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MALARIA IN EARLY INFANCY: EFFECT OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTp) AND MATERNALLY TRANSFERRED ANTIBODIES IN THE KASSENA-NANKANA DISTRICT.

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DECLARATION

I hereby declare that except for references to other people's work, which have been duly acknowledged, this thesis is based on secondary data derived from a primary study I helped to conduct at the Navrongo Health Research Centre, Navrongo and under the supervision of Prof. Fred Binka and Prof. Daniel Dodoo and neither all nor part of this thesis has been presented for another degree elsewhere.

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DEDICATION

To my parents Robert & Janet Atuguba (of blessed memory)-I wonder what their comments would have been.

To Uncle Nicho & Adam of blessed memory

To my family & malaria prone infants

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ABSTRACT

Background

Beneficial effects of Intermittent Preventive Treatment in pregnancy (IPTP-SP) on the mother and birth outcomes are known. The few studies on the effects of IPTp-SP beyond *in utero* have been contradictory and tended to focus on late infancy and beyond. Mechanisms and targets explaining effects of IPTp-SP beyond *in utero* remain largely unknown. This study determined if IPTp-SP use was associated with risk of malaria and all cause mortality in early infancy and if maternally transferred antibody levels (Total IgG) to malarial antigens could explain observed IPTp-SP effects or were independently associated with the aforementioned outcomes.

Methods

This study was undertaken by conducting secondary analyses of the whole data from a five year cohort of 2279 newborns enrolled between 2006 and 2007 in the Kassena-Nankana Districts. Analysis was restricted to early infancy. Survival techniques (Cox Proportional hazards and Kaplan-Meier plots) were used to estimate frequency of IPT-SP doses and risk of malaria and all cause mortality. ELISA assays performed on a subset of the cohort (672), measured total IgG antibody titres to selected malaria antigens and frequency of IPTp-SP doses. Analyses of variance (ANOVA), simple regression techniques and box plots were employed to determine associations between antibody titres and frequency of IPTp-SP doses. IPTp-SP dosing frequency, Antibody types and risk of malaria and all cause mortality were also measured. Statistical significance was set at 5% with 95% confidence interval.

Results

Infants of mothers who had one dose of IPTp-SP had 44% less risk of parasitaemia [Hazards Ratio (HR) =0.60(95%CI= 0.45, 0.79), P<001], 41% less riskof uncomplicated malaria, [HR=0.59(95%CI =0.40, 0.86), P=005], and 41% less risk of severe malaria

[HR=0.58(95%CI =0.41, 0.80), P<0.001]. Higher dosing frequencies of IPTp-SP did not significantly impact risk of malaria in early infancy, compared to none. None of the dosing frequencies of IPTp-SP was significantly associated with a decrease in all cause mortality. Antibody titres to malarial antigens (MSP3, N-MSP3, C-MSP3, GLURP-R0, GLURP-R2, and GMZ2) did not differ with frequency of IPTp-SP dosing. . Antibodies to GLURP-R2 were associated with less risk of parasitaemia [HR=0.65 (95%CI =0.50,0 .84), P=001], uncomplicated malaria [HR=0.66 (95%CI =0.45,0.95), P=0.026] and severe malaria [HR=0.66 (95%CI= 0.49,0.90), P=0.009] . Antibodies to N-MSP3 and C-MSP3 were associated with less risk of uncomplicated malaria [HR=0.76 (95%CI= 0.62, 0.93), P=0.007] and [HR=0.64(95%CI= 0.46, 0.90), P=0.009], and respectively. Antibodies to MSP3 were associated with less risk of severe malaria [HR=0.80, (95%CI =0.67, 0.96), P=0.016].

Conclusions

IPTp-SP beyond *in utero* reduces risk of malaria in early infancy in the KNDs but dosing frequency of IPTp-SP does not significantly alter titres of maternally transferred antibodies (total IgG) to the malaria antigens MSP3, N-MSP3, C-MSP3, GLURP-R0, GLURP-R2 and GMZ2. However, total IgG to N-MSP3, C-MSP3 and GLURP-R2 are independently associated with less risk of malaria in early infancy.

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LIST OF ABBREVIATIONS

ADCI:	Antibody dependent cellular immunity
AU:	Arbitrary units
BCR:	B cell receptor
CSP:	Circumsporozoite protein
CD4:	Cluster of differentiation 4
CD8:	Cluster of differentiation 8
CM:	Cerebral malaria
C-MSP:	Carboxyl terminal of merozoite surface protein
CTL:	Cytotoxic lymphocyte
ELISA:	Enzyme linked Immunosorbent Assay
ERC:	Ethical Review Committee
G6PD:	Glucose 6 phosphate dehydrogenase
GHS:	Ghana Health Service
GLURP R0:	Glutamine Rich protein region 0
GLURP R2:	Glutamine Rich protein region 2
IgA:	Immunoglobulin A
IgD:	Immunoglobulin D
IgE:	Immunoglobulin E
IgG:	Immunoglobulin G
IgM:	Immunoglobulin M
IPT:	Intermittent preventive treatment
IPTc:	Intermittent preventive treatment in children
IPTi:	Intermittent preventive treatment in infancy
IPTp:	Intermittent preventive treatment in pregnancy

IPTp-SP:	Intermittent Preventive Treatment in pregnancy using Sulphadoxine-Pyrimethamine
IRB:	Institutional Review Committee
KND:	Kassena-Nankana District
LAMP:	Loop Mediated Amplification Test
MHC:	Membrane Histocompatibility Complex
MSP:	Merozoite Surface Protein
NAAT:	Nucleic Acid Amplification Tests
NHRC:	Navrongo Health Research Centre
NMIMR:	Noguchi Memorial Institute for Medical Research
NMSP:	Amino terminal of merozoite surface protein
OD:	Optical Density
PBS:	Phosphate Buffered Saline
PCR:	Polymerase chain reaction
QT-NASBA:	Quantitative nucleic acid sequence-based amplification
RDT:	Rapid Diagnostic Test
SMA:	Severe Malarial Anaenia
SP:	Sulphadoxine-Pyrimethamine
WHO:	World Health Organization
1^0:	Primary
2^0:	Secondary

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The incidence of malaria has been on the decline globally since 2001 (Griffin et al., 2016; Bhatt et al., 2015). Incidence declined by 21% both worldwide and in the African Region. In the same time frame, death rates due to malaria declined by about 29% and 31% globally and in the African Region respectively (WHO, 2016a). This has been mainly attributed to increased coverage of effective malaria interventions (insecticide treated nets, indoor residual spraying, intermittent preventive treatment, case management). The proportion of women receiving the recommended 3 or more doses of intermittent preventive treatment increased five-fold in 20 African countries from 6% in 2010 to 31% in 2015. Compared to 30% in 2010, over half (53%) of the population at risk in sub-Saharan Africa slept under a treated net in 2015. The proportion of the population at risk of malaria protected by indoor residual spraying (IRS) reduced from a height of 5.7% globally in 2010 to 3.1% in 2015. The proportion of febrile children who sought care at a public health facility in 22 African countries and who received a malaria diagnostic test rose from 29% in 2010 to 51% in 2015. Between 2010 and 2015, the malaria mortality rate among children under 5 declined by an estimated 35%. Revelations are that children and pregnant women in Africa south of the sahara have greater access to effective malaria control (WHO, 2016b).

Despite these impressive results, malaria remains a serious public health problem, especially in sub-Saharan Africa. About 3.2 billion people remain at risk of malaria the world over (WHO, 2016b). Per the WHO 2016 report, there were 212 million new cases

of malaria and 429,000 deaths worldwide in 2015. The WHO African Region accounted for the majority of cases worldwide (90%). The more vulnerable groups to malaria are children under 5 years, pregnant women, non-immune travelers and refugees/displaced persons entering into malaria endemic areas. Children under 5 years are especially vulnerable to malaria infection, illness and death. In 2015, malaria killed an estimated 303 000 under-fives worldwide. Of that number 80% was in the African Region (WHO, 2016b). This translates to claiming the life of 1 child every 2 minutes. Nearly 85% of malaria is caused by *Plasmodium falciparum* accounting for about 90% of deaths (‘WHO, 2015).

In several countries, progress in the fight against malaria is threatened by the development and spread of mosquito resistance to insecticides and parasite resistance to antimalarial medicines. Considerable gaps in the coverage of principal malaria control tools remain; health systems are under-resourced and poorly accessible to those most at risk of malaria. At the 2015 World Health Assembly, member states adopted the *Global Technical Strategy for Malaria 2016-2030* with a renewed commitment to fighting the malaria scourge. The World Health Organization (WHO) recommends a combination of early diagnosis and effective treatment, use of long lasting insecticide treated nets, indoor residual spraying for vector control, environmental management, increased public health advocacy and in endemic areas intermittent preventive treatment (IPT), as a combined strategy for malaria control. The RTS,S malaria vaccine might be added to the armamentarium in the foreseeable future (Gosling & Seidlein, 2016; Cibulskis et al., 2016; WHO, 2016b; Mbachu et al., 2012).

In Ghana, malaria remains a leading cause of illness and death accounting for some 38% of outpatient clinic visits and 27% hospital admissions (Nonvignon et al., 2016). It

accounts for 48.5% of admission in children under five years and 25% of deaths (NMCP, 2017).

In spite of the shifts in populations at risk for malaria (Cotter et al., 2013) and the fact that high transmission shifts clinical malaria towards younger age groups irrespective of seasonality (Cotter et al., 2013; Carneiro et al., 2010), the effects of malaria in early infancy (the first six months of exta uterine life) have been largely ignored. The explanation often given is that malaria is rare in this age-group as a result of protection from maternally transferred antibodies and presence of foetal Haemoglobin (Ceesay et al., 2015). However recent evidence suggests otherwise; infection rates of up to 21.7% have been reported in this age group in Guinea (Ceesay et al., 2015). In a birth cohort study in Burkina Faso 20% of infants experienced their first clinical episode of malaria before 6 months of age (Natama et al., 2018). Infection rates of up to 13.6% have been recorded in Ghanaian children under six months of age (Wagner et al., 1998).

Malaria in early infancy (the first six months of extra uterine life) is not uncommon in the Kassena-Nankana District where the primary study on which this thesis (secondary study) is based was carried out. Indeed, in the primary cohort study from which this thesis (secondary study) derives, parasite rates of about 16% in early infancy were found. Malaria parasite prevalence increased with age in the first six months of extra uterine life (early infancy) and could be ascribed to waning of the much touted maternally transferred antibodies (appendix Ai). Incidence rate per person-day (IR) of slide confirmed uncomplicated malaria was 1.58 [95%CI = (1.42, 1.76)] (data from the primary study).

There is scanty evidence that in addition to reducing preterm delivery, low birth weight, maternal malaria and anaemia, intermittent preventive treatment of malaria in pregnancy with sulphadoxine-pyrimethamine (IPTp-SP), affects susceptibility to malaria and all cause mortality in infants of these mothers (Eisele, Larsen, & Steketee, 2010).

Beneficial effects of SP in IPTp beyond birth on infancy, have been reported in the few studies carried out; ranging from a reduction in all cause neonatal mortality, in trial as well as Programme conditions (Eisele et al., 2012a; Menéndez et al., 2010a), through reduced infection rates in infancy (Le Port et al., 2011) to decreased risk of parasitaemia, uncomplicated malaria and severe malaria (Atuguba, 2012) [Appendices tables Aiii, Aiv Av & figures Ai, Aii and Aiii]. On the other hand, other studies have reported increased susceptibility to infections and to severe malaria.

As the benefits of IPTp-SP *in utero* are reported to occur in a dose frequency dependent manner (Chico et al., 2017), it is reasonable to suspect that, same applies as regards its benefits or otherwise in early infancy (Harrington et al., 2013). The harmful effects of malaria in their offspring, is said to be triggered by heavy parasitization and a buildup of infected red blood cells in the placentae of pregnant women (Avril et al., 2012). SP's mechanism of action, is to competitively inhibit enzymes involved in the synthesis of malaria parasite and other pathogen cell wall constituents (Yaro, 2009). As a result of the disruption in the synthesis of parasite cell wall constituents, parasite growth is arrested. SP by this mechanism clears peripheral and placental malaria parasitaemia and prevents reinfection (Radeva-Petrova et al., 2014).

On the one hand, the clearance of parasites from the placental bed, can reduce inflammatory processes and lead to an improvement of materno-foetal exchanges across the placenta (including antibody transfer) (Gutman & Slutsker, 2017; Malek, Sager, & Schneider, 1998; Walter, Garin, & Blot, 1982). The aforementioned mechanism could explain the lower placental and infant infection rates that Le port and his colleagues (2011) have alluded to. On the other hand, clearance of malaria parasites by SP, could lead to increased antigenaemia peripherally and in the foeto-placental circulation. Antigens could then cross over to the foetus, leading to priming and immune tolerance (Broen et al.,

2007a; Duffy, 2007). Infants or offspring of such mothers display a “tolerant” phenotype and the risk of malaria in infancy in these offspring maybe increased (Apinjoh et al., 2015).

Similarly, clearance of parasites from the peripheral circulation by SP could result in two scenarios: maternal antibody production could actually fall as a consequence of reduced exposure to antigen. By extension, there will be less antibody production by mother and less transfer to the foetus (Staalsoe et al., 2004). This could then result in increased risk of malaria in infancy. However, in a recently published cohort study in coastal Ghana, looking at antibody levels in both mothers and their infants, IPTp-SP did not appear to influence the magnitude of transplacental transfer of antibodies even though it might decrease exposure to malaria (Stephens et al., 2017a). Conversely, increased peripheral antigenaemia from malaria parasite demise could stimulate an increased production of maternal antibodies and subsequently, transfer of same to the foetus. It has been reported that, maternal antibody levels influence transplacental antibody transfer (Palmeira et al., 2012). In this case, risk of malaria in the foetus will be expected to be less and a protective effect as seen in the primary study will be observed (appendix tables Aii, Aiv, Av). Taken together, the protective effect of SP on risk of malaria seen in this cohort, may be explained by suggesting that, antigenaemia resulting from the process of parasite clearance from the periphery, stimulates more maternal antibody production; clearance of parasites from the placental bed by SP results in improved foeto-maternal exchanges. Consequently, there is increased antibody transfer to the foetus that subsequently reduces the risk of malaria in early infancy.

Greater understanding of the targets and mechanisms may throw more light on and give insights into novel strategies for malaria control, especially as the malaria control agenda increasingly enters elimination and eradication mode (Alonso et al., 2011)

1.2 Problem Statement

A lot of immuno-epidemiological studies often ignore the first 6 months of extra uterine life (early infancy). The explanation often advanced is that malaria is rare in this age-group but recent evidence suggests otherwise (Natama et al., 2018; Ceesay et al., 2015).

Malaria in the Kassena-Nankana Districts (KNDs) is intense, especially in the high transmission season, such that infants get infected and do come down with the disease (Oduro et al., 2010, 2007; Koram et al., 2003; Baird et al., 2002; Binka et al., 1995). Control strategies have been anchored on early diagnosis and prompt treatment. This may not be available to rural populations where most early infants may be far away from health facilities. Even where health facilities are available, limited capacity in the rural areas (both clinical and laboratory) to diagnose malaria (Hailegiorgis et al., 2010; Ishengoma, Derua, & Rwegoshora, 2010) may result in inaccurate and misdiagnoses challenges, with the attendant waste of resources. This adds to the cycle of poverty in resource poor areas, such as the KNDs (Bell et al., 2016). IPTp-SP, given usually to protect pregnant women and their unborn fetuses, may lower the risk of malaria in early infancy (Menéndez et al., 2010b), in which case it will be a useful adjunct to current strategies for protecting the infant in his /her early life. However, studies to the contrary have also been reported (Harrington et al., 2013). In the midst of contradictory evidence on the benefits of IPTp-SP beyond foetal life, few studies looking exclusively at the effect of IPTp-SP beyond *in utero* have been carried out in country and none in the study area.Yet fewer studies, have attempted to elucidate targets and mechanisms of action of IPTp-SP, in early infancy, in the country (Stephens et al., 2017a). The specific role of protective maternal antibodies remains a question for additional investigation (Moya-Alvarez, Abellana, & Cot, 2014a). In the midst of contradictory evidence about the benefits or otherwise of IPTp-SP on the risk of malaria in early infancy, this secondary study seeks to demonstrate how the use of IPTP-SP impacts on the magnitude of maternally transferred antibodies in their offspring

(early infants) and to establish if IPTp-SP and these antibodies are associated with lower risk of malaria and all-cause mortality in early infancy in the Kassena-Nankana Districts (KNDs). This study also sought to adduce evidence as regards mechanism of action of IPTp-SP beyond life *in utero*.

1.3 Conceptual framework

1.3.1 Relationship between extrinsic and intrinsic factors influencing Risk of malaria in humans

Factors that may affect the risk of malaria in early infancy, maybe divided broadly into intrinsic and extrinsic factors. Intrinsic factors include those pertaining to the host (human), the parasite (*Plasmodium sp*) and the vector (female anophiline mosquito)-biology. The extrinsic or external factors include environmental factors, control and prevention measures, as well as social, behavioural, economic and political factors (Breman, 2001). Maternal factors such as age, educational level, parity, nutritional state, bednet use, IPTp-SP (Port et al., 2012) and genetic factors such as blood type O, or presence of variant Haemoglobin alleles such as haemoglobins S and C, have been reported to have a protective effect on malaria risk (Amaratunga et al., 2011). Glucose 6-phospate dehydrogenase enzyme (G6PD) deficiency may influence maternal susceptibility to malaria (Manjurano et al., 2015). Taken together these factors may impact on the quality and magnitude of maternal antibody transfer to the infant. Similarly, environmental factors such as transmission season, residence, location, indoor residual spraying (IRS) may act through degree of exposure to infective bites and influence maternal antibody production and transfer to the infant (Port et al., 2012). Similarly, behavioural patterns that expose mother and infant to mosquito bites, could modify their exposure, experience and development of immunity. Social, behavioural, economic, educational status and political factors (individual, household & health system) could conspire to influence access to IPTp-SP by mother. Drug resistance could also potentially influence the risk of malaria in

infancy, by selecting for resistant strains in the placenta and ultimately influence the infant responses to malaria (Harrington et al., 2009). Difficulty in clearing these resistant or tolerant parasitaemia may lead to accumulation in the placental bed. Such a development can impair transplacental exchanges, including antibody transfer. The accumulation of parasites and parasite products in the placental bed, can increase the chances of immune priming of the foetus (Metenou et al., 2007). The risk of immunological tolerance and neonatal parasitaemia may then be increased. The phenomena of immunological tolerance and neonatal parasitaemia are associated with increased risk of clinical malaria in infancy (Moya-Alvarez et al., 2014a). For the infant, use of bednets (behaviour), their season of birth, residence and location (environment) could impact on their risk of malaria. Infant's age, maturity at birth, (biology) mother's IPTp-SP use, and nutritional status (behaviour) could potentially affect the quality and magnitude of maternally transferred antibodies and ultimately impact on infants' risk of malaria (Kampmann & Jones, 2015; Dobaño et al., 2012). Infant (host) genetic factors (biology) can also potentially affect the risk of malaria in infancy; blood group O, Haemoglobin S, C as well as G6PD deficiency alleles (biology) are reported to have protective effects on risk of malaria (Amaratunga et al., 2011). From the foregoing, the contextual framework is illustrated schematically in figure 1 below.

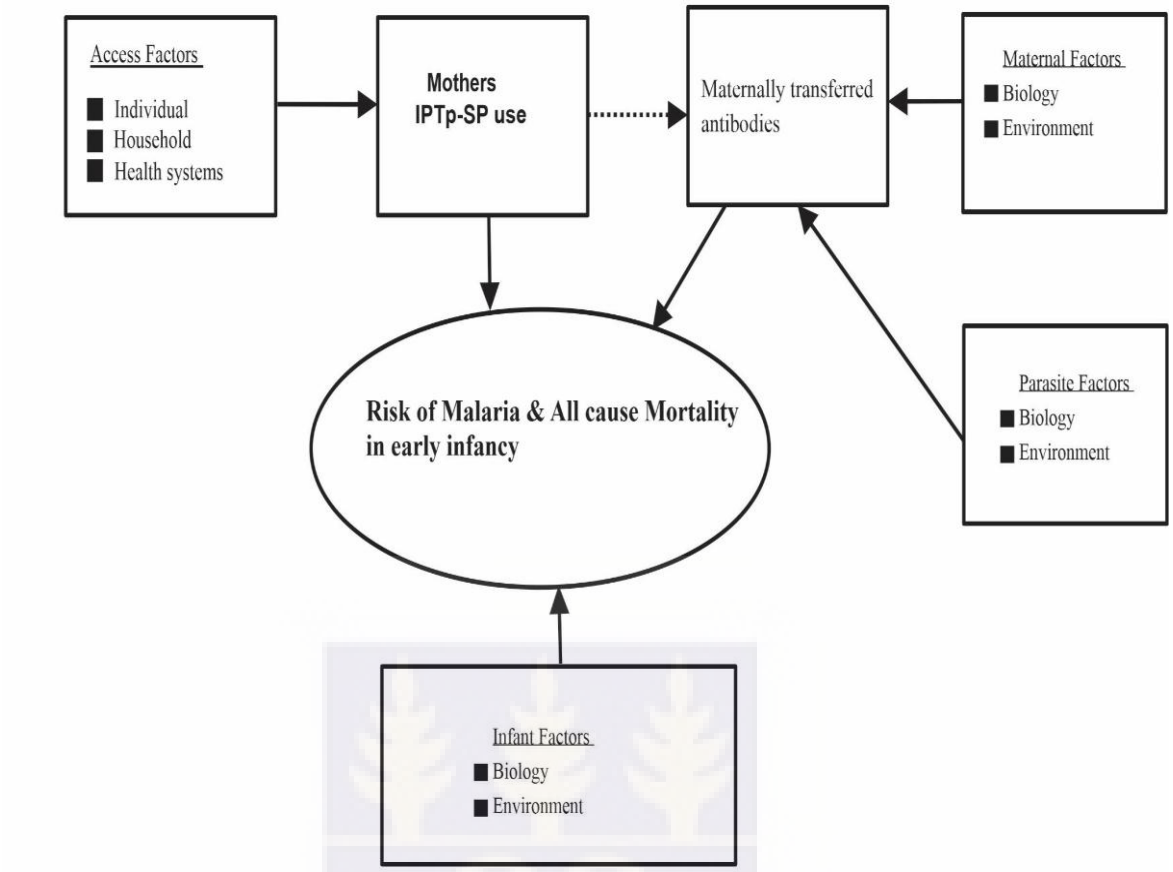


Figure 1: Showing the conceptual framework

1.4 Justification

In light of the renewed call for use of 'granular'data for targeted interventions, there is the need to generate evidence for adjunct interventions for malaria morbidity & mortality. Also with the re-kindling of interest in malaria elimination, early infants as a parasite reservoir is increasingly important for targeted interventions.

The evidence generated on the role of these maternally transferred antibodies, will find application in the evaluation of malaria vaccines such as the RTS-S. This the study will do by generating information on potential confounders and effect modifiers.Additionally the study will throw more light on important malaria antigens of protective and exposure value. This study may also contribute to our understanding of and potentially generate novel ideas for new drug development by homing in on important malaria antigens that

pharmacologic agents could target. To not investigate this phenomenon further, is to rob policy makers of evidence required to support the continued use of IPT-SP even in the face of SP resistance as an adjunct tool to reduce morbidity and mortality in early infants. .

1.5 Research Questions

1. What is the association between mothers' IPTp-SP use, maternally transferred antibodies and time to first parasitaemia in their infants (offspring) in the KNDs?

2. What is the association between mothers' IPTp-SP use, maternally transferred antibodies and uncomplicated malaria in their infants (offspring) in the KNDs?

3. What is the association between mothers' IPTp-SP use, maternally transferred antibodies and severe malaria in their infants (offspring) in the KNDs?

4. What is the association between mothers' IPTp-SP use, maternally transferred antibodies and all cause mortality in their infants (offspring) in the KNDs?

5. What are the differences in the magnitudes of maternally transferred antibodies to selected malaria antigens amongst infants (offspring) born to mothers who had IPTp-SP and infants born to mothers who did not in the KNDs?

1.6 Hypothesis

$H_{A:}$ IPTp-SP use increases maternally transferred (transplacental or cord blood) antibody titres and this lowers risks of malaria in early infants (offspring) born to these mothers compared to mothers who had no IPTp-SP.

$H_{0:}$ There is no difference in maternally transferred (transplacental or cord blood) antibody titres in early infants born to mothers who had IPTp-SP compared to mothers who had no IPTp-SP.

1.7 Objectives

1.7.1 General objective

To determine the relationship between IPTp-SP use, maternal malaria antibody transfer to their offspring and risk of malaria and all cause mortality in early infancy in the KNDs.

1.7.2 Specific objectives

1. To determine the association between mothers' IPTp-SP use, maternally transferred antibodies and time to first malaria parasitaemia, in their infants in the KNDs.
2. To determine the association between mothers' IPTp-SP use, maternally transferred antibodies and uncomplicated malaria, in their infants in the KNDs.
3. To determine the association between mothers' IPTp-SP use, maternally transferred antibodies and severe malaria, in their infants in the KNDs.
4. To determine the association between mothers' IPTp-SP use, maternally transferred antibodies and all cause mortality, in their infants in the KNDs.
5. To compare the magnitude of maternally transferred antibodies to selected malaria antigens, amongst infants born to mothers who had IPTp-SP and infants of mothers who did not in the KNDs.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Brief History of Malaria

Malaria is among the oldest of diseases infecting and affecting human forebears in one form or another way back in time. Perhaps, more than any other infectious agent, during our recent evolution, its effect has been the highest (Carter & Mendis, 2002a); this has positioned malaria as the most important parasitic disease of humans, threatening nearly half the world's population in areas with potential for transmission (WHO, 2016b).

For almost 5000 years, malaria seems to have been no strange disease in China. The Nei Ching (The Canon of Medicine) from 4,700 years ago chronicles symptoms, highly suggestive of malaria with a propensity for epidemics (Hoeppli, 1959). Evidence from Sumerian and Egyptian texts dating between 3,500 to 4,000 years back suggest these areas were not spared the malaria scourge (Sargon, 1975; Ebbell, 1937). Vedic and or Hindu scripture contain several references to fevers, some of which in almost all certainty were malaria and strongly suggest malaria had beseeched India some 3,000 years ago. By 500 BC malaria had appeared in Greek writings; coincidental with human pioneering and husbandry (Sargon, 1975; Hippocrates, 1923).

By the second century B.C., malaria announced its presence in Italy. The marshy areas around Rome, Sicily and Sardinia were infamous for summer and autumnal fevers. However, under the affluence of the Roman Empire (circa 50 B.C. to 400 A.D.), using environmental management, malaria was kept at bay for several centuries from the Roman Campagna itself. As the power of the empire ebbed and with the advent of the dark ages, there was a reversal to the status ante - unsafe and largely uninhabitable marshes recognized and dreaded by later generations (Celli, 1977).This development underscores the influence of socio-economic and political stability on malaria control. Indeed, so

associated was malaria with marshy areas that inhabitants incorrectly attributed the cause of the disease to the malodourous air emanating from the marshes hence the name "mal'aria" Italian for "bad air"(Cox, 2010). The Arabian peninsula was not spared and Prophet Mohammad's followers were attacked by an epidemic of 'Yethrib' fever in 622 AD (Snow et al., 2013).

By then, malaria had extended its reach from the Mediterranean as far as Japan. At end 15th century A.D, after sneaking into northern Europe probably during the dark ages, *P. vivax*, *P. falciparum*, and maybe, *P. malariae*, were introduced by Europeans and West Africans into the New World. Malaria reached its global limits sometime in the 19th century, exacting its highest impact on the health of all under its shadow; that was no less than half of the world's population, claiming perhaps every tenth life in its wake. The wellbeing of all who lived within its shadow was greatly threatened.

On the 6th of November 1880, the French physician Alphonse Laveran detected a male gametocyte exflagellating in a blood smear from a French Foreign Legion trooper with malaria stationed in Algeria. The epoch of scientific comprehension of malaria had begun-documenting plasmodia as the cause of malaria (Bruce-Chwatt, 1981). Progress accelerated and while working in Sierra Leone in 1897, the British surgeon, Ronald Ross identified plasmodia oocysts in the guts of mosquitoes fed on parasitaemic birds, implicating mosquitoes in the transmission and as the vector of malaria (Snow et al., 2012). William George McCallum confirmed plasmodia exflagellation as a process of sexual reproduction in 1897 and Batistta Grassi and his colleagues confirmed the anopheline mosquito, as the vector of human malaria in 1900. In 1948 Henry Shortt and Cyril Garnham confirmed the development of malarial parasites in the liver before their *entre* into the blood stream. The presence of the hypnozoite stage in the *Plasmodium* life cycle was demonstrated in 1982 by Wojciech Krotoski (Cox, 2010).

The control of malaria has been dodgy in course of time. That quinine was discovered, is considered one of the luckiest occurrences in medical discoveries of the 17th century. Quinine was the first chemical compound to be used to treat an infectious disease. As bark of the cinchona (quina-quina) tree, it was used to treat malaria in the 1600s, when it was referred to as the "Jesuits' bark," "cardinal's bark," or "sacred bark." These names emanate from its use by Jesuit missionaries, who in turn were inspired by earlier use of the bark by the native Peruvian population to treat febrile ailments (Achan et al., 2011). Quinine was used as the second major way to prevent malaria among non immunes in Africa, after environmental sanitation, commencing before the First World War (Brabin, 2014). Quinine was the backbone of malaria chemotherapy until other effective medications came onstream. Chloroquine, beginning in the 1940s, was extensively used (Achan et al., 2011). Amidst heavy use, chloroquine resistance slowly developed. By the late 1950s chloroquine resistance had appeared in parts of south America and southeast Asia, and by the 1980s almost all areas with falciparum malaria were affected (Moran & Bernard, 1989). With chloroquine failing, quinine was fallen on once more, particularly in the management of severe malaria until the dawn of the Artemisinins.

At the 8th World Health Congress in 1955, malaria was the first human disease penciled for eradication (Nájera, González-Silva, & Alonso, 2011). Global Malaria Eradication Programme (GMEP) was subsequently launched armed with two major tools Chloroquine and dichlorodiphenyltrichloroethane (DDT) (Russell et al., 2013). By 1969, however, the GMEP was on its knees and the World Health Organization (WHO) capitulated and altered the malaria agenda for countries from imminent elimination to indefinite control. For decades, malaria was to be neglected. In 1978 the historian Gordon Harrison lamented of malaria's persistence against the backdrop of spirited efforts to combat it,

> *"Failure so universal, so apparently ineluctable, must be trying to tell us something. The lesson could be of course that we have proved incompetent warriors. It could also be that we have misconstrued the problem"*

Three main factors conspired to explain this failure: (i) parasite resistance to effective antimalarials, (ii) near collapse of vector control programs in developing countries, and (iii) the delay in the development of practical vaccines perhaps as a result of insufficient comprehension of the mechanisms of naturally acquired clinical immunity against plasmodia. Bill and Melinda Gates' call for elimination and eradication has re-ignited interest in and re-established as the long-term policy goal by a consensus decision of the Roll Back Malaria Partnership (RBMP) in 2008 (Smith et al., 2013; Greenwood & Targett, 2011; Harrison, 1978).

2.2 Causative agents and Epidemiology

Malaria is known to be caused in humans naturally, by seven parasites of the genus Plasmodium: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale curtisi, Plasmodium ovale wallikeri, Plasmodium knowlesi and Plasmodium cynomolgi* (Rénia & Goh, 2016). These parasites of the obligate intracellular Apicomplexa family, are transmitted through bites of infected female Anopheline mosquitoes that bite between dusk and dawn. Transmission of malaria parasites can also to a lesser degree be through blood transfusion, vertically (congenital malaria), sharing contaminated needles and organ transplantation (Mace & Arguin, 2017; Owusu-Ofori et al., 2013). Malaria parasites can result in asymptomatic infection in humans with existing immunity, or a range of clinical disease spanning mild to severe disease and death in those lacking appreciable immunity (Crompton et al., 2014). *P. falciparum,* the deadliest, prevails in Africa and causes majority of illness and death (Crompton et al., 2014). However, other parasites like *P. vivax* also lead to appreciable symptomatic disease (Wirth & Alonso, 2017; Rénia & Goh, 2016; Crompton et al., 2014).

P. falciparum is widely distributed in the tropical, subtropical and warm temperate regions of the world, with a preponderance in tropical Africa, South East Asia and the western pacific (Autino et al., 2012; Carter & Mendis, 2002b). *P. malariae* thrives under both tropical and temperate areas with transmission conditions adapted to sparse and mobile human populations and generally exhibits low prevalences (Autino et al., 2012). *P. o. curtisi* and *P. o. wallikeri* are strictly warm-climate parasites, today found commonly throughout Africa but limited in distribution elsewhere-New Guinea, Philippines (Western Pacific). Their transmission is more dependent on denser and less mobile populations (Oguike et al., 2011; Lysenko & Beljaev, 1969). *P. vivax* is the most tenacious survivor of all the malaria species and its transmission is more dependent on denser and less mobile populations. In South America *P.vivax* prevails over *P. falciparum* (Autino, Noris, et al., 2012; Carter & Mendis, 2002b). *P. knowlesi* and *P. cynomolgi* are limited in distribution to certain forested areas in south east Asia (Rénia & Goh, 2016; De Silva, Lau, & Fong, 2014).

The endemicity of malaria was initially described in India using spleen rates and subsequently terms such as hyperendemic (51%-75%), holoendemic (>75%) and mesoendemic (11-50%) and hypoendemic (<10%) were adopted. This classification has been generally abandoned (except for Hyper and holo endemic that still see some use) in favour of the "stable-unstable classification". This classification based on vector indices, makes use of a dynamic model of transmission first developed by Sir Ronald Ross and refined by McDonald (Hay, Smith, & Snow, 2008).

In endemic areas humans are exposed to more than one infective bite per day and repeatedly infected throughout life (Appawu et al., 2004a). This state, with frequent forceful, perennial transmission, is termed *stable malaria*. In such areas in early life because of a lack of effective immunity death and illness reign (Perkins et al., 2011). With

appropriate treatment, uncomplicated falciparum malaria largely resolves. With vital organ dysfunction or when the proportion of infected erythrocytes rises above 3%, death as an outcome rises steeply. Coma is typical of falciparum malaria and even with aggressive management is associated with death rates of about 10- 25% among children but rare in adults in areas of stable transmission (Sahu et al., 2015). Seizures, usually generalized and often recurrent, occur in up to 50% of children with cerebral malaria. Neurological sequelae are rarer in the few adults who suffer cerebral malaria (less than 3%) compared to 10-20% of children- notably those with complications of hypoglycemia, severe malaria anaemia (SMA), recurrent seizures, and deep coma (Birbeck et al., 2010; Caulfield et al., 2004). Anaemia is commonly the norm among young children living in areas with stable transmission (Novelli et al., 2010; Koram et al., 2003), especially where parasite resistance to the recommended drugs exits. However survivors develop a good degree of immunity against the disease in areas of stable transmission and by adulthood exhibit mostly asymptomatic malaria (Barry et al., 2011). In places where transmission is low, irregular, or focal, protective immunity is not acquired and symptomatic disease including cerebral malaria may occur at all ages. This state of affairs is referred to as *unstable malaria* (White et al., 2014). This picture of susceptibility to malaria in all ages is shared with nonimmune from areas where natural malaria transmission is nonexistent.

Epidemics or complex emergency may ensue with environmental, economic, or social upheavals; situations such as heavy downpours or floods following drought or migration of workers or displacement of refugees from a non-malarious to an endemic region. A breakdown in malaria control and prevention services exaggerates epidemic conditions. Places with unstable malaria, such as Ethiopia, northern India, Madagascar, Sri Lanka, semi arid areas in eastern, southern Africa and some sahelian areas of west Africa are epidemic prone (Djimdé et al., 2004; Worrall, Rietveld, & Delacollette, 2004).

Protein-energy and micronutrient deficiencies, in particular zinc and vitamin A, add greatly to the malaria burden (Darling et al., 2017; Crookston et al., 2010). Infections such as HIV infection make pregnant women more prone to severe and frequent malaria attacks (González et al., 2014) and the reverse may also be true of *P. vivax* (Ramsuran et al., 2011).The phenomenon of urban malaria is also making considerable contribution to the malaria burden with up to 28% of the burden in Africa taking place in fast developing urban hubs (Kudom, Mensah, & Agyemang, 2012).

2.3 The Plasmodium life cycle

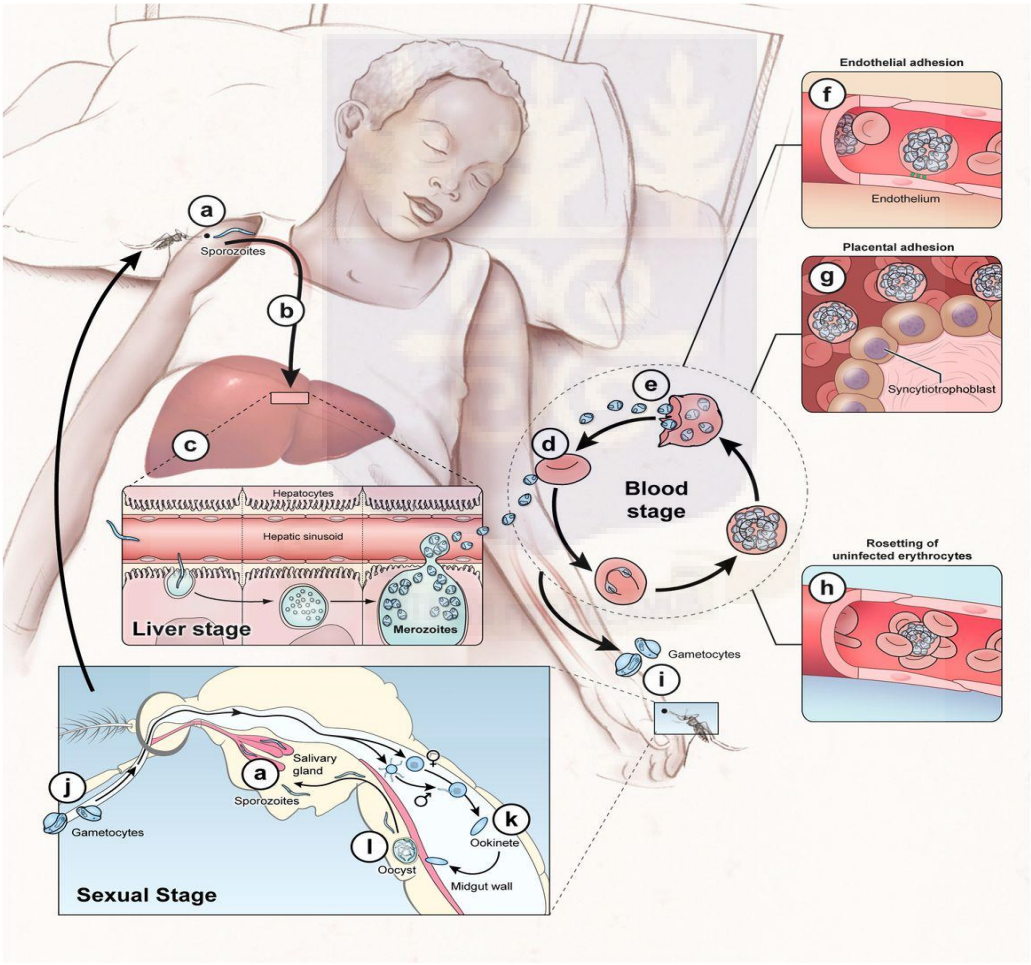


Figure 2 The plasmodium life cycle
Source: (Portugal, Pierce, & Crompton, 2013)

The Plasmodium life cycle in humans involves the asymptomatic liver stage (also called pre-erythrocytic stage), the blood-stage which causes disease and the sexual gametocyte blood-stage which infects mosquitoes that transmit the parasite (Kaddumukasa et al.,

2015) (Figure 2). Infection commences, when a female Anopheles mosquito injects saliva containing sporozoites into the skin and or blood in the process of taking a blood meal (a). Sporozoites may also enter draining lymph nodes from the skin where they are presented by dendritic cells (DCs) and prime CD8+ T cells. The exceedingly motile sporozoites travel to the liver, transverse Kupffer cells (macrophages in the liver) and invade a few liver cells (hepatocytes) (b). In humans, the infection remains clinically quiescent at the liver stage. Each sporozoite infected liver cell (hepatocyte) gives rise to many thousands of asexual parasites called merozoites (c). About a week after liver cell (hepatocyte) invasion, merozoites exit the liver into the bloodstream and commence progressive 48 or 72 hour cycles (d) of red blood cells (RBC) invasion, replication, RBC rupture, and merozoite release (e) according to the species. A few *P. vivax P. ovale wallikeri* and *P. ovale curtisi* sporozoites become non-dividing hypnozoites that remain dormant in liver cells for weeks, months or years before reactivating. Clinical symptoms of malaria, only arise during the blood stage and can commence as early as three days subsequent to the release of merozoites from the liver, which go on to invade the RBCs. Inside the RBCs, the parasite remodels the RBC dramatically and exports variant surface antigens (VSAs) such as *P. falciparum* erythrocyte membrane 1 (PfEMP1) to the RBC surface. VSAs, pose as receptors for a diversity of endothelial cell ligands and enable binding of infected red blood cells (iRBCs) to the microvascular endothelium of various organs (f), letting parasites evade clearance by the spleen. The sequestration of iRBCs in the microvasculature encourages the inflammation and circulatory impedance associated with clinical syndromes of severe malaria, including cerebral malaria and pregnancy-associated malaria with iRBCs in the placenta (g). VSA-mediated rosetting of iRBCs to uninfected RBCs, may also contribute to disease (h). Coincident with the rupture of iRBCs and the release of merozoites and assortment of merozoite products are, inflammation and the clinical symptoms of malaria. Through poorly understood mechanisms, a few trophozoites

differentiate into sexual forms (gametocytes), which are taken up by mosquitos in blood meals. (i) Without treatment, most people with *P. falciparum* malaria will have gametocytaemia within 10–40 days after the onset of parasitaemia. In the mosquito, the gametes fuse, eventually forming sporozoites, that pass into the mosquito salivary gland to complete the life cycle (Arama & Troye-Blomberg, 2014; Crompton et al., 2014; Greenwood & Targett, 2011).

2.4 Pathogenesis

2.4.1 Uncomplicated malaria

Malaria symptomatology, following infection, can only commence in any ill patient with the first liver schizont rupture and release of merozoites into the peripheral circulation-a silent event for the vast majority of patients, who will become clinically ill. As the cycle of red blood cell invasion and re-invasion by merozoites continues, the level of parasitaemia parallels the level of human response (that is fever, C-reactive protein (CRP) and tumor necrosis factor a [TNF-α]), until the patient crosses a threshold of awareness and "feels ill" (Oakley et al., 2011). Uncomplicated malaria is defined as symptoms present (fever) but no clinical or laboratory signs to show severity or vital organ dysfunction (WHO, 2015). Within the human host, during an initial infection, macrophage ingestion of merozoites, ruptured schizonts, or antigen-presenting trophozoites in the circulation or spleen leads to release of TNF-α (Randall et al., 2010). The molecule, along with others in a cascade, is responsible for fever during infection. Other important molecules found during active infection include, interleukin 10 (IL-10) and interferon (IFN-Ɣ) among others (Gun et al., 2014). Some degree of antibody production by the prior macrophage–T-cell–B-cell axis of the immune system confers additional macrophage activity in subsequent infections. This leads to a more efficient clearance of parasites and production of new antibodies in later encounters with the parasite (Hviid, Barfod, & Fowkes, 2015;

Krzych et al., 2012). In the milieu of continuously presented parasite protein repertoire, the host immune system produces additional antibodies and confers additional protection (Kaddumukasa et al., 2015). During each symptomatic episode, uncomplicated malaria is easily treated with antimalarials specific to the parasite and the vast majority of patients make uneventful recoveries when treated appropriately (WHO, 2015).

2.4.2 Severe malaria

The inability to contain the multiplication of the infecting parasites, heralds severe malaria in the host (White et al., 2014; Chan et al., 2012). The adherence or sticking of parasitized red blood cells (RBCs) to other cells, is fundamental to the pathophysiology of severe malaria syndromes such as cerebral malaria, respiratory failure, multiorgan failure, and death (Laishram et al., 2012). Parasitized RBCs, stick to the vasculature via "sequestration," closely imitating inflammatory leukocyte attachment. Half of infected RBC isolates form aggregates capable of blocking microvasculature, which are made up of both infected RBCs bound to each other ("auto agglutinates") and infected RBCs bound to uninfected RBCs ("homotypic RBC rosettes") and or to platelets ("heterotypic RBC rosettes")-Figure 2**(h).** Sequestration and rosette formation impede blood flow, resulting in tissue ischaemia, endothelelial damage and cell demise. It is therefore not surprising that, in vitro rosetting is greater in parasite strains from patients with severe disease, especially in cases of cerebral malaria (Kuadzi, Ankra-Badu, & Addae, 2011).

P. falciparum uniquely causes infected erythrocytes to expresse *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) on their outer surface. PfEMP-1 binds to a diversity of target molecules found on erythrocytes, platelets, vascular endothelial cells-CD36 (platelet glycoprotein IV) and ICAM-1, thus targeting platelets and endothelium. Inflammation, endothelial activation, nitrous oxide (NO) depletion and procoagulant microparticles may contribute to cytoadhesion. Responses from both adaptive and innate immune systems are

readily detected, with the main immune effector being antibodies at this stage (Crompton et al., 2014; Portugal et al., 2013; Smith et al., 2013).

2.4.3 Placental malaria

In placental malaria, infected red blood cells (iRBCs) with mature trophozoites and schizogony gather in the intervillous spaces often times to high densities. Pregnancy associated malaria, maybe accompanied by intervillous infiltrates of monocytes and macrophages, some with hemozoin; Hemozoin (indicating previous infection) maybe present in fibrin deposits. P. *vivax* exibits no such behaviour and show no pathologic changes. Sequestration in the placenta occurs, when infected erythrocytes (IEs) stick to chondroitin sulphate A (CSA) and hyaluronic acid (HA), expressed by syncytiothrophoblast lining the placental intervillous spaces. CSA binding iRBCs unlike other iRBCs adsorb IgM which may encourage sequestration. This is a departure from the mechanism in the non-pregnant states where adhesion is to ICAM-1 and CD36 (Adukpo et al., 2013). Another major difference in placental malaria is that rosette formation (stacking of 2 or more uninfected erythrocytes) plays little role in the pathology as opposed to cerebral malaria. Parasitized erythrocytes express unique variant surface antigens(VSA) principally the VAR2CSA protein. The scantiness of immunity to these pregnancy specific VAR antigens, explains some of the proneness of pregnant women to placental malaria. Placental malaria affects the normal immune balance in the placenta resulting in greater synthesis of inflammatory cytokines like TNFα, IL-2, IFN^{ϋ}. High TNFα is associated with foetal growth restriction, low birth weight (LBW) and anaemia. High IFN^{ϋ} is associated with reduced prevalence of placental malaria. Chemokines produced by monocytes and syncytiotrophoblasts may be important in attracting monocytes to placenta. Infiltrates include a lot of neutrophils and T cells (Djabanor, Quansah, & Asante, 2017; Dong et al., 2012).

2.5 Presentation of malaria

When an individual has been inoculated with a plasmodium parasite, a number of clinical effects may ensue sequentially: infection, asymptomatic parasitaemia, uncomplicated illness, severe malaria and death. Several factors affect the disease manifestations of the infection and the chances of progression to the last two categories, including species of infecting parasite, the levels of immunity of the host, and the timing and efficacy of treatment, if any (Wassmer et al., 2015; WHO, 2012). Flu-like symptoms that can mimic other febrile illness are common to all malaria species, and may include: headache, fever, shivering, joint pain, vomiting, haemolytic anaemia, jaundice, haemoglobin in the urine, retinal damage, and convulsions. The classic symptom of malaria is paroxysms. These paroxysms are cyclical bouts of chills, rigors, then fever and sweating, occurring every two days (tertian fever) in *P. vivax*, *P. ovale wallikeri* and *P ovale curtisi* infections and every three days (quartan fever) for *P. malariae*. For *P. falciparum,* infections can result in recurrent fever every 36– 48 hours (malignant subtertian) or a less marked and almost continuous fever. *P. knowlesi* infections mimic severe *P. malariae* infections. Severe malaria is usually caused by *P. falciparum*. These symptoms of falciparum malaria arise 9-30 days after infection. Individuals with cerebral malaria, frequently exhibit neurological symptoms including: abnormal posturing, nystagmus, conjugate gaze palsy, opisthotonus, seizures, or coma (Enden, 2013; Laishram et al., 2012; Gill & Beeching, 2009).

2.6 Complications of Malaria

There are several grave complications of malaria. Respiratory distress, which occurs in up to 25% of adults and 40% of children with severe *P. falciparum* malaria may develop; possible causes include, respiratory compensation of metabolic acidosis, pulmonary oedema which is non cardiogenic in origin, concomitant pneumonia, and severe anaemia. Acute respiratory distress syndrome may ensue in 5-25% of adults and up to 29% of pregnant women, although it is rare in young children with severe malaria. Malaria maybe

complicated by coinfections such as HIV with attendant increases in mortality. Renal failure is a feature of blackwater fever, where there is haemoglobinuria and associated with *P. malariae* infection. Infection with *P. falciparum* may result in cerebral malaria (Glasgow coma scale less than 9 in adults or Blantyre coma of less than 3 in children and no other cause for the coma) -enlarged spleen(splenomegaly), severe headache, enlarged liver (hepatomegaly), hypoglycemia (blood sugar less than 2.2mmol/l) and hemoglobinuria with renal failure may occur (Trampuz et al., 2003). Malaria in pregnant women can result in stillbirths, infant mortality and low birth weight, particularly in *P. falciparum* infection, but also with *P. vivax* (Rogerson, 2017)

2.7 Diagnosis of malaria

WHO recommends swift malaria diagnosis, either by microscopy or malaria rapid diagnostic test (RDT), in all suspected malaria cases before treatment is started. To confirm clinical malaria, the gold standard is microscopic demonstration of trophozoites in stained peripheral blood smears. The mainstay of malaria diagnosis in most large health establishments is microscopy but the quality of microscopy based diagnosis might pose a challenge (Falade et al., 2016). The sensitivity of blood films ranges from 75-90% in optimum conditions, to as low as 50% depending on the skill of the examiner, as well as parasite concentration on the blood film. Malaria rapid diagnostic tests (RDTs) have the potential to greatly improve the quality of diagnosis, especially in remote areas where access to good quality microscopy services is limited. This is because, RDTs are relatively simple to perform and interpret, require limited training, rapidly provide results and allow for the diagnosis of malaria at the community level (Masanja et al., 2010).

Microscopy and rapid diagnostic tests (RDTs) are the main choices for diagnosing malaria in the field. However, neither method is capable of detecting low density malaria infections (Umbers et al., 2015). Nucleic acid amplification tests (NAATs) obviate this

weakness by allowing the detection of low density infections. Their limit of detection can be as low as <1 parasite/µL. NAATs can be broadly divided into 4 roles: qualitative or quantitative parasite detection, determination of the multiplicity of infection, genotyping to distinguish recrudescence from reinfection, and detection of drug resistance mutations. Polymerase chain reaction (PCR) – including nested, quantitative or real-time reverse transcription (RT-PCR), loop mediated isothermal amplification (LAMP) and quantitative nucleic acid sequence-based amplification (QT-NASBA) are examples of the main NAATs developed to detect malaria. Currently, WHO recommendation is that NAATs be considered only for epidemiological research or surveys. (Tegegne et al., 2017).

2.8 Management of Malaria

Management of malaria hinges on vector control with indoor residual spraying, use of insecticide treated nets and larvicides (Ghosh, Chowdhury, & Chandra, 2012; Okumu & Moore, 2011). In areas of high transmission, Intermittent Preventive Treatment (IPT) is advocated for highly vulnerable populations (pregnant women and children under five years of age) (Wilson et al., 2011). In infants its known as IPTi (Pearce et al., 2013) and in pregnancy IPTp (Tagbor et al., 2015). A variation known as seasonal malaria chemotherapy (SMC), is in the below 5 years age group (Tagbor et al., 2016). Additionally, chemotherapy to manage symptomatic cases is also actively championed, with oral artemisinin combined therapies for uncomplicated cases in areas of high chloroquine resistance. For cases of severe malaria parenteral artemisinins or parenteral quinine are recommended (WHO, 2015).

2.9 Malaria in Ghana

Malaria has been known in Ghana to be a common cause of febrile illness for a long time, even if that knowledge is to varying degrees. Evidence from northern and southern Ghana

supporting this, are in the use interchangeably of names for fever and malaria (Adongo, Kirkwood, & Kendall, 2005; Ahorlu et al., 1997).

Malaria is endemic with year round transmission in all parts of the country with the entire population of 27m at risk (Awine et al., 2017) (figure 4).

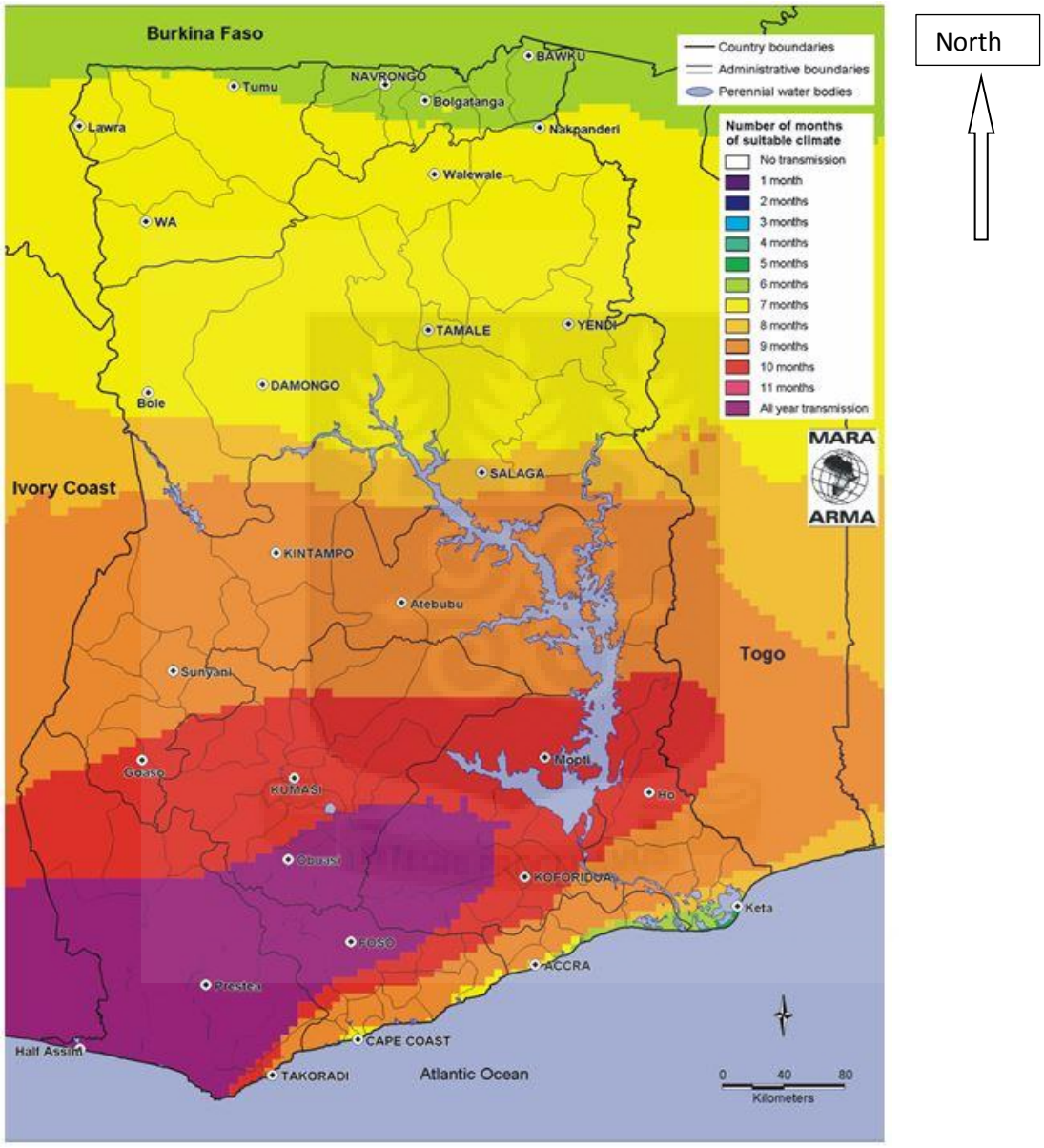


Figure 3 showing malaria epidemiological strata in Ghana
Source:http://www.ghanahealthservice.org/malaria/nmcp-imgs/vector-control-map.JPG

Under-fives and pregnant women are more at risk due to less than optimal immunity (USAID, 2017). Malaria accounts for about 38% of outpatient illness and 27.3% of all admissions (Nonvignon et al., 2016). Out of the over 3 million cases of clinical malaria encountered, nearly 1million occur in underfives with between 3000 and 4000 deaths

(Afoakwah, Nunoo, & Andoh, 2015). Majority of these deaths occur at home. However, in twelve years (2000-2012), case fatalities have dropped from 14.1% to 0.6% (USAID, 2016a). Seasonal variations in transmission of malaria exist in the country, that are increasingly pronounced, the further north one travels; ranging from 6-7 months in the northernmost part of the country (May-October) to 10-11 months in the forest zone; a small peak between May and June and a bigger peak between October and November (Ghana Statistical Service, 2012). *P. falciparum* accounts for up to 90% of all infections *P. malariae* less than 10% and *P. ovale* at less than 1%. Mixed infections of *P. falciparum* and *P. malariae* are not uncommon but *P. vivax* infections have not been recorded in Ghana (USAID, 2017). Major vectors are of the *An. gambiae* species complex and *An. funetus. An. arabiensis* is to be found in the savannah region whilst *An. malas* nestles in the mangrove swamps of the south west part of the country. These vectors have distribution in the rural and peri-urban areas, prefer to bite late at night and are indoor resting. Outdoor biting tends to be common in the northern savannah. Transmission is less intense in large urban centres compared to rural areas (parasite prevalences in 6-59month age group of 6.6% in Accra and Kumasi compared to over 80% in rural areas); this phenomenon holds true even in the same ecological zone (USAID, 2016a). However between 2011 and 2016 there have been changes in prevalence of parasitaemia with most areas that recorded high prevalences seeing appreciable falls and areas with low prevalences chalking marginal increases thus: upper east region 44% to14.5%, Upper West Region 51% to 22.0%, Northern Region from 48% to 24.6%, Brong Ahafo Region from 37% to 22.9%, Ashanti Region from 22% to 15.7%, Eastern Region from 22% to 31.1%, Western region from 36% to 22.8%, Central Region from 32% to 30.2%, Greater Accra region from 4% to 4.9% and Volta region from 17% to 27.3%. Though the boundaries have not been delineated precisely, nevertheless three malaria epidemiological

zones are recognized: the northern savannah, the tropical rain forest and the coastal savannah/mangrove belt (Ghana Statistical Service, 2012, 2016).

In Ghana, malaria may present as asymptomatic, uncomplicated or severe. “Asymptomatic” individuals are otherwise normal individuals who carry parasites in their blood. A person presenting with a history of fever within the preceding 2-3 days, or with fever on examination (axillary temperature 37.5°C or rectal temperature 38.5°C), in the absence of any other cause for the fever will be considered a suspected case of malaria. In the absence of signs of severe disease, a case of suspected malaria confirmed by parasitological investigation is said to be "uncomplicated" malaria.

A person with suspected uncomplicated malaria commonly complains of: fever or a history of fever within the preceding 2-3 days, chills, rigors and or headache. Other complaints may include: Myalgia, nausea and/or vomiting, anorexia, sweating, abdominal pain (especially in children), bitterness in the mouth, irritability and refusal to feed (in infants). At presentation symptoms maybe indistinguishable from other infectious diseases such as pneumonia, meningitis, enteric fever or septicaemia (Maitland, 2015). Malaria is said to be severe, if in addition to any of the symptoms for uncomplicated malaria, the individual has at least one of the following danger signs or laboratory findings: altered consciousness, generalized convulsions (fits) lasting less than 30 minutes; repeated generalized convulsions-2 or more within 24 hours; difficulty in breathing or pulmonary oedema; disseminated intravascular coagulation; marked jaundice; prostration; hyperpyrexia (axillary temperature over 38.5°C); inability to take anything orally; repeated profuse vomiting; circulatory collapse or shock; severe normocytic anaemia; hypoglycemia (blood sugar< 2.2mmol/L); renal impairment (serum creatinine >265umol/l); haemoglobinuria or hyperparasitaemia (250,000 parasites/microlitre). Again,

these signs maybe indistinguishable from other infectious diseases such as pneumonia, meningitis, enteric fever or septicaemia (Maitland, 2015; WHO, 2012; Oduro et al., 2007).

The policy of the National Malaria Control Programme (NMCP), requires that suspected cases of malaria be confirmed before treatment. Diagnosis based solely on clinical grounds is frowned upon. This is in keeping with WHO recommendations (WHO, 2016b). Priority here is to underfives. Diagnosis maybe confirmed with either a rapid diagnostic test or microscopy. The thrust of the NMCP, is to improve quality of microscopy diagnosis in the higher level facilities and scale up use of RDTs in the periphery. Four levels of diagnosis Pertain: community level, primary health care level (CHPS compounds), the secondary and tertiary levels. RDTs are recommended at the community and primary health care levels. A mix of RDTs and microscopy for the health centres and polyclinics and microscopy for the hospitals. Alternate malaria diagnostic tests are quantitative buffy coat (QBC), thin film acridine orange (Kawamoto), immunological tests other than RDTs and Polymerase chain reaction (PCR). These alternate tests, tend to be employed in research as opposed to service settings. (NMCP, 2015; USAID, 2016b).

Malaria control in Ghana began in the 1950s and was aimed to reduce the malaria burden till it was no longer of public health significance. However, it was realised that the disease could not be controlled by the health sector alone. A multi pronged approach had to be pursued with other health related sectors. With this in mind, interventions were fashioned to control this wily foe; residual insecticide spraying against mosquitoes, mass chemoprophylaxis with Pyrimethamine, medicated salt and improvement of drainage system. Inspite of this spirited effort, malaria continued to be the leading cause of ill health in Ghana. The situation was not helped with the collapse of the Global eradication campaign. Malaria was neglected in country and subsequently malaria parasite resistance to chloroquine, the first line drug at the time, developed (Moran & Bernard, 1989;

Neequaye et al., 1986). In 1999 Ghana then committed to the Roll Back Malaria (RBM) initiative. In the process, she developed a strategic framework for the commencement of the rollback malaria initiative.

> *"The Ghana RBM emphasizes the strengthening of health services through multi and inter-sectoral partnerships and making treatment and prevention strategies more widely available".*

Control thus hinges on use of insecticide treated nets (ITNs), indoor residual spraying (IRS) for vector control, prompt confirmed diagnosis, appropriate and adequate treatment, the use of intermittent Preventive treatment in pregnancy (IPTp) and a recent addition, seasonal malaria chemotherapy (SMC) (MOH/GHS, 2013).

Vector control remains an important part of malaria control programmes in Ghana, with the main aim of reducing malaria illness and death via reduction of transmission levels. To this end, an integrated approach of using ITNs, adulticiding (in partnership with AngloGold and USAID president's malaria initiative or (PMI), larviciding (and environmental management and intersectoral collaboration is being pursued. IRS in targeted areas with over 40% parasite prevalence) (MOH/GHS, 2013; USAID, 2014)

Amongst students in boarding schools, there existed a culture of mosquito net use prior to the introduction of treated nets in 1998, although usage was low. This introduction followed randomized controlled studies in northern Ghana, that demonstrated a 17% reduction in all cause mortality in underfives, who slept under bednets compared to those who did not (Binka et al., 1996). A 2003 survey put the Figure at 3.5% but this had improved to about 43% in 2008 and in 2016 this had appreciated to 52.3% per the Ghana malaria indicator survey (Ghana Statistical Service, 2017). This increase in coverage largely, can be attributed to greater sensitization and a bed net distribution drive by the national malaria control programme targeting, antenatal clinics, child welfare clinics, as

well as pupils in schools in collaboration with PMI, Global Fund, and DFID (MOH/GHS, 2013).

Following the failure of chloroquine as a first line drug for treatment of uncomplicated malaria (Koram et al., 2005) the country adopted Artesunate- Amodiaquine (AQ) as the first line therapy for uncomplicated malaria in 2005 (Koram, Quaye, & Abuaku, 2008). In 2008/9, the policy was revised to include Artemether-Lumefantrine (AL) and Dihydroartemisinin-Piperaquin as alternate first line drugs. To improve access to these antimalarials in country, ACTs have been classified as over the counter medications since 2009 (Malm et al., 2013). For uncomplicated malaria in pregnancy either quinine alone or in combination with clindamycin is recommended in the first trimester. Artemisinin combination therapies (ACTs) may be used in the first trimester if quinine is unavailable or compliance cannot be guaranteed. Artemisinin combination therapies (ACTs), are the recommendation for malaria in pregnancy in the second or third trimester. For severe malaria, intravenous Artesunate, intravenous Quinine or intramuscular Artemether maybe used. Rectal Artesunate is endorsed for pre-referral use but this is yet to gain widespread use (NMCP, 2015; USAID, 2016b)

In Ghana, Intermittent Preventive Treatment in Pregnancy with SP (IPTp-SP) is one of the three pronged approaches in The National Guidelines for Malaria in Pregnancy (MIP) informed by WHO's policy. The others are, promoting the use of ITNs at the first ANC visit, and effective case management of malaria during pregnancy. IPTp-SP was introduced in 2005 (Duah et al., 2012). At the time the main cohort study on which this thesis is based was carried out between 2006 and 2011, a minimum of two doses of Sulphadoxine-Pyrimethamine (SP) administered orally, one month apart starting from week 16 of gestation and not exceeding week 36, was the recommendation for IPTp-SP (WHO, 2016b) . IPTp-SP coverage amongst mothers in this cohort was 38% (Oduro et

al., 2010). In 2012, the guidelines were revised and Ghana has since revised the regimen to reflect the current WHO recommendation (USAID, 2014). The updated recommendations advocate SP for all pregnant women to be administered at each scheduled ANC visit, with the first dose administered as early as possible during the second trimester and subsequently, provided the doses are separated by at least one month. The last dose may be administered up to the time of delivery without safety concerns. In addition folic acid at a daily dose equal or above 5mg should not be given together with SP (WHO Malaria Policy Advisory Committee and Secretariat, 2015). The 2016 Ghana malaria indicator survey report a national coverage of 78%, with the upper east region where the study site is located recording over 90% coverage.

2.10 Immunology Immunity and the Immune system

The main subject areas in immunology are: cell mediated host defense functions, antibody related defense mechanisms, hypersensitivity reactions (including allergy), autoimmunity, immunodeficiency and transplantation (Tauber, 2017).

Immunity refers to the protective adaptations (structural and functional) in higher organisms to rid the body of foreign particles (microbial and otherwise) and abnormal cells. Immunity might also be said to refer to resistance of an organism (host) to disease causing organisms (pathogens) and their toxic effects. The *immune system* is the name used to describe the organs, tissues, cells, and molecules involved in the totality of host defense mechanisms (Sehgal & Berger, 2000). The *immune response* is the response (collective and coordinated) made by the host's immune systems cells and molecules to defend itself against a pathogen or foreign material or altered self (Chaplin, 2010).

An *antigen* is defined as any substance that can bind to a specific *antibody*. All antigens have the potential to elicit specific antibodies, but some need to be attached to an immunogen in order to do so (Prechl, 2017). *Antibodies* are immunoglobulins produced by

plasma cells (activated B lymphocytes) in reaction to infection or immunization. Immunoglobulins are antigen binding molecules of B-cells (Laffy et al., 2017).

The human immune system involves the interplay between our non-specific immunities (collectively referred to as our innate or in-born immunity) and our specific immune responses (adaptive or acquired immunity), for which there are 2 branches:

1. Humoral immunity (antibody production) and
2. Cell mediated immunity (T cell activation) (Netea, Quintin, & van der Meer, 2011).

These 2 branches can be naturally acquired or by vaccination**.**

Examples of naturally acquired passive adaptive immunity is passively transferred maternal antibodies to the foetus predominantly through the placenta (Dobbs & Dent, 2016) and transfer of IgA to infant through human colostrum (Ballard & Morrow, 2013). Examples of naturally acquired active adaptive immunity is resistance to tuberculosis following exposure to *M. tuberculosis* or the resistance to *P. falciparum* by adults after years of exposure to malarial parasites in endemic areas. On the other hand, examples of artificially acquired passive adaptive immunity is administration of anti-tetanus toxoid and artificially acquired active adaptive immunity is administration of oral polio vaccine (Chu & Englund, 2014).

2.10.1 Innate or Nonspecific Immunity

Innate immunity is an ancient form of host defense common to almost all eukaryotic cells. Innate immune response is resistance (physical, cellular & biochemical that is pre-existing or inborn and not acquired) through contact with a microorganism, foreign substance or antigen or immunization. The individual has innate immunity by genetic or constitutional makeup. Its dependent on species, race and individual and a function of age, hormonal influences and nutrition. The response is rapid (first line of defense) and to microbes and not to non-infectious substances. Innate immunity does not recognize every possible

antigen. Instead it recognizes pathogen associated molecular patterns (PAMPs), binding to endocytic pattern recognition receptors on phagocytes, indicating that the pathogen is an invader rather than a part of the host. These signals activate various killing mechanisms, including the nonspecific complement system (via the classical, alternate or lectin mediated pathways) which disrupt cell membranes of attacking cells. The signals also activate phagocytes (such as macrophages and neutrophils), which engulf invaders. When invaders get bound to mannose or toll like receptors on the phagocytes, various signals (TNFα, IL1, IL6) tell the phagocytes that cells in the vicinity are invaders (Janeway & Medzhitov, 2002). The complement system is made up of different proteins that function in unison to disrupt cell walls. Host cells possess various surface molecules that turn off complement attack, so that complement is directed only against attacking cells (like plasmodia), whilst structural carbohydrates on many pathogen cell walls trigger complement attack. Nonspecific or innate defense by itself may not completely clear an infection and in some cases pathogens can evade innate defenses-as malaria parasites often do (Carton, Daly, & Ramani, 2007; Frank, 2002).

2.10.2 Specific or adaptive or acquired Immunity

Nonspecific immunity identifies common repetitive structural features that differentiate pathogens from self cells. Specific immunity identifies minor regions of particular pathogen molecules. The *adaptive immune response* or *adaptive immunity* is the response of antigen specific *lymphocytes* to antigen. It involves the development of immunological memory. After the initial encounter with an antigen (for instance a malaria parasite), the adaptive immune reaction to each succeeding encounter with the same antigen is quicker and more effective, a vital feature of protective immunity referred to as *immunological memory*. Immunological memory is specific and long lived for a particular antigen. Clonal selection of lymphocytes generates adaptive immune responses. Specific recognition may depend on a few amino acids sequences of a pathogen protein, such that molecules of the

host immune system bind to a molecular shape (the epitope) on the pathogen that is not shared by other pathogens (Akira, Uematsu, & Takeuchi, 2006). Pathogen genotypes can so differ such that, a host can recognize particular sites on one genotype but not others. At times, all pathogens of the same species share the specificity, and recognition between different kinds of pathogens. Other times, different pathogen genotypes may differ in molecular shape, so that the host molecules that bind precisely to one pathogen molecule or are unable to identify and bind another pathogen molecule that varies a little. This is Antigenic variation and is common with malaria infections. Adaptive immune reactions can be naturally or artificially acquired. The lymphocyte is the main cell in adaptive immunity and there are two main types-B and T cells (Carton et al., 2007; Frank, 2002).

2.10.3 B Cells and Antibodies (Humoral Immunity)

B cells or B lymphocytes mature in the bone marrow and then circulate in the blood and lymph systems as immune cells**.** B cells (plasma cells when activated) express globular proteins (immunoglobulins) or B cell receptors (BCRs) on their cell surfaces in response to antigen challenge for instance, in infection with malaria or immunization. These BCRs bind to either neutralize pathogens or package them for phagocytosis. These same BCRs when secreted into the circulation are called antibodies (Carton et al., 2007). Antibodies which belong to the globulin fraction of serum proteins each has an exclusive structure that allows precise binding to its epitope on the corresponding antigen. This is the basis of the humoral or antibody dependent immune system.

Overall, all antibodies have the same structure (figure 5); Y or T-shaped polypeptides with a couple each of identical heavy chains and light chains (lamda and kappa). These four chains are held by disulphide bonds. The Fab portion of the antibody is that region that binds to epitopes. The Fc portion determines the biological activity of the antibody (Hoffman, Lakkis, & Chalasani, 2016).

The effector functions of antibodies in the humoral system are four-fold: neutralization, opsonization, antibody dependent cellular cytotoxicity (ADCC) or inhibition and compliment fixation (Gomes et al., 2016). Via specially controlled recombination and mutation processes, the B cells generate alternative antibody specificities. The host preserves a huge diversity of antibody specificities each of which are initially few. Novel pathogen epitopes (such as malaria parasite epitopes) bind to rare antibody specificity. B cell division is stimulated post binding and there is expansion of the clonal lineage with a boost in production of matching antibody. The matching B cells intensify their mutation rate, producing many antibodies that are a little dissimilar, that differ in their affinity to the attacker, bind more firmly and are encouraged to divide faster. This refinement of the B cells is known as affinity maturation. There are five classes of antibody: immunoglobulin A (IgA), immunoglobulin D (IgD), immunoglobulin M (IgM), immunoglobulin G (IgG), and immunoglobulin E (IgE) (Hoffman et al., 2016; Janeway, 2001).

2.10.4 Immunoglobulins (Igs)

Immunoglobulin A (IgA), has 2 subclasses and composes about 6% of the serum antibodies. The Fc portion of secretory IgA binds to mucous components and aids mucous trap microbes. IgA is found mostly in body secretions including breast milk as secretory IgA (sIgA), exerting its effects by protecting internal body surfaces, exposed to the environment, via blocking the attachment of pathogens to mucous membranes. IgA is capable of activating the alternative complement pathway (Xiong & Hu, 2015).

Immunoglobulin D (IgD), composes about 0.2% of the serum antibodies. IgD is on the surface of B-lymphocytes (together with monomeric IgM) as a B-cell receptor (BCR) or sIg where it may regulate B-lymphocyte activity or play a role in removing B-lymphocytes that generate self-reactive autoantibodies (Chen & Cerutti, 2010).

Immunoglobulin E (IgE), composes about 0.002% of the serum antibodies. Most IgE is firmly bound to basophils and mast cells via its Fc region. The Fc portion of IgE made against parasitic worms and arthropods, can either bind to eosinophils facilitating opsonization or it can bind to mast cells and basophils and mediate a lot of allergic reactions. Antigens can cross link cell-bound IgE, triggering the release of vasodilators for an inflammatory response (Gadermaier et al., 2014).

Immunoglobulin G (IgG), makes up about 80% of the serum antibodies. The Fc portion of IgG, is capable of activating the classical complement pathway and can bind to macrophage and neutrophils for heightened phagocytosis and to natural killer (NK) cells for antibody-dependent cytotoxicity (ADCC). The Fc portion also enables IgG to cross the placenta. IgG is the only class of antibody that traverses the placenta into the fetal circulation (Vidarsson, Dekkers, & Rispens, 2014). IgG has 4 subclasses (IgG_1, IgG_2, IgG_3 and IgG4 in order of increasing abundance). Each subclass, with respect to antigen binding, immune complex formation, complement activation, triggering of effector cells, half-life and placental transport has a unique profile (Scott-Taylor et al., 2017).

Immunoglobulin M (IgM), composes about 13% of the serum antibodies and during an immune response is the first antibody to be produced. IgM is a pentamer and therefore has 10Fab sites and 5 Fc sites. The Fc portions of IgM are capable of activating the classical complement pathway. On the surface of B-lymphocytes, monomeric forms of IgM are found as B-cell receptors. IgM activates the classical complement pathway the most among the antibodies (Nguyen & Baumgarth, 2016).

Naive B cells produce IgM (Seifert et al., 2015). By altering the constant region post stimulation and affinity maturation, B cells can produce several types of immunoglobulins, the commonest being IgG and IgA. On first encounter with a novel pathogen such as a malaria parasite or part of it thereof, the rare matching antibodies cannot control infection

and so while the host boosts matching antibody production, the infection festers. By and large the host may produce enough antibodies to clear pathogens that carry the matching epitope (primary response). On the other hand, if the pathogens vary the matched epitope (as the malarial parasite is prone to doing), the host has to produce novel antibody types to get rid of the variant pathogens (Taher et al., 2017).

A memory of an epitope is maintained once the host produces an antibody specificity against a matching epitope. The host can then rapidly produce a lot of matching antibodies (secondary response), upon re-exposure to the same epitope-priming. The response to each antigen challenge is dose dependent and increasingly intense, so that succeeding responses are of higher strength. This memory lets the host get rid of successive reinfection, without obvious symptoms (as happens to many individuals living in malaria endemic areas) (Hoffman et al., 2016).

Antibodies typically bind to surface epitopes of pathogens (such as the PfEMP1 antigens on red cells infected by malaria parasite). So, antibodies support clearance of parasites or pathogens in circulation or otherwise exposed to direct attack. Once a pathogen enters a host cell (intracellular), the host ought to draw on other defenses such as T cells (Nogaro et al., 2011; Carton et al., 2007; Frank, 2002; C. Janeway, 2001).

2.10.5 T Cells

T cells or T lymphocytes, develop and mature in the thymus gland. T-cells can be divided into T cells bearing T-cell receptors (detected with anti-CD3 antibodies), and null cells including natural killer (NK) cells. Further divisions are CD4+ and CD8+ cells α: β T cells and γ: δ T cells. Selfcells constantly degrade intracellular proteins into small peptides which bind with the hosts' major histocompatibility complex (MHC) molecules and are transported to the cell surface for the attention of roving T cells. T cells possess receptors capable of binding only to specific peptide-MHC complexes and different clones produce

different receptors. T cells may in addition, receive stimulatory signals suggestive of pathogen invasion and activate processes leading to the death of the infected cell. This way, T cells aid in getting rid of intracellular infections (Alberts, Johnson, & Lewis, 2002).

Antigen-presenting cells (APCs) process external proteins including malarial parasite proteins, break them down into smaller peptides, and display the peptide- MHC class II complex on their cell surfaces. CD4+ T cells, can bind to the presented class II peptide-MHC complexes if they have matching TCRs; These CD4+ cells (helper T cells) may deliver a helping signal (cytokines) required to stimulate an antibody or CTL response. It is another mechanism at play in the destruction of malaria parasites (Moro-García et al., 2018).

In sum, cell mediated immunity encompasses induction and proliferation of antigen specific T cells that get rid of infection by direct cytotoxic killing (CD8+ cells) or aiding antibody formation (stimulatory cytokines to B cells from CD4+ cells) to mediate parasite clearance, followed by a contraction phase where antigen-specific T cells survive as memory cell exhibiting a behaviour similar to B cells. This is one mechanism at play in the destruction of malaria parasites (Alberts et al., 2002; Moormann, 2009).

2.10.6 Transfer of immunity by antibodies or by lymphocytes.

Immunity by serum transfer, confers humoral immunity (circulating antibodies) and provides immediate but short-lived immunity as antibodies decay over time. A natural form of this passive immunity is the transfer of protective antibodies from the mother to the foetus in pregnancy (Baxter, 2007). Long lived immunity is gotten via adoptive transfer by lymphoid cells. Immunity thus conferred is referred to as cell-mediated immunity (Garcia et al., 2018).

This thesis focuses mainly on the subject of antibody related defense mechanisms, infectious diseases (malaria) and draws on the adaptive and innate immunity divisions of immunology to varying extents

2.11 Immunity to Malaria

Malaria immunity may be defined as the state of resistance to the infection occasioned by the interplay of factors and processes which are involved in destroying the plasmodia or by curtailing their growth and development. Innate or nonspecific immunity to malaria is an intrinsic part of the host, an immediate reaction to the presence of the pathogen, not reliant on any preceding infection with it. Acquired immunity may be either active or passive and it is an augmentation of the defense mechanism self, as a result of a prior interaction with the malarial parasites (or parts thereof). Passive acquired immunity is conferred by the prenatal or postnatal transfer of protective antibodies from mother to child or by the injection, diffusion or active transport of such substances thereof (Kutty, 2014; Doolan, Dobaño, & Baird, 2009). The time needed to develop resistance to malaria is relatively lengthy when compared to the swift acquisition of immunity to other diseases (Yazdanbakhsh & Sacks, 2010).The development of immunity to malaria is characterized by the capacity to keep disease and parasite density in check.The major factor driving protection from disease may be specific to effectors that reduce parasite density. However, other effectors, for instance reactions that decrease proinflammatory cytokines, may also play a role (Korir et al., 2012). In humans, various types of adaptive immunity against malaria parasites have been defined: (i) anti disease immunity, conferring protection against clinical disease, (ii) anti parasite immunity, conferring protection against parasitaemia and (iii) premunition, providing protection against novel infections by keeping a low-grade and largely asymptomatic parasitaemia (López et al., 2017; Moormann, 2009).

2.11.1 Malaria immunity in adults

Adults rarely have high-density parasitaemia in areas of intense transmission. The prevalence of parasitaemia and the risk of illness and death as a result of malaria decrease sharply with age after early childhood (Pinkevych et al., 2012). In these holoendemic areas, most people are almost perennially exposed to infective bites and the bulk of them seldom experience patent disease. They pursue their lives feeling essentially healthy despite parasites loads that would almost definitely be catastrophic to a malaria-naive visitor. This tenacity in the midst of infection is naturally acquired immunity to malaria. There are a few adults, who do have symptoms that look severer than those in children with similar parasite profiles. These adults, may represent the few who are either removed from their repetitive infections or resist the odds of reinfection for a duration that makes their immunity to fade (Owusu-Agyei, Koram, & Baird, 2001). Much as resistance to disease in the long run is gotten with cumulative exposure to malaria, resistance to liver infection is hardly ever attained, so adults in endemic areas tend to display non sterilizing immunity. So protective malaria immunity in humans is intricate, beginning with resistance early to severe disease, then to uncomplicated disease but hardly involves resistance to infection. Repeated infections with Plasmodium variants are reportedly responsible for this kind of protection employing cytophilic antibodies and memory cells (Rogerson et al., 2007a).

2.11.2 Malaria immunity in infants and young children

Infants and young children as opposed to adults, at least occasionally, do not have naturally acquired immunity or might possess a nascent one (Odorizzi & Feeney, 2016; Portugal et al., 2014). An “anti disease immunity” is shown by young children which is a function of illness associated with a given parasite density. This protection appears to be gained rapidly resulting in decreased deaths or less severe clinical disease. However, the

seemingly gradually acquired "anti parasite immunity" offers protection against dense parasitaemias with the risk of severe disease (Cowman et al., 2016; Doolan et al., 2009). The risk of clinical disease is thought to be low in very early infancy. Depending on the degree of transmission and from about the third month of life onwards, infants become increasingly prone to high parasitaemia, severe disease and death. This protection in early infancy is thought to be attributable to maternally transferred antibodies which tend to wane over time and thought to be a function of transmission type (McLean et al., 2017; Moormann, 2009). The persistence of foetal haemoglobin has also been implicated in the protection against malaria during early infancy (Billig, McQueen, & McKenzie, 2012). However, at least one study has refuted the explanation that maternally transferred antibodies against malaria antigens are responsible for the protective effect seen in early infants (Riley et al., 2000). The protective effect seen in infants may be related to parasite growth-inhibitory factors such as lactoferrin and secretory IgA found in breast milk and in maternal and infant sera (Namrata Anand et al., 2015; Shi et al., 2011). Additionally, host genetic factors such as Haemoglobin AS , CC genotypes as well as G6PD deficiency have been shown to confer some protection (Kutty, 2014). With the waning of passive immunity, children initially become increasingly vulnerable to severe disease (Dobbs & Dent, 2016). The mechanisms of immunity to severe malaria entail the attainment of 'strain-specific' antibodies to counteract important *P. falciparum* variant antigens driving the pathogenesis of severe disease. Examples of these are antibodies to subsets of PfEMP1s-CD36 and ICAM-1 that play a role in sequestration and the induction of 'strain-transcendent' regulatory mechanisms. These mechanisms, keep overzealous malaria parasite-induced inflammation in check (Keegan & Dushoff, 2013). Both of these mechanisms may rely on repetitive infective bites to be maintained (Crompton et al., 2014). In young children, specific antibody responses to acute malaria infection are on the whole brief. However, there is a slow rise in antigen and IgG specificity yearly, with

exposure. This phenomenon perseveres, in the absence of transmission (i.e. during the dry or low transmission season in the case of seasonal malaria); giving reliable defense against malaria symptoms when a certain threshold is exceeded (Keegan & Dushoff, 2013). The occurrence of low-level submicroscopic parasitaemia may be vital in the onset and maintenance of immunity or premunition. From age two the incidence of clinical disease starts to lessen and the risk of death drops sharply but in particular children are more at risk of cerebral malaria within this period (Aponte et al., 2007). Children remain vulnerable to recurrent malaria attacks into puberty, eventually acquiring a malaria resistant state in adulthood but remain disposed to infection with blood-stage parasites (Crompton et al., 2014; Doolan et al., 2009). Studies suggest that the onset of puberty itself, acting through the modulation of the immune system by steroid hormones, rather than collective exposure associated with age, may be an overriding factor in the commencement of protective immunity (Griffin et al., 2015; Pathak et al., 2012; Klein et al., 2008; Kurtis et al., 2001). Acquisition of immunity, albeit non sterile, is dependent on repetitive exposure to parasites. Therefore, interventions that effectively curtail exposure, may have deleterious effects on immunity acquisition in areas with intense transmission (Bretscher et al., 2015).

2.11.3 Malaria immunity in pregnancy

The characteristics of naturally acquired immunity to malaria in pregnancy substantially changes. Inspite of the effective immunity associated with adulthood in areas of stable transmission, expectant mothers are at higher risk of malaria than their non-expectant counterparts especially paucigravidae (Brabin, 1983).This is so for a number of reasons. Their behaviour and physiology appear to put them at increased risk of infective bites-waking up more frequently to urinate, higher metabolic rates that result in higher body temperatures and faster and deeper exhalations. Their ability to limit parasite replication

also appears dampened as serum cortisol levels increase and proinflammatory cell mediated immunity is downgraded to tolerate foetus and placenta (Sharma & Shukla, 2017). Cortisol is known to bias the immune system towards a T-helper 2 response (hormonal immunosuppression) (Spellberg & Edwards, 2001). Immunity to malaria in pregnancy has been reported to be acquired in a parity dependent manner. This is because the targets of adhesion molecules of the *Pf*EMP-1 on malarial parasites in the placenta are different (chondroitin sulphate A-CSA, specifically var2csa) from those in the peripheral circulation (CD-36 and ICAM-1) (Adukpo et al., 2013; Salanti et al., 2004). So even though the pregnant woman might have considerable immunity against adhesion molecules in the peripheral circulation, she needs to build the required repertoire for parasites in the placenta over successive pregnancies-hence the parity dependent nature of immunity acquisition (Sharma & Shukla, 2017). Also, for some primigravidae with severe anemia, cytokines and increased cortisol levels (which suppress T cell mediated immunity) in the placenta, have been demonstrated to be markedly higher among them, than among other primigravidae.This finding correlates well with malarial parasitaemia and intervillous monocyte infiltrates in the placenta; (Crompton et al., 2014; Doolan et al., 2009; Rogerson et al., 2007b). Groups in low transmission settings and non-immunes, are at higher risk of severe disease irrespective of parity, as result of paucity of pre-existing immunity. This narrative is with respect to *P falciparum* because it leads to the greatest disease in infant as well as maternal deaths. *P vivax* may also cause low birth weight and anaemia and until recently was not known to sequester in the placenta. To the best of present knowledge, susceptibility to *P malariae* and both species of *P ovale* does not alter in pregnancy. *P. falciparum* infection may also play a role in the acquisition of immunity in the offspring due to sensitization to parasite antigens during foetal life and this area of research has gained interest of late (Djabanor et al., 2017; Duffy, 2007; Malhotra et al., 2005)

2.12 Blood stage malaria antigens

Blood stage malaria antigens are circulating antigens from erythrocytic or blood stage (asexual stages) of the parasite in the human host.The development of immunity targeting these circulating asexual stages or their antigenic products, is the basis of protection from malaria in endemic settings (Healer et al., 2013). Antibodies to some of these antigens, have been shown to be associated with some protection against clinical malaria in previous studies (Daou et al., 2015; Dodoo et al., 2011a).

Glutamate-rich protein (GLURP) is a 220- kilodalton (kDa) frame of 1,271 amino acids including an amino-terminal non-repetitive region (R0) and two blocks of repetitive sequences R1(Central repeat region) and R2 (carboxy-terminal repeat region (Theisen et al., 1995). The protein is present in all the developmental stages of *P. falciparum* in humans, including on the surface of newly released merozoites. Furthermore, GLURP has been identified as a target antigen for antibodies involved in antibody-dependent cellular inhibition (ADCI), which is reported to be involved in acquired protective immunity to malaria amongst Ghanaian children (Kana et al., 2017). Levels of GLURP-specific antibodies are known to correlate with protection against hyperparasitaemia and clinical disease (Dodoo et al., 2000; Theisen et al., 1998). Anti-GLURP R2 antibodies have been found to be associated with lower risk of symptomatic malaria infection in Burkinabe and Ghanaian children (Adu et al., 2016). The GLURP-R0 contains the conserved non-repeat N-terminal region, (amino acids 25-514), and GLURP-R2 (amino acids 705-1178) of the carboxy-terminal repeat region, (Dodoo et al., 2011a). Merozoite surface protein 3 (MSP3) is a 48kDa long synthetic peptide called LR55 (amino acids 181-276) of (Dodoo et al., 2008). It also has N and C terminals.

GMZ2 the N-terminal region of the glutamate-rich protein fused in frame to the C-terminal region of merozoite surface protein 3 (MSP3) is a hybrid protein (GMZ2). GMZ2

is formulated in Al(OH)3 (Jepsen et al., 2013; Noor et al., 2010). GMZ2 was reported in a phase 2b trial to be well tolerated, immunogenic, and reduced incidence of malaria in Ghanaian, Burkinabe, Gabonese and Ugandan children (Sirima et al., 2016).

2.13 Intermittent Preventive Treatment (IPT)

Intermittent Preventive Treatment (IPT), is a control strategy for malaria in vulnerable groups (pregnant women and children under 5 years) in malaria endemic areas to help reduce the burden of *P. falciparum* malaria. Intermittent preventive treatment (IPT) of malaria involves the administration of therapeutic doses of antimalarials at predetermined intervals, irrespective of parasitaemia or symptoms to reduce incidence of malaria and prevalence of malaria anaemia. In infants, its known as IPTi delivered in the context of the expanded programme on immunization (Cibulskis et al., 2016; Beeson et al., 2011). IPTi is yet to have widespread implementation. For selected countries and for young children between ages 3 months and 5 years, commencing 2012, IPT is delivered in the high transmission season as seasonal malaria chemotherapy (SMC) (Cibulskis et al., 2016; NDiaye et al., 2016).

For pregnant women IPTp is delivered through routine health visits-antenatal clinics. Since the strategy was recommended in late 1998 by the WHO (d'Almeida et al., 2011), there have been several significant changes to the context in which IPTp is being deployed. IPTp using Sulphadoxine Pyrimethamine(SP)-IPTp-SP, is recommended for all pregnant women at each scheduled antenatal care (ANC) visit until the time of delivery, provided that the doses are given at least one month apart (or as early as possible from the 2nd trimester) regardless of whether or not the pregnant women are infected with malaria. Ideally it should be administered as directly observed therapy (DOT) of three tablets Sulphadoxine/pyrimethamine (each tablet containing 500mg/25mg SP) giving the total required dosage of 1500mg/75mg (WHO, 2016b)

Use of IPTp decreases the incidence of low birthweight by 42%, neonatal death by 38%, placental malaria by 65% and antenatal parasitaemia (Sangaré et al., 2010). Main barriers to the provision of IPTp are; stock outs and user fees; poor health facility organization, resulting in poor quality of care; poor provider performance, including mix-ups over the timing of each IPTp dose; and poor antenatal attendance, affecting IPTp uptake. Education, knowledge about malaria/IPTp, socio-economic status, parity, number and timing of antenatal clinic visits, principally determine IPTp coverage (Hill et al., 2013).

2.14 IPTp-SP, maternally transferred antibodies and risk of malaria in early infancy.

The effectiveness of IPTp-SP in preventing malaria related adverse pregnancy outcomes is well established even in areas with drug resistance (Mpogoro et al., 2014). SP is thought to exert its beneficial effects, by reducing or clearing blood malarial parasites and other bacterial load both peripherally and in the placenta (Gosling et al., 2010). Peripheral clearance of parasitaemia results in less maternal malaria and improved maternal haemoglobin profiles. Improvements in transplacental exchanges (including nutrients and antibodies) by IPTp-SP, results in improved birth weights, reduced intra uterine growth retardation and preterm births (Chico et al., 2017). The beneficial effects of IPTp-SP beyond *in utero,* may have their antecedents from mothers' malaria experience during pregnancies with reports of associations between mothers' malaria experiences with increased malaria risks in infancy-pregnancy associated malaria (PAM) (Schwarz et al., 2008; Le Hesran et al., 1997). PAM determines foetal exposure to *Plasmodium sp in utero* and is correlated to early development of clinical episodes during infancy (Moya-Alvarez et al., 2014a). By reducing or clearing placental infection rates and thence the risk of parasitaemia in their infants IPTp-SP may effectively lower risk of clinical malaria in early infancy (Le Port et al., 2011). Alternatively, or in concert, reduction in inflammatory reactions as a result of placental clearance of malarial parasitaemia and other pathogens by IPTp-SP, may facilitate greater transfer of relevant maternal antibodies to infants born to

such mothers. Potentially, this could result in a lower risk of malaria in early infancy; for it is been reported that a higher clearance of placental malaria parasites and other pathogens, is thought to result in facilitation of transplacental exchanges including antibodies (Mace et al., 2015; Umbers, Aitken, & Rogerson, 2011). These views are supported by studies that have shown that infections and inflammation impair antibody transfer across the placental barrier (Palmeira et al., 2012). Further support comes from earlier studies that have reported pregnancy associated malaria with reduction in maternal antibody transfer to infants (Okoko et al., 2001; Branch et al., 1998), increased risk of developing parasitaemia in infants born to such mothers (Broen et al., 2007b), and increased risk of disease and mortality in such infants (Schwarz et al., 2008; Mutabingwa et al., 2005). However, soluble malaria antigens may also be transferred transplacentally and some foetuses can mount immune responses to this prenatal exposure to these antigens (Metenou et al., 2007). In the latter case, there could be immune priming or premonition (Malhotra et al., 2009a, 2005), with the anticipated development of immune tolerance (Bonner et al., 2005; Broen et al., 2007b) and increase in the risk of malaria subsequently in infancy (Sadissou, d'Almeida, & Cottrell, 2014). By reducing or clearing placental infections, IPTp-SP may also reduce transplacental transfer of soluble malaria antigens or less commonly malarial parasites (Danquah et al., 2016) and reduce the chances of immune priming and subsequently the risk of malaria. However, in a cohort study in the study area it was reported there was no such association between the presence of placental malaria and risk of malaria in infants of such mothers (Awine et al., 2016).

Having said this, there is the alternate view that, clearance of peripheral parasitaemia could actually result in lower antibody titres in mothers who are given IPTp-SP (Anchang-Kimbi et al., 2016). The explanation advanced is that IPTp-SP effect is akin to reduced exposure. That being the case, IPTp-SP could result in less transfer of antibodies to the foetus and subsequent increase in risk of malaria in early infancy. However, in a recent

cohort study in coastal Ghana where mothers and their infants had their antibodies compared, IPTp-SP did not appear to negatively impact on transplacental transfer of antibodies to the early infant (Stephens et al., 2017b).

There is growing body of evidence in support of high antibody titres protecting against malaria by clearing malarial parasites and preventing clinical disease (Murungi et al., 2013, 2017). Passively transferred and naturally acquired antibodies have been demonstrated to be protective of clinical malaria in humans (Boyle et al., 2015; Sabchareon et al., 1991; Cohen, McGregor, & Carrington, 1961). These antibodies (IgG) traverse the placenta to the foetus (Williams & McFarlane, 1969) mainly in the third trimester of pregnancy. It has been demonstrated that, antibodies that were protective of malaria could be obtained from cord blood, lending support to the view that at least in part, passively acquired maternal immunity to malaria in infants is antibody mediated (Edozien, 1961). Antibodies are considered to act in synergy with foetal haemoglobin to reduce the risk of clinical malaria in early infancy (Kangoye et al., 2014; Sabchareon et al., 1991). This they do, even as endogenous production of IgM and IgG get underway (Nhabomba et al., 2014). In early infancy (first six months of life), antibodies are believed to play a critical role in reducing the risk of malaria. The antibodies passed from the presumably immune mother to infant could function in several ways: inhibit sporozoites invasion of liver cells (Dent et al., 2008), prevent merozoites invasion of erythrocytes (Tham et al., 2009), dampen parasite development (Crompton et al., 2010), opsonization or complement-mediated lysis (Portugal et al., 2013), facilitate parasite phagocytosis by macrophages (Groux & Gysin, 1990); disrupt rosettes (Vigan-Womas et al., 2010) and the sequestration of infected erythrocytes (Ricke et al., 2000). Rosetting and sequestration are implicated in the pathogenesis of severe malaria **(**Figure 2**).** Non-neutralizing antibodies may contribute to reduction in risk of malaria through antibody-dependent monocyte or NK cell mediated cytotoxicity (Portugal et al., 2013). So, any perturbations that influence

these transplacental antibody transfers can potentially impact on the risk of malaria in the first 6 months of life (Wilcox, Holder, & Jones, 2017).

In any case, the antigenic targets and mechanisms of protection are not well understood and are likely to be multifaceted. IPTp-SP, might affect immune responses of the early infant, by influencing transplacental passage of maternal antibodies, or foetal in utero exposure to malaria antigens (Moya-Alvarez, Abellana, & Cot, 2014b). Sulphadoxine-Pyrimethamine (SP), is a combination antibiotic, with known antimalarial and antibacterial properties. SP is recommended for use in pregnant women as a prophylaxis against malaria in pregnancy in areas of moderate to high transmission as intermittent preventive treatment of malaria in pregnancy (IPTp-SP) (WHO, 2016b). IPTp-SP achieves its *in utero* effect by clearing peripheral and placental malarial parasites but also has biological activity on other pathogens (Gutman & Slutsker, 2017). Via this mechanism IPTp-SP's beneficial effects *in utero* (reduced stillbirths, reduced intrauterine growth retardation and improved birth weights) are realized (Vallely et al., 2007). However, evidence continues to build up that suggests that benefits of IPTp-SP extend into infancy by reducing risk of malaria and all cause mortality (Menéndez et al., 2010a; Moya-Alvarez et al., 2014a). Since SP has antimalarial as well as anti-bacterial properties, IPTp-SP might be influencing the risk of morbidity and mortality in early infancy by clearing parasites, reducing placental bed inflammation, improving placental bed perfusion and facilitating substance exchanges across the placenta including antibodies (Desai et al., 2015; Gutman & Slutsker, 2017). However other investigators have reported increased susceptibility to malaria and mortality in infancy with IPTp-SP in cohort studies in Cameroon and Tanzania (Apinjoh et al., 2015; Harrington et al., 2013, 2011) Analysis of data from the primary cohort study on which the secondary study is based, reported an over 40% reduction in the risk of malaria in early infants of mothers who had at least one dose of IPTp-SP compared to early infants of mothers who had no IPTp-SP, after adjusting for

potential confounders (appendix Aviii). This phenomenon seen in the primary study could be explained by suggesting two mechanisms: that IPTp-SP maybe acting by reducing placental malarial parasite densities, inhibiting maternal placental inflammatory response to infection and resulting in greater protective antibody transfer and less soluble antigen transfer (Chico et al., 2017); alternatively, by clearing peripheral parasitaemia, the placental bed is less likely to be infected. Less placental infections would reduce the chances of transplacental transfer of soluble antigens. Presumably, this will result in less immunotolerant states in utero and more immunocompetent states in extra uterine life (Broen et al., 2007b; Duffy, 2007).

An alternate mechanism, by which IPTp-SP is said to exact its effect, is by improving on birth weight. This IPTp-SP is believed to do, via its antibacterial properties. Placental bed is cleared of bacterial infections, reducing inflammation and improving on transfer of nutrients and other substances across the placental bed to the foetus (Bulmer et al., 1993; Walter et al., 1982). Simply by improving on birth weight all cause morbidity and mortality will be improved; as low birth weight is generally associated with higher morbidity and mortality (Chico et al., 2015; Mace et al., 2015; Walker et al., 2017) in an apparent downplay of the role of antibodies. But then in a Mozambican study, serum reactivity to infected erythrocytes from placentae and children were at delivery associated with higher birth weights (Mclean et al., 2015; Mayor et al., 2013). In a Kenyan study levels of anti adhesion antibodies to placental infected erythrocytes was associated with higher birth weights and in Benin reduced the likelihood of low birth weight (Ndam et al., 2015).This puts the role of antibodies back squarely on the radar.

Chico and colleagues (2017) have also suggested in their aforementioned work that the protection against adverse birth outcomes by IPTp-SP is dependent on the frequency of dosing. This argument is taken further here; it is suggested the protective effect by IPTp-

SP beyond *in utero* in extra uterine life (early infancy) is not only dependent on the frequency of dosing of IPTp-SP but also on the quantity of maternally transferred antibodies (Cord blood IgG) which is in turn is influenced by the frequency of dosing of IPTp-SP. The aforementioned argument is arrived at thus-IPTp-SP causes a decline in placental malaria (Hommerich et al., 2007). Placental malaria predicts susceptibility to parasitaemia in a dosing frequency (frequency) dependent fashion in infancy. It can then be inferred that IPTp-SP would affect susceptibility to malaria infection and disease in infancy (Harrington et al., 2013) in a dose frequency dependent fashion.

CHAPTER THREE

3.0 METHODS

3.1 Study design

Secondary data was analysed retrospectively in the context of a five year birth cohort study (primary study) that sought to document malaria experiences in the Kassena-Nankana District. So data sources were essentially secondary and retrospective.

Two sources of data were employed: First, all of the enrolment, morbidity and mortality data from the primary study, was used to assess relationships between the dosing frequencys (frequency) of IPTp-SP (independent variables) and the risk of parasitaemia, uncomplicated malaria, severe malaria (including severe malarial anaemia as a subset analysis) and all cause mortality in early infancy, as dependent variables. The exposed group was infants of mothers who had IPT-SP and the unexposed group was infants of mothers who never had IPTp-SP. The duration of follow-up was the first six months of life (early infancy).

A second data source came from a subset of the baseline archived plasma samples of newborns from the primary study. Again, accompanying enrolment (baseline), morbidity and mortality data was used, to assess the relationship between maternally transferred antibodies to selected malaria antigens and the risk of parasitaemia, uncomplicated malaria, severe malaria (including severe malarial anaemia as a subset analysis) and all cause mortality in early infancy. This same subset of archived baseline plasma samples, was used to assess the magnitude of maternally transferred total IgG in newborns to selected malarial antigens, in relation to differing IPTp-SP dosing frequencys (frequency).

3.2. The main or primary (1^0) study

This section outlines the aims, recruitment, enrolment, followup and data capture strategies for the primary cohort study "MALARIA MORTALITY AND MORBIDITY IN

THE FIRST FIVE YEARS OF LIFE IN A BIRTH COHORT OF CHILDREN IN NORTHERN GHANA" from which this secondary study derives.

I was initially a co-investigator and study physician on the study, from proposal writing through scientific reviews and ethical/regulatory approvals to study commencement in 2006. Subsequently in December 2007 and for the rest of this five year project, I was the site principal investigator and study physician with oversight responsibility for the day to day running of the project in Navrongo. This included but was not limited to data collection, entry, validation, healthcare of study participants and project administrative work.

3.2.1 Recruitment & Enrolment in the primary study

For the primary study a cohort of 2279 (from amongst all newborns in the KNDs) newborns was enrolled between March 2006 and March 2007 in the Kassena-Nankana Districts of Northern Ghana. The main aim was to obtain malaria data in a stable long-term cohort that enables one to adequately characterize malaria clinical outcomes and important epidemiological and immunological questions related to the development and testing of candidate malaria vaccines in Kassena-Nankana Districts. The primary study inclusion criteria were:

1. All healthy male and female newborn infants whocouldbe enrolled at birth or within the first 28 days of extra uterine life.
2. Willingness of parent or guardian to give witnessed parental informed consent.
3. The expectation of continuous residency of the child in the KNDs for the study duration.

The exclusion criteria for the primary study were:

1. Inability of the child's parent or guardian to give informed consent.
2. Expectation of possible unavailability to continue participation in the study.

Enrolment of newborns of mothers recruited into the study was carried out after written informed consent had been sought and obtained from mothers.

3.2.2 Follow-up in the primary study

A passive follow up approach was employed in the primary study. Malaria was treated as per the prevailing standard treatment guidelines of the Ministry of Health/Ghana Health Service (Ministry of Health (GNDP) Ghana, 2004) and was free of charge for acute illnesses. Details of how the primary study was carried out, are captured elsewhere (Oduro et al., 2012; Oduro et al., 2010).

3.2.3 Laboratory work in the Primary study

Malaria slides were made by trained study staff, air dried stained in 10% Giemsa and read independently by two experienced microscopists. Parasites were enumerated per 200 white blood cells (WBCs) assuming an average white blood cell count of 8000/µl of blood. The counts were then averaged. In the event that there was more than 50% discordance in the two readings, then a third reader acted as a tie breaker, with the final reading being the average of the two most concordant readings. Haemoglobin was measured using automated Hemocue machines (HemoCue AB, Sweden).). Heel prick or cord blood (0.5ml) as well as filter paper blood blots were taken to study immune correlates of protection and host genetic factors of protection or susceptibility to malaria. The Plasma from lithium heparinized whole blood, were collected in keeping with established standard operating procedures and stored in a minus 80 degrees centigrade (-80ºC) freezer (Revco, USA) whose temperature was monitored twice daily.

3.2.4 Data Capture in the primary study

Each study participant had a unique personal identifier (study identification number). Obstetric and antenatal history, practices leading up to birth, including IPTp use, selected

newborn physical characteristics and other socio-demographic characteristics were captured on structured case report forms (appendix B).

Biological samples similarly had the unique identifiers of study participants. Laboratory procedures and results were captured on structured case report forms bearing these unique identifiers (appendix B). The unique identifiers allowed for the linking of laboratory to socio-demographic and other data. The original data was double entered into a Visual Foxpro database. Data capture in the primary study are detailed elsewhere (Oduro et al., 2010)

3.2.5 Data extraction manipulation and capture in the secondary study.

The original data for the primary study was captured in Visual Foxpro. So the primary datasets were then imported into STATA intercooled version 13.2 for manipulation.

Table 1 below shows the types of variables extracted and the manipulation of the variables if any for the statistical analysis.

Table 1 showing variable manipulation for secondary study

Variable	Initial capture in primary study (data type)	Manipulation in secondary study	Remarks
IPTp-SP use	Continuous discrete	Recoded to ordinal: 0,1,1+,2,3+	Analysed as ordinal (categorical)
Birth weight	Continuous	Categorized into dichotomous/binary	Analyzed as weight ≥ 2.5kg or <2.5kg
Parasitaemia of newborns	1.Continous (count) 2. Nominal (present or absent)	1.geometrically transformed 2.Maintained as dichotomous	Analysed using both data types
Gender of newborns	Nominal	Nil	Analysed as dichotomous/binary (categorical)
Birth season of newborns	Nominal, month of birth	Recoded into high transmission (wet)-May to October / low transmission (dry) season-November to April.	Analysed as dichotomous/binary (categorical)
Residence of Newborns	Nominal (compound identification number)	Urban/Rural clusters generated and Recoded as urban/rural	Analysed as dichotomous/binary (categorical)
Location of newborns	Nominal (compound identification number)	Irrigated/Non irrigated clusters generated and Recoded as Irrigated/Non irrigated	Analysed as dichotomous/binary (categorical)
Bednet use	Nominal-dichotomous	Nil	Analysed as dichotomous (categorical)
Gravidity	Continuous discrete	Recoded to ordinal: primigradae (1), secondigravidae (2), multigravidae (3+)	Analysed as ordinal(categorical)
Mother's education	Continuous discrete (years of schooling)	Recoded to ordinal: basic education (up to 9 years), secondary education and above (> 9 years	Analysed as ordinal (categorical)
Body temperature	Continuous	Recoded to binary/dichotomous	Analysed as temperature ≥ 37.5^{o}C (fever present) or <37.5^{o}C (fever absent)

Antibody titres for this secondary study were initially entered in excel spreadsheets (Microsoft windows 2013) for the subset (sample) of participants that were randomly generated for each dosing frequency of IPTp-SP.

So in carrying out the secondary analysis in this thesis, use was made of the unique identifier for each study participant to link immunological data generated in the secondary study, to other laboratory, socio-demographic, morbidity and mortality data already collected in the primary study (figure 4).

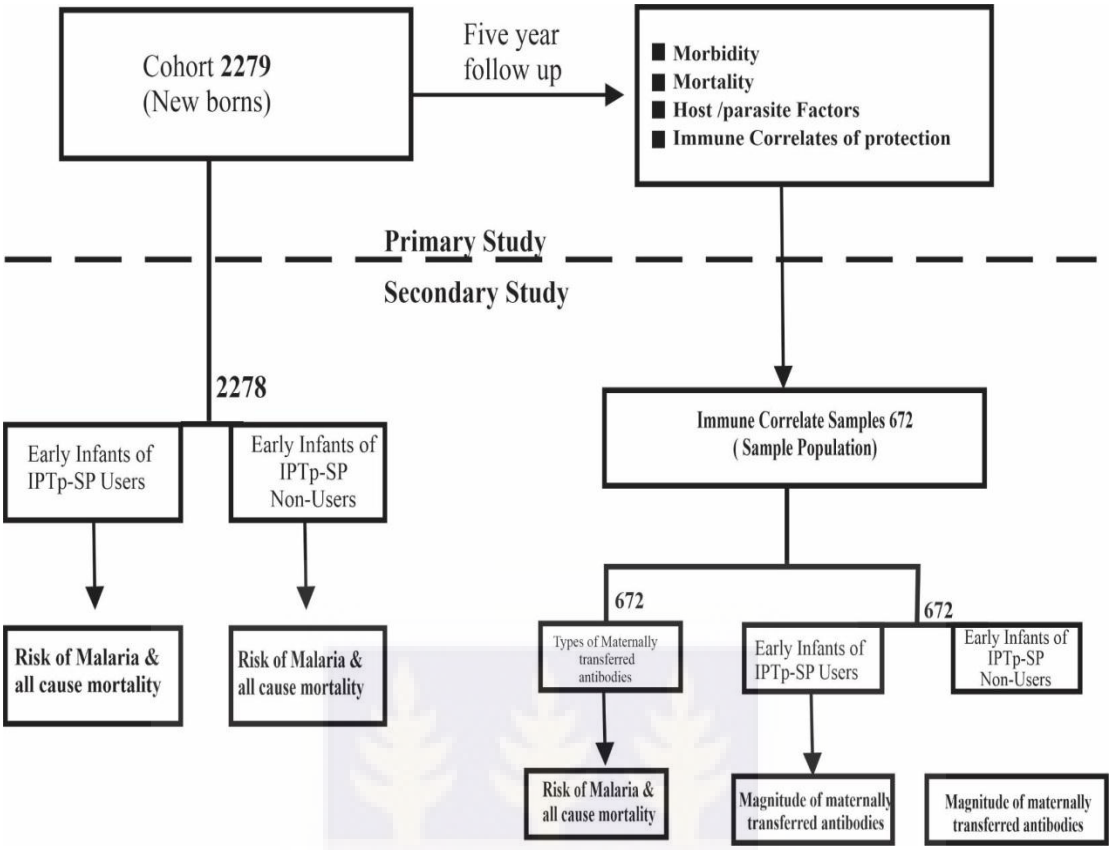


Figure 4 Relationship between main or primary (1^0) study and secondary (2^0) study

3.3 The Study area

The KND has been divided into two districts (KNDs) (Dalaba et al., 2014). The KNDs are about 1679km^2 and located between latitudes 10~30 and 11'00'North of the equator and between longitudes 1'00' and 1' 30 west of the zero meridian (Figure 5) and up to 400m above sea level. The area is of the guinea savanna vegetation type, hyperendemic for malaria with marked seasonal variations (Koram, et al., 2003; Binka et al., 1995). Malaria attack rates at the time of the study averaged 3.5 per child per year (Oduro et al., 2007) with incidence density in children less than five years being 1.56 per p-yr at risk. The area has an annual rainfall of about 850mm most of which falls between the months of May and October, with a relatively dry epoch between November and April. A large irrigation Dam and several small water impoundments, hand dug wells and boreholes allow for year round transmission of malaria. The area has a population of about 153000 inhabitants living in approximately 15000 dispersed compounds in 46 villages with 4000 live births

per annum. The population is mainly rural with subsistence farming being the main occupation.There are three main ethnic groups; Nankanis, Kassenas and Bulsas (Oduro et al., 2010).

Seroprevalence of Human immunodeficiency virus (HIV) amongst antinatal care attendees was 3.2% and intermittent preventive treatment in pregnancy (IPTp-SP) coverage was 38% amongst mothers of the cohort in 2006 (Oduro et al., 2010). Oduro and colleagues (2004) reported 20% clinical resistance to SP in the study area. The area has a district hospital, eight health centers and two private clinics, which provide curative and preventive health care services to the community. There are as many as 28 Community-based Health Planning and Services (CHPS) compounds located in various communities that provide primary health care services including reproductive health care. Births, deaths, in and out migrations are monitored and updated quarterly, by the Navrongo Demographic and Health Surveillance System (NDSS) (Chatio et al., 2015; Oduro et al., 2010).

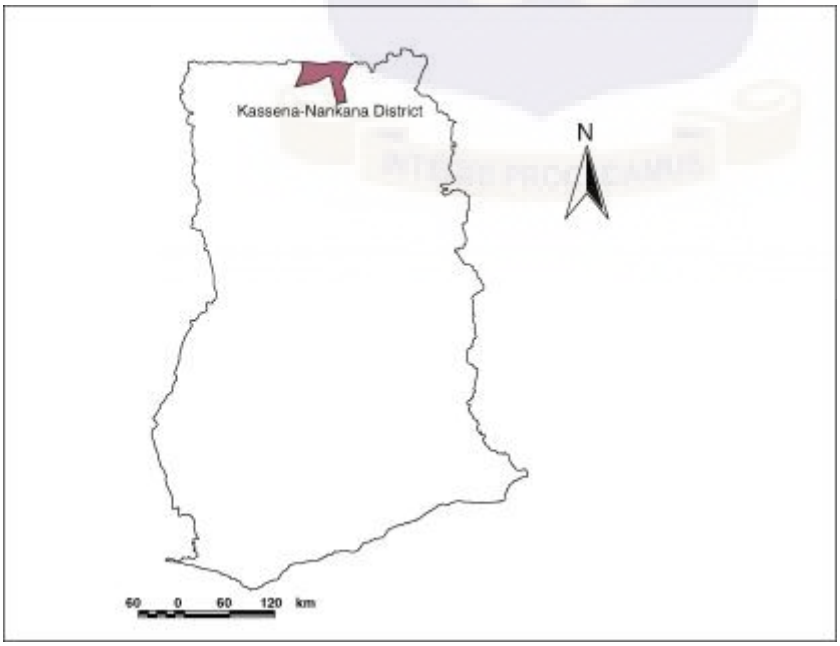


Figure 5 location of study area
Source:(Adjuik et al., 2010)

3.4 Study population

The study population consisted of newborns between March 2006 and March 2007 in the KNDs.

3.5. Sample size determination & Sampling

To estimate risk of malaria and all cause mortality in early infants and IPTp-SP use for this secondary study, data for the whole cohort of 2279 was analyzed. However to determine differences in antibody titres and IPTp-SP use a sample population of the cohort was used for logistic reasons. To calculate sample size for determining differences in antibody titres with IPTp-SP use in this secondary study, reference is made to previous immunoepidemiological work in the study site where Dodoo and colleagues (2011) reported prevalences of antibody up to 30%. Assuming an elevated prevalence of 20% in the newborns of mothers of the IPTp-SP group, using a two sample t-test with 5.0% level of significance, a power of 80%, then a minimum sample of 94 per group was required (Charan & Biswas, 2013). The formula is represented below.

$$\text{Sample size} = \frac{2(Z_{\alpha/2}+Z_{\beta})^2 P(1-P)}{(P1-P2)^2}$$

$Z_{\alpha/2}=Z_{0.05/2}=Z_{0.025}=1.96$ (from Z table) @ type1 error of 5%

$Z_{\beta}=Z_{0.02}=0.842$(from Z table) @ 80% power

(P1-P2) = difference in proportion of events in two groups

P= pooled prevalence [prevalence in infants of mothers who used IPTp-SP+ prevalence in infants of mothers who did not use IPTp-SP]/2

Therefore, the minimum sample size to allow for between group comparisons for the four groups was 376 (94 for each of the four groups below):

1. Newborns whose mothers never had IPTp-SP.

2. Newborns of mothers who had one dosing frequency of IPTp-SP

3. Newborns of mothers who had two doses r of IPTp-SP.

4. Newborns of mothers who had three or more doses of IPTp-SP.

Allowing for 50% losses in processing and inadequate sample volumes, 143 samples (rounded to 200) were required for each of the four arms.

Using the existing database and unique Identifiers of participants, random samples of 200 participants were generated for each of the four groups (random sampling stratified on IPT-SP use). Sampling was done using Stata statistical software (version 13.2) by the unblinded statistician.The technicians and investigators were however blinded to the IPTp-SP groups.

Unique identifiers of this random sample of 800 were then used to retrieve and analyze archived plasma of newborns enrolled into the birth cohort study.

3.6 Laboratory work in the secondary study

Antibodies to the following antigens, were considered; merozoite surface protein 3 (MSP3), amino terminal of the merozoite surface protein 3 (N-MSP3), carboxy terminal of the merozoite surface protein 3 (C-MSP3), Glutamate rich protein-region 0 (GLURP-R0), Glutamate rich protein-region 2 (GLURP-R2), and blood stage malaria vaccine candidate GLURP-R0 fused to MSP3 (GMZ2). The choice of antigens was based on availability and prior published work on their protective effect on malaria. A list of the generated 800 unique identifiers was transferred to a spreadsheet and visual comparisons of these unique numbers were made with those of the archived samples. When the identifiers matched, the samples were retrieved from cold storage in batches and analyzed. 713 were assayed.

Total IgG levels to the aforementioned antigens were measured by indirect ELISA (Enzyme linked immunosorbent assay). Briefly, 100µl/well of a 0.5µg/ml antigen solution

in coating buffer (plain PBS, pH 7.4) was added to wells of a 96-well microtitre ELISA plate (Maxisorp Nunc, Denmark). Coated plates were kept in a refrigerator at 4°C overnight. Plates were then washed four times in washing buffer (PBS with 0.1% Tween-20) with 30 seconds incubation between each wash using the Biotek ELx 405 automated ELISA plate washer (Biotek Instruments, Winooski, VT; USA). The washed plates were padded dry on a tissue paper and blocked with 200µl of blocking buffer (PBS with 5% milk powder, 0.1% Tween-20) and incubated at room temperature for 1 hour. Plates were washed four times in washing buffer as described above and diluted plasma samples (1:1000 for C-MSP3, 1:500 for Glurp-R0, Glurp-R2, GMZ2, N-MSP3 and MSP3 samples) in sample dilution buffer (PBS with 1% milk powder, 0.1% Tween-20 and 0.02% Na-azide) was added at 100µl/wells in duplicates. To control for inter-assay and day-to-day variations in the procedure, each assay (ELISA plate) included a calibration curve obtained by a 3-fold titration of a pool of hyper immune plasma (Standard pool) known to be positive for total IgG to the specific malaria antigens tested. For antigens GMZ-2, Glurp-R2, N-MSP3, the standard pool was diluted 1:1000, and for Glurp-R0, C-MSP3, MSP3 it was diluted at 1:500. A buffer blank (dilution buffer) served as control for detection of background responses. The plates with the samples and standard pool plasma were incubated at room temperature for 2 hours in a humidified chamber after which they were washed four times in washing buffer as described.

Secondary antibody, goat anti-human IgG (H+L), HRPO conjugated (KPL, CA; USA), diluted at 1: 3,000 in conjugate dilution buffer (PBS with 1% milk powder, 0.1% Tween-20) was added to plates at 100µl/well. The plates with the conjugates were incubated for 1 hour at room temperature after which they were washed four times in washing buffer and padded dry. Bound secondary antibody was quantified by incubation with ready to use TMB plus2 (3, 3', 5, 5'-Tetramethylbenzidine) substrate (Kem-En-Tec Diagnosis A/S,

Taastrup, Denmark) for 5 minutes in the dark. The reaction was stopped with 100ul/well of 0.2 M H_2SO_4.

Optical density (OD) was read at 450 nm with a reference wavelength of 630 nm using a Biotek EL 808 ELISA plate reader (Biotek Instruments, Winooski, VT; USA). Optical density (OD) values for the test samples were converted into antibody units (AU) with the standard reference curves generated for each ELISA plate using a Microsoft Excel-based four parameters logistic curve-fitting application (ADAMSEL b040, Ed Remarque© 2009). 'Lows' were replaced with half of the lowest measured value for each antigen and 'highs' were replaced with twice the measured value for each antigen.

3.7 Quality control

The archived plasma for the secondary (2^0) study was analyzed in the Immunological laboratories of the Noguchi Memorial Institute for Medical Research (NMIMR) using established Standard Operating Procedures. Instruments were caliberated, controls were run and equipment validated. Data entry into spreadsheets conformed to good laboratory practices.

3.8 Operational definitions

Time to first parasitaemia was defined as the time from enrolment to the time a first slide positive malaria diagnosis was made for study participant at a health facility for perceived illness.

- A case of uncomplicated malaria was defined as axillary temperature of ≥37.5^{o}Cc or a history of fever in the preceding 48 hours and presence of malarial parasitaemia and no other obvious cause for the fever at presentation (NMCP, 2016).
- Severe malaria was defined as axillary temperature of ≥37.5^{o}C or a history of fever in the preceding 48 hours and presence of malarial parasitaemia and no other

obvious cause for the fever at presentation, plus at least one of the danger signs for severe malaria in table 3 below (NMCP, 2016).

- Severe malaria anaemia was defined as parasitaemia with Haemoglobin <6.0g/dl and no other obvious cause for the anaemia at presentation (Ansah et al., 2009).

Table 2 Danger signs of severe malaria

Danger signs of severe malaria	
• Impaired consciousness (including unrousable coma). • Prostration, i.e. generalized weakness so that the patient is unable to sit, stand or walk without assistance. • Multiple convulsions: more than two episodes within 24h. • Deep breathing/ respiratory distress (acidotic breathing). • Acute kidney injury. • Clinical jaundice plus evidence of other vital organ dysfunction. • Abnormal bleeding.	• Circulatory collapse or shock, systolic blood pressure < 80mm Hg in adults and < 50mm Hg in children. • Acute pulmonary oedema/acute respiratory distress syndrome • Hypoglycaemia (< 2.2mmol/l or < 40mg/dl) • Metabolic acidosis (plasma bicarbonate < 15mmol/l) • Severe normocytic anaemia (haemoglobin < 5g/dl, packed cell volume < 15% in children Haemoglobinuria; • Hyperlactataemia (lactate > 5mmol/l) • Renal impairment (serum creatinine > 265µmol/l)

(WHO, 2012)

3.9 Key variables

Table 3 Key Variables

Independent/Exposure variables	Dependent/outcome variables	Control/Co -variates
• IPTp-SP use • Types of maternally transferred antibodies	• Malaria -parasitaemia -uncomplicated malaria -complicated/severe malaria • All cause mortality • Magnitude of maternally transferred antibodies	• Sex • Age • Birthseason • Bednet use • Mothers parity • Location • Residence

3.10 Statistical methods

Data was examined for normal distributions and skewedness via histogram distributions. Continuous variables were then summarized as means, with their accompanying standard deviations, where the distributions of these variables were normal (birth weight, haemoglobin). In the case of skewed continuous data (antibody titres, parasite counts),

variables were either summarized and reported as medians with interquartile ranges (antibody titres) or manipulated by transformation to mimic a normal distribution (antibody titres to log10 values and malaria parasite counts to geometric mean values). Variables were then compared for statistical significance using the student's t-test or F-test.

Categorical variables were summarized as frequencies or proportions (gender, birth season, parasitaemia status, residence, location, bednet use, parity, education level, IPTp-SP use). To test for associations between these categorical variables, Pearson Chi squared tests were employed.

For the multivariate model, all participants were censored at age 6 months after birth (data analyses restricted to first 6 months of extra uterine life-early infancy, whether outcomes of interest were realised or not) or date of death or date of loss to follow up. The person time at risk was then calculated for each infant and the crude rate of incidence was estimated for parasitaemia, uncomplicated malaria, severe malaria, (including severe malaria), and mortality by mothers IPTp-SP status. These incidence rates were important in incorporation into the hazards model and in calculating risk of the outcomes. For time to first parasitaemia, early infants with parasitaemia at birth were dropped from the analysis. One mother, whose IPTp-SP status could not be determined from the database, was also dropped from the analyses.

The proportional hazards model (cox regression) was used to compare the risk of time to parasitaemia, uncomplicated malaria, severe malaria, (including severe malaria anaemia) and mortality amongst infants born to mothers with differing dosing frequency of IPTp-SP and those who did not.

Informed by the pathobiology of malaria, prior exploratory analyses (appendix A) and published literature, age, gender, location, residence, bednet use, parity, season of birth,

and birth weight were considered in the model as potential confounders *apriori*. The survival analyses techniques employed, found expression in Kaplan-Meier (KM) plots. Proportional Hazards tests were performed using the Log rank tests for the KM plots and the Cox models for goodness of fit model.

These KM plots were tested for significance, using the Log rank tests to test the constant of proportionality assumption (chi square test for trends). Where variables failed the cox proportional hazards test, stratified results were reported as well; risk of parasitaemia was stratified by bednet use, birth season; risk of uncomplicated malaria was stratified by birth season and residence; risk of severe malaria was stratified by birth season; risk of severe malaria anaemia stratified by bednet use, birth season, mothers's education and risk of all cause mortality stratified by location. Up to this point, all the 2279 study participants in the primary study were considered in the secondary analysis.

For the immunological component of this secondary study, the subset (sample population) of the data (672) was used. Median titres of maternally transferred antibodies were calculated over all and then by IPTp-SP dosing frequency and displayed graphically. One-way analysis of variance (ANOVA) was performed to determine if median levels of antibody were different between the various SP groups (IPTp-SP dosing frequency) and Kruskal-Wallis test was used to test for statistically significant differences between groups. Simple linear regression was used to assess the correlation between log transformed antibody titres and IPTp-SP dosing frequency. Antibody titres were log transformed (base 10), box plots generated and comparisons made between log transformed antibody titres for the malaria antigens and IPTp-SP dosing frequency. Similarly, comparisons were made between log transformed antibodies and mothers' education, bednet use, infants' sex, birth weight and birth season. The choice of these covariates was informed by the results from prior analyses of the primary cohort study

(appendix A) and published literature as espoused in the conceptual framework (section 1.3). Antibody titres were log transformed (base 10), so that the effect on risk of malaria and all-cause mortality could be associated with a ten-fold increase in the titres. The likelihood of high and low antibody levels by SP use (IPTp-SP dosing frequency) and the risk of parasitaemia, uncomplicated malaria, severe malaria and all-cause mortality were again determined, using cox regression modeling (survival analyses techniques).

Only covariates at a significance level of at least 5% in the unadjusted model (crude rates) were included in the final adjusted model. Adjusted rates were determined by controlling for gender (sex), season of birth, bednet use and informed by results of prior analyses from the primary study (appendix A) and published literature as espoused in the conceptual framework (section1.3). The significance level of 5% and a 95% confidence interval were employed throughout the analyses. Table 4 below shows a summary of variable associations investigated and appropriate tests of significance applied.

Table 4 Variable associations with tests of statistical significance

Variable associations	Type of analysis	Test statistic	Level of statistical significance (α) and confidence interval (CI)
Exploratory analyses			
IPTp-SP dosing frequency and Birthweight, IPTp-SP dosing frequency and parasitaemia, IPTp-SP dosing frequency and gender, IPTp-SP dosing frequency and birth-season, IPTp-SP dosing frequencys and residence, IPTp-SP dosing frequency and location, IPTp-SP dosing frequency and bednet use, IPTp-SP dosing frequency and gravidity, IPTp-SP dosing frequency and formal education	Cross tabulations	Pearson χ^2	0.05 and 95%CI
Multivariate analyses for the whole cohort (N=2278)			
Association between ITPp-SP dosing frequencys and risk of parasitaemia, Association between ITPp-SP dosing frequency and uncomplicated malaria, Association between ITPp-SP dosing frequency and severe malaria Association between ITPp-SP dosing frequency and all cause mortality	Cox proportional hazards model Kaplan-Meier (KM) Plots	Wald test for crude and adjusted rates Log rank test for KM plots	0.05 and 95%CI
Multivariate analyses for immunological study (N=672)			
Association between tenfold increase in antibody titre (log10) and risk of parasitaemia. Association between tenfold increase in antibody titre and uncomplicated malaria. Association between tenfold increase in antibody titre and severe malaria. Association between tenfold increase in antibody titre and all cause mortality.	Cox proportional hazards model	Wald test for crude and adjusted rates	0.05 and 95%CI

3.11 Ethical considerations

In the primary study, which was carried out between 2006 and 2011, ethical approvals were obtained from the Navrongo Health Research Centre Institutional Review Board (NHRC-IRB) -IRB No: **NHRC045** (appendix D_i) & Noguchi Memorial Institute for

Medical Research-Institutional Review Board (NMIMR-IRB) - IRB No: **NMIMR-IRB CPN 006/05-06** (appendix D_{ii}). Written informed consent was sought from mothers of newborns before enrolment into the study (Appendix C). Treatment was free for acute illnesses and according to Ghana Health Service treatment guidelines and the study was conducted in the spirit of Good clinical and laboratory practices.

As the original consent form (Appendix C) did not include the use of data and samples of study participants for purposes other than the primary study, ethical clearance was again sought for this secondary study as well. Ethical clearance for this study was sought and obtained from the Ghana Health Service-Ethics Review Committee (GHS-ERC) - ERC No: **GHS-ERC:04/09/15** (appendix D_{iii}), Navrongo Health Research Centre-Institutional Review Board (NHRC-IRB) - IRB No: **NHRCIRB208** (appendix D_{iv}) and the Noguchi Memorial Institute for Medical Research-Institutional Review Board (NMIMR-IRB) -: **NMIMR-IRB CPN 001/15-16** (appendix D_{v}). The unique personal identifiers and not participant names were employed in the analysis. Anonymized samples were used.

CHAPTER FOUR

4.0 RESULTS

This chapter presents results for this secondary study, by first reporting summary statistics and frequency distributions of some characteristics of both the whole cohort and the subset (sample population). This is followed by a description and analyses of some background characteristics of the whole dataset (as a secondary study) in an exploratory fashion.

Next to be reported are the descriptive and then the analytical aspects of the whole cohort (as a secondary study), looking at the associations between the IPTp-SP dosing frequency and the risk of malaria and all cause mortality in early infancy.

Results for the immunological component of the study (employing a sample population) then follow looking at the associations between IPTp-SP dosing frequency and magnitude of maternally transferred antibody as well as the associations between these antibodies (irrespective of IPTp-SP use) and risk of malaria and all cause mortality in early infancy.

4.1 Exploratory Analyses

4.1.1 Baseline characteristics for the cohort

For the whole dataset, out of a total of 2279 newborns that were enrolled, one mother's IPTp-SP status could not be ascertained. She was therefore dropped from the secondary analyses and the final sample size analyzed was 2278.

With reference to table 5, about 17% (391) of newborns were born to mothers who never had IPTp-SP. Female newborns enrolled were about 51% (1155). Over 80% (1577) of the enrolled newborns were normal weight (2.5kg) or above. Another 2% (47) were parasitaemic at birth, with more than half of study participants enrolled in the high transmission season (1325). Over 85% (1968) of the enrolled newborns were rural dwellers and most were from the non irrigated parts of the district (1897). Only about 16% (381) were from the irrigated areas of the district and about that same proportion of

mothers had had no IPT-SP. Bednet use amongst the mothers of these newborns was over 50% (1225). About a quarter of these mothers were primigravidae and newly half (47.37%) had had no formal education. These findings are comparable to those of the subset (sample population) in table 6, suggesting strongly that the sample population for the immunological component of this secondary study is representative of the primary cohort.

Mean enrolment weight was 2.88± 0.49 kg. Mean haemoglobin levels amongst the cohort at enrolment were 14.59±2.29 g/dl and geometric mean parasitaemia at enrolment was [296.3(95%CI: 183.69, 478.00)].

Table 5 Baseline characteristics in the birth cohort enrolled between 2006 and 2007 in the KNDs. N=2278

Characteristic		**[n]%**
Female		[1155]50.70
Birthweight ≥2.5kg		[1577]81.61
Born in High transmission season		[1325]58.17
Parasitaemic at enrollment		[47]2.06
Rural residence		[1968]86.39
Located in Irrigated area		[381]16.73
Bednet use		[1220]53.56
primigravidae		[551]24.19
No formal education (none)		[1079]47.37
IPTp-SP dosing frequency		
	0	[391]16.73
	1	[516]22.65
	2	[640]22.09
	3+	[731]32.00

Table 6 Baseline characteristics in a subset (sample population) of the birth cohort enrolled between 2006 and 2007 in the KNDs. N=672

Characteristic		[n]%
Female		[351]52.23
Birthweight ≥2.5kg		[547]81.40
Born in High transmission season		[406]60.42
Parasitaemic at enrollment		[15]2.23
Rural residence		[564]83.93
Located in Irrigated area		[124]18.45
Bednet use		[376]55.95
primigravidae		[175]26.04
No formal education (none)		[310]44.49
IPTp-SP dosing frequency		
	0	[163]24.26
	1	[165]24.55
	2	[170]25.30
	3+	[174]25.89

Baseline characteristics were comparable between the two groups (tables 5 & 6) suggesting strongly the sample population was representative of the whole cohort.

There were significant differences between the numbers enrolled in the high transmission season and the low transmission season (table 7); with significantly more newborns enrolled in the high transmission season compared to the low transmission season. Of those infants whose mothers had never had IPTp-SP, 70% (1325) of them were born in the high transmission season compared to 30% (953) in the low transmission season. Fewer mothers of infants born in the high transmission season were making it to the higher dosing frequency of IPTp-SP and the converse was true for mothers of newborns in the low transmission season. Similarly, there was a significant difference in the residence status of study participants. Of those infants whose mothers had never had IPTp-SP, rural dwellers constituted over 90% (395). Fewer rural dwellers were making it to higher dosing frequency for IPTp-SP compared to urban dwellers. Educational level and mothers' SP use also registered statistical significance. Amongst those mothers who never had IPTp-SP, less than half had had any formal education (197). In this group those making it for one

dose of IPTp-SP were about 23% and only just improved to about 30% and fell a notch below to 29% for the second and third doses of IPTp-SP. For mothers with basic education, even though the proportion not receiving any IPTp-SP was lower than for the non-educated group (44.5%) those mothers who received IPTp-SP for each of one, two or three or more doses was over 40%. For mothers with secondary education and above, there was a steady increase in the proportions receiving higher dosing frequency even though this was the smallest group. There were no significant associations between Birth weight (<2.5kg and ≥2.5kg), gender, location, parasitaemia status at birth, bednet use, gravidity and IPTp-SP use. These results are comparable to earlier analyses of the associations between these characteristics and the 0, 1+ IPTp-SP groups (appendix Aii).

Table 7 Associations between some maternal and infant characteristics and IPT-SP use in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	0 IPTp-SP [n]%	1 IPTp-SP [n]%	2 IPTp-SP [n]%	≥3+IPTp-SP [n]%	χ^2	P value
Birth weight>2.5kg	[391] 80.56	[515]82.56	[640]79.37	[31] 83.45		
Birth weight<2.5kg[n]%	[76]19.44	[90]17.44	[132]20.63	[121]16.55	4.37	0.224
Parasitaemic at birth[n]%	[8]2.05	[11]2.13	[14]2.19	[14]1.92		
Aparasitaemic at birth[n]%	[383]97.95	[505]97.87	[626]97.81	[717]98.08	0.14	0.987
Sex[n]%						
male	[192]49.10	[237]45.93	[331]51.72	[363]49.66		
female	[199]50.90	[279]54.07	[309]48.28	[368]50.34	3.89	0.274
Birth season [n]%						
High transmission	[271]69.31	[301]58.33	[379]59.22	[374]51.16		
Low transmission	[120]30.69	[215]41.67	[261]40.78	[357]48.84	34.98	<0.001
Residence [n]%						
urban	[32]8.18	[59]11.43	[81]12.66	[138]18.88		
rural	(359]91.82	[457]88.57	[559]87.34	[593]81.12	0.22	<0.001
Location[n]%						
Non-irrigated	[312]79.80	[436]84.50	[537]83.91	[612]83.72		
irrigated	[79]20.20	[80]15.50	[103]16.09	[119]16.28	4.24	0.237
Bednet use[n]%						
No	[194]49.62	[255]49.42	[291]45.47	[318]43.50		
Yes	[197]50.38	[261]50.58	[349]54.53	[413] 56.50	6.21	0.102
Maternal education [n]%						
Primigravidae	[86]21.99	[114]22.09	[158]24.69	[193]26.40		
secundigravidae	[81]20.72	[112]21.71	[118]18.44	[162]22.16		
Multigravidae	[224]57.29	[290]56.20	[364]56.88	[376]51.44	8.44	
Formal Education[n]%						
None	[194]49.62	[246]22.8	[322]29.84	[(317]29.38		
Basic	[174]44.50	[235]45.54	[272]42.50	[308]42.13		
≥ secondary	[23]5.88	[35]6.78	[46]7.19	[106]14.50	38.16	<0.001

4.2 IPTp-SP use (dosing frequency) and incidences of parasitaemia, uncomplicated malaria, severe malaria and all cause mortality in their early infants (offspring) in the KNDs.

Table 8 IPTp-SP use (dosing frequency) and incidence of malaria parasitaemia in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time/years	Rate/1000-person years(95%CI)
0 IPTp-SP	132	2.24	58.89 (49.66,69.85)
1 IPTp-SP	95	2.96	32.09 (26.24,39.23)
2 IPTp-SP	119	3.67	32.38 (27.06,38.76)
3+IPTp-SP	120	4.21	28.53 (23.72,33.98)

Compared to early infants of mothers who had no IPTp-SP, incidence of parasitaemia in early infants of those who had 1, 2, or 3 or more dosing frequency of IPTp-SP was about twice less. Notably, there were no significant differences in incidence of parasitaemia with increasing dosing frequency (table 8 above).

Table 9 IPTp-SP use (dosing frequency) and incidence of uncomplicated malaria in early infants and in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time/years	Rate/1000-person years(95%CI)
0 IPTp-SP	71	2.29	31.02 (24.59,39.15)
1 IPTp-SP	51	3.03	16.86 (12.81,22.18)
2 IPTp-SP	68	3.75	18.12 (14.28,22.98)
3+ IPTp-SP	65	4.28	15.16 (11.89,19.33)

Here too, there was about a 50% reduction in incidence of uncomplicated malaria in early infants of those who had any of the dosing frequencies, compared to early infants whose mothers had no IPTP-SP. The decrease in incidence as dosing frequency of IPTp-SP increased only reached statisitical significance with the three or more doses **(table 9**).

Table 10 IPTp-SP use (dosing frequency) and incidence of severe malaria in early infants and in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time/years	Rate/1000-person years(95%CI)
0 IPTp-SP	103	2.29	45.00 (37.10,54.59)
1 IPTp-SP	70	3.03	23.14 (18.30,29.24)
2 IPTp-SP	84	3.8	22.38 (18.07,27.72)
3+ IPTp-SP	87	4.3	20.29 (16.44,25.03)

The incidence of severe malaria for early infants of mothers who had no IPTp-SP, was twice that of early infants of those who had any of the dosing frequency. Again, there were no significant differences in incidence of severe malaria between those who had 1, 2 or 3+ doses, even though there was a decreasing trend as IPTp-SP dosing frequency increased (Table 10)

Table 11 IPTp-SP use (dosing frequency) and Incidence of severe malaria anaemia in early infants in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time/years	Rate/1000-person years(95%CI)
0 IPTp-SP	5	2.29	2.29 (0.91,5.25)
1 IPTp-SP	3	3.03	0.99 (0.32,3.07)
2 IPTp-SP	2	3.75	0.53 (0.13,2.13)
3+ IPTp-SP	2	4.29	0.47 (0.11,1.86)

A subset analysis on the incidence of severe malaria anaemia for early infants of mothers who had no IPTp-SP, showed that the incidence of severe malaria anaemia was two fold in the 0 IPTp-SP group, while there appeared to to less risk of severe malaria anaemia by 1%, 47% and 53% amongst those whose mothers had the 1, 2, 3+ dosing frequency respectively. Even though incidence decreased with increase in dosing frequency of IPTp-SP, this was not statistically significant (table 11).

Table 12 IPTp-SP use (dosing frequency) and incidence of all cause mortality in early infants in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	no of events	Person time/years	Rate/1000-person years(95%CI)
0 IPTp-SP	7	2.29	3.06 (1.46,6.42)
1 IPTp-SP	5	3.03	1.65 (0.69,3.97)
2 IPTp-SP	9	3.75	2.39 (1.24,4.60)
3+ IPTp-SP	9	4.29	2.10 (1.09,4.03)

There was an initial reduction in the incidence of all cause mortality at one IPTp-SP dosing frequency compared to early infants who had no IPTp-SP but subsequently increased with no clear pattern at the 2, and 3+dosing frequencies. However, these findings were not statistically significant (table 12).4.3 Mothers use of IPTp and hazards of parasitaemia, uncomplicated malaria, severe malaria and all cause mortality in their early infants (offspring) in the KNDs.

Table 13. Hazard ratios for malaria parasitaemia in early infancy for a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Crude hazard ratio (95%CI)	p-Value[w]	Adjusted[a] hazard ratio (95%CI)	p-Value[w]
IPTp-SP dosing frequency					
0 IPTp-SP	[383]	1.0			
1 IPTp-SP	[505]	0.55 (0.41,0.72)	<0.001	0.60 (0.45,0.79)	<0.001
2 IPTp-SP	[626]	0.55 (0.42,0.72)	<0.001	0.60 (0.47,0.81)	<0.001
3+IPTp-SP	[717]	0.48 (0.37,0.63)	<0.001	0.62 (0.34,0.55)	0.001
Maternal education					
None	[1055]	1.0		1.0	
Basic	[937]	0.75 (0.61,0.93)	0.008	0.83 (0.68,1.03)	0.092
≥secondary	[203]	0.37 (0.23, 0.60)	<0.001	0.67 (0.42,1.08)	0.101
Gravidity					
Primigravidae	[539]	1.0			
Secundigravidae	[462]	1.20 (0.86,1.63)	0.260	-	-
Multigravidae	[1230]	1.18 (0.91,1.51)	0.207	-	-
Bednet use					
yes	[1192]	1.0		1.0	
no	[1039]	1.88 (1.54,2.31)	<0.001	1.80 (1.47,2.21)	<0.001
Sex					
Male	[1101]	1.0		1.0	
Female	[1130]	1.35(1.10,1.65)	0.004	1.32 (1.10,1.61)	0.005
Birth weight					
≥2.5kg	[1825]	1.0			
<2.5kg	[406]	1.05(0.80,.1.39)	0.683	-	-
Birth season					
High transmission	[1291]	1.0		1.0	
Low transmission	[940]	0.41 (0.32,0.52)	<0.001	0.41 (0.32,0.53)	<0.001
location					
Non-irrigated	[1856]	1.0		-	-
irrigated	[375]	1.02 (0.78,1.32)	0.907	-	-
Residence					
Urban	[305]	1.0			
Rural	[1926]	3.95 (2.32,6.72)	<0.001	3.59 (2.06,6.29)	<0.001

W=Wald p value a=Adjusted for residence, birth season, sex, bednet use, maternal education, IPTp-SP. 1.0=referent

After adjusting for covariates (residence, birth season, sex, bednet use, maternal education) in the cox model, use of IPTp-SP was associated with 40% less hazards of parasitaemia, [HR=0.60(95%CI= 0.45,0.79), P<001]. Higher dosing frequency of 3+IPTp-SP did not confer added protection at 38% less hazards. Mothers' education beyond the secondary level, bednet use, being male and being born in the low transmission season were also significantly associated with reduced hazards of parasitaemia (table 13).

Table 14 Hazard ratios for uncomplicated malaria in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Crude hazard ratio (95%CI)	p-Value[w]	Adjusted[a] hazard ratio(95%CI)	p-Value[w]
IPTp-SP dosing frquency					
0 IPTp-SP	[391]	1.0			
1 IPTp-SP	[516]	0.54 (0.38,0.78)	<0.001	0.59 (0.40,0.86)	0.005
2 IPTp-SP	[640]	0.58 (0.41,0.82)	<0.002	0.63 (0.44,0.90)	0.011
3+IPTp-SP	[731]	0.49 (0.34,0.69)	<0.001	0.61 (0.42,0.88)	0.008
Maternal education					
None	[1079]	1.0		1.0	
Basic	[989]	0.72 (0.55, 0.95)	0.019	0.84 (0.63,1.10)	0.200
≥Secondary	[21]	0.50 (0.28,0.87)	0.015	1.00 (0.56,1.81)	0.674
Gravidity					
Primigravidae	[551]	1.0			
Secundigravidae	[473]	1.07 (0.73,1.57)	0.724	-	-
Multigravidae	[1254]	1.02 (0.74,1.40)	0.892	-	-
Bednet use					
yes	[1220]				
no	[1058]	1.51 (1.18, 1.96)	0.001	1.47(1.14,1.91)	0.003
Sex					
Male	[1123]	1.0			
Female	[1155]	1.46 (1.13, 1.89)	0.003	1.45 (1.13,1.87)	0.004
Birth weight					
≥2.5kg	[1859]	1.0			
<2.5kg	[419]	1.11 (0.77,1.60)	0.565	-	-
Birth season					
High transmission	[1325]	1.0			
Low transmission	[953]	0.43 (0.32,0.59)	<0.001	0.40 (0.29,0.54)	<0.001
location					
Non-irrigated	[1879]	1.0			
Irrigated	[381]	1.23 (0.89,1.71)	0.208	-	-
Residence					
Urban	[310]	1.0			
Rural	[1968]	4.87 (2.43,9.79)	<0.001	4.37 (2.10,9.08)	<0.001

W=Wald p value a=Adjusted for residence, birth season, sex, bednet use, maternal education, IPTp-SP. 1.0=referent

After adjusting for covariates (residence, birth season, sex, bednet use, maternal education) in the cox model, use of IPTp-SP was associated with 41% [HR=0.59(95%CI 0.40,0.86), p=005], 36% [HR=0.63(95%CI=0.41,0.84), P=011] and 39% [HR=0.61(95%CI =0.42,0.88), P=008] less hazards of uncomplicated malaria for early infants of mothers who had 1,2 and 3+ dosing frequency of IPTp-SP respectively, compared to those who had none. Beyond the first dose the decrease in risk of uncomplicated malaria was not significant with higher dosing frequencies of IPTp-SP. Bednet use, being male and being born in the low transmission season were also

significantly associated with less hazards of uncomplicated malaria. Here again, maternal education was not significantly associated with reduced hazards of uncomplicated malaria in their early infants (table 14).

Table 15 Hazard ratios for severe malaria in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Crude hazard ratios (95%CI)	p-Value	Adjusted[a] hazard ratios (95%CI)	p-Value[w]
IPTp-SP dosing frequency					
0 IPTp-SP	[391]	1.0			
2 IPTp-SP	[516]	0.51 (0.37,0.71)	<0.001	0.58 (0.41,0.80)	<0.001
2 IPTp-SP	[640]	0.50 (0.37,0.67)	<0.001	0.52 (0.38,0.71)	<0.001
3+IPTp-SP	[731]	0.45 (0.33,0.61)	<0.001	0.52 (0.38,0.71)	<0.001
Maternal education					
None	[1079]	1.0			
Basic	[989]	0.70 (0.56,0.90)	0.004	0.82 (0.64,1.04)	0.107
≥Secondary	[21]	0.47 (0.29,0.76)	0.002	0.74 (0.45,1.23)	0.251
Gravidity					
Primigravidae	[551]	1.0			
Secundigravidae	[473]	1.03 (0.73,1.44)	0.882	-	-
Multigravidae	[1254]	1.01 (0.77,1.33)	0.915	-	-
Bednet use					
yes	[1220]	1.0			
no	[1058]	1.68 (1.34,2.10)	<0.001	1.34 (1.06,1.67)	0.014
Sex					
Male	[1123]	1.0			
Female	[1155]	1.53(1.22,1.92)	<0.001	1.49 (1.19,1.86)	<0.001
Birth weight					
≥2.5kg	[1859]	1.0			
<2.5kg	[419]	1.10 (0.81,1.51)	0.536		
Birth season					
High transmission	[1325]	1.0			
Low transmission	[953]	0.42 (0.32,0.55)	<0.001	0.44 (0.34,0.58)	<0.001
location					
Non-irrigated	[1879]	1.0			
Irrigated	[381]	1.02 (0.75,1.38)	0.895	-	-
Residence					
Urban	[310]	1.0			
Rural	[1968]	4.73 (2.65,8.64)	<0.001		

W=Wald p value a=Adjusted for residence, birth season, sex, bednet use, maternal education, IPTp-SP. 1.0=referent

After adjusting for covariates (residence, birth season, sex, bednet use, maternal education) in the cox model, compared to 0 IPTp-SP dosing frequency, use of IPTp-SP was significantly associated with less hazards of severe malaria in their early infants for IPTp-SP dosing frequency 1, 2, and 3+.Increasing dosing frequencies beyond 1 IPTp-SP did not significantly reduce the hazards of severe malaria amongst their early infants. Also

significantly associated with reduced hazards of severe malaria, were bednet use, being male and being born in the low transmission season (Table 15).

Table 16 Hazard ratios for severe malaria anaemia in early infancy for a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Crude hazard ratios (95%CI)	p-Value[w]	Adjusted[a] hazard ratios (95%CI)	p-Value[w]
IPTp-SP dosing frequency					
0 IPTp-SP	[391]	1.0			
1 IPTp-SP	[516]	0.45 (0.11,1.88)	0.278	-	-
2 IPTp-SP	[640]	0.24 (0.05,1.25)	0.091	-	-
3+ IPTp-SP	[731]	0.21 (0.04,1.09)	0.064	-	-
Maternal education					
None	[1079]	1.0		1.0	
Primary	[989]	0.36(0.10,1.34)	0.129	-	-
≥Secondary	[21]	3.36e-16(1.73e-16, 6.53e-16)	<0.001	7.28e-20(-)	-
Gravidity					
primigravidae	[551]	1.0			
secundigravidae	[473]	0.29 (0.03,2.60)	0.270	-	-
Multigravidae	[1254]	0.76 (0.23,2.60)	0.671	-	-
Bednet use					
yes	[1220]	1.0			
no	[1058]	3.45 (0.94,12.72)	0.063	-	-
Sex					
Male	[1123]	1.0			
Female	[1155]	1.37 (0.44,4.30)	0.592	-	-
Birthweight					
≥2.5kg	[1859]	1.0			
<2.5kg	[419]	0.66 (0.18,2.42)	0.528	-	-
Birth season					
High transmission	[1325]	1.0		1.0	
Low transmission	[953]	0.13 (0.16,0.97)	0.047	0.13 (0.016,0.1.10)	0.062
location					
Non-irrigated	[1879]	1.0		-	-
Irrigated	[381]	0.99 (0.22, 4.49)	0.985	-	-
Residence					
Urban	[310]	1.0			
Rural	[1968]	$2.01e^{+15}$ ($9.46e+^{14}$,$4.26e+^{15}$,)	<0.001	2.60e+16(-)	-

W=Wald p value a=Adjusted for birth season, mothers' education, residence. 1.0=referent

After adjusting for covariates (residence, birth season, sex, maternal education) IPTp-SP use was not associated with risk of severe malaria anaemia. Neither were the covariates adjusted for associated with a decrease or increase in risk of severe malaria anaemia (Table 16).

Table 17 Hazard ratios for all cause mortality in early infancy for a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Crude hazard ratios (95%CI)	p-Values[w]	Adjusted[a] hazard ratios (95%CI)	p-Values[w]
IPTp-SP dosing frequency					
0 IPTp-SP	[391]	1.0		1.0	
1 IPTp-SP	[516]	0.54 (0.17,1.70)	0.292	-	-
2 IPTp-SP	[640]	0.78 (0.29,2.10)	0.628	-	-
3+IPTp-SP	[731]	0.69 (0.19,1.84)	0.455	-	-
Maternal education					
None	[1079]	1.0		-	-
Basic	[989]	1.46 (0.69,3.08)	0.324		
≥Secondary	[21]	0.86 (0.19,3.82)	0.838		
Gravidity		1.0			
Primigravidae	[551]	1.04 (0.40,2.70)	0.937	-	-
Secundigravidae	[473]	0.63 (0.27,1.48)	0.294	-	-
Multigravidae	[1254]				
Bednet use		1.0			
yes	[1220]	1.00 (0.49, 2.06)	0.986	-	-
no	[1058]				
Sex		1.0			
Male	[1123]	1.11 (0.54,2.28)	0.767		
Female	[1155]				
Birth weight		1.0			
≥2.5kg	[1859]	0.22 (0.11,0.45)	<0.001	0.25 (0.13,0.57)	<0.001
<2.5kg	[419]				
Birth season		1.0			
High transmission	[1325]	0.80 (0.38,1.68)	0.559	-	-
Low transmission	[953]				
location					
Non-irrigated	[1879]	0.18 (0.02,1.25,)	0.083	-	-
Irrigated	[381]				
Residence		1.0			
Urban	[310]			-	-
Rural	[1968]	1.03 (0.35,2.93)	0.962		

W=Wald p value a=Adjusted for birth weight. 1.0=referent

After adjusting for birth weight as a covariate, birthweight greater than or equal to 2.5kg was the only variable independently and significantly associated with reduction in all cause mortality in early infants in the KNDs by about 75% (table 17).

4.3 Tests of proportional hazards assumption for IPTp-SP use and hazards of parasitaemia, uncomplicated malaria, severe malaria and all cause mortality in their early infants (offspring) in the KNDs.

Table 18 Tests of proportional hazards assumption for malaria parasitaemia in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	χ^2 test statistic	P value
IPTp-SP dosing frequency		
1 IPTp-SP	0.34	0.560
2 IPTp-SP	3.16	0.075
3+ IPTp-SP	1.89	0.169
Transmission season	59.41	<0.001
Maternal education		
Basic education	0.03	0.856
≥Secondaryeducation	0.13	0.719
Bednet use	4.25	0.039
sex	0.22	0.640
Residence	0.00	0.967

P≤0.05 indicates variable in the hazards model failed the PH assumption test and had to be stratified on.

Table 19 Tests of proportional hazards assumption for uncomplicated malaria in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	χ^2 test statistic	P value
IPTp-SP dosing frequency		
1 IPTp-SP	2.02	0.155
2 IPTp-SP	1.58	0.209
3+ IPTp-SP	0.03	0.869
Transmission season	36.32	<0.001
Maternal education		
Basic education	1.09	0.297
≥Secondary education	0.02	0.882
Bednet use	0.61	0.434
sex	0.15	0.702
Residence	4.84	0.028

P≤0.05 indicates variable in the hazards model failed the PH assumption test and had to be stratified on.

Table 20 Tests of proportional hazards assumption for severe malaria in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	test statistic χ^2	P value
IPTp-SP dosing frequency		
1 IPTp-SP	0.02	0.893
2 IPTp-SP	3.42	0.064
3+ IPTp-SP	0.46	0.500
Transmission season	39.79	<0.001
Maternal education		
Basic education	0.38	0.536
≥Secondaryeducation	0.00	0.997
Bednet use	1.27	0.259
sex	0.02	0.896
Residence	0.42	0.517

P≤0.05 indicates variable in the hazards model failed the PH assumption test and had to be stratified on.

Table 21 Tests of proportional hazards assumption for severe malaria anaemia in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	χ^2 test statistic	P value
IPTp-SP dosing frequency		
1 IPTp-SP	1.19	0.275
2 IPTp-SP	0.37	0.545
3+ IPTp-SP	4.34	0.037
Transmission season	3.85	0.050
Maternal education		
Basic education	4.53	0.033
≥Secondaryeducation	1.0	_
Bednet use	11.26	0.001
Residence	1.0	_

P≤0.05 indicates variable in the hazards model failed the PH assumption test and had to be stratified on.

Table 22 Tests of proportional hazards assumption for all cause mortality in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	χ^2 test statistic	P value
IPTp-SP dosing frequency		
1 IPTp-SP	0.39	0.531
2 IPTp-SP	0.29	0.592
3+ IPTp-SP	0.01	0.913
Bednet use	1.62	0.203
location	4.57	0.033

P≤0.05 indicates variable in the hazards model failed the PH assumption test and had to be stratified on.

4.4 IPTp-SP use (dosing frequency) and stratified hazards of parasitaemia, uncomplicated malaria, severe malaria and all cause mortality in their early infants (offspring) in the KNDs.

Table 23 Stratified hazard ratios for malaria parasitaemia in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Stratified hazard ratios (95%CI)	p-Value[w]
IPTp-SP dosing frequency			
0 IPTp-SP	[391]	1.0	
1 IPTp-SP	[516]	0.60 (0.45,0.79)	<0.001
2 IPTp-SP	[640]	0.60 (0.46,0.79)	<0.001
3+ IPTp-SP	[731]	0.61 (0.47,0.81)	<0.001
Maternal Education			
None	[1079]	1.0	
Basic	[989]	0.84 (0.68,1.03)	0.092
Secondary+	[21]	0.67 (0.42,1.08)	0.103
Sex			
Male	[1123]	1.0	
Female	[1155]	1.32 (1.09,1.61)	0.005
Residence			
Urban	[310]	1.0	
Rural	[1968]	3.60 (2.06,6.30)	<0.001

Stratified by birth season, bednet use 1.0=referent

After stratifying by birthseason and bednet use, IPTp-SP, being male and living in the urban part of the district were still significantly associated with reduced hazards of parasitaemia amongst early infants in the KNDs (table 23 above).

Table 24 Stratified hazard ratios for uncomplicated malaria in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Stratified hazards(95%CI)	p-Value[w]
IPTp-SP dosing frequency			
0 IPTp-SP	[391]	1.0	
1 IPTp-SP	[516]	0.59 (0.44,0.86)	0.005
2 IPTp-SP	[640]	0.63 (0.44,0.90)	0.011
3+ IPTp-SP	[731]	0.61 (0.42,0.88)	0.008
Maternal Education			
None	[1079]	1.0	
Basic	[989]	0.84 (0.62,1.00)	0.203
Secondary+	[21]	1.02 (0.52,1.40)	0.946
Bednet Use			
Yes	[1220]	1.0	
No	[1058]	1.48 (1.14,1.91)	0.003
Sex			
Male	[1123]	1.0	
Female	[1155]	1.45 (1.13,1.87)	<0.004

Stratified by birth season and residence. 1.0=referent

After stratifying by birth season and residence, use of IPTp-SP, being male and bednet use in the district were still significantly associated with reduced hazards of uncomplicated malaria amongst early infants in the KNDs (table 24).

Table 25 Stratified hazard ratios for severe malaria in early infancy for a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Stratified hazards(95%CI)	p-Value[w]
IPTp-SP dosing frequency			
0 IPTp-SP	[391]	1.0	
1 IPTp-SP	[516]	0.58 (0.41,0.80)	0.001
2 IPTp-SP	[640]	0.52 (0.38,0.71)	<0.001
3+ IPTp-SP	[731]	0.52 (0.38,0.71)	<0.001
Maternal Education			
None	[1079]	1.0	
Basic	[989]	0.80 (0.62,1.00)	0.083
Secondary+	[21]	0.88 (0.52,1.40)	0.647
Bednet Use			
Yes	[1220]		
No	[1058]	1.60 (1.27,2.00)	<0.001
Sex			
Male	[1123]	1.0	
Female	[1155]	1.51 (1.21,1.88)	<0.001
Residence			
Urban	[310]	1.0	
Rural	[1968]	4.61 (2.48,8.55)	<0.001

Stratified by birth season. 1.0=referent

After stratifying by birthseason, use of IPTp-SP, being male, and living in the urban part of the district were still significantly associated with reduced hazards of severe malaria amongst early infants in the KNDs (table 25).

Table 26 Stratified hazard ratios for severe malaria anaemia in early infancy for a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Stratified hazard ratios (95%CI)	p-Values[w]
IPTp-SP dosing frquency			
0 IPTp-SP	[391]	1.0	
1 IPTp-SP	[516]	0.60 (0.14,2.60)	0.491
2 IPTp-SP	[640]	0.33 (0.06,1.68)	0.181
3+IPTp-SP	[731]	0.37 (0.07,1.99)	0.245
Residence			
Urban	[310]	1.0	
Rural	[1968]	3.37e+16(-)	-

Stratified by mbednet, birth season, mothers' education. 1.0 =referent

After stratifying by birthseason, bednet use and mothers' education, no significant associations were found between IPTp-SP use, residence and the risk of severe malaria anaemia amongst early infants in the KNDs (table 26).

Table 27. Stratified Hazard ratios for all cause mortality in early infancy for a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Statified hazard ratios (95%CI)	p-Values[w]
IPTp-SP dosing frequency			
0 IPTp-SP	[391]	1.0	
1 IPTp-SP	[516]	0.69 (0.23,2.13)	0.522
2 IPTp-SP	[640]	0.92 (0.34,2.47)	0.872
3+IPTp-SP	[731]	0.92 (0.35,2.41)	0.864
Bednet use			
Yes	[1220]	1.0	
No	[1058]	0,76 (0.35,2.47)	0.492
Birth weight			
<2.5kg	[1859]	1.0	
≥ 2.5kg	[419]	0.27 (0.13,0.57)	0.001

Stratified on location 1.0=referent

After stratifying by location, only birth weight greater than 2.5kg was significantly associated with the risk of all cause mortality amongst early infants in the KNDs (table 27).

4.5 IPTp-SP use (dosing frequency) and Kaplan -Meier (K-M) plots (survival curves) of parasitaemia, uncomplicated malaria, severe malaria and all cause mortality in their early infants (offspring) in the KNDs.

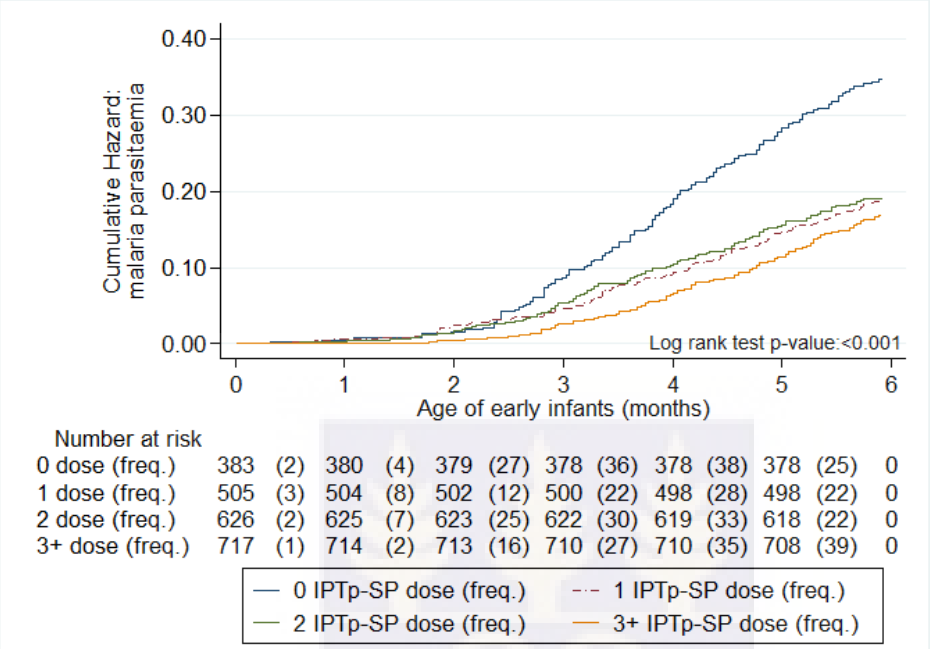


Figure 6 IPTp-SP use (dosing frequency) and risk of malaria parasitaemia in early infancy in a birth cohort enrolled between 2006 and 2007 in the KNDs.
Legend: freq= dosing frequency

The KM plot showed a significant chi square for trends (<0.001) reinforcing the finding in the cox model that risk of parasitaemia was reduced in early infants of mothers who had at least one IPTp-SP dose (frequency) compared to early infants of those who did not (Figure 6 above).

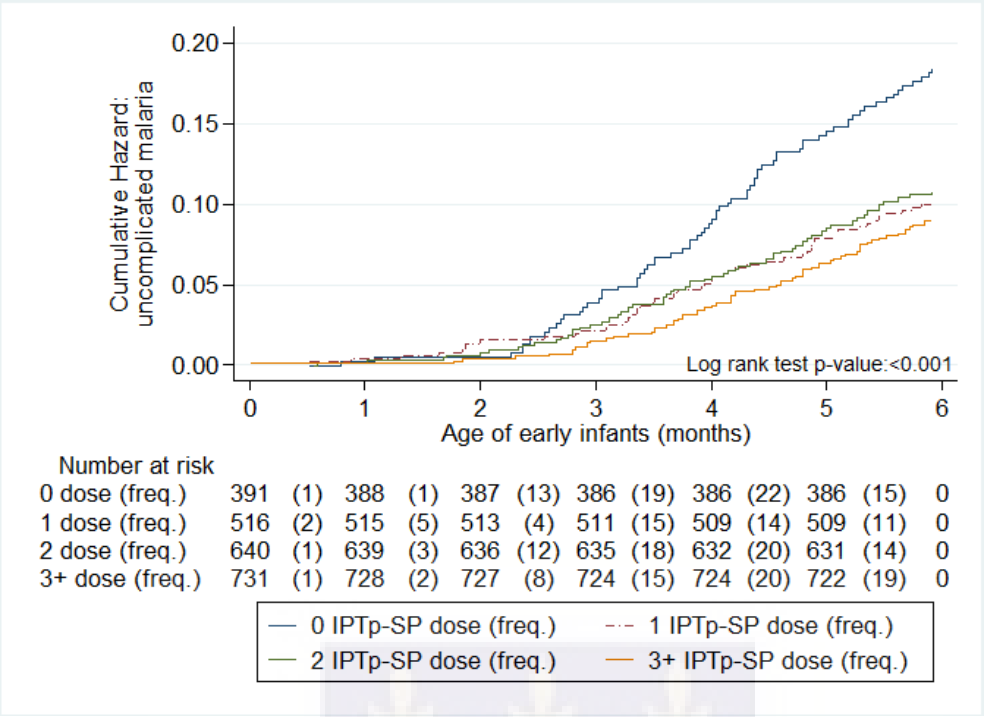


Figure 7 IPTp-SP use (dosing frequency) and risk of uncomplicated malaria in early infants in a birth cohort enrolled between 2006 and 2007 in the KNDs.
Legend: Freq=dosing frequency

The KM plot here showed a significant chi square for trends (<0.001) reinforcing the finding that risk of uncomplicated malaria was reduced in early infants of mothers who had at least one IPTp-SP dose (frequency) compared to early infants of those who did not (Figure 7 above).

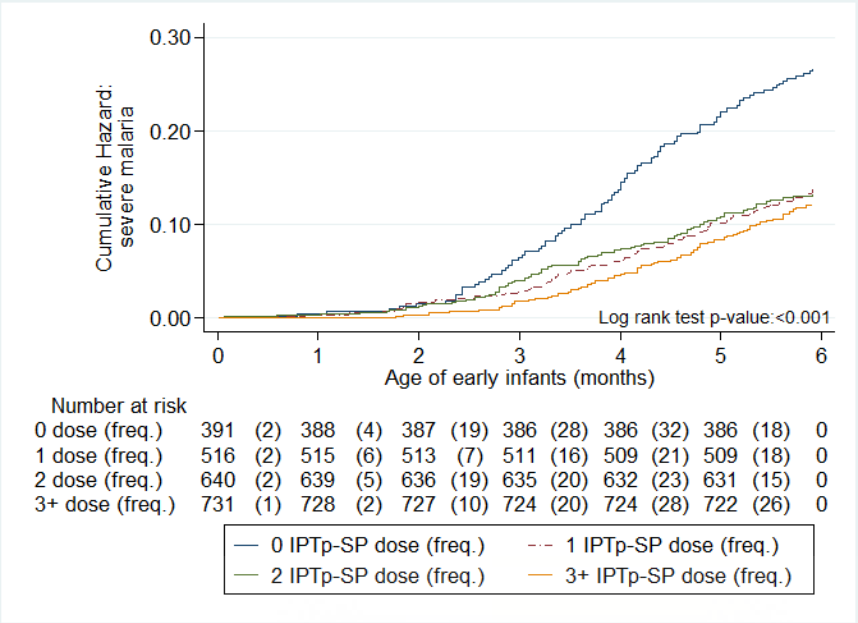


Figure 8 IPTp-SP use (dosing frequency) and risk of severe malaria in early infants in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Legend: Freq=dosing frequency

The KM plot for differing IPTp-sp dosing frequency and risk of severe malaria demonstrates a highly significant association between increasing dosing frequency of IPTp-sp and a reduction in hazards of severe malaria amongst off spring or early infants with a long rank p value of less than 0.001 (figure 8 above).

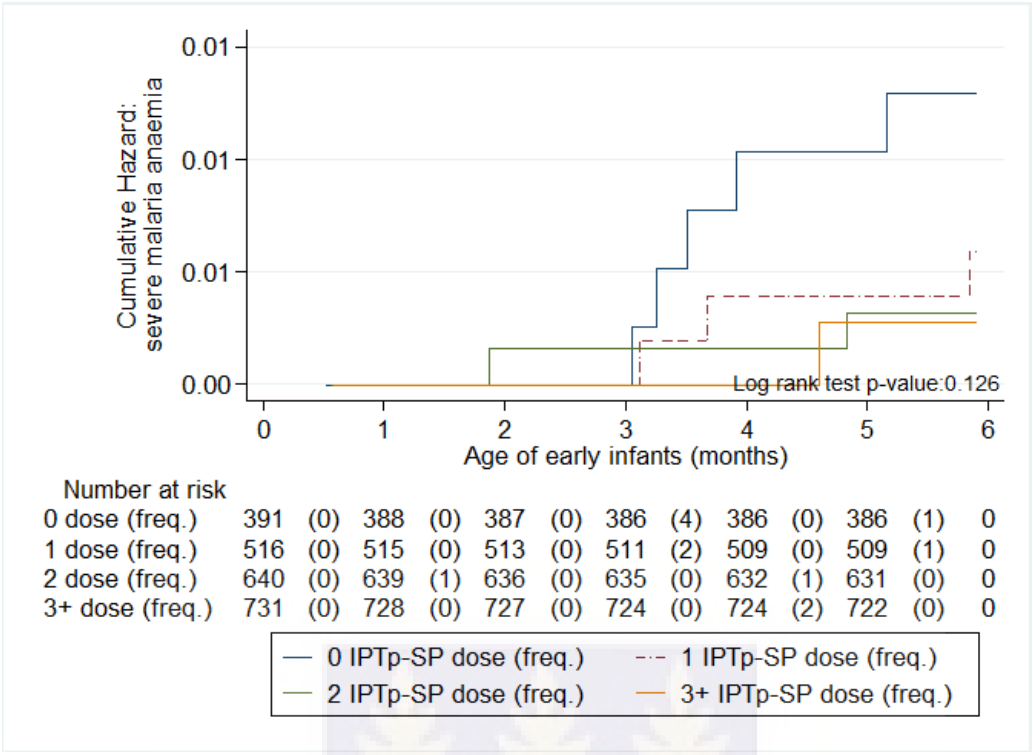


Figure 9 IPTp-SP use (dosing frequency) and incidence of severe malaria anaemia in their early infants in a birth cohort enrolled between 2006 and 2007 in the KNDs.
Legend: Freq=dosing frequency

Conversely for severe malaria anaemia, the KM plot showed a non-significant chi square for trends (0.126) reinforcing the finding that reduction in hazards of severe malaria anaemia in early infants of mothers who had at least one IPTp-SP compared to early infants of those who did not, was not statistically significant (Figure 9).

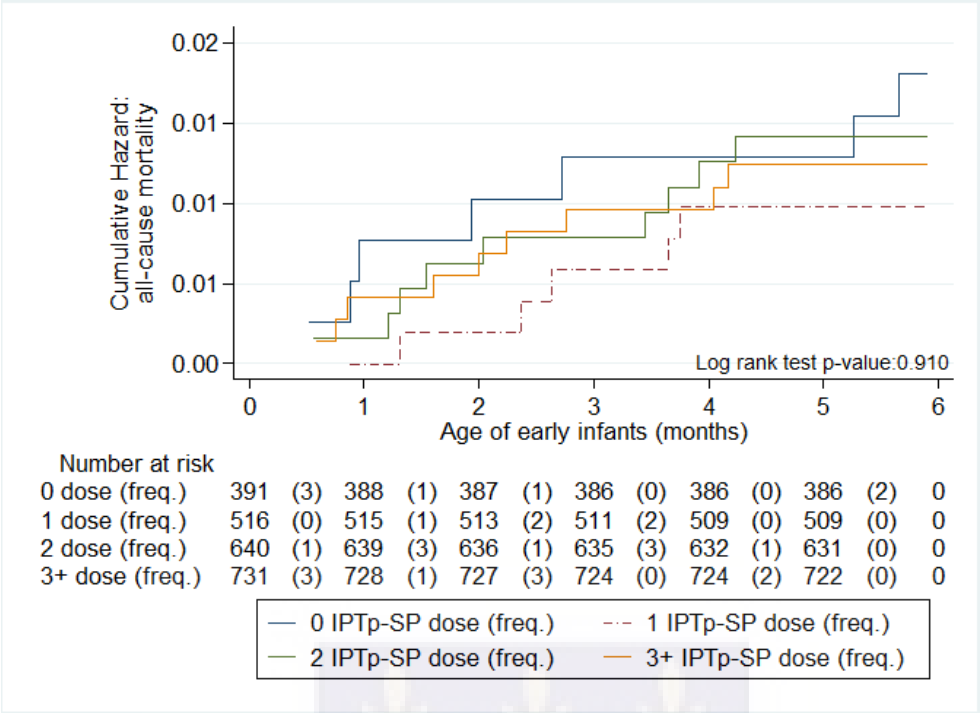


Figure 10 IPTp-SP use (dosing frequency) and risk of all cause mortality in early infants in a birth cohort enrolled between 2006 and 2007 in the KNDs.
Legend: Freq=dosing frequency

There was no significant association between differing doses of IPTp-SP and risk of all cause mortality amongst their offspring or early infants in the KNDs (figure 10).

4.6 Analyses: immunological component of the secondary study

A total of 713 archived samples were retrieved from storage for analysis. Out of which 41 were dropped because they were duplicate identifications. A total 672 were then analyzed after data cleaning (Figure 11).

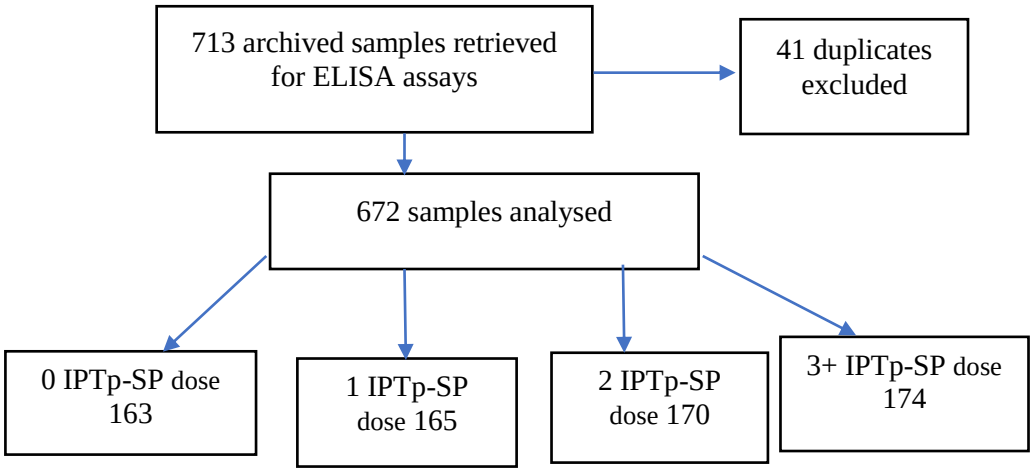
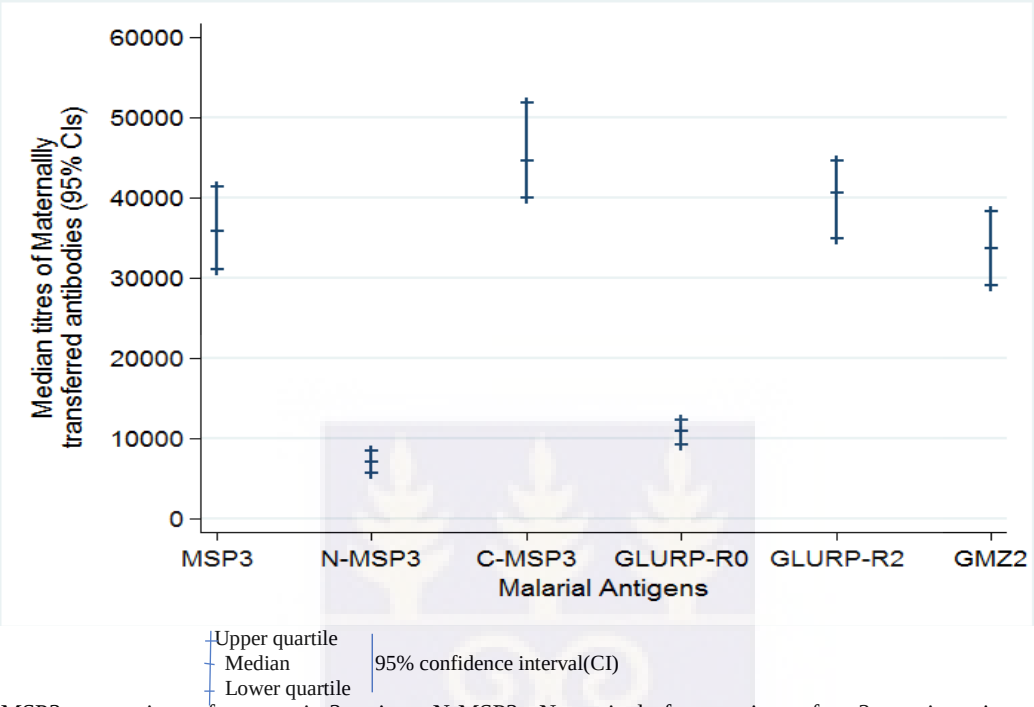


Figure 11 schema showing how sample numbers were derived

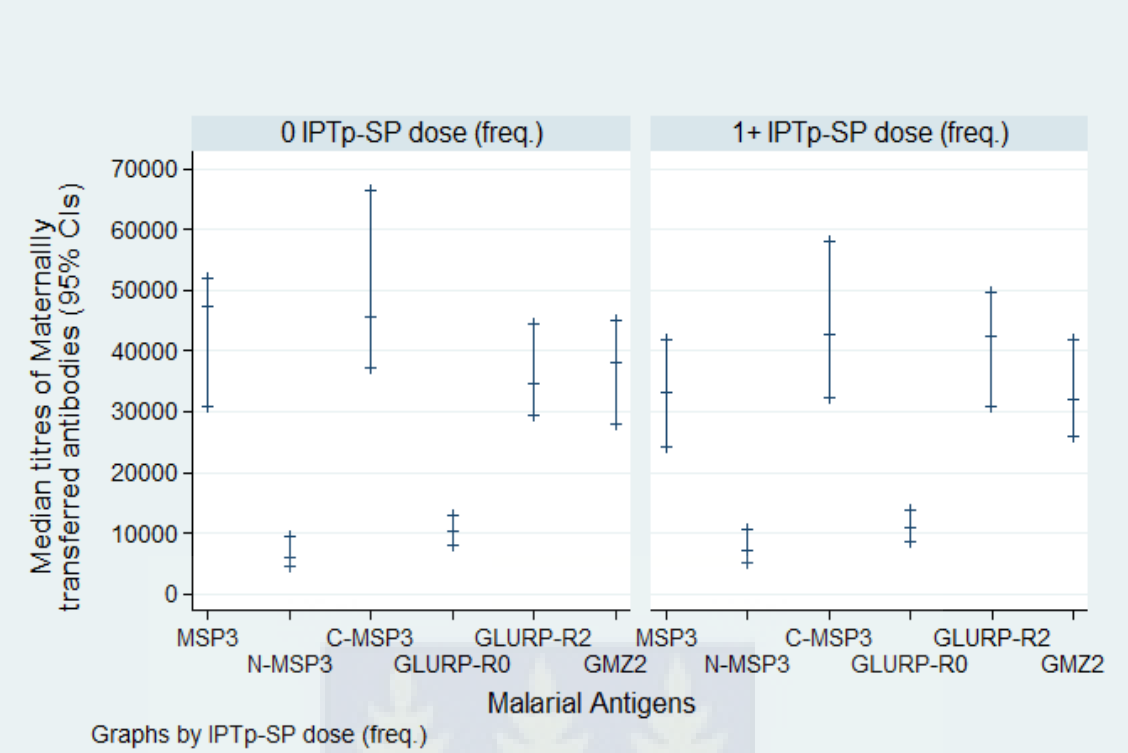
4.6.1 Magnitude of maternally transferred antibodies (median titres) to malaria antigens at enrolment in a subset (sample population) of the birth cohort in the KNDs.



MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen.

Figure 12 showing median levels of maternally transferred antibody titres (with min, max) to malaria antigens at enrolment in a subset (sample population) of the birth cohort in the KNDs.

On the whole the results show that, median titre levels of MSP3, C-MSP3, GLURP-R2 and GMZ2 were not significantly different but were higher than the mean titres of N-MSP3 and GLURP-RO at enrollment amongst early infants in the KNDs (figure 12).

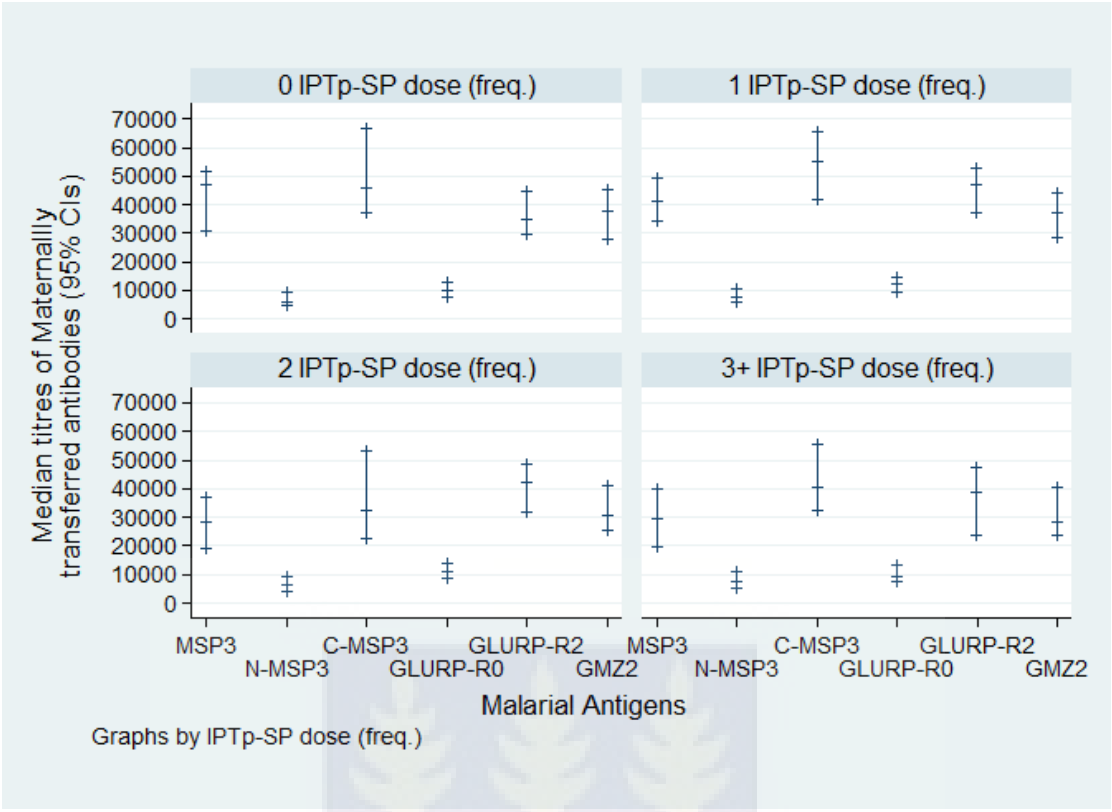


Upper quartile
Median
Lower quartile
95% confidence interval(CI) freq=dosing frequency

MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen Freq=frequency of dosing

Figure 13 showing median levels of maternally transferred antibody titres (with min, max) to malaria antigens by IPTp-SP dosing frequency (0, 1+) in a subset (sample population) of the birth cohort in the KNDs.

When median titres were stratified by IPTp-SP dosing frequency(0,1+), no significant differences were seen in median antibody titres (total IgG) to malaria antigens amongst early infants, for the different dosing frequency of IPTp-SP. Irrespective of of dosing frequency, median titre levels of MSP3, C-MSP3, GLURP-R2 and GMZ2 were each higher than the mean titre levels of either N- MSP3 and GLURP-RO at enrollment amongst early infants in the KNDs. However these were statistically insignificant (figure 13).



Upper quartile
Median
Lower quartile
95% confidence interval (CI) freq=dosing frequency

MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface protein 3 antigen,C-MSP= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen freq= dosing frequency

Figure 14 showing median levels of maternally transferred antibody titres (with min, max) to malaria antigens by IPTp-SP dosing frequency (0, 1, 2, and 3+) at enrolment in a subset (sample population) of a birth cohort in the KNDs.

When median titres were stratified by IPTp-SP dosing frequency (0, 1, 2, 3+), no significant differences were seen in median antibody titres (total IgG) to malaria antigens amongst early infants for the different dosing frequencies of IPTp-SP. Again irrespective of dosing frequency, median titre levels of maternally transferred antibodies to MSP3, C-MSP3, GLURP-R2 and GMZ2 were similar and each was higher than the median titres of either N- MSP3 or GLURP-RO at enrollment, amongst early infants in the KNDs (figure 14).

Table 28 ANOVA showing association between median levels of maternally transferred antibody titres to malarial antigens and different IPTp-SP dosing frequency (0,1+) at enrolment in a subset (sample population) of a birth cohort in the KNDs.

Median titres of maternally transferred (cord blood) total IgG antibodies (min, max) [n]						
IPTp-SP dosing frequency	MSP3	N-MSP3	C-MSP3	GLURP R0	GLURP R2	GMZ2
0	47186 (240-203608) [159]	6022 (82-1246310) [158]	45577 (58-1360984) [163]	10197 (9-211982) [158]	34608 (97-214845) [160]	38026 (270-135198) [160]
1+	34855 (240-203608) [499]	7306 (82-1250000) [492]	43225 (117-1360984) [509]	11052 (9-211981) [485]	42579 (97-214845) [497]	32628 (271-135198) [499]
p-value[k]	0.214	0.730	0.462	0.768	0.090	0.553

k=Kruskal-Wallis ANOVA= Analysis of variance
IgG= immunoglobulin G antibodies, MSP3=merozoite surface protein antigen, N-MSP3= N-terminal of merozoite surface protein 3 antigens, C-MSP3= C-terminal of merozoite surface protein 3 antigens, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen.

Median titres were stratified by IPTp-SP dose (0,1+). Using ANOVA, no significant differences were seen in median antibody titres (total IgG) to malaria antigens amongst early infants between the 1+ dosing frequency and 0 dosing frequency (table 28).

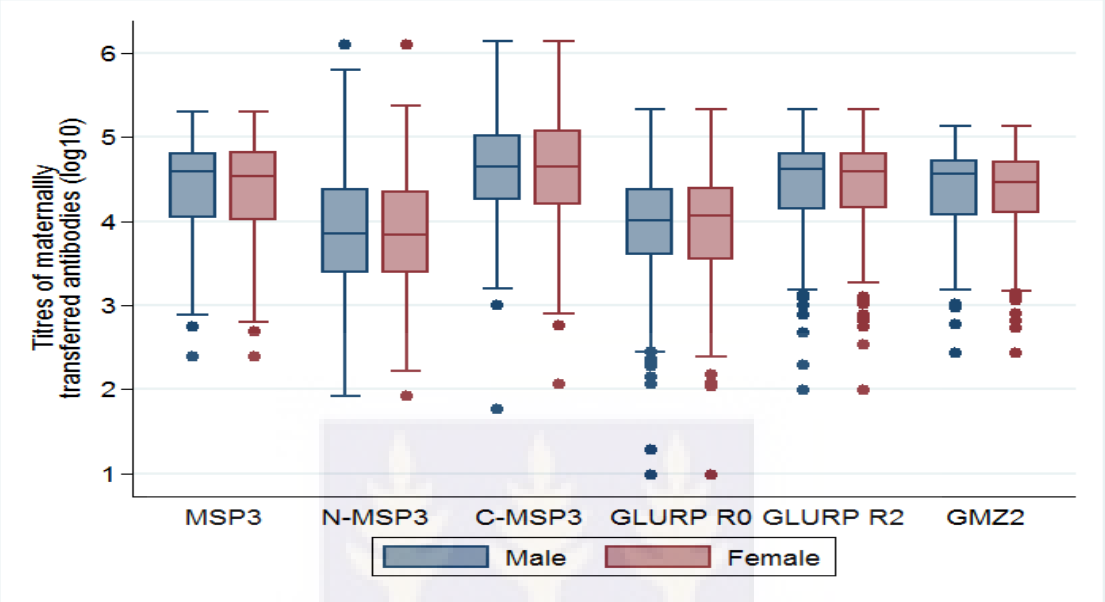
Table 29 ANOVA showing association between median levels of maternally transferred antibody tires to malarial antigens and different IPTp-SP dosing frequency (0,1,2,3+) at enrolment in a subset (sample population) of the birth cohort in the KNDs.

Characteristic	Median maternally transferred (cord blood) total IgG Antibody titres (min, max) [n]					
IPTp-SP dosing frequency	MSP3	N-MSP3	C-MSP3	GLURP R0	GLURP R2	GMZ2
0	47186 (240-203608) [159]	6022 (82-1246310) [158]	45577 (58-1360984) [163]	10197 (9-211982) [158]	34608 (97-214,845) [160]	38026 (270-135,198) [160]
1	41414 (240-203608) [161]	7794 (821-246310) [158]	55106 (5,661-360984) [165]	12383 (227-211981) [158]	46847 (470-214845) [161]	36948 (587-135198) [162]
2	28448 (240-203608) [166]	6162 (821-246310) [166]	32375 (18641-360984) [170]	11173(9-211981) [161]	42094 (729-214845) [167]	30625 (792-135198) [166]
3+	29404 (240-203608) [172]	7496 (821-250000) [168]	40791 (1,171-360984) [174]	9474 (9-211981) [166]	38535 (97-214845) [169]	28580 (270-135198) [171]
p-value[k]	0.209	0.643	0.504	0.721	0.204	0.578

k=Kruskal-Wallis
IgG= immunoglobulin G antibodies, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface protein 3 antigens, C-MSP3= C-terminal of merozoite surface protein 3 antigens, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen

Again using ANOVA, median titres were stratified by IPTp-SP dose (0,1,2,3+) No significant differences were seen in median antibody titres (total IgG) to the six selected malaria antigens amongst early infants for the different dosing frequency of IPTp-SP at enrolment (table 29).

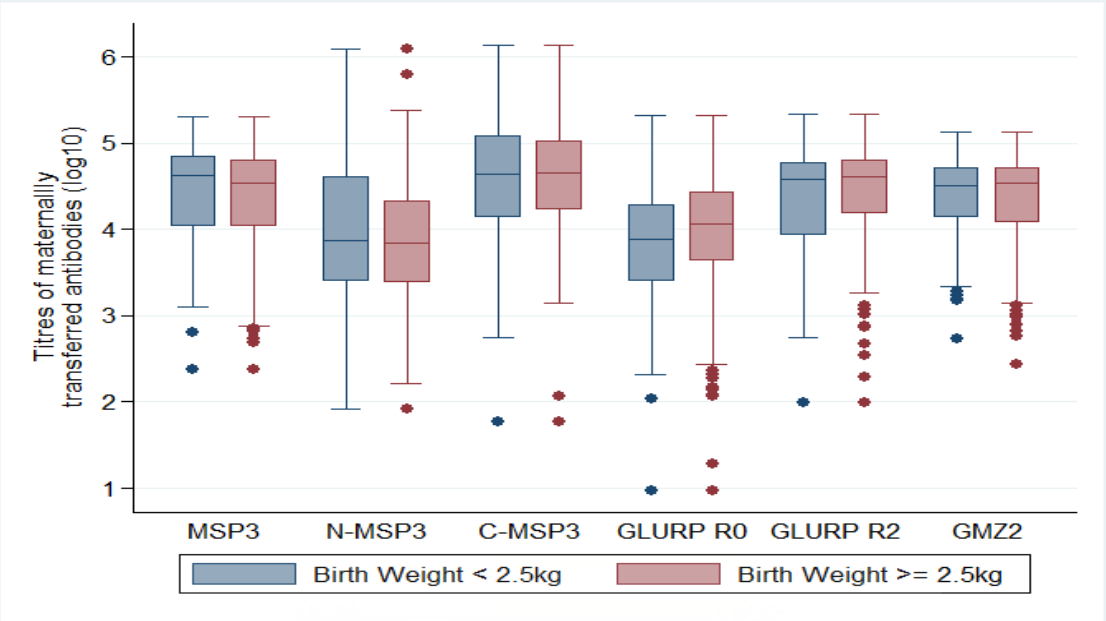
4.6.2 Magnitude of maternally transferred antibodies (log10 transformed titres) to malaria antigens at enrolment in a subset (sample population) of the birth cohort in the KNDs.



IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen
Boxes indicate the median (central line) and the interquartile range. The error bar indicates the 90th percentile, and points beyond the 95th percentile (outliers) are shown as circles.

Figure 15 showing log10 transformed maternally transferred antibody titres at enrolment to malaria antigens by gender in a subset (sample population) of a birth cohort in the KNDs.

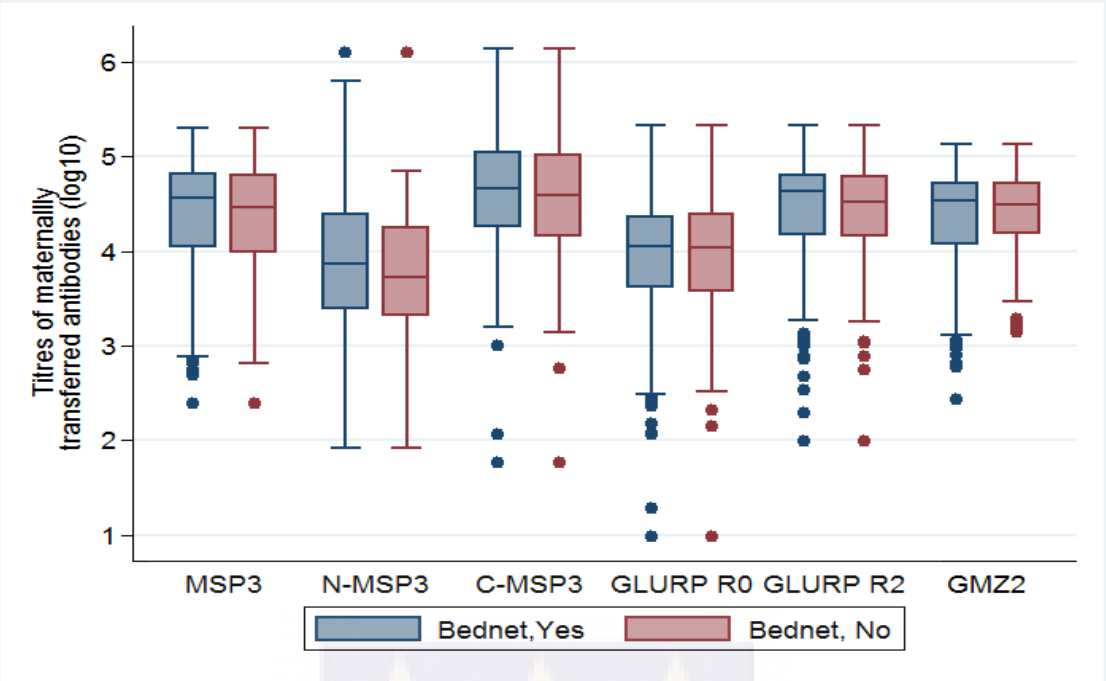
In the log transformed antibody titres to the selected malaria antigen none of the six antigens assayed (MSP3, N-MSP3, C-MSP3, GLURP-R0, GLURP-R2, GMZ2), showed any significantly consistent change with sex in the sample population (early infant) at enrolment in the KNDs (figure 15 above).



IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigenBoxes indicate the median (central line) and the interquartile range. The error bar indicates the 90th percentile, and points beyond the 95th percentile (outliers) are shown as circles.

Figure 16 showing log10 transformed maternally transferred antibody titres at enrolment to malaria antigens by birth weight in a subset (sample population) of a birth cohort in the KNDs.

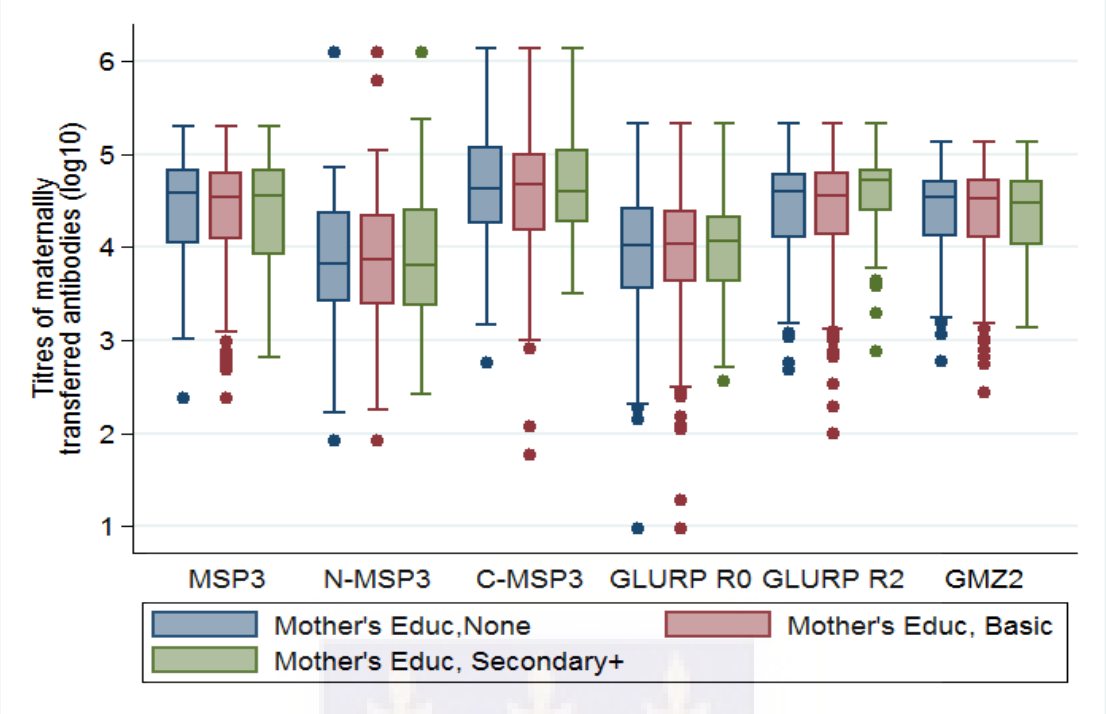
In the log transformed antibody titres to the selected malaria antigen none of the six antigens assayed showed any significantly consistent change with birth weight above or below normal in the sample population (early infant) at enrolment in the KNDs (figure 16 above).



IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigenBoxes indicate the median (central line) and the interquartile range. The error bar indicates the 90th percentile, and points beyond the 95th percentile (outliers) are shown as circles.

Figure 17 showing log10 transformed maternally transferred antibody titres at enrolment to malaria antigens by bednet use in a subset (sample population) of a birth cohort in the KNDs.

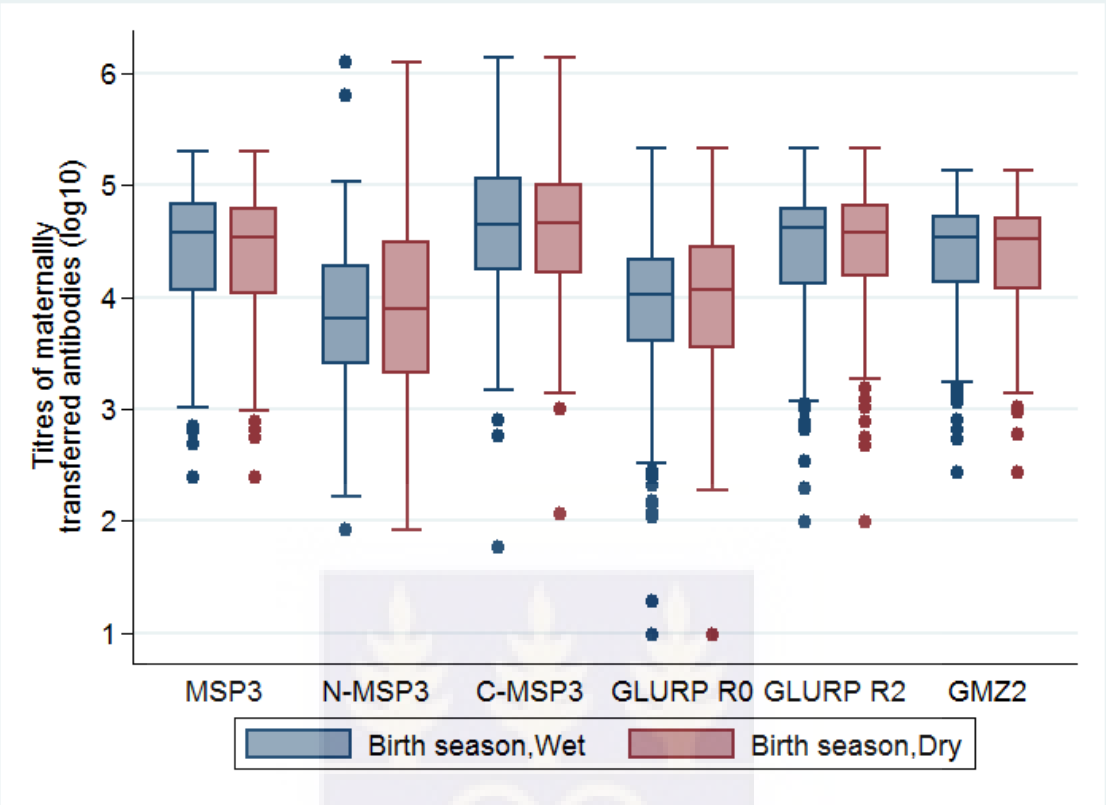
In the log transformed antibody titres to the selected malaria antigen none of the six antigens showed any significantly consistent change with bednet use in the sample population (early infant) at enrolment in the KNDs (figure 17 above).



IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen. Boxes indicate the median (central line) and the interquartile range. The error bar indicates the 90th percentile, and points beyond the 95th percentile (outliers) are shown as circles.

Figure 18 showing log10 transformed maternally transferred antibody titres at enrolment to malaria antigens by mothers' formal education status in a subset (sample population) of a birth cohort in the KNDs.

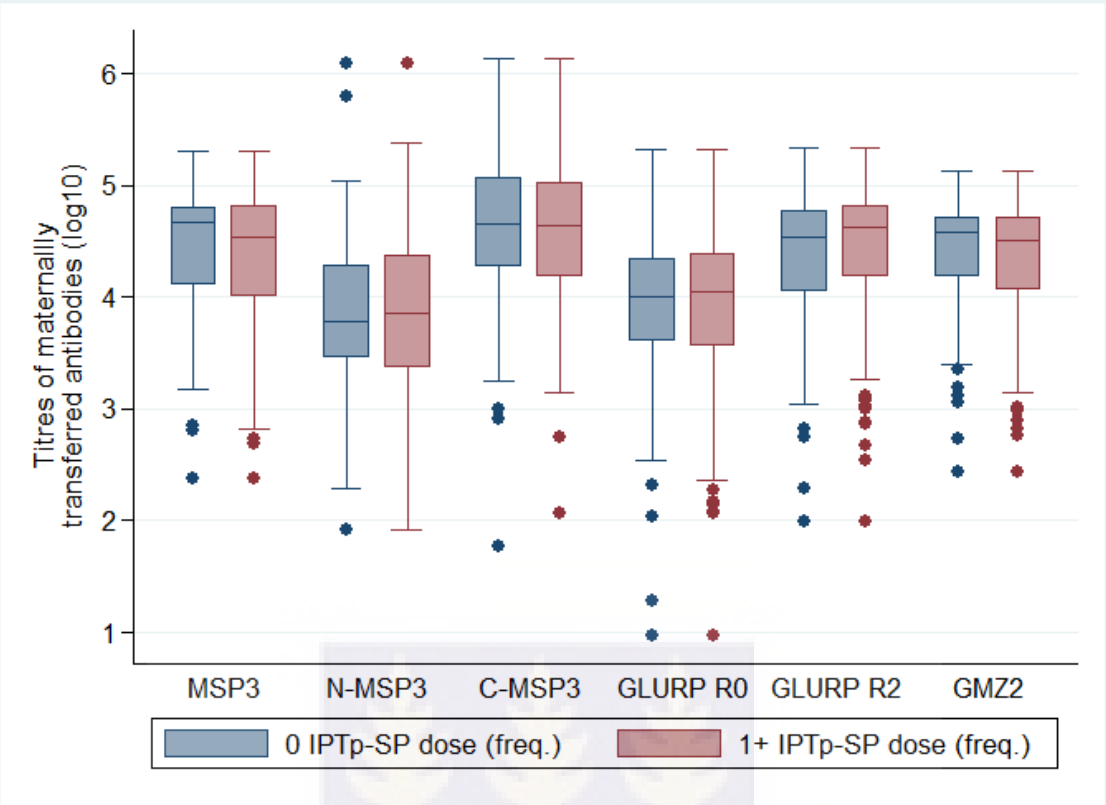
In the log transformed antibody titres to the selected malaria antigen none of the six antigens showed any significantly consistent change with mothers' education in the sample population (early infant) at enrolment in the KNDs (figure 18 above).



IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigenBoxes indicate the median (central line) and the interquartile range. The error bar indicates the 90th percentile, and points beyond the 95th percentile (outliers) are shown as circles.

Figure 19 showing log10 transformed maternally transferred antibody titres at enrolment to malaria antigens by early infants' birth season, in a subset (sample population) of a birth cohort in the KNDs.

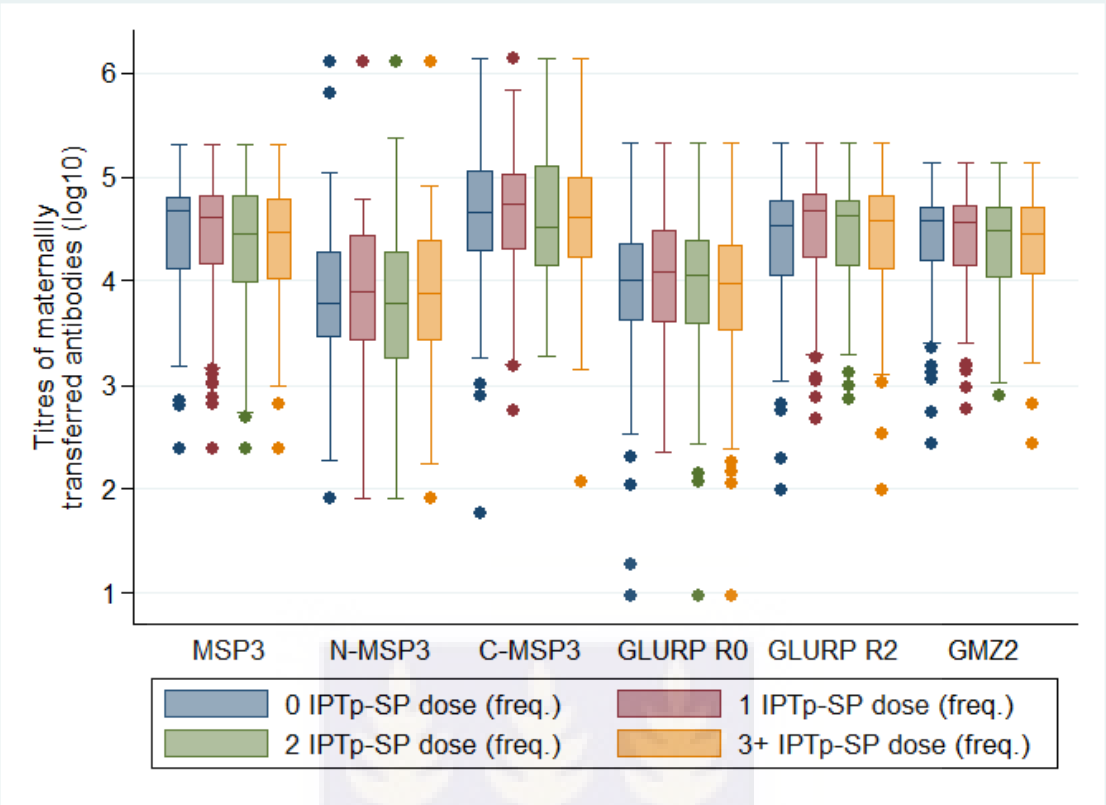
In the log transformed antibody titres to the selected malaria antigens, none of the six antigens showed any significantly consistent change with birth season in the sample population (early infant) at enrolment in the KNDs (figure 19 above).



IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigenBoxes indicate the median (central line) and the interquartile range. The error bar indicates the 90th percentile, and points beyond the 95th percentile (outliers) are shown as circles.
Freq=dosing frequency

Figure 20 showing log10 transformed maternally transferred antibody titres at enrolment to malaria antigens by IPTp-SP dosing frequency (0, 1+) in a subset (sample population) of a birth cohort in the KNDs.

In the log transformed antibody titres to the selected malaria antigen, none of the six antigens showed any significantly consistent change with different dosing frequencyof IPTp-SP (0,1+) in the sample population (early infant) at enrolment in the KND (figure 20 above).



IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen. Boxes indicate the median (central line) and the interquartile range. The error bar indicates the 90th percentile, and points beyond the 95th percentile (outliers) are shown as circles.
Freq=dosing frequency

Figure 21 showing log10 transformed maternally transferred antibody titres at enrolment to malaria antigens by IPTp-SP dosing frequency (0, 1, 2, and 3+) in a subset (sample population) of a birth cohort in the KNDs.

In the log transformed antibody titres to the selected malaria antigen none of the six antigens showed any significantly consistent change with varying dosing frequency of IPTp-SP (0,1,2,3+) in the sample population (early infants) at enrolment in the KNDs.

Table 30 Regression showing relationship between log10 transformed total IgG and IPTp-SP use (dosing frequency) in the subset (sample population) of the birth cohort enrolled between 2006 and 2007 in the KND.

Characteristic [n]	[n]	co-efficient(95%CI)	*p*-value	Adjusted R^2
MSP3				0.0012
1 IPTp-SP	[161]	0.009 (-0.118,0.136)	0.886	
2 IPTp-SP	[166]	-0.089 (0.216,0.037)	0.164	
3+ IPTp-SP	[172]	-0.076 (-0.200,0.049)	0.236	
N-MSP3				-0.0027
1 IPTp-SP	[158]	0.118 (-0.113,0.350)	0.314	
2 IPTp-SP	[166]	0.016 (-0.212,.245)	0.889	
3+ IPTp-SP	[168]	0.064 (-0.163,0.292)	0.550	
C-MSP3				-0.0027
1 IPTp-SP	[162]	0.028 (-0.106,0.162)	0.678	
2 IPTp-SP	[170]	-0.012 (-0.145,0.121)	0.863	
3+ IPTp-SP	[174]	0.044 (-0.177,0.088)	0.509	
GLURP-R0				-0.0021
1 IPTp-SP	[158]	0.078 (-0.086,0.243)	0.348	
2 IPTp-SP	[161]	0.023 (-0.140,0.186)	0.783	
3+ IPTp-SP	[166]	-0.023 (-0.184,0 .140)	0.791	
GLURP-R2				0.0033
1 IPTp-SP	[162]	0.136 (0.009,0.263)	0.035	
2 IPTp-SP	[167]	0.109 (-0.016,0.235)	0.088	
3+ IPTp-SP	[169]	0.063 (-.063,0.188)	0.327	
GMZ2				-0.0030
1 IPTp-SP	[161]	0.015 (-0.088,0.117)	0.778	
2 IPTp-SP	[166]	-0.033 (-0.135,0.069)	0.53	
3+ IPTp-SP	[171]	-0.023 (-0.125,0.078)	0.651	

IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen.

In the regression analysis no correlation was found between doses of IPTp-SP and at least a tenfold increment in antibody titres to the malaria antigens at enrolment in the sample population (early infants) of the birth cohort in the KND. In other words, IPTp-SP dosing

frequency did not predict a tenfold increase in maternally transferred antibodies in their early infants.

From the foregoing analyses we therefore fail to reject the null hypothesis **(**$H_{0:}$ There is no difference in maternally transferred (transplacental or cord blood) antibody titres in early infants born to mothers who had IPTp-SP compared to mothers who had no IPTp-SP) and say that there are no significant differences in maternally transferred antibodies of early infants to the selected malaria antigens with dosing frequency of IPTp-SP.

4.6.3 IPTp-SP use (dosing frequency) and incidences of parasitaemia, uncomplicated malaria, severe malaria and all cause mortality in early infancy in a sample population of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Table 31 IPTp-SP use (dosing frequency) and incidence of parasitaemia in early infancy in a sample of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time/years	Rate/1000-person years(95%CI)
0 IPTp-SP	50	0.92	54.22 (41.10-71.54)
1 IPTp-SP	27	0.95	28.24 (19.37-41.18)
2 IPTp-SP	37	0.98	37.57 (27.22-51.86)
3+ IPTp-SP	30	1.01	29.67 (20.74-42.43)

The incidence rates of parasitaemia in the sample population in table 31**,** demonstrate a fairly representative sample from the main cohort study.

Table 32 IPTp-SP use (dosing frequency) and incidence of uncomplicated malaria in early infancy in a sample of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time/years	Rate/1000-person years(95%CI)
0 IPTp-SP	27	0.92	29.28 (20.08,42.70)
1 IPTp-SP	11	0.96	11.50 (6.37,20.77)
2 IPTp-SP	17	0.98	17.26 (10.73,27.77)
3+ IPTp-SP	17	1.01	16.81 (10.45,27.04)

The incidence rates of uncomplicated malaria in the sample population in table 32 demonstrate a fairly representative sample from the main cohort study.

Table 33 IPTp-SP use (dosing frequency) and incidence of severe malaria in early infancy in a sample of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time/years	Rate/1000 person-years(95%CI)
0 IPTp-SP	33	0.92	35.79 (25.44-50.34)
1 IPTp-SP	21	0.96	21.96 (14.32-33.68)
2 IPTp-SP	21	0.98	21.32 (13.90-32.70)
3+ IPTp-SP	22	1.01	21.76 (14.33-33.04)

Table 33 incidence rates of severe malaria show the sample population was fairly representative of the primary cohort.

Table 34 IPTp-SP use (dosing frequency) and incidence of severe malaria anaemia in early infancy in a sample of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time/years	Rate/1000-person years (95%CI)
0 IPTp-SP	2	0.92	2.17 (0.52,8.67)
1 IPTp-SP	1	0.96	1.05 (0.15,7.42)
2 IPTp-SP	1	0.98	1.02 (0.14,7.21)
3+ IPTp-SP	-	-	-

The incidence of severe malaria anaemia in table 34 show sample population was fairly representative of the main or primary cohort.

Table 35 IPTp-SP use (dosing frequency) and incidence of all cause mortality in early infancy in a sample of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time/years	Rate/1000person- years (95%CI)
0 IPTp-SP	4	0.93	4.25 (1.60,11.35)
1 IPTp-SP	2	0.96	2.06 (0.52,8.25)
2 IPTp-SP	3	0.99	3.02 (0.97,9.35)
3+ IPTp-SP	1	1.03	0.98 (0.14,6.92)

The incidence rates in table 35 demonstrate a fairly representative sample from the main or primary cohort.

4.6.4 Hazard ratios of parasitaemia, uncomplicated, severe malaria, all cause mortality and log10 transformed maternally transferred antibodies (total IgG) in a sample population of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Table 36 Hazard ratios of malaria parasitaemia and Log10 transformed maternally transferred antibodies (total IgG) in a sample population of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[N]	Crude hazard ratios (95%CI)	p-Values[w]	[a]Adjusted hazard ratios (95%CI)	p-Values[w]
Total IgG to MSP3	[657]	0.95 (0.72,1.28)	0.736	-	-
Total IgG to N-MSP3	[649]	0.84 (0 .71,1.00)	0.048	0.85 (0.71,1.00)	0.058
Total IgG to C-MSP3	[657]	0.76 (0.56,1.03)	0.072	-	-
Total IgG to GLURP-R0	[631]	0.93 (0.74-1.17)	0.525	-	-
Total IgG to GLURP-R2	[642]	0.65 (0.50,0.84)	0.001	0 .65 (0.50,0 .84)	0.001
Total IgG to GMZ2	[658]	0.89 (0.60,1.30)	0.540	-	-

W=Wald p value [a]Adjusted for mother's education, birth season, bednet use, IPTp-SP use
IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen.

After adjusting for potential confounders, a tenfold increase in maternally transferred antibodies (Cord blood total IgG) to GLURP-R2 antigen was significantly associated with a 36%, [0.64 (95%CI =0.50, 0.83), P<001] less hazards of parasitaemia compared to the other antigens. The other five antigens were not associated significantly with protection against malaria parasitaemia (table 36 above).

Table 37 Hazard ratios of uncomplicated malaria and Log10 transformed maternally transferred antibodies (total IgG) in a sample population of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[N]	Crude hazard ratios (95%CI)	p-Value[w]	[a]Adjusted hazard ratios (95%CI)	p-Value[w]
Total IgG to MSP3	[658]	0.84 (0.58,1.21)	0.344	-	-
Total IgG to N-MSP3	[650]	.75(0.61,0.92)	0.006	0.76 (0.62,0.93)	0.007
Total IgG to C-MSP3	[672]	0.63 (0.44,0.90)	0.012	0.64 (0.46,0.90)	0.009
Total IgG to GLURP-R0	[643]	0.99 (0.72,1.36)	0.938	-	-
Total IgG to GLURP-R2	[657]	0.64 (0.44,0.92)	0.017	0.66 (0.45,0.95)	0.026
Total IgG to GMZ2	[659]	0.85 (0.52,1.40)	0.527	0.85 (0.51,1.41)	0.524

W=Wald p value aAdjusted for, birth season, bednet use, IPTp-sp use
IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen.

.

After adjusting for confounders tenfold increase in maternally transferred antibody (Cord blood total IgG) titres to C-MSP3 and GLURP-R2 antigens were significantly associated with 20% [HR= 0.64(95%CI= 0.45,0.989) P=0.009] and 34% [HR= 0.65(95%CI= 0.45,0.94), P=0.024] less hazards respectively of severe malaria, compared to the other antigens (Table 37 above).

Table 38 Hazard ratios of severe malaria and Log10 transformed maternally transferred antibodies (total IgG) in a sample population of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[N]	Crude hazard ratios (95%CI)	p-Values[w]	[a]Adjusted hazard ratios (95%CI)	p-Values[w]
Total IgG to MSP3	[657]	0.86 (0.61,1.19)	0.356	-	-
Total IgG to N-MSP3	[649]	0.80 (0.67,0.96)	0.016	0.80 (0.67,0.96)	0.016
Total IgG to C-MSP3	[671]	0.73 (0.50,1.06)	0.095	-	-
Total IgG to GLURP-R0	[642]	0.98 (0.74,1.31)	0.897	-	-
Total IgG to GLURP-R2	[656]	0.65 (0.48,0.89)	0.007	0.66 (0.49,0.90)	0.009
Total IgG to GMZ2	[658]	0.91 (0.56,1.46)	0.688	-	-

W=Wald p value [a]Adjusted for sex, birth season, bednet use, IPTp-sp use
IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen.

A tenfold increase in the IgG antibody titres to N-MSP3 and GLURP-R2 conferred significantly a 20% and 34% less hazards for severe malaria (table 38 above)

Table 39 Hazard ratios of severe malaria anaemia and Log10 transformed maternally transferred antibodies (total IgG) in a sample population of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic (n)	[N]	Crude hazard ratios (95%CI)	p-Values[w]	[a]Adjusted hazard ratios (95%CI)	p-Values[w]
Total IgG to MSP3	[658]	4.89 (2.84,8.40)	<0.001	4.63 (2.80,7.78)	<0.001
Total IgG to N-MSP3	[650]	1.34 (1.12,1.60)	0.001	1.36 (1.09,1.70)	0.006
Total IgG to C-MSP3	[672]	0.72 (0.20,2.66)	0.627	-	-
Total IgG to GLURP-R0	[643]	1.17 (0.65,2.11)	0.604	-	-
Total IgG to GLURP-R2	[657]	1.95 (1.54,2.48)	<0.001	2.05 (1.35,3.14)	0.001
Total IgG to GMZ2	[659]	4.32 (1.09,17.16)	0.037	4.05 (1.05,15.60)	0.042

W=Wald p value a=Adjusted for sex, birth season, bednet use, IPTp-sp use IgG= immunoglobulin G antibodies, IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen.

A tenfold increase in the IgG antibody titres to N-MSP3, C-MSP3 were associated with 4.53 and 1.34 greater hazards of severe anaemia while GLURP R2 was significantly associated with 34% less hazards of severe anaemia (table 39 above).

Table 40 Hazard ratios of all cause mortality and Log10 transformed maternally transferred antibodies (total IgG) in a sample population of a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic [n]	[N]	Crude hazard(95%CI)	p-Values[w]	[a]Adjusted hazard(95%CI)	p-Values[w]
Total IgG to MSP3	[658]	0.65 (0.25,1.72)	0.396	-	-
Total IgG to N-MSP3	[650]	1.40 (0.82,2.39)	0.217	-	-
Total IgG to C-MSP3	[672]	0.57 (0.21,1.34)	0.180	-	-
Total IgG to GLURP-R0	[643]	0.45 (0.18,0.91)	0.025	0.46 (0.18,1.19)	0.110
Total IgG to GLURP-R2	[657]	0.46 (0.20,1.20)	0.118	-	-
Total IgG to GMZ2	[659]	0.42 (0.14,1.25)	0.119	-	-

W=Wald p value a=Adjusted for sex, birth season, bednet use, IPTp-sp use IgG= immunoglobulin G antibodies, IgG=immunoglobulin G, MSP3=merozoite surface protein 3 antigen, N-MSP3= N-terminal of merozoite surface 3 protein antigen, C-MSP3= C-terminal of merozoite surface protein antigen, GLURP-R0=Region 0 of Glutamine rich protein antigen, GLURP-R2= Region 2 of Glutamine rich protein antigen, GMZ2= candidate malaria vaccine (fusion of MSP3 and GLURP-R0) antigen.

After adjusting for covariates, a tenfold rise in titres of maternally transferred antibodies to all six malaria antigens were insignificantly associated with risk of all cause mortality amongst early infants in the KNDs (table 40).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Introduction

This study was carried out by analyzing secondary data from a large birth cohort (2279 newborns) that was enrolled between March 2006 and March 2007 aimed at documenting their malaria morbidity and mortality experiences in the first five years of life. This secondary analysis was restricted to the first six months of life (early infancy). The main dataset was analyzed for associations between the use of intermittent preventive treatment in pregnancy (IPTp-SP) and the risk of malaria (parasitaemia, uncomplicated malaria, and severe malaria) and all-cause mortality utilizing a retrospective cohort approach. A subset of the main cohort was randomly selected stratified on dosing frequency of IPTp-SP and analyzed for differences in antibody titres to selected malarial antigens and different dosing frequencies of IPTp-SP. Associations between titres of antibodies to these malaria antigens were also analyzed in relation to the risk of malaria (parasitaemia, uncomplicated malaria, severe malaria) and all-cause mortality. Cross sectional and cohort approaches were adopted in the analysis.

In the midst of contradictory evidence about the benefits or otherwise of IPTp-SP on the risk of malaria in early infancy, the general aim of this secondary study was to demonstrate how the use of IPTP-SP impacts on the magnitude of maternally transferred antibodies in their offspring (early infants) and to establish if IPTp-SP and these antibodies are associated with reduced risk of malaria and all-cause mortality in early infancy in the Kassena-Nankana Districts (KNDs).

5.2 Socio-demographic factors and IPTp-SP usage in the KNDs

Baseline findings show a higher enrolment of newborns in the high transmission season compared to the low transmission season. This is as a result of the timing of enrolment in

the primary study; enrolment commenced in March 2006 and so most of the enrolment drive spanned the high transmission season of May-October. After this period, the enrolment into the primary cohort was deliberately slowed so as to have representation for both the high transmission as well as the low transmission seasons.

There appears to be a fall in proportion of mothers making it for higher frequency of dosing in the high transmission season; for such mothers, the second and third doses would have coincided more with the high transmission season. These are times when as a mainly farming community (Chatio et al., 2015) individuals are more likely to be busy on their farms and may choose that activity over and above attendance at an antenatal clinic (opportunity cost). It should be noted also that the decision to embark on such an enterprise might have to get the blessing of the head of household who almost invariably will be the male partner. A scenario like that could impact on access to IPTp-SP (Hurley et al., 2016). By virtue of the bad nature of roads during the high transmission season (Aryeetey & Kanbur, 2017), geographical access to IPTp-SP may be restricted either because mothers cannot get to health facilities or health workers cannot also get to facilities to discharge their duties. So individual, household and health facility factors could conspire to produce this picture. On the other hand, the increase in proportions of mothers receiving IPTp-SP for children born in the high transmission season may be explained thus- those pregnancies especially for the second and third doses likely traversed the low transmission season when there was less farming activities. There is less of a dilemma in making the decision to attend antenatal clinic; there is better geographical access to health facilities.

In keeping with the largely rural nature of the study area the majority of mothers of infants enrolled into the study are from the rural areas. Of interest too, is the finding that majority of those who have no IPTp-SP are from the rural areas; less than 10% are from the urban

area. The urban newborns or infants have greater proportion of their mothers being able to get higher doses of IPTp-SP and the converse is true for the rural newborns. The urban area, which is the central township, has more health facilities within reach, including the district hospital than the more dispersed rural settlements in the periphery. Also, this is where more mothers are more likely to have formal education, do less farm work and be able to take independent decisions to access antenatal care compared to mothers of infants in the rural areas. Financial and geographic accessibility are known to affect uptake of antenatal care services and by extension IPTp-SP (Hurley et al., 2016)

Amongst mothers who have no IPTp-SP, over half of them have no formal education. Proportions of mothers with no formal education who have higher dosing frequency of IPTp-SP appears to increase and for those with basic education, this appears to decrease. For those with secondary education or more, proportions who have higher dosing frequency of IPTp-SP appears to increase. It is not immediately clear why this phenomenon is observed amongst mothers with only basic education. It would appear in the Kassena-Nankana District a threshold exists at the secondary education level, beyond which education impacts positively on IPTp-SP uptake. In any case, education has been reported to be important in improving uptake of IPTp-SP (Anchang-Kimbi et al., 2014). A cross-sectional study from Tanzania concluded that education beyond the basic level was associated with optimal uptake of IPTp-SP (Exavery et al., 2014).

5.3 IPTp-SP use (dosing frequency), maternally transferred antibodies and time to first parasitaemia in their early infants in the KNDs.

IPTp-SP reduces risk of time to first parasitaemia amongst early infants of mothers who took SP as intermittent prevention of malaria during pregnancy in the KNDs. This finding is not isolated. In a cohort study in Benin that looked at placental malaria and the risk of malaria in infancy, use of SP for IPTp by the mothers was associated with lower

placental infections and less risk of infection in offspring (Le Port et al., 2011). IPTp-SP might employ two mechanisms with two contrasting outcomes for the early infant: by influencing transplacental passage of maternal antibodies or foetal in utero exposure to malaria antigens (Moya-Alvarez et al., 2014a). The former by facilitating antibody transfer, would result in less risk of malaria in the infant (Griffin et al., 2015) and the latter in greater risk of malaria. There have been fears that by clearing parasitaemia, IPTp-SP would effectively be reducing exposure and slowing the acquisition of a threshold and relevant antibody repertoire (Chico et al., 2017). This is because maternal antibody levels or concentrations are important in transplacental antibody transfer and do influence antibody transfer (Dechavanne et al., 2015; Palmeira et al., 2012) If that is the case, by extension less antibodies would be produced by the mother (Mayor et al., 2015). If less antibodies are produced by the mother, arguably there will be less antibodies transferred to the foetus, increasing the risk of malaria in early infancy. Even though this secondary study (thesis) did not measure antibody levels of mothers of infants, (and so the assertion could not be refuted or corroborated) the findings of a protective effect with IPTp-SP use would suggest that in the study area, IPTp-SP use does not reduce or interfere with relevant antibody transfer. Indeed a recent cohort study in the coastal belt of Ghana reported no evidence to support the assertion that IPTp-SP reduces antibody titres to malarial antigens in mothers and their infants (Stephens et al., 2017a). One can posit then that, by reducing or clearing placental infections, IPTp-SP may also reduce transplacental transfer of soluble malarial antigens or less commonly transplacental transfer of malarial parasites. This reduces premunition and the "tolerant phenotype" and thence the risk of malaria in infancy (Malhotra et al., 2009b).

Additionally it can be suggested that in the study area, IPTp-SP reduces placental parasitaemia (Anchang-Kimbi et al., 2014) and inflammation, not only by its antimalarial effect but also its antibacterial effect. This allows for transfer of the threshold titres of

immunoglobulin G (IgG) antibody to the unborn foetus (Gutman & Slutsker, 2017; Stanisic et al., 2015). This transfer then augments parasitaemia reduction in infancy. Antibodies may clear merozoites and infected erythrocytes (IEs) through opsonization; or complement-mediated lysis; inhibition of merozoite invasion; or blocking the adhesion of IEs to vascular endothelium (Gomes et al., 2016). Non-neutralizing antibodies may contribute to reduction in risk of malaria parasitaemia through Antibody-dependent monocyte or natural killer (NK) cell–mediated cytotoxicity (Gomes et al., 2016; Portugal et al., 2013). The proposed explanation above gets traction from the reports that passively transferred and naturally acquired antibodies are protective of clinical malaria in humans (Boyle et al., 2015; Cohen et al., 1961); the demonstration that these antibodies (IgG) traverse the placenta to the foetus (Williams & McFarlane, 1969) mainly in the third trimester of pregnancy; and growing body of evidence in support of high antibody titres protecting against malaria (Murungi et al., 2013). Having said that, opsonized antigen may also be taken up by antigen presenting cells and presented to appropriate T cells triggering a T cell mediated response (adaptive) to reduce parasitaemia (Portugal et al., 2014). Given that the findings of this study do not support a dosing frequency dependent mechanism by IPTp-SP, the effect might be by a threshold mechanism. For beyond the first dose, even though risk of parasitaemia continues to decrease with higher dosing frequencies of IPTp-SP, it is not significant. The significant protection offered by use of bednets (almost twice the hazards of parasitaemia for not using one) to parasitaemia is not unexpected as bednets provide a mechanical barrier to infective bites and have been known to reduce maternal parasitaemia by as much as 38% (Takem & D'Alessandro, 2013). This being the case placental malaria is reduced, chances of transplacental transfer of parasites and soluble antigens are lowered, placental bed perfusion and exchanges are improved and ultimately, so are birth outcomes.

This not withstanding, there is at least one trial from the study site that did not find an association between maternal bednet use and reduced risk of malaria parasitaemia in mothers (Browne, Maude, & Binka, 2001). Similarly, the significant protection offered by being born in the low transmission season is probably because of reduced vector proliferation in the low transmission season and the attendant fall in the number of infective bites encountered by the infant. Another plausible explanation is that for infants born in the low transmission season, a good part of their *in utero* life is in the high transmission season, when there is greater exposure of mothers to infective bites (Kasasa et al., 2013; Appawu et al., 2004b). The greater exposure to infective bites could have resulted in a greater breadth and magnitude of transferred antibodies to the infant, conferring protection. Per this argument the converse holds true for infants born in the high transmission season. The lack of association here between mothers' education and risk of parasitaemia in the adjusted model is unexpected. Higher education attained by mothers, correlates well with household wealth that results in better living standards and thence to better means and methods of malaria prevention and treatment. (Wanzira et al., 2017). In this study, the majority of mothers who have had no IPTp-SP are from the rural areas where entomological inoculation rates are higher (Appawu et al., 2004b) and dwellings are less likely to be constructed in a manner that will impede mosquito access compared to their urban counterparts (Wanzirah et al., 2015). They are also less likely to have the wherewithal to augment the use of bednets with other forms of mosquito control such as insecticide sprays and topical applications compared to their urban counterparts. It might well be that the lumping of the secondary and tertiary education groups in the analyses conflated this association.

Similarly, the observation that risk of parasitaemia is higher in the rural compared to the urban setting is not unexpected, as other studies have supported this finding (Kweku et al., 2017; Wanzira et al., 2017; Iqbal et al., 2016). Possible explanations include, better

breeding sites for the vector, poorer housing contruction and a relative lack of alternate sources of vector control such as insecticides and mosquito repellants in the rural areas. This results in greater infective bite rates in the rural areas.

The protective effect seen with the female gender is intriguing but gender differences in disease have been documented (Adam et al., 2017; Ruel et al., 2011). Gender disparities in the global burden of disease have been reported severally and Lim and colleagues in a systematic analysis for the global burden of disease, reported a total morbidity and mortality which was higher in males than in females throughout life (Lim et al., 2012). Indeed, there is growing evidence that, these disparities start *in utero* as evidenced in a recent cross-sectional study in Sudan that reported that carrying female foetuses increased the risk of placental malaria in their mothers (Adam et al., 2017). In their work, Muenchhoff and Goulder (2014) attest that, during infancy and childhood, males have a higher susceptibility to and greater severity of infectious diseases including malaria. Females are reported to mount stronger humoral and cellular immune responses to infections or antigenic stimulation than males. This greater immunoreactivity can be beneficial in clearing pathogens including malaria parasites (Amur, Parekh, & Mummaneni, 2012) . Occasionally though, this spirited performance can have a down side as observed in the higher mortality amongst females in the RTS, S/AS01 vaccine trial (Klein et al., 2016) or higher incidence of autoimmune disorders in females. In any case, the reason advanced for this edge of the female infant over the male maybe in the operation of the immune system; in the “minipuberty” experienced by infants there is a surge in sex steroid hormones (Hirsch et al., 2014; Kuiri-Hänninen, Sankilampi, & Dunkel, 2014). In males naturally, testosterone is the major hormone. But then testosterone has an overall immunosuppressive action biasing the immune system towards a T-helper 2 response (more immunotolerant state). Oestrogen on the other hand, which is the main female hormone supports a T-helper1 response (more immunoreactivity state) at

low doses and enhances humoral immunity and T-helper 2 responses at higher doses (Prahl et al., 2017). The reason why the females in this cohort did not appear to have a competitive advantage is unclear but might be due to deleterious effects of a suboptimally primed and nascent immune status. However, in the Sudanese study where Adam and his colleagues (2017) found a higher risk of placental malaria amongst mothers carrying female foetuses, one could argue that these female foetuses stood a greater chance of being sensitized by soluble antigens *in utero,* compared to their male counterparts. If this argument holds, it can be proffered that, the mothers of females in this birth cohort from Navrongo probably have more placental malaria than the mothers of their male counterparts, ultimately resulting in immunotolerant phenotypes that were more susceptible to malaria (Sylvester et al., 2016). Unfortunately, this study did not do placental smears and so this hypothesis cannot be tested in this cohort. Another explanation that maybe advanced is that the "minipuberty" that females are thought to benefit from does not occur within the first six months of life or in early infancy or even in the study site. The effect of oestrogen at higher doses (that of T-helper 2 effect) might be at play here, offsetting the cell mediated immunity and dampening a vigorous immunological response (Danesh & Murase, 2015; Kissick et al., 2014; Roberts, Walker, & Alexander, 2001; Kanda & Tamaki, 1999). Lastly could the male children have been receiving preferential treatment from parents and guardians? Male children being cherished in this area as potential heirs could explain the phenomenon seen.

Stratifying the results by bednet use and birthseason (the variables that failed the proportional hazards assumption test in time to first parasitaemia) did not alter significantly the intepretations of the results as regards IPT-use and the risk of parasitaemia in early infancy.

Humoral responses are mounted against all development stages of the malaria parasite (Gomes et al., 2016). Merozoite surface antigens are strongly targeted by naturally acquired humoral immunity (Deshmukh et al., 2018; Beeson et al., 2016). Opsonizing antibodies against merozoites require interactions with neutrophils or monocytes to trigger an anti-pathogenic response via antibody dependent cellular inhibition (ADCI), respiratory burst and phagocytosis (Osier et al., 2014).

ADCI and respiratory burst require the release of soluble mediators which kill parasites or inhibit their growth, while merozoite phagocytosis involves the active removal of merozoites by phagocytic cells following schizont rupture (Hill et al., 2013). ADCI, respiratory burst and phagocytosis exhibit different anti-pathogenic effector mechanisms. Cytophilic antibodies may be able to interact with both monocytes and neutrophils and result in the destruction of opsonized merozoites via multiple effector mechanisms (Hill et al., 2013). Out of the antibodies to the six antigens assayed (MSP3, N-MSP3, C-MSP3, GMZ2, GLURP-R0 and GLURP-R2), only antibodies to GLURP-R2 (Glutamine rich protein-region 2) is significantly associated with protection against malaria parasitaemia independent of IPTp-SP use. A tenfold increase in antibody titre to GLURP-R2 is significantly associated with a 35% less hazards of parasitaemia amongst early infants after adjusting for potential confounders. This protective effect of the highly immunogenic R2 repeat region of GLURP (Kumar et al., 2014) has been reported before in Ghanaian and Burkinabe children (Adu et al., 2016; Dodoo et al., 2011b). Even though Kangoye and colleagues (2014) have reported no association between antibodies to GLURP and protection from malaria in Burkinabe children, Kana and colleagues (2017) have gone further to demonstrate that, not only is GLURP-R2 associated with protection against malaria but that antibodies to GLURP specifically recognize merozoites and mediate opsonizing phagocytic activity-suggesting a mechanism of action that might be employed to reduce parasitaemia *in vivo*.

5.4 IPTp-SP use (dosing frequency), maternally transferred antibodies and uncomplicated malaria in their early infants in the KNDs.

In the case of uncomplicated malaria, compared to no IPTp-SP, infants whose mothers took one dose of IPT-SP have significantly 42% less hazards of developing uncomplicated malaria. Higher dosing frequency did not significantly alter the risk, suggesting that IPTp-SP might not be working in a manner dependent on dosing frequency. However, the mechanisms at play in conferring protection are similar to those suggested for reducing risk of parasitaemia (section 5.0.3 above). This finding of IPTp-SP's protective effect on uncomplicated malaria is at variance with findings from another cohort study in Cameroun that reported an increased susceptibility to malaria amongst infants of mothers who had two or more IPTp-SP in the first year of life (Apinjoh et al., 2015). Their study however did not make the distinction between uncomplicated malaria and severe malaria. What is more, the authors chose to lump none and one dose together as a group and to compare this to two or more doses of IPTp-SP. This study has sought to compare the various dosing frequencies of IPTp-SP and attempted a comparison thereafter.

Again, bednet use, being male and being born in the low transmission season are protective of uncomplicated malaria. Reasons have already been advanced or suggested for these findings in the section preceding this and are similar to those for risk of parasitaemia (preventing infective bites from mosquitoes with bednets, fewer infective bites in the low transmission season and likely preferential treatment for male infants). Apinjoh and colleagues (2015) however corroborate the finding in this study by reporting that infants were more susceptible to malaria in the high transmission season. Again too, risk of uncomplicated malaria was higher amongst early infants in the rural areas compared to the urban town and probably for reasons espoused for the risk of parasitaemia in section 5.0.3 above.

Stratifying the results by residence and birth season (the variables that failed the proportional hazards assumption test in time to first uncomplicated malaria episode), here too, did not alter significantly, the intepretations of the results as regards IPT-use and the risk of uncomplicated malaria in early infancy.

Tenfold total IgG titre increases to the N terminal of MSP3, the C terminal of MSP3 and region 2 of GLURP are significantly associated with less uncomplicated malaria in early infants at 24%, 36% and 34% less hazards respectively. Osier and his colleagues (2014) in a cohort study have demonstrated that these antibodies to MSP3 are associated with protection against malaria- working via the opsonic phagocytic pathway in Kenyan children, to clear merozoites from the blood stream. This might well be the mechanism employed by these antibodies in this study. The C terminal of MSP3 has consistently been associated with protection against malaria in several studies (Fowkes et al., 2010) and the finding in this study corroborates this.

5.5 IPTp-SP use (dosing frequency), maternally transferred antibodies and severe malaria in their early infants in the KNDs.

For severe malaria, after adjusting for possible confounders and covariates, use of one dose of IPTp-SP is associated with 42% less hazards of severe malaria; yet again higher dosing frequency did not significantly affect the risk of severe malaria lending further support that the action of IPTp-SP in this study is independent of dosing frequency. This protective effect of IPTp-SP on Ghanaian infants is at variance with what Harrington and his colleagues (2013) reported in Tanzanian children-an increased risk of severe malaria in the first two years of life, with two or more doses of IPTp-SP. Bednet use here was significantly associated with less hazards of severe malaria. Indeed, non users of bed nets had over one and a half times the hazards of developing severe malaria compared to those that used bed nets. This finding is not unexpected. Female infants yet again are at greater

risk of contracting severe malaria probably for reasons espoused already in the discussion on risk of parasiteamia above (section 5.0.3). The phenomenon seen with bednet use is supported by a Kenyan study that looked at bednet use and malaria hospital admissions. In that Kenyan study, greater use of bednets resulted in fewer malaria hospitalizations (Kamau et al., 2017). Again, being born in the low transmission season is associated with 59% less hazards of severe malaria in infants. Yet again early infants in rural areas were at higher risk of developing severe malaria compared to their urban counterparts. This is to be expected as these rural infants are already at greater risk of parasitaemia and uncomplicated malaria.

Again too, stratification by birth season (the variable that failed the proportional hazards assumption test in time to first severe malaria episode) did not alter significantly the intepretations of the association between IPT-SP use (dosing frequency) and the risk of severe malaria in early infants.

In this present study, tenfold increase in antibody titres to N-MSP3 and GLURP-R2 are significantly associated with less hazards of severe malaria by 20% and 34% respectively. This suggests that, antibodies still play a significant role in mitigating the deleterious effects of say, pro-inflammatory cytokines in severe malaria (Oyegue-Liabagui et al., 2017). Anti MSP3 and anti GLURP-R2 antibodies might do this by triggering an antibody dependent T cell regulatory response to counter a T-helper1 immunoreaction (Figueiredo et al., 2017).

5.6 IPTp-SP use (dosing frequency), maternally transferred antibodies and severe malaria anaemia in their early infants in the KNDs.

A subgroup analysis was carried out on severe malaria anaemia. Incidence of severe malaria anaemia amongst infants of mothers who had no IPT-SP is twice that of infants of mothers who had one dose of IPTp-SP, then proceeds to decrease with increasing

frequency of IPTp-SP, but these findings are insignificant. In the cox model, after adjusting for potential confounders, risk of severe malarial anaemia is not associated with dosing frequency of IPTp-SP. Similarly, maternal education and transmission season are not associated with severe malaria anaemia. The lack of association between the aforementioned factors and severe malaria anaemia might be due to the complex interplay of factors in the pathogenesis of severe malaria anaemia-the destruction of parasitized and unparasitized erythrocytes, born marrow suppression and dysregulation of the erythropoietin system (Djabanor et al., 2017). Here male gender is insignificantly associated with risk of severe anaemia but a case control study of severe malaria in the study area before this cohort was enrolled, reported an increased risk of severe malaria anaemia amongst males (Oduro et al., 2007) .

Yet again, stratefying the results by bednet use, birthseason, mothers' education (the variables that failed the proportional hazards assumption test in time to first severe malaria anaemia) did not alter significantly the intepretations of the results as regards IPT-use and the risk of malaria anaemia in early infancy. The Tenfold increase in the total IgG antibodies to MSP3, GMZ2, N-MSP3 and GLURP-R2 are associated with by over 4.5 fold, 4.0 fold 1.3 fold and 2.0 fold greater hazards of developing severe malaria anaemia respectively. This seeming oddity here suggests the protection conferred supposedly by an antibody dependent and opsonizing phagocytic mechanisms may not be operating in isolation. These findings perhaps underscore the inherently diverse nature of the immune effector mechanisms involved in the pathogenesis of severe malaria (Autino, Corbett, et al., 2012; Buffet et al., 2011) and encourages one to invoke the conspiracy of host genetic factors, the ADCI and "respiratory burst" pathways including balance of T-helper1 and T-helper 2 responses (Haque et al., 2014) as likely explanations. The pathophysiology of severe malaria anaemia might explain what is playing out here (White, 2018). Higher antibody titres for antigens that are known to protect against clinical malaria; MSP3, N-

MSP3, GLURP-R2) could translate into higher opsonization and splenic clearance of parasitized and antigen coated erythrocytes resulting in anaemia. This way, high antibody titres will be associated with severe malarial anaemia (Buffet et al., 2011). In addition, activation of other mechanisms like ADCI and "respiratory burst" could have deleterious effects on otherwise normal erythrocytes (collateral damage) (Deroost et al., 2016). On the other hand, they could simply be reflecting mothers' exposure to malaria antigens. Again too, nutritional anaemia could potentially confound this state of affairs (Engle-Stone et al., 2017). However, these interpretations are made with caution because of the small sample size and presumable lack of power in the severe malaria anaemia subgroup.

5.7 IPTp-SP use (dosing frequency), maternally transferred antibodies and all cause mortality in their early infants in the KNDs.

All-cause mortality chose to depart from the general rule where at least one dose of IPTp-SP has a significant protective effect on parasitaemia, uncomplicated and severe malaria. Most studies have tended to focus on the protective effect of IPTp-SP on maternal malaria and anaemia, low birth weight, preterm delivery and neonatal mortality (Eisele et al., 2012b; Mace et al., 2015; Menéndez et al., 2010a). Also, superiority of the 3 doses over the 2 doses at improving maternal anaemia and birth outcomes has been demonstrated and resulted in revisions to the IPTp-SP dosing frequency by the World Health Organization (WHO) in 2012 (Diakite et al., 2011; Isah et al., 2017). Compared to fewer dosing frequency a third dose among other benefits reduces placental parasitaemia, low birth weight and maternal anaemia as reported by Isah and his colleagues in Nigeria (2017). In this study, it is found that beyond *in utero,* hazards of all cause mortality in early infancy (first six months of life) amongst infants of mothers who had differing dosing frequency of IPTp-SP were not significantly different from those who never had IPTp-SP. In other words in this study IPTp-SP has no significant effect on survival in early infancy. A study in Benin reported that the effect of IPTp-SP when taken earlier on in pregnancy

predominantly improves birth weight (Huynh et al., 2012) whereas Bardaji and colleagues (2011) argue IPTp-SP given towards term directly improves on chances of survival in infancy. We cannot corroborate or refute these findings as we cannot determine with certainty the periods in their pregnancies that mothers of these infants took their IPTp-SP. It is conceivable that the effect of IPTp-SP on reducing all cause mortality in the study area kicks in during the second half (semester) of the first year of life. We document in this study enrolment weight over 2.5kg as independently associated with less hazards of all cause mortality in early infancy. Birth weight is an indicator of foetal growth and low birth weight has been shown to predict short term survival of the newborn (Risnes et al., 2011). Additonally, low birth weight is reported to be associated with increased death in infancy (Mayor, 2016). One major mechanism advanced to explain the effect of IPTp-SP on survival beyond *in utero* is by improving on birth weight. We do not demonstrate an association between IPTp-SP and a reduction in all cause mortality in this study even though higher birth weight is found to be associated with less risk of dying in early infancy. It is cogent then to advance that, IPTp-SP in this study site may not be engaging in the mechanism of improved birth weight. Again given the rather few deaths recorded in early infancy this study, there may not be adequate power to detect an association where there is one. In other words this could be a type 2 error at play.

Similarly, there was a lack of association between tenfold increase in total IgG titres to the six malaria antigens and mortality. Here, the lack of a significant association between IPTp-SP and all cause mortality could have arisen from the lack of specificity in the outcome (all cause mortality) biasing the point estimates towards the null hypothesis.

5.8 Comparison of magnitude of maternally transferred antibodies to selected malaria antigens amongst their early infants in the KNDs.

When these maternally transferred antibody titres to malaria antigens, MSP3, C-MSP3, N-MSP3, GLURP-R0, GLURP-R2 and GMZ2 were stratified on IPTp-SP use (dosing frequency), no significant differences were seen between the median titres in analyses of variance; neither are any significant differences seen in the log 10 transformed titres and IPTp-SP use. Indeed, in a simple linear regression run, IPTp-SP use does not predict a tenfold rise in titre of total IgG for any of the six malaria antigens under investigation. In the light of this, the null hypothesis [$H_{0:}$ There is no difference in maternally transferred (transplacental or total cord blood IgG) antibody titres in early infants born to mothers who had IPTp-SP compared to early infants of mothers who had no IPTp-SP] cannot be rejected. So we fail to reject and uphold the null hypothesis in this study. Thus, in this study no significant differences exist in total IgG antibodies to MSP3, C-MSP3, N-MSP3, GLURP-R0, GLURP-R2 and GMZ2 in early infants, as a result of differing doses of IPTp-SP. The closest study to this has been a recent cohort study in the coastal belt of Ghana where the investigators could not demonstrate that mothers' IPTp-SP influenced infants' antibody titres (Stephens et al., 2017a). Similarly, Log10 transformed titres and birthweight, birth season, gender, bednet use and mother's education do not yield any statistically significant differences. Consistently however, the IgG antibody titres to MSP3, C-MSP3, GLURP-R2 and GMZ2 were higher than GLURP-R0 and N-MSP3, even if not significantly so**.** This observation, maybe a result of exposure to malaria antigens, rather than possessing protective value against malaria. As malaria continues to decline globally more sensitive tools for monitoring and surveillance are required for the elimination and eradication drive (Kusi et al., 2014). In that regard, MSP3, C-MSP3, GLURP-R2 may warrant further investigations as alternate markers of exposure and transmission.

5.9 Limitations of the study

This study has some limitations to it:

1. This was a secondary one was compelled to work with data already collected, variables decided *apriori*. Potential confounders such as Haemoglobin C, S, and G6PD status were not controlled for.

2. IPTp-SP use was reported and not directly observed and so recall bias by the mothers cannot be ruled out.

3. There were no placental smears and mothers' sera to test the relationship between maternal and infant pathologies and antibody responses.

4. Total IgG was assayed and so IgG subclasses and the effect of cytophilic and non cytophilic antibodies could not be probed.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Key findings

1. Dosing frequency of IPTp-SP did not significantly influence the median antibody titres to malarial blood stage antigens MSP3, N-MSP3, C-MSP3, GLURP-R0, and GLURP-R2 GMZ2 in their infants or offspring.

2. Tenfold increase in maternally transferred antibodies to GLURP-R2 is associated with reduced risk of parasitaemia, uncomplicated and severe malaria in early infancy irrespective of IPTp-SP.

3. Tenfold increase in maternally transferred antibodies to N-MSP3 and C-MSP3 are associated with reduced risk of uncomplicated and severe malaria in early infancy irrespective of IPTp-SP.

4.Tenfold increase in maternally transferred antibodies to malarial blood stage antigens MSP3, N-MSP3, C-MSP3, GLURP-R0, GLURP-R2 GMZ2 are not associated significantly with all cause mortality.

5. Compared to mothers who had no IPTp-SP, mothers' use of at least one dose (frequency of use) of IPTp-SP significantly reduces risk of malaria parasitaemia, uncomplicated malaria and severe malaria in early infancy (first six months of life). More frequent dosing does not significantly improve on risk of malaria.

6. Mothers' IPTp-SP dosing frequency is not significantly associated with all-cause mortality in early infants compared to offspring of mothers who had none.

7. Bednet use, being born in the low transmission season and being male are significantly associated with less risk of parasitaemia, uncomplicated malaria and severe malaria but not with all-cause mortality.

8. Higher formal education is independently significantly associated with less risk of parasitaemia but not with uncomplicated, severe malaria or all-cause death.

9. Majority of mothers who have no IPTp-SP are from the rural setting and over half of mothers who have no IPTp-SP have no formal education.

10. IPTp-SP uptake is reduced during the high transmission season.

6.2 Conclusions

The study findings provide evidence that even though IPTp-SP beyond *in utero* reduces risk of malaria in early infancy in the KNDs, the dosing frequency of IPTp-SP does not significantly alter titres of maternally transferred antibodies (Cord blood total IgG) to the malaria antigens MSP3, N-MSP3, C-MSP3, GLURP-R0 GLURP-R2 GMZ2. However, maternally transferred antibodies (total IgG) to N-MSP3, C-MSP3 and GLURP-R2 are independently associated with less risk of malaria in early infancy.

6.2.1 Contributions to Knowledge

1. Titres of maternally transferred antibodies to MSP3, N-MSP3, C-MSP3, GLURP-R0 GLURP-R2 and GMZ2 are independent of IPT-SP dosing frequency in the KNDs.

2. This study contributes to the scanty literature that IPTp-SP beyond *in utero* reduces risk of malaria in early infancy.

6.3 Recommendations

TO THE DISTRICT HEALTH MANAGEMENT TEAM:

1. As at least one dose of IPTP-SP is associated with reduced risk of malaria so existing mechanisms of delivery of IPTp-SP ought to be strengthened in line with the revised WHO policy.

2. Novel ways ways of promoting delivery of IPTp-SP as an adjunct tool to reducing early infant mortality have to be explored.

TO POLICY MAKERS:

1. IPTp-SP might be a useful adjunct tool to reducing malaria morbidity in the KNDs.

TO THE SCIENTIFIC COMMUNITY:

1. Functional assays on antibodies and better designed and well powered studies should be carried out to unravel the targets and mechanisms of action of IPTp-SP on risk of malaria in the early infancy.

2. Malaria antigens N-MSP3, C-MSP3 and GLURP-R2 as single or composite antigens should be investigated further as potential vaccine candidates.

3. Malaria antigens MSP3, C-MSP3, GLURP-R2 may warrant further investigations as alternate markers of exposure and transmission.

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APPENDICES

Appendix A: Supplemental Tables & Figures (Appendix A)

Table Ai. Age specific parasitaemia in a birth cohort enrolled between 2006 and 2007 in the KNDs.

	Age			
Parasitaemia status	**<1 month**	**1-3months**	**3-5 months**	**5-6 months**
Aparasitaemic[n]%	[328]97.62	[1152]91.43	[1269]83.32	[603]84.34
Parasitaemic[n]%	[8]2.28	[108]8.57	[254]16.68	[112]15.66

Table Aii. Associations between some maternal, infant characteristics and IPTp-SP use (dosing frequency) in the birth cohort enrolled between 2006 and 2007 in the KND

Characteristic	**0 IPTp-SP**	**1+ IPTp-SP**	χ^2	p-value
Birth weight>2.5kg**[n]%**	[391] 80.56	[343]18.18		0.558
Birth weight<2.5kg[n]%	[315]80.56	[1544]82.82		
Parasitaemic at birth[n]%	[8]2.05	[39]2.07		0.979
Aparasitaemic at birth[n]%	[383]97.95	**[**1848]97.93	0.00	

Table Aii continued				
Characteristic	**0 IPTp-SP**	**1+ IPTp-SP**	χ^2	p-value
Gender[n]%				0.933
Male	[192]49.10	[931]49.34		
Female	[199]50.90	[956]50.70	0.01	
Birth season [n]%				
High transmission	[271]69.31	[1054]55.86		<0.001
Low transmission	[120]30.69	[833]44.14	24.09	
Residence [n]%				
Urban	[32]8.18	[278]89.68		0.001
Rural	(359]91.82	[1609]85.27	11.81	
Location[n]%				
Non-irrigated	[312]79.80	[1585]84.00		0.043
Irrigated	[79]20.20	[302]16.00	4.10	
Bednet use[n]%				0.167
Yes	[197]50.385	[1023]54.21		
No	[194]49.62	[864]45.79	1.91	
Gravidity[n]%				
Primigravidae	[86]21.99	[465]24.19		0.504
Secundigravidae	[81]20.72	[392]82.8820.77		
Multigravidae	[224]57.29	[1030]82.1454.58	1.37	
Formal Education[n]%				
None	[194]49.62	[885]46.90		0.042
Basic	[174]44.40	[815]43.19		
≥ secondary	[23]10.955.88	[187]9.91	6.33	

Table Aiii. IPT-SP use (dosing frequency) and incidence of malaria parasitaemia in their early infants and in the primary birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time	Rate/1000-person years(95%CI)
0 IPTp-SP	131	2.22	58.84 (49.59,69.84)
1+ IPTp-SP	330	10.76	30.57 (27.45,34.06)

Table Aiv. IPTp-SP use (dosing frequency) and incidence of uncomplicated malaria in their early infants in the primary cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time	Rate/1000-person years(95%CI)
0 IPTp-SP	71	2.24	31.67 (25.09,39.96)
1+ IPTp-SP	184	10.96	16.78 (14.53,19.39)

Table Av. IPTp-SP use (dosing frequency) and incidence of severe malaria in their early infants in the primary cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	No of events	Person time	Rate/1000-person years(95%CI)
0 IPTp-SP	103	2.24	45.94 (37.86,55.72)
1+ IPTp-SP	241	10.96	21.98 (19.38,24.94)

Table Avi. IPTp-SP use (dosing frequency) and incidence of severe malaria anaemia in their early infants in a birth cohort enrolled between 2006 and 2007 in the KNDs.

characteristic	no of events	Person time	Rate/1000-person years(95%CI)
0 IPTp-SP	5	2.2	2.22 (0.92-5.36)
1+ IPTp-SP	7	10.96	0.63 (0.30,1.34)

Table Avii. IPTp-SP use (dosing frequency) and incidence of all cause mortality in their early infants in the primary birth cohort enrolled between 2006 and 2007 in the KNDs.

characteristic	no of events	Person time	Rate/1000-person years(95%CI)
0 IPTp-SP	6	2.28	2.63 (1.18,5.85)
1+ IPTp-SP	23	11.06	2.08 (1.38,3.13)

Table Aviii. IPTp-SP dosing frequency (0, 1+) and hazards for malaria parasitaemia in their early infants in the primary study enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Crude hazard(95%CI)	p-Values	Adjusted hazard(95%CI)	p-Values
IPTp-SP use					
0 IPTp-SP	[383]	1.0		1.0	
1+ IPTp-SP	[1848]	0.52 (0.42,0.65)	<0.001	0.60 (0.48,0.76)	<0.001
Maternal education					
None	[1055]	1.0		1.0	
Primary	[937]	0.76 (0.62,0.93)	0.010	0.85 (0.69,1.04)	0.131
≥Secondary	[203]	0.37 (0.23, 0.60)	<0.001	0.69 (0.43,1.11)	0.129
Gravidity					
Primigravidae	[539]	1.0			
Secundigravidae	[462]	1.17 (0.86,1.60)	0.319	-	-
Multigravidae	[1230]	1.17 (0.90,1.50)	0.220	-	-
Bednet use					
Yes	[1192]	1.0			
No	[1039]	1.90 (1.55,2.00)	<0.001	1.83 (1. 50,2.24)	<0.001
Sex					
Male	[1101]	1.0		1.0	
Female	[1130]	1.32 (1.08,1.60)	0.006	1.32 (1.09,1.60)	0.004
Birth weight					
≥2.5kg	[1825]	1.0		-	
<2.5kg	[406]	1.04 (0.79,1.35)	0.797	-	-
Birth season					
High transmission (wet)	[1291]	1.0		1.0	
Low transmission (dry)	[940]	0.39 (0.31,0.50)	<0.001	0.41 (0.32,0.53)	<0.001
Location					
Non-irrigated	[1856]	1.0		-	-
Irrigated	[375]	1.02 (0.78,1.33)	0.876	-	-
Residence					
Urban	[305]	1		-	
Rural	[1926]	4.16 (2.45, 7.03)	<0.001	3.80 (2.19,6.59)	<0.001

W=Wald p value a=Adjusted for residence, birth season, sex, bednet use, maternal education. 1.0=referent

Table Aix. IPTp-SP dosing frequency (0, 1+) and hazards for uncomplicated malaria in their early infants in the primary cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Crude hazard(95%CI)	p-Values	Adjusted hazard(95%CI)	p-Values
IPTp-SP use					
0 IPTp-SP	[391]	1.0		1.0	
1+ IPTp-SP	[1887]	0.53 (0.40,0.70)	<0.001	0.58 (0.43,0.78)	<0.001
Maternal education					
None	[1079]	1.0		1.0	
Primary	[989]	0.72 (0.60,0.91)	0.017	0.85 (0.64,1.18)	0.239
≥Secondary	[21]	0.49 (0.28, 0.88)	<0.016	0.82 (0.46,1.47)	0.510
Gravidity					
Primigravidae	[551]	1.0			
Secundigravidae	[473]	1.05 (0.72,1.584	0.790	-	-
Multigravidae	[1254]	1.00 (0.73,1.38)	0.993	-	-
Bednet use					
Yes	[1220]	1.0			
No	[1058]	1.52 (1.18,1.96)	<0.001	1.46 (1.19,1.79)	<0.001
Sex					
Male	[1123]	1.0		1.0	
Female	[1155]	1.36 (1.11,1.66)	0.002	1.45 (1.13,1.88)	0.003
Birth weight					
≥2.5kg	[1859]	1.0		-	
<2.5kg	[419]	1.04 (0.79,1.35)	0.797	-	
Birth season					
High transmission	[1325]	1.0		-	
Low transmission	[953]	0.43 (0.32,0.59)	<0.001	0.40 (0.29,0.54)	<0.001
Location					
Non-irrigated	[1879]	1.0		-	-
Irrigated	[381]	1.22 (0.88,1.70)	0.216	-	-
Residence					
Urban	[310]	1.0			
Rural	[1968]	4.83 (2.14,9.69)	<0.001	4.39 (2.12,9.11)	<0.001

W=Wald p value a=Adjusted for residence, birth season, sex, bednet use, maternal education. 1.0=referent

Table Ax. IPTp-SP dosing frequency (0,1+) and hazards for severe malaria in their early infants in the primary birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Crude hazard(95%CI)	p-Values	Adjusted hazard(95%CI)	p-Values
IPTp-SP use					
0 IPTp-SP	[391]	1.0		1.0	
1+ IPTp-SP	[1887]	0.48 (0.38,0.61)	<0.001	0.56 (0.43,0.73)	<0.001
Maternal education					
None	[1079]	1.0		1.0	
Basic	[989]	0.36 (0.10,1.34)	0.128	0.82 (0.65,1.04)	0.107
≥Secondary	[21]	3.35e-16 (1.73e-16 6.52e-16)	<0.001	0.92 (0.57,1.50)	0.754
Gravidity					
Primigravidae	[551]	1.0			
Secundigravidae	[473]	0.29 (0.32,2.56)	0.264	-	-
Multigravidae	[1254]	0.75 (0.22,2.55)	0.646	-	-
Bednet use					
Yes	[1220]	1.0		1.0	
No	[1058]	3.46 (0.94,12.77)	0.062	1.61 (1.28,2.01)	<0.001
	[1123]				
	[1155]				
Sex					
Male	[1859]	1.0			
Female	[419]	1.53 (1.28,1.93)	<0.001	1.51(1.21,1.89)	<0.001
Birthweight					
≥2.5kg	[1325]				
<2.5kg	[953]	1.12 (0.81,1.54)	0.495	-	-
Birth season					
High transmission	[1879]	1.0		1.0	
Low transmission	[381]	0.40 (0.30,0.53)	<0.001	0.42 (0.32,0.56)	<0.001
Location					
Non-irrigated	[310]	1.0		-	-
Irrigated	[1968]	1.02(0.75, 1.39)	0.892	-	-
Residence					
Urban				1.0	
Rural		4.73(2.62,8.56)	<0.001	4.40(2.36,8.19)	<0.001

W=Wald p value a=Adjusted for residence, birth season, sex, bednet use, maternal education. 1.0=referent

Table Axi. IPTp-SP dosing frequency (0,1+) and hazards for severe malaria anaemia in their early infants in a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Crude hazard(95%CI)	p-Values	Adjusted hazard(95%CI)	p-Values
IPTp-SP use					
O IPtp-SP	[391]	1.0		1.0	
1+ IPTp-SP	[1887]	0.29 (0.09,0.90)	0.032	0.40 (0.12,1.37)	0.144
Maternal Education					
None	[1079]	1.0		1.0	
Primary	[989]	0.36 (0.10,1.34)	0.129	0.81 (0.64,1.04)	0.109
≥Secondary	[21]	3.35e-08 (-)	1.00	-	-
Gravidity					
Primigravidae	[551]	1.0			
Secundigravidae	[473]	0.29 (0.03, 2.57)	0.265	-	-
Multigravidae	[1254]	0.75 (0.22,2.57)	0.648	-	-
Bednet use					
Yes	[1220]				
No	[1058]	3.46 (0.94,12.79)	0.062	-	-
Sex					
Male	[1123]	1.0			
Female	[1155]	1.35 (0.43,4.30)	0.596	-	-
Birthweight					
≥2.5kg	[1859]				
<2.5kg	[419]	1.09 (0.80,1.49)	0.588	-	-
Birth season					
High Transmission	[1325]	1.0		1.0	
Low transmission	[953]	0.13 (0.02,0.97)	0.047	0.16 (0.02,1.27)	0.083
location					
Non-irrigated	[1879]	1.0		-	-
Irrigated	[381]	0.98 (0.22, 4.48)	0.982	-	-
Residence					
Urban	[310]	1.0			
Rural	[1968]	9.93e+15 (-)	-	-	-

W=Wald p value a=Adjusted for birth season, mothers' education, residence. 1.0=referent

Table Axii. 1+IPTp-SP dosing frequency (0, 1+) and hazards for all cause mortality in early infancy for a birth cohort enrolled between 2006 and 2007 in the KNDs.

Characteristic	[n]	Crude hazard(95%CI)	p-Values	Adjusted hazard(95%CI)	p-Values
IPTp-SP use					
0 IPTp-SP	[391]	1.0		-	
1+ IPTp-SP	[1887]	0.79 (0.32,1.94)	0.609	-	-
Maternal education					
None	[1079]	1.0		1.0	
Primary	[989]	1.37 (0.63,2.91)	0.421	-	-
Secondary	[21]	0.85 (0.19,3.81)	0.837	-	-
Tertiary					
Gravidity	[551]				
Primigravidae	[473]	1.0		-	
secundigravidae	[1254]	1.04 (0.40,2.70)	0.933	-	-
Multigravidae		0.59 (0.251.39)	0.225	-	-
Bednet use	[1220]				
No	[1058]	1.0			
Yes		0.93(0.44, 1.94)	0.855		
Sex	[1123]				
Male	[1155]	1		-	-
Female		1.38 (0.66,2.89)	0.393	-	-
Birth weight	[1859]				
<2.5kg	[419]	1.0		1.0	
≥2.5kg		0.21(0.10,0.42)	<0.001	0.25 (0.12,0.52)	<0.001
Birth season	[1325]				
High transmission	[953]				
Low transmission		0.73 (0.34,1.56)	0.418	-	-
Location	[1879]				
Irrigated	[381]	1.0			
Non-irrigated		0.17 (0.02, 1.30)	0.905	-	-
Residence	[310]				
Urban	[1968]	1.0		1.0	
Rural		0.99 (0.34,2.82)	0.979	-	-

W=Wald p value a=Adjusted for birth weight. 1.0=referent

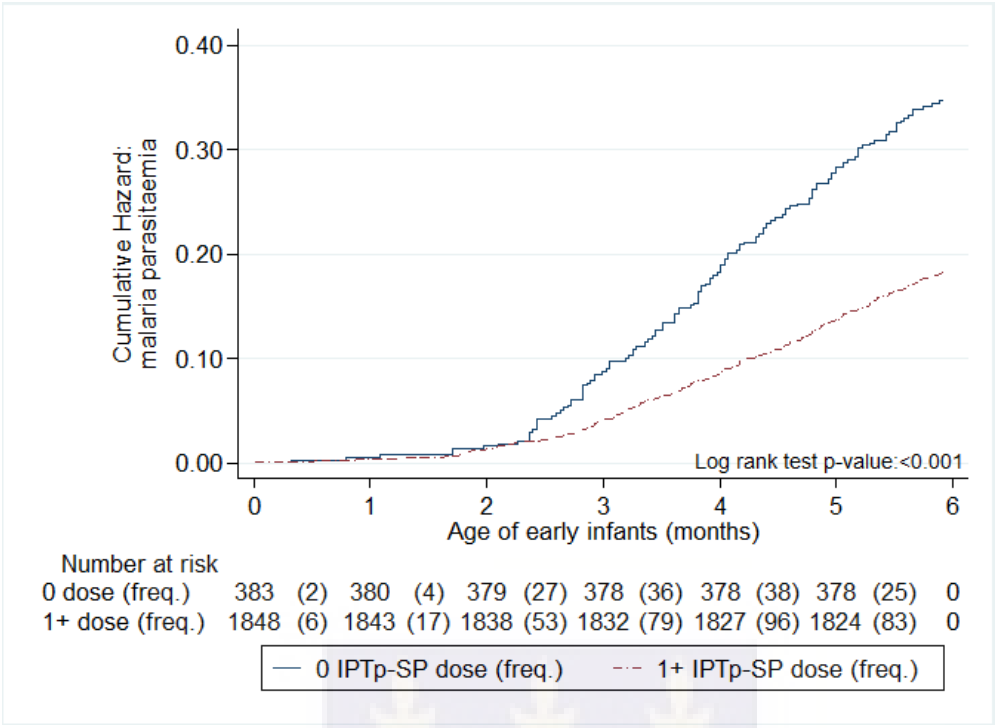


Figure Ai. Hazard Ratio estimates for the use of at least one dose of IPTp-SP and Risk of malaria parasitaemia in their early infants in the KNDs.
Legend: Freq=dosing frequency

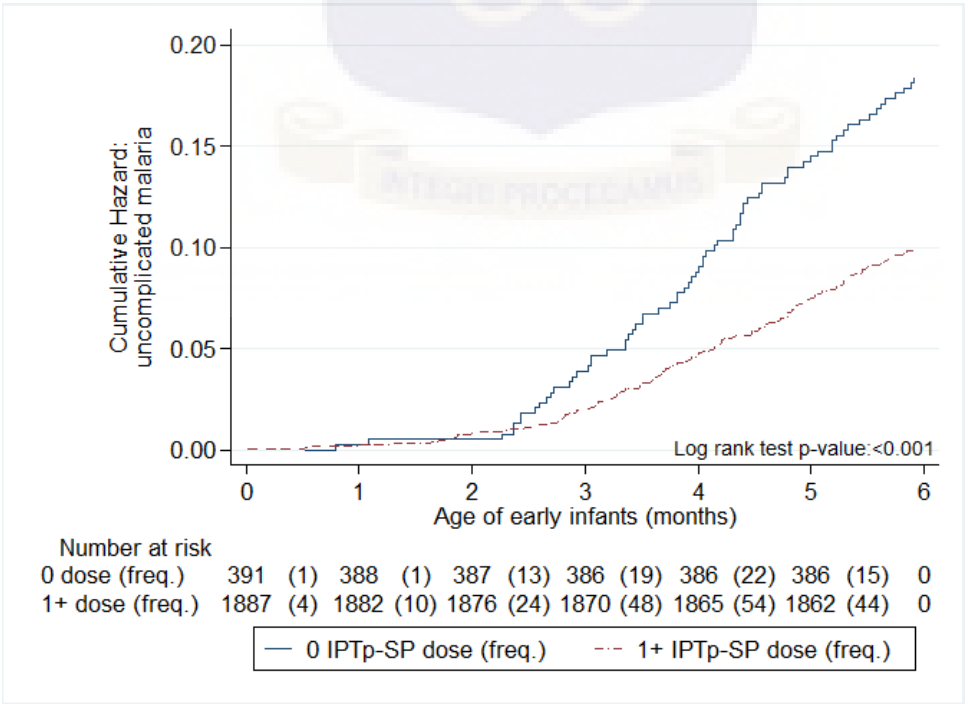


Figure Aii. Hazard Ratio estimates for the use of at least one dose of IPTp-SP and Risk of uncomplicated malaria in their early infants in the KNDs.
Legend: Freq=dosing frequency

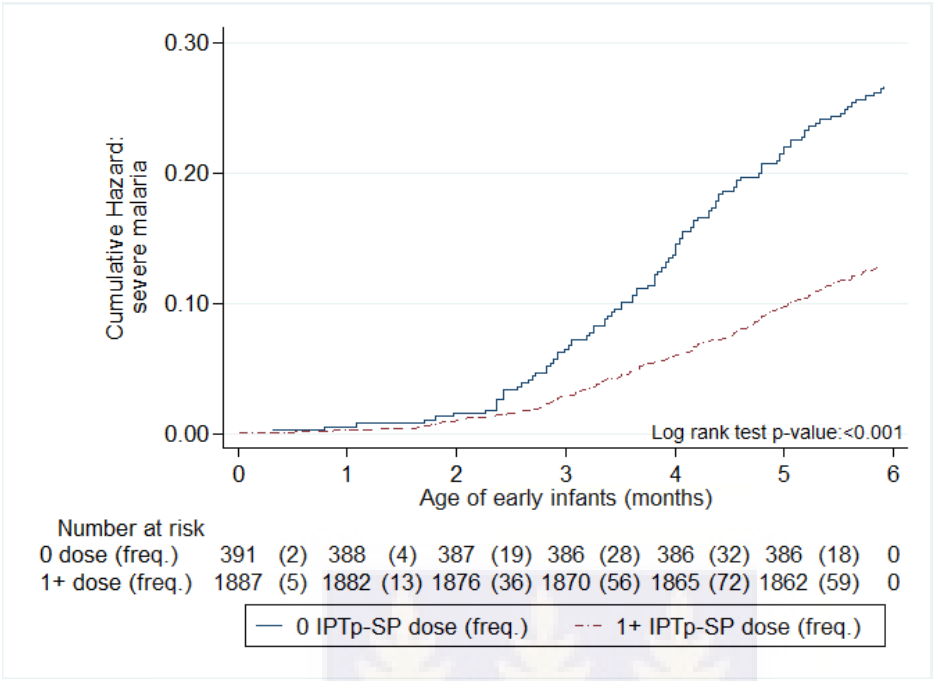


Figure Aiii. Hazard Ratio estimates for the use of at least one dose of IPTp-SP and Risk of severe malaria in their early infants in the KNDs.
Legend: Freq=dosing frequency

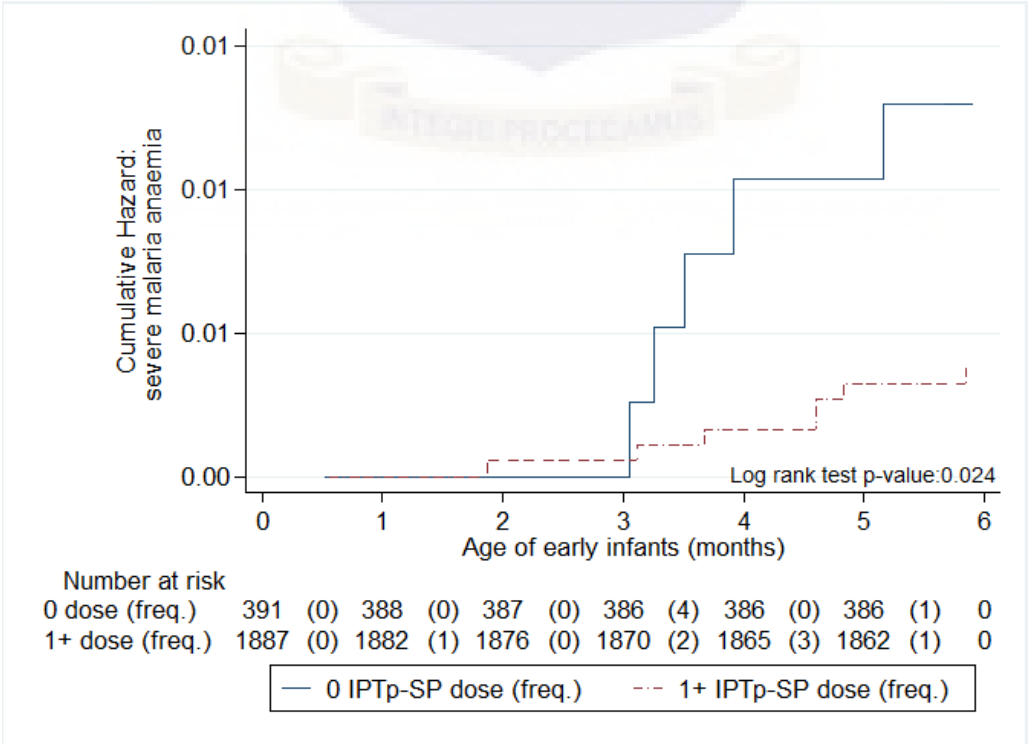


Figure Aiv. Hazard Ratio estimates for the use of at least one dose of IPTp-SP and Risk of severe malaria anaemia in their early infants in the KNDs.
Legend: Freq=dosing frequency

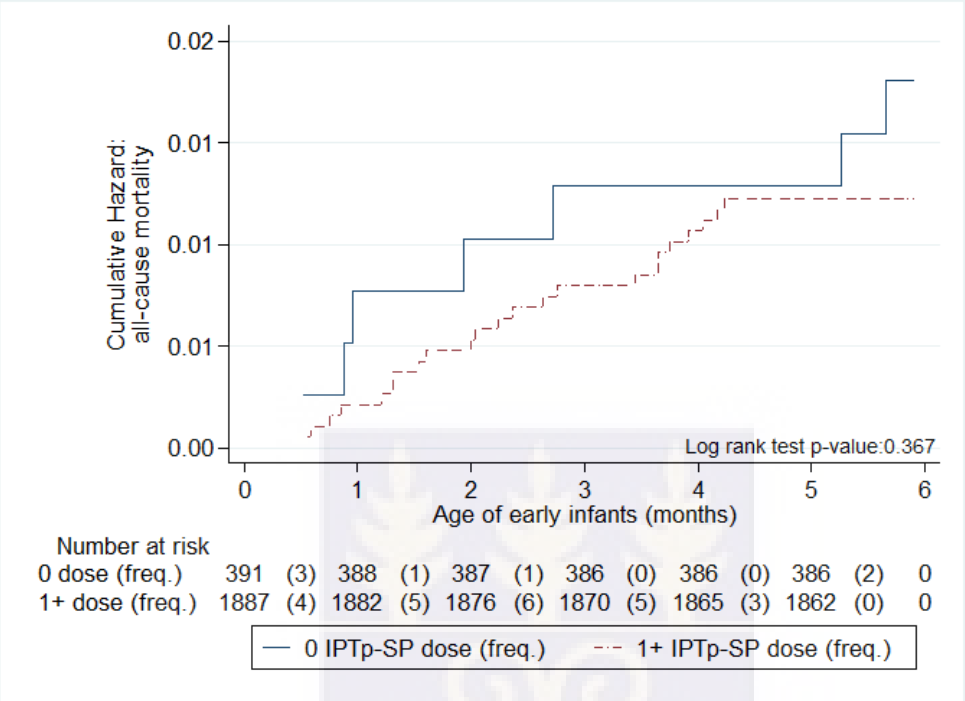


Figure Av. Hazard Ratio estimates for the use of at least one dose of IPTp-SP regimen and Risk of all cause mortality in their early infants in the KNDs.
Legend: Freq=dosing frequency

Appendix B -Questionnaire Forms for Main (1^0) Study

bcs enrolment visit form **1-4**

General

1. STUDY ID:	B	C	S					STUDY_ID

2. Date of Enrolment: (dd/mm/yyyy)								DATE

3. Name of delivery attendant:	ATTNAME

4. Delivery attendant	1. Midwife	2. Nurse	3.TBA	4. Other	ATTEND

1.Hospital	2. Health Centre	3. TBA Home	4. Home	5. CHC	6.Other	FACILITY

5. Delivery facility	

6.Type of delivery	1. Spontaneous	2. Assisted	3. Caesarean	DTYPE

7. Compound ID of Study Child:						COMPID

8. Compound Name of study child:	COMPNAM

9. Name of Village/Locality of study child:	VILLNAME

10. Name of the Section:		SECTION

11. Name of Mother:					MANAME
12. Name of Father:					FANAME
13. Paternal Ethnicity	1.Kasem	2.Nankam	3. Buli	4.Other	PAETHN
14. Maternal Ethnicity	1.Kasem	2.Nankam	3. Buli	4.Other	MAETHN

bcs enrolment visit form 2-4

Fetal Characteristics

1. **STUDY ID**: | B | C | S | | | | | STUDY_ID

2. Date of Birth (DOB): (dd/mm/yyyy) | | | | | | | | | DOB

3. Name of Study Child: CNAME

4. Sex of Study Child: 1. Male | 2. Female SEX

5. Weight at birth (Kg): | | . | | BWEIGHT

6. Weight at enrolment (Kg): | | . | | EWEIGHT

7. Number of babies during this delivery | | NCHILD

8. Maturity of study child | 1. Term | 2. Small for date | 3. Preterm | MATURE

9. Physical Appearance of study child at birth | 1. Normal | 2. Deformed | APPEAR

10. ***if deformed (specify***) | | DEFORM

11. Birth order of study child | | B_ORDER

12. Is the study child well today | 1. YES | 2. NO | 9.NK | SC_WELL

13. **If No, specify**		SC_SICK

14. Is the study child sucking well today	1. YES	2. NO	9.NK	SUCK

15. Is the study child yellow today	1. YES	2. NO	9.NK	JAUND

bcs enrolment visit form 3-4

Maternal characteristics

STUDY ID:	B	C	S					STUDY_ID

Name of Respondent | RNAME

2. Date of Birth (if not known ask Age (years): | | | | | | | | | MDOB

3. Age in years (if DOB is unknown) | | | MAGE

4. Number of years of formal education: | | | EDUC

5. Occupation:

1. House wife	2. Farming	3. Trading	4. Public service	5. Other	OCCUP

6. Marital Status:

Married	2. Single	3. Divorced	4. Separated	5. Widowed	MARISTA

7. Is mother alive: | 1. Yes | 2. No | MOTALIVE

8. Number of pregnancies: | | | NPREG

9. Number of siblings of Study Child still alive: | | | SALIVE

10. Was the Study Child born at term: | 1. Yes | 2. No | 9. NK | BORNTERM

11. Did the mother attend antenatal clinic during the pregnancy | | | ANC

12. Where was the mother attending antenatal clinic, specify | 1.WMH | 2.Health Centre | 3.CHC | 4.Others (specify) | PLANC

13. Was the mother sleeping under bed net during this pregnancy | 1.Yes | 2.No | BEDNET

14. How many SP (fansidar) was given at antenatal during this pregnancy | | FANSIDAR

15. Did the mother fall sick during this pregnancy	1.Yes	2.No	SICK

16. What was the type of sickness (list all , if many)	1.	2.	L-SICK
	3.	4.	

17. Field Worker Code			FWCODE

bcs enrolment visit form 4-4

1. **STUDY ID**:

B	C	S					STUDY_ID

Enrolment completed:

1. Blood specimen collected

1.Yes	2. No	CBLOOD

2. Source of blood specimen collected

1.Heel	2. Cord	3.Other	SBLOOD

3.Specimen processed into:

1. Serum	1.Yes	2. No	SERUM
2. Filter paper	1.Yes	2. No	FPAPER
3. Blood smear	1.Yes	2. No	BSMEAR
4. Whole blood	1.Yes	2. No	WBLOOD

State the Haemoglobin at birth

		.		HAEBIR

Date: (dd/mm/yyyy)

								OUTDATE

If not enrolled, state reasons

Field CCB:

Investigators Name: _____

Investigator's Signature: _____

bcs out-patient visit form **1-1**

1. **STUDYCHILD ID**:

B	C	S					STUDY_ID

2. Visit Date: (dd/mm/yyyy)		VDATE
3. Name of Study Child:		NAME
4. Sex of Study Child:	1. Male 2. Female	SEX
5. Name of Respondent:		RNAME

PRESENTING COMPLAINTS

Has the Study child any of the following during this illness?			**Duration (in days)**	
6. Fever	1. Yes	2. No		FEVER
7. Shaking chills/rigors	1. Yes	2. No		SHAKING
8. Diarrhoea	1. Yes	2. No		DIARR
9. Vomiting	1. Yes	2. No		VOMIT
10. Headache	1. Yes	2. No		HEAD
11. Abdominal Pains	1. Yes	2. No		APAINS
12. Bodily Pains	1. Yes	2. No		BPAINS
13 Cough	1. Yes	2. No		COUGH
14. Chest pain	1. Yes	2. No		CPAINS
15. Difficulty in breathing	1. Yes	2. No		BREATH
16. Bloody or Coca-Cola Urine	1. Yes	2. No		BURINE
17. Inability to suck /drink /eat	1. Yes	2. No		SDE
18. Rashes	1. Yes	2. No		RASH
19. Sleeping all the time	1. Yes	2. No		SLEEP
20. Convulsions	1. Yes	2. No		CONV
21.Other (specify)				OTHER1
FW/FS/CRC CODE:				CODE

bcs out-patient visit form 1-2

1. **STUDY CHILD ID**:	B C S	STUDY_ID
2. Visit Date: (dd/mm/yyyy)		VDATE
3. Axillary Temperature (^{o} C):	.	TEMP
4. Weight (Kg):	.	WEIGHT

5.	Malaria blood smear taken?	1. Yes	2. No		BSMEAR
6.	Filter paper	1. Yes	2. No		FPAPER
7.	Haemocue Hb (g/dl):			.	HAEMO
8.	Rapid malaria diagnostic test	1. Positive	2. Negative	3. Not Done	RAMAT

Hospital Clinician's summary:

9	Final diagnosis	1.Malaria	2.AR1/cough	3.Diarrhoea/dysentery	DIAG
		4. Malnutrition	5. Accidents	6. Others	
10	Treatment (Multiple answers allowed)	1. Antimalaria	2.Antibiotics	3.Antipyretics	TREAT
		4.Hematinics		5. Others (specify)	
11	Outcome	1. Treated and discharge		2. Admitted / 3.Died	OUTCOM

Blood smear microscopy Results

12	Malaria Parasites	1. Positive		2. Negative		PARAST
13	Species	1. PF	2.	3. PO	4.	SPECIES

			PM		Mixed	
14	Trophozoite Counts/ (200 wbc)					TCOUNT
15	Gametocytes Counts / (200 wbc)					GAMET

Checked and certified by: | | | CODE

bcs in-patient visit form **1-1**

1. **STUDY ID** | B | C | S | | | | | STUDY_ID

2. In-patient number: | | | | | | | | INPAT

3. Date of Admission: (dd/mm/yyyy) | | | | | | | | | DATE

4. Time of Admission: (24hr clock) | | | : | | | TIME

5. Name of Study Child: NAME

6. Sex of Study Child: 1. Male | 2. Female | SEX

7. Name of Respondent: NAMEREP

RECENT MEDICAL HISTORY

8. Has Study Child fallen **ILL** recently: | 1. Yes | 2. No | 9. NK | ILL

(if no, skip to 12)

9. What was the ILLNESS?	1. Malaria / Paa /Pua	2. ARI/ Chest problem		ILLNAM
	3. Diarrhoea	4. Others	9.NK	

10. Was the child hospitalised, if yes which hospital	1. Navrongo	2. Sandema	3. Bolga	4.Other	ADMITED

11. If Yes, reason for the hospitalisation:

1. Short of	2. Convulsion	3. Chest	4. Diarrhoea	5. Malaria	6. Other	TRANFUSE

13. Name of malaria drug administered:	1. Amodiaquine /Artesunate	2. Chloroquine	3. Fansidar	4. Other	DRUGNAM

blood		Problem			

12. Has the child received malaria treatment in the last one month:	1. Yes	2. No	9. NK	MALTREAT

14. Have you sought treatment for the child somewhere before coming to the Navrongo hospital/ward?

1. Home	2. Herbalist /traditional healer	3. Health Facility	4. Others	5. No	ORIGIN

15. Has this study child been sleeping under **Bednet?**	1.Yes	2.No	9.NK	BEDNET

bcs in-patient visit form 1-2

SYMPTOMS AT ADMISSION

STUDY ID:

B	C	S					STUDY_ID

Does Studychild have any of the following symptoms during this illness?	**Present**	**Absent**	**Duration (in days)**	
1. Fever	1.Yes	2. No		FEVER
2. Shaking chills/rigors	1.Yes	2. No		SHAKING
3. Diarrhoea	1.Yes	2. No		DIARR
4. Vomiting	1.Yes	2. No		VOMIT
5. Joint pains	1.Yes	2. No		JPAINS
6. Bodily pains	1.Yes	2. No		BPAINS
7. Headache	1.Yes	2. No		HEADA
8. Cough	1.Yes	2. No		COUGH
9. Difficulty in breathing	1.Yes	2. No		BREATH
10. Bloody /Coca-cola Urine	1.Yes	2. No		BURINE
11. Inability to suck /drink /eat	1.Yes	2. No		SDE
12. Inability to sit/stand unaided	1.Yes	2. No		SSU
13. How many convulsions did the study child have in the last 24 hours?				CONVUL
14. How is the Baby Crying	1. Appropriate	2. In Appropriate		BCRY
15. Can Baby open the eyes?	1. Yes	2. No		BEYE
16. Can the Baby move their Arms?	1. Yes	2. No		BARM
MO/SN/FS/FW CODE:				CODE

.

bcs in-patient visit form 1-3

Physical Examination

STUDY ID	B C S	STUDY_ID
1. Respiration (number/minute):		RESRATE
2. Axillary Temperature (°c):	.	TEMP
3. Pulse (heartbeat)/ (minute):		HEARTRATE
4. Systolic blood pressure (mmHg):		SBP
5. Diastolic blood pressure (mmHg):		DBP
6. Weight (Kg):	.	WEIGHT

7. Hydration Status:	1. None	2. Some	3. Severe	DEHYDRA
8. Jaundice*:*		1.Yes	2.No	JAUND
9. Respiratory distress:		1. Present	2. Absent	RESDISTRES
10. Splenomegaly:		1. Present	2. Absent	SPLEENSIZE
11. Hepatomegaly:		1. Present	2. Absent	LIVERSIZE
12. Lethargy		1. Present	2. Absent	LETHARGY
13. Restlessness		1. Present	2. Absent	RESTLESS
14. Unconsciousness/Semi-consciousness:		1. Present	2. Absent	UNCONSCI

15. Blantyre Coma Score:	**Feature**	Eye	Verbal	Motor	Total	TOTAL
	Score =					
16. **Clinical Diagnoses**	1. Cerebral Malaria	2.Severe Anaemia	3.Febrile Convulsion	4. Others		CDIAG

bcs in-patient visit form 1-4

LABORATROY RESULTS :

STUDY ID | B | C | S | | | | | STUDY_ID

Date: (dd/mm/yyyy) | | | | | | | | | DATE

Blood glucose (mmol/l): | | | . | | BGLUCOSE

Haemocue Hb (g/dl): | | | . | | HAEMO

PARASITOLOGY

1.	Parasitaemia	1. Positive		2. Negative		PARAST
2.	Species	1. Pf	2. PM	3. PO	4. Mixed	SPECIES
3	Trophozoite Counts/ (200 wbc)					COUNT
7.	Gametocytes Count/ (200 wbc)					GAMET
8.	Lab. Tech's Code					LCOD

OTHER LABORATORY TESTS, SPECIFY

bcs in-patient visit form 1-5

STUDY ID | B | C | S | | | | | STUDY_ID

HAEMATOLOGY RESULTS

#	Parameter	Results				Units	Range	
1	WBC			.		$X10^3$/ul	4.5-10.5	WBC
2	RBC		.			$X10^6$/ul	4.00-6.00	RBC

3	**HGB**			•			g/dl	11.0-18.0	HGB
4	HCT			•			%	35.0-60.0	HCT
5	MCV			•			fL	80.0-99.9	MCV
6	MCH			•			pg	27.0-31.0	MCH
7	MCHC			•			g/dl	33.0-37.0	MCHC
8	PLT						$X10^3$/ul	150-450	PLT
9	LYM			•			%	20.5-51.1	LY
10	MON			•			%	1.7-9.3	MO
11	GRA			•			%	42.2-75.2	GR
12	LYM#			•			$X10^3$/ul	1.2-3.4	LY_N
13	MON#			•			$X10^3$/ul	0.1-0.6	MO_N
14	GRA#			•			$X10^3$/ul	1.4-6.5	GR_N
15	RDW			•			%	11.6-13.7	RDW
16	PDW			•			fl	15.5-17.1	PDW
17	MPV			•			fL	7.8-11.0	MPV

MO/SN/FS/FW CODE: | | | CODE

bcs in-patient visit form 1-6

BIOCHEMISTRY

STUDY ID: | B | C | S | | | | | STUDY_ID

1.	**BLOOD LACTATE (mmol/l) (0-6)**				•			BLACTATE
2.	TOTAL BILIRUBIN (µmol/l) [0-17]				•			BILITOTAL
3.	**CREATNINE (µl) [44-97]**				•			CREATIN
4	AST (GOT) (µmol/l) [up to 37]				•			AST-GOT
5	ALK. .PHOS (µ/l) [117-390]				•			ALK-PHOST
6	**POTASSIUM (mmol/l) [3.6-5.5]**				•			POTASS
7	TOTAL PROTEIN (g/l) [56-85]				•			TPROTEIN
8	ALBUMIN (g/l) [38-44]				•			ALBUMIN
9	UREA/BUN (mmol/l) [1.7-9.1]				•			UREA
10	SODIUM (mmol/l) [136-146]				•			SODIUM
11	ALT/GPT (µ/l) [0-40]				•			ALT
12	DIRECT BILIRUBIN (µmol/l) [0-41]				•			BILDIRECT
13	BLOOD GLUCOSE				•			BGLUCOSE
14	G6PD							G6PD

MO/SN/FS/FW CODE: | | | CODE

bcs in-patient visit form 1-7

STUDY COMPLETION

STUDY ID:

B	C	S					STUDY_ID

Treatment:

Haemotransfusion	1. Yes	2. No	BLOOD

Treatments	1. Antimalaria	2.Antibiotics	3. Other	TREATS

Final Outcome:

Final outcome of case:

1. Survived	2. Died	3. Absconded	OUTCOME

Date: (dd/mm/yyyy)								OUTDATE

If withdrawn, state reasons

Investigators Name: _____

Investigator's Signature: _____

TWICE-YEARLY SURVEYS

bcs twice-yearly contact form 1-1

1. **STUDY CHILD ID**:	B	C	S					STUDY_ID

2. Contact Number			CONTACT

3. Expected Date: (dd/mm/yyyy)									EDATE

4. Contact Date: (dd/mm/yyyy)									CDATE

5. Name of Study Child:	NAME

6. Sex of Study Child:	1. Male	2. Female	SEX

7. Respondent relationship to Study Child:

1. Mother	2. Father	RELATION
3. Other (specify)		

8. Has Study Child fallen **ILL** since the last contact or within the past 6 months:

1. Yes	2. No	8. NA	9.NK	ILL

(If 8. is No or NK skip to 10)

9. What was the ILLNESS?

1. Malaria / Paa /Pua	2. Diarrhoea	3. ARI/ Cough	4. Convulsion	ILLNAM
5. Others (specify)	9. NK			

10. Has the child been hospitilised since the last contact, if yes where	1. Navrongo	2. Sandema	3. Bolga	4.other	ADMIT

11. Has the study child received malaria treatment since the last contact/within the past six months:

1. Yes	2. No	9. NK	MALTREAT

12. What was the name of the drug/treatment given to the study child?

1. Camoquine/ Artesunate	2. Chloroquine	3. Fansidar/ Malafan	DNAME

4. Herbs	5. Other	9. NK

13. Is the study child currently sleeping under insecticide treated **Bednet?**	1.Yes	2.No	9.NK	BEDNET

bcs twice-yearly contact form 1-2

1. **STUDY CHILD ID**:	B	C	S					STUDY_ID

2. Contact Date: (dd/mm/yyyy)									CDATE

CURRENT COMPLAINTS

Has the Study child any of the following complaints?			**Duration (in days)**	
3. Fever	1. Yes	2. No		FEVER
4. Shaking chills/rigors	1. Yes	2. No		SHAKING
5. Diarrhoea (3 or more watery stools)	1. Yes	2. No		DIARR
6. Vomiting	1. Yes	2. No		VOMIT
7. Headache	1. Yes	2. No		HEAD
8. Abdominal Pains	1. Yes	2. No		APAINS
9. Bodily Pains	1. Yes	2. No		BPAINS
10. Cough	1. Yes	2. No		COUGH
11. Difficulty in breathing	1. Yes	2. No		BREATH
12. Bloody urine	1. Yes	2. No		BURINE
13. Inability to suck /drink /eat	1. Yes	2. No		SDE
14.Others 1 (specify)				OTHER14
15. Others 2 (specify				OTHER15
FW /FS /RA CODE				CODE

bcs twice-yearly contact form 1-3

1. **STUDY CHILD ID**: | B | C | S | | | | | STUDY_ID

2. Contact Date: (dd/mm/yyyy) | | | | | | | | | CDATE

PHYSICAL EXAMINATION

3. Axillary Temperature (^{o} c): | | | • | | TEMP

4. Pulse (heartbeat)/ (minute): | | | | HEARTRATE

5. Systolic blood pressure (mmHg): | | | | SBP

6. Diastolic blood pressure (mmHg): | | | | DBP

7. Weight (Kg): | | | • | | WEIGHT

8. Height (cm) | | | • | | HEIGHT

9. MUAC (cm) | | | • | | MUAC

10. MO/MA CODE: | | MCOD

11. Spleen size (cm): | | • | | SPLEEN

12. Liver size (cm) | | • | | LIVER

bcs twice-yearly contact form 1-4

1. **STUDY CHILD ID**: | B | C | S | | | | | STUDY_ID

2. Contact Date: (dd/mm/yyyy) | | | | | | | | | CDATE

SPECIMEN COLLECTION:

3. Haemocue Hb (g/dl): | | | . | | HAEMO

4.	Rapid malaria diagnostic test	1. Positive	2. Negative	3. Not Done	RAMAT

CHECKLIST DURING CONTACT VISIT

	SPECIMEN			
5.	Immunological specimen	1. Yes	2. No	IMSPECI
6.	Blood smear	1. Yes	2. No	BSMEAR
7.	Filter paper	1. Yes	2. No	FPAPER
8.	Haemacue Hb	1. Yes	2. No	HBTEST
9.	Rapid malaria test	1. Yes	2. No	RMTEST

Microscopy Results

10.	Malaria Parasites	1. Positive		2. Negative		PARAST
11.	Species	1. Pf	2. Pm	3. Po	4. Mixed	SPECIES
12.	Trophozoite Counts/ (200 wbc)					TCOUNT
13.	Gametocytes/ (200 wbc)					GAMET

LAB. TECH. CODE: | | | CODE

bcs twice-yearly contact form 1-5

1. **STUDY CHILD ID:**	B	C	S					STUDY_ID

2. Date: (dd/mm/yyyy)									DATE

3. Status:

1. Available	2. Missed	3. Lost to Follow-up	4. Withdrawn	OUTCOME

If withdrawn, state reasons

Certified Correct by: _____

Investigator's Name: _____

Investigator's Signature: _____

4. Date certified: (dd/mm/yyyy)									OUTDATE

Appendix C- Informed Consent Form for Primary Study

PARENTAL CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Protocol Title: MALARIA MORTALITY AND MORBIDITY IN THE FIRST FIVE YEARS OF LIFE IN A BIRTH COHORT OF CHILDREN IN NORTHERN GHANA

(This parental consent form will be translated into the main languages of the local people, Kassem and Nankam)

INTRODUCTION

We are from the Navrongo Health Research Centre conducting studies into malaria in this community and would like to invite your newborn child/ward to take part in this study. This study is being conducted in close collaboration with Noguchi Memorial Institute for Medical Research (NMIMR) in Accra, the US Naval Medical Research Centre (NMRC) and the US Naval Medical Research Unit #3 in Cairo, Egypt.

PURPOSE OF THE STUDY

Malaria is caused by a germ that is passed from one person to the other by the bite of a mosquito that carries the malaria germ. Malaria is a very serious health problem in Ghana, as it is in many African countries. The disease strikes people of all ages, male or female. It can be particularly severe in children and may cause death. There is no vaccine that can prevent malaria now, but many scientists throughout the world are working to find one. The information we will get from this study will help us plan later studies to test malaria vaccines. To plan those studies, we need to know how often children get sick from the malaria germ and how often the germ weakens their blood. If we can find a malaria vaccine that works, we hope that the whole community will benefit. This study is not a vaccine study, but it will help us prepare for vaccine studies in the future.

Your newborn child will be one of 2000 or more healthy infants born in communities around the Navrongo War Memorial Hospital, Biu and the Kassena-Nankana East Health Centres during the study period who will be participating in the study. The study will last for 6 years but the participation of each child will last for 5 years.

Taking part is completely voluntary and so you are not obliged to allow your newborn child or ward to participate. You have every right to refuse. If you should refuse it is all right. You may withdraw your child from the study at any time without penalty or loss of any benefits s/he may otherwise be entitled to. Whether or not your child/ward participates in the study s/he will receive the same, appropriate medical care for malaria at all the health facilities when s/he is not well. The study is sponsored by the United States National Institutes of Health.

INCLUSION/EXCLUSION

Your child can participate in the study if you are resident in the Kassena-Nankana district and if you agree to have your child take part in the study. Your child cannot participate in the study if you plan on leaving the area in the next five years, or if you do not agree to have your child take part in the study.

STUDY PROCEDURE / WHAT WILL HAPPEN

If you agree for your child to be part of the study, he/she will be examined by a health professional at birth and at the close of the study. A small sample of blood 5-10 drops or 0.5-1.0ml), will be collected from the umbilical cord of the child during delivery or from the heel of the child if the child is enrolled after delivery to determine the baseline protection and resistance to malaria. . The child will then be visited twice in a year, in April-May (beginning of the high malaria transmission season) and in October-November, (beginning of the low transmission season). At each visit a small quantity (5-10 drops or 0.5-1.0ml) of blood will be taken to see if the malaria germ is in the body, measure how his/her body is responding to the malaria germ and see how the germ has weakened the blood. The child will also be examined by a clinician. Between these wet and dry season visits you will have to bring the child to the health facility anytime s/he is not well. These visits to the health facility will enable us measure the number of times your child has mild or severe malaria.

A member of the study team will be available to take some blood (5 drops or 0.5ml) to see if there are malaria germs in the body. Treatment will then be provided by medical personnel in the health facility according to Ghana Ministry of Health guidelines.

BENEFITS

Your child will benefit from participating in the study. S/he will have the opportunity to be seen quickly and receive rapid treatment when s/he is not well. Malaria treatment will be provided without cost to you or your family. In addition to the direct benefits, your child and other participants will be contributing to the ultimate aim of finding and testing a vaccine for malaria.

RISKS

The examinations may be inconvenient to your child and s/he will experience a small degree of pain when the finger or heel is pricked. There is also the remote possibility of the site of the finger or heel prick becoming infected and also of excessive bleeding from the site. To minimize this happening all materials used in obtaining the blood samples from your child will be used only once and only for your child. Also the blood will be taken by well trained personnel who will ensure that blood lost is minimal and the site does not become infected.

CONFIDENTIALITY

The information collected from your child shall be used only for the purposes of this study. We will collect data on the forms provided specifically for this study. We will keep the forms for your child in a file and keep it in a locked cabinet. The forms will have personal identifiers. The only people allowed to see the records will be the study investigators and members of review committees at the investigating institutions. We will not release any data that would allow identification of you or your child/ward to a third party without your consent. Samples collected will only be used to address the scientific objectives of this protocol. All samples will be stored at NHRC or in the laboratories of the institutions participating in the protocol. In the event that the samples would be used to address other objectives, prior approval would be sought from the Host IRB (NHRC-IRB).

COMPENSATION

We will not pay you for your child's or ward's participation in this study. However, in the event that injury is caused to your child as a result of participation in this research project, we will arrange for his/her medical care at the Navrongo War Memorial Hospital without charge.

The attendance and treatment at the health facility will not cost you or your family anything. When the child receives treatment the health care provider will ask you to bring the child to the health facility either on the 3rd, 7th and /or 14th day after treatment begins to find out if s/he is well and to find out whether the treatment given is working well. The day(s) for the review(s) will be at the discretion of the health care provider. If it is found that the treatment is not working well he/she will be reviewed at the health facility and may be given an alternate treatment. If your child is admitted for severe malaria and discharged from the hospital we will ask you to bring him/her for review in the Navrongo War memorial hospital a week after discharge. Whether you bring your child or not for these visits we shall follow up on your child on day 7 or a week after discharge to find out how your child is responding to the treatment.

WITHDRAWAL FROM STUDY

Your child's or ward's participation in this study is completely voluntary. You can refuse to let your child or ward participate or may withdraw him or her from the study at any time without fear of being pressured or coerced to continue to participate in the study. In the event that you decide to withdraw your child from the study, the treatment available to your child in any health facility will not be affected in any way.

If we the study investigators believe that your child's participation in the study or his/her continuation in the study might harm him or her, we will withdraw your child from the study. However, we will make certain that your child continues to receive appropriate treatment.

QUESTIONS

You are encouraged to ask questions at any time before and during the study. If you have any questions concerning study information you may contact the following persons;

Dr. Frank Atuguba and Dr. Abraham V. O. Hodgson of
Navrongo Health Research Centre
P. O. Box 114
Navrongo
Tel: 0742 22380 /22496

OR:

Dr. Kwadwo A. Koram and Prof. Francis K. Nkrumah of
Noguchi Memorial Institute for Medical Research
University of Ghana
P. O. Box LG 581
Legon
Tel: 021 501178 /501179

INSTITUTIONAL APPROVAL

The Institutional Review Boards (IRBs) at Navrongo Health Research Centre, and the Noguchi Memorial Institute for Medical Research, Accra, Ghana and the Ghana Health Service Ethical Review Committee (GHS-ERC) have reviewed this study, evaluated the potential risks and benefits and have granted approval for us to carry out this study. You may therefore also direct any additional questions you may have regarding your rights as a participant in this study to the chairmen of these boards, namely;

Dr. John Awoonor Williams (Chair, NHRCIRB)

Regional Director of Health Services
Regional Directorate of Health

Bolgatanga, Upper East Region

Phone:07222335

Rev. Dr. Samuel Anyete-Nyampong
IRB Chair
Noguchi Memorial Institute for Medical Research
University of Ghana
Legon
Tel: 021 501178

Prof. A.G.B Amoah
Chair, GHS-ERC
C/o Health Research Unit
Ghana Health Service
Accra
Tel; 021 679323

If you have difficulty contacting these individuals you should contact one of the study field workers. They will be able to put you in touch with any of the individuals listed above.

SIGNATURE OF PARTICIPANT WILLING TO PARTICIPATE

I have read or have had someone read all of the above for me, asked questions, received answers concerning areas I did not understand, and am willing to give consent for my child/ward to participate in this study. I will not have waived any of my rights by signing this consent form. Upon signing this form, I will receive a copy of this consent document for my personal records.

Printed name of participant (child or ward)
……

Printed name of parent or guardian:
……..…….……

Signature/Left Thumb Print of parent/guardian
……..……:……

Printed name of witness: ……..…….……

Signature/Left Thumb Print of witness ……..……:…….

Printed name of person administering consent:
……..
Language:……

Investigator or attending Health Care Professional's Affidavit:

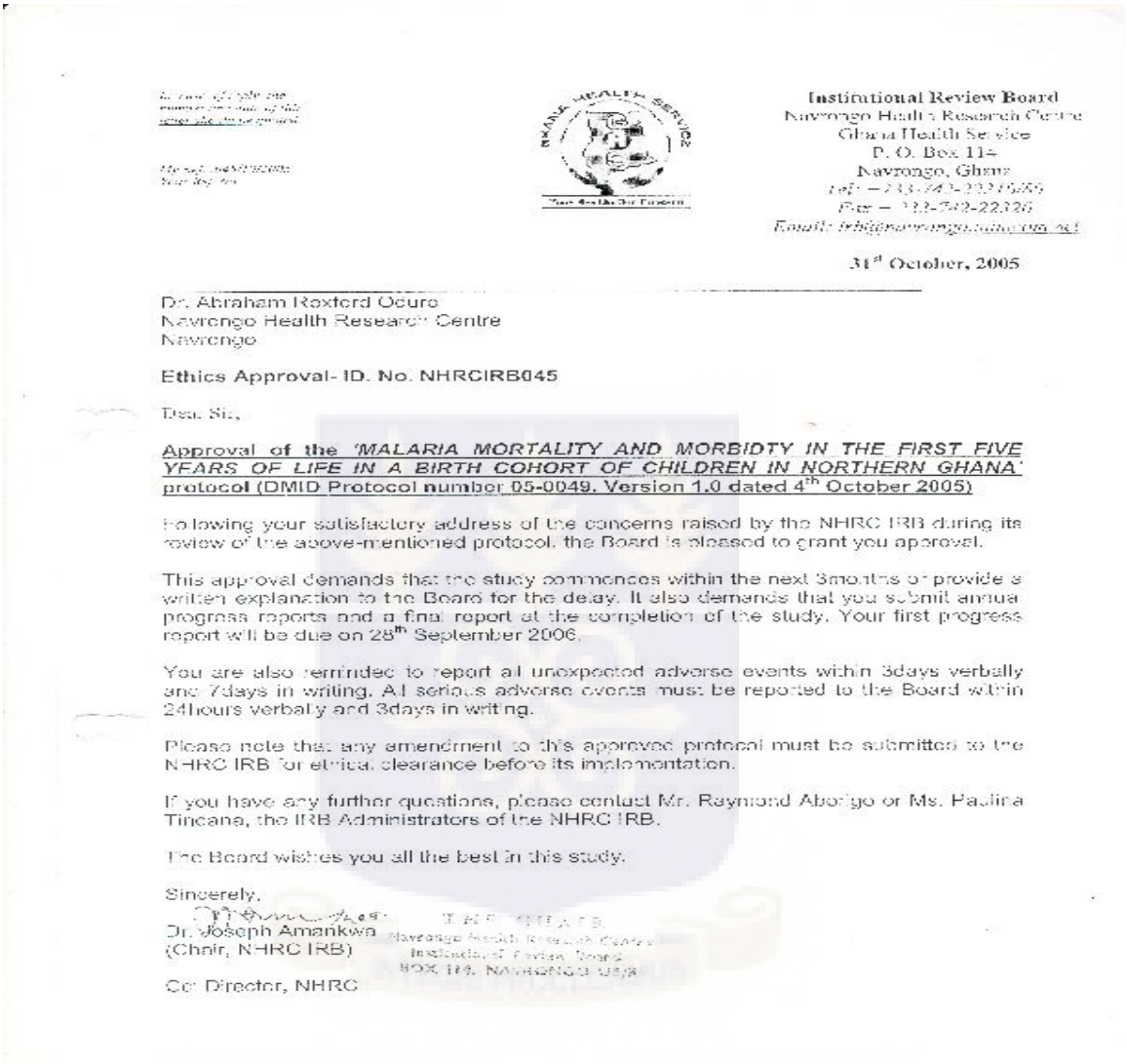
I certify that I have explained to the above individual(s) the nature and purpose of the study, potential benefits and possible risks associated with the participation in this research project. I have answered any questions that have been raised and have witnessed the above signature on the date indicated below:

Printed name of the investigator: ……..……..
Position:……
Signature:…….

Date:……
[dd /mm /yyyy]

Appendix D- ERC/IRB Approvals

NHRC approval for main study (appendix D_i)



Institutional Review Board
Navrongo Health Research Centre
Ghana Health Service
P. O. Box 114
Navrongo, Ghana
Tel: +233-(0)72-22206/22369
Fax: +233-(0)72-22206
Email: irb@navrongo.gha.net

31st October, 2005

Dr. Abraham Rexford Oduro
Navrongo Health Research Centre
Navrongo

Ethics Approval- ID. No. NHRCIRB045

Dear Sir,

Approval of the 'MALARIA MORTALITY AND MORBIDTY IN THE FIRST FIVE YEARS OF LIFE IN A BIRTH COHORT OF CHILDREN IN NORTHERN GHANA' protocol (DMID Protocol number 05-0049, Version 1.0 dated 4th October 2005)

Following your satisfactory address of the concerns raised by the NHRC IRB during its review of the above-mentioned protocol, the Board is pleased to grant you approval.

This approval demands that the study commences within the next 3months or provide a written explanation to the Board for the delay. It also demands that you submit annual progress reports and a final report at the completion of the study. Your first progress report will be due on 28th September 2006.

You are also reminded to report all unexpected adverse events within 3days verbally and 7days in writing. All serious adverse events must be reported to the Board within 24hours verbally and 3days in writing.

Please note that any amendment to this approved protocol must be submitted to the NHRC IRB for ethical clearance before its implementation.

If you have any further questions, please contact Mr. Raymond Aborigo or Ms. Paulina Tincana, the IRB Administrators of the NHRC IRB.

The Board wishes you all the best in this study.

Sincerely,

Dr. Joseph Amankwa
(Chair, NHRC IRB)

Navrongo Health Research Centre
Institutional Review Board
BOX 114, NAVRONGO-UER

Cc Director, NHRC

NMIMR approval for main study (appendix D_{ii})

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
INSTITUTIONAL REVIEW BOARD

(UNIVERSITY OF GHANA)

Phone: +(233) 21 500374 /501178
Fax: +(233) 21 502182
Email: Director@noguchi.mimcom.net
Telex No: 2556 UGL GH

P.O. Box LG581
Legon
Ghana

My Ref. No: DF.22

22nd November 2005.

Your Ref. No:

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 0001276**

NMIMR-IRB CPN 006/05-06 **IORG 0000908**

On 16th November, 2005 the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB), conducted a full board review and approved your protocol titled:

TITLE OF PROTOCOL : **Malaria mortality and morbidity in the first five years of Life in a birth cohort of children in Northern Ghana**

PRINCIPAL INVESTIGATOR : **Dr. K. A. Koram et al**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 15th November, 2006. You are to submit annual reports for continuing review.

Signature of Chairman: .....
Rev. Dr. Samuel Ayete-Nyampong
(NMIMR – IRB, Chairman)

cc: Professor David Ofori-Adjei
(MB CHB, FRCP, FWACP)
Director, Noguchi Memorial Institute
for Medical Research, University of Ghana, Legon.

Ghana Health Service- ERC approval for secondary study (appendix Diii)

GHANA HEALTH SERVICE ETHICS 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: +233-302-681109
Fax + 233-302-685424
Email: Hannah. Frimpong@ghsmail.org

27th October, 2015

Dr. Frank Achembona Atuguba
Department of Epidemiology and Disease Control
School of Public Health
College of Health Sciences
University of Ghana

ETHICS APPROVAL - ID NO: GHS-ERC: 04/09/15

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

"Malaria in Early infancy: Effect of Maternally Transferred Antibodies and Intermittent Preventive Treatment in the Kassena-Nankana Districts"

This approval requires that you inform the Ethics Review Committee (ERC) when the study begins and provide Mid-term reports of the study to the Ethics 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 three days verbally and seven 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 note that this approval is given for a period of 12 months, beginning October 27th, 2015 to October 26th, 2016.

However, you are required to request for renewal of your study if it lasts for more than 12 months.

Please always quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.....
DR. CYNTHIA BANNERMAN
(GHS-ERC CHAIRPERSON)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

1

NHRC IRB approval for secondary study (appendix D_{iv})

In case of reply the number and date of this letter should be quoted.

My Ref. App/InfIPTp/08/2015
Your Ref:

Navrongo Health Research Centre
Institutional Review Board
Ghana Health Service
P. O. Box 114
Navrongo, Ghana
Tel/Fax: +233-3821-22348
Email: irb@navrongo-hrc.org

28th August, 2015.

Dr. Frank Atuguba
Navrongo Health Research Centre
Ghana Health Service
P.O. Box 114
Navrongo, UER

ETHICS APPROVAL ID: NHRCIRB208

Dear Dr. Atuguba,

Approval of protocol titled *Malaria in early infancy: Effect of maternally transferred antibodies and use of intermittent preventive treatment of malaria in pregnancy (IPTp) in the Kassena Nankana districts*

I write to inform you that following your satisfactory address of the concerns raised by the Navrongo Health Research Centre Institutional Review Board (NHRCIRB) during its full Board review of the above-mentioned protocol, the Board is pleased to grant you approval.

The following documents were reviewed and approved:

- Completed New Protocol submission forms
- Study protocol Version 1.0 June, 2015
- Consent forms used in Primary study
- Field guides used in Primary study (Questionnaire, enrolment forms and Case Report forms
- Curriculum Vitae of Investigators

Please note that any amendment to these approved documents must receive ethical clearance from the NHRCIRB before implementation.

Should you require a renewal of your approval, a progress report should be submitted two (2) months before the expiration date. This approval expires on **27th August, 2016.**

The Board wishes you all the best in this project.

Sincerely,

Dr. John Koku Awoonor-Williams
(Chair, NHRC IRB)

Cc: The Director, NHRC

NMIMR IRB approval for secondary study (appendix D_v)

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979
A Constituent of the College of Health Sciences
University of Ghana

INSTITUTIONAL REVIEW BOARD

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E-mail: nirb@noguchi.mimcom.org
Telex No: 2556 UGL GH

Post Office Box LG 581
Legon, Accra
Ghana

My Ref. No: DF.22
Your Ref. No:

2nd September, 2015

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 00001276**

NMIMR-IRB CPN 001/15-16 **IORG 0000908**

On 2nd September 2015, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB) at a full board meeting reviewed and approved your protocol titled:

TITLE OF PROTOCOL : **Malaria in early infancy: Effect of maternally transferred antibodies and use of intermittent preventive treatment of malaria in pregnanacy (IPTp) in the Kassena-Nankana District**

PRINCIPAL INVESTIGATOR : **Dr. Frank A. Atuguba, PhD Cand.**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 1st September, 2016. You are to submit annual reports for continuing review.

Signature of Chair: .....
Mrs. Chris Dadzie
(NMIMR – IRB, Chair

cc: Professor Kwadwo Koram
Director, Noguchi Memorial Institute
for Medical Research, University of Ghana, Legon