti-malarial drug for use as IPTp. The following properties are required of any drug that will replace SP in future when it will no longer be useful for IPTp:

• It must have a long-half life, as it has been suggested that the duration of prophylaxis rather than the treatment effect is the most important determinant of IPTp efficacy.

• It must be safe during pregnancy, and well-tolerated to ensure high compliance with treatment in women who are often asymptomatic when infected with malaria.

• The administration should be easy (ideally a single dose).

• It should be readily available and at an affordable cost.

Considering these criteria, Mefloquine (MQ) is probably one of the most attractive options. It has a long half-life and is still highly efficacious in African countries (Briand et al., 2007). Most studies have shown that MQ is safe for use in pregnancy (Mount et al., 2004; Sketetee et al., 1996; Vanhauwere et al., 1998). A study showed that women who received MQ treatment had a significantly higher risk of stillbirth than women not treated or treated with other anti-malarials (WHO, 1993). There is a need to further confirm this finding. However, MQ has been associated with a range of side-effects that could impede compliance treatment. In a clinical trial, in which Beninese women were randomized to receive either SP or MQ for IPTp, the mild adverse events observed were: vomiting, nausea and dizziness, and these resolved fast (National Institute of Health, USA, 2006). Very few women have refused to take the second dose because of a poor tolerance of the first intake. The drug has been well-accepted in spite of its bitter taste, and there has beenless than 1% of early vomiting (within 1 h) after giving a fat snack before the women took the drug (as recommended by the manufacturer). Whatever the alternative drug, the cost is likely to behigher than the cost of SP or CQ.

Mefloquine remains expensive even if the cost has recently declined. In the near future, lower cost and higher availability of the drug in African countries should make this option even more realistic.

Artemisinin combination therapies (ACTs) are also being evaluated for IPTp. They have been shown to be highly efficacious and safe during pregnancy except when used in the first trimester. However, if the effect of IPTp is mainly prophylactic (White, 2005), then short-acting drugs would be expected to provide little benefit. Moreover, ACTs are still very expensive and less easily deliverable as they require multiple treatment doses that cannot be given as a directly observed therapy in the ANC clinic. This could result in low compliance with treatment regimen. There are also concerns of embryo-toxicity with artemisinin derivatives in animal studies (Clark, 2009). Other potential candidates, such as SP plus azithromycin and SP plus Artesunate have been evaluated and found to be safe and effective (Kalilani et al, 2007). Kalilani et al. (2007) reported that though the parasite clearance time was significantly faster in the SP-Artesunate group, recrudescent episodes of malaria were less frequent with SP-azithromycin and SP-Artesunate compared with SP monotherapy. Azithromycin, a macrolide antibiotic, which has been found to have antimalarial effect (Andersen et al., 1998), has been used in pregnant women to treat sexually transmitted infection (STIs), and other infections with a good safety profile (Gray et al., 2001; Sakar et al., 2006). SP plus amodiaquine, and chlorproguanil-dapsone are also being assessed for IPTp. Piperaquine (used in combinations with other anti-malarials rather than used alone) might be one of the very promising options for IPTp (Briand et al., 2007).

Review of Methods

The WHO is yet to standardize any method(s) for the systematic evaluation of the effectiveness of SP as IPTp. However, in 2012, it proposed, through its Evidence Review Group (ERG) on MiP that the potential core elements of monitoring studies for SP-IPTp should include:

a. Review of ANC (number and timing of IPTp-SP doses) and birth weight data through routine health system records. Use of data on trends of birth weight and neonatal mortality and their association with IPTp-SP coverage (adjusting for other potential confounders routinely collected during ANC visits)

b. Specific studies to evaluate IPTp-SP effectiveness controlling for multiple factors (age, gravidity, HIV status, ANC visits, number of SP doses received, etc) such as: (i) Cross-sectional studies at delivery units (ii) Case-control studies of women delivering LBW babies or with maternal anemia

c. Monitoring of prevalence of SP molecular resistance markers, preferably at first ANC (pre-SP administration) although the association of resistance markers with SP effectiveness requires further investigation

d. Collection of dried blood spots for analysis of molecular markers of SP resistance

e. Assessment of *in vitro* SP efficacy

f. Assessment of 42 day *in vivo* SP efficacy in asymptomatic parasitaemic pregnant women

Again, it proposed amongst other things, that when implementing and monitoring the effectiveness of SP-IPTp, the following should be ascertained: (i) the quality of the SP tablets as determined by international standards such as international pharmacopoeia (ii) its efficacy in clearing *in vivo* parasitaemia and protecting against malaria infection and its consequences during pregnancy and (iii) the prevalence of molecular markers of SP resistance (WHO, 2013). The methods of evaluation of the effectiveness of SP-IPTP employed in this study were therefore based on the above proposals.

Evaluation of quality of SP tablets

Conventionally, tests for the quality of a drug are performed using procedures outlined in official monographs such as the European, British, the United States and the International Pharmacopoeia. Such monographs prescribe the most basic aspects of drug quality that need to be assessed for a particular drug and formulation, and the criteria to be used in the assessment. In this study, protocols prescribed by the United States Pharmacopoeia, 2015, volume 3 were used. This was because it was readily available and was the Pharmacopoeia being used by the Food and Drugs Authority which is the drug regulatory body in the country.

The tests for the determination of the quality of drugs such as SP can broadly be classified as physical tests (those that include visual inspection), chemical tests (for content of stated active pharmaceutical ingredients (SAPIs) and impurities under normal and simulated storage conditions), *in vitro*disintegration and dissolution tests and *in vivo* bioavailability studies (Amin and Kokwaro, 2007).

Visual inspection of SP tablets includes checking for (i) clear labeling of the drug (both the brand and generic names (ii) statement of strength of the active principles (iii) statement on dosage forms (iv) registration with regulatory authority (v) batch or lot numbers (vi) dates of manufacture and expiry as well as (vii) conditions of storage of the drug. It also involves looking out for characteristics such as uniformity of colour, shape, size, and textureof the tablets. Breaks and cracks as well as embedded spots or contaminations must also be looked out for in the tablets. Physical tests may include tests for uniformity of weight, friability (how well a tablet holds under normal conditions of transportation, measured by the proportion of the tablet that is lost as powder) and tablet hardness (Aulton, 2001).

Chemical tests for SP quality include test of identity, which first tests for the presence of the SAPIs; the test for quantification of content, which determines the amount of SAPIs in the tablets expressed as a percentage of the label claim; and dissolution test which determines the amount of active ingredient that is released from the tablets (compared to a reference standard) and available for absorption. This is used as surrogate marker of *in vivo* bioavailability for poorly aqueous-soluble drugs such as sulfadoxine-pyrimethamine (Amin and Kokwaro, 2007).

Field methods that can be used to screen for the identity of the SAPIs include, (i) visual examination of packaging (ii) the use of hand-held RAMAN spectroscopy and (iii) the use of Counterfeit Detection device (CD3): Others are (iv) refractometry and (v) colometry. Meanwhile, specialized mass spectrometry (MS) techniques and Desorption electrospray ionization (DESI) can be used for the confirmation of the identity of the SP. (Lalani, Kitutu,

Clarke, & Kaur, 2017; Hajjou, Qin, Bradby, Bempong, & Lukulay, 2013; Green et al., 2007). The high-performance Liquid chromatography (HPLC) can also be used to confirm the identity of the SAPI. The quantification of the SAPIs is done in research and drug quality assurance laboratories, using methods such asthe Semi-quantitative thin layer chromatography (TLC) and the quantitative HPLC alone or coupled to a mass spectrometer (LC–MS) (Lalani et al, 2017). The Laser-induced breakdown spectroscopy (LIBS) is a newer method of quantification of SAPIs (Myakalwar et al., 2011). In this study, visual examination of packaging and HPLC were used for screening and confirming the identity of SP as the active principles respectively. The HPLC was also used to quantify the amount of sulphadoxine and pyrimethamine against their stated strengths on the label following protocols described in the US pharmacopoeia, 2015. This was because of its reliability and availability (compared to the other methods of quantification). The investigations were done at the analytical laboratory of the Food and Drug Authority (FDA) of Ghana.

The dissolution tests used to determine the amounts of sulphadoxine and pyrimethamine that dissolves into solution in an appropriate medium such as phosphate buffer under appropriate conditions of temperature of 37°C and pH of 6.8. It is expected that not less than 60% of the stated label of both sulphadoxine and pyrimethamine must be dissolved in the solution after 30 minutes of the dissolution process (USP, 2015). The dissolution is aproxy measure of bioavailability (extent to which sulphadoxine and pyrimethamine molecules will dissolve in the human body). It is done using a dissolution apparatus.

For any dissolution study, the dissolution apparatus employed is determined by the dosage form characteristics and the intended route of administration. For solid dosage forms, such SP tablets, the standard dissolution testing methodologies are the United States Pharmacopoeia (USP) Apparatus 1 (basket) and the USP Apparatus 2 (paddle). Immediate-release, modified-release and extended release tablets are usually tested in classical dissolution baths with USP 2 paddles. Apparatus 2 was therefore used in this study. Floating capsules and tablets generally use USP 1 baskets (Practical Science, 2010). Other dissolution techniques and equipment include USP 3 (reciprocating cylinders), USP 4 (flow-through-cell), USP 5 (paddle-over-disk), USP 6 (cylinder) and USP 7 (reciprocating holders) (USP, 2015). The development of a dissolution procedure involves selecting the dissolution media, apparatus type and hydrodynamics (agitation rate) appropriate for the product. After the dissolution process, the amount of the SAPI, in this case SP, in solution is then quantified after an assay with the appropriate methods of quantification described previously.We again employed the HPLC method of quantification in this study because it was available.

The test for bioavailability is an *in vivo* test. It measures the rate and extent of availability of drug in systemic circulation. Bioavailability tests are important, especially for drugs with limited aqueous solubility (such as generic SP, especially its pyrimethamine components) that canlead to erratic, incomplete and unpredictableabsorption profiles (Amin, 2007). Bioavailability studies involve serially taking plasma samples of subjects who have received the drug under testing and a reference drug. The concentrations of the drugs at the various times are then quantified using the methods of quantitation of drug

concentration as described previously. The simultaneous quantitation of SP from plasma in a bioavailability studies requires the use of liquid chromatography–isotopic dilution mass spectrometry methods (Liu et al., 2012; Matar, Awad & Elanim, 2014). Bioavailability studies were not carried out in this research work because of the non-availability of a LC-MS system.

SP as IPTp efficacy studies

In evaluating the efficacy of SP as IPTp, both the therapeutic efficacy and protective or prophylactic efficacy of the drug should be assessed (WHO, 2013). This is because, SP given as IPTp is expected to not only to clear *Plasmodium* parasitaemia if any, but also to protect against the risk of malaria infection (either as a result of recrudescence or reinfection) and consequences of the infection both to mother and foetus. A number of studies have therefore employed different scientific methodologies to achieve this aim. Firstly, there have been randomized control trials where the efficacy of SP as IPTp has been compared to other measures of control of MiP such as IPTp with mefloquine, and other antimalarials. SP-IPTp has also been compared with Intermittent Screening and Treatment (IST) of malaria in pregnancy (Tagbor, Bruce Agbo, Greenwood & Chandramohan, 2010; Gonzalez et al., 2014; Briand et al., 2015). In these studies, pregnant women that met the inclusion criteria were followed up till delivery to assess how SP as IPTp influences adverse maternal and birth outcomes such as low birth weight, placental and peripheral parasitaemia and maternal anaemia compared to the other MiP control measures. Randomized placebo-controlled trials have also been conducted in determining the efficacy of SP-IPTp in preventing adverse outcomes (Menendez et al., 2008; Menendez et al., 2010). Conducting a placebo-

controlled trial with an intervention such as SP-IPT in an area of malaria endemicity raises ethical concerns, since SP-IPTp is a standard intervention recommended by the WHO for control of MiP in these areas. To mitigate the ethical concern, LLINs were made available to all study participants for usage.In these studies, diagnoses of placental and peripheral blood parasitaemia were by both microscopy and by PCR.

Secondly cross-sectional methods have also been used to evaluate the effects of SP-IPTp on birth outcomes (Arinatwe et al., 2013; Harrington et al., 2011; Gutman et al., 2013). In these studies, adverse maternal and birth outcomes described previously, are assessed amongst delivering mothers and correlated with the use of SP as IPTp during the antenatal period. The use of SP during the antenatal period can be ascertained by verbal recall by the mothers, reference from antenatal record and by measuring plasma serum content of sulphur as an index of SP usage (Harrington) given that no other sulphur containing has been used over the antenatal period.

The third approach in evaluating the effectiveness of SP-IPTp is the *in vivo* therapeutic efficacy study. Pregnantwomen who are parasitaemic yet asymptomatic of malaria (usually having a temperature of below 37.5°C), are HIV negative, presenting after quickening (between 16-26 weeks of gestation) and have received no prior anti-malarials including IPTp-SP during the current pregnancy are eligible for enrollment (Tan et al., 2014). MRDTs are used to screen for *Plasmodium* parasitaemia. Parasitaemia is then confirmed using microscopy and or PCR. Enrolled pregnant women receive under direct observation, therapeutic doses of SP (1500mg of sulphadoxine and 75mg of pyrimethamine) and followed up weekly for 42 days to determine the rate of

parasite clearance. The re-emergence of parasitaemia as a result of recrudescence or reinfection is also assessed by amplification of molecular markers such as *msp-1, msp-2 and glurp* (Gutman et al., 2015). The therapeutic failure rate is then compared to the 10% failure rate beyond which the WHO calls for the withdrawal of a drug as a first line anti-malarial.

In this study, the therapeutic efficacy of SP as IPTp was evaluated using an *in vivo* SP efficacy study and a cross-sectional study of the effects of SP on maternal and birth outcomes. A randomized control trial was not employed because of limited time, human and financial resource.

Surveillance of the prevalence of molecular markers of SP resistance

The methods applied in this aspect of an SP-IPTp effectiveness study are aimed at determining the prevalence of mutations in the *dhfr* and *dhps* genes that are known to confer on the *Plasmodium* parasites, resistance to SP. Parasites isolates are taken from both participating pregnant women and the general population being studied. Parasite DNA can be extracted from whole blood, frozen red cell pellets, or dry blood spots (DBS) using appropriate extraction protocolssuch as chelex method of DNA extraction (Siame, Mharakurwa, Chipeta, Thuma, & Michelo, 2015) or the commercial DNA purification kit, QIAamp® (Iriemenam et al., 2012). Detection of mutations in the *dhfr* and *dhps* genes can be by (i) a *dhfr*and *dhps* mutation specific PCR (Wang, Brooks, Sims, & Hyde, 1995; Gyang, Peterson, & Wellems, 1992; Biswas, Escalante,Chaiyaroj, Angkasekwinai, & Lal, 2000; Tahita et al., 2015) (ii) PCR followed by Enzymatic Restriction Digestion (Duraisingh, Curtis & Warhurst, 1998; Eberl et al, 2001; Siame et al., 2015) and (iii) PCR followed Direct DNA sequencing or Pyrosequencing. (Iriemenam, et al, 2012; Teik,

Mersumpin, & William, 2013; Zhou et al, 2006). Other techniques include (iv) dot blot/probe hybridization techniques (v) real-time PCR (vi) the SNaP shot primer extension method, and (vii) sequence-specific oligonucleotide probe–enzyme-linked immunosorbent assay (Abdel-Mushin et al., 2002; Alifrangis et al., 2005; Durand et al., 2000). Conventionally, these molecular methods focus on evaluating individual mutations in the *dhfr* codons **50, 51, 59, 108**, and **164** and *dhps* codons **436, 437 540, 581**, and **613** (Durand et al., 2000; Kublin et al., 2002, Plowe et al., 1997; Reeder et al., 1996). Individual or combination of codons to be studied may however be varied in a study according to the observed trend of prevalence of mutations in a geographical study area.

In this study, the chelex method was used for DNA extraction. Detection of mutations on the *dhfr* and *dhps* genes conferring parasite resistance to SP was done by a nested PCR followed by enzymatic restriction digestion (Duraisingh et al., 1998). This was because the set up for mutation detection for the other techniques were not readily available. Again, the restriction digestion is also known to be a high-throughput technique of detection of mutations which has been well studied.The molecular basis for enzymatic restriction digestion is that, difference in homologous DNA sequences can be detected by the presence of fragments of different lengths after digestion of the DNA samples in question with specific restriction endonucleases (RE) which cleave DNA at specific restriction sites: Fragments, as molecular markers, are therefore specific to a single clone/restriction enzyme combination. The principle underpinning its usage in detection of mutations in genes (in this case *dhfr* and *dhps*) is that, Single Nucleotide Polymorphisms (SNPs)or Insertions/Deletions (INDELs) can create or abolish

restriction endonuclease (RE) recognition sites, thus affecting quantities and length of DNA fragments resulting from RE digestion.

We focused on mutations at codons **I51, R59** and **N108** of the *dhfr* gene and codons **G437** and **E540** on the *dhps* gene using the enzymes **Tp509I, XmnI, BsrI, MwoI** and **FokI** respectively. This was because, these mutations have been reported to be most prevalent in the West African sub-region; the others have relative rarity in the sub-region. The WHO recommends that IPTp-SP should be used in regions with a prevalence rate of **K540E** less than 50% and **A581G** less than 10% (WHO, 2013a). Since we did not detect the prevalence of mutations in A581G, comparison was prevalence of mutations was made to **K540G**only. Prevalence ofmutations in *dhfr* genes were also compared to that of previous studies in Ghana for trend analysis.

Lot quality assurance sampling

The lot quality assurance sampling (LQAS) methodology was originally developed in industry for quality control purposes. It is currently however employed in healthcare surveys. It makes use of small sample sizes when conducting surveys in small geographical or population-based areas (lots) (Lanata & Black, 1991).

Using LQAS in health surveys requires that, firstly, an upper and lower performance levels should be set. This categorizes a lot into two groups; low or high performers. The low performers fall below the threshold value or limit and their experiences can be deemed to be unacceptable, poor or defective whilst the high performers can be said to have acceptable experience or coverage.

The required sample size, n for a LQAS is calculated as shown elsewhere (Food and Nutrition Technical Assistance [FANTA], 2012) as follows:

$d = [n \times p/100]$

n is therefore $= \frac{100 \times d}{P}$

Where n= number of cases to be foundor the sample size

d = threshold value

p = coverage standard.

The small sample size required means LQAS can be used as frequently as needed. Compared to traditional survey methods, which are deemed costlier and time-consuming, LQAS is less expensive, less time consuming and can be managed relatively more efficiently (Lantana & Black, 1991; Robertson, Anker, Roisin, Macklai, & Engstrom, 1997). It is also possible to obtain relatively precise estimates of the performance level for the entire area sampled. Again, unlike traditional surveys methods that usually provide information at the regional or national level, LQAS provides information at the local level and therefore allows for direct effective local supervision and improvements in on-going health programmes (Valadez, Hage, & Vargas, 2005). In this study, the LQAS was used as a sampling method in the SP efficacy trial for the reasons stated above.

CHAPTER THREE

MATERIALS AND METHODS

Study Area

Sekondi-Takoradi is the administrative capital of the Western Region of Ghana. It is located in the south-eastern part of the Western Region. The Metropolis is bordered to the west by Ahanta West District and to the east by Shama District (Figure 6). At the south of the Metropolis is the Atlantic Ocean and at the northern part is Wassa East District. It covers a land size of 191.7 km^2 (Ghana Statistical Service (GSS), 2014). Though the smallest district in terms of land size, the Sekondi-Takoradi Metropolis is the most urbanized among the 22 districts in the region. It has few suburban areas. According to the 2010 population and housing census, Sekondi-Takoradi has a population of 559,548 (GSS).

It lies on latitude 4.91°N and longitude 1.77°W. Sekondi-Takoradi Metropolis has three main vegetation types, namely, mangrove, savannah woodland and tropical forest. The tropical forest is predominately found around the northern parts of the Metropolis and stretches to the east covering a large part. The savannah woodland is dotted around the middle belt and mangrove vegetation is found along the southern portion

The climate of the Metropolis is equatorial, with an average annual temperature of about 22°C, experienced between January and March. Rainfall is bi-modal, with the major season occurring between March and July and the

minor season occurring between August and November. The mean annual rainfall is about 1,380 mm, covering an average of 122 rainy days.

Malaria transmission rate is nearly stable all year round with rates increasing in the rainy seasons mainly in the months of May, June and July. The incidence of malaria in the general population is 28.7 per cent and the incidence in pregnant woman was 1.84 % in 2014 and 1.74 % in 2015. (Unpublished data, Metropolitan Disease Control Unit, Sekondi-Takoradi Metropolitan Assembly, 2015). There were no records available on the prevalence of subclinical malaria neither in the metropolis nor the region.

Study Sites

Three health facilities were chosen. The first was the Effia Nkwanta Hospital, the regional referral centre for the Western region of Ghana. The second was Takoradi Hospital which serves as the Metropolitan Hospital and Essikado Hospital which serves mainly the suburban areas of the Sekondi-Takoradi Metropolis. These study facilities were chosen not with the aim of making any comparison of the variations in data outcomes, if any, but rather for the convenience of obtaining adequate data within a very limited time and space of the research work: In any case, there are essentially no differences in the ecological and socio-cultural settings in their locations.The study facilities where all located along the costal belt of the metropolis.

Effia Nkwanta Regional Hospital

Effia Nkwanta is the regional hospital. It is located in Sekondi and has a total bed capacity of 329 and an obstetric bed capacity of 48. Average monthly antenatal attendance for 2014 at Effia Nkwanta is 1176, with 124 being average new attendants per a month and 1052 being average old

attendants per month. The total annual deliveries stood at 3,169 with a monthly average delivery of 264 (Records and Statistics Department of Effia Nkwanta Regional Hospital, 2014).

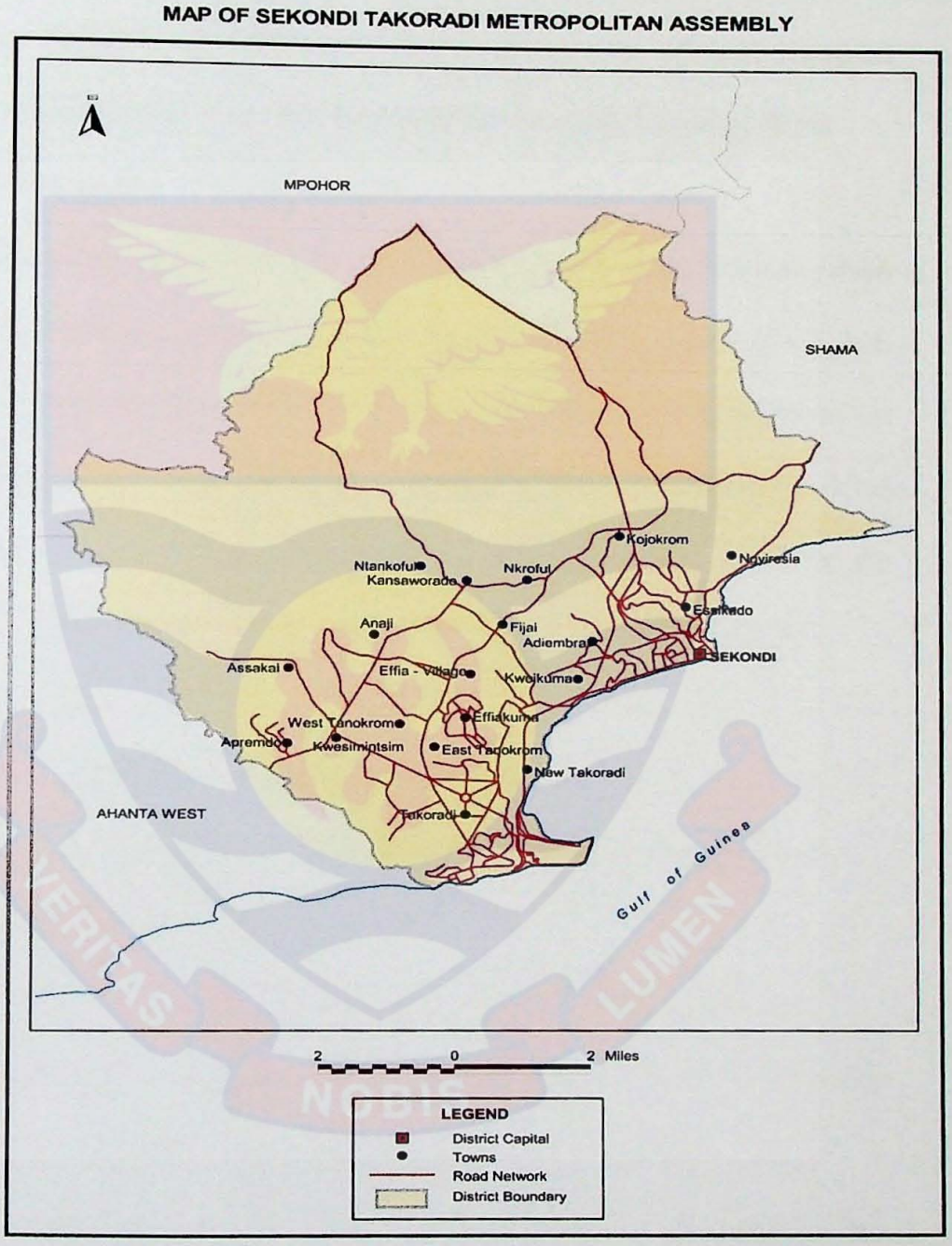

Figure 6: Map of Sekondi Takoradi metropolis. (Source: Ghana Statistical Service district reports, 2014 Accessed December 2016).

Takoradi Hospital

The Takoradi Hospital is the metropolitan hospital. It is located at south-western part of the metropolis in Takoradi. It has a total bed capacity of 104 and an obstetric bed capacity of 18. The average monthly antenatal attendance for 2015 was 1,028 and average monthly new antenatal attendees being 114 (Unpublished data, Record unit of the Takoradi Hospital, 2014).

Essikado Hospital

Essikado hospital is a very well attended government hospital which runs regular antenatal clinics. It is also located in the eastern parts of Sekondi. It mostly serves women in the relatively lower socioeconomic brackets of the metropolis. Average monthly ANC attendance for 2014 was 1005, with 103 of them being new attendees. Average monthly deliveries stood at 214 in the same year. (Unpublished data, Records unit of Essikado Hospital, 2014).

Study Design

The research was a four-part study comprising:

1. *In vivo* sulphadoxine-pyrimethamine therapeutic efficacy study
2. Maternal and Birth outcome study
3. Characterization of molecular markers of SP resistance and
4. Evaluation of the quality of SP tablets used in the SP-IPTp programme in the Sekondi-Takoradi Metropolis.

It employed both institutional-based prospective study and cross-sectional study. While the therapeutic efficacy study was a prospective study, the birth outcome and the characterization of molecular markers studies were cross-sectional. The procedures leading to the evaluation of the quality of SP tablets in use for IPTp were partly observational and partly experimental.

Study Methods

The study methods employed in this study were mixed. The SP efficacy trial, the birth outcome study, the molecular surveillance and the laboratory experiments in the evaluation of the quality of SP tablets employed mainly quantitative methods to generate data. The observational tours of the Regional Medical stores, hospitals' stores and pharmacies as well as interactions with staff of these outfits were qualitative methods of data generation.

Sampling Method and Sample Size Estimation

A passive case finding method was used to recruit participants into the SP Efficacy study, the birth outcome study and the molecular surveillance study. Tablets for the evaluation of the quality of SP in use were however randomly selected from the study sites.

A sample size of 40 was calculated for the SP efficacy study using the LQAS recommended by the WHO, (WHO, 1996) taking an estimated prevalence of 25% treatment failure as indication of replacement of the first-line antimalarial drug, a 10% failure rate as acceptable with a power of 80% and a 95% confidence interval.

$$d = [n \times p/100]$$

$$n \text{ is therefore} = \frac{100 \times d}{P}$$

Where n = number of cases to be foundor the sample size

d = threshold value

p = coverage standard.

Sample size, n = $\frac{100x10}{25}$

n = **40**

Forty-two (42) pregnant women were however recruited into the study through an active and adaptive case finding method (FANTA, 2012)

For the birth outcome study and the study characterizing mutant *dhfr* and *dhps* genes, a minimum sample size of 384 was calculated as shown elsewhere (Ovenseri-Ogbomo, & Omuemu, 2010). Thus, for a population of more than 10,000;

Sample size, $N = z^2 (1–p) (p)/b^2$, where

N = minimum sample size

z = the standard normal deviation, usually set at 1.96 which corresponds to the 95% confidence interval.

p = the proportion in the target population estimated to have the infection. With no reasonable estimate, it is set at 50%.

b = degree of accuracy desired, usually set at 5%

Inclusion and Exclusion Criteria

Those included the *in vivo* SP efficacy study were pregnant women of any age and parity who were at 16-26 weeks of gestation based on last menstrual period (LMP) or have experienced quickening (feeling of first foetal movements) and are yet to receive their first SP dose; those with axillary temperatures less than 37.5°C and had signed an informed consent. Meanwhile pregnant women with a history of hypersensitivity reaction to SP or components of SP, had Glucose-6-Phosphate Dehydrogenase defect (Full or Partial), had Sickle Cell Disease, with axillary temperatures greater than 37.5°C, those with a history of receipt of antimalarial in the past month, those

stool evidence of helminth infection and those known to have HIV infection were excluded from the study.

The inclusion criteria for the birth outcome study were: a singleton pregnancy, available history of usage of SP-IPTp. (This was ascertained by a verbal questioning and from antenatal booklets). Delivering mothers who have had blood transfusion after the 16th gestational age, those who have taken any other antimalarial apart from SP for IPTp after the 16th gestational age, those with stool evidence of helminth infection and those with multiple pregnancies were excluded from this study.

All non-pregnant attendees at the general OPD of the study sites, regardless of demographic characteristics with microscopy confirmed clinical malaria were included in the study characterizing the molecular markers for SP resistance.

Ethical Considerations

The study was conducted according to the Helsinki Declaration on Research regarding human subjects. The protocol for this study was reviewed and approved by the Ghana Health Service's Ethical Review Committee (GHS-ERC). Ethical clearance certificate with identification number GHS-ERC: 78/03/13 was issued by the GHS-ERC before commencement of the study (See appendix A for copy of certificate). Approval was also sought and given by respective Medical Superintendents of the three health facilities used in the study.

Before any data was collected in any of the study, the rationale and procedures of the study was fully explained to participants in their own language. They were then given the chance to ask questions if any. Individuals

who agreed to participate then signed consent forms attached to the questionnaires. For participants below the age of 18 years whose assent was sought, their parents/guardians signed the consent forms on their behalf. Confidentiality and safety were ensured at all times.

Questionnaires

Participants in the therapeutic efficacy and birth outcome studies answered questionnaires that sought information on: a) vital signs: 1.Weight 2. Height and 3. Temperature: b) Demographic characteristics: 4. age, 5. Educational level, 6. Occupation, 7. Partner's occupation and 8. Partner's level of education: Others questions included 9. Parity: 10. Date of last delivery; 11. History of neonatal and or perinatal death: 12. History of low birth weight: 13. History of prematurity in previous pregnancies 14. Malaria control measure used (i) Insecticide treated net (ii) Indoor residual spraying (iii) others. 15. Usage of an antimalarial in the last four weeks 16. Use of any sulphur containing drugs in the last four weeks 17. G6PD and HIV status were asked and responses were verified from antenatal booklets.

Collection and Preparation of Blood Samples

For the SP efficacy study, three different kinds of samples were collected and prepared. These were: Finger pricking for MRDT, venipuncture for venous blood as well as blotting of filter papers with venous blood. In the birth outcome study, both peripheral venous and placental villous blood (from the maternal surface of the placenta) were taken from delivering mothers and blotted on filter papers as well. For the study characterizing the mutant genes for SP resistance, filter papers were blotted with venous blood from microscopy confirmed clinical malaria in outpatient attendees. Blood sample

collection and preparation in the therapeutic SP efficacy study and in the study characterizing the molecular markers of SP resistance was done by trained laboratory technologists. Thatfor the birth outcome study was done by attending midwives who were so trained to do. In the preparation of sample for MRDT, participants were given a pin prick (using a lancet provided by the manufacturer) on the palmar aspect of any of their fingers (usually their thumb) after the area was cleaned and sterilized with methylated spirit. Micro Pasteur pipettes provided in the test kits were used totake about 5ul of blood from the pricked point and delivered into the sample well of the kits for buffer addition.In all cases of venous and placenta villous blood collection, about 3 ml of the venous and or placenta villous blood were obtained from each participant using a sterile disposable hypodermal syringe fitted with a 23-gauge needle and dispensed from the syringe barrel into a sterile EDTA tubes for furtherprocessing. Venous blood samples in the EDTA tubes where used for malaria diagnosis by microscopy and determination of haemoglobin levels. While dispensing the blood from the syringe barrel, about 5-10µl of the blood from participants was used to blot already cut filter papers into circular blots, usually about 3cm in diameter. These (the blotted filter papers) were allowed to air dry labeled and put into individual plastic envelopes stored at room temperature awaiting DNA extraction and amplification.

Haematological Analysis

The haematological parameters investigated for this study included determination of haemoglobin levels and thin and thick film microscopy for malaria parasites. These were determined only in women who participated in the therapeutic efficacy and birth outcome studies.

Determination of Haemoglobin Levels

Haemoglobin levels for participating women were estimated using a Mindray® automated haematology analyzer. Anaemia was categorized using haemoglobin levels as follows: mild; 9.0.1-10.9 g/dl, moderate; 7.0-8.9 g/dl and severe; less than 7.0g/dl (WHO, 2011).

Diagnosis of *Falciparum* Malaria

Three methods were employed in the diagnosis of malaria. These were rapid diagnostic test (RDT), microscopy and polymerase chain reaction (PCR). All three methods were employed in the therapeutic efficacy study whilst two; microscopy and PCR were in diagnosing malaria in the birth outcome and molecular surveillance for mutant genes for SP resistance studies.

Diagnosis by Rapid Diagnostic Test

A First Response® *Pf*HRP-2 MRDT was used in the recruitment of participants into the therapeutic efficacy studies. Both negative and positive controls were set for each box of test kits to ensure reliability of kits. Processes of blood sample taking were as already described. About 60µl of assay buffer provided was then delivered into the buffer well. Test was allowed to run for about 20 minutes at room temperature. A test was said to be positive when two rose-pink colour bands were seen at both the control (C) and test (T) labels. If the colour band was seen at only the control label, then the test was said to be a negative test. Test was said to be invalid if there were no bands at both the control and test labels.

Diagnosis by microscopy

Microscopy for *P. falciparum* was done according to a protocol described elsewhere (van Eijk et al., 2004). Thick and thin blood smears of both peripheral venous as well as placental villous (where applicable) blood were prepared and stained with Geimsa. The thin blood films were used for speciation. The thick films were used to estimate the parasite density. Malaria parasites were counted against 300 leucocytes. Parasite densities were expressed per microlitre using an assumed count of 8000 white blood cells per microlitre of blood.

$$\text{Parasite Density} = \frac{8000\text{WBC}/\mu\text{l} \times \text{Parasites counted against 300 WBC}}{300\ \text{WBC}}$$

To declare a film as negative (without parasites), minimum of 100 high power fields would were examined. Slides were read independently by two experienced microscopists. If there were disparities in any result, a third and a more experienced microscopist read the slide. His result was deemed final.

Polymerase Chain Reaction (PCR)

Nested PCR methods were used to monitor treatment outcomes as well as distinguish between reinfection and recrudescence amongst samples with parasitaemia during the SP efficacy study. They were also used in the molecular diagnosis of peripheral and placental *Plasmodium* infection in the birth outcome studies. PCR methods were also used in amplifying *dhfr* and *dhps* genes in the study characterizing the markers of SP resistance in both pregnant women and the non-pregnant OPD attendees. The PCR machine used is the Techne TC-412 maufactured by Barloworld Scientific Ltd, Staffordshire, UK. (Details of specific primers and reaction conditions used for each aspect of the study has been described accordingly).

Detection of PCR products

Detection of PCR products was done using Agarose gel electrophoresis. The gel documentation machine used was EBOX VX5, Vilber Lourmat, Marnela-Vallee cedex 3, France. A 1.5% agarose gel was prepared by weighing a 1.5g of agarose into a 100ml of 1X TBE and microwaving until total dissolution of the agarose. About 3µL of Ethidium Bromide was added, swirled to mix, poured into a gel cast and allowed to stand (with a 16-well comb(s) inserted) till the gel is formed The formed gel was put into an electrophoresis chamber filled with 1X TBE buffer. PCR products were loaded into the wells with a 100kb ladder as well and the chamber was connected to a 95-100V constant power source. The PCR products together with the DNA ladder were allowed to run appreciably. The gel was then placed in a gel imaging cabinet and digitally photographed under UV light.

DNA Extraction

Dried blood spots were cut to appropriate size (approximately 3mm x 3mm) using disposable razor blades or a pair scissors. The pair of scissors used was cleaned with hydrochloric acid in between each cut whilst razor blades used were disposed off after each cut. DNA extraction, in all cases, was done using the Chelex method of DNA Extraction as described by Dokomajilar and Greenhouse (2006) (Appendix E).

The Therapeutic Efficacy Study

The aim of this part of the research was to determine the therapeutic efficacy of SP-IPTp in pregnant women attending antenatal clinics at Essikado and Takoradi Hospitals using a prospective *in vivo* study.

Study protocol

Recruitment for this aspect of the study was done based on the inclusion and exclusion criteria previously described. Pregnant women who met the inclusion criteria were screened for malaria using the MRDT after a written informed consent was sought. Their socio-demographic and obstetric characteristics were also assessed using a questionnaire. Venous blood was then taken from both pregnant women who were MRDT positive and those who were not. This blood sample was used for confirmation of parasitaemia, determination of parasite density by microscopy and determination of haemoglobin. Participants then received three tablets of SP (500mg sulphadoxine and 25mg pyrimethamine each) under direct observation of the healthcare providers. If a sample was MRDT positive yet was microscopy negative, the sample was deemed negative and the participant was withdrawn from the study. Those whose parasitaemia were confirmed by microscopy and were yet asymptomatic for malaria were then enrolled into the study. Their samples deemed to be day 0 samples. These participants were followed up on days 3, 7, 14, 21 and 28, 35 and 42. Venous blood was used to blot already cut filter papers for molecular analysis by PCR. All the participants continued taking all other haematinics apart from folic acid.

To encourage commitment to the follow-up visits, participantswere given an equivalent of $3 (USD) at each follow-up visit to cover travel expenses. Where possible, mobile phone numbers of participants were taken. They were called and reminded of their visit a day or two prior to their day of follow up. Each follow-up visit included an interview for fever and recent medication history including anti-malarials, and measurement of axillary

temperature. Blood samples were also taken for diagnosis of malaria parasitaemia by microscopy as well as PCR. Women found to be symptomatic on follow-up were treated according to national guidelines and excluded from the study.

Molecular analysis

PCR was done for the amplification of the *msp2* protein of the *Plasmodium falciparum* parasite on all day 0 samples and on each day of follow up. A 25µl PCR reaction mixture was prepared in all cases.This consisted of 12.5ul of the DreamTaq green® premix; 0.3µl each of the forward and reverse primers; 10.2µl nuclease free water and 2µl of extracted DNA samples or controls as the case may be. Primers and reaction conditions for the PCR were as followed in table 1.

Table 1: Primers and reaction conditions for amplification of *msp2* proteins.

Primers	**Reaction conditions**
Nest1 **S1**:5'GAGTATAAGGAGAAGTATG3' **S4**:5'CTAGAACCATGCATATGTCC3'	Initial denaturation at 94°C for 3 minutes; 30 cycles of denaturation at 94°C for 25 seconds, annealing at 42°C for 1 minute and extension at 65°C for 2 minutes. Final extension was at 72°C for 3 minutes.
Nest 2 **S2** 5'GAGGGATGTTGCTCCACAG3' **S3** 5'GAAGGTAATTAAAACATTGT3'	Initial denaturation at 94°C for 3 minutes; 30 cycles of denaturation at 94°C for 2 minutes. Annealing was at 50°C for 1 minute and extension at 70°C for 2 minutes. Final extension was at 72°C for 3 minutes.

Distinguishing between recrudescence and reinfection

To distinguish recrudescence from new infection, *msp2* PCR products of paired pre-treatment sample and samples which showed presence of parasites after treatment were run side by side on a 1.5% Agarose gel and with a 100bp DNA ladder. Paired DNA bands that moved at the same rate were determined as being from the same parasite strain and were therefore classified as cases of recrudescence: Paired bands that moved at different rates on the gel were considered to be from different strains of parasite and therefore classified as being cases of re-infection.

The Maternal and Birth Outcome Study

The objectives of this study were (i) to determine the peripheral and placental blood prevalence of *Plasmodium* parasites in delivering mothers (ii) to determine the relationship between the number of SP doses and the prevalence of peripheral and placental parasitaemia in delivering mothers (iii) to measure birth weight of babies and correlate it with peripheral and placental parasitaemia, IPTp-SP usage and maternal anaemia and ITN usage and (iv) to determine the specific mutation of *Plasmodium falciparum* in peripheral and placental blood samples. It was a retrospective cohort study. It involved women of all parity and of all ages delivering at the study facilities.

Study protocol

Delivering mothers at the selected health facilities were recruited into the study based on inclusion criteria previously described. Their informed consent was sought. Those who agreed to be involved had their demographic and obstetrics characteristics taken using the questionnaire. Their peripheral venous and placental villous blood were taken at delivery and examined for

the presence of the malaria parasite by both microscopy and PCR. Maternal haemoglobin levels were also determined from the peripheral venous blood. Anaemia was categorized as describedpreviously. Birth weights of babies were also measured to the nearest 10g. Low birth weight was defined as a birth weight less than 2.5 kg. The number of doses of SP taken by the delivering mothers was determined by asking mothers. This was confirmed from ANC booklets. Micronutrients (such as iron, multivitamins and folic acid) supplementation was also confirmed from the ANC booklets. Detection of *Plasmodium* parasitaemia was by both microscopy and PCR. Detection of parasitaemia by microscopy has been previously described.

Detection of *Plasmodium* parasitaemia by PCR

Detection by PCR followed the protocol described by Singh et al, (1999). It was a nested PCR and involved the use of both genus and species-specific primers in a 25µl reaction mixture in all cases. This consisted of 12.5µl of the DreamTaq green® premix; 0.3µl each of the forward and reverse primers; 10.2µl nuclease free water and 2µl of extracted DNA samples or controls as the case may be. Controls were 3D7 laboratory strains which was supplied by Professor Neils Quashie of the Noguchi Memorial Institute of Medical Research. The primers involved and the corresponding reaction conditions were as shown in table 2 below:

Table 2: *Plasmodium* genus specific PCR primers and reaction conditions

Primers	Reaction Conditions
rPLU1: 5'TCA AAG ATT AAG CCA TGC AAG TGA3' ***rPLU5***: 5'CCT GTT GTT GCC TTA AAC TCC3'	Initial denaturation was at 94°C for 4 min; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 1 min and extension at 72°C for 1 min.Final elongation was at 72°C for 4 min.
rPLU3: 5' TTT TTA TAA GGA TAA CTA CGG AAA AGC TGT3' ***rPLU4***: 5' TAC CCG TCA TAG CCA TGT TAG GCC AAT ACC3'	Initial denaturation was at 94°C for 4 min; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 62°C for 1 min and extension at 72°C for 1 min.Final elongation was at 72°C for 4 min.

Products of the nest 2 PCR were then separated in 1.5% agarose gel by electrophoresis after staining with ethidium bromide and read under UV light.. If a sample showed DNA bands, its nest 1 PCR product was used as DNA template for another nest 2 PCR, this time involving species-specific primers for genotyping the *Plasmodium* species type as shown in table 3 below.

Table 3: Species specific PCR primers and reaction conditions for *Plasmodium*

Primers	Reaction Conditions
P. falciparum	Initial denaturation was at 94°C for 4 min; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 1 min and extension at 72°C for 1 min.Final elongation was at 72°C for 4 min.
rFAL1: 5' TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT3'	
rFAL2: 5' ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC3'	
P. vivax	
rVIV1: 5' CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC3'	
rVIV2: 5' ACT TCC AAG CCG AAG CAA AGA AAG TCC 3'	

Product of species specific PCR were also detected in a detection chamber under UV light after 1.5% agarose gel electrophoresis with ethidium bromide staining.

Surveillance and Characterization of Molecular Markers of SP Resistance

The aim of this aspect of the study was to determine prevalence and characterize the markers of SP resistance both in participating pregnant women and in *Plasmodium* positive attendees at the general OPD. Isolates used were form:

1. Samples from attendees at the general OPD who have been confirmed, using microscopy as having clinical malaria.
2. All microscopyconfirmed day 0 samples as well as specimens which the *Plasmodium falciparum* fail to clear in the therapeutic efficacy study.
3. *Plasmodium falciparum* positive peripheral and placental specimens in the birth outcome study.

Study protocol

Blood samples of attendants at the general OPD of the study sites with microscopy confirmed clinical malaria were blotted on filter papers after informed consent was sought. These attendees were categorized into two groups; 0-59 months and those above 59 months. DNAs extracted from these samples were used toanalyze and characterize mutations in *dhfr* and *dhps* genes as an index for the general population studied. DNA was extracted using the Chelex method of DNA extraction as described previously. In the case of the categories of pregnant women described above, DNAs already extracted from the previous studies (therapeutic efficacy and birth outcome studies) were used to determine the specific mutations, if any, in these participants. Extracted DNAs were all stored at -32°C pending usage.

Detection of the mutations in the *dhfr* and *dhps* genes

The detection of mutations in the *dhfr* and *dhps* genes was done by modification of a protocol described elsewhere (Duraisingh et al., 1998). It involved a nested system of amplifying the region of a gene of interest by PCR, followed by restriction digestion.

The PCR protocols

Primers used for the PCR included those so designed to be able to create restriction sites so that even polymorphisms not described by natural restriction sites can be detected to distinguish between all of the polymorphisms in the *dhfr* and *dhps* genes known. However, for this study, the interest was in mutations in **S108N**, **N51I** and**C59R;** the so called triple mutations in the *dhfr* gene responsible for pyrimethamine resistance and **K540E** and **A437G** mutations in *dhps*gene responsible for sulphadoxine

resistance. All primers were purchased from The Midland, Certified Reagent Company, Texas, USA.

The PCR reaction mixtures for both nest 1 and 2 were made to a volume of 25µl; comprising 12.5µl of the Dream taq green® premix, 10.2µl of nuclease free water, 0.3µl of both the forward and reverse primers and2µl of the DNA template. Primers and reaction conditions were as shown in table 4 and 5 below.

Table 4: Primers and reaction conditions for *dhfr* amplification

Primers	Reaction Conditions
Nest 1 ***M1***5'TTTATGATGGAACAAGTCTGC 3' ***M5***5'AGTATATACATCGCTAACAGA 3'	Initial denaturation at 94°C for 3 min; then 94°C for 1 min, annealing at 45°C for 1 min and extension at 72°C for 1 min, repeated for 40 cycles, with a final extension step at 72°C for 10 min
Nest 2 **M35'**TTTATGATGGAACAAGTCTGC GACGTT3' ***F/5***'AAATTCTTGATAAACAACGGA ACCTttTA3' ***F5***'GAAATGTAATTCCCTAGATATG gAATATT3' ***M45***'TTAATTTCCCAAGTAAAAC TATTAGAgCTTC3'	Reaction conditions for nest 1 were same as above, only that in this case, the first five annealing steps were carried out for 2 minutes.

Table 5: Primers and reaction conditions for *dhps* amplification

Primers	Reaction Conditions
Nest 1 ***R2***5'AACCTAAACGTGCTGTTCAA3' ***R/***5'AATTGTGTGATTTGTCCACAA3'	Initial denaturation at 94°C for 3 min; then 94°C for 1 min, annealing at 45°C for 1 min and extension at 72°C for 1 min, repeated for 40 cycles, with a final extension step at 72°C for 10 min
Nest 2 ***K***5'TGCTAGTGTTATAGATATAGGatGAG cATC3' ***K/***5'CTATAACGAGGTATTgCATTTAATg CAAGAA3'	Reaction conditions for nest 1 were same as above, only that in this case, the first five annealing steps were carried out for 2 minutes.

Restriction Fragment Length Polymorphisms (RFLP)

Enzymatic restriction digestions were carried out overnight with the various restriction enzymes using the protocols described by the manufacturer (Midland Certified Reagents Company, Texas USA). The enzymes **Tsp509I** and **BsrI** were used to digest at codon 51 and 108 on the *dhfr* gene respectively, each at 65°C for 16 hours after nest 2 PCR amplifications with the primers M3 and F/. **XmnI** was used for digestion at codon 59 on the *dhfr* gene at 37°C after nest 2 PCR amplifications with the primers F and M4. Codons 437 and 540 on the *dhps* genes were digested by enzymes **MwoI** and **FokI** respectively, both at 37°C after nest 2 amplification with the primers K and K/. A reaction volume of 20µl was used for all digestions. This comprised 10µl of the PCR product, 8.5µl of nuclease free water, 1µl of buffer and 0.5µl of the restriction enzyme.

Controls

The external control used was the 3D7 laboratory strain supplied by Professor Neils Quashie of Noguchi Memorial Institute of Medical Research. However, the primer designs used provided for internal controls in the PCR products after Nest 2 amplifications. For instance, PCR product following nest 2 amplification with M3-F/ contained an additional Tsp509I restriction site which served as an internal control for the digestion of codon N51I. The PCR product after Nest 2 amplification with the primers F-M4 also contained an extra XmnI restriction site which served as an internal control for the digestion of C59R as well as an extra site for the enzyme AluI in the digestion of S108N. The primer K/ created an additional restriction site in its product for the enzyme MwoI as internal control for the digestion of A437G whilst the primer K created an internal control for the digestion of K540E by FokI.

Gel electrophoresis

This was done on 2.5% agarose gel stained with ethidium bromide. 100bp markers were used to size the DNA bands and visualized under a digital UV light gel documentation system.

Evaluation of the Quality of SP Tablets in Use as SP-IPTp

Since procurement and storage of a drug affects the quality of the drug given to the end user, the evaluation of the quality of SP tablets being used as IPTp at the time of the study begun by firstly, assessing the supply chain and storage conditions of these drugs. This was then followed by identifying the active compounds, SP, in the sampled tablets, then assaying the amount of these active compounds in the tablets using the United States Pharmacopoeia (USP). The solubility of the sampled tablets was also evaluated as a measure of quality using a dissolution method.

Storage and supply chain of the SP tablets

SP is a programme drug. It is therefore supplied to most public medical facilities at no cost by the central medical stores through the regional medical stores of the ministry of health. A visit was therefore paid to the western regional medical stores to ascertain on first-hand the supply and storage processes. At the store, there was an interaction with the store managers to find out on issues bordering on the supply chain. There was also a visit to the pharmacy unit of the studies facilities to interact with the managers there and to ascertain at first hand their storage conditions as well as how the drugs eventually gets to the end user, the pregnant woman. SP tablets for analysis of solubility and USP were sampled from all the three study facilities and from the regional medical stores and analysis were done at the Analytical Laboratory of the FDA, Accra, Ghana.

Identification of the active principles and quantities of sulphadoxine and pyrimethamine in the tablets sampled

The identification of the sulphadoxine and pyrimethamine and quantification of same, in the sampled tablets, followed a method described in the US pharmacopoeia National Formulary, 2015.Volume 3 with some modifications. The method involved preparation of the SP tablets into solution and subsequent injection into a High-Performance Liquid Chromatography (HPLC) machine.

The High-Performance Liquid Chromatography (HPLC) equipment

The HPLC equipment used was the Agilent® 1260 Infinity Series fitted with a Quaternary pump, an Auto-sampler, Column Temperature Compartment and a Variable Wavelength Detector (VWD).The column for the

HPLC for the experiments was Phenomenex lung Phenyl-Hexyl 5u 150 x 4.6mm; serial number: 512900-8. The mobile phase was Acetonitrile: 0.1% Phosphoric Acid (17:83, v: v). All HPLC experiments were maintained at ambient temperature at a flow rate of 2.38ml/min using a 20-µL injection loop. Detection was under UV light at 230nm.

Preparation of reference standard

The reference standard used was 0.4mg/ml Sulfadoxine and 0.02mg/ml Pyrimethamine. This was prepared by transferring 40mg Sulfadoxine Reference Standard and 2mg Pyrimethamine Reference Standard (procured from the US Pharmacopoeial Convention™) into a 100ml volumetric flask.17ml of Acetonitrile was added and sonicated to dissolve. Solution was further diluted with 0.1% Phosphoric acid solution to volume.

Sample preparation

Twenty tablets, randomly selected from the study facilities were weighed individually and ground into powder form. To the accurately weighed powder equivalent to 200mg of sulphadoxine and 10mg pyrimethamine in a 100mL volumetric flask, 28ml of acetonitrile was added and the sample was then sonicated for 30 minutes and allowed to stand for some time. The sample was diluted with 0.1% Phosphoric acid to volume and mixed. Then 5 ml of the resulting solution was pipetted into a 25ml volumetric flask and diluted with the mobile phase to volume.The solution was filtered and 5mls of the filtrate was taken for the injection into the HPLC for analysis.

Identification of active principles in the tablets sampled

The separation and subsequent identification of sulphadoxine and pyrimethamine, using the USP method was first verified by injecting a mixture

of reference standard sulphadoxine and pyrimethamine solutions on the HPLC column with detection at 230 nm under conditions described previously. The reference standard used was 0.4mg/ml and 0.02mg/ml of Sulfadoxine and pyrimethamine respectively. Six (6) injections of 20µl of the standard were made in all. The average retention times of sulphadoxine and pyrimethamine in the standard injections were determined. Six injections were also made for the sample and the average retention times for sulphadoxine and pyrimethamine were determined. The average retention times of the sulphadoxine and pyrimethamine in the samples were compared with the average retention times of the sulphadoxine and pyrimethamine in the standard.

Determination of quantities of sulphadoxine and pyrimethamine in the SP tablets (Assay).

For the assay of the active principles in SP, the active principle assayed is expected not to be less than 90% or more than 110% of the amount on the label.

The percentage assay was calculated as followed:

Percentage Assay equals

$$\frac{\text{Sample Response}}{\text{Standard Response}} \times \frac{\text{Standard Concentration}}{\text{Sample Concentration}} \times \text{Standard Potency} \times 100\%$$

Where:

Sample Response is the area under the curve of the peaks of sulphadoxine and pyrimethamine in the sample. It is equivalent to the amount of the active principle (sulphadoxine and pyrimethamine) that eluted in HPLC the analysis and therefore the amount of the active principle of the sample that dissolved in the buffer.

Standard Response is the area under the curve of the peaks of sulphadoxine and pyrimethamine in the reference standard used. It is equivalent to the amount of sulphadoxine and pyrimethamine that eluted in the HPLC analysis.

Standard Concentration is the concentration of sulphadoxine or pyrimethamine in the standard solution used.

Sample concentration is the concentration of sulphadoxine or pyrimethamine in the sample used.

Standard Potency is the potency or strength of the standard solution to be used as reference.

Determination of the solubility of SP tablets

The solubility of SP tablets in use at the time of the study was determined using dissolution tests described in the US Pharmacopoeia (2015), USP 38, National Formulary 33rd edition, Vol 3. A Dissolution test is done to determine the amount of active ingredient that is released from the dosage form. The test result can be used as surrogate marker of *in vivo* bioavailability. It involves the dissolution of formulations of the drug in given buffer using a dissolution apparatus and subsequent determination of the amount of the active principles that goes into solution within a specific time using the HPLC.

The Dissolution apparatus

The apparatus used was the paddle apparatus. The essential parts of the apparatus are; a paddle, vessels (six of them) and a motor. The paddle (made of stainless steel type 316) formed from a shaft and a blade was used as a stirring element. The shaft is positioned such that its axis is not more than 2mm from the vertical axis of the vessel at any point and it rotates smoothly without any significant wobble that could affect the results. A distance of

25+/- 2 mm is allowed between the bottom of the blade and the inside bottom of the vessel and this is maintained throughout the test. The vessels are cylindrical with a hemispherical bottom and are made of a transparent glass material (to allow for observation). They are fitted with a heating jacket which allows holding the temperature inside the vessels at 37 +/- 0.5°C during the test and keeping the fluid medium used in constant smooth motion.

The dissolution procedure

A phosphate buffer dissolution medium (pH: 6.8) was prepared. Each of the six glass vessels of the dissolution apparatus were filled with the dissolution medium to a volume of 1000ml. The heating jacket was then turned on and the dissolution medium was equilibrated to 37+/-0.5°C and was covered. One dosage unit of Sulphadoxine-Pyrimethamine tablet was placed in each vessel. Care was taken to exclude air bubbles from the surface as the dosage unit was being placed into the medium. Operation of the apparatus then begun immediately after placing the dosage unit into the vessel. This was done by starting the paddle to rotate at a speed of 75rpm. At 30 minutes, 5ml of the sample from each vessel was taken and 20µl of each was injected into the HPLC machine for determination of the amount of Sulphadoxine and Pyrimethamine dissolved in the dissolution medium. There were two injections from each vessel. A Standard Solution of 0.4mg/ml and 0.02mg/ml of Sulfadoxine and pyrimethamine was used respectively.

Analysis of outcomes of dissolution

For immediate-release dosage forms such as Maladar™ the US pharmacopoeia dissolution requirements for Sulphadoxine and Pyrimethamine is that not less than 60% of the labeled amount of Sulphadoxine and

Pyrimethamine should be dissolved in a dissolution medium in 30 minutes (United States Pharmacopoeia, 2015 & Minzi et al., 2003)

The percentage release of each active principle was calculated using the formulae below:

$$\frac{\text{Sample Response}}{\text{Standard Response}} \times \frac{\text{Standard Concentration}}{\text{Sample Concentration}} \times \text{Standard Potency} \times 100\%$$

Definitions of the above parameters are as described previously.

Data Analysis

Data for the *in vivo* SP efficacy study was entered into STATA, version 13.0. Prevalence of anaemia and usage of ITN amongst participants were determined. Associations between (i) maternal age and parasite density (ii) parity and parasite density and (iii) parasite density and anaemia were determined using a Pearson correlation analysis.Percentage parasitic clearance on days 28 and 42 was also calculated and compared with the WHO expected for continuous use of a drug.

Data from maternal and birth outcome study was entered into SPSS version 21. Prevalence was calculated for the following; (i) peripheral and placental parasitaemia by both microscopy and by PCR in delivering mothers (ii) mutant genes in the *Plasmodium* parasites responsible for resistance (iii) anaemia in delivering mothers and (iv) low birth weight.

A Chi-square test was used to test how low birth weight was associated with (i) number of SP doses taken (ii) peripheral and placental parasitaemia, malaria vector control measures (iii) and (iv) maternal haemoglobin. A multivariate regression analysis was used to assess the risk posed by these factors to low birth weight. Chi-square test was also used to determine theassociation between placental *Plasmodium* parasitaemia and (i) number of

SP doses taken (ii) parity of delivering mothers and (iii) the use of malaria control measures. A multivariate regression analysis was further used to test the risk posed these factors to prevalence of placental *Plasmodium* parasitaemia. A chi-square again tested for the association between maternal anaemia and SP dosages taken, *Plasmodium* parasitaemia and use of ITNs.

Data from the molecular markers of SP resistance surveillance was entered also entered into STATA version 13.0. Prevalence for single, double, triple, quadruple and quintuple mutations were determined at *dhfr* **I51, R59, N108,** and *dhps* **G437** and **E540** amongst outpatients at the general OPD and participating pregnant women. Significance in the difference in the prevalence of mutations in isolates from adults and children was also determined using the z-test score.

In identifying sulphadoxine and pyrimethamine as the active principles in the SP tablets, mean retention time for sulphadoxine and pyrimethamine were compared to that of their respective standard. The tablets were deemed to contain Sulphadoxine and pyrimethamine if their mean retention time calculated were +/- 10% of their respective standard preparations. A t-test was also done to compare the mean retention times of the samples and standards. Quantities of Sulphadoxine and pyrimethamine assayed were compared to the expected 90% lower limit or 110% upper limit of the labeled amounts.

CHAPTER FOUR
RESULTS

SP Efficacy Study

General characteristics of participating women

A total of Four hundred and thirteen (413) ANC attendees who met the inclusion criteria for the study were screened for malaria using MRDTs as as described on page 98 above. Out of these, fifty-seven (57) representing 13.8% were found to be positive. Microscopy after Geimsa staining, however confirmed *P. falciparum* infection in forty-two (42) representing 10.2% of the samples. The species-specific PCR detecting *Plasmodium falciparum* later confirmed fifty-four (54) of samples, representing 13.1%. This put the prevalence of asymptomatic malaria amongst the population studied at 13.8% by MRDT, 10.2% by microscopy and 13.1% by PCR.

The mean age of the participating pregnant women was 28.02 years. Age, eighteen (18) was the least and thirty-nine (39) was the oldest. The mean gravidity was 2.0. Eleven participants (11), representing 26.19% were primigravidae. The mean gestational age was 19.7 weeks. Though 73.81% of the participating women owned ITNs, only 57.14% of them were actually using it as a malaria control measure. The reasons for not using the nets included; rooms being too hot, not really appreciating the need for its use, the perception that there are no mosquitoes in the environment they live in and inability to mount nets amongst others. Seven (7), representing 16.67% out of

CHAPTER FOUR
RESULTS

SP Efficacy Study

General characteristics of participating women

A total of Four hundred and thirteen (413) ANC attendees who met the inclusion criteria for the study were screened for malaria using MRDTs as as described on page 98 above. Out of these, fifty-seven (57) representing 13.8% were found to be positive. Microscopy after Geimsa staining, however confirmed *P. falciparum* infection in forty-two (42) representing 10.2% of the samples. The species-specific PCR detecting *Plasmodium falciparum* later confirmed fifty-four (54) of samples, representing 13.1%. This put the prevalence of asymptomatic malaria amongst the population studied at 13.8% by MRDT, 10.2% by microscopy and 13.1% by PCR.

The mean age of the participating pregnant women was 28.02 years. Age, eighteen (18) was the least and thirty-nine (39) was the oldest. The mean gravidity was 2.0. Eleven participants (11), representing 26.19% were primigravidae. The mean gestational age was 19.7 weeks. Though 73.81% of the participating women owned ITNs, only 57.14% of them were actually using it as a malaria control measure. The reasons for not using the nets included; rooms being too hot, not really appreciating the need for its use, the perception that there are no mosquitoes in the environment they live in and inability to mount nets amongst others. Seven (7), representing 16.67% out of

the forty-two participants included in the study were sickling positive; all of whom had the genotype AS when Hb electrophoresis was done.

The mean haemoglobin was 10.73g/dl. Prevalence of anaemia among participants was 76.19%: 42.86% being mild; 30.95% being moderate and 2.38% being severe. The mean parasite density was 149.6 parasite/µl. Five (5; 11.9%) of the sample showed presence of gametocytes.

Association between parasite density and anaemia

There was a statistically significant negative correlation between parasite density and anaemia (r=0.0028, p=0.02) as shown in Figure 7.

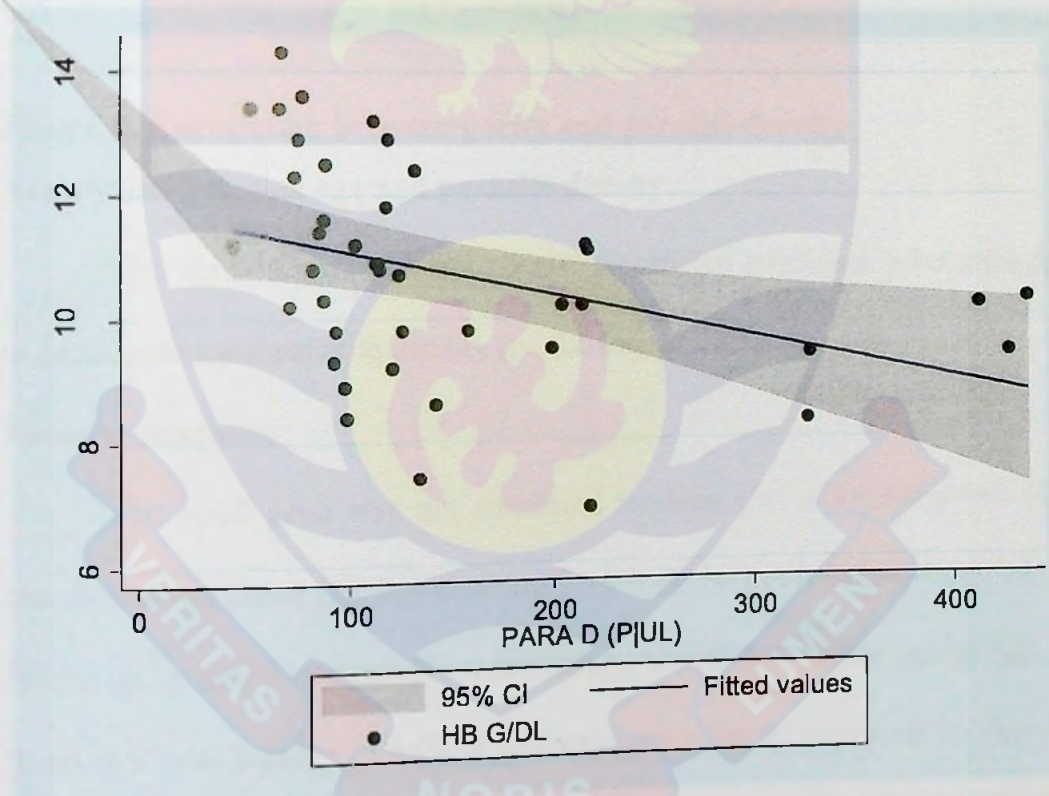

Figure 7: Correlation between parasite density and haemoglobin

Association between parity and parasite density

There was also a significant negative correlation between participants' parity and parasite density (r =-0.0053; p< 0.001) as shown in figure 8.

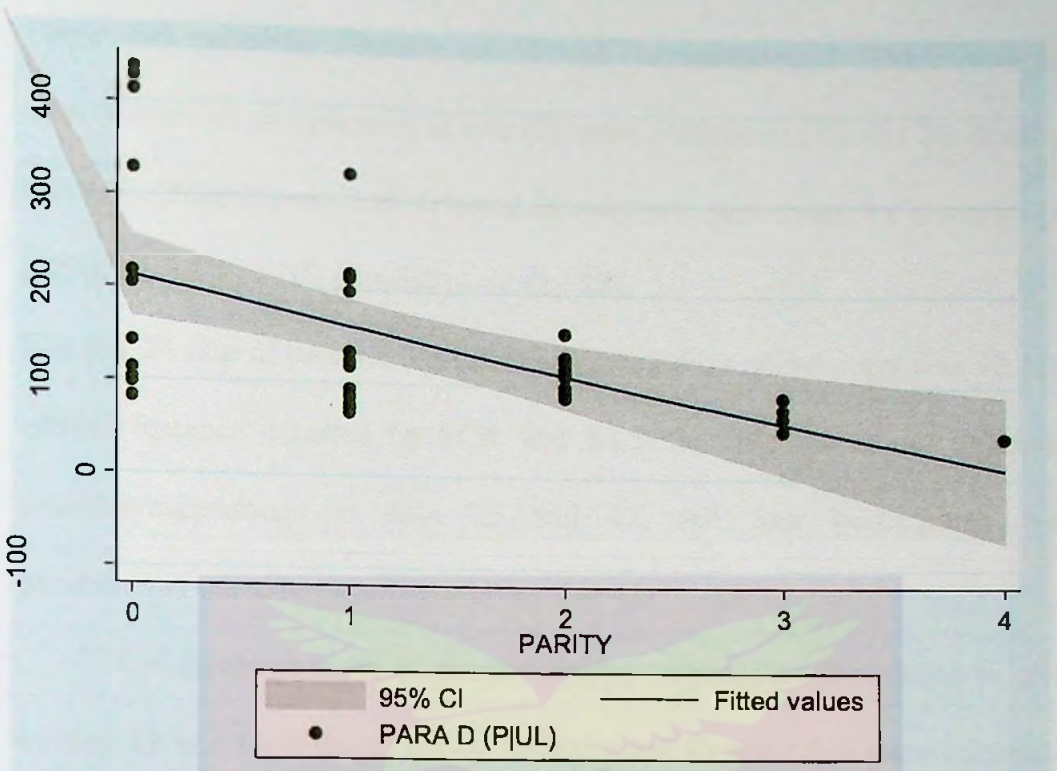

Figure 8: Correlation between parity and parasite density

Association between age and parasite density

This study observed a statistically significant relationship between age of participants and parasite density (r=-0.0166, p=0.05).

Parasite clearance

The *msp2* gene was not amplified in three (3) of the forty-two (42) samples taken. Three (3), representing 7.14% of the participants, were lost to follow up by day 7 and one (1) more person was lost to follow up by day 14 (making a total number of 4 (9.52%) participants lost to follow up by day 14. Only one (1) participant, representing 2.38% as detected by microscopy 2.56% by PCR failed to clear parasites by day 3. The day 3 parasite clearance rate was therefore 97.62% as by microscopy and 97.44% by PCR. By day 7, all participants had cleared parasitaemia. Giving a parasite clearance rate of 100% as detected both microscopy and PCR. By day 21, there was a reappearance of parasitaemia in two (2) participants detected by both microscopy and PCR.

These had parasites densities of 58 and 183parasites/µl. There was the reappearance of parasitaemia in two (2) more participants by day 28 detected by PCR. (Parasitaemia was detected in only one participant by microscopy: parasite density of 102 parasite/µl on day 28).

The day 28 rate of parasite clearance as detected by microscopy was 92.11% whilst clearance detected by PCR was 88.57%. There were no additional parasitesreappearing on days 35 and 42. All four participants with parasitaemia failed to clear parasites by day 42.

Gel electrophoresis of paired samples of parasites that failed to clear by day 42 and their pre-treatment counterparts showed that parasitaemia in one of the samples was as a result of recrudescence whilst that in the remaining three were as result of re-infection: The reinfection rate by day 28 was therefore 7.89%. Two (2) of these three women who got reinfected did not use ITN as a malaria control measure. One (D2, as shown in Figure 9) of these had reinfection by two (2) different strains of the parasite.

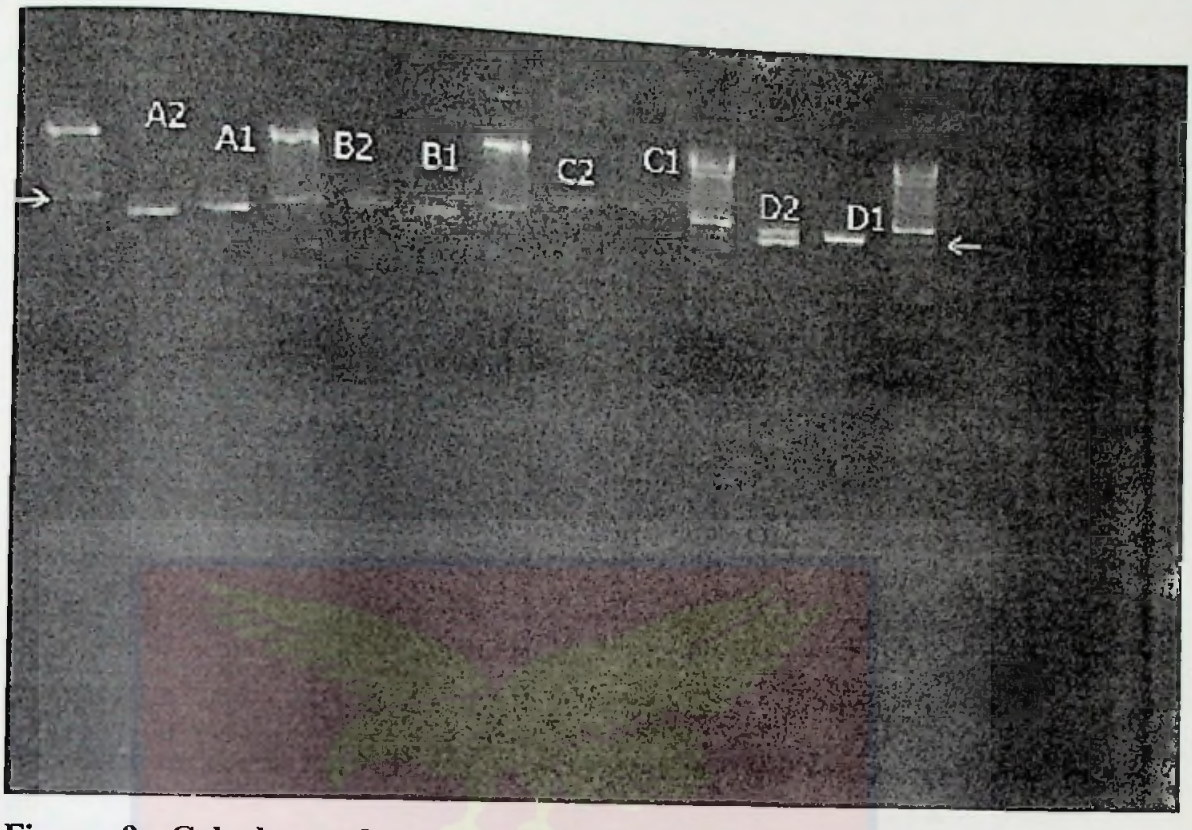

Figure 9: Gel electrophoresis pattern of msp2 genes of *P. falciparum* isolates that failed to clear by day 28 and isolates from their paired pre-treatment samples. (A1, B1, C1, and D1 are msp2 genes from pretreatment isolates while A2, B2, C2 and D2 are from isolates of day 28 samples that failed to clear.

Prevalence of mutations in *dhfr* and *dhps* genes

Enzymatic restriction fragmentation was done to detect mutations **N108**, **R59** and **I51** on the *dhfr* gene and **E540** and **G437** on the *dhps* gene for day 0 parasites which *dhfr* and *dhps* genes were detectable. *Dhfr* and *dhps* genes were amplified in all 42 day 0 samples and in samples that showed the presence of parasites on any other day. Table 6 is a summary of the prevalence of the mutations detected.

Prevalence of single mutations

Single mutations in the *dhfr* and *dhps* genes were found as follows: **N108** (36, 85.7%); **R59** (35, 83.3%); **I51** (31, 73.8%) for *dhfr* gene and **E540** (3, 7.1%); **G437** (8, 19.1%) for *dhps* genes.

Prevalence of double and multiple mutations

Prevalence of double mutations was as follows: **IN**: (33, 78.6%); **RN**: (34, 81%); **IR**: (31, 73.8%); GE: (0, 0.0%). Triple mutations **IRN**: (30, 71.4%). Quadruple mutations **IRNG**: (5, 11.9%) The quadruple mutation, **IRNE** was not found. There were no quintuple mutations as well.

Table 6: Prevalence of double and multiple mutations on *dhfr* and *dhps* genes of *Plasmodium falciparum* isolates from day 0 samples

Mutations	Frequency	Percentage
IN	33	78.6
RN	34	81.0
IR	31	73.8
GE	0	0.0
IRN	30	71.4
IRNG	5	11.9
IRNE	0	0.0
IRNGE	0	0.0

Note

IN refers to double mutations I51 and N108

RN refers to double mutations R59 and N108

IR refers to double mutations I51 and R59

IRN refers to mutations I51, R59 and N108

IRNG refers to quadruple mutations I51, R59, N108 and G437

IRNE refers to quadruple mutations I51, R59, N108 and E540

IRNGE refers to quintuple mutations I51, R59, N108, G437 and E540

The parasite that failed to clear (recrudescence) by day 28 had triple mutation **IRN**. Two (2) of the other three (3) which were determined to be as result of reinfection had double mutations **IN.**

Maternal and Birth Outcome Study

Demographic characteristics of study participants

A total number of four hundred and twenty-five (425) delivering mothers participated in this aspect of the study. The modal age group was 20-29 years and the mean age was 28.7 years. Meanwhile, 7.6% of the participants were in their teen ages and 3.4% were 40 years and above.

Thirty-one of the participants had no form of formal education whatsoever while majority, two hundred and fifteen were (215) representing 52.2% had had education to the basic level; primary, junior high or middle school: Only sixty-four participants (15.5%) have tertiary level education. Thirty-one participating women (7.4%) had no form of employment. Of those employed, 70.6% were in the informal sector whilst 22.0% were in the formal sector. Sixty-nine percent (69.0%) of the participants lived in the urban centres of the metropolis whilst 31% lived in sub-urban areas. The mean gravidity of the women was 2.19 whilst the modal gravidity was 3. The mean birth weight was 3.38kg.

Study outcomes

Frequency of IPT doses taken

During the period of the study, there were shortages of the SP drugs in some of the facilities because of a general national shortage. All participants recruited from the Takoradi hospital (152) had therefore not taken any dose of SP. These were considered as IPT0 or control group. Of those who had taken

any SP dose, thirty-six, representing 8.5% had taken only one dose of SP, eighty-five of them, representing 20.0% of the participants took 2 doses and one hundred and fifty-two (representing 35.8% of the total but 55.7% of the group that took SP) of the participants took 3 doses. No participant took more than 3 doses of SP. These were as shown in table 7 and figure 10 below.

Table 7: Frequencies doses of IPT taken by delivering mothers.

IPT Dose	Frequency	Percentage (%)
IPT0	152	35.8
IPT1	36	8.5
IPT2	85	20.0
IPT3	152	35.8

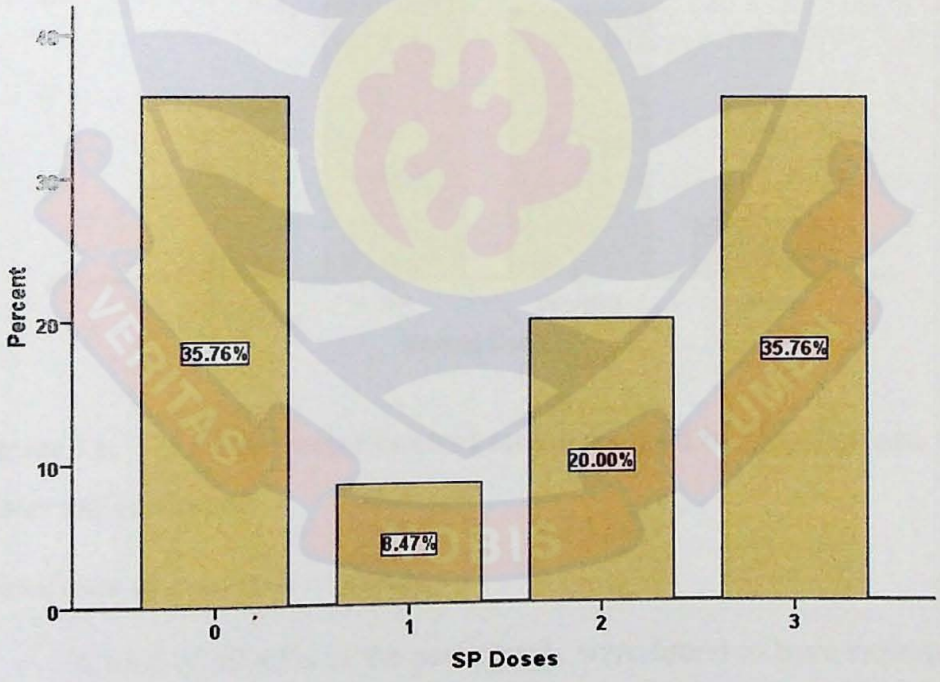

Figure 10: IPT usage.

Malaria vector control measures

It was observed that 64.8 percent of the delivering mothers recruited were using ITNs as their mode of malaria vector control. Of the 35.2% who were not using the ITNs, 13.3% owned the nets but failed to use tham. The

total ITN coverage in terms of ownership was therefore 78.1%. Twenty-eight percent (28.3%) used indoor spraying of insecticides as their measure of mosquito control. Four of the participants were not using any form of vector control measure and about twenty-six (6.12%) were using other forms of mosquito control such as mosquito repellents, mosquito coils, wearing of protective clothing. These were as represented in figure 11 below.

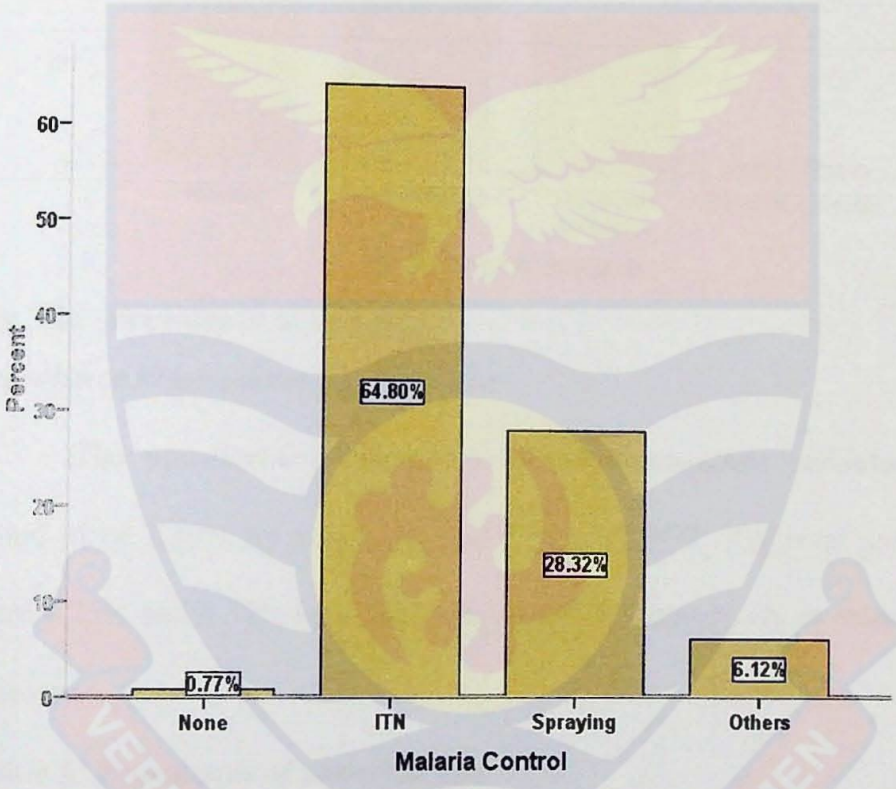

Figure 11: Malaria vector control measures used by participants.

Maternal outcomes

Prevalence of maternal anaemia.

A total of 40.47% of the participants were found to have various levels of anaemia (haemoglobin (Hb) levels less than 11.0g/dl) as shown in figure 12. Severe anaemia accounted for 1.18% (Hb less than 7g/dl); moderate anaemia, 14.82% (Hb of 7.0-8.9 g/dl) and mild anaemia, 24.27% (Hb of 9.0-10.9g/dl).

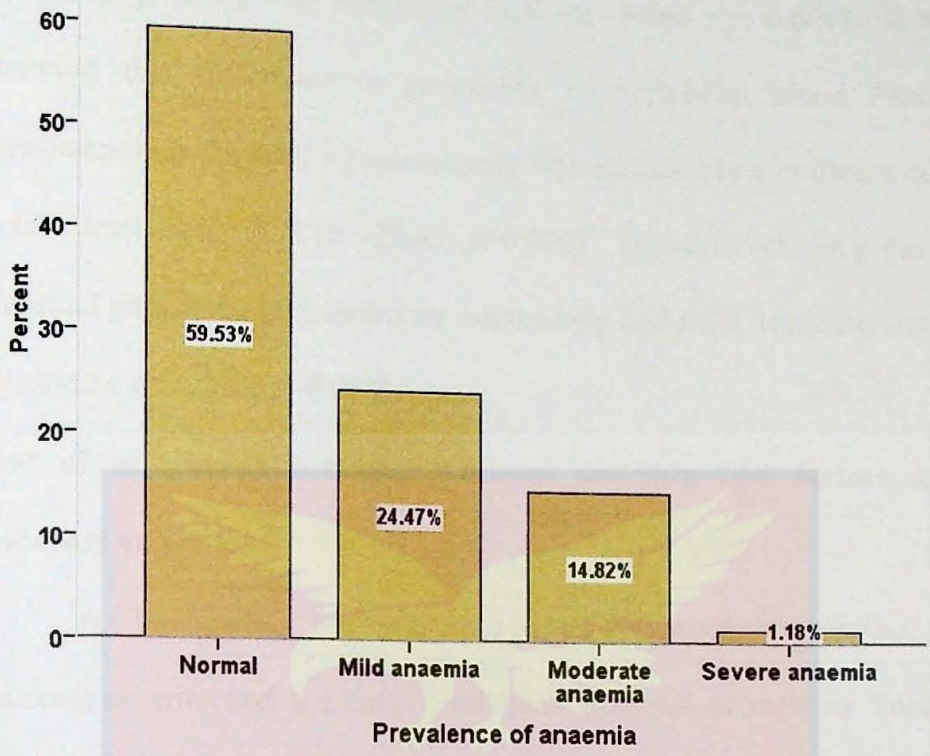

Fig. 12: Prevalence of anaemia

Prevalence Plasmodium parasitaemia

The prevalence of peripheral blood *Plasmodium* parasitaemia was found to be 1.89% by micrscopy and 3.54% by PCR. Placental parasitaemia were 4.25% and 6.37% by microscopy and PCR respectively as shown in table 8 below.

Table 8: Prevalence of parasitaemia

	Microscopy			PCR		
	N	**Positives**	**Prevalence**	**N**	**Positives**	**Prevalence**
Peripheral Blood	424	8	1.89%	424	15	3.54
Placental Blood	424	18	4.25%	424	27	6.37

A Wilcoxon Signed Rank test was performed: Itwas observed that there was a statistically significant difference between the prevalence of peripheral and placental *Plasmodium* parasitaemia as detected by microscopy

(z: -3.162; p=0.002) as well as by PCR (z: -3.464 ; p: 0.001). It was also observed that difference in prevalence of peripheral blood *Plasmodium* parasitaemia as detected by microscopy was statistically significant compared to that detected by PCR (z: -20.42; p: 0.000). The difference in prevalence of placental parasitaemia detected by microscopy and PCR, was also statistically significant (z: -20.32 p: 0.000).

Test of association between martenal anaemia and factors affecting maternal anaemia.

A Chi-square (χ^2) test was used to test the association between maternal anaemia and the factors influencing the risk of anaemia. Factors considered were number of SP doses taken and the use of malaria control measures. Maternal anaemia was not significantly associated with the number of SP doses taken (χ^2= 8.37, p=0.5) nor use of malaria control measures such as ITN and Indoor spraying with insecticides (χ^2=6.62, p=0.7) as shown in Table 9.

Table 9: Test of association between maternal anaemia and factors affecting the risk of anaemia.

Factors	No. Tested	Mild Anaemia No. (%)	Moderate Anaemia No. (%)	Severe Anaemia No. (%)	x^2	P-value
Doses of SP					8.37	0.5
Nil	168	36 (21.4)	26 (15.5)	1 (0.6)		
1	44	10 (22.7)	10 (22.7)	0 (0.0)		
2	80	21 (26.3)	13 (16.3)	2 (2.5)		
3	133	38 (28.6)	14 (10.52)	2 (1.5)		
Vector Control					6.62	0.7
None	15	4 (26.7)	3 (20.0)	0 (0.0)		
ITN	211	82 (38.9)	48 (22.7)	5 (2.4)		
Indoor Spraying	33	10 (30.3)	8 (24.2)	0 (0.0)		
Others	16	6 (37.5)	2 (12.5)	0 (0.0)		

Test of association between peripheral *Plasmodium* parasitaemia and factors influencing the risk of parasitaemia

A Chi-square test showed that peripheral *Plasmodium* parasitaemia was significantly influenced by both the number of doses of SP taken (χ^2= 10.59, p=0.01) and the use of a malaria control measure (χ^2=49.34, p< 0.001) as shown in Table 10 below.

Table10: Test of association between peripheral *Plasmodium* parasitaemia and factors influencing the risk of parasitaemia.

Factors	No. Tested	Positive No. (%)	χ^2	p-value
SP Doses			10.59	0.01
Nil	172	12 (7.0)		
1	41	1 (2.4)		
2	72	0 (0.0)		
3	132	2 (1.5)		
Vector Control			49.34	<0.001
None	15	5 (33.3)		
ITN	345	5 (14.5)		
Indoor Spraying	33	2 (6.1)		
Others	16	2 (12.5)		

Multivariate regression analysis between peripheral *Plasmodium* parasitaemia and factors that affect the risk of the infection.

A multivariate regression analysis done showed that not taking SP at all was associated with a four-fold increase risk of malaria infection as compared to taking 3 doses of SP (AOR=4.88, p=0.04). Meanwhile, the use of ITNs reduced by 9-fold the risk of peripheral *Plasmodium* infection (AOR=9.71, p=0.01) as shown in table 11 below.

Table11: A multivariate regression analysis between peripheral *Plasmodium* parasitaemia and factors affecting the risk of infection.

Factors	No. Tested	Positives No. (%)	Adjusted OR (95% CI)	p-value
SP Doses				
Nil	172	12 (6.9)	4.89	0.04
1	41	1 (2.4)	1.63	0.7
2	79	0 (0.0)	4.64E-9	<0.001
3	132	2 (1.5)	Reference	
Vector Control				
None	15	5 (33.3)	Reference	
ITN usage	345	5 (1.5)	9.71	0.001
Indoor Spraying	33	2 (6.1)	2.21	0.5

Association between placental *Plasmodium* parasitaemia and factors affecting the risk of infection.

A Chi-square test was done to determine the association between placental *Plasmodium* parasitaemia and risk factors affecting placental infection. It showed that number of SP doses taken by the pregnant woman and the use of other malaria control measures (such as ITN and Indoor spraying) were significantly associated with placental parasitaemia ($\chi^2 = 13.86$, $p = 0.003$) and ($\chi^2 = 23.99$, $p < 0.001$) respectively (Table 12). Placental *Plasmodium* infection was however not significantly associated with parity of the delivering mother ($\chi^2 = 2.73$, $p = 0.44$).

Table 12: Test of association between placental *Plasmodium* parasitaemia and factors affecting the risk of infection

Factors	No. Tested	Positives N (%)	χ^2	p-value
SP Doses			13.86	0.003
Nil	168	12 (7.1)		
1	44	8 (18.1)		
2	79	2 (2.5)		
3	132	5 (3.8)		
Vector Control			23.99	<0.001
None	11	4 (36.4)		
ITN	47	15 (4.3)		
Indoor Spraying	34	5 (14.7)		
Others	16	2 (12.5)		
Parity			2.73	0.44
0	117	11(9.4)		
1	118	6 (5.1)		
2	88	6 (6.8)		
3 and Above	92	4 (4.3)		

Multivariate regression analysis between placental *Plasmodium* parasitaemia and factors that affect the risk of the infection.

This was done to test the risk posed by factors which were significantly associated (by χ^2 test) with placental *Plasmodium* infection. Mothers that did not use any malaria control measure had the highest risk of placental infection (AOR= 8.4, p=0.03). Those who used ITNs had the lowest risk of placental infection (AOR=0.25, p=0.88). A pregnant woman who had not taken any dose of SP had a 4 times increased risk of placental *Plasmodium* infection (AOR=4.2, p=0.03). Meanwhile a pregnant woman who had taken only a dose of the SP had an 8 time increased risk of placental infection (AOR=8.14, p=0.03). Subsequent higher doses of SP reduced the risk of placental infection (Table 13).

Table 13: Multivariate regression analysis between placental *Plasmodium* parasitaemia and factors affecting the risk of the infection

Factors	No. Tested	Positive No. (%)	Adjusted OR (95% CI)	*p*-value
SP Doses				
Nil	168	12(7.1)	4.2	0.03
1	44	8(18.1)	8.14	0.03
2	79	2(2.5)	1.1	0.9
3	132	5(3.8)	**Reference**	
Vector control measures				
None	11	4(36.4)	8.4	0.03
ITN	347	15(4.3)	**Reference**	
Indoor Spray	34	5(14.7)	1.21	0.8

Birth outcomes

Prevalence of low birth weight

The mean birth weight of babies was calculated as 3.38kg (SD +/- 0.307). Prevalence of low birth weight (birth weight < 2.5kg) was found to be 11.06% as shown in table 14.

Table 14: Frequency table of the birth weights categories of babies.

	Frequency	Percent
Less than 2.5kg	47	11.06
2.5kg to 4.5kg	376	88.47
More than 4.5kg	2	0.47
Total	425	100.00

Association between low birth weight and factors affecting the risk low birth weight

A Chi-square test (χ^2) was used to determinethe association between low birth weight and factors affecting the risk of low birth weight. The factors considered were; number of SP doses taken, the use or otherwise of other malaria control measures (such as ITN and IRS), maternal haemoglobin and placental paracetaemia.

Low birth weight was significantly associated with the number of SP doses taken; the use of other malaria control measures and the presence of *Plasmodium* placental parasitaemia (Table15). There was however no significant association between maternal anaemia and low birth weight (p= 0.29).

Table 15: Test of association between low birth weight and the factors influencing the risk of low birth weight.

Factors	No. Tested	Low Birth Weight No. (%)	χ^2	p-value
SP Doses			47.93	<0.001
Nil	168	43 (25.6)		
1	44	13 (29.5)		
2	80	2 (2.5)		
3	133	4 (3.0)		
Vector Control			1.13	<0.001
None	11	9 (81.8)		
ITN	349	28 (8.0)		
Indoor	34	15 (44.1)		
Others	16	8 (50.0)		
Maternal Haemoglobin			3.72	<0.3
Normal Haemoglobin	252	34 (13.5)		
Mild Anaemia	105	13 (12.4)		
Moderate Anaemia	63	14 (22.2)		
Severe Anaemia	5	1 (20.0)		
Placental Parasitaemia			71.57	<0.001
Positive	27	19 (70.4)		
Negative	396	396 (10.9)		

Multivariate logistic regression analysis between low birth weight and factor affecting the risk of low birth weight

A multivariate regression analysis was done for the factors that were significantly associated with low birth weight to determine the risk each of these factors posed to low birth weight.As shown in table 16, the presence of placental parasitaemia in the delivering mother posed the highest risk of low birth weight (**AOR**=27.2, p<0.001). Pregnant women who took one dose of SP had 13 times increased risk of low birth weight (**AOR**= 13.52, p<0.001) whilst those who took no SP tablets at all as IPTp had 11 times risk of low birth weight (**AOR**=11.09, p< 0.001). Taking 2 doses or more of SP was not associated with a risk of low birth weight (**AOR**= 0.83, p= 0.8).

Compared to those who did not use any other form of malaria control measure, pregnant women who used ITNs where significantly protected from low birth weight (**AOR**=11.46, p< 0.001). Meanwhile indoor residual spraying did not give significant protection against low birth weight (**AOR**=1.27, p< 0.001).

Table 16: Multivariate logistic regression analysis between low birth weight and factors affecting the risk of infection.

Factors	No. Tested	Low birth weight	Adjusted OR (95% CI)	p-value
SP Doses				
Nil	168	43 (25.6)	11.09	<0.001
1	44	13 (29.5)	13.52	< 0.00!
2	80	2 (0.83)	0.83	0.83
3	130	4 (3.0)	**Reference**	
Vector Control				
None	11	9 (81.8)	**Reference**	
ITN	349	28 (8.0)	11.46	<0.001
IRS	34	15 (44.1)	1.27	0.7
Placenta parasitaemia				
Yes	27	19 (70.4)	27.2	<0.001
No	397	43 (10.9)	**Reference**	

Prevalence of mutations in the *dhfr* and *dhps* genes.

Prevalence of single mutations

The prevalence of mutations in the *dhfr* genes were as follows: **N108** (26, 92.3%); **I51** (23, 85.2%); **R59** (24, 88.89%). Those on the *dhps* genes were; **E540** (2, 7.40%) and **G437** (6, 22.22%).

Prevalence of double mutations

Double mutations observed in *P. falciparum* isolated from placenta of delivering mothers were as followed: **IN**: (19, 70.3%); **RN**: (21, 77.8); IR: (19, 70.3%); **GE**: (1, 3.7%).

Prevalence of multiple mutations

Triple mutations were as followed **IRN:** (20, 74.1%) There were three (3, 11.1%) quadruple mutations **IRNG** and one (1, 3.70%) quadruple mutations **IRNE.** There were no quintuple mutations observed in the parasites isolated from the delivering mothers. Table 17 summarizes prevalence of mutations at the various codons.

Table 17: Prevalence of double and multiple mutations in the *dhfr* and *dhps* genes of *P. falciparum* placental isolates.

Mutations	Frequency	Percentage (%)
DHFR		
Double mutations		
IN	19	70.3
RN	21	77.8
IR	19	70.3
Triple Mutations		
IRN	20	74.1
DHPS		
GE	1	3.7
Quadruple Mutations		
IRNG	3	11.1
IRNE	1	3.7
Quituple Mutations		
IRNGE	0	0.0

Surveillance and Characterization of Molecular Markers of SP Resistance

General outcomes

A total of Five hundred and twenty-nine (529) samples were obtained from individuals attending the general outpatient department of the study facilities. The *dhfr* and *dhps* genes were not amplified in all the samples: Sixty-one (61) of the *dhfr* genes failed to amplify whilst forty-eight (48) of the *dhps*genes did not amplify. The Four hundred and twenty (420) isolates that amplified both the *dhfr* and the *dhps* genes were therefore used for further analysis. One hundred and forty-two (142, 33.8%) of these were from children under five years whilst Two hundred and seventy-eight (278, 66.2%) of these were from participants five years and above.

Prevalence of single mutations

Three hundred and ninety (390), representing 92.9% of the isolates studied had mutations N108 of the *dhfr* gene. Meanwhile prevalence of mutations**I51** and **R59** were 82.3% and 87.6% respectively.Twenty-six representing 6.7% had mutations **E540** of the *dhps* gene and eighty seven (87) representing 20.6% of the samples had mutations **G437**.

Prevalence of double and multiple mutations

The prevalence of double mutations were as follows: **IN** (310, 73.8%); **RN** (316, 75.3%); **IR** and **GE** (4, 1.0%). The prevalence of the triple mutation **IRN** was (299, 71.1%); ninety-eight of it (98, 32.7%) being in isolates from under-fives and two hundred and one (201, 67.2%) being from participants above five years. There was also no statistically significant difference in the prevalence of triple mutations at **IRN** in isolates from children compared to adults (z=-0.704, p=0.48). Fifty six (56), representing 13.3%, quadruple

mutations **IRNG** were found in the sample set. Fifteen (15) representing 3.7% of **IRNE** were also found. The highly resistant quintuple mutations **IRNGE** were observed in two isolates. Prevalence of all mutations is summarized in table 18 below (Images are as shown Appendix F-Laboratory Photo Gallery).

Table18: Prevalence of mutations in the *dhfr* and *dhps* genes of *P. falciparum* isolates from general outpatient attendees.

Mutations	Frequencies	Percentages
DHFR GENE		
Single Mutations		
I51	346	82.3
R59	368	87.6
N108	390	92.2
Double Mutations		
IN	310	73.8
RN	316	75.3
IR	284	67.6
Triple Mutations		
IRN	299	71.1
DHPS GENE		
Single Mutations		
G437	87	20.6
E540	26	6.3
Double Mutations		
GE	4	0.95
Quadruple Mutations		
IRNG	56	13.3
IRNE	15	3.7
Quintuple Mutations		
IRNGE	2	0.5

Evaluation of the Quality of SP Tablets in Use

General observations

The brand of SP drugs in use at the time of the study was Maladar®, a product manufactured by Ernest Pharmaceutical Limited, a leading local pharmaceutical company which is into manufacturing, importing and exporting of pharmaceutical products. Being a programme (SP-IPTp programme) drug, SP is supplied at no cost to the user facility and ANC clients by the Central Medical Stores of the Ministry of Health of Ghana, located in Accra, through its Regional Medical Stores (in this case the Western Regional Medical Store) which are sited at Sekondi.

Maladar® comes in a white immediate-release tablet formulation. It is stated to contain 500mg of Sulphadoxine and 25mg of Pyrimethamine. The tablets were preserved in a well closed foil into blisters; each blister made up of six tablets. These blisters were in turn packaged in smaller card boxes and each box contains two blisters. These smaller boxes were packed into bigger boxes in cartons. At the Central and Regional Medical Stores these boxes were packed and stored on wooden pallets and shelves at room temperature. At all the studyfacilities, however, the boxes of SP tablets were kept in air-conditioned stores on shelves. A pharmacist or an appointee of the pharmacy unit supplied the SP tablets in the smaller boxes to the antenatal units of the medical facilities, where they were usually kept under room temperature. Three tablets were given according to schedule to pregnant women without any cost and were observed directly to swallow with water.

Identification of the active principle in the tablets sampled

Sulphadoxine

The average retention time for sulphadoxine standard after six injections was 29.8835 minutes. The average retention time for the test sulphadoxine after six injection of the sample was 29.908 minutes. This average retention of 29.908 minutes was comparable to that of the standard (within the +/- 10% limit). A t-test also done to compare the two mean-retention times showed that there was no significant difference in the mean retention time for sulphadoxine samples compared to the standard ($t=0.862$; $p=0.41$).

Pyrimethamine

The average retention time for pyrimethamine standard after six injections was 39.3101 minutes. The average retention time for the test pyrimethamine after six injection of the sample was 39.3972 minutes. This average retention of 39.3972 minutes was comparable to that of the standard (within the +/- 10% limit). A t-test done to compare the mean retention time for pyrimethamine samples with the standard showed no statistically significant difference between the two means ($t=1.02$; $p=0.329$).

Based on the above findings, the active principles of the tablets in used were determined to be sulphadoxine and pyrimethamine.

Determination of quantities of sulphadoxine and pyrimethamine in the SP tablets

Percentage assay (USP) =

$$\frac{\text{Sample response}}{\text{Standard response}} \times \frac{\text{Standard concentration}}{\text{Sample concentration}} \times \text{Standard Potency} \times 100\%$$

Standard concentration was calculated as 0.4M for sulphadoxine and 0.02M for pyrimethamine. Standard potency for sulphadoxine pyrimethamine was 0.999.

Using the above the average percentage assay for sulphadoxine using 3 samples was determined to be 95.25% and that of pyrimethamine was 92.75%. Per the US pharmacopoeia used, the percentage assay (USP) of the active principle of sulphadoxine and pyrimethamine should not be less than 90% and more than 110%. A USP of 95.25% and 92.75% for sulphadoxine and pyrimethamine respectively implies that adequate quantities of the active principles of sulphadoxine and pyrimethamine were in the tablets of SP (as claimed by the manufacturer).

Determination of the dissolution of SP tablets.

The amount of the active principle dissolved in after 30 mins was also determined using methods described above. The percentage dissolution was calculated as below:

$$\frac{\text{Sample response}}{\text{Standard response}} \times \frac{\text{Standard concentration}}{\text{Sample concentration}} \times \text{Standard Potency} \times 100\%$$

Standard concentration of sulphadoxine was 0.4M and that of pyrimethamine was 0.02M. Standard potency was 0.99%. The average percentage dissolution of SP in all the 6 dissolution vessels was determined as follows:

Sulphadoxine: $\frac{94.64+96.11+94.29+95.71+94.87+95.75}{6} = 95.22\%$

Pyrimethamine: $\frac{78.174+83.982+83.651+85.8153+82.1965+84.35}{6} = 83.03\%$

A dissolution rate of 95.22% and 83.03% after 30 minutes for sulphadoxine and pyrimethamine respectively were considered good since they exceeded the minimum expected dissolution of 60% recommendation.

CHAPTER FIVE

DISCUSSION

SP Efficacy Study

The aim of this aspect of the study was to assess the *in vivo* efficacy of SP by determining the rate of parasitic clearance (parasitological response) of *Plasmodium falciparum* amongst parasitaemic yet asymptomatic pregnant women attending ANC after receipt of SP and comparing it to the WHO standard. Currently, the WHO recommends a change in the use of an antimalarial when treatment failure rates go beyond 10% (WHO, 2009).

Validity of study

According to the WHO, if 20% or more of enrolled patients are lost to follow-up or are otherwise excluded due to protocol violations, validity of a study can be questionable (WHO, 2003): A 9.52% loss to follow up recorded in this study may imply this study had a rather high validity.

Prevalence of asymptomatic malaria amongst the pregnant women

Asymptomatic or subclinical malaria is said to be human *Plasmodium* infections which do not lead to clinical symptoms and therefore remain undetected by fever based surveillance systems (Galataz et al., 2016). Prevalence of asymptomatic malaria amongst pregnant women was found to be 13.8% by MRDT, and 13.1% by PCR and 10.2% using microscopy. The higher observed difference in prevalence of asymptomatic malaria by the MRDT than by microscopy, could be because, MRDT detection of

Plasmodium parasitaemia is generally known to be more sensitive than malaria parasite detection by microscopy (Abba et al., 2011). Asymptomatic malaria tends to have very low parasitaemia. This was the case in this study with a mean parasite density of 149.6 parasites/µl. With a realistic parasite density threshold of microscopy under field conditions being 50-100 parasites/µl (WHO, 1988; Milne et al 1994), chances are that there were some levels of parasitaemia which were below the detection limit of microscopy (sub-microscopic) but not below the detection by the MRDTs which detects only the presence of antigens. Also, MRDTs can detect parasite antigen (*Pf*HRP-2) even after 30 days of effective treatment thereby increasing the numbers diagnosed by the kits relativemicroscopy. Similarly, the higher prevalence of asymptomatic *Plasmodium* parasitaemia detected by PCR relative to microscopy could be due to the fact that PCR is generally more sensitive, being able to detect very low levels of parasitaemia. Some PCR assays are said to be able to detect parasitaemia less than 1 parasite/µl (Cnops et al., 2011). Thus said, microscopy, especially when serially done by independent microscopists, remains the gold standard in diagnosing malaria, though in research settings, molecular methods such as the PCR are preferred. The problem with using MRDT, as alluded to earlier is that, it might not always give you a true reflection of the prevalence or incidence of malaria since the antigen *pf*HRP-2 tested when detecting *P. falciparum* positivity can remain in the blood about 30 days after treatment (Bell et al, 2006; Murray et al, 2008 & Abba et al, 2011) and therefore lead to an increase in the number of positives. In this study, therefore, malaria detection by the MRDT was

usedonly as a screening tool to recruit participants. Parasitaemia was later confirmed by microscopy before participant was included.

The prevalence rate by microscopy observed in this study, though lower, is comparable to the 15% prevalence recorded in a similar study in Offinso district in the Ashanti region of Ghana (Tutu et al., 2011). It was also lower than the prevalence of 19.4% observed in a similar study in Bobo-Dioulasso, a peri-urban area in neighbouring Burkina Faso (Cisse et al., 2014). It was comparable to the prevalence of 10.9% found in Luanda, Angola, an urban site with similar malaria epidemiological characteristics (Campos et al., 2012). A prevalence of 10.1% by microscopy was however twice as higher as the 5%recorded in a similar study in Madina, in the Ga East Municipality, about 13.5km from Accra, the national capital of Ghana (Stephens et al 2014). These figures, though varied, all emphasize the significant need of the use of IPTp-SP which has as one of its aims to clear *Plasmodium* parasitaemia and thereby eliminating the adverse effects of pregnancy associated malaria: In the absence of SP-IPTp therefore, there would be an increased risk of maternal anaemia and low birth weight, which is one of the most important causes of infant morbidity and mortality. The persistence of asymptomatic parasitaemia would also result in the reservation of a pool of parasites which would sustain malaria transmission thereby thwarting the efforts at malaria elimination. Asymptomatic malaria is one of the feared obstacles to malaria elimination globally.

ITN coverage and usage

Though 78.81% of the participants owned ITNs, only 57.14% were sleeping in them. This coverage of 78.81% is lower than the projected 80% for

endemic regions as far back as 2010 (WHO, 2010) and the current expected 100% universal coverage in the same region (WHO, 2015). It is also lower than the current national ITN coverage of 96% in Ghana (WHO, 2015). There is a very strong evidence for the efficacy of ITNs in preventing malaria infection and its consequences in pregnancy, as reported by Gamble and others in a Cochrane review in 2009 and in a more recently by Eisele and others (2012) in meta-analysis. The WHO also recognizes ITNs as one of the most cost effective ways of controlling malaria in vulnerable groups such as pregnant women and children under-five years of age. There is the need therefore, to strengthen efforts to scale up ITN coverage amongst pregnant women in Ghana, since they together with children under-five years are at most risk of suffering from malaria and its consequences. Availability of the ITNs does not seem to be the problem in most endemic regions of the world, because ITNs are given mainly through the antenatal clinics either free or at very subsidized prices. Reasons why most of these women who had the nets but were not using them chiefly included, lack of appreciation of its importance; feeling warm when sleeping in it; not knowing how to mount it and the perception of lack of mosquitoes in their neighbourhood. All these barriers can be surmounted by intensified user education.

Prevalence of anaemia in pregnant women.

A rather high prevalence of anaemia (76.19%: 42.86% being mild; 30.95% moderate and 2.38% severe) amongst the pregnant women is disturbing. This prevalence rate is comparable to a rate of 73.4% recordedelsewhere (Agyiri, 2011) in a similar study in South Suntreso,a peri-urban area in the Ashanti region of Ghana. It is however higher than the 62.0%

quoted by the WHO as the prevalence of anaemia amongst pregnant women in Ghana (WHO, 2013). It is also higher than the prevalence of 54.5% observed recently in a similar study in Nigeria (Olatunbosun et al., 2014).

Malaria infections in the first or second trimester of pregnancy increase the risk of anaemia (Kalilani et al., 2011). All the participants in this study were in their second trimester (mean gestational age of 19.7 weeks) and they therefore have an increased risk of anaemia from the malaria infection. This could be the reason for the higher prevalence of anaemia relative to other studies. Infections with geo-helminths and the very physiology of pregnancy could also have contributed to the anaemia. Anaemia has dire consequences on the pregnant woman and the developing foetus. Maternal anaemia is a direct or indirect contributor to maternal mortality. For instance, in India, anaemia is reported to be responsible directly or indirectly to 40% of maternal death. Maternal death is also known to rise by 8 to 10 fold when maternal haemoglobin drops to < 5g/dl (Kalaivani, 2009). It has also been implicated as a cause of low birth weight (which is an important contributor to perinatal and infant mortality) by IUGR and prematurity (Suguitan et al., 2003). In order to reduce maternal morbidity and mortality and minimize the untoward birth outcomes, there is the need to intensify efforts at malaria control during pregnancy using SP-IPTp and other prescribed measures such as the use of LLINs and prompt diagnosis and treatment whilst tackling other contributors to maternal anaemia such as micronutrient supplementation and treatment of geohelminths.

Parasite density and maternal anaemia

The study observed a statistically significant negative relationship between parasite density and level of haemoglobin amongst participants (r= -0.0028, p=0.02). Participants with high parasite densities had relatively lower levels of haemoglobin. Similar findings were observed in Tanzania by Marchant et al, in 2015 and in the Democratic Republic of Congo by Matangila and others in 2014 (Matangila et al., 2014).

Low-level asymptomatic malaria is known to result in chronic, low-grade haemolysis (Douglas et al., 2013). The higher the parasitaemia, the greater the degree of red cell destruction and the greater the fall in haemoglobin; the longer this goes on the greater the impairment of red cell production (Phillips et al., 1992), the more extensive the destruction of non parasitized red cells (Looareesuwan et al., 1987; Gwamaka et al., 2011) and the greater the cumulative burden of anaemia (Douglas et al., 2012). This therefore explains the finding in this study that increasing parasite density worsens anaemia. Malaria, whether symptomatic or asymptomatic is therefore a threat to maternal and foetal health. IPTp-SP has been proven to reduce the burden of malaria infection in pregnancy and thereby significantly reducing the risk of anaemia in pregnancy (Kayentao et al 2005; Gies et al., 2009 & Wilson et al., 2011). There is the need therefore, to intensify the use of IPTp-SP alongside other malaria control measures to reduce the prevalence of maternal anaemia and its consequences on both mother and child.

Parity and parasite density

The study also observed a significant negative correlation between participants' parity and parasite density (r= -0.0053, $p<0.001$). In other words, pregnant women with lower parity had relatively, significantly higher parasitaemia. This confirms a well established fact that in malaria endemic regions of the world, primigravida and secundigravida women have higher risk of malaria infection and suffer the most adverse effects of *P. falciparum* infection in pregnancy. The reason for this observation is that nulliparous and primiparous pregnant women have developed little or no immunity to the pregnancy selected strains of the parasite. (Fleming, 1989; Matteeli et al., 1994; Tutu et al., 2010; Guyatt et al., 2001& Poespoprodjo et al., 2008). For nulliparous` and primiparous therefore, ensuring with certainty the institution and patronage of malaria control measures such as IPTp-SP and LLINs must be non- negotiable and policies should be targeted as such. It must be noted however that, Suate et al., (2002); Adam et al., (2005) and Campos et al., (2012) all found evidences contrary to this observation in Mozambique, Eastern Sudan and Luanda in Angola respectively. Factors that may account for this disparity in the two observations may include differences in malaria transmission intensity, prevalence of mutant strains of *Plasmodium* parasite and coverage and usage of key malaria control measures such as the use of LLINs.

Age and parasite density

It has been reported in other studies that age influences the risk of malaria infection. Females who get pregnant at a younger age and especially are primigravidae have a higher risk of malaria parasitaemia than their older counterparts. For instance, Uneke and Falade reported that primiparous pregnant women below age 25 years and in their first trimester of pregnancy have even a more heightened risk of developing placental malaria (Uneke, 2008; Falade et al., 2010) compared to their multiparous and older ones. This study also observed a statistically significant negative correlation between age of participants and parasite density (r=-0.0166, p=0.05). Younger pregnant mothers in the study had a higher likelihood of higher parasite densities compared to their older counterparts. Younger pregnant women are most likely nulliparous and therefore have little or no exposure to pregnancy associated *Plasmodium* parasites and are therefore not pre-immuned to these parasites. This increases their risk of higher parasite numbers. Risk and consequences of MiP may therefore be dire in these women. This significant association between age and parasite density contrasts reports in similar studies in nearby Burkina Faso by Cisse and others in 2014 and in eastern Sudan by Adam and others in 2005. It is not clear why neighbouring Burkina Faso with similar malaria transmission patterns would have contrasting outcomes compared to this study. However the reported contrast in the eastern Sudan study could be due to their unstable malaria transmission patterns. In unstable malaria transmission areas pregnant women of all ages have similar risk to *Plasmodium* infection and the consequences of the infection compared those in stable transmission area like Ghana.

Rate of parasitological clearance

The study observed that, SP when given as IPTp to asymptomatic parasitaemia pregnant women was associated with a high cure rate of 97.62% by day 3 and 100% by day 7. An early (day3) parasitological failure rate of 2.38% and a late (day 28) failure rate of 9.52% are both below the threshold of 10% failure rate for which the WHO calls for the withdrawal of a drug for first line treatment (WHO, 2010). These observations therefore mean that the SP drug used has very significant therapeutic efficacy in clearing existing *Plasmodium* parasitaemia in the study population. This high *in vivo* efficacy observed could have been contributed to by the high quality of tablets in use as IPTp (USP of 95.3% and 92.8% for sulphadoxine and pyrimethamine respectively as well as dissolution of 95.2% and 88.0% for sulphadoxine and pyrimethamine respectively). Of the four (4) samples that showed reappearance of parasitaemia, three (3) were as result of reinfection and one (1), recrudescence. The true late parasitological treatment failure can be said to be 2.38%. However, because one of the aims of giving SP as IPTp is for the drug to remain in significant therapeutic threshold concentrations over a longer period of time to prevent even new infections, the reappearance of the parasites as a result of reinfection would still have to be included in classification of parasitological failure. Thus said, the average time to failure amongst those who failed, was 26.25 days. The current Ghanaian IPTp-SP policy recommends SP doses to be spaced at least 4 weeks apart at every antenatal care visit after quickening until delivery, suggesting that women could receive the next dose at approximately the average time of parasitological failure. Participants may therefore have another chance of clearing parasites, This

study did not include measurement of plasma concentrations of SP over the days of follow up, hence we are unable to ascertain how changing plasma drug concentrations influences the risk of reinfection or recrudescence. However the evaluation of the solubility (which is a surrogate marker for bioavailabity of a drug) and assay concentration of SP tablets in use for the IPTp programme revealed that the drugs were of high quality. This means adequate amounts of the active principle were made available in plasma to achieve this high therapeutic efficacy.

Prevalence of *dhfr* and *dhps* mutations

This study observed a very high prevalence of single mutations at codons 108 (85.73%), 59 (83.33%) and 51(73.81%) on the *dhfr* gene. Prevalence of mutations at codons 540 and 437 on the *dhps* gene were 7.14% and 19.05% respectively. Single mutations were therefore much commoner at codons 108, 59 and 51 on the *dhfr* genes than codons 540 and 437 on the *dhps* genes as was the case in a recent study in Burkina Faso (Tahita et al., 2015). In Ghana, Mockenhaupt and others also found the prevalence of triple mutations at codons 108, 59 and 51 to have increased from 36% in 1998 to 73% in 2006. They attributed this rise in prevalence to the introduction of SP as IPTp in Ghana. They also reported the association of *dhfr* mutations alone with SP treatment failures in northern Ghana, while suggesting a minor role of mutations in the *dhps* gene. The observed prevalence of 71.42% of triple mutations in *dhfr* 108, 59 and 51 was 71.42 %, is comparable to that observed by Mockenhaupt and others in 2006; comparable to the 71.1% prevalence observed in the general public in molecular surveillance aspect of this study but much higher than the 22% observed by Duah and others in 2008 in Tarkwa

(closest major town to Takoradi) and the 11.4% observed by Tahita and others in Burkina Faso in 2015. Reasons for these observed differences in prevalence are not quite clear, but could be attributed to factors such as geography, drug pressure, and sample size. This study observed the prevalence of quadruple mutations to be 7.14% which was higher compared to the 0.8-1.4% found in northern Ghana (Mockenhaupt et al., 2005 & Marks et al., 2005).

Association between parasite mutations and risk of treatment failure

Though single mutations at codon 108 has been associated with a 10 fold increase in the risk of parasite resistance to SP (Mockenhaupt et al., 2005) and *dhfr* triple mutation is known be associated with *in vitro* pyrimethamine resistance (Gregson & Plowe, 2005); a 1,000-fold reduction in pyrimethamine susceptibility (White, 2005) and an increased risk of SP treatment failure in children (Kublin et al., 2002; Mockenhaupt et al., 2005 & Picot et al 2009), this study observed thatSP still maintained a high rate of parasitological clearance (97.6% by day 3 and 100% by day 7) in spite of the high prevalence of single mutation at codons 108 (85.7%) and a high prevalence of triple mutations at codons 108, 59 and 51 (71.42%) recorded.

This high rate of parasite clearance in the face of high prevalence of mutations at the *dhfr* genes could have been contributed to by the relatively lower parasite densities observed and acquired immunity of the pregnant women. A key question however is whether this situation (of high rate of parasitic clearance in the face of high prevalence of mutations in the *dhfr* and *dhps* genes) can be sustained or whether further development of SP drug resistance is inevitable in Ghana and in West Africa in general. Though the level of resistance at which IPTp-SP becomes ineffective is unknown, the WHO

recommends that IPTp-SP should be used in regions with a prevalence rate of K540E less than 50% and A581G less than 10% (WHO, 2013a). This calls for a regular systematic monitoring of prevalence trends of mutations in the *dhfr* and *dhps* genes and treatment outcomes so as to inform policy makers for possible action.

Maternal and Birth Outcome Study

This aspect of the study, aimed at (i) Determining the prevalence of maternal anaemia and correlating it with factors such as IPTp-SP usage and other factors used in the control of malaria (ii) determining the peripheral and placental blood prevalence of *Plasmodium* in delivering mothers by both microscopy and PCR (ii) determining the specific mutation of *Plasmodium falciparum* in peripheral blood and placental samples (iii) measuring birth weights of babies delivered and correlating it with the levels of peripheral and placental parasitaemia, IPTp-SP usage, maternal anaemia and ITN usage (iv) determining the association between peripheral and placental parasitaemia and the number of SP doses taken, usage of malaria vector control measures and parity of the delivering mother.

ITN coverage

Of the 425 participants sampled in this part of the study, 332 (78.1%) owned ITNs but only two hundred and seventy-five (275) representing 64.8% were actually using the ITNs as a malaria control measure. As noted in the therapeutic efficacy study, this coverage is also lower than the projected 80% for endemic regions as far back as 2010 (WHO, 2010) and the current expected 100% universal coverage in the same region (WHO, 2015).

It is also lower than the current national ITN coverage of 96% in Ghana (WHO, 2015). Up-scaling of the coverage of ITN would require involvement of all stakeholders such as the MOH, the GHS, district assemblies, chiefs and opinion leaders in the study area and for that matter the whole of Ghana to overcome the barriers that militate against increasing the coverage and usage of ITNs especially amongst vulnerable groups such as pregnant women (as in this case) and children under five years of age.

Availability of the nets does not seem to be a barrier. This is because the nets are given at ANCs either free of charge or at very subsidized cost. Identified barriers to ITN ownership and usage from this study included: (i) lack of appreciation of its importance, (ii) feeling warm when sleeping in the nets (iii) not knowing how to mount the net and (iv) the perception of lack of mosquitoes in their neighbourhood. Most of these barriers can be overturned by educating the clients. If distribution via the ANC does not seem adequate, there is the need to do a regular mass distribution to scale up coverage.

IPTp-SP coverage

Coincidentally, during the period of study, there had been a shortage of SP drugs for the IPTp nationwide. One of the study facilities, Takoradi Hospital did not have any stock of the SP tablets. Most women who attended antenatal clinic and delivered in that facility therefore had not taken any dose whatsoever of the SP drug. As many as 152 women fell into this category. They were categorized as IPT0.

Owing to the importance of SP-IPTp described in many studies (Kayentao et al 2005; Rogerson et al, 2007; Gies et al., 2009 & Wilson et al., 2011), every effort must be made both by national, regional and local procurements units to ensure constant supply of the SP drugs. In the event that these efforts at these various levels fail, it is imperative for antenatal attendees, if possible be made to purchase their own SP tablets on the open market (since SP is quite cheap) and be supervised to take the tablets. Of those who had taken any SP dose, thirty-six, representing 8.5% had taken only one dose of SP, eighty-five of them, representing 20.0% of the participants took 2 doses and one hundred and fifty-two (35.8% of the total but 55.7% of the group that took SP) of the participants took 3 doses. These figures are lower than the range of 60-79% (WHO, 2015) quoted as the range of IPTp-SP coverage for Ghana for 2015. It must however be said that IPTp coverage in most malaria endemic region remains low. For instance, van Eijk et al (2013) reported that of the 21·4 million estimated malaria-exposed births across 27 countries in 2010, only an estimated 4·6 million (21·5%, 95% CI 19·3–23·7) were born to mothers who received intermittent preventive treatment. Doku et al (2016) also reported that factors hampering the IPTp3 coverage in Ghana included; Shortage of the SP tablets (which was confirmed by the findings in this study); inadequate supply of portable water for administration of SP; unavailability of IPT during outreach services; lack of knowledge by ANC staff about the dropout rate in their area of jurisdiction and poor attitude of some health workers. Though these reasons were not elicited in this study, the shortage of the SP drug at the time of the study was an obvious observation.

Maternal outcomes

Prevalence of anaemia

The prevalence of anaemia amongst delivering mothers, in this aspect of the study was found to be 40.47%: Severe anaemia accounted for 1.18%; moderate anaemia, 14.82% and mild anaemia, 24.27%. This prevalence is lower than the 76.19% observed in the *in vivo* therapeutic efficacy study of this research. It was also lower than the 62.0% quoted by the WHO as the prevalence of anaemia amongst pregnant women in Ghana (WHO, 2013). This difference could be attributed to the fact that compared to antenatal attendees in the therapeutic efficacy study, delivering mothers would have fully benefited from interventions such as iron and folic acid supplementation, protection from ITNs and IPTp-SP, which protects against anaemia. The delivering mothers are therefore better protected against anaemia compared to their antenatal counterparts.

Factors affecting the risk of maternal anaemia.

Factors tested for included the usage and frequency of dosing of IPTp-SP as well as the use of malaria vector control measures. It has been reported by a number of studies that maternal anaemia is significantly controlled by IPTp-SP (Kayentao et al., 2005, Gies et al., 2009 & Wilson et al., 2011) and use of malaria vector control measures especially, ITNs (Gamble et al., 2009 & Eisele et al., 2012). This study however observed no significant association between maternal anaemia and use of IPTp-SP ($\chi2$= 8.37, p=0.5) and use of other malaria vector control measures such as ITNs and IRS ($\chi2$=6.62, p=0.7). This implies that, controlling malaria (using IPTp-SP and vector control measures such as ITNs) amongst study participants is not associated with

asignificant reduction in the risk of maternal anaemia. This was evident by the lack of significant association between peripheral *Plasmodium* parasitaemia and maternal anaemia ($\chi 2$= 0.634, p=0.9) in the study. The risk of maternal anaemia could have been contributed to by other factors such and variations in socio-demographic, economic and obstetric characteristics in the study participants. Though all the pregnant women sampled were on one form of micronutrient supplementation or the other, variations in the type and hence the components of these supplements as well as varying participants' compliance in taking the supplements could have influenced the risk of maternal anaemia.

Prevalence of peripheral and placental parasitaemia.

The prevalence of *Plasmodium falciparum* parasitaemia in placental bloodamongst delivering mothers were found to be 4.25% and 6.37% by microscopy and PCR respectively and in peripheral blood,1.89% and 3.45% by microscopy and PCR respectively. The prevalence of placental *Plasmodium falciparum* parasitaemia observed is much lower than the prevalence of 11% (by microscopy) and 26% (by PCR) reported by Hommerich et al, (2007) in Ghana. They are however comparable to the 2.5% (by microscopy) and 5% (by PCR) recently observed by Stephens et al., (2014) in a similar study in Accra, Ghana. IPTp-SP was implemented in 2005 in Ghana; comparing the observed prevalence made by Hommerich et al in 2006 (and published in 2007) to that observed in this study (in 2014) and by Stephens et al, it can be said that the prevalence of placental malaria over this 7 year period is on a downward trend. The use of SP as IPTp and other control measures such as ITNs, IRS over the period as well as the use of the highly

effective artemisinin based combination therapy can be said to have significantly reduced the overall *Plasmodium* parasite burden leading to this overall observed reduction. Again, relative lower placental and peripheral parasitaemia observed in this study could be due to the relative higher modal gravidity (3 pregnancies) and mean age (28.9 years) of the sampled women. This is because the prevalence of malaria in pregnancy is known to be higher in primigravidae (than in multigravidae) and in pregnant women with younger ages (than in older women) (Uneke, 2008; Falade et al., 2010 & Adegnika et al., 2011). The study also observed a significantly higher prevalence of placental *Plasmodium falciparum* parasitaemia compared to peripheral blood. This higher prevalence observed in the placenta compared to peripheral blood is due to the fact that *P. falciparum* infected erythrocytes tend to have a predilection for the placenta bed in pregnant women. They sequester by adhering to chondroitin sulphate A (CSA) expressed in placenta. The placenta therefore selects for strains of *P. falciparum* with a CSA-binding phenotype. *P. falciparum* infected erythrocytes in placenta therefore remains the most important index of measuring the risk of the consequences of pregnancy associated malaria.

There was also a significant difference in observed prevalence of parasitaemia detected by PCR compared to microscopy. PCR significantly yielded higher prevalence compared to microscopy. This buttresses the point that PCR is more sensitive (than microscopy) in diagnosing malaria and it is the gold standard for malaria diagnosis in research and reference laboratory settings (Brozan et al., 2008).

Effects of SP doses and ITN on peripheral *Plasmodium* infection

In this study, we observed that peripheral *Plasmodium* parasitaemia was significantly associated with both number of doses of IPTp-SP (x^2=10.6, p=0.01) and the usage of malaria vector control measures(x^2=49.3, p<0.001): Mothers who did not take any SP dose at all had a 5-fold increased risk of peripheral parasitaemia compared to those who took 3 doses of SP (AOR=4.89, p=0.04). Those who used ITN had a 10-fold reduction in the risk of peripheral *Plasmodium* parasitaemia compared to those who did not use any form of malaria vector control measure. Whilst usage of SP is associated with clearing of *Plasmodium* parasitaemia (if any) and prevention of subsequent infection, ITNs prevent malaria in pregnancy by preventing bites of the pregnant woman by the infective female *Anopheles* mosquito and therefore preventing malaria in pregnancy. These findings agree with reports from a number studies which confirm the benefits of IPTp-SP (Rogerson et al., 2007; Geis, Coulibaly, Ouattara & D'Alessandro, 2009; Tan, et al., 2014) and ITNs (Eisele et al., 2012; Gimnig et al., 2016; Gamble et al 2009) to the pregnant woman in controlling malaria infection in pregnancy and its consequences.

Factors affecting the prevalence of placental *Plasmodium* parasitaemia

SP doses and prevalence of placental parasitaemia

There was an observed significant association between the number of SP doses taken and the prevalence of placental *P. falciparum* parasitaemia as determined by PCR (x^2= 13.8, p= 0.003). Risk of placental parasitaemia was higher in those who did not take any SP at all (AOR=4.2, p=0.03) and those who took only one dose of SP (AOR=8.14, p=0.03). This association of SP and risk of placental *P. falciparum* parasitaemia has been amply elucidated by

Kayentao et al in 2103 when they compared the effects on the use of 3 doses of IPT-SP and 2 doses in a systematic review. They established that women who took ≥ 3 doses of SP were approximately half as likely to have placental malaria compared with those who took 2 doses, regardless of HIV status (RR=0.51 [95% CI, 0.38–0.68]; I2=0%). These findings amongst others were the basis for the current recommendations by the WHO Evidence Review Group for SP for IPTp to be taken till delivery so as to increase the number of doses taken (WHO, 2012).

Compared to those who took three doses of SP, pregnant women who did not take any SP at all had 4 times the risk of placental parasitaemia whilst who took only one dose of SP had an 8 times increased risk of placental parasitaemia. Those who took 2 doses had a further reduced risk (AOR=1.1, p=0.9). This observation implies that at least 2 doses of SP as IPTp is required to offer some protection against placental *Plasmodium* parasitaemia to pregnant women studied. One dose does not; and may rather increase the risk of placental *Plasmodium* parasitaemia. Similar findings like this informed the basis for which, the WHO recommended at least 2 doses of SP as IPTp (WHO, 2004). In Takoradi Hospital, where most participants who did not take any SP dose attended antenatal clinic, it was observed that there was much emphasis (during educational talks by healthcare givers) on the use of other malaria control measures such as ITN. Indeed, 68.2% of those who used ITNs for malaria control belonged to this category. This could explain why in spite of not having taken any dose of SP, this category of attendees had a much lower risk of placental parasitaemia compared to those who took one dose of SP.

ITN usage and placental parasitaemia

The use of ITNs is one of the recommendations of the WHO in the control of malaria in pregnancy. Indeed, usage of ITNs has been associated with preventing malaria in pregnancy and its consequences (Gamble et al., 2009). It was observed in this study that, compared to those who used ITNs participants who did not use ITNs had 8 timesthe risk of placental parasitaemia (AOR= 8.4, p=0.03). This is a proof of how effective ITNs are in controlling malaria in pregnancy. There is therefore the need to intensify ITN coverage in pregnant women and in the general population as a whole because of its enormous benefit of reducing the incidence of pregnancy associated malaria and its consequences.

Parity and prevalence of placental parasitaemia

Literature abounds to support reports that increasing parity reduces the risk of placental parasitaemia. (Fleming, 1989; Matteeli et al., 1994; Tutu et al., 2010; Guyatt et al., 2001& Poespoprodjo et al., 2008) ; the so-called parity-dependent immunity against malaria infections in subsequent pregnancies.Primigravidae are therefore at a higher risk of placental malaria. This is because, they have no previous exposure to pregnancy associated *P. falciparum* infection and therefore lack protective antibodies that block the adhesion of parasitized erythrocytes to CSA in the placental inter-villous space to promote clearance of parasites. In this study however, it was observed that placental *P. falciparum* parasitaemia was not significantly associated with parity (x^2=2.73, p=0.4). Findings of this study is however consistent with a few studies that have reported parity as not being significant in determining the risk of malaria infection in pregnancy in Africa (Dickoa et al., 2003;

Anagos et al., 1986 & Jeane et al., 2008). The reason for the lack of significant association between the parity and prevalence of placental *Plasmodium* infection could be due to the parity distribution of the participating women. As much as 77.9% of participating women were either primiparous or secundiparous, the rest being multiparous. This gives little variation for comparing prevalence of placental parasitaemia by parity. Implementation of IPTp and other malaria control measures could have led to a reduction in the overall malaria in pregnancy burden. This could have further led to a delay in the acquisition of parity-dependent immunity amongst the pregnant women in the area hence the lack of significant disparities in the risk of placental *Plasmodium* parasitaemia based on parity.

Birth Outcomes

Prevalence of low birth weight.

The prevalence of low birth weight, (birth weight below 2.5kg) one of the most important contributors of neonatal and infant morbidity and mortality was found to be 11.06%. This is a little above the national average of 11% and the expected WHO figure of below 10% (UNICEF, 2013). It is however lower than the prevalence of 14% and 17% for neighbouring Burkina Faso and Côte d'Ivoire respectively (UNICEF, 2013). Abubakari et al, (2015) also found the prevalence of low birth weight in northern Ghana to be 29.6%. This near-target low birth weight prevalence might mean policies such as improving antenatal attendance; prevention of anaemia by iron and folic acid supplementation, IPTp-SP, use of ITN and the overall reduction of poverty levels put in place by the MOH and the GHS and District and Metropolitan Assemblies targeted at better birth outcomes are yielding the desired results.

Low birth weight is usually as result of IUGR and prematurity. It is influenced by a number of factors which include: Access to antenatal care; maternal under-nutrition which is directly linked to poverty (Abubakari et al., 2015); maternal anaemia which is also influenced by maternal under-nutrition; maternal age; placental malaria and other conditions during pregnancy such as hypertension disorders in pregnancy. The lower prevalence of low birth weight in this study compared to that carried out by Abubakari and others in Northern Ghana could be attributed to the relatively poorer socioeconomic environment with its consequent maternal under-nutrition in the northern region compared to our area of study: A relatively better access to health services in general and antenatal services in particular could have also contributed to thisrelatively lower low birth weight prevalence observed in the study area.

Risk factors for low birth weight

It was observed in this study that low birth weight was associated with (i) use of SP (x^2=47.9, p<0.001) (ii) use of malaria control measures, particularly ITNs(x^2=1.13, p< 0.001) and (iii) placental parasitaemia (x^2=71.6, p<0.001). Low birth weight was however not associated with maternal anaemia (x^2= 3.72, p= 0.29).

SP doses and low birth weight

IPTp-SP is known to decrease peripheral and placental parasitaemia, and to increase maternal haemoglobin and infant birth weight, especially in primi and secundigravidae.

Using a multivariate regression analysis, it was observed that compared to those who took 3 doses of the SP, those who did not take any

dose of SP had an 11-fold increase in the risk of delivering babies with low birth weight (AOR=11.1, $p<0.001$). Pregnant women who took only a single dose of SP increased their risk of low birth weight 13 times (AOR=13.52, $p<0.001$). Meanwhile taking 2 doses of SP was not associated with an increased risk of low birth weight (AOR=0.83, $p=0.8$). In this study therefore, SP can be said to have only offered any protection whatsoever against delivering of a baby with low birth weight if the pregnant woman received at least 2 doses: Increasing the number of doses of IPTp-SP was therefore more beneficial in improving birth outcomes. This SP-dose dependent protection against low birth weighthas been observed in several similar studies (Titaley et al., 2010; Menendez et al., 2010; Kayentao et al., 2005; Kayentao et al.,2013; Rogerson et al, 2007; Gies et al., 2009 & Wilson et al., 2011): Such findings informed the current update of the WHO policy on IPTp-SP to allow pregnant women to take as many scheduled doses as possible of SP during the antenatal period, even till delivery (WHO, 2012).

Antenatal attendees who did not take any SP at all (mainly as a result of shortage of SP as programme drug) had a lower risk of delivering low birth weight compared to those who took just a dose of SP. This was because, at Takoradi hospital, where majority of those who did not take any dose of SP, healthcare givers were seen more intensely educating attendees on the use of other interventions (such as the use of ITNs and micro-nutrients supplementation) that improve birth outcomes. Indeed, 68.2% of all ITN users attended Takoradi Hospital. Thus, even in the absence of IPTp with SP, these pregnant women were better protected.

Malaria vector control and low birth weight

It was observed in this study that the use or otherwise of malaria vector control measures such as ITNs and Indoor spraying significantly influenced the risk of low birth weight. Compared to those who did not use any malaria vector control measure, the use of ITNs, but not Indoor Sraying protected significantly against the delivery of low birth weight babies. (AOR= 11.5, p< 0.001) and (AOR=1.27, p=0.7) respectively. There is a very strong evidence for the efficacy of ITNs in preventing malaria infection and its consequences in pregnancy, as reported by Gamble et al in a Cochrane review in 2009 and in a more recently by Eisele et al in 2012. Findings of this study therefore add to these available evidences.

Anaemia and low birth weight

A number of studies have reported that anaemia in pregnancy significantly increases the risk of low birth weight (Feresu et al., 2010; Ticconi et al., 2005; Mumbare et al., 2011). We found in this study however, that low birth weight was not significantly associated with maternal anaemia (x^2= 3.72, p= 0.29). This was because, severe anaemia which is known to be responsible for all anaemia related poor birth outcomes had a very low prevalence of 1.18% amongst the study participants. This rather low prevalence of anaemia therefore influenced the risk of low birth weight insignificantly. Severe anaemia causes low birth weight by inducing maternal and foetal stress. This stimulates the synthesis and release of corticotrophin releasing hormone (CRF). Elevated CRF increases foetal cortisol production. Cortisol may inhibit longitudinal growth of the foetusleading to IUGR and therefore low birth weight (Allen, 2001).

Anaemia, if present in these participants was mainly mild (24.27%) or moderate (14.82%). Whilst mild anaemia may not have any effect on pregnancy and labour, moderate anaemia may only cause easy fatigability in the pregnant woman without affecting foetal outcomes.

Placental parasitaemia and low birth weight

Malaria in pregnancy resulting in placental parasitaemia is known to increase the risk of low birth weight (Kassam et al., 2006; Cottrel et al., 2007; Huynh et al., 2011; Ayoola et al., 2012 & Valea et al., 2012). This study observed that placental *Plasmodium* parasitaemia was significantly associated with low birth weight ($x^2 = 71.57$, $p< 0.001$). It increased the risk of delivery of low birth weight amongst participants by 27- fold (AOR=27.2, p<0.001). The possible explanation to this phenomenon is that, placental *Plasmodium* parasitaemia is known to trigger a cascade of placental events that culminates is a reduction in placental growth; reduction of amino acids transport to the foetus; placental hypoxia and a reduction in the activities of Glucose Transporter 1 (GLUT1): These are thought to be the final pathways leading to IUGR (Rogerson et al., 2007).

Prevalence of mutations in the *dhfr* and *dhps* genes

Prevalence of mutations were found (as in the *in vivo* efficacy study and in the molecular surveillance) were found to be higher in the *dhfr* gene than in the *dhps* genes: The highest prevalence being 92.25% at codon 108, the core codon.This situation seems to be the case in quite a number of studies conducted in the West African sub-region (Falade et al., 2007; Aziken et al., 2011; Tahita et al., 2015; Duah et al., 2012 & Mockenhaupt et al., 2005). Reason for this phenomenon has been explained in the study analyzing the

molecular surveillance for mutant genes in the general population studied. The prevalence of triple mutation at codons 108, 59 and 51 (known to increase parasite resistance by a 1000-fold) was significantly higher (74.07%) in parasites isolates compared to parasites who did not have the triple mutations (x^2: 12.283, P< 0.001). This implies that though the prevalence of placental parasitaemia was low, it was observed that a significant number of the parasites that were found in the placenta of delivering mothers had triple mutations.The *dhfr* triple mutations therefore influenced the prevalence of placental parasitaemia significantly. This they did by conferring resistance to the *Plasmodium falciparum* parasite and thereby preventing clearance by SP. Only 4 isolates had quadruple mutation. Quintuple mutations, known to confer super resistance of the parasite to SP were not found in any of the parasites isolated.

Surveillance and Characterization of Molecular Markers of SP Resistance

Molecular markers for drug resistant malaria are based on genetic changes that confer parasite resistance to drugs used to treat and prevent malaria. These markers have been validated as tools for surveillance of resistance (Djimde et al., 2001) and their potential value to policymakers has been demonstrated by their use to help control a malaria epidemic (Djimde et al 2004), to guide national malaria treatment policies (Mugittu et al., 2004) and to monitor changes in parasite drug susceptibility following changes in malaria drug treatment policy (Kublin et al., 2004). In this aspect of the research, we set out to determine by PCR followed by RFLP, the background prevalence of mutations in the *dhfr* and *dhps* responsible for parasite resistance to sulphadoxine and pyrimethamine respectively amongst attendees

at the general OPD as a pointer to the potential of SP resistance amongst the general population and by extension, amongst pregnant women.

General outcomes

Out of the four hundred and twenty (420) isolates analyzed, one hundred and forty-two (142) representing 33.8% were from children under five years and two hundred and seventy-eight (278) representing 66.2% were from patients' ages five years and above. The study found no statistically significant difference in the prevalence of triple mutations **IRN** in samples from under-fives compared to those above five years ($z= -0.704$, $p= 0.48$). This means that risk of SP resistance conferred by triple mutations does not vary in children under five years compared with the populations above five years. This was important to ascertain because, in endemic regions of the world, preimmunity from repeated exposure helps the adult population to fight off the *Plasmodium* infection to some extent even without medication: Children under-five years of age lack this preimmunity and therefore miss out on this advantage. Assuming the parasite sub-population in children under-five years (the most vulnerable to *P. falciparum* infection) showed higher prevalence of mutations than in the adult population, it might mean that the risk of parasite resistance to SP would be higher in the children (who are usually used in efficacy trials) than in the adult population.

This study results show that there was generally a high prevalence of strains of *P. falciparum* with the resistance alleles of the *dhfr* or *dhps* genes in the area of study. This observation is as a result of the increased use of SP in terms of its use for IPTp and the unauthorized use for the treatment of uncomplicated malaria. On a whole, prevalence of mutations was higher in the

dhfr genes than in the *dhps* genes. This scenario was also reported by Duah and others in 2012 after a molecular surveillance of *dhfr* and *dhps* genes in 9 sentinel sites in Ghana (Duah et al., 2012), as it was the case in a study in Burkina Faso (Tahita et al., 2015). This relative higher prevalence of mutations in the *dhfr* gene compared to the *dhps* gene observed, might be due to usage of pyrimethamine as in combination with dapsone as chemoprophylactic agents for control of malaria in pregnancy in West Africa some years prior to its combination with sulphadoxine for usage in IPTp: The relative longer use of pyrimethamine for malaria control in pregnancy compared to sulphadoxine might have therefore selected, with much intensity, for mutant parasites at the *dhfr* gene due to a relatively longer drug pressure. This study also observed triple and quadruple mutations. Quintuple mutations in the *dhfr* and *dhps* genes were only observed in two isolates. Although triple, quadruple and quintuple mutants were detected in the samples analyzed in this study, it must be noted that the predictive value of these markers varies geographically and involves a complex interplay between host immunity, parasites, and drug (Omar et al., 2001; Alifrangis et al., 2003 & Staedke et al., 2004).

Mutations in *dhfr* genes

Mutations in both*dhfr* and *dhps* genes develop in a stepwise fashion, each added mutation further increase the intensity of parasite resistance to SP. In Ghana, Mockenhaupt et al (2005) and others reported that, point mutations in *dhfr* but not *dhps* are responsible for reduced *P. falciparum* susceptibility to SP in a study conducted among children under 5 years in Tamale. Subsequently, there has not been any other study discounting this report. In

that same study, they reported the prevalence of mutations in *dhfr* gene **N108**, **I51** and **R59** to be 72%, 56%, and 65% respectively just before the withdrawal of SP as use for treatment of uncomplicated malaria and before its usage as SP-IPTp. We observed, 9 years thereafter, the prevalence of mutations **N108**, **I51** and **R59** to be 92.9%, 82.35% and 87.6% respectively, an obvious increase in the prevalence single mutations in the *dhfr* gene.

This study did not examine the additional mutation at codon 164 (I164L) which has been associated with high grade pyrimethamine resistance (Plowe et al., 1997) because of its observed rarity in the West African sub-region. Reasons for the increase in the frequencies of mutations at these codons in this study compared to the findings by Mockenhaupt et al (2005) could be due to drug pressure from the usage of SP as the drug for IPTp nine years prior to the time of this study and the unauthorized use of SP for the treatment of uncomplicated malaria in urban centres such as this study area because of its low cost and convenient dosing. Further, other antifolates such as co-trimoxazole (trimethoprim and sulfamethoxazole) are used in the treatment of respiratory tract infectionsin children, HIV and AIDS prophylaxis against opportunistic infections. This may also contribute to the selection of resistant parasites because co-infections of bacteria and malaria are common (Jelinek et al., 1999). To what extent geographical and socioeconomic variations in the two study sites could have influenced this increase would be difficult to say since the two studies were carried out at different times.

The prevalence of mutations in *dhfr* is however lower when compared with observations from eastern Africa where the general increase in the prevalence of *dhfr* and *dhps* mutant genes and SP resistance is rife. For

instance, in Angola, in recent surveys, mutations in **N108**, **I51**, and R**59** were evident in 100%, 93%, and 57% of isolates, respectively (Gama et al., 2011) and in Tanzania, 100%, of isolates in each case for **R59**, **N108, S436/G437**, and **E540**, respectively, in 2007 from a low transmission area (Alifrangis et al., 2011). Data from Uganda also showed within 99%, near 99%, and 57–94% of isolates respectively for the **N108, I51**, and **R59** mutations (Francis et al., 2006).

As mentioned earlier, for mutations in *dhfr* and *dhps* genes every added single mutation increases the intensity of resistance to SP. Double mutations therefore confer more intense resistance to the parasite than single mutations. The prevalence of double mutations in the *dhfr* gene were found to be (310, 73.8%) for **IN**; (316, 75.3%) for **RN** and (284, 67.6%) for **IR**. The highest prevalence of double mutation was therefore at codons **RN** as was the case in the study in Burkina Faso (Tahita et al., 2015).

The prevalence of triple mutation in the *dhfr* gene (**IRN**) in the population studied was 71.1%. Eighteen samples (18) from Tarkwa (the closest bigger town to Sekondi-Takoradi in the Western region of Ghana) in 2007-2008 and fifteen (15) samples from Begoro (a suburban district in the Ashanti region of Ghana) collected in 2010 and examined by Duahet al (2012) showed a prevalence of the triple mutation at codons **IRN** to be 22% and 80% respectively. Comparison of these findings with what was observed in this study is difficult because of the small sample size in these two other studies. However, it suffices to say that there is evidence to support the rising presence of *dhfr* triple mutation in Ghana. As alluded to earlier, triple mutations in the *dhfr* gene confers on the parasites about 1000-fold intensity to SP resistance *in*

vitro. Such a high prevalence of triple mutation at the *dhfr* gene observed means the risk of resistance to SP in the population studied and in Ghana for that matter is also high. This high level of triple mutation does not necessarily mean the use of SP as IPTp is compromised, because, the efficacy of SP, *in vivo*, is a complex interplay of a number of host factors, parasite factors, drug quality, folate levels and parasite density (Omar et al., 2001; Alifrangis et al., 2003 & Staedke et al., 2004; van Hensbroek et al. 1995; Dzinjalamala et al. 2005 & Ehrhardt et al. 2002).

Mutations in *dhps* genes

The prevalence of mutations at codons of the *dhps* were observed as 20.6% for **G437** and 6.3% for **E540** and the prevalence of double mutations **GE** was 1.0%. These frequencies were lower compared to the observations made in the *dhfr* genes. These relatively lower frequencies seem to be the trend in West and Central Africa (Falade et al., 2007; Aziken et al., 2011), for which reasons have already been ascribed. In Eastern and Southern Africa however, *dhps* mutations have similar or sometimes higher prevalence compared to *dhfr* mutations (Gama et al., 2011; Alifrangis et al., 2011 & Francis et al., 2011). The WHO recommends that prior to implementation of IPTp-SP in any region with moderate to high malaria transmission, the prevalence of E540 and G581 should be determined. IPTp-SP should be used in regions with a prevalence rate K540E less than 50% and A581G less than 10% (WHO, 2013a). With a prevalence of mutations in E540 in this study being 6.3%, SP can be said to be still useful in the population studied. Mutations at codon 581, on the *dhps* gene, known to confer super resistance to SP in eastern Africa (Minja et al., 2013 & Naidoo and Roper, 2013), were not

determined because of its relative rarity in the West African sub-region (Falade et al., 2007; Aziken et al., 2011).

The contributions of mutations at the *dhps* to parasite resistance to SP in Ghana have been questioned by Mockenhaupt et al in 2005. Why this is so, is unclear. They further postulated that their observation gives credence to the concept that in essence, SP resistance is mediated only secondarily by *dhps* variants once *dhfr* triple mutant parasites predominate (Watkins et al. 1999; Nzila et al. 2000a). Nonetheless, reports from other studies especially from East Africa, are at variance with this concept (Nzila et al., 2000a; Kublin et al., 2002; Kyabayinze et al. 2003). However, this aspect of the study did not set out to prove or disprove the assertions by Mockenhaupt et al, but to give a background prevalence of mutations in *dhfr* and *dhps* in the study population.

Prevalence of quadruple and quintuple mutations

Fifty-six (56), representing 13.3%, quadruple mutations were found in the sample set of codons **I51, R59, N108** and **G437 (IRNG)** and seven (15) representing 3.7% were found at codons **I51, R59 N108** and **E540 (IRNE)**. The highly resistant quintuple mutations were observed in only two isolates. Since mutations in *dhfr* and *dhps* and subsequent conferred resistance is stepwise, the 13.31% prevalence of quadruple mutations is high and therefore increases the risk of resistance of parasites to SP. It is however lower than the 44% and 25% reported by Duah et al (2012) for forest and savannah zones respectively for parasites isolated between 2007 and 2010 respectively in Ghana. However as noted earlier SP efficacy may not only be dependent on the prevalence of mutations in the *dhfr* and the *dhps* genes but several other factors. Thus said, this high prevalence of mutations are contributors of

treatment failures with SP and therefore should alert researchers and public health policy makers as to the need for preparedness for options to SP as IPTp.

Quintuple mutation **(IRNGE)** is known to be associated with high grade parasite resistance to SP (Plowe et al., 1997). In this study, two (2) of the parasites isolated showed the presence of quintuple just as in the study by Duah et al (2012). The Mockenhaupt study also reported the presence of quintuple mutation in one isolate. It does not appear therefore that the prevalence of the quintuple mutation is fixed or increasing significantly across the country as it is the case in most places in eastern and southern Africa (Matondo et al., 2014 & Siame et al., 2015).

Evaluation of the Quality of SP Tablets in Use

The effectiveness of an anti-malaria drug in achieving therapeutic targets is not only dependent on susceptibility of parasite to drugs but also on host factors as well as the quality of drug being used. Therapeutic failures may therefore occur in situations where you have a susceptible parasite yet a poor-quality drug or a resistant parasite and a very good quality drug. Having looked at the therapeutic efficacy of the SP tablet in terms of its ability to clear parasites; its effectiveness in terms of controlling malaria during pregnancy and the prevalence of parasites mutation conferring resistance to SP, this aspect of the study used observational and laboratory methods to evaluate the quality of SP tablets being used for SP-IPTp at the time of the study.

Physical properties of the SP tablets

As mentioned previously, SP is a programme drug. Its procurement is therefore centralized. It was observed that at the Regional Medical Stores, SP was stored in cartons on wooden pallets at room temperature. Whilst at the

pharmacy stores and dispensing units they were stored on shelves at temperatures below 20°C. These storage arrangements meet the WHO requirements of storing SP below 30°C in a cool dry place away from light (WHO, 2011 & WHO, 2104). Relative humidity was not determined objectively. The Maladar® tablets were formulated as white, hard rounded tablets with a score line on one side for breaking for ease of swallowing rather than dividing the tablets in equal doses. They were well sealed in aluminum foils, as recommended by WHO with each foil containing 6 tablets.

Simulated determination of tablets stability and shelf-life were not also done. However, all the Maladar® tablets used were well within their shelf life (stated by the manufacturer) and have been duly registered with the Food and Drugs Authority of Ghana. The tablets were found not to be friable in that no portions of the tablets were lost in powder during transportation under normal conditions of transportation. The average weight of one tablet which contains 500mg sulphadoxine and 25mg of pyrimethamine was determined to be 0.6385g (638.5mg) and all the sample tablets used showed acceptable uniformity in that none of them had percentage weight deviating more than 5% as recommended by the US Pharmacopoeia. It can therefore be said that the physical characteristics of the tablets in use met acceptable national and international standards and so did their storage condition. What was worrying however, was the observation that for about 6 months prior to the birth outcome study there was a national shortage of the SP drug. This greatly affected supply to most publicly run medical facilities. The shortage was so intense to the extent that none of the pregnant women delivering at one of the study facilities had receive even a single dose of the SP during pregnancy.

This is unacceptable under any circumstance, given the proven benefit of SP-IPT to both the pregnant woman and the unborn child.

Identification of active principle in Maladar®

The mean retention times for both sulphadoxine and pyrimethamine samples were both comparable to that of the standard sulphadoxine ($t = 0.862$; $p = 0.41$) and the standard pyrimethamine ($t = 1.03$; $p = 0.329$) respectively. The active principle in the Maladar® tablets sampled can therefore be identified to be sulphadoxine and pyrimethamine.

Determination of quantities of sulphadoxine and pyrimethamine in Maladar®

According to the US pharmacopoeia used, the determined percentage assay (USP) of the active principle of sulphadoxine and pyrimethamine should not be less than 90% and more than 110% of the quantities stated on the label. In this study, we observed a USP of 95.25% and 92.75% for sulphadoxine and pyrimethamine respectively. This means that there are adequate quantities of both sulphadoxine and pyrimethamine in the tablets in use to achieve the desired therapeutic target. This is important not only because use of sub-standard drugs which fail to achieve therapeutic targets on such a large scale as SP-IPTp can be very costly to users and the health system, but also, can lead to an increase in the prevalence of mutant variants of the parasite due to the drug's inability to clear parasites completely: There is an intuitive link between drug quality and drug resistance. When anti-malarials such SP are substandard, they contribute to creation of resistant parasite strains by selecting for susceptible parasite strains (because of their sub-therapeutic content) and allowing resistant strains of the parasites to flourish at their

expense. Meanwhile, sub-standard drugs which contain too much of the active ingredients (more than 110% of what is stated on the label) makes too much of the active principle available to the body and therefore precipitating toxic or adverse effects. Quality of antimalarial drugs such as SP is therefore important as it affects the ability to effectively manage one of the most important diseases in SSA and (in the case of SP) in one of the most vulnerable group at risk of malaria, the pregnant women.

Dissolution of Maladar®

The US Pharmacopoeia recommends that the amount of active ingredients of SP should not be less than 60%, 30 minutes after dissolution in an appropriate medium under appropriate conditions of temperature and pH. The dissolution of sulphadoxine and that of Pyrimethamine were found to be 95.22% and 83.03% respectively in this study. The tablets used therefore have adequate dissolution of both sulphadoxine and pyrimethamine in solution.In 2011, the WHO reported of a number of SP brands on the Ghanaian and the West African sub-region that failed the dissolution test (WHO, 2011).

The dissolution of a drug is used as surrogate marker of *in vivo* bioavailability (the measure of the rate and extent of availability of drug in systemic circulation) for oral dosage forms containing poorly aqueous-soluble drugs such as SP (Amin et al 2005). With this good dissolution determined, all other things been equal, the SP tablets in use are expected tohavegood bioavailability for the desired therapeutic and protective effects in the pregnant women. SP tablets have notoriously poor *in vitro* dissolution profiles, especially with regard to the pyrimethamine component. This is mostly a problem with the generic products rather than the originator. It is thought that

this poor aqueous solubility of pyrimethamine is occasioned by the use of poor quality raw materials or poor choice of excipients in the formulation (Kibwage et al., 2000 & Risha et al., 2002). We however found that, the SP tablets in use, though generic, have good solubility. These tablets might have been therefore properly formulated.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary of Findings

This was a four-part facility-based cross-sectional and longitudinal study to investigate the effectiveness of sulphadoxine-pyrimethamine as intermittent preventive therapy of malaria in pregnancy in the Sekondi-Takoradi metropolis of the Western region of Ghana. The SP efficacy study investigated the rate of parasite clearance in parasitaemic yet asymptomatic antenatal attendees; the birth outcome study examined the effect of taking SP or not on placental parasitaemia and birth weights. Meanwhile the molecular surveillance investigated mutations in the *dhfr* and *dhps* genes responsible for SP resistance in *Plasmodium* parasites isolated from pregnant women as well as the attendees at the general OPD. Finally, observational and laboratory methods were used to evaluate the quality of SP drug in usage as IPTp.

The rate of parasite clearance was found to be 97.62% on day 3 of follow up and 92.11% on day 28 of follow up. There was no significant correlation between the observed treatment failure and the prevalence of mutation in *dhfr* and *dhps* genes. Parasite density of *Plasmodium falciparum in* infectedasymptomatic antenatal attendants had significant association with the prevalence of anaemia and attendants' parity and age. Though 78.81% of these pregnant women owned ITNs only 57.14% were actually using them. Reasons for not using the ITN included lack of appreciation of its importance; feeling warm when sleeping in it; not knowing how to mount it and the perception of lack of mosquitoes in their neighbourhood.

The prevalence of placental parasitaemia was found to be 6.37% by PCR and 4.25% by microscopy. Placental parasitaemia showed significant association with number of SP doses taken, ITN usage but not parity of the delivering mother. Parasites isolated from the placenta also showed a significantly high prevalence (74.07%) of *dhfr* triple mutation at codons **IRN** but not in the *dhps* gene. The prevalence of low birth weight was found to be 11.06%. Low birth weight showed significant association with placental parasitaemia, number of SP doses taken, use of ITNs but not maternal anaemia.

In the molecular surveillance study, we found a relatively higher prevalence of mutations in *dhfr* genes than in *dhps*. *Dhfr*: **N108** (92.9%); **R59** (87.6%) and **I51** (82.35%): *Dhps*: **G437** (20.6%) and **E540** (6.3%). There was also a high (71.1%) prevalence of triple mutations at the *dhfr* **IRN**. Quadruple mutations were found to be for **IRNG** (13.3%) and **IRNE** (3.7%). Only two of the parasites showed the presence of quintuple mutations at **IRNGE**. There were no significant variations in the prevalence of the mutations observed in parasites isolated from adults and that isolated from children.

The quality of the SP tablets in use was found to be good. It had a 30-minute dissolution of 95.2% for sulphadoxine and 83.0% for pyrimethamine and a USP of 95.2% for sulphadoxine and 92.8% for pyrimethamine. The physical properties and storage conditions of these drugs were also good.

Conclusions

In spite of the high prevalence of mutations responsible for SP resistance observed in parasite isolates from the pre-treatment samples and in the attendants at the general OPD, SP still showed a high rate of parasite clearance and therefore can be said to be effective. *Plasmodium falciparum*

parasite density had a significant influence on maternal anaemia and was itself significantly influenced by maternal parity but not age.

The prevalence of placental malaria observed in this study was lower than that reported in earlier studies but comparable to prevalence observed in recent studies since the inception of IPTp-SP. Placental parasitaemia was significantly influenced by the number of SP doses taken and the use of ITNs but not parity of the delivering mother.

The prevalence of low birth weight was just a little higher than the national average and the WHO expected. It was however lower than figures recorded in neighbouring Burkina Faso and Côte d'Ivoire. Significant risk factors for low birth weight were the prevalence of placental parasitaemia, number of SP doses taken by the pregnant woman, ITN usage but not maternal anaemia. Placental parasitaemia is also influenced by the presence of *dhfr* triple mutation.

The prevalence of mutations responsible for SP resistance especially in the *dhfr* gene has risen compared to previous studies carried out in Ghana and in neighbouring Burkina Faso but lower than prevalence recorded in most parts of eastern Africa. The highly resistant quintuple mutation was found in only two isolates and its frequency does not appear to be rising in the country when compared to previous studies.

The quality of SP tablets used was determined to be good in terms of its dissolution, USP and physical properties.

Recommendations

IPTp-SP still maintains high effectiveness in clearing parasites and preventing placental parasitaemia and reducing the prevalence of low birth

weight in antenatal attendees and delivering mothers respectively, it is therefore imperative that government acting through the Ministry of Health and the Ghana Health Service improve the coverage of IPT-SP so that every pregnant woman can benefit from this all-important programme.

There is the need for ministry of health acting in conjunction with research institutions to carry out such studies nation-wide to ascertain on a much broader scale the effectiveness of the SP as IPTp so as to inform stronger policies.

Considering the high prevalence of mutations responsible for SP resistance, there is the need to institute a more systematic surveillance, both facility base and in the general public, so as to monitor, on a more regular basis, trends in the prevalence of these mutations in order to promptly inform health policy makers. There is also the need for international health bodies to standardized methods of evaluation of *in vivo* SP efficacy to be used globally to allow for standard comparison of outcomes.

ITN usage was observed to contribute significantly to the reduction of prevalence of placental parasitaemia and low birth weight yet it had low coverage and usage. It is therefore important for the Ministry of Health acting through the Ghana Health Service and its partnersto step up activities to improve the coverage and usage of ITNs.

The finding of the shortage of SP as a programme drug was unacceptable. It is imperative that the Ministry of Health put every measure in place to ensure a regular and sustained supply of the drugs to facilities.

REFERENCE

Abba, K., Deeks, J. J., Olliaro, P., Naing, C. M., Jackson, S. M., Takwoingi, Y....& Garner, P. (2011). Rapid diagnostic tests for diagnosing uncomplicated *P. falciparum* malaria in endemic countries. *Cochrane Database of Systematic Review*, (7), 122

Abdalla, H., Matambo, T. S., Koekemoer, L. L., Mnzava, A. P., Hunt, R. H., & Coetzee M. (2008). Insecticide susceptibility and vector status of natural populations of *Anopheles arabiensis* from Sudan. *Transactions of the Royal Society Tropical Medicine and Hygiene*, 102, 263–271.

Abdalla, S., Weatherall, D. J., Wickramasinghe, S. N., & Hughes, M. (1980). The anaemia of *P. falciparum* malaria. *British Journal of Haematology,* 46, 171–183.

Abdel-Muhsin, A. M., Ranford-Cartwright, L. C., Medani, A. R., Ahmed, S., Suleiman, S., Khan, B.,...& H. A. Babiker. 2002. Detection of mutations in the *Plasmodium falciparum dihydrofolate reductase (dhfr)* gene by dot-blot hybridization. *American Journal Tropical Medicine and Hygiene,* 67, 24–27.

Abrams, E.T, Brown, H., Chensue, S. W., Turner, G. D., Lema, V. M., & Rogerson, S. J. (2003). Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated beta chemokine expression. *Journal of Immunology*, 170, 2759–2764.

Abubakari, A., Kynast-Wolf, G., & Jahn, A. (2015). Prevalence of abnormal birth weight and related factors in Northern region, Ghana. *BMC Pregnancy and Childbirth,* 15, 335.

Achidi, E. A., Kuoh, A. J., Minang, J.T., Ngum, B., Achimbom, B. M., Motaze, S. C.,... & Troye-Blomberg, M. (2005). Malaria infection in pregnancy and its effects on haemoglobin levels in women from a malaria endemic area of Fako Division, South West Province, *Cameroon. Journal of Obstetrics and Gynaecology*, 25 (3), 235-40.

Adam, I., Elhassan, E. M., Mohammed, A. A., Salih, M. M., & Elbashir, M. I. (2011). Malaria and pre-eclampsia in an area with unstable malaria transmission in Central Sudan. *Malaria Journal*, 10, 258.

Adam, I., Khamis, A. H., & Elbashir, M. I. (2005). Prevalence and risk factors for *Plasmodium falciparum* malaria in pregnant women of eastern Sudan. *Malaria Journal,* 4, 4-7.

Adegnika, A. A., Luty, A. J., Grobusch, M. P., Ramharter, M., Yazdanbakhsh, M., Kremsner, P. G., & Schwarz, N. G. (2011). AB0 blood group and the risk of placental malaria in sub-Saharan Africa. *Malaria Journal*, 10, 101.

A-Elgayoum, S. M. E., El-Feki, A. E. A., Mahgoub, B. A., El-Rayah, A. E., & Giha, H. A. (2009). Malaria over diagnosis and burden of malaria misdiagnosis in the suburbs of central Sudan: special emphasis on artemisinin-based combination therapy era. *Diagnostic Microbiology and Infectious Diseases*, 10, 16.

Agbozo, F., Abubakari, A., Der, J., & Jahn, A. (2016). Prevalence of low birth weight, macrosomia and stillbirth and their relationship to associated maternal risk factors in Hohoe Municipality, Ghana. *Journal of Midwifery*, DOI: http://dx.doi.org/10.1016/j.midw.2016

Agyiri, J. (2011). Determinants of anaemia in pregnant women in peri-urban Kumasi in the Ashanti Region. Unpublished master's thesis. Kwame Nkrumah University of Science and Technology. Kumasi, Ghana. Retrieved on 21st November 2016. http://hdl.handle.net/123456789/45

Ali, A. A., Okud, A., Khojali, A., & Adam, I. (2012). High incidence of obstetric complications in Kassala Hospital, Eastern Sudan. *Journal of Obstetrics and Gynaecology*, 32(2): 148-9.

Alifrangis, M., Enosse, S., Khalil, I. F., Tarimo, D. S., Lemnge, M. M., Thompson, R...& Ronn, A.M. (2003). Prediction of *Plasmodium falciparum* resistance to sulfadoxine/pyrimethamine in vivo by mutations in the dihydrofolate reductase and dihydropteroate synthetase genes: a comparative study between sites of differing endemicity. *American Journal of Tropical Medicine and Hygiene* 69: 601–606.

Alifrangis, M., Enosse, S., Pearce, R., Drakeley, C., Roper, C., Khalil, I. F...& Bygbjerg C. (2005). A simple, high-throughput method to detect *Plasmodium falciparum* single nucleotide polymorphisms in the *dihydrofolate reductase, dihydropteroate synthase,* and *P. falciparum* chloroquine resistance transporter genes using polymerase chain reaction and enzyme-linked immunosorbent assay-based technology. *American Journal of Tropical Medicine and Hygiene.* 72, 155–162.

Alifrangis, M., Lusingu, J. P., Mmbando, B., Dalgaard, M. B., Vestergaard, L. S., Ishengoma...& Bygbjerg, I. C. (2011). Five-year surveillance of molecular markers of *Plasmodium falciparum* antimalarial drug resistance in Korogwe District, Tanzania: accumulation of the 581G

mutation in the *P. falciparum* dihydropteroate synthase gene. *American Journal of Tropical Medicine and Hygiene,* 80, 523–527.

Allen, L. H. (2001). Biological mechanisms that might underline iron's effect on foetal growth and preterm birth. *Journal of Nutrition*, 131, 581-89.

Alonso, P. L., Lindsay, S. W., Amstrong, J. R. M., de Francisco, A., Shenton, F. C., Greenwood, B. M.,...& Hall, A. J. (1991). Effects of insecticide treated bed nets on mortality of Gambian children. *Lancet*, 333 (8756), 1499-1502.

Alweis, R. L., DiRosario, K., Conidi, G., Kain, K. C., Olans, R., & Tully, J. L. (2004). Serial nosocomial transmission of *Plasmodium falciparum* malaria from patient to nurse to patient. *Infection Control Hospital Epidemiology*. 2004, 25: 55-59.

American Society of Health-System Pharmacists (2012). *Drug Information* 2012. Bethesda, Maryland, USA. Retrieved on 8th December 2016. http://www.worldcat.org/title/ahfsdrug.information2012/oclc/774043698

Amin, A. A., & Kokwaro, G. O. (2007). Antimalarial drug quality in Africa. *Journal of Clinical Pharmacy and Therapeutics,* 32, 429–440

Amin, A. A., Snow, R. W., & Kokwaro, G. O. (2005). The quality of sulfadoxine-pyrimethamine and amodiaquine in the Kenyan retail sector. *Journal of Clinical Pharmacy and Therapeutics,* 30, 559–565.

Andersen, S. L., Oloo, A. J., Gordon, D. M., Ragama, O. B., Aleman, G. M., Berman, J. D.,...& Shanks, G. D. (1998). Successful double blinded, randomized, placebo-controlled field trial of azithromycin and

doxycycline as prophylaxis for malaria in western Kenya. *Clinical Infectious Diseases*, 26(1), 146–150.

Andrade, B. B., Santos, C. J. N., Camargo, L. M., Souza-Neto, S. M., Reis-Filho, A., Clare^ncio, J.,...& Barral-Netto, M. (2011). Hepatitis B Infection Is Associated with Asymptomatic Malaria in the Brazilian Amazon: *PLoS ONE* 6(5), 19841.

Ansell, J., Hamilton, K. A., Pinder, M., Walraven, G. E., & Lindsay, S. W. (2002). Short-range attractiveness of pregnant women to Anopheles gambiae mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 96(2), 113-6.

Antinori, S., Galimberti, L., Milazzo, L. & Corbellino, M. (2012). Biology of Human Malaria *Plasmodia* Including *Plasmodium knowlesi. Mediterranean Journal of Haematology and Infectious Diseases, 4 (1),* 4084.

Arinaitwe, E., Ades, V., Walakira, A., Ninsiima, B., Mugagga, O., Patil, T., & Dorsey, G. (2013). Intermittent Preventive Treatment with sulphadoxine–pyrimethamine for Malaria in Pregnancy: a cross-sectional study from Tororo, Uganda. *PLOS ONE*. 8 (9), 73073.

Asundep, N. N., Jolly, P. E., Carson, A. P., Turpin, C. A., Zhang, K., Wilson, N. O.,...& Tameru, B. (2015). Effect of Malaria and Geohelminth Infection on Birth Outcomes in Kumasi, Ghana. *International Journal of Tropical Disease and Health*. 2014; 4(5), 582–594.

Ataíde, R., Mayor, A., & Rogerson, S. J. (2014). Malaria, primigravidae, and antibodies: knowledge gained and future perspectives. *Trends in Parasitology*. 30 (2), 85–94.

Autino, B., Corbett, Y., Castelli, F., & Taramelli, D. (2013). Pathogenesis of Malaria in Tissue and Blood. *Mediterranean Journal of Haematology and Infectious Diseases*, 4(1), 61

Awolola, T. S., Brooke, B. D., Koekemoer, L. L., & Coetzee, M. (2002). Resistance of malaria vector *Anopheles gambiae s.s* to pyrethroid insecticidesin south-western Nigeria.Annals of Tropical Medicine and Parasitology, 96, 849-852.

Ayoola, O. O., Omotade, O. O., Gemmell, I., Clayton, P. E., & Cruickshank, J.K. (2014) The impact of malaria in pregnancy on changes in blood pressure in children during their first year of life. *Hypertension,* 63, 167–172.

Ayoola, O. O., Whatmore, A., Balogun, W. O., Jarrett, O. O., Cruickshank, J. K., & Clayton, P. E. (2012). Maternal malaria status and metabolic profiles in pregnancy and in cord blood: relationships with birth size in Nigerian infants. *Malaria Journal*, 11, 75.

Baliraine, F. N., Afrane, Y. A., Amenya, D. A., Bonizzoni, M., Menge, D. M., Zhou, G....&Yan, G. (2009). High prevalence of asymptomatic *Plasmodium falciparum* infections in a highland area of western Kenya: a cohort study. *Journal Infectious Disease.* 200, 66–74.

Balkew, M., Gebre-Michael, T., & Hailu A. (2003). Insecticide susceptibility level of *Anopheles arabiensis* in two agro-development localities in eastern Ethiopia. *Parasitologia*, 45, 1–3

Balkew, M., Ibrahim M., Koekemoer, L. L., Brooke, B. D., Engers, H., Aseffa, A., Gebre-Michael, T., & Elhassen I. (2010). Insecticide resistance in *Anopheles arabiensis* (Diptera: Culicidae) from villages

in central, northern and south west Ethiopia and detection of *kdr* mutation. *Parasites and Vectors,* 3, 40.

Barat, L. M., & Bloland, P. B. (1997). Drug resistance among malaria and other parasites. *Infect Dis Clin North Am*. 11:969–987.

Barat, L., Chipipa, J., Kolczak, M, & Sukwa, T. (1999). Does the availability of blood slide microscopy for malaria at health centres improve the management of persons with fever in Tanzania? *American Journal of Tropical Medicine and Hygiene*; 60, 1024–30.

Bardaji, A., Sigauque, B., Sanz, S., Maixenchs, M., Ordi, J., Aponte, J. J.,...& Menendez, C. (2011). Impact of malaria at the end of pregnancy on infant mortality and morbidity. *Journal of Infectious Diseases*, 203(5), 691-9.

Basco, L. K., & Ringwald, P. (1998). Molecular epidemiology of malaria in Yaoundé, Cameroon. II. Baseline frequency of point mutations in the dihydropteroate synthase gene of *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene*, 58, 374–377.

Basco, L. K., de Pecoulas, P. E., Le Bras, J., & Wilson, C. M. (1996). *Plasmodium falciparum*: Molecular characterization of multidrug-resistant Cambodian isolates. *Experimental Parasitology*, 82, 97-103.

Bayoumi, N. K., Bakhet, K. H., Mohammed, A. A., Eltom, A. M., Elbashir, M. I., Mavoungou E., & Adam, I. (2009). Cytokine profiles in peripheral, placental and cord blood in an area of unstable malaria transmission in eastern Sudan. *Journal of Tropical Paediatrics* 55, 233–237.

Bell, D., Wongsrichanalai, C., & Barnwell, J.W. (2006). Ensuring quality and access for malaria diagnosis: how can it be achieved? *National Review of Microbiology* 4,682–95.

Bertagnolli, M., Luu, T. M., Lewandowski, A. J., Leeson, P., & Nuyt, A. M. (2016). Preterm birth and hypertension: is there a link? *Current Hypertension Reports*, 18, 28.

Bhattarai, A., Ali, A. S., Kachur, S.P., Mårtensson, A., Abbas, A. K., Khatib, R.,...& Gerstenmaier, J. F. (2007). Impact of artemisinin based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Med* 4, 309.

Binka, F. N., Indome, F., & Smith, T. (1998). Impact of spatial distribution of permethrin impregnated bed nets on child mortality in rural northern Ghana. *American Journal of Tropical Medicine Hygiene*, 59, 80-85.

Biswas, S., Escalante, A., Chaiyaroj, S., Angkasekwinai, P., Lal, A. A. (2000). Prevalence of point mutations in the dihydrofolate reductase and dihydropteroate synthetase genes of *Plasmodium falciparum* isolates from India and Thailand: a molecular epidemiologic study. *Tropical Medicine and International Health*, 5 (10), 737-43.

Blackley, R. L., Dihydrofolate Reductase. In: Blackley, R. L., & S. J. Benkovic, (Eds.) *Folates and Pteridines*, Vol.I, New York, John Wiley and Sons Inc., 191-253.

Boeuf, P., Hasang, W., Hanssen, E., Glazier, J. D., & Rogerson, S. J. (2011). Relevant assay to study the adhesion of *Plasmodium falciparum* infected erythrocytes to the placental epithelium. *PLoS One*, 6, 21126.

Bouyou-Akotet, M. K, Adegnika, A. A., Agnandji, S. T., Ngou-Milama, E., Kombila, M., Kremsner, P. G., & Mavoungou, E. (2005). Cortisol and susceptibility to malaria during pregnancy. *Microbes and Infections,* 7(11-12), 1217-23

Bouyou-Akotet, M. K., Ionete-Collard, D. E., Mabika-Manfoumbi, M., Kendjo, E., Matsiegui, P. B., Mavoungou, E., & Kombila, M. (2003). Prevalence of *Plasmodium falciparum* infection in pregnant women in Gabon. *Malaria Journal*, 2: 18.

Branch, O., Udhayakumar, V., Hightower, A. W., Oloo, A. J., Hawley, W. A., Nahlen, B. L,...& Lal, A. A. (1998). A longitudinal investigation of IgG and IgM antibody responses to the merozoite surface protein-119-kilo Dalton domain of *Plasmodium falciparum* in pregnant women and infants: associations with febrile illness, parasitaemia, and anaemia. *American Journal of Tropical Medicine and Hygiene*, 58, 211–19.

Briand, V., Cottrell, G., Massougbodji, A., & Cot, M. (2007). Intermittent preventive treatment for the prevention of malaria during pregnancy in high transmission areas. *Malaria Journal*, 6,

Briand, V., Escolano, S., Journot, V., Massougbodji, A., Cot, M., & Jubert-Bitter, P. (2015). Mefloquine versus Sulphadoxine-Pyrimethamine for Intermittent Preventive treatment in pregnancy: A joint analysis on efficacy and tolerability. *American Society of Tropical Medicine and Hygiene*, 93 (2), 300-304.

Brooks, D. R., Wang, P., Read, M., Watkins, WM., Sims, P.F., & Hyde, J. E. (1994). Sequence variation of the hydroxymethyldihydropterin pyrophosphokinase: Dihydropteroate synthase gene in lines of the

human malaria parasite, *Plasmodium falciparum,* with differing resistance to sulfadoxine. *European Journal of Biochemistry*, 2, 397-405.

Brozan, R. N., McMorrow, M. L., & Kachur, S. P. (2008). Diagnosis of Malaria: challenges for clinicians in endemic and non-endemic regions. *Molecular diagnosis Therapy, 12*(15), 299-306.

Buffet, P. A., Gamain, B., Scheidig, C., Baruch, D., Smith, J. D., Hernandez-Rivas, R.,...Scherf, A. (1999). *Plasmodium falciparum* domain mediating adhesion to chondroitin sulfate A: a receptor for human placental infection. *Proceedings from the National Academy of Science USA* 96,12743–12748.

Buffet, P. A., Safeukui, I., Deplaine, G., Brousse, V., Prendki, V., Thellier, M.,…& Mercereau-Puijalon, O. (2011). The pathogenesis of *Plasmodium falciparum* malaria in humans: insights from splenic physiology. *Blood,* 117, 381–392.

Bzik, D. J., Li, W. B., Horii, T., & Inselburg, J. (1987). Molecular cloning and sequence analysis of the *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase gene. *Proceedings of the National Academy of Sciences of the United States of America*, 84, 8360–8364.

Campos, P. A., Valente, B., Campos, R. B., Gonçalves, L., & Rosário, V. E. (2012). *Plasmodium falciparum* infection in pregnant women attending antenatal care in Luanda, Angola. *Revista da Sociedade Brasileira de Medicina Tropical,* 45(3), 369–374.

Carvalho, B. O., Lopes, S. C., Nogueira, P. A., Orlandi, P. P., Bargieri, D. Y., Blanco, Y. C.,…&Costa, F. T. (2010). On the cyto-adhesion of

Plasmodium vivax-infected erythrocytes. *Journal Infectious Disease*, 202, 638-647.

Carvalho, B. O., Matsuda, J. S., Luz, S. L., Martinez-Espinosa, F. E., Leite, J. A., Franzin, F.,...& Costa, F. T. (2011). Gestational malaria associated to *Plasmodium vivax* and *Plasmodium falciparum* placental mixed-infection followed by foetal loss: a case report from an unstable transmission area in Brazil. *Malaria Journal.* 10, 178.

Chang, K-H., & Stevenson, M. M. (2004). Malarial anaemia: mechanisms and implications of insufficient erythropoiesis during blood-stage malaria. *International Journal of Parasitology, 34*, 1501–1516.

Chen, X. & Wang, Y. (2008). Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. *Circulation,* 117, 3171–3180.

Chico, R. M., Canol, J., Ariti, C., Collier, T. J., Chandramohan, D., Roper, C., & Greenwood, B. (2015). Systematic Review: Influence of malaria transmission intensity and the 581G mutation on the efficacy of intermittent preventive treatment in pregnancy: systematic review and meta-analysis. *Tropical Medicine and International Health*, 20 (12), 1621–1633.

Chico, R. M., Dellicour, S., Roman, E., Mangiaterra, V., Coleman, J., Menendez, C.,...& Hill, J. (2015). Global Call to Action: maximize the public health impact of intermittent preventive treatment of malaria in pregnancy in sub-Saharan Africa *Malaria Journal*, 14, 207.

Chico, R. M., Mayaud, P., Ariti, C., Mabey, D., Ronsmans, C., Chandramohan, D. (2012). Prevalence of Malaria and Sexually

Transmitted and Reproductive Tract Infections in Pregnancy in Sub-Saharan Africa: A Systematic Review *JAMA*, 307 (19), 2079-2086.

Christensen, D. L., Kapur, A., & Bygbjerg, I. C. (2011). Physiological adaption to maternal malaria and other adverse exposure: low birth weight, functional capacity, and possible metabolic disease in adult life. *International Journal of Gynaecology and Obstetrics*, 115 (1), 16-19.

Cisse, M., Sangare, I., Lougue, G., Bamba, S., Bayane, S., D., & Guiguemde, R., T. (2014). Prevalence and risk factors for *Plasmodium falciparum* malaria in pregnant women attending antenatal clinic in Bobo-Dioulasso (Burkina Faso). *BMC Infectious Diseases*, 14,631

Clark, R. L. (2009). Embryotoxicity of the artemisinin anti-malarials and potential consequences for use in women in the first trimester. *Reproductive Toxicology*, 28, 285–296.

Cnops, L., Jacobs, J., & Van Esbroeck, M. (2011). Validation of a four-primer real-time PCR as a diagnostic tool for single and mixed *Plasmodium* infections. *Clinical Microbiology and Infection,* 17 (7), 1101–1107.

Coleman, M., Casimiro, S., Hemingway, J., & Sharp, B. (2008). Operational impact of DDT reintroduction for malaria control on *Anopheles arabiensis* in Mozambique. *Journal of Medical Entomology*, 45, 885–890

Coleman, R. E., Sattabongkot, J., Promstaporm, S., Maneechai, N., Tippayachai, B., Kengluecha, A.,…& Khuntirat, B. (2006). Comparison of PCR and microscopy for the detection of asymptomatic

malaria in a *Plasmodium falciparum/ vivax* endemic area in Thailand. *Malaria Journal*, 5, 121.

Conroy, A., Serghides, L., Finney, C., Owino, S. O., Kumar, S., Gowda, D. C.,...& Kain, K. C., (2009). C5a enhances dysregulated inflammatory and angiogenic responses to malaria in vitro: potential implications for placental malaria. *PLoS One*, 4, 4953

Corbel, V., N'Guessan, R., Brengues, C., Chandre, F., Djogbenou, L., Martin, R. M,...& Rowland, M. (2007) Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. *Acta Tropica*, 101, 207–216

Costa, F. T., Lopes, S. C., Ferrer, M., Leite, J. A., Martin-Jaular, L., Barnabeu, M.,...& del Portillo, H. (2011). On cytoadhesion of *Plasmodium vivax*: raison d'être? *Memórias do Instituto Oswaldo Cruz*, 106, 79-84

Cottrell, G., Mary, J. Y., Barro, D., & Cot, M (2007). The importance of the period of malarial infection during pregnancy on birth weight in tropical Africa. *American Journal of Tropical Medicine and Hygiene*, 76(5), 849-54.

Coulibaly, S. O., Nezien, D., Traore, S., Kone, B., & Magnussen, P. (2006). Therapeutic efficacy of sulphadoxine-pyrimethamine and chloroquine for the treatment of uncomplicated malaria in pregnancy in Burkina Faso. *Malaria Journal*, 5, 49.

Cowman, A. F., Morry, M. J., Biggs, B. A., Cross, G. A., & Foote, S. J. (1988). Mutations in dihydropteroate synthase are responsible for sulfone and sulphonamide resistance in *Plasmodium falciparum*. *Proceedings of the National Academy of Science*, U S A, 85, 9109-1

Cox-Singh, J., Davies, T. M. E., Lee, K, Shamsul, S. S. G., Matusop, A., Ratman, S.,...& Singh, B. (2008). *Plasmodium knowlesi* Malaria in Humans Is Widely Distributed and Potentially Life Threatening. *Clinical Infectious Diseases*, 46 (2), 165–171.

Crooker, I. P., Tanner, T. M., Myers, J. E., Baker, P. N. (2004). Syncitiotrophoblast degradation and the pathophysiology of malaria infected Placenta. Placenta, 25 (4), 273-82.

Cruickshank, J. K., Mzayek, F., Liu, L., Kieltyka, L., Sherwin, R., Webber, L.... & Berenson, G. S. (2005). Origins of the "black/white" difference in blood pressure: roles of birth weight, postnatal growth, early blood pressure, and adolescent body size: the Bogalusa heart study. *Circulation,* 111, 1932–1937.

Curtis, C. F., & Mnzava, A. E. (2000). Comparison of house spraying and insecticide treated nets for malaria control. *Bull World Health Organization,* 78, 1389-1400.

Dagne, G. (2002). Weekly chloroquine prophylaxis and the effect on maternal haemoglobin status at delivery. *Tropical Medicine and International Health*, 7, 29–34.

D'Alessandro, U., Olaleye, B. O., McGuire, W., Thomson, M. C., Langerock, P., Bennett, S.,... & Greenwood, B. M. (1995). A comparison of the efficacy of insecticide-treated and untreated bed nets in preventing malaria in Gambian children. *Transactions of the Royal Society Tropical Medicine Hygiene*, 89, 596–598

Das, B. S., & Nanda, N. K. (1999). Evidence for erythrocyte lipid peroxidation in acute *falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 93, 58–62.

de Moraes-Pinto, M. I., Verhoeff, F., Chimsuku, L., Milligan, P. J. M., Wesumperuma, L., Broadhead, R. L...& Hart, C. A. (1998). Placental antibody transfer: influence of maternal HIV infection and placental malaria. *Archives of Disease in Childhood - Foetal and Neonatal Edition,* 79, 202–05.

De- zuleta, J., Kafuko, G. W., McCrae, A. W. R., Cullen, J. R., Pedrsen, C. K., & Wasswa, D. F. (1964). A malaria eradication experiment in the highlands of Kigezi, Uganda. *East African Medical Journal*, 41, 102-120.

Delfino, R. T., Santos-Filho, O. A., & Figueroa-Villar, J. D. (2002). Molecular modeling of wild-type and antifolate resistant mutant *Plasmodium falciparum dhfr*. *Biophysical Chemistry*, 98:287–300.

Dellicour, S., Tatem A. J., Guerra C. A., Snow R. W., & ter Kuile F. O. (2010). Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. *PLoS Med*., 7(1), 221.

Denoeud, L., Fievet, N., Aubouy, A., Ayemonna, P., Kiniffo, R., Massougbodji, A., & Cot, M. (2007). Is chloroquine chemoprophylaxis still effective to prevent low birth weight? Results of a study in Benin. *Malaria Journal*, 6, 27.

Desai, M., Kuile, F. O., Nosten, F., McGready, R., Asamoa, K., Brabin, B. J., & Newman, R. D. (2007). Review: epidemiology and burden of malaria in pregnancy. *Lancet Infectious Disease*, 7:93–104.

Diakite, O. S., Kayentao, K., Traore, B. T., Djimde, A., Traore, B., Diallo, M...& Doumbo, O. K. (2011). Superiority of 3 over 2 doses of intermittent preventive treatment with sulfadoxine-pyrimethamine for the prevention of malaria during pregnancy in Mali: a randomized controlled trial. *Clinical Infectious Disease,* 53, 215–223.

Diamond-Smith, N., Singh, N., Gupta, R. K., Dash, A., Thimasarn, K., Campbell, O. M., & Chandramohan, D. (2009). Estimating the burden of malaria in pregnancy: a case study from rural Madhya Pradesh, India. *Malaria Journal,* 8, 24.

Dickoa, A., Mantelb, C., Theraa, M. A., Doumbiaa, S., Dialloa, M., Diakitéa, M... & Doumbo, O. K. (2003). Risk factors for malaria infection and anaemia for pregnant women in the Sahel area of Bandiagara, Mali. *Acta Tropica,* 89, (1), 17–23

Djimde, A. A., Dolo, A., Ouattara, A., Diakite, S., Plowe, C. V., & Doumbo, O. K. (2004). Molecular diagnosis of resistance to antimalarial drugs during epidemics and in war zones. *Journal of Infectious Disease*, 190, 853-855.

Djimde, A., Doumbo. O. K., Steketee, R. W., & Plowe, C. V. (2001). Application of a molecular marker for surveillance of chloroquine-resistant *falciparum* malaria. *Lancet,* 358, 890-891.

Dokomajilar, C., Lankoande, Z. M., Dorsey, G., Zongo, I., Ouedraogo, J. B., & Rosenthal, P. J. (2006). Roles of specific *Plasmodium falciparum* mutations in resistance to amodiaquine and sulfadoxine-pyrimethamine in Burkina Faso. *American Journal of Tropical Medicine and Hygiene,* 75, 162–165.

Doku, D. T., Zankawah, M. M., & Adu-Gyamfi, A. B. (2016). Factors influencing dropout rate of intermittent preventive treatment of malaria during pregnancy. *BioMed Central Research Notes*, 9, 460.

Doolan, D.L., Dobano, C., & Baird, J. K. (2009). Acquired immunity to malaria. *Clinical Microbiology Review*, 22, 13-36.

Douglas, N. M., Anstey, N. M., Buffet, P. A., Poespoprodjo, J. R., Yeo, T. W., White, N. J., & Price R. N. (2012). The anaemia of *Plasmodium vivax* malaria. *Malaria Journal*, 11, 135.

Douglas, N., Lampah, D., Kenangalem, E., Simpson, J., Poespoprodjo, J., & Sugiarto, P. (2013). Major burden of severe anemia from non-*falciparum* malaria species in southern Papua: a hospital-based surveillance study. *PLoSMed*, 10, 12.

Drugs .com. Sulphadoxine-pyri-methamine. Retrieved on 6th December 2016 from https://www.drugs.com/monograph/pyrimethamine-sulfadoxine-and-pyrimethamine.html.

Duffy, P. E. (2007). *Plasmodium* in the placenta: parasites, parity, protection, prevention and possibly preeclampsia. *Parasitology*, 134, 1877–1881.

Dunyo, S., Milligan, P., Edwards, T., Sutherland, C., Targett, G., & Pinder, M. (2006). Gametocytaemia after drug treatment of asymptomatic *Plasmodium falciparum*. *PLoS Clinical Trials*, 1, 20

Duraisingh, M. T., Curtis, J., Warhurst, D. C. (1998). *Plasmodium falciparum*: Detection of Polymorphisms in the Dihydrofolate Reductase and Dihydropteroate Synthetase Genes by PCR and Restriction Digestion *Experimental Parasitology*, 89, (1) 1-8

Durand, R. E., Sayen, J., Delabre, J-F., Marmorat-Khuong, A. Di Piazza, J. P., & Le Bras. J. (2000). Use of molecular beacons to detect an anti-folate resistance associated mutation in *Plasmodium falciparumAntimicrobial. Agents and Chemotherapy*, 44, 3461–3464.

Dzinjalamala, F. K., Macheso, A. M., Kublin, J. G., Taylor, T. E., Barnes, K. I., Molyneux, M. E… & Smith P. J. (2005). Association between the pharmacokinetics and *in vivo* therapeutic efficacy of Sulphadoxine-Pyrimethamine in Malawian children. *Antimicrobial Agents and Chemotherapy*, 49(9), 1-6

Dzinjalamala, F. K., Macheso, A., Kublin, J. G., Taylor, T. E., Barnes, K. I., Molyneux, M. E… & Smith P. J. (2005) Blood folate concentrations and in vivo sulfadoxine-pyrimethamine failure in Malawian children with uncomplicated *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene*, 72, 267–272.

Eberl, K. J., Jelinek, T., Aida A. O., Peyerl-Hoffmann, G., Heuschkel, C., el Valy, A. O., & Christophel, E. M (2001). Prevalence of polymorphisms in the dihydrofolate reductase and dihydropteroate synthetase genes of *Plasmodium falciparum* isolates from southern Mauritania. *Tropical Medicine and International Health,* 6, (10), 756-760.

Echeverry, D. F., Deason, N. A., Davidson, J., Makuru, Honglin Xiao, H., Niedbalski, J…& Lobo, N. F. (2016). Human malaria diagnosis using a single step direct PCR based on the *Plasmodium* cytochrome oxidase III gene. *Malaria Journal*, 15,128

Ehrhardt, S., Mockenhaupt, F. P., Agana-Nsiire, P., Mathieu, A., Anemana, S. D., Stark, K....& Bienzle, U. (2002). Efficacy of chloroquine in the treatment of uncomplicated, *Plasmodium falciparum* malaria in northern Ghana. *Annals of Tropical Medicine and Parasitology, 96, 239–247.*

Eijla, A. M., Ayisi, G. J., & Kuile, F. O. (2002). Implementation of IPT with SP for control of malaria in Kisumu, Kenya. *Malaria Journal*, 265–6.

Eisele, T. P., Larsen, D. A., Anglewicz, P. A., Keating, J., Yukich, J., Bennett, A...& Steketee, R. W. (2012). Malaria prevention in pregnancy, birth weight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. *Lancet Infectious Disease*, 12 (12), 942–949.

Enweronu-Laryea, C. C., Adjei, G. O., Mensah, B., Duah, N., & Quashie, N. B. (2013). Prevalence of congenital malaria in high-risk Ghanaian new borns-a cross sectional study. *Malaria Journal*, 12, 17

Esposito, F., Habluetzel, A. (1997). Chapter 3: The Anopheles vector. pp. 30-41. In "The Handbook of Malaria Infection in the Tropics". AIFO, Bologna.

Falade, C. O., Tongo, O. O., Ogunkunle, O.O., & Orimadegun, A. E. (2010). Effects of malaria in pregnancy on new-born anthropometry. *Journal of Infections in Developing Countries*, 4: 448-53.

FANTA (2012). Semi-quantitative evaluation of Access and Coverage (SQUEAC)/ Simplified Lot Quality assurance Sampling Evaluation of Access and Coverage (SLEAC) Technical Reference. Washington DC: FANTA.

Feresu, S. A. (2016). Annual distribution of births and deaths outcomes at Harare Maternity Hospital, Zimbabwe. *The Central African journal of medicine*, 56, 30–41.

Ferone, R., Burchall, J. J., & Hitchings, G. H. (1969). *Plasmodium berghei* dihydrofolate reductase. Isolation, properties and inhibition by antifolates. *Molecular Pharmacology*, 5:49–59.

Filler, S., Causer, L. M., Newman, C. D., Barber, A. M., Roberts, J. M., MacArthur, J., Praise, M. E., & Steketee, R. W. (2003). Malaria Surveillance-United States. *Centre for Disease Control Surveillance Summaries.*

Finlayson, K., & Downe, S. (2013). Why do women not use antenatal services in low- and middle-income countries? A meta-synthesis of qualitative studies. *PLoS Med*, 10: e1001373.

Fleming, A. F. (1989). Tropical obstetrics and gynaecology. Anaemia in pregnancy in Tropical Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 83, 441–448.

Francis, D., Nsobya, S. L., Talisuna, A., Yeka, A., Kamya, M.R., Machekano, R...& Dorsey, G. (2006). Geographic differences in antimalarial drug efficacy in Uganda are explained by differences in endemicity and not by known molecular markers of drug resistance. *Journal of Infectious Disease*, 193, 978 –986.

Galatas, B., Bassat, Q., & Mayor, A. (2016). Malaria Parasites in the Asymptomatic: Looking for the Hay in the Haystack: *Trends in Parasitology*, 32 (4), 296–308.

Gama, B. E., Pereira-Carvalho, G., Kosi, F., Oliverira, A., Fortes, F., Rosenthal, P. J...& Ferreira-da-Cruz, M. (2011). Molecular markers of antifolate resistance in *Plasmodium falciparum* isolates from Luanda, Angola. *Malaria Journal,* 10, 248.

Gamble, C. L., Ekwaru, J. P., & ter Kuile, F. O. (2009). Insecticide-treated nets for preventing malaria in pregnancy. *Cochrane Database Systematic Review*, 2:CD003755

Gamboa, D., Ho, M. F., Bendezu, J., Torres, K., Chiodini, P. L., Barnwell, J. W...& Cheng, Q. (2010). A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack *pfhrp2* and *pfhrp3*: implications for malaria rapid diagnostic tests. *PLoS One,* 5, 8091.

Ganguly, S., Saha, P., Guha, S. K., Biswas, A., Das, S., Kundu, P, K., & Maji, A, K. (2013). High prevalence of asymptomatic malaria in a tribal population in eastern India. *Journal of Clinical Microbiology*, 51, 1439–1444.

Garner, P., Gulmezoglu, A. M. (2003). Drugs for preventing malaria-related illness in pregnant women and death in the newborn. *Cochrane Database of Systematic Reviews,* 1, 10.

Garret-Jones, C. (1964). Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. *Nature*, 204, 1173-1174.

Gatton, M. L., Hogarth, W., & Saul, A. (2001). Time of treatment influences the appearance of drug-resistant parasites in *Plasmodium falciparum* infections. *Parasitology*, 123, 537-46.

Geiger, C., Compaore, G., Coulibaly, B., Sie, A., Dittmer, M., Sanchez, C... & Jänisch, T. (2014). Substantial increase in mutations in the genes *pfdhfr* and *pfdhps* puts sulphadoxine-pyrimethamine-based intermittent preventive treatment for malaria at risk in Burkina Faso. *Tropical Medicine and International Health,* 19(6), 690-697.

Gesase, S., Gosling, R.D., Hashim, R., Ord, R., Naidoo, I., Madebe, R.,...& Chandramohan, D. (2009). High resistance of *Plasmodium falciparum* to sulphadoxine/pyrimethamine in northern Tanzania and the emergence of *dhps* resistance mutation at Codon 581. *PLoS One* 4, 4569.

Ghai, R. R., Thurber, M. I., Bakry, A. E., Chapman, C. A., & Goldberg, T. L. (2016). Multi-method assessment of patients with febrile illness reveals over-diagnosis of malaria in rural Uganda. *Malaria Journal,* 15, 1502-1504

Ghana Health Service (GHS). Intermittent preventive treatment of malaria in pregnancy: training manual for health providers, participants guide. Accra: GHS/MOH; 2005

Ghana Health Service. (2009). *Intermittent Preventive Treatment of malaria in pregnancy: Training manual for health providers, participants guide 2009.* Accra. GHS/MOH.

Ghana Health Service. (2014). *Annual Report, 2014.*GHS/MOH, Accra Ghana.

Ghana Statistical Service (2014). *District reports 2014.* Accra, Ghana.

Gies, S., Coulibaly, S. O., Ouattara F. T., & D'Alessandro, U. (2009). Individual efficacy of intermittent preventive treatment with

sulfadoxine–pyrimethamine in primi- and secundigravidae in rural Burkina Faso: impact on parasitaemia, anaemia and birth weight. *Tropical Medicine and International Health*, 14(2), 174-182

Gimnig, J. E., Otieno, P., Were, V., Marwanga, D., Abong'o, D., Wiegand, R.,&...Hamel, M. J. (2016). The Effect of Indoor Residual Spraying on the Prevalence of Malaria Parasite Infection, Clinical Malaria and Anemia in an Area of Perennial Transmission and Moderate Coverage of Insecticide Treated Nets in Western Kenya. *PLOS ONE*, 11(1): e0145282. doi:10.1371/journal.pone.0145282

Gimnig, J. E., Vulule, J. M., Lo, T. Q., Kamau, L., Kolczak, M. S., Phillips-Howard, P. A...& Hightower, A. W (2003). Impact of permethrin-treated bed nets on entomological indices in an area of intense year-round malaria transmission. *American Journal Tropical Medicine Hygiene*, 68, 16-22.

González, R., Ataíde, R., Naniche, D., Menéndez, C., & Mayor, A. (2102). HIV and Malaria Interactions. Where Do We Stand? *Expert Review of Anti-Infective Therapy*, 10 (2), 153-165.

Gonzalez, R., Monbo-Ngoma, G., Ouedraogo, S., Kakolwa, M., Abdallah, S., Acrombessi, M., & Menedez, C. (2014). Intermittent Preventive Treatment of Malaria in pregnancy with Mefloquine in HIV-negative women: A multicentre Randomized Control Trial. *PlosMed* 11 (9), 1733.

Gray, R. H., Wabwire-Mangen, F., Kigozi, G., Sewankambo, N. K., Serwadda, D., Moulton, L. H...& Wawer, M. J. (2001). Randomized trial of presumptive sexually transmitted disease therapy during

pregnancy in Rakai, Uganda. *American Journal of Obstetrics and Gynaecology*, 185(5), 1209–1217.

Green, M. D., Nettey, H., Rojas, O. V., Pamanivong, C., Khounsaknalath, L., Ortiz, M. G...& Manolin, O. (2007). Use of refractometry and colorimetry as field methods to rapidly assess antimalarial drug quality. *Journal of Pharmaceutical and Biomedical Analysis,* (43), 105-110

Greenwood, B. (2004). The use of antimalarial drugs to prevent malaria in the population of malaria-endemic areas. *American Journal of Tropical Medicine and Hygiene,* 70, 1–7

Gregson, A., & Plowe, C.V. (2005). Mechanisms of resistance of malaria parasites to antifolates. *Pharmacology Review*, 57, 117–145.

Gude, N.M., Roberts, C. T., Kalionis, B. & King, R. G. (2004) Growth and function of the normal human placenta. *Thrombosis Research*, 114, 397–407.

Guerra, C. A., Gikandi, P. W., Tatem, A. M., Noor, A. M., Smith, D. L., Hay, S. I., & Snow, R. W. (2008). The limits and intensity of *Plasmodium falciparum* transmission: implications for malaria control and elimination worldwide. *PloSmedicine* 5 (2), e38

Gutman, J., Mwandama, D., Wiegand, R. E., Abdallah, N. C., Iriemenan, N. C., Shi, Y. P., & Skarbinski, J. (2015). *In vivo* efficacy of sulphadoxine-pyrimethamine for the treatment of asymptomatic parasitaemia in pregnant in Machinga district, Malawi. *Malaria Journal,* 14: 197.

Gutman, J., Mwandama, J., Wugard, R. E., Ali, D., Mathanga, D. P., & Skarbinski, J. (2013). Effectiveness of Intermittent Preventive Treatment with SP during pregnancy on maternal and birth outcomes in Machinga district, Malawi. *Journal of Infectious Disease,* 208 (6), 907-916.

Guyatt, H. L., & Snow, R. W., (2001). The epidemiology and burden of *Plasmodium falciparum*-related anaemia among pregnant women in sub-Saharan Africa. *American Journal of Tropical Medicine and Hygiene,* 64(1-2), 36-44.

Gwamaka, M., Fried, M., Domingo, G., Duffy, P. E. (2011). Early and extensive CD55 loss from red blood cells supports a causal role in malarial anaemia. *Malaria Journal*, 10,386.

Gyang, F. N., Peterson, D. S., & Wellems, T. E. (1992). *Plasmodium falciparum:* rapid detection of *dihydrofolate reductase* mutations that confer resistance to cycloguanil and pyrimethamine. *Experimental Parasitology,* 74,470–472.

Hajjou, M., Qin, Y., Bradby, S., Bempong, D., &Lukulay, P. (2013). Assessment of the performance of a handheld Raman device for potential use as a screening tool in evaluating medicines quality. *Journal of Pharmaceutical and Biomedical Analysis*, 74 (23), 47-55

Haldar, K., & Mohandas, N. (2009). Malaria, erythrocytic infection, and anaemia. *American Society of Haematology EducationProgram*: 87–93

Hamer, D. H., Ndhlovu, M., Zurovac, D., Fox, M., Yeboah-Antwi, K., Chanda P.,...& Snow, R. W. (2007) Improved diagnostic testing and malaria treatment practices in Zambia. *JAMA*, 297:222731.

Harbach, R. E. (2009). Mosquito taxonomic inventory (http://mosquito-taxonomic-inventory.info). *Accessed* 29 September 2009.

Harrington, W. E., Mutabingwa, T. K., Kabyemela, E., Fried, M., & Duffy, P. E. (2011). Intermittent treatment to prevent pregnancy malaria does not confer benefit in an area of widespread drug resistance. *Clinical Infectious Disease*, *53*,224–230.

Harrington, W. E., Mutabingwa, T. K., Muehlenbachs, A., Sorensen, B., Bolla, M.C., Fried, M., & Duffy, P. E. (2009). Competitive facilitation of drug-resistant *Plasmodium falciparum* malaria parasites in pregnant women who receive preventive treatment. *Proceedings of National Academy of Science of the United States of America*, 106, 9027–9032.

Harrison, G. (1978). Mosquitoes, Malaria, and Man: A History of the Hostilities Since 1880, John Murray, ISBN 0-71953-780-8, London, UK.

Hastings, I. M., Watkins, W. M., & White, N. J. (2002). The evolution of drug-resistant malaria: the role of drug elimination half-life. *Philos Transactions of the Royal Society of London*, 357, 505-19.

Hawley, W, A., Phillips-Howard, P.A., Ter Kuile, F.O., Terlouw, D.J., Vulule. J., Ombok, M.,...& Kolczak, M. S. (2003). Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in Western Kenya. *American Journal Tropical Medicine and Hygiene*, 68:121-127.

Hay, S. I., Sinka, M. E., Okara, R. M., Kabaria, C. W., Mbithi, P. M., Tago, C.C...& Godfray, H. C. J. (2010). Developing Global Maps of the Dominant Anopheles Vectors of Human Malaria. *PLOS*, 10, 1371

Himeidan, Y.E., Chen, H., Chandre, F., Donnelly, M. J., & Yan, G. (2007). Short report: permethrin and DDT resistance in the malaria vector Anopheles arabiensis from eastern Sudan. *American Journal Tropical Medicine Hygiene.* 77, 1066–1068

Hommerich, L., von Oertzen, C., Bedu-Addo, G., Holmberg, V., Acquah, P. A., Eggelte, T. A… & Mockenhaupt, F. P. (2007). Decline of placental malaria in southern Ghana after the implementation of intermittent preventive treatment in pregnancy. *Malaria Journal*, 6, 144

Howard, S. C., Omumbo, J., Nevil, C., Some, E. S., Donnelly, C. A., & Snow, R. W. (2000). Evidence for a mass community effect of insecticide-treated bed nets on the incidence of malaria on the Kenyan coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94, 357-360.

Huynh, B. T., Fievet, N., Gbaguidi, G., Dechavanne, S., Borgella, S., Guezo-Mevo, B…& Cot M. (2011) Influence of the timing of malaria infection during pregnancy on birth weight and on maternal anaemia in Benin. *American Journal of Tropical Medicine and Hygiene,* 85(2), 214-20.

Hyde, J. E., (1990). The dihydrofolate reductase-thymidylate synthetase gene in the drug resistance of malaria parasites. *Pharmacology and Therapeutics,* 48:45-59.

Igweh, J. C. (2012). Biology of Malaria Parasites, Malaria Parasites, Dr. Omolade Okwa (Ed.), ISBN: 978-953-51-0326-4.

IPCA. (2006). Sulfadoxine-pyrimethamine-Laridox. Accessed January 3, 2007, from www.malaria-ipca.com

Iriemenam, N. C., Shah, M., Gatei, W., van Eijk, A. M., Ayisi, J., Kariuki, S...& Shi., Y. P. (2012). Temporal trends of sulphadoxine-pyrimethamine (SP) drug-resistance molecular markers in *Plasmodium falciparum* parasites from pregnant women in western Kenya.*Malaria Journal*, 11,134.

Ismail, M. R., Ordi, J., Menendez, C., Ventura, P. J., Aponte, J. J, Kahigwa, E...&Alonso, P. L. (2000) Placental pathology in malaria: a histological, immune-histochemical, and quantitative study. *Human Pathology* 31, 85–93.

Jelinek, T., Kilian, A. H. D., Curtis, J., Duraisingh, M. T., Kabagambe, G., von Sonnenburg, F., & Warhurst, D. C., (1999). *Plasmodiumfalciparum*: selection of serine 108 of dihydrofolate reductase during treatment of uncomplicated malaria with co-trimoxazole in Ugandan children. *American Journal of Tropical Medicine and Hygiene* 61: 125–130

Joint Formulary Committee (2013). *British National Formulary (BNF)* (65 ed.). London, UK: Pharmaceutical Press. p. 429. ISBN 978-0-85711-084-8.

Juhola, J., Magnusson, C. G., Viikari, J. S., Kähönen, M., Hutri-Kähönen, N., Jula, A...& Juonala, M. (2011). Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: The Cardiovascular Risk in Young Finns Study. *Journal of Paediatrics*, 159, 584–590.

Kalaivani, K. (2009). Prevalence & consequences of anaemia in pregnancy. *Indian Journal Medical Research*, 130(5), 627-33.

Kalilani, L., Mofolo, I., Chaponda, M., Rogerson, S. J., & Meshnick, S. R. (2010). The effect of timing and frequency of *Plasmodium falciparum* infection during pregnancy on the risk of low birth weight and maternal anaemia. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 104 (6), 416-22.

Kalilani, L., Mofolo, I., Chaponda, M., Rogerson, S. J., Alker, A. P., Kwiek, J. J., & Meshnick, S. R. (2007). a randomized controlled pilot trial of azithromycin or artesunate added to sulfadoxine-pyrimethamine as treatment for malaria in pregnant Women. *PLoS One*, 2, e1166.

Kalilani, L., Taylor, S., Madanitsa, M., Chaluluka, E., Kalanda, G., Rogerson, S… & Ter Kuile, F. O. (2011). Waning effectiveness of SP IPTP in the presence of high SP resistance in Malawi. *Tropical Medicine and International Health* 16, 34–35.

Kamwendo, D. D., Dzinjalamala, F. K., Snounou, G., Kanjala, M. C. C., Mhango, C. G., Molyneux, M. E., & Rogerson, S. J. (2002). *Plasmodium falciparum*: PCR detection and genotyping of isolates from peripheral, placental, and cord blood of pregnant Malawian women and their infants. *Transactions of Royal Society Tropical Medicine and Hygiene,* 96, 145–49.

Kang, H., Kreuels, B., Adjei, O., Krumkamp, R., May, J., & Small, D. S. (2013). The causal effect of malaria on stunting: a Mendelian randomization and matching approach. *International Journal of Epidemiology,* 42, 1390–1398.

Kassam, S. N., Nesbitt, S., Hunt, L. P., Oster, N., Soothill, P., & Sergi, C., (2006). Pregnancy outcomes in women with or without placental

malaria infection. *International Journal of Gynaecology and Obstetrics*, 93(3), 225-32

Kayentao, K., Garner, P., van Eijk, A. M., Naidoo, I., Roper, C., Mulokozi, A...& ter Kuile, F. O. (2013). Intermittent preventive therapy with sulfadoxine-pyrimethamine for malaria in pregnancy in Africa using monthly regimens with 3 or more doses versus the standard 2-dose regimen: a systematic review and meta-analysis. *JAMA*, 309: 594–604.

Kayentao, K., Kodio, M., Newman, R. D., Maiga, H., Doumtabe, D., Ongoiba, A...& Doumbo, O. (2005). Comparison of intermittent preventive treatment with chemoprophylaxis for the prevention of malaria during pregnancy in Mali. *Journal of Infectious Diseases*, 191 (1), 109-16.

Kayentao, K., Mungai, M., Parise, M., Kodio, M., Keita, A. S., Coulibaly, D...& Doumbo, O. K. (2007). Assessing malaria burden during pregnancy in Mali. *Acta Tropica,* 102, 106–112.

Kayser, F., H., Bienz, Bienz, K.A., Eckert, J., Zinkernagel, R. M. (2005). Medical Microbiology, Thieme. pp 234-237

Kibwage, I. O., & Ngugi JK (2000). Sulphadoxine /pyrimethamine tablet products on the Kenyan market: quality concerns. *East and Central African Journal of Pharmaceutical Sciences,* 3, 14–19.

Killeen, G. F., Smith, T. A., Ferguson, H. M., Abdulla, S., Mshinda, H., Lengeler, C., & Kachur, S. P. (2007). Preventing childhood malaria in Africa by protecting adults from mosquitoes with insecticide treated-nets. *PLoS Med*, 4, 229.

Kleinschmidt, I., Schwabe, C., Shiva, M., Segura, J. L., Sima, V., & Mabunda, S. J. (2009) Combining indoor residual spraying and insecticide-treated

net interventions. *American Journal Tropical Medicine Hygiene,* 81, 519–24.

Kouznestov, R., L. (1977). Malaria control by application of indoor spraying of residual insecticides in tropical Africa and its impact on community health. *Tropical Doctor,* 7(2), 81-91.

Kritsiriwuthinan, K., & Ngrenngarmlert, W. (2011). Asymptomatic malaria infections among foreign migrant workers in Thailand. *Asian Pacific Journal Tropical Medicine*, 4, 560–563.

Krungkrai, J., Webster, H. K., & Yuthavong, Y. (1989). De novo and salvage biosynthesis of pteroyl penta-glutamates in the human malaria parasite, *Plasmodium falciparum. Molecular and Biochemical Parasitology,* 32, 25–37.

Kublin, J. G., Cortese, J. F., Njunju, E. M., Mukadam R. A. G., Wirima, J. J., Kazembe, P. N.,...& Plowe, C. V. (2004). Re-emergence of chloroquine-sensitive *Plasmodium falciparum* malaria after cessation of chloroquine use in Malawi. *Journal of Infectious Diseases,* 187, 1870-1875.

Kublin, J. G., Dzinjalamala, F. K., Kamwendo, D. D., Malkin, E. M., Cortese, J. F., Martino, L. M.,...& Plowe, C. V. (2002). Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of *Plasmodium falciparum* malaria. *Journal of Infectious Diseases,* 185, 380–388.

Kulkarni, M.A. Malima, R., Mosha, F. W., Msangi, S., Mrema, E., Kabula, B., Lawrence, B... & Drakeley, C. (2007). Efficacy of pyrethroid-treated nets against malaria vectors and nuisance-biting mosquitoes in

Tanzania in areas with long-term insecticide-treated net use. *Tropical Medicine and International Health,* 12, 1061–1073

Kyabayinze, D., Cattamanchi, A., Kamya, M. R., Rosenthal, P. J. & Dorsey, G. (2003). Validation of a simplified method for using molecular markers to predict sulfadoxine-pyrimethamine treatment failure in African children with *falciparum* malaria. *American Journal of Tropical Medicine and Hygiene,* 69, 247–252.

LaBeaud, A.D., Malhotra, I., King, M. J., King, C. L., & King, C. H. (2009). Do antenatal parasite infections devalue childhood vaccination? *PLoS Negleted Tropical Disease,* 3, 442

Lalani, M., Kitutu, F. E., Clarke, S., & Kaur, H. (2017). Anti-malarial medicine quality field studies and surveys: a systematic reviewof screening technologies used and reporting of findings.*Malaria Journal,* 16, 1852-6

Lanata, C. F., & Black, R. E. (1991). Lot quality assurance sampling techniques in health surveys in developing countries: advantages and current constraints. *World Health Statistics Quarterly.* 44(3), 133-9.

Lengeler, C. (2004) Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Systematic Review*,2, 363.

Leshem, E., Keller, N., Guthman, D., Grossman, T., Solomon, M., Marva, E., & Schwartz, E. (2011). False positive *Plasmodium falciparum* histidine-rich protein 2 immunocapture assay in acute schistosomiasis caused by Schistosoma mekongi. *Journal of Clinical Microbiology,* 49, 2331–2.

Leslie, T., Mikhail, A., Mayen, I., Nader, M., Chandler, C., Whitty, C. J. M., & Rowland, M. (2012). Over-diagnosis and mistreatment of malaria among febrile patients at primary healthcare level in Afghanistan: observational study. *British Medical Journal*, 345.

Lewandowski, A. J., Davis, E. F., Yu, G., Digby, J. E., Boardman, H., Whitworth, P...& Leeson, P. (2015). Elevated blood pressure in preterm-born offspring associates with a distinct antiangiogenic state and microvascular abnormalities in adult life. *Hypertension,* 65, 607–614.

Lindblade, K. A., Eisele, T.P., Gimnig, J. E., Alaii, J. A., Odhiambo, F., ter Kuile, F. O...& Rosen, D. H. (2004). Sustainability of reductions in malaria transmission and infant mortality in western Kenya with use of insecticide-treated bed nets: 4-6 years of follow-up. *JAMA*, 291, 2571-2580.

Lindsay, S. W., & Gibson, M., E. (1988). Bednets revisited-old idea, new angle. *Trends in Parasitology*. 4, (10), 270–272.

Lindsay, S., Ansell, J., Selman, C., Cox, V., Hamilton, K., & Walraven, G. (2000). Effect of pregnancy on exposure to malaria mosquitoes. *Lancet*. 355 (9219).

Liu, Y., Zhang, K. E., Liu, Y., Zhang, H., Song, Y., Pu, H...& Lu, C. (2012) Pharmacokinetic Properties and Bioequivalence of Two Sulfadoxine/Pyrimethamine Fixed-Dose Combination Tablets: A Parallel-Design Study in Healthy Chinese Male Volunteers. *Clinical Therapeutics*, 34 (11), 2212-2220.

Looareesuwan, S., Merry, A. H., Phillips, R. E., Pleehachinda, R., Wattanagoon, Y., & Ho, M., (1987). Reduced erythrocyte survival following clearance of malarial parasitaemia in Thai patients. *British Journal of Haematology*. 67 (4), 473–8.

Lucchi, N. W., Demas, A., Narayanan, J., Sumari, D., Kabanywanyi, A., Kachur, S. P...& Udhayakumar, V. (2010). Real-Time Fluorescence Loop Mediated Isothermal Amplification for the Diagnosis of Malaria. *PLoS ONE* 5 (10), 1371.

Luxemburger, C., McGready, R., Kham, A., Morison, L., Cho, T., Chongsuphajaisiddhi, T...& Nosten, F. (2001). Effects of malaria during pregnancy on infant mortality in an area of low malaria transmission. *American Journal Epidemiology*, 154(5), 459-65.

Luyckx, V. A., & Brenner, B. M. (2015). Birth weight, malnutrition and kidney-associated outcomes–a global concern. *National Reviews in Nephrology*, 11, 135–149.

Luyckx, V. A., Bertram, J. F., Brenner, B. M., Fall, C., Hoy, W. E., Ozanne, S. E., & Vikse, B. E. (2013). Effect of foetal and child health on kidney development and long-term risk of hypertension and kidney disease. *Lancet*, 382: 273-283.

Mabunda, S., Aponte, J. J., Tiago, A, & Alonso, P. (2009). A country-wide malaria survey in Mozambique. II. Malaria attributable proportion of fever and establishment of malaria case definition in children across different epidemiological settings. *Malaria Journal*. 8, 74.

MacDonald, G. (1957). Epidemiology and Control of Malaria. London: Oxford University Press.

Malhotra, I., Dent, A., Mungai, P., Wamachi, A., Ouma, J. H., Narum, D. L.,...& King, C. L. (2009). Can prenatal malaria exposure produce an immune tolerant phenotype? A prospective birth cohort study in Kenya. *PLoS Med.* 6,116

Malhotra, I., Mungai, P., Muchiri, E., Kwiek, J. J., Meshnick, S. R., & King, C. L. (2006). Umbilical cord-blood infections with *Plasmodiumfalciparum* malaria are acquired antenatally in Kenya. *Journal of infectious Diseases*, 194, 176–83.

Maltha, J., Gillet, P., Cnops, L., van den Ende, J., van Esbroeck, M., & Jacobs, J. (2010). Malaria rapid diagnostic tests: *Plasmodium falciparum* infections with high parasite densities may generate false positive *Plasmodium vivax* pLDH lines. *Malaria Journal*, 9, 198.

Marchant, T., Armstrong-Schellenberg, J. R. M., Edgar, T., Ronsmans, C., Nathan, R., Abdulla, S...& Lengeler, C. (2105). Anaemia during pregnancy in southern Tanzania. *Annals of Tropical Medicine and Parasitology,* 477-487

Marks, F., Evans, J., Meyer, C. G., Browne, E. N., Flessner, C., von Kalckreuth, V...& May, J. (2005). High prevalence of markers for sulfadoxine and pyrimethamine resistance in *Plasmodium falciparum* in the absence of drug pressure in the Ashanti region of Ghana. *Antimicrobial Agents and Chemotherapy*, 49, 1101–5.

Massaga, J. J., Kitua, A. Y., Lemnge, M. M., Akida, J. A., Malle, L. N., Ronn, A. M...& Bygbjerg, I. C. (2003). Effect of intermittent treatment with amodiaquine on anaemia and malarial fevers in infants in Tanzania: A randomized placebo-controlled trial. *Lancet*, 361, 1853–1860.

Matangila J. R., Lufuluabo, J., Ibalanky, A. L., Inocêncio da Luz, R. A., Lutumba, P., & Van Geertruyden, J. (2014). Asymptomatic *Plasmodium falciparum* infection is associated with anaemia in pregnancy and can be more cost-effectively detected by rapid diagnostic test than by microscopy in Kinshasa, Democratic Republic of the Congo. *Malaria Journal* 13, 132

Matar, K. M., Awad, A., & Elamin, S. B. (2014). Pharmacokinetics of Artesunate Alone and in Combination with Sulfadoxine-Pyrimethamine in Healthy Sudanese Volunteers.*American Journal of Tropical Medicine Hygiene,* 90(6), 1087-1093

Matondo, S. I., Temba, G. S., Kavishe, A. A., Kauki, J. S., Kalinga, A., van Zwetselaar, M...& Kavish, A. (2014). High levels of sulphadoxine-pyrimethamine resistance *Pfdhfr-Pfdhps* quintuple mutations: a cross sectional survey of six regions in Tanzania. *Malaria Journal*, 13,152.

Matteeli, A., Donato, F., Shein, A. (1994). Malaria and anaemia in pregnant women in urban Zanzibar, Tanzania. *Annals of Tropical Medicine and Parasitology*, 88(5), 475–483.

Maxwell, C. A., Msuya, E., Sudi, M., Njunwa, K. J., Carneiro, I. A., & Curtis, C. F. (2002). Effect of community-wide use of insecticide-treated nets for 3-4 years on malarial morbidity in Tanzania. *Tropical Medicine and International Health*, 7, 1003-1008.

Mayor, A., Bardají, A., Felger, I., King, C. L., Cisteró, P., Dobaño, C...& Rogerson, S. (2012). Placental infection with *Plasmodium vivax*: a histopathological and molecular study. *Journal of Infectious Diseases*, 206, 1904–1910.

McGready, R., Boel, M., Rijken, M. J., Ashley, E. A., Cho, T., Moo, O...& Nosten, F. H., (2012). Effect of early detection and treatment on malaria related maternal mortality on the north-western border of Thailand 1986-2010. *PLoS ONE*, 7(7), 40244.

McGready, R., Lee, S. J., Wiladphaingern, J., Ashley, E.A., Rijken, M. J., Boel, M...& Nosten, F. H. (2012). Adverse effects of *falciparum* and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population-based study. *Lancet Infectious Disease Journal*. 12 (5), 388-96.

Menendez, C., Bardaji, A., Sigauque, B., Romagosa, C., Sanz, S., Casas-Serra, E., & Alonso, P. L. (2008). A randomized Placebo-Controlled trial of Intermittent Preventive Treatment in pregnant women in the context of Insecticide Treated Nets delivered through Antenatal Clinics. *PLOS ONE*. 3 (4), 1934.

Menendez, C., Bardaji, A., Sigauque, B., Sarz, S., Aponte, J. J., Mabunda, S., & Alonso, P. L. (2010). Malaria prevention with intermittent preventive treatment during pregnancy reduces neonatal mortality. *PLOS ONE* 5 (2) e9438.

Menendez, C., Ordi, J., Ismail, M. R., Ventura, P. J., Aponte, J. J., Kahigwa, E...& Alonso, P. L. (2000). The impact of placental malaria on gestational age and birth weight. *Journal of Infectious Diseases*, 181: 1740–45.

Menendez, C., Serra-Casas, E., Scahill, M. D., Sanz, S., Nhabomba, A., Bardaji, A...&Mayor, A. (2011). HIV and placental infection modulate the appearance of drug-resistant *Plasmodium falciparum* in pregnant

women who receive intermittent preventive treatment. *Clinical Infectious Disease*, 52, 41–48.

Mercé, L.T. (2009). Inter-villous and utero-placental circulation in normal early pregnancy and early pregnancy loss assessed by 3-dimensional power Doppler angiography. *American Journal of Obstetrics and Gynaecology*. 315, 1–8

Metz, J. (2007). Folic acid metabolism and malaria. *Food and Nutrition Bulletin*, 28, 540–549.

Miller, L. H., Ackerman, H. C., Su, X., & Wellems, T. E., (2013). Malaria biology and disease pathogenesis: insights for new treatments. *Nature Medicine* 19, 156-167.

Milne, L. M., Chiodini, P. L., & Warhurst, D. C. (1994). Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. *Journal of Clinical Pathology*, 47,740–742.

Milne, L. M., Kyi, M. S., Chiodini, P. L, Warhurst, D. C. (1994). Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. *Journal Clinical Pathology*, 47,740–742.

Ministry of Health (2009). Training manual for the management of malaria at health facilities in Ghana: participant's guide. Accra: MOH; 2009.

Minja, D.T., Schmiegelow, C., Mmbando, B., Bostrom, S., Oesterholt, M., Magistrado, P...& Alifrangis, M. (2013). *Plasmodium falciparum* mutant haplotype infection during pregnancy associated with reduced birth weight, Tanzania. *Emerging Infectious Disease*, 19, 1909.

Mockenhaupt, F. P., Bedu-Addo, G., Eggelte, T. A., Hommerich, L., Holmberg, V., von Oertzen, C., & Bienzle, U. (2008). Rapid Increase

in the Prevalence of Sulfadoxine-Pyrimethamine Resistance among *Plasmodium falciparum* Isolated from Pregnant Women in Ghana. *Journal of Infectious Diseases*, 198, 1545–9

Mockenhaupt, F. P., Bousema, T. J., Eggelte, T. A., Schreiber, J., Ehrhardt, S., Wassilew, N., Otchwemah, R. N... & Bienzle, U. (2005). *Plasmodium falciparumdhfr* but not *dhps* mutations associated with sulphadoxine-pyrimethamine treatment failure and gametocyte carriage in northern Ghana. *Tropical Medicine and International Health*, 10, 901– 8.

Mol, B. W., Roberts, C. T., Thangaratinam, S., Magee, L. A., de Groot, C. J., & Hofmeyr, G. J. (2016). Pre-eclampsia. *Lancet,* 387, 999–1011.

Moody, A. H. & Chiodini P. L. (2002). Non-microscopic method for malaria diagnosis using OptiMAL IT, a second-generation dipstick for malaria *p*LDH antigen detection. *British Journal of Biomedical Science,* 59, 228-231.

Mpungu, S. K., & Mufubenga, P. (2008). Use of antenatal care, maternity services, intermittent presumptive treatment and insecticide treated bed nets by pregnant women in Luwero district, Uganda. *Malaria Journal*, 7, 44.

Muehlenbachs, A., Mutabingwa, T. K., Edmonds, S., Fried, M., & Duffy, P. E. (2006). Hypertension and maternal-foetal conflict during placental malaria. *PLoS Medicine*, 3, 446.

Mugittu, K., Ndejembi, M., Malisa, A., Lemnge, M., Premji, Z., Mwita, A., Nkya, W...& Mshinda, H. (2004). Therapeutic efficacy of sulfadoxine-pyrimethamine and prevalence of resistance markers in Tanzania prior to revision of malaria treatment policy: *Plasmodiumfalciparum*

dihydrofolate reductase and dihydropteroate synthase mutations in monitoring in vivo resistance. *American Journal of Tropical Medicine and Hygiene*, 71, 696-702.

Mumbare, S. S., Maindarkar, G., Darade, R., Yenge, S., Tolani, M. K., & Patole, K. (2012). Maternal Risk Factors Associated with Term Low Birth Weight Neonates: A Matched-Pair Case Control Study *Indian Paediatrics* Volume 49.

Munhenga, G., Masendu, H. T., Brooke, B. D., Hunt, R. H., Koekemoer L. K. (2008). Pyrethroid resistance in the major malaria vector *Anopheles arabiensis* from Gwave, a malaria-endemic area in Zimbabwe. *Malaria Journal*, 7, 247

Murray, C. K., Gasser, R. A. Jr., Magill, A. J., & Miller, R. S. (2008) Update on rapid diagnostic testing for malaria. *Clinical Microbiology Review* 21, 97-110.

Myakalwar, A. k., Sreedhar, S., Barman, I., Dingari, N., Rao, V. S…& Kumar, M. (2011). Laser-induced breakdown spectroscopy-based investigation and classification of pharmaceutical tablets using multivariate chemometric analysis. *Talanta,* 87, 53-59.

Nahlen, B. L., Alakija, T., Ogunbode, O., Adetoro, O., Akintunde, A., Nguyen-Dinh, P…&Breman, J. G. (1989) Lack of efficacy of pyrimethamine prophylaxis in pregnant Nigerian women. *Lancet*, 8667, 830–834.

Naidoo, I., & Roper, C. (2011). Drug resistance maps to guide intermittent preventive treatment of malaria in African infants. *Parasitology,* 138, 1469–1479.

Naidoo, I., & Roper, C. (2013). Mapping 'partially resistant', fully resistant' and 'super resistant' malaria. *Trends in Parasitology*, 29, 505-515

National Institutes of Health USA. (2006). Intermittent preventive treatment during pregnancy in Benin: A randomized, open, and equivalent trial comparing sulfadoxine–pyrimethamine with mefloquine. National Institutes of Health USA.

Ndao, C. T., Dumont, A., Fievet, N., Doucoure, S., Gaye, A., & Lehesran, J. Y. (2009). Placental malarial infection as a risk factor for hypertensive disorders during pregnancy in Africa: a case-control study in an urban area of Senegal, West Africa. *American Journal of Epidemiology*, 170, 847–853.

Nduati, E., Diriye, A., Ommeh, S., Mwai, L., Kiara, S., Masseno, V... & Nzila, A. (2008). Effect of folate derivatives on the activity of antifolate drugs used against malaria and cancer. *Parasitology Research*, 102, 1227–1234.

Newman, R. D., Parise, M. E., Slutsker, L., Nahlen, B., & Steketee, R. W. (2003). Safety, efficacy and determinants of effectiveness of antimalarial drugs during pregnancy: Implications for prevention programmes in *Plasmodium falciparum* endemic sub-Saharan Africa. *Tropical Medicine and International Health*, 8, 488–506.

Nosten, F., McGready, R., & Mutabingwa, T. (2007). Case management of malaria in pregnancy. *The Lancet Infectious Diseases*,7, 118–125.

Nosten, F., McGready, R., Simpson, J. A., Thwai, K. L., Balkan, S., Cho, T... & White, N. J. (1999). Effects of *Plasmodium vivax* malaria in pregnancy. *Lancet*, 354, 546–49.

Nussenblatt, V., Mukasa, G., Metzger, A., Ndeezi, G., Garrett, E, Semba, R. D. (2001). Anaemia and interleukin-10, tumour necrosis factor alpha, and erythropoietin levels among children with acute uncomplicated *Plasmodium falciparum* malaria. *Clinical Diagnosis Laboratory for Immunology* 8, 1164–1170.

Nzila, A. M., Mberu, E. K., Sulo, J., Dayo, H., Winstanley, P. A., Sibley, C. H., & Watkins, W. M. (2000a) Towards an understanding of the mechanism of pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum:* Genotyping of dihydrofolate reductase and dihydropteroate synthase of Kenyan parasites. *Antimicrobial Agents and Chemotherapy* 44, 991–996.

O'Donnell, A., Raiko, A., Clegg, J. B., Weatherall, D. J., & Allen, S. J. (2007). Southeast Asian ovalocytosis and pregnancy in a malaria-endemic region of Papua New Guinea. *American Journal of Tropical Medicine and Hygiene,* 76(4), 631-3.

Ohrt C., Sutamihardja, M. A., Tang, D., & Kain, K.C. (2002). Impact of microscopy error on estimates of protective efficacy in malaria prevention trials. *Journal Infectious Disease,* 186, 540-6.

Okoko, B. J., Wesumperuma, L. H., Ota, M. O., Pinder, M., Banya, W., Gomez, S. F… & Hart, A. C. (2001). The influence of placental malaria infection and maternal hypergammaglobulinemia on trans-placental transfer of antibodies and IgG subclasses in a rural west African population. *Journal of Infectious Diseases*, 184, 627–32

Olatunbosun, O. A., Abasiattai, A. M., A. Bassey, E. A., James, R. S., Ibanga, G. & Morgan, A. (2014). Prevalence of Anaemia among

Pregnant Women at Booking in the University of Uyo Teaching Hospital, Uyo, Nigeria. *Biomed Research International*, 849, 80-8.

Omar, S. A., Adagu, I. S., & Warhurst, D. C. (2001). Can pre-treatment screening for dhps and dhfr point mutations in *Plasmodium falciparum* infections be used to predict sulfadoxine-pyrimethamine treatment failure? *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 95, 315–319.

Onchiri, F. M., Pavlinac, P., Singa, B. O., Mulongo, J. N., Farquhar, C., John-Stewart, G. C., & Walson J. (2015). Frequency and correlates of malaria overdiagnosis and treatment in western Kenya. *Lancet,* 109(15), 70067-7

Orish, V, N., Onyeabor, O. S., Boampong, J. N., Afoakwah, R., Nwaefuna, E., Acquah, S.,...& Iriemenam, N. C. (2015)._Prevalence of intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) use during pregnancy and other associated factors in Sekondi-Takoradi, Ghana. African Health Science, 15 (4), 1087-1096.

Ouma, P. O., van Eijk, A. M., Hamel, M. J., Sikuku, E., Odhiambo, F., Munguti, K.… & Slutsker, L. (2007). The effects of health care worker training on the use of intermittent preventive treatment for malaria in pregnancy in rural western Kenya. *Tropical Medicine and International Health*, 12, 953–61

Ouma, P., Parise, M. E., Hamel, M. J., ter Kuile, F. O., Otieno, K., Ayisi, J. G…& van Eijk, A. M. (2006). A randomized controlled trial of folate supplementation when treating malaria in pregnancy with sulphadoxine-pyrimethamine. *PLoS Clinical Trials*, 1, e28.

Oyibo, W. A. & Agomo, C. O. (2010). Scaling Up of Intermittent Preventive Treatment of Malaria in Pregnancy Using Sulphadoxine–Pyrimethamine: Prospects and Challenges *Maternal and Child Health Journal* 15, 542–552.

Particle Sciences (2010). In Vitro Dissolution Testing for Solid Oral Dosage Forms Technical Brief, Volume 5.

Pearce, R.J., Drakeley, C., Chandramohan, D., Mosha, F., & Roper, C. (2009). Molecular determination of point mutation haplotypes in the dihydrofolate reductase and dihydropteroate synthase of *Plasmodium falciparum* in three districts of northern Tanzania. *Antimicrobial Agents and Chemotherapy,* 47, 1347-1354.

Peters, P. J., Thigpen, M. C., Parise M. E., & Newman, R. D. (2007). Safety and toxicity of sulphadoxine-pyrimethamine: Implications for malaria prevention in pregnancy using intermittent preventive treatment. *Drug Safety*, 30(6), 481–501.

Phillips, R. E, Pasvol, G. (1992). Anaemia of *Plasmodium falciparum* malaria. *Baillieres Clinical Haematology*. 5(2), 315–30.

Picot, S., Olliaro, P., de Monbrison, F., Bienvenu, A. L., Price, R. N., & Ringwald, P. (2009). A systematic review and meta-analysis of evidence for correlation between molecular markers of parasite resistance and treatment outcome in *falciparum* malaria. *Malaria Journal*, 8, 89.

Plowe, C. V., Cortese, J. F., Djimde, A., Nwanyanwu, O. C., Watkins, W. M., Winstanley, P. A…& Doumbo, O. K.(1997). Mutations in *Plasmodium falciparumdihydrofolate reductase* and *dihydropteroate synthase* and

epidemiologic patterns of pyrimethamine-sulfadoxine use and resistance. *Journal of Infectious Diseases,* 176, 1590–1596.

Poespoprodjo, J. R., Fobia, W., Kenangalem, E., Lampah, D. A., Warikar, N., Seal, A…& Price, R. N., (2008). Adverse pregnancy outcomes in an area where multidrug-resistant *Plasmodium vivax* and *Plasmodium falciparum* infections are endemic. *Clinical and Infectious Disease*, 46(9), 1374-81.

Raghavendra, K., Tapan K., Barik, B. P., Reddy, N., Sharma, P., & Aditya, P. D. (2011). Malaria vector control: from past to future. *Parasitology Research*, 108,757–779

Ratovonjato, J. Le Goff, G., Rajaonarivelo, E., Rakotondraibe, E. M., & Robert V. (2003). Recent observations on the sensitivity to pyrethroids and DDT of *Anopheles arabiensis* and *Anopheles funestus* in the central Highlands of Madagascar; preliminary results on the absence of the *kdr* mutation in *Anopheles. arabiensis*. *Archives of the. Institute of Pasteur, Madagascar* 69, 63–69

Recker, M., Bouma, M.J., Bamford, P., Gupta, S., & Dobson, A.P. (2009) Assessing the burden of pregnancy-associated malaria under changing transmission settings. *Malaria Journal* 8, 245.

Reeder, J. C., Rieckmann, K. H., Genton, B., Lorry, K., Wines, B., & Cowman, A. F. (1996). Point mutations in the dihydrofolate reductase and dihydropteroate synthetase genes and in vitro susceptibility to pyrimethamine and cycloguanil of *Plasmodium falciparum* malaria in Africa. *American Journal of Tropical Medicine and Hygiene*, 55, 209-13.

Reyburn, H., Mbatia, R., Drakeley, C, Carneiro, I., Mwakasungula, E., Mwerinde, O...& Whitty, C. J. (2004). Over-diagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *British Medical Journal,* 329, 1212–17.

Reyburn, H., Ruanda, J., Mwerinde, O., & Drakeley, C. (2006). The contribution of microscopy to targeting antimalarial treatment in a low transmission area of Tanzania. *Malaria Journal*, 5, 4.

Rijken, M. J., Mcgready, R., Boel, M. E., Poespoprodjo, R., Singh, N., Syafruddin, D.,...& Nosten, F. (2012). Malaria in pregnancy in the Asia-Pacific region. *Lancet Infectious Diseases*, 12:75–88.

Riley, E. M., Wagner, G. E., Akanmori, B. D., & Koram, K. A. (2001). Do maternally acquired antibodies protect infants from malaria infection? *Parasite Immunology*, 23, 51–59.

Risha, P. G., Shewiyo, D., Msami, A., Masuki, G., Vergote, G., & Vervaet, C. (2002). *In vitro* evaluation of the quality of essential drugs on the Tanzanian market. *Tropical Medicine and International Health*, 7, 701–707.

Robertson, S. E., Anker, M., Roisin, A. J., Macklai, N., & Engstrom, K. (1997). The lot quality technique: a global review of applications in the assessment of health services and diseases surveillance. *World Health Stat Quarterly,* 7, 50,199–209

Roche, (2008). FANSIDAR® (sulfadoxine and pyrimethamine)" (PDF). Medicines.org.au. Roche Products Pty Limited. Retrieved 9 January 2014.

Rodriguez-Morales, A. J., Sanchez, E., Vargas, M., Piccolo, C., Colina, R., Arria, M., & Franco-Paredes, C. (2006). Pregnancy outcomes associated with *Plasmodium vivax* malaria in north-eastern Venezuela. *American Journal of Tropical Medicine Hygiene*,74 (5), 755-7.

Rogerson, S. J., Hviid, L., Duffy, P. E., Leke, R. F., & Taylor, D. W. (2007). Malaria in pregnancy: pathogenesis and immunity. *Lancet Infectious Diseases*, 7, 105-117.

Rogerson, S. J., Pollina, E., Getachew, A., Tadesse, E., Lema, V. M., & Molyneux, M. E. (2003). Placental monocyte infiltrates in response to *Plasmodium falciparum* infection and their association with adverse pregnancy outcomes. *American Journal of Tropical Medicine and Hygiene,* 68, 115–19.

Roll Back Malaria/WHO. The Abuja declaration and the plan of action. An extract from the African Summit on Roll Back Malaria, Abuja. Geneva: WHO; 2000

Romagosa, C., Ordi, J., Saute, F., Quintò, L., Machungo, F., Ismail, M. R...&Menendez, C. (2007). Seasonal variations in maternal mortality in Maputo, Mozambique: the role of malaria. *Tropical Medicine and International Health*, 12 (1), 62-7.

Rovira-Vallbona, E., Dobaño, C., Bardajì, A., Cisterò, P., Romagosa, C., Serra-Casass, E., Quintò, L...&Mayor, A. (2011). Transcription of *var* genes other than var2csa in *Plasmodium falciparum* parasites infecting Mozambican pregnant women. *Journal of Infectious Disease*, 204: 27-35

Russell P. F. (1955). Man's Mastery of Malaria. London: oxford University Press.

Russell, T. L., Lwetoijera, D. W., Maliti, D., Chipwaza, B., Kihonda, J., Charlwood, D...& Killeen, G. F. (2010). Impact of promoting longer-lasting insecticide treatment of bed nets upon malaria transmission in a rural Tanzanian setting with pre-existing high coverage of untreated nets. *Malaria Journal*, 9, 187

Sakar, M., Woodland, C. C., Koren, G., & Einarson, A. R. (2006). Pregnancy outcome following gestational exposure to azithromycin. *BMC Pregnancy and Childbirth*, 6, 18

Salgame, P., Yap, G. S., & Gause, W. C. (2013). Effect of helminth-induced immunity on infections with microbial pathogens: *Nature Immunology*, 14 (11), 1118–1126.

Sander, A. F., Salanti, A., Lavstses, T., Nielsen, M. A., Theander, T. G., Lehe, R. G...& Taylor, D. W. (2011). Positive selection of *Plasmodium falciparum* parasites with multiple var2csa-type PfEMP1 genes during the course of infection in pregnant women. *Journal of Infectious Diseases*; 203: 1679-85.

Santolamazza, F. et al. (2008). Distribution of knock-down resistance mutations in *Anopheles gambiae* molecular forms in west and central Africa. *Malaria Journal.* 7, 74

Santos-Filho, O. A., de Alencastro, R. B., & Figueroa-Villar JD (2001) Homology modeling of wild type and pyrimethamine/cycloguanil-cross resistant mutant type *Plasmodium falciparum* dihydrofolate

reductase. A model for antimalarial chemotherapy resistance. *Biophysical Chemistry,* 91:305–317.

Schaible, U. E., & Kaufmann, S. H. E., (2007). Malnutrition and Infection: Complex Mechanisms and Global Impacts: *PLoS Medicine*. 2007 May; 4(5), 115.

Scherf, A., Pouvelle, B., Buffet, P. A., & Gysin, J. (2001). Molecular mechanisms of *Plasmodium falciparum* placental adhesion. *Cellular Microbiology,* 3,125–131

Scho¨nfeld, M., Miranda, I. B., Schunk, M., Maduhu, I., Maboko, L., Hoelscher, M…& Löscher, T. (2007). Molecular surveillance of drug resistance associated mutations of *Plasmodium falciparum* in south-west Tanzania. *Malaria Journal*, 6, 2.

Schultz, L. J., Steketee, R. W., Macheso, A., Kazembe, P., Chitsulo, L., & Wirima, J. J. (1994). The efficacy of antimalarial regimens containing sulphadoxine–pyrimethamine and/or chloroquine in preventing peripheral and placental *Plasmodium falciparum* infection among pregnant women in Malawi. *American Journal of Tropical Medicine and Hygiene,* 51, 515–522.

Shah, M., Omosun, Y., Lal, A., Odero, C., Gatei, W, Otieno, K…&Shi Y. P. (2015). Assessment of molecular markers for anti-malarial drug resistance after the introduction and scale-up of malaria control interventions in western Kenya. *Malaria Journal*, 14:75

Shiff, C. J., Minjas, J. & Premji, Z. (1994). The ParaSight-F test: a simple rapid manual dipstick test to detect *Plasmodial falciparum* infection. *Parasitology Today, 10*, 494-495.

Shulman, C. E., & Dorman, E. K. (2003). Importance and prevention of malaria in pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 97, 30–35.

Shulman, C. E., Marshall, T., Dorman, E. K., Bulmer, J. N., Cutts, F., Peshu, N., & Marsh, K., (2001). Malaria in pregnancy: adverse effects on haemoglobin levels and birth weight in primigravidae and multi-gravidae. *Tropical Medicine and International Health*, 6 (10), 770-8.

Siame, M. N. P., Mharakurwa, S., Chipeta, J., Thuma, P., & Michelo, C. (2015). High prevalence of dhfr and dhps molecular markers in *Plasmodium falciparum* in pregnant women of Nchelenge district, Northern Zambia *Malaria Journal*, 14,190-5

Sibley, C. H., Hyde, J. E., Sims, P. F., Plowe, C. V., Kublin, J. G., Mberu, E. K., Cowman, A. F...& Nzila A. M. (2001). Pyrimethamine-sulfadoxine resistance in *Plasmodiumfalciparum*: What next? *Trends in Parasitology,* 17, 582–588

Simira, S. B., Sawadogo, R., Moran, A. C., Konate, A., Diarra, A., Yameogo, M...&Newman, R. D. (2003). Failure of a chloroquine chemoprophylaxis program to adequately prevent malaria during pregnancy in Koupela District, Burkina Faso. *Clinical Infectious Diseases*, 36, 1374–1382.

Simpson, J. A., Watkins, E. R., Price, R. N., Aarons, L., Kyle, D. E., & White, N. J. (2000). Mefloquine pharmacokinetic-pharmacodynamic models: implications for dosing and resistance. *Antimicrobial Agents Chemotherapy*, 44, 3414-24.

Singh, N., Mehra, R. K. & Srivastava, N. (2001). Malaria during pregnancy and infancy, in an area of intense malaria transmission in central India. *Annals of Tropical Medicine and Parasitology,* 95, 19–29.

Sinka, M., Bangs, M., Manguin, S., Coetzee, M., Mbogo, C., Hemingway, J., Patil, A… & Hay, S. (2010). The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: Occurrence data, distribution maps and bionomic précis. *Parasites & Vectors*, 3, 117-120

Sirawaraporn, W., Prapunwattana, P., Sirawaraporn, R., Yuthavong, Y., & Santi, D.V. (1993). The dihydrofolate reductase domain of *Plasmodium falciparum* thymidylate synthase-dihydrofolate reductase: Gene synthesis, expression, and anti-folate resistant mutants. *Journal of Biological Chemistry,* 268, 21637-44.

Sirawaraporn, W., Sathitkul, T., Sirawaraporn, R., Yuthavong, Y., & Santi, D. V. (1997a). Antifolate-resistant mutants of *Plasmodium falciparum* dihydrofolate reductase. *Proceedings from the National Academy of Science of the United States of America*, 94:1124-9.

Sirawaraporn, W., Yongkiettrakul. S., Sirawaraporn, R., Yuthavong, Y., & Santi, D. V. (1997b). *Plasmodium falciparum*: Asparagine mutant at residue 108 of dihydrofolate reductase is an optimal antifolate-resistant mutant. *Experimental Parasitology*, 87, 245-52.

Snow, R. W., Molyneux, C. S., Njeru, E. K., Omumbo, J., Nevill, C. G., Muniu, E., & Marsh, K. (1997). The effects of malaria control on nutritional status in infancy. *Acta Tropica*, 65, 1–10.

Spencer, H. C., Collins, W. E., Chin, W, & Skinner, J. C. (1979). The enzyme linked immunosorbent assay (ELISA) for malaria. The use of in-vitro cultured *Plasmodiumfalciparum* as antigen. *American journal of Tropical Medicine and Hygiene, 28,* 927-932.

Staedke, S. G., Sendagire, H., Lamola, S., Kamya, M. R., Dorsey, G., & Rosenthal, P. J. (2004). Relationship between age, molecular markers, and response to sulphadoxine-pyrimethamine treatment in Kampala, Uganda. *Tropical Medicine and International Health* 9: 624–629.

Steketee, R. W., Nahlen, B. L., Parise, M, E., & Menendez, C. (2001).The Burden of Malaria in Pregnancy in Malaria-Endemic Areas. The Intolerable Burden of Malaria: A New Look at the Numbers: Supplement to Volume 64(1) of the *American Journal of Tropical Medicine and Hygiene.*

Stephens, J. K., Ofori, M. F., Quakyi, I. A., Wilson, M. L., & Akanmori, B. D. (2014). Prevalence of peripheral blood parasitaemia, anaemia and low birth weight among pregnant women in a suburban area in coastal Ghana. *The Pan African Medical Journal* - ISSN 1937-8688.

Stephenson, L. S., Latham, M. C., Ottesen, E. A. (2000). Malnutrition and parasitic helminth infections. *Parasitology* 121 (Suppl) : S23–S38

Suate, F., Menendez, C., Mayor, A., Aponte, J., Gomez-olive, X., Dgedge, M., & Alonso, P. (2002). Malaria in pregnancy in rural Mozambique: the role of parity, sub-microscopic and multiple *Plasmodium falciparum* infections. *Tropical Medicine and International Health.* 2002, 7 (1): 19-28. 10.1046/j.1365-3156.2002.00831. x.

Suguitan Jr, A. L., Leke, R. G., Fouda, G., Zhou, A., Thuita, L., Metenou, S...& Taylor D. W. (2003). Changes in the levels of chemokines and cytokines in the placentas of women with *Plasmodium falciparum* malaria. *Journal of Infectious Diseases,* 188, 1074–1082.

Suguitan, A. L. Jr., Cadigan, T. J., Nguyen, T. A., Zhou, A., Leke, R. J., Metenou, S., Thuita L...& Taylor, D. W. (2003). Malaria-associated cytokine changes in the placenta of women with pre-term deliveries in Yaoundé, Cameroon. *American Journal of Tropical Medicine and Hygiene,* 69, 574–81.

Sullivan, A. D., Nyirenda, T., Cullinan, T., Taylor, T., Harlow, S. D., James, S. A., & Meshnick, S. R. (1999). Malaria infection during pregnancy: intrauterine growth retardation and preterm delivery in Malawi. *Journal of Infectious Diseases* 179: 1580–83.

Sulzer, A. J., Wilson, M. & Hall, E. C. (1969). Indirect fluorescent antibody for parasitic diseases. V. An evaluation of a thick-smear antigen in the IFA test for malaria antibodies. *American Journal of Tropical medicine and Hygiene, 18*, 199-205.

Sutherland, C. J., Tanomsing, N., Nolder, D., Oguike, M., Jennison, C., Pukrittayakamee, S...& Polley, S. D. (2010). Two non-recombining sympatric forms of the human malaria parasite *Plasmodium ovale* occur globally. *Journal of Infectious Disease.* 201(10), 1544–50.

Tagbor, H., Bruce, J., Agbo, M., Greenwood, B., & Chandramohan, D. (2010). Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: a randomized controlled non-inferiority trial. *PLoS ONE*, 5(12), 14425.

Tagbor, H., Bruce, J., Ord, R., Randall, A., Browne, E., Greenwood, B., & Chandramohan, D. (2007). Comparison of the therapeutic efficacy of chloroquine and sulphadoxine-pyrimethamine in children and pregnant women. *Tropical Medicine and International Health*, 12:1288–1297.

Tahita, M. C., Tinto, H., Erhart, A., Kazienga, A., Fitzhenry, R., VanOvermeir, C...& D'Alessandro. (2015). Prevalence of the dhfr and dhps Mutations among Pregnant Women in Rural Burkina Faso Five Years after the Introduction of Intermittent Preventive Treatment with Sulfadoxine-Pyrimethamine. *PLoSONE*, 10(9), 1371-1374

Takem, E. N., & D'Alessandro, U. (2013). Malaria in pregnancy. *Mediterranean Journal of Haematology and Infectious Diseases*, 5(1), 4084

Tako, E. A., Zhou, A., Lohoue, J., Leke, R., Taylor, D. W., & Leke, R. F. (2005). Risk factors for placental malaria and its effect on pregnancy outcome in Yaoundé, Cameroon. *American Journal of Tropical Medicine and Hygiene*, 72, 236–242.

Tamaru, T., & Picciano, M. F. (2006). Folate and human reproduction. *American Journal of Clinical Nutrition,* 83, 993–1016.

Tan, K. R., Katalenich, B. L., Mace, K. E., Nanbozi, M., Taylor, S. M., Meshnick, R. S., & Craig, A. S. (2014). Efficacy of sulphadoxine pyrimethamine for IPT of malaria in pregnancy, Mansa, Zambia. *Malaria Journal*, 13, 227.

Tarimo, S.D. (2007). Appraisal on the prevalence of malaria and anaemia in pregnancy and factors influencing uptake of intermittent preventive

therapy with sulfadoxine-pyrimethamine in Kibaha district, Tanzania. *East African Journal of Public Health*, 4 (2), 80-83.

Taylor, R. B., Shakoor, O., Behrens, R. H., Everard, M., Low, A. S., Wangboonskul, J...& Kolawole, J. A. (2001). Pharmacopoeial quality of drugs supplied by Nigerian pharmacies. *Lancet,* 357, 1933–1936.

Taylor, S. M., Antonia, A., Feng, G., Mwapasa, V., Chaluluka, E., Molyneux, M.,...& ter Kuile, F. O. (2012). Adaptive evolution and fixation of drug-resistant *Plasmodiumfalciparum* genotypes in pregnancy-associated malaria: 9-year results from the QuEERPAM study. *Infections and Genetic Evolution*, 12, 282–290.

Teik, Y. L., Mersumpin, S, & William, T. (2013). Mutational Analysis of *Plasmodium falciparum dhfr* and *dhps* genes in the interior division of Sabah, Malaysia. *Malaria Journal*, 12, 445

ter Kuile, F. O., & Rogerson S. J. (2008). *Plasmodium vivax* Infection during Pregnancy: An Important Problem in Need of New Solutions. *Clinical Infectious Disease* 46, 1382-1384.

ter Kuile, F. O., Parise, M. E., Verhoeff, F. H., Udhayakumar, V., Newman, R. D., van Eijk, A. M...& Steketee, R. W. (2004). The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-Saharan Africa. *American Journal of Tropical Medicine and Hygiene*, 71, 41–54.

Ticconi, C., Arpino, C., Longo, B., & Mapfumo, M. (2005). Prevalence and risk factors for low birth weight in Northern Zimbabwe. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*, 88, 146–7.

Titaley, C. R., Dibley, M. J., Roberts, C. L. & Agho, K. (2010). Combined iron/folic acid supplements and malaria prophylaxis reduce neonatal mortality in 19 sub-Saharan African countries. *American Journal of Clinical Nutrition*, 92(1), 235-43.

Tobian, A. A. R., Mehlotra, R. K., Malhotra, I., Wamachi, A., Mungai, P., Koech, D.,...& Ouma, J. (2000). Frequent umbilical cord-blood and maternal-blood infections with *Plasmodium falciparum, P. malariae* and *P. ovale* in Kenya. *Journal of Infectious Diseases*, 182, 558–63.

Tran, V., Weckman, A. & Kain, K. C. (2016). Malaria in Pregnancy: An Unrecognized Risk Factor for Neuropsychiatric Disease in Offspring? *Paediatric Infectious Diseases*, 1,14-17

Triglia, T., & Cowman, A.F. (1994). Primary structure and expression of the dihydropteroate synthetase gene of *Plasmodiumfalciparum*. *Proceedings of the National Academy of Science United States America*, 91, 7149-7153.

Triglia, T., Menting, J. G., Wilson, C., & Cowman, A. F. (1997). Mutations in dihydropteroate synthase are responsible for sulfone and sulphonamide resistance in *Plasmodium falciparum*. *Proceedings from the National Academy of ScienceUnited States of America* , 94, 13944-9.

Triglia, T., Wang, P., Sims, P. F., Hyde, J. E., Cowman, A. F. (1998). Allelic exchange at the endogenous genomic locus in *Plasmodium falciparum* proves the role of dihydropteroate synthase in sulfadoxine-resistant malaria. *EMBO Journal*, 17, 3807–3815

Tukur, I. U., Thacher, T. D., Sagay, A. S., & Madaki, J. K. (2007). A comparison of sulfadoxine-pyrimethamine with chloroquine and

pyrimethamine for prevention of malaria in pregnant Nigerian women. *American Journal of Tropical Medicine and Hygiene*, 76, 1019–1023.

Tutu, E. O., Lawson, B., & Browne, E. (2011). The effectiveness and perception of the use of sulphadoxine-pyrimethamine in intermittent preventive treatment of malaria in pregnancy programme in Offinso district of Ashanti region, Ghana. *Malaria Journal,* 10, 385.

Tutu, E. O., Otupiri, E., Larbi, J., Brown, C., Browne, E., & Lawson, B. (2010). The effect of intermittent preventive treatment using sulphadoxine pyrimethamine in control of malaria in pregnancy: A cross-sectional study in the Offinso district of Ghana. *Journal of Public Health and Epidemiology,* 2, 53-59.

Umbers, A. J., Aitken, E. H., & Rogerson, S. J. (2011). Malaria in pregnancy: small babies, big problem. *Trends in Parasitology*, 27 (4).

UN (2006). L'ame´lioration de la durabilite´ de la production du coton eb afrique de l'ouest et du centre. In Confe´rence des Nations Unies Sur le Commerce et le De´veloppement: Fonds Commun pour les Produits de Base Consultatif Internationnal du Coton, Nations Unies.

UNAIDS/WHO. Global report: UNAIDS report on the global AIDS Epidemic 2013. *WHO Library Cataloguing- in-Publication Data* 2013; UNAIDS (JC2502/1/E).

Uneke, C. J. (2008). Impact of Placental *Plasmodium falciparum* malaria on pregnancy and perinatal outcome in Sub-Saharan Africa. Part III: placental malaria, maternal health and public health. *Yale Journal of Biology and Medicine*, 81, 1-7.

UNICEF, The state of the world's children. New York, USA: UNICEF; 2013

USP (2015). *United States Pharmacopoeia, USP 38, National Formulary 33rd Edition, Vol 3*. Rockville: Pharmacopoeial Convention INC.

Vafa, M., Troye-Blomberg, M., Anchang, J., Garcia, A., & Migot-Nabias, F. (2008). Multiplicity of *Plasmodium falciparum* infection in asymptomatic children in Senegal: relation to transmission, age and erythrocyte variants. *Malarial Journal,* 7, 17.

Valadez, J. J., Hage, J., & Vargas, W. (2005). Understanding the relationship of maternal health behaviour change and intervention strategies in a Nicaraguan NGO Network. *Soc Sci Med,*61, 1356–68.

Valea, I., Tinto, H., Drabo, M. K., Huybregts, L., Sorgho, H., Ouedraogo, J. B., Guiguemde, R. T... & D'Alessandro, U., (2012). An analysis of timing and frequency of malaria infection during pregnancy in relation to the risk of low birth weight, anaemia and perinatal mortality in Burkina Faso. *Malaria Journal*, 11, 71.

van Eijk, A. M., Ayisi J. G., ter Kuile, F. O., Otieno J. A., Misore, A. O., Odondi, J. O...&Nahlen B. L., (2004). Effectiveness of intermittent preventive treatment with sulphadoxine-pyrimethamine for control of malaria in pregnancy in western Kenya: a hospital-based study. *Tropical Medicine and International Health,* 9 (3), 351–360.

van Eijk, A. M., Ayisi, J. G., Slutsker, L., Ter Kuile, F. O., Rosen, D. H., Otieno, J. A...& Shi, Y. P. (2007). Effect of haematinic supplementation and malaria prevention on maternal anaemia and malaria in western Kenya. *Tropical Medicine and International Health*, 12(3), 342-52.

van Hensbroek, M. B. S., Morris-Jones, S., Meisner, S., Jaffar, S., Bayo, L., Dackour, R... & Greenwood, B. M. (1995). Iron, but not folic acid, combined with effective antimalarial therapy promotes haematological recovery in African children after acute *falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 89, 672–676.

Vanhauwere, B., Maradit, H., & Kerr, L. (1998). Post-marketing surveillance of prophylactic mefloquine (Lariam) use in pregnancy. *American Journal of Tropical Medicine and Hygiene,* 58, 17–21.

Verhaeghen, K., Bortel, W. V., Roelants, P., Backeljau, T., & Coosemans, M. (2006). Detection of the East and West African *kdr* mutation in *Anopheles gambiae* and *Anopheles arabiensis* from Uganda using a new assay based on FRET/Melt Curve analysis. *Malaria Journal*, 5, 16.

Verhaeghen, K., Bortel, W. V., Roelants, P., Okello, P. E., Talisuna, A., & Coosemans, M. (2010). Spatio-temporal patterns in *kdr* frequency in permethirn and DDT resistant *Anopheles gambiae s. s.* from Uganda. *American Journal of Tropical Medicine and Hygiene*, 82, 566–573.

Wadsworth, M. E., Cripps, H. A., Midwinter, R. E., & Colley, J. R. (1985). Blood pressure in a national birth cohort at the age of 36 related to social and familial factors, smoking, and body mass. *British Medical Journal (Clinical Research Edition),* 291, 1534–1538

Walter, P. R, Garin, Y., & Blot, P. (1982). Placental pathologic changes in malaria. A histologic and ultrastructural study. *American Journal of Pathology,* 109, 330–42.

Walther, B., Miles, D. J., Crozier, S., Waight, P., Palmero, M. S., Ojuola, O., Touray, E... & Flanagan, K. L. (2010). Placental malaria is associated with reduced early life weight development of affected children independent of low birth weight. *Malaria Journal*, 9, 16

Wang, P., Brooks, D. R., Sims, P. F. G., and Hyde, J. E. (1995). A mutation-specific PCR system to detect sequence variation in the dihydrofolate reductase *dihydropteroate synthetase* gene of *Plasmodium falciparum. Molecular and Biochemical Parasitology,* 71, 115–125.

Wang, P., Sims, P. F., & Hyde, J. E. (1997). A modified in vitro sulfadoxine susceptibility assay for *Plasmodium falciparum* suitable for investigating Fansidar resistance. *Parasitology,* 115, 223–230.

Wang, P., Wang, Q., Aspinall, T. V., Sims, P. F., & Hyde, J. E. (2004). Transfection studies to explore essential folate metabolism and antifolate drug synergy in the human malaria parasite. *Plasmodium falciparum. Molecular Microbiology*, 51, 1425–1438.

Warhurst, D. C. (1998). Antimalarial drug discovery: development of inhibitors of dihydrofolate reductase active in drug resistance. *Drug Discovery Today*, 3:538–546.

Watkins, W. M., & Mosobo, M. (1993). Treatment of *Plasmodium falciparum* malaria with pyrimethamine-sulfadoxine: Selective pressure for resistance is a function of long elimination. *Transactions Royal Society Tropical Medicine and Hygiene*, 87 (1) 75-78.

Watkins, W. M., Mberu, E. K., Winstanley, P. A., & Plowe, C. V. (1999). More on the efficacy of antifolate antimalarial combinations in Africa. *Parasitology Today,* 15, 131–132.

Watkinson, M., Rushton, D. I., & Lunn, P. G. (1985). Placental malaria and foeto-placental function: low plasma oestradiols associated with malarial pigmentation of the placenta. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 79, 448–50.

WebMD, (2014). Fansidar®, Pyrimethamine-sulfadoxine dosing, indications, interactions, adverse effects, and more". *Medscape Reference.* Retrieved 6th December 2016.

Weidekamm, E., Plozza-Nottebrock, H., Forgo, I., & Dubach, U. C. (1982). Plasma concentrations in pyrimethamine and sulfadoxine and evaluation of pharmacokinetic data by computerized curve fitting. Bulletin of the World Health Organization, 60, 115–122.

Weidekamm, E., Plozza-Nottebrock, H., Forgo, I., & Dubach, U.C. (1982). Plasma concentrations in pyrimethamine and sulfadoxine and evaluation of pharmacokinetic data by computerized curve fitting. *Bulletin of the World Health Organization*, 60, 115–122.

White N. (1999). Antimalarial drug resistance and combination therapy. *Phil Transactions of the Royal Society. London Biological Science,* 141, 1413-22.

White, N. J. (2005). Intermittent presumptive treatment for malaria. *PLoS Medicine*, 2, 28–33

White, N. J. (2008). *Plasmodium* knowlesi: The Fifth Human Malaria Parasite. *Clinical Infectious Diseases*, 46(2), 172–173,

White, N. J. (2009). Malaria. In G. C. Cook and I. A. Zumla (Eds), *Manson's tropical Disease* (1201-1300) Saunder Elsevier Ltd.

White, N. J., & Pongtavornpinyo, W. (2003). The de novo selection of drug-resistant malaria parasites. *Proceedings of Biological Science,* 270, 545-54.

Wickramasinghe, S. N., & Abdalla, S. H. (2000). Blood and bone marrow changes in malaria. *Baillieres Best Pract Res Clin Haematol,* 13, 277–299.

Wilson, M. L. (2013). Laboratory Diagnosis of Malaria Conventional and Rapid Diagnostic Methods. *Archives of Pathology and Laboratory medicine,* 137, 805–811.

Wilson, N. O., Ceesay, F. K., Obed, S. A., Adjei, A. A., Gyasi, R. K., Rodney, P.,...& Stiles, J. K. (2011). Intermittent preventive treatment with sulfadoxine-pyrimethamine against malaria and anaemia in pregnant women. *American Journal of Tropical Medicine and Hygiene,* 85(1), 12-21.

Wongsrichanalai, C., Barcus, M. J., Muth, S., Sutamihardja, A., & Wernsdorfer, W. H. A. (2007). Review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). *American Journal of Tropical Medicine Hygiene,* 77:119–127.

Woodruff, A. W., Ansdell, V. E., & Pettitt, L. E. (1979). Cause of anaemia in malaria. *Lancet,* 1, 1055–1057.

World Health Organization (1988). Malaria diagnosis: memorandum from a WHO meeting. *Bull World Health Organization* 66: 575– 594.

World Health Organization (1993). Prevention and management of severe anaemia in pregnancy. Geneva, Switzerland: World Health Organization. WHO/FHE/MSM/93.5.

World Health Organization (2003). Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated *falciparum* malaria. Geneva, Switzerland: WHO

World Health Organization (2005). *Malaria- a Human Burden: Roll Back Malaria Department.* Geneva, Switzerland: WHO.

World Health Organization (2005). *Recommendations on the use of sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment during pregnancy (IPT) in areas of moderate to high resistance to SP in the African Region.* Brazzaville, Congo: WHO.

World Health Organization (2006). *Indoor residual spraying: use of indoor residual spraying for scaling up global malaria control and elimination.* Geneva, Switzerland: The WHO/Global Malaria Control.

World Health Organization (2010). *World Malaria Report,* 2010. Geneva, Switzerland: WHO

World Health Organization (2011). *Haemoglobin concentration for the diagnosis of anaemia and assessment of severity.* Geneva, Switzerland: The WHO.

World Health Organization (2011). Survey of the quality of selected antimalarial medicines circulating in six countries of sub-Saharan Africa. Geneva, Switzerland: The World Health Organization.

World Health Organization (2012). *World malaria report, 2012.* Geneva, Switzerland: The World Health Organization.

World Health Organization (2015). *World Malaria Report 2015.* Geneva, Switzerland: The World Health Organization.

World Health Organization. (1996). *Assessment of Therapeutic Efficacy of Antimalarial Drugs for Uncomplicated Falciparum Malaria in Areas with Intense Transmission*. Geneva, Switzerland: World Health Organization.

World Health Organization. (1998). *HIV in Pregnancy: a review*. Geneva, Switzerland: WHO/UNAIDS.

World Health Organization. (2000). *Severe falciparum malaria*. Geneva, Switzerland: World Health Organization, Communicable Diseases Cluster.

World Health Organization. (2004). *A strategic framework for malaria prevention and control during pregnancy in the African region*. WHO Regional Office for Africa, Brazzaville, Republic of Congo: WHO.

World Health Organization. (2007). *Technical expert group meeting on intermittent preventive treatment in pregnancy (IPTp)*. Geneva, Switzerland: Global Malaria Programme/World Health Organization.

World Health Organization. (2009). *Methods for Surveillance of Antimalarial Drug Efficacy*. Geneva, Switzerland: The World Health Organization.

World Health Organization. (2011). *Malaria Rapid Diagnostic Test Performance. Results of WHO product testing of malaria RDTs: round 3*. Geneva, Switzerland: The World Health Organization.

World Health Organization. (2011). *Working document QAS/07. 218/FINAL*. Geneva, Switzerland: The World Health Organization.

World Health Organization. (2012). *Drug Information*.Geneva, Switzerland: The World Health Organization.

World Health Organization. (2012b). *Updated WHO policy recommendation: intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP).* Geneva, Switzerland: The World Health Organization.

World Health Organization. (2013). *Global anaemia prevalence trends.* Geneva, Switzerland: The World Health Organization.

World Health Organization. (2013). *Global Malaria Programme. World Malaria Report 2010.* Geneva, Switzerland: The World Health Organization.

World Health Organization. (2013b). *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach.* Geneva, Switzerland: The World Health Organization.

World Health Organization. (2014). *World malaria report 2014.* Geneva: Switzerland: World Health Organization.

WorldHealthOrganization.(2016).http://www.who.int/nutrition/publications/globaltargets_policybrief_lbw/en2025. Retrieved 8th December 2016.

Yawson, A.E., McCall, P. J., Wilson, M. D., & Donnelly, M. J. (2004). Species abundance and insecticide resistance of *Anopheles gambiae* in selected areas of Ghana and Burkina Faso. *Medical Veterinary Entomology.* 18, 372–377.

Yuvaniyama, J., Chitnumsub, P., Kamchonwongpaisan, S., Vanichtanankul, J., Sirawaraporn, W., Taylor, P., & Yuthavong, Y. (2003). Insights into antifolate resistance from malarial DHFR-TS structures. *Nature Structural Biology,* 10:357–365.

Zaim, M., Aitio, A., & Nakashima, N. (2000). Safety of pyrethroid-treated mosquito nets. *Medical and Veterinary Entomology*. 14, 1–5

Zhou, Z., Poe, A. C., Limor, J., Grady, K. K., Goldman, I., McCollum, A. M...& Udhayakumar, V. (2006). Pyrosequencing, a High-Throughput Method for Detecting Single Nucleotide Polymorphisms in the *Dihydrofolate Reductase* and *Dihydropteroate Synthetase* Genes of *Plasmodium falciparum*. *Journal of Clinical Microbiology*, 44 (11) 3900–3910.

APPENDICES

APPENDIX – A: ETHICAL CLEARANCE CERTIFICATE

GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted

My Ref GHS-ERC: 3
Your Ref No

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Tel: +233-302-681469
Fax + 233-302-685424
Email: nanatwesakyiad@yahoo.com

12th June, 2013

Dr. Leslie Larry Afutu
University of Cape Coast
Department of Human Biology
Cape Coast

ETHICAL APPROVAL - ID NO: GHS-ERC: 78/03/13

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

"Efficacy of Sulphadoxine-pyrimethamine as intermittent preventive treatment of Malaria in Pregnancy in Sekondi-Takoradi Metropolis, Ghana"

This approval requires that you inform the Ethical Review Committee (ERC) when the study begins and provide Mid-term reports of the study to the Ethical Review Committee (ERC) for continuous review. The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Please note that any modification without ERC approval is rendered invalid.

You are also required to report all serious adverse events related to this study to the ERC within seven days verbally and fourteen days in writing.

You are requested to submit a final report on the study to assure the ERC that the project was implemented as per approved protocol. You are also to inform the ERC and your sponsor before any publication of the research findings.

Please always quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.....
DR. CYNTHIA BANNERMAN
(GHS-ERC VICE-CHAIRPERSON)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

APPENDIX-B: INFORMED CONSENT FORM

GHANA HEALTH SERVICE

ETHICAL REVIEW COMMITTEE ON RESEARCH INVOLVING HUMAN SUBJECTS (ERCRIHS)

INFORMED CONSENT FORM

Title: Evaluation of the Effectiveness of sulphadoxine-pyrimethamine as intermittent preventive treatment of malaria in pregnant women in selected health facilities in Sekondi-Takoradi, Ghana.

Principal Investigator: Dr. Leslie Larry Afutu

Name of the Organization: Department of Biomedical Science University of Cape Coast, School of Biological Sciences, U.C.C

General Information about Research

Purpose of the Study

The purpose of this study is to evaluate the effectiveness of SP as IPTp in the control of malaria in pregnancy in selected health facilities in the Western Region of Ghana.

Study objectives

1. To determine the efficacy of SP by a trial in asymptomatic yet parasitaemic pregnant women.
2. To determine the peripheral and placental blood prevalence of malaria parasites in delivering mothers.
3. To determine the specific mutation of *Plasmodium falciparum* in peripheral and placental blood samples.
4. To measure birth weight of babies and correlate it with placental parasitaemia, IPTp-SP usage and maternal anaemia

5. To determine the relationship between the number of SP doses and peripheral and placental parasitaemia in delivering mothers.
6. To determine the prevalence of the mutations in *dhfr* and *dhps* genes of *Plasmodium falciparum* in parasitaemic pregnant women involved in study and in outpatient attendees with malaria infection.
7. To determine the quality of the SP tablets in use for IPTp by determining the concentration of the active principle and solubility of the tablets.

Procedures

To enable the successful completion of this research you are being invited to take part in this research by providing us answers to some specific questions. If you accept, you will be required to fill out a survey which will be provided by the principal investigator, Dr. Leslie Larry Afutu. If you do not wish to answer any of the questions included in the survey, you may skip them and move on to the next page. The study is a four-part one and is expected to last for to last six months. The parts are; the efficacy study, the birth outcome study, the SP molecular surveillance study, the SP concentration study and the drug quality study. A routine finger prick for capillary blood would be done to determine parasitaemic yet asymptomatic patients who would be enrolled into the study. Two millilitres of venous blood would be taken from enrolled participants. This would be used for efficacy study, molecular surveillance study and the SP concentration study. For the birth outcome study, 2mls of blood would be taken from both the peripheral veins and the placenta villi of delivering women.

After using samples for the index study, what remains would be stored for about 6-12 months during which time they may be used for related studies. Left-over specimens, would, afterwards, be disposed by burning them in an incinerator or by deep burying.For the efficacy study, participants would be followed on days 3, 7, 14, 21, 28 and 42.

Possible risks and discomforts

Though taking of peripheral venous sample may be a source of discomfort, the discomfort is temporal. There is no physical risk to patients since syringes to be used would be sterile ones.

Possible benefits

You may not have a direct benefit from the research, but your contributions to this research will serve as a basis to influence the possible review of the policy of intermittent preventive treatment with sulphadoxine-pyrimethamine.

Voluntary participation and right to leave the research

You are being asked to take part in this research by volunteering to be a subject. You are to understand that:

- Taking part in this research is entirely voluntary.
- You may refuse to take part or withdraw from the study at any time without anyone objecting and without penalty or loss of any benefit to which you are otherwise entitled to.

Confidentiality

All information collected for this study will be kept strictly confidential and will not be shown to anyone. We will not release any information about you to anyone.

Contacts for additional information

Further information pertaining to this research can be obtained from the supervisors, Prof. Nyarko Boampong on the telephone number 0208154078 and Prof. Neils Quashie on telephone number 0545507575 or Dr. Leslie Larry Afutu on 0208389750.

Your rights as a participant

This research has been reviewed and approved by the Institutional Review Board of University of Cape Coast (UCC-IRB). If you have any questions about your rights as participants, you can contact that IRB office between the hours of 8:00am and 4:30pm through the landlines 0332135351/0289670793 (4) or email address: irb@ucc.edu.gh. You may also contact the chairman, Prof. Albert A. Addo-Quaye through the number 0243-189593 when necessary.

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research title: *"Evaluation of the effectiveness of sulphadoxine-pyrimethamine as intermittent preventive treatment of malaria in pregnancy in selected health facilities in Sekondi-Takoradi, Ghana"*.has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

....

Date Name and signature or mark of volunteer

If volunteers cannot read the form themselves, a witness must sign here: I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

....

Date Name and signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

....

Date Name and Signature of Person Who Obtained Consent

APPENDIX-C

UNIVERSITY OF CAPE COAST

DEPARTMENT OF HUMAN BIOLOGY

PhD PARASITOLOGY RESEARCH.

RESEARCH QUESTIONNAIRE

TITLE: Evaluation of the Effectiveness of Sulphadoxine-Pyrimethamine as intermittent preventive treatment of malaria in pregnancy in selected health facilities in Sekondi-Takoradi, Ghana.

Questionnaire

1. Code: ..... 2. Age: .....

3. Weight (kg)..... 4. Height (m).....

5. Axillary Temperature.....

6. Locality.....

7. Highest educational qualification: 1. Tertiary.....

2. Secondary.....

3. Basic.....

4. Others (specify).....

8. Occupation.....

9. Partner's occupation.....

10. Partner's level of education.....

11. Gestational Age..... (Using LMP)

12. Parity.....

13. Date of last delivery (if applicable) .....

14. Do you have any history of neonatal or perinatal death? Yes or No.

15. What do you think was the cause of death? .....

16. Do you have any history of low birth weight? Yes or No.

17. Any history of prematurity? Yes or No.

18. What malaria control measure are you using currently?

a. Insecticide Treated Nets.....

b. Indoor Residual spraying.....

c. Others (specify).....

19. Have taken any antimalarial in the last 4 weeks? Yes or No

20. Have you taken any sulphur containing drugs (eg. co-trimoxazole) in the last 4 weeks? Yes or No

21. Have you had any blood transfusion in the last 4 weeks? Yes or No

22. What is your G6PD status? a. No defect.....

b. Partial defect.....

c. full defect.....

(To be confirmed from ANC records booklet).

23. HIV status? ..... (To be confirmed from ANC records.)

THANK YOU

APPENDIX– D

PREPARATION OF REAGENTS

Preparation of 0.5M EDTA solution at pH 8.0

1. Weigh 186.1g disodium ethylenediamine tetraacetate 2H2O into 800 ml of deionized water.
2. Stir the solution vigorously using a magnetic stirrer.
3. Add NaOH solution to adjust the pH to 8.0.
4. Adjust the volume to 1L with deionized water.
5. Filter the solution through a 0.5 micron filter.
6. Sterilize solution in an autoclave.
7. Store at room temperature for further use.

Preparation of 1 litre of 10X TAE Running Buffer, pH 8

1. Dissolve 48.5g Tris (hydroxymethyl amino methane) in about 800 mL of deionized water.
2. Add 11.4 mL glacial acetic acid and 2 mL 0.5M EDTA
3. Add deionized water to 1L.
4. Store at room temperature.
5. Dilute stock solution 10:1 to make a 1X working solution.

APPENDIX E

Chelex DNA Extraction

Materials for DNA extraction using Chelex Method.

Dried, blood-blotted filter papers; 10% saponin in water (stored at -20°C); 1XPBS (Calcium and Magnesium Free pH7.4); 20% Chelex (stored at room temperature); a heat block at 95°C

Materials: Dried, blood-blotted filter papers; 10% saponin in water (stored at -20°C); 1XPBS (Calcium and Magnesium Free pH7.4); 20% Chelex (stored at room temperature); a heat block at 95°C

1. Cut the filter paper to appropriate size using a scissors or hole puncher. The same blade or hole puncher can be used after wiping with an alcohol swab.

2. Combine the dried, blood blotted filter papers with 1ml of PBS and 50ul of 10% saponin in a 1.5 ml Microfuge tube, invert several times, and store over night at 4°C.

3. Microfuge tubes for 5 seconds and aspirate the now reddish PBS/saponin from the tubes with a clean non-barrier tip attached to a Pasteur pipette at the end of a vacuum assembly using a new tip for each tube.

4. Add 1 ml of PBS/tube (no saponin), invert several times and incubate at 4 °C for 15-30min. Turn on the heat block at this time to allow time for 95 °C to be reached.

5. Microfuge and aspirate (as above) as much fluid as possible and afterwards use the tip to press the filter paper down into the lower third of the tube without packing it excessively.

6. Transfer 1ml of vortexed chelex stock solution to a 1.5 ml microfuge tube and using a tip with tapered end cut off. Dispense 50 ul to each sample,

vortexing or inverting the tube every two or three transfers (to be sure you are not just transferring water with all the chelex settled to the bottom of the dispensing tube).

7. Add 100ul of sterile water to each tube.

8. Extract the parasite DNA by incubating the tubes for 10 min in a 95 °C heat-block, vigorously vortexing each sample every 2 minutes or so throughout the incubation. It is best to briefly uncap each tube every two minutes or so in the block to release pressure, or else the tubes will "pop"

9. After incubation, microfuge the tubes for 5 minutes at high speed. Meanwhile, label two sets of 0.6mL microfuge tubes for transfer, the second set for final storage of the extracted DNA samples.

10. Transfer as much solution as possible from the spun tubes to the first set of microtubes with a 200uL barrier-tip, not worrying if chelex is carried over as well.

11. Spin tubes for 10 minutes at high speed and then transfer the final, white-to-yellowish supernatant (avoiding the pelleted chelex) to the final set of labeled tubes. Store at -20C.

APPENDIX – F

LABORATORY PHOTO GALLERY

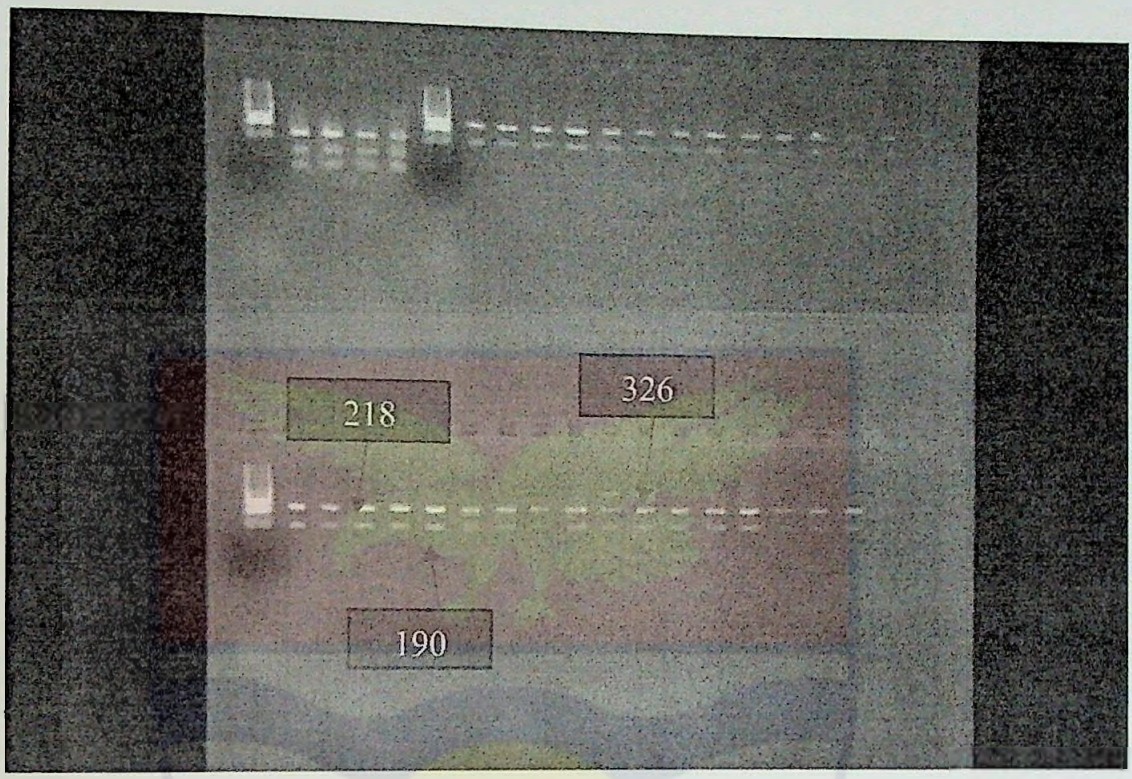

PLATE A: Ethidium Bromide-stained agarose gel showing fragments after PCR-RFLP at codon 108 of the *dhfr* gene with the enzyme, BsraI.

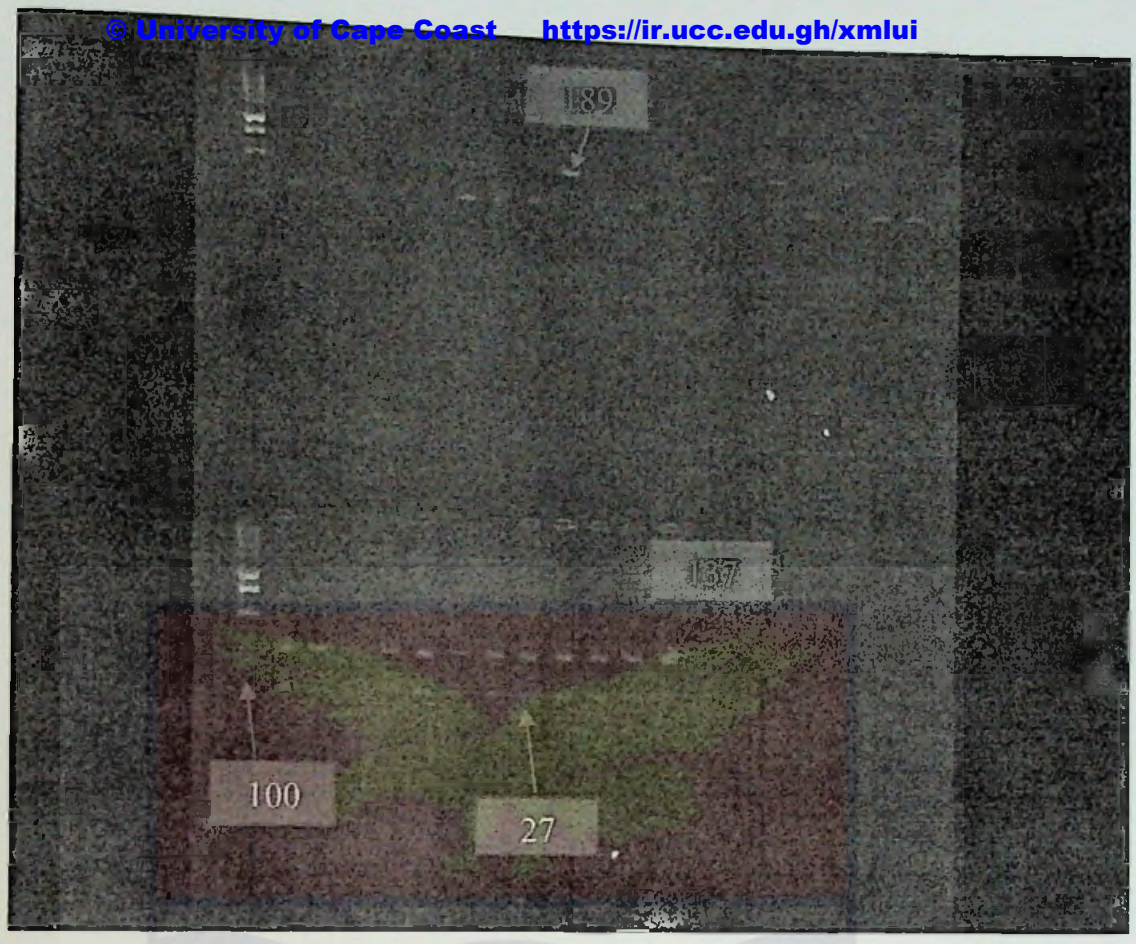

PLATE B: Ethidium Bromide-stained agarose gel showing DNA fragments after PCR-RFLP at codon 59 of the *dhfr* gene with the enzyme, XmnI

PLATE C: Ethidium Bromide-stained agarose gel showing DNA fragments after PCR-RFLP at codon 51 of the *dhfr* gene with the enzyme, Tsp5091

PLATE C: Ethidium Bromide-stained agarose gel showing DNA fragments after PCR-RFLP at codon 437 of the *dhps* gene with the enzyme, MwoI

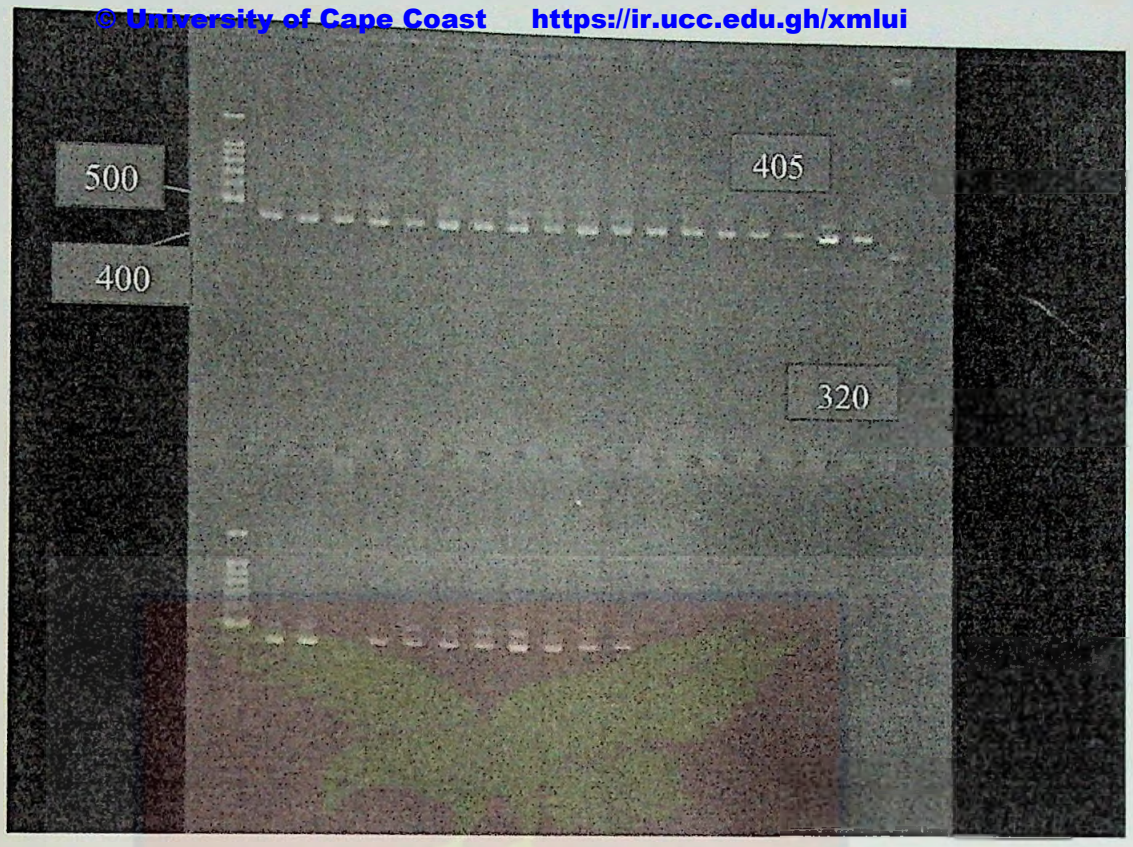

PLATE C: Ethidium Bromide-stained agarose gel showing DNA fragments after PCR-RFLP at codon 540 of the *dhps* gene with the enzyme FokI.