PREVENTIVE ASPECTS OF MALARIA IN PREGNANCY IN BADAGRY LOCAL GOVERNMENT AREA OF LAGOS STATE, NIGERIA

BY

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DECLARATION

We hereby declare that this thesis titled Preventive Aspects of Malaria in Pregnancy in Badagry Local Area of Lagos State, Nigeria is the original research work carried out by Nneka Josephine Chukwurah in the Department of Zoology, University of Lagos, Nigeria.

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CERTIFICATION

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This thesis is dedicated to my very dear parents, Ezennia Paul Chukwuemeka and Mrs. Floxy Ekwunife Nwokedi, who never lived to see the fulfillment of this dream.

TABLE OF CONTENTS

LIST OF TABLES

PAGES

LIST OF FIGURES

PAGES

LIST OF PLATES

PAGES

LIST OF APPENDICES

PAGES

LIST OF ABBREVIATIONS/ACRONYMS

AES	Average enlarged spleen
A. gambiae sl	*Anopheles gambiae senso latu*
CQ	Chloroquine
DALYS	Disability adjusted life years
DNA	Deoxyribonucleic acid
EIR	Entomological inoculation rate
FMoH	Federal Ministry of Health
GPS	Global positioning system
HTD	Human therapeutic dose
IPTp-SP	Intermittent preventive treatment in pregnancy using Sulfadoxine- pyrimethamine
ITNs	Insecticide treated mosquito nets
IUGR	Intra-uterine growth retardation
IVM	Integrated vector management
LBW	Low birth weight
LGA	Local Government Area
LLNs	Long lasting insecticidal nets
LMP	Last Menstrual Period
MBR	Man biting rate
PCR	Polymerase Chain Reaction
PCV	Packed cell volume
PSC	Pyrethrum spray collection
RDT	Rapid diagnostic test
SP	Sulfadoxine-pyrimethamine
SR	Sporozoite rate
WHO	World Health Organization

ABSTRACT

The study focused on the Preventive aspects of malaria in pregnancy in Badagry Local Government Area, one of the 20 LGAs in Lagos State in Southwestern Nigeria. The objectives of the study were to determine the knowledge, attitude and practices of the community on malaria; determine the effectiveness of Intermittent Preventive Treatment in pregnancy (IPTp) using Sulfadoxine-pyremethamine (SP) [IPTp-SP] in the prevention of malaria; and ascertain the safety of SP during pregnancy by administering SP to pregnant laboratory bred albino mice. Data were obtained using semi-structured questionnaire and focus group discussion on beliefs, knowledge and practices of community members in Ikoga ward and in pregnant women attending antenatal clinics. Microscopic diagnosis using Giemsa stain was conducted to establish the baseline parasitological prevalence of malaria amongst 3,978 participants in the community. Entomological data on the vector population was collected by Pyrethrum Spray Collection (PSC). The mosquitoes were identified morphologically and the *A. gambiae* complex by Polymerase Chain Reaction (PCR). IPTp-SP intervention was evaluated in 426 pregnant women who received one or two doses of SP (1500mg sulfadoxine + 75 mg pyrimethamine), depending on when they registered. Fifty seven pregnant women did not take the SP, because of reaction to SP. The pregnant women comprised 183 of primigravidae, 135 of secundigravidae and 165 of multigravidae. Parameters used to evaluate the effectiveness of the SP included parasite rate determination, parasitaemia, anaemia, clinical malaria and determination of Low Birth Weight (LBW). The safety of SP was experimentally evaluated from the histopathological examination of foetuses of SP treated pregnant mice and by sperm test assay of male mice administered SP. Analysis of the questionnaire on malaria prevention revealed malaria to be a serious health problem; 48% of community members use Raid insecticide spray; 30% take herbs while 21% use mosquito coil. Malaria prevention by pregnant women included taking of herbs by

30%, use of chloroquine (30%) and daraprim (23%) while 48% and 21% use raid insecticide spray and mosquito coils respectively to control mosquito. Parasitological results revealed the predominant parasite to be *P. falciparum* with malaria prevalence of 23.3%. The *Anopheles* mosquitoes were predominantly *A. gambiae sensu stricto* and *A. arabiensis* in the ratio of 2:1. There was a reduction in malaria prevalence from 15.7% to 2.1% in pregnant women administered SP and showed statistical significance, ($P<0.05$); parasitaemia decreased from 44,650 per µl of blood to 14,085 per µl of blood, but showed no statistical significance among the different gravida. The percentage of pregnant women that had PCV of less than 33% decreased from 44.7% to 37.9% with SP administration and showed statistical significance, ($P<0.05$). Four percent of the pregnant women who received SP had LBW compared with 3.5% of those who did not receive SP, and was not statistically significant, ($P>0.05$). Morphological and histopathological examination of foetuses of mice treated with SP and control confirmed the absence of abnormality. Sperm head abnormalities were observed in both test (5.2%) and control (3.1%). Sperm head abnormality in test animals were not statistically significant over the negative control values, ($P<0.05$), was not dose dependent and did not increase with the duration of exposure. Therefore SP is not mutagenic at the recommended dose levels and can be considered effective. Sperm abnormalities observed included pin head, round head, bent and coiled tail.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Malaria is an infectious disease caused by *Plasmodium* species and is transmitted from one person to another by the bite of infected female *Anopheles* mosquito (WHO, 2010). Approximately 40% of the world's populations are affected. The disease is endemic in over 106 countries and about 3.3 billion people are at risk (WHO, 2010). Malaria remains one of the leading health problems of the developing world and it is estimated that there are approximately 225 million cases of malaria, caused by *P. falciparum* and *P. vivax* within the African Region with over 85% of cases (WHO, 2010; Yeka *et al*., 2011). Malaria kills about one million people annually and 89% of the death due to malaria occurs in Africa South of the Sahara (WHO, 2009).

Two factors are largely responsible, first, the majority of infections in the region are caused by *Plasmodium falciparum*, the most dangerous of the four human malaria parasites; and secondly, the most effective malaria vector — the mosquito *Anopheles gambiae,* which is the most widespread in the region and the most difficult to control (Uneke, 2007). The majority of malaria victims are children under the age of five and pregnant women who die from severe malaria (Desai *et al*., 2007). Malaria is the only vector borne disease on the World Health Organization's Disability Adjusted Life Years (DALYS) list (Carrington, 2001) and tops the list of the first cause of DALYS in Africa (Breman *et al*., 2004). DALYS is an aggregate measure of premature mortality, morbidity and disability which can also be used to analyze the cost effectiveness of major interventions. When the burden is measured as DALYs, 58% of the total global

burden due to malaria is concentrated among the poorest nations. Globally, almost 3% of disability adjusted life years are due to malaria mortality while it is 10% in Africa (Breman *et al*., 2004; Breman and Holloway, 2007).

Malaria has continued to form the single largest component of the disease burden in Africa. The economic burden of malaria is estimated at an average annual reduction in economic growth of 1.3% for the African countries with the highest burden. An estimated 12 billion US dollars are lost to the African continent's GDP annually due to malaria (Sachs, 2001). In addition to economic and neurological disabilities, there are social costs (Murphy and Breman, 2001; Chima *et al.,* 2003). Sachs and Malaney, (2002) demonstrated a correlation between the presence of malaria in a country and that country's per capita GDP, and shows an inverse relationship between the two and that malaria causes underdevelopment. Najera (1994) attributed the disappearance of malaria in parts of Europe with economic development related to agricultural expansion rather than vector control or chemoprophylaxis. One of the major challenges in malaria control is parasite resistance with affordable monotherapies as seen in the case of CQ, which spread from South America to Asia, to East Africa and finally to West Africa (Wernsdorfer, 1994; Marsh, 1998; White, 1999; Bloland, 2001; WHO, 2003).

In Africa, malaria causes around 20% of all deaths in children under five. It is responsible for 24% to 40% of all outpatient hospital attendance and 20% to 50% of hospital admission in Africa. Children experience four or more febrile episodes yearly, resulting in billions of febrile episodes meriting antimalaria drug treatment if no precise diagnostic tool is available (WHO, 2009). Malaria is highly endemic in Nigeria and remains a major cause of morbidity and mortality leading to hospital attendance in

all age groups (Anumudu *et al*., 2006; Kuti *et al*., 2006; Akinleye, *et al*., 2009; Abasiattai *et al*., 2009; Okwa *et al*., 2011). In Nigeria, malaria is responsible for 25% infant mortality and 30% childhood mortality and is the leading cause of anaemia in children in rural areas (Smith *et al*., 1998). It is responsible for the death of 300,000 thousand people annually and is associated with about 11% of maternal mortality, claiming the lives of as much as 10,000 pregnant women annually (FMOH, 2005).

Malaria in pregnancy causes significant maternal and perinatal morbidity and mortality in sub-Saharan Africa (Schulman *et al.,* 2002; Kapito-Tembo *et al*., 2011). Malaria in pregnancy has been associated with increased incidence of anaemia, spontaneous abortions, preterm labour, foetal distress, congenital infections, foetal death *in-utero*, still births and intra-uterine growth restriction (IUGR) (Mutabingwa, 2003; Okoko *et al*., 2003; Recke *et al*., 2009). In addition, placental parasitaemia, severe anaemia and IUGR all contribute to LBW which is the single greatest risk factor for neonatal and infant mortality (Ouma *et al*., 2007). Newborn with LBW are four times more likely to die as infants than are babies born with normal birth weight (Yartey, 2006).

The World Health Organization (WHO) has a set of recommendations for effective malaria control and include the following: facilitate access of the populations at risk to effective treatment of malaria; promote the applications of preventive measures such as Insecticides Treated Nets (ITNs); Intermittent Preventive Treatment (IPT) using SP and Integrated Vector Management (IVM) against malaria for populations at risk; build capacity for malaria control; strengthen malaria surveillance and monitoring and evaluation systems, (Okonofua, 2004; WHO, 2005).

1.2 STATEMENT OF THE PROBLEM

Malaria has continued to be a serious health problem particularly among pregnant women (Steketee *et al*., 2001; Desai *et al*., 2007). In Nigeria, malaria is endemic in all the 36 states of the country including the Federal Capital Territory. Malaria parasitaemia and anaemia are common among pregnant women with a prevalence of over 50% (Idowu *et al*., 2005; Adefioye *et al.,* 2007; Enato *et al*., 2009). Asymptomatic malaria parasitaemia is more common in primigravidae in the second trimester and in the younger age group. This leads to maternal morbidity and anaemia resulting in LBW and Pre-term delivery (WHO, 2004; van Eijk, 2004).

1.3 THE PURPOSE OF THE STUDY

The purpose of the study is to determine malaria endemicity, the knowledge, attitude and practices of the community in Ikoga ward on malaria prevention and treatment as well as evaluate the acceptability and effectiveness of IPTp-SP in pregnancy outcome in peri-urban area of Badagry Local Government Area of Lagos State, Nigeria.

1.4 OBJECTIVES OF THE STUDY

The objectives of the study are to:

1. Determine the knowledge, attitude and practices (KAP) of the peri-urban study community on malaria.
2. Determine the knowledge of pregnant women attending antenatal clinics, on malaria and IPT, using SP for malaria prevention.
3. Determine the level of malaria endemicity in the study community and among pregnant women attending antenatal clinics.
4. Compare OptiMAL Diamed Rapid Diagnostic Test (RDT) and microscopy in malaria diagnosis.

5. Determine the effectiveness of IPT in reducing maternal parasitaemia, placenta parasitaemia, anaemia and fever among pregnant women including preterm delivery and LBW.
6. Identify the major malaria vectors in the study community and determine the entomological inoculation rate (EIR).
7. Assess the mutagenic potential of SP on the foetuses of laboratory bred pregnant albino mice and spermatozoa in male albino mice

1.5 SIGNIFICANCE OF STUDY

The study is designed to provide information on knowledge, attitude and practices on malaria and its control in peri-urban community; knowledge of malaria in pregnant women and malaria prevention in pregnant women attending antenatal clinics in Badagry LGA and the use of IPTp-SP. This study will provide information on the acceptability and effectiveness of IPTp-SP in reducing malaria morbidity and parasitaemia among pregnant women attending ANC clinics in Badagry LGA and the awareness of IPTp-SP in malaria prevention among pregnant women. It will assess the safety of SP by mutagenicity studies using animal model. IPTp-SP has been adopted as a component of malaria policy by many countries in sub-Saharan Africa including Nigeria. This recommendation is based on studies conducted mainly in East African countries, namely Kenya and Tanzania. Data on post-implementation effectiveness of this measure are scarce particularly in Nigeria. While SP resistance has been documented mainly in Southern part of Nigeria, no resistance to SP has been documented in Badagry LGA; this study will therefore provide the needed data on SP usage and its effectiveness in IPT in the region.

1.6 RESEARCH QUESTIONS

1. How much knowledge do the community members have on malaria?
2. How much knowledge do pregnant women have on malaria as well as their knowledge on IPTp-SP?
3. What is the level of malaria endemicity in the study community and among pregnant women?
4. How useful is the OptiMAL Rapid Diagnostic Test (RTD) compared to microscopy in malaria diagnosis?
5. What is the effectiveness of intermittent preventive treatment in pregnancy using Sulfadoxine-pyrimethamine (IPTp-SP)?
6. What are the malaria vectors present in Ikoga ward and what is the Entomological Inoculation Rate (EIR)?
7. Does Sulfadoxine-pyrimethamine (SP) have any mutagenic effect on the foetus of pregnant albino mice and spermatozoa of male albino mice?

1.7 LIST OF OPERATIONAL DEFINITION OF TERMS

CQ	Chloroquine is a 4 amino quinolinine antimalarial medicine used in the treatment of malaria.
CRPF	Resistance of *Plasmodium falciparum* Parasite to Chloroquine.
SP	A combination of Sulfadoxine and Pyrimethamine recommended for Prevention of Malaria in Pregnancy.
IPT	Intermittent Preventive Treatment is the intermittent administration of Sulfadoxine-pyrimethamine antimalarial medicine to prevent malaria.

IPTp-SP	Administration of two doses of Sulfadoxine-pyrimethamine intermittently at least one month interval to prevent malaria in pregnancy.
LBW	Babies born with birth weight of less than 2.5kg.
PRE-TERM	Babies delivered before 37 weeks of pregnancy are referred to as pre-term.
EIR	Entomological Inoculation Rate is the number of infective mosquito bites received by an individual in a malaria endemic area usually annually.
MBR	Man Biting Rate is the number of mosquitoes biting man per night.
PCR	The Polymerase Chain Reaction (PCR) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence
PCV	Packed Cell Volume is the portion of whole blood volume occupied by erythrocytes (red blood cells).
ANC	Antenatal Clinics are clinics visited by pregnant women for care to monitor the woman and the baby before the delivery of the baby.
KAP	Knowledge, Attitude and Practices studies, tell us what people know about certain things, how they feel, and how they behave.
FGD	Focus Group Discussion is discussion held among a group of people with common interest to obtain information about a particular problem.

DSN	Disease Surveillance Notification is a systematic collection, collation, analysis and storage of data for decision making.
PSC	Pyrethrum Spray Collection is an entomological method used in the collection of mosquitoes (mosquitoes that rest indoors).
RDT	Rapid Diagnostic Test are quick alternatives to diagnosis based on clinical grounds or microscopy, particularly where good quality microscopy services cannot be readily provided.
IRB	Institutional Review Board is concerned with ethical, regulatory and policy concerns with human subjects research.
UNICEF	United Nations Children Educational Funds is an organization that works for children's rights, their survival, development and protection, guided by the Convention on the Rights of the Child.
GPS	Global Positioning System is a radio navigation system that allows land, sea, and airborne users to determine their exact location.
IUGR	Intrauterine Growth Restriction refers to the poor growth of a baby while in the mother's womb during pregnancy. Specifically, it means the baby is not developing normally.
IRS	Indoor Residual Spraying is the application of residual insecticide inside houses in order to control mosquitoes and other insects.
GDP	Gross Domestic Product (GDP) refers to the market value of all goods and services produced within a country in a given period.

It is often considered an indicator of a country's standard of living.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

Malaria has continued to be a public health problem. Up to 600 million cases and more than 2 million deaths are caused by *P. falciparum* and 400 million cases of *P. vivax* malaria occur annually (Ademowo, 2000; Breman *et al*., (2007). In sub-Saharan Africa, where *P. falciparum* malaria is pervasive and the major killer of children less than five years, children experience four or more febrile episodes yearly; this results in billions of febrile episodes meriting anti-malaria drug treatment if no precise diagnostic tool is available. However, evidence showed that between 2000 and 2009, that the prevalence of malaria has declined markedly in eleven countries in sub-Saharan Africa. These countries are Algeria, Cape Verde, Eritrea, Madagascar, Namibia, Rwanda, Sao Tome and Principe, South Africa, Swaziland, United Republic of Tanzania and Zambia. Morocco and Turkmenistan were certified by the Director General of World Health Organization in 2009 to have eliminated malaria (WHO, 2010). The reduction in malaria prevalence has been attributed to scaling-up of control interventions including more efficient treatment regimens (e.g. artemisinin-based combination therapy) and insecticide-treated bed nets (WHO, 2010; Ishengoma *et al*., 2011).

Annually, approximately 25 million pregnant women are at risk of *Plasmodium falciparum* infection in sub-Saharan Africa with high parasitaemia and one in four women have evidence of placental infection at the time of delivery (Steketee *et al*., 2001; Desai *et al*., 2007; Uneke, 2008). Falciparum malaria in pregnancy is an important cause of maternal and perinatal morbidity and mortality in malaria endemic

areas. In pregnancy, malaria is more common, more severe, more atypical, and more fatal (Shulman and Dorman; 2003). Pregnant women in malarious areas may experience a variety of adverse consequences from malaria infection, including anaemia and placental accumulation of parasites, while their newborns may have LBW from prematurity and IUGR (McGregor *et al*., 1983; Brabin, 1983; Steketee *et al*., 1996; Desai *et al.,* 2007). Other consequences of malaria during pregnancy for the newborn include congenital infection and increased infant mortality linked either to preterm-LBW or IUGR-LBW (Mokuolu *et al*., 2009). The occurrence of these problems underscores the importance of malaria prevention in pregnancy (Shulman and Durman, 2003).

Studies have shown that administering two doses of IPTp-SP at least one month interval is more effective than chemoprophylaxis or case management in the prevention of malaria in pregnancy (Challis *et al*., 2004; Falade *et al*., 2007). Several other studies have indicated that IPTp-SP to be highly efficacious and more effective than chloroquine prophylaxis (van Eijk *et al*., 2004). The World Health Organization currently recommends a three-prong approach to the control of malaria during pregnancy. These are IPTp-SP, LLINs usage and effective case management of clinical infection (Okonofua, 2004; FMOH, 2005; WHO, 2010). The cost effectiveness of delivering three single doses of intermittent preventive treatment in infants (IPTi) using SP in controlling malaria in the first year has been demonstrated by studies conducted in Republic of Tanzania and Mozambique (Schellenberg *et al*., 2001; Hutton *et al*., 2009). However, WHO recommendations for malaria control in children in endemic areas rely on case management, use of ITNs and vector control, none of

which has proved fully efficacious for controlling infections (Mabaso *et al*., 2004; Baird, 2005; Binka and Akweongo, 2006).

In Nigeria, six million pregnant women are at risk of malaria infection each year (FMOH, 2005). Malaria during pregnancy is a major cause of foetal and maternal morbidity and mortality and claims the lives of about 10,000 pregnant women every year, especially in primigravida (in the second trimester); in the younger age group and is still associated with complications (Ekejindu *et al.,* 2006; Savage *et al*., 2007; Nnaji and Ikechebelu, 2007; Nwagha *et al*., 2009; Nnaji *et al*., 2009). Malaria in pregnancy has been associated with increased incidence of anaemia, spontaneous abortions, preterm labour, foetal disresss, congenital infections, foetal death *in-utero*, stillbirths and IUGR (Adams *et al*., 2011). Neonatal and infant mortality are the resultant effect (Figure 1).

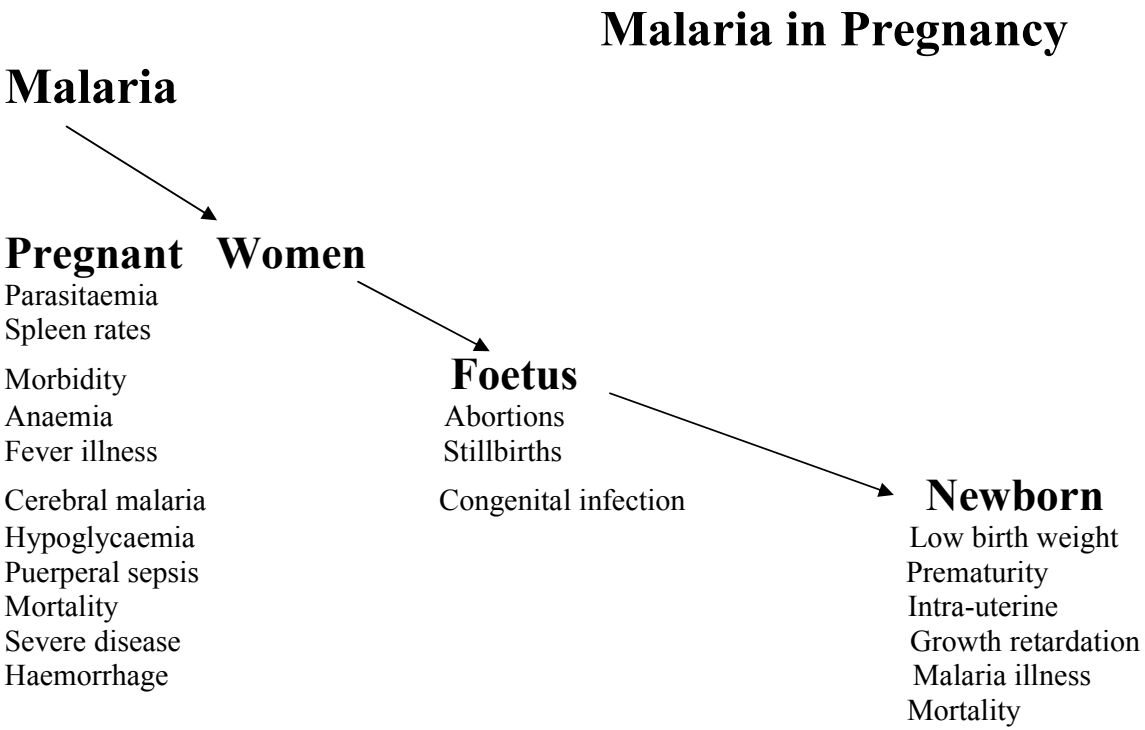

Figure 1: Consequence of malaria in pregnancy.

Source: WHO Regional Office for Africa (2004)

Babies born with LBW are four times more likely to die as infants than are babies born with normal birth weight (Mutabingwa, 2003; Yartey, 2006). Studies conducted in endemic countries including Nigeria have shown that pregnant women particularly in their first pregnancy are at risk from malaria infection and that the prevalence of *Plasmodium* parasitaemia is still very high (Steketee, 2001; Dim and Onah, 2007; Nnaji *et al*., 2009; Enato *et al*., 2009).

2.2 MALARIA PARASITE

Malaria parasites are members of the genus *Plasmodium* (phylum *Apicomplexa*). Four species of *Plasmodium* infect man and can be transmitted by humans. These are *Plasmodium falciparum. Plasmodium malariae, Plasmodium ovale and Plasmodium vivax* (Oyala *et al*., 1999). A fifth species, *Plasmodium knowlesi*, is a zoonosis that causes malaria in macaques but can also infect humans (Fong *et al*., 1971). Malaria disease is classified into uncomplicated and complicated or severe malaria. Severe malaria results from uncomplicated malaria disease that is not treated promptly and adequately (Mwenesi *et al*., 1995). *P. falciparum* which accounts for more than 90% of the infection is responsible for the majority of malaria infections. The disease if not treated promptly results in severe malaria, which includes anaemia, cerebral malaria, coma and death (Mendis *et al*., 2001; Seal *et al*., 2010).

Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* is generally a milder disease which is rarely fatal. Malaria produces symptoms indistinguishable from many other diseases, particularly fever, rigours and headache. Accurate diagnosis to distinguish it from other febril diseases is required in order to urgently commence treatment to prevent severe disease and death (WHO, 2003).

2.3 THE MALARIA VECTOR

The malaria vectorial system in Africa south of the Sahara is the most powerful because of climatic conditions which favours the *A. gambiae* sensu sticto, *A. arabiensis*, and *A. funestus* the most efficient vectors in the world (White, 1974; Colluzzi, 1984; Pertrarca *et al*., 1991; Lindsay *et al*., 1998; Beier *et al.*, 1999; Awolola *et al*., 2007; Djenontin *et al.,* 2010). *A. gambiae ss* is the major vector of human malaria throughout sub-Saharan Africa. Its remarkable preference for human blood, its ability to feed and rest inside human dwellings, together with its high longevity, allowing sustainable development of *Plasmodium* parasites under a wide variety of ecological settings, makes it the most proficient malaria vector in the world (Garret-Jones, 1980; Hunt *et al*., 1998). *A. gambiae* is present virtually everywhere in sub-Saharan Africa, populating the array of environments, typically found on this continent and transmitting malaria to humans in remote rural areas as well as in large cities. Other secondary vectors, such as *A. mouchetti* and *A. nili* have also been reported in some parts of Africa (Coetzee *et al.*, 2000).

In Nigeria, out of the 37 documented *Anopheles* species, the main vectors include *An. funestus, An. gambiae ss, An. pharoensis, An. arabiensis, An. nili, An. coustani, An. moucheti moucheti, A. paludis, An. hargreavesi, An. melas, An. hancocki, An. brohieri,* and *An. flavicosta* (Coetzee *et al*., 2000; Awolola *et al.,* 2002). However, only four species namely *An. gambiae ss, An. melas, An. arabiensis and An. funestus* are the important vectors in Nigeria, with other vectors playing a minor or local role (Gillet and Smith, 1972). Vectors can be identified morphologically using microscopy and molecularly by Polymerase Chain Reaction (PCR) particularly for the *Anopheles gambiae* complex (Scott *et al*., 1993).

2.4 PATHOGENESIS OF MALARIA

The pathology and clinical manifestations associated with malaria are almost exclusively due to the asexual erythrocytic stages of the parasites (Dondorp *et al*., 2004). Tissue schizonts and gametocytes cause little, if any, pathology. *Plasmodium* infection causes an acute febrile illness which is most notable for its periodic fever paroxysms occuring at either 48 or 72 hour intervals. The severity of the attack depends on the *Plasmodium* species as well as other circumstances, such as the state of immunity, the general health and nutritional status of the infected individual. Malaria is a chronic disease which has a tendency to relapse or recrudesce over months or even years (Trampuz *et al*., 2003).

Malaria is naturally transmitted through the bite of infected female anopheline mosquitoes. It can also be transmitted via blood transfusions or sharing syringes (Marcucci *et al*., 2004; Greenwood *et al*., 2005). Mechanical transmission of infected blood will result in a shorter incubation period since there will be no liver stage and the risk of fatality with mechanically-transmitted *P. falciparum* is increased (Bruce-Chwatt, 1993). The lack of the liver stage infection also precludes relapses in *P. vivax* or *P. ovale* infections. Congenital transmission has also been documented, but relatively rare despite the heavy parasite infection of the placenta (Mokuolu *et al*., 2009**).**

Symptoms of malaria usually commence 10-15 days after the bite of an infected mosquito. The typical prepatent and incubation periods following sporozoite inoculation vary according to species. The prepatent period is defined as the time between sporozoite inoculation and the appearance of merozoites in the blood.

Incubation periods tend to be a little longer and are defined as the time between sporozoite inoculation and the onset of disease symptoms. Sometimes the incubation periods can be 8- 30 days (Bledsoe, 2007) or prolonged for several months in *P. ovale,* and *P. malariae* (Cogswell, 1992) and up to 30 years in *P. vivax* (Trampux *et al*., 2003). All four species produce 'flu-like' symptoms and include: headache, slight fever, muscle pain, anorexia, nausea and lassitude. The symptoms tend to correlate with increasing numbers of parasites. These symptoms will be followed by febrile attacks also known as the malarial paroxysms which will exhibit periodicities of 48 hours for *P. vivax, P. ovale*, and *P. falciparum*, and a 72-hour periodicity for *P. malariae.* Initially the periodicity of these paroxyms may be irregular as the broods of merozoites from different exoerythrocytic schizonts synchronize. This is especially true in *P. falciparum* which may not exhibit distinct paroxysms, but exhibit a continuous fever, daily attacks or irregular attacks of 36-48 hour periodicity.

Patients may also exhibit splenomegaly, hepatomegaly (slight jaundice), and haemolytic anaemia during the period in which the malaria paroxysms occur. The malarial paroxysm will usually last 4-8 hours and begins with a sudden onset of chills in which the patient experiences an intense feeling of cold despite having an elevated temperature. This is often referred to as the cold stage which is characterized by a vigorous shivering which is followed by the hot stage. The patient feels an intense heat accompanied by severe headache, fatigue, dizziness, anorexia, myalgia, and nausea. The periodicity of these paroxysms is due to the synchronous development of the malarial parasite within the human host, with all of the parasites within a host at approximately the same stage (ie, ring, trophozoite, and schizont) as they proceed through schizogony. The malarial paroxysm corresponds to the rupture of the infected

erythrocytes and the release of merozoites. The 72 hour periodicity in *P. malariae* is due to its slower growth and maturation during the blood-stage schizogony. Studies in *P. vivax* have demonstrated a correlation between fever and serum TNF-α (tumor necrosis factor-alpha) level. Presumably antigens or toxins are released when the infected erythrocyte ruptures and lead to the production of TNF-α and the febrile attacks (Bruce Chwatt, 1993).

The severity of the paroxysms and duration of the symptoms varies according to species and correlates with the average and maximum parasitemia exhibited by the various species. *P. falciparum* is capable of producing a severe and lethal infection, whereas the other species are rarely mortal. Patients infected with *P. vivax*, especially for the first time, can be quite ill. However, *P. vivax* rarely causes complications or results in death although severe malaria involving multiple organs has also been noted in *P. vivax* infections (Kochar *et al*, 2009). Relapses to the activation of *P. vivax* hypnozoites can occur for several years. *P. ovale* is the most benign in that the paroxysms tend to be mild and of short duration and relapses seldom occur more than one year after the initial infection. *P. malariae* generally produces a mild disease, but the initial paroxysms can be moderate to severe. It is the most chronic and recrudescences have been documented several decades after the initial infection. This chronicity is sometimes associated with renal complications, which are probably due to the deposition of antigen-antibody complexes in the glomeruli of the kidney. The malarial paroxysms will become less severe and irregular in periodicity as the host develops immunity.

This immunity, however, is not a sterilizing immunity in that the infection persists longer than the symptoms and individuals can exhibit relapses or recrudescences or become re-infected. If untreated, all forms of malaria tend to be chronic. In contrast to the other three species, *P. falciparum* can produce serious disease with mortal consequences. This increased morbidity and mortality is due in part to the high parasitemias associated with *P. falciparum* infections. These potentially high parasitemias are due in part to the large number of merozoites produced and the ability of *P. falciparum* to invade all erythrocytes. In contrast, *P. vivax* and *P. ovale* prefer reticulocytes (i.e., immature erythrocytes), whereas *P. malariae* prefers senescent erythrocytes. The parasitaemia can also rapidly increase due to the cytoadherence and sequestration of *P. falciparum*. This sequestration in the tissues minimizes removal of infected erythrocytes by the spleen and allows for a more efficient erythrocyte invasion (Chen *et al*., 2000).

The high parasitaemia and sequestration result in other complications associated with *falciparum* malaria, the most notable being anaemia and cerebral malaria (Maude *et al*., 2009). The anaemia is due in part to the destruction of erythrocytes during blood-stage schizogony. Furthermore, non-infected erythrocytes are destroyed at higher rates during the infection and there is a decreased production of erythrocytes. The pathology associated with all malarial species is related to the rupture of infected erythrocytes and the release of parasite material and metabolites, haemozoin (malaria pigment) and cellular debris. In addition to the paroxysms discussed above, the deposition of hemozoin has long been known as a characteristic feature of malaria. There is an increased activity of the reticulo-endothelial system, particularly in the liver and spleen and thus their enlargement, as evidenced by macrophages with

ingested infected and normal erythrocytes and hemozoin. Except for *P. falciparum*, the pathology associated with malaria tends to be benign. Several severe complications can be associated with Falciparum malaria, with cerebral malaria being the most notable and a frequent cause of death (Boivin, 2002).

Cerebral malaria is characterized by an impaired consciousness. The presenting symptoms are severe headache followed by drowsiness, confusion, and ultimately coma. Convulsions are also frequently associated with cerebral malaria. Malaria has been found to cause cognitive impairments, especially in children. This neurologic damage is as a result of cerebral malaria to which the children are more vulnerable (Holding *et al*., 2001; Boivin, 2002). Cerebral malaria is associated with retinal whitening (Maude *et al*., 2009) which may be a useful clinical sign in distinguishing malaria from other causes of fever (Beare *et al*., 2006). These neurological manifestations are believed to be due to the sequestration of the infected erythrocytes in the cerebral microvasculature. Sequestration refers to the cytoadherence of trophozoite- and schizont-infected erythrocytes to endothelial cells of deep vascular beds in vital organs, especially brain, lung, gut, heart and placenta. This sequestration provides several advantages for the parasite. The major advantage is the avoidance of the spleen and the subsequent elimination of infected erythrocytes. In addition, the low oxygen tensions in the deep tissues may provide a better metabolic environment.

The human placenta is an ideal site for the accumulation of *Plasmodium falciparum* malaria parasites, and as a consequence serious health problems arise for the mother and the foetus (Brabin *et al*., 2004; Ekejindu, 2006; Savage *et al*., 2007; Bako *et al*., 2009). Although pathogenesis of placental malaria is only partially understood, *Plasmodium falciparum*-infected erythrocytes sequester in the intervillous space of

the placenta causing placental malaria, with consequent anaemia, (Walter and Bolt, 1982; Steketee *et al*., 1996; Sullivan *et al*., 1999) a condition that increases a woman's chances of having a LBW baby (Archibald, 1958; Cannon, 1958; Jilly, 1969; van Geertruyden *et al*., 2004; Desai *et al*., 2007; Thévenon *et al*., 2010). The sequestration of the *Plasmodium falciparum*-infected erythrocytes leads to undetection of the parasite in peripheral blood smears, resulting in misdiagnosis of the parasite and therefore are not treated (Desowitz and Alpers, 1992; Marchesini and Crawley, 2004; WHO, 2004; Desai *et al*., 2007; Brabin *et al*., 2008). The altered placental integrity results in impairment of foetal nutrition leading to reduction in birthweight which is a leading cause of poor infant survival and development (Yamada, 1989; Brabin *et al*., 2008; Uneke, 2008). In areas of Africa with stable malaria transmission, *P. falciparum* infection during pregnancy is estimated to cause as many as 10,000 maternal deaths each year, 8% to 14% of all LBW babies (defined as birth weight of less than 2,500g) and 3% to 8% of all infant death (Desai *et al.,* 2007; Uneke, 2008). Outside Africa, malaria infection rates in pregnant women are much lower but are more likely to cause severe disease, preterm births, and foetal loss. *Plasmodium vivax* is common in Asia and the Americas and, unlike *P falciparum*, does not cytoadhere in the placenta, but, is associated with maternal anaemia and low LBW.

Maternal malarial infection during pregnancy is associated with maternal anemia, LBW and maternal mortality. World Health Organization estimates that more than half of pregnant women in the world have a haemoglobin level indicative of anaemia (<11.0/dl), the prevalence may however be as high as 56 to 61% in developing countries (Idowu *et al.*, 2005; Bukar *et al*., 2008; Enato *et al*., 2009). Women often become anaemic during pregnancy because the demand for iron and other vitamins is

increased due to the associated physiological burden of pregnancy. The inability to meet the required level for these substances either as a result of dietary deficiencies or infection gives rise to anaemia (van den Broek, 1996; 2003; Idowu *et al*., 2005). Anaemia ranges from mild, moderate to severe and the World Health Organization has categorized the haemoglobin level in pregnant women as follows: anaemia in pregnancy at 10.0-10.9g/dl (mild anaemia); 7-9.9g/dl (moderate anaemia) and < 7g/ dl (severe anaemia) (WHO, 1989). Early antenatal care attendance can reduce anaemia during pregnancy (Adinma *et al*., 2002). Malaria is responsible for between 2-15% of maternal anaemia. Severe anaemia has been estimated to be between 5 and 10% (Shulman, 1999; Menendez *et al*., 2000; Shulman *et al*., 2002).

Malaria is responsible for 5-14% of LBW which is the single greatest risk factor for neonatal and infant mortality (Menendez, 1995; Ezugwu, 2006). Analysis showed that a baby is twice as likely to be born with a LBW if the mother has an infected placenta at delivery (Gyatt and Snow, 2001). The analysis further showed that, one-fifth of the LBWs of babies born to mothers in areas where malaria is endemic are due to malarial infection of the placenta during pregnancy (Gyatt and Snow, 2001). LBW is the single greatest risk factor for neonatal and infant mortality (McCormick, 1985). Malaria infection in pregnancy which accounts for between 3-5% of all newborn deaths has been attributed to between 75,000 and 200,000 infant deaths (Steketee *et al*., 2001; Guyatt and Snow, 2001; WHO, 2004). Cross-sectional data on birth weight and survival from five areas in sub-Saharan Africa showed that infant mortality is three times higher for LBW babies than for those of normal weight (Guyatt and Snow 2001). The effects on neonatal mortality are even more marked, with a LBW baby being nine times more likely to die in the first month of life than a normal-weight

baby. The risks for mortality increase steadily as the birth weight decreases to below the LBW threshold, though the data are limited. The best example is from Malawi, where the infant death rate per 1,000 live births was 650 for babies with birth weights of less than 1,500 g; 276 for babies with birth weights of 1,500 to 1,999 g; 58 for babies with birth weights of 2,000 to 2,499 g, and 24 for babies with normal birth weights (>2,499 g) (Guyatt and Snow, 2004).

Malaria in pregnancy causes preterm delivery and still birth (Menendex *et al*., 2007; Brabin *et al*., 2008). Primigravidae and Secundigravidae are most at risk, but in areas with moderate to intense transmission, or a high prevalence of infection with Human Immunodeficiency Virus (HIV), women of higher gravidity are also affected (Schulman *et al.,* 2001; Beck *et al.,* 2001). Studies suggest that HIV infection may diminish a pregnant woman's capacity to control *P. falciparum* infection and thus lead to decreased efficacy of antimalarial interventions (Konde–Lule *et al*., 1991; Steketee *et al.,* 1996). The presence of placental malaria in HIV-positive women may also increase the risk of vertical transmission of HIV (Bloland *et al.*, 1995). These deleterious effects of malaria on pregnancy led to the recommendation that pregnant woman in malaria endemic countries take chemoprophylaxis during pregnancy (WHO, 2004). Although primigravidae are most affected by malaria, the consequences for infants born to multigravid women in Africa may be greater than previously appreciated. This is because HIV increases the risk of malaria and its adverse effects, particularly in multigravidae, and recent observational studies show that placental infection almost doubles the risk of malaria infection and morbidity in infants born to multigravidae (Reinhardt *et al*., 1978; Diagne *et al*., 1997; Perrault *et al*., 2009).

2.5 SOCIAL AND ECONOMIC EFFECTS OF MALARIA

Malaria affects the health and wealth of nations and individuals alike. In Africa, malaria is classified to be both a disease of poverty and a cause of poverty (Sanh *et al*., 2008). A high percentage of the people live in extreme poverty in rural areas (Otubanjo and Mafe, 2002). Malaria has significant measurable direct and indirect costs, and has been shown to be a major constraint to economic development. For developing economies, this has meant that the gap in prosperity between malarious and non-malarious countries has become wider over the years. Annual economic growth in countries with high malaria transmission has historically been lower than in countries without malaria. Economists believe that malaria is responsible for a 'growth penalty' of up to 1.3% per year in some African countries (Sachs, 2001; Chima *et al.,* 2003). When compounded over the years, this penalty leads to substantial differences in GDP between malarious and non-malarious countries and severely restrains the economic growth of the entire region (Sachs and Malaney, 2002; Chima *et al.,* 2003).

The direct costs of malaria include a combination of personal and public expenditures on both prevention and treatment of the disease. Personal expenditures include individual or family spending on Insecticide Treated Mosquito Nets (ITNS), doctors' fees, anti-malarial drugs, transport to health facilities, support for the patient and sometimes accompanying family member during hospital stays. Public expenditures include spending by Government on maintaining Health Facilities and Health care infrastructure, publicly managed vector control, education and research. In some countries with a heavy malaria burden, the disease may account for as much as 40% of public health expenditure, 30-50% of in-patient admissions, and up to 50% of out-

patient visits. The indirect costs of malaria include lost productivity or income associated with illness or death. This might be expressed as the cost of lost work-days or absenteeism from formal employment and the value of unpaid work done in the home by both men and women. Malaria has a greater impact on Africa's human resources than simple lost of earnings. An indirect cost of malaria is the human pain and suffering caused by the disease. Malaria also hampers children's schooling and social development through both absenteeism and permanent neurological and other damage associated with severe episodes of the disease (Elyazar *et al*., 2011).

2.6 DIAGNOSIS OF MALARIA

The gold standard for diagnosis of malaria is microscopy; however various rapid diagnostic tests have been developed for the rapid diagnosis of *Plasmodium* malaria parasite (WHO, 1993a; WHO, 2000a; 2000b; Oyibo *et al*., 2009; Baker *et al*., 2010; Hopkins *et al*., 2011). A key feature of the World Health Organization Global Malaria Control Strategy is rapid diagnosis of malaria, so that effective treatment can be administered quickly, in order to reduce morbidity and mortality (WHO, 1993b; WHO, 1993c; WHO, 2000c; 2000d; WHO, 2006; Kyabayinze *et al*., 2010).

Prompt and accurate diagnosis of malaria is essential for prompt and appropriate treatment of malaria in order to reduce the risk of severe disease in malaria endemic regions, (Kyabayinze 2010). Presumptive treatment of malaria is widely practised where microscopy or rapid diagnostic tests (RDTs) are not readily available. Rapid diagnostic tests (RDT) for malaria diagnosis, detect either histidine-rich protein-2 (HRP-2) or parasite lactate dehydrogenase enzyme (pLDH) (Leke *et al*., 1999; Piper *et al*., 1999; WHO, 2000b). The use of OptiMAL rapid diagnostic test has been well

documented (Hunt-Cooke *et al*., 1999; Agomo *et al.*, 2003; 2004; Oyibo *et al.,* 2009). It is a simple detection assay that uses a dipstick technique which was developed and introduced by Diamed AG. The OptiMAL RDT detects metabolic enzyme of *Plasmodium* lactate dehydrogenase (pLDH) produced by viable malaria parasites and also released from parasite infected erythrocytes. Several studies have evaluated HRP-2 based RDTs (Singh and Valecha 2000; Tarimo *et al.,* 2001; Forney *et al.,* 2003) for the diagnosis of malaria in children and non-pregnant adults. The use of RDTs for diagnosing malaria in pregnant women attending antenatal clinics has been reported (Singer *et al*., 2004; VanderJagt *et al.,* (2005).

The use of RDT will promote rational use of drug, particularly with the introduction of artemisinin-based combination therapy (ACT) for treatment of malaria. The use of RDT will target treatment of patients with parasitologically confirmed malaria in order to improve quality of care, reduce over consumption of anti-malarials, reduce drug pressure and in turn delay development and spread of drug resistance (WHO, 2000b; Murray *et al*., 2008; Samane *et al*., 2010). Several studies have shown that the decline in pLDH activity has also been shown to parallel the decline of viable parasites during therapy, (Oduola *et al.,* 1997). This assay may therefore, be used to monitor patient progress during therapy and serves as an indication of recrudescence and possible drug-resistant infections, assuming that pLDH levels persist in these conditions. However RDT-supported malaria diagnosis may have led to the overprescription of ACTs, with the drug being prescribed to people with RDT-negative results (Uzochukwu *et al*., 2011). One possible factor contributing to variable test performance is the diversity of parasite antigens (Baker *et al*., 2010). Some of the RDTs include Parasight F, OptiMAL DIAMED and

Immunochromatographic test (ICT) (Tagbor *et al*., 2008). RDT have also been used in Primary Health care Facilities and in some remote communities for the diagnosis of malaria (Kyabayinze *et al*., 2010; Hopkins *et al*., 2011).

2.7 PREVALENCE OF MALARIA IN PREGNANCY

Studies conducted by various researchers have shown the prevalence of malaria during pregnancy to be between 7.7% and 79.3%; (Okwa, 2003; Adefioye *et al*., 2007; Kagu *et al*., 2007; Nnaji *et al*., 2009; Gajida *et al*., 2010). Studies were conducted in Gabon by Marielle *et al*., 2003 and Bouyou-Akotet *et al.*, (2003) which recorded malaria prevalence of 57%, out of which majority (64%) were primigravida. High malaria prevalence rate have been recorded in different parts of Nigeria: 44%, 60% and 52% respectively in Lagos State (Anorlu, 2001; Okwa, 2003; Raimi and Kanu, 2010); 72% in Oshogbo (Adefioye *et al*., 2007); 20% in Edo (Enato *et al*., 2009). Nnaji *et al*., (2009) recorded as high as 79.3% malaria prevalence among women attending ANC in Nnewi, South East Nigeria. Malaria infection during pregnancy is associated with poor maternal and foetal outcomes including LBW. In malaria-endemic areas, LBW is primarily a consequence of intra-uterine foetal growth restriction (Rogerson *et al*., 2007).

2.8 MALARIA CONTROL STRATEGIES

Malaria control is based on four strategies which include: chemotherapy, malaria prevention [IPT and Insecticide Treated Nets (ITNs)/LLINs], chemoprophylaxis for non-immune visitors and Integrated Vector Management (IVM) (WHO, 1993a; WHO, 2000c; Omo Aghoja *et al.,* 2008; WHO, 2009). Treatment of uncomplicated malaria is based on prompt diagnosis and early treatment, using oral Artemisinin-

based combination therapy (ACT). Treatment of complicated or severe malaria requires the use of intra-venous quinine or parenteral administration of arthemeter. In order to prevent the deleterious effects of malaria during pregnancy, control strategies for malaria in pregnancy are formulated in relation to the epidemiological patterns of the infection. The current emphasis in stable transmission areas, is on IPTp-SP during pregnancy, combined with the use of ITNs and case management while, in low transmission areas, emphasis is primarily on case management (ter Kuille *et al*., 2003; WHO, 2004; Gikandi, 2008). IPTp-SP has been a key component of the focused antenatal care package, for nearly a decade, reducing the burden of LBW attributable to malaria in sub-Saharan Africa (Chico and Chanramohan, 2011). Increasing IPTp dosing from two to more frequent doses may be beneficial in some circumstances (HIV positive women), but in view of increasing parasite resistance to SP, the currently recommended antimalarial for IPTp, appropriate monitoring, evaluation and research is required, in order to establish optimal control strategies (Menendex *et al*., 2007).

ITNs used throughout pregnancy, or from mid-pregnancy onwards, improve pregnancy outcomes particularly in the first few pregnancies in women living in malaria endemic countries in Africa as recommended by WHO (Gamble *et al*., 2007). Their use is a pivotal control option, although further research on their potential benefits in women living in areas outside Africa and under conditions of lower transmission is required. The non-immune visitors are required to take prophylaxis while visiting malaria endemic areas (WHO, 2010). Vector control methods aimed at both adult vectors and the larvae, including the removal of breeding places are recommended. Transgenic strains of mosquitoes have been developed and evaluated

to replace or suppress wild vector populations and thereby reduce transmission and deliver public health gains (Breman *et al*., 2007; UNICEF, 2007).

Various malaria intervention efforts are producing a measurable public health impact. The annual number of malaria cases and deaths has continued to decline, especially in Africa (WHO, 2010). The number of countries that have successfully reduced their malaria burden, by half over the past decade has continued to increase. For the first time, not a single case of Falciparum malaria was reported in the WHO European Region in 2009. A total of 11 countries in the WHO African Region showed a reduction of more than 50% in the number of confirmed cases of malaria between 2000 and 2009. Downward trends of 25%–50% were seen in 8 other countries outside Africa. Morocco and Turkmenistan were certified by the Director-General of WHO in 2009 as having eliminated malaria (WHO, 2010).

2.9 CHEMOPROPHYLAXIS WITH PALUDRINE AND PYRIMETHAMINE

The first drug developed principally for chemoprophylaxis was Proguanil (Paludrine). Clinical trials of this drug was conducted in Nigeria and its efficacy led immediately to its use in Nigerian troops in 1944 (Salako, 2006). The drug became available to the entire population soon after the war, although it was later superseded by the equally effective Pyrimethamine which had the advantage of a weekly administration (WHO, 1986; Nahlen, 1989). Pyrimethamine was given weekly for chemoprophylaxis in pregnant women against P. *falciparum* malaria. It had slow blood schizontocidal activity, but considerable activity on the primary tissue forms of *P. falciparum* and to lesser extent, on *P.vivax*. It has also a pronounced sporontocidal effect so that an

individual with gametocytes in the blood is non-infectious to mosquitoes. Pyrimethamine, when taken at adult dose of 25mg once a week prevented infections with *P. falciparum* malaria, and suppresses *P. vivax* malaria.

However, concerns about the rapid development of parasite resistance to pyrimethamine and its slow schizontocidal activity were raised shortly after its introduction (Coatney *et al.,* 1952; Goodwin, 1952; Wilson and Edeson, 1953; Petersen, 1987). Resistance to pyrimethamine has been reported since the 1950s and the resistance initially spread to parts of East Africa, West Africa and the Far East but gradually more areas became involved and the drug became ineffective in preventing malaria (Coartney *et al*., 1952; Wilson and Edeson, 1953; Avery-Jone, 1958; Bruce-Chwatt, 1993; Peterson, 1987; Durand *et al*., 2000). Studies carried out showed decreased effectiveness of Pyrimethamine in Mali, (Plowe *et al*., 1996) and in Nigeria, (Nahlen *et al*., 1989).

A study was conducted in Ilorin, Nigeria where pregnant women who had *P. falciparum* were given 25 mg pyrimethamine weekly for suppressive prophylaxis (Nahlen *et al.*, 1989). *In vivo* and *in vitro* tests conducted showed 67% (59/88) and 60% (6/10) pyrimethamine resistance. A second group of parasitaemic and parasite free pregnant women having been enrolled were treated with curative dose of 25mg/kg. Half of them were given 25mg pyrimethamine weekly while the other half received no prophylaxis. Parasitological failure rates did not differ between the pyrimethamine-treated and those who did not receive pyrimethamine during the 16-week follow up (Nahlen *et al.*, 1989). Research has shown that Pyrimethamine acts by selectively inhibiting dihydrofolate reductase in the parasite thus preventing the

synthesis of purines and pyrimidines (Mckie *et al*., 1998). Further study by Lozovsky *et al*, (2009) showed four key amino acid replacements were implicated in pyrimethamine resisstance.

2.10 CHEMOPROPHYLAXIS USING CHLOROQUINE (CQ)

The consequences of malaria due to *P. falciparum* in pregnancy led to the recommendation that pregnant women receive full dose of antimalarial treatment on their first contact with antenatal service. This was followed by weekly chemoprophylaxis of frequent or regular use of an antimalarial drug given at less than a therapeutic dose during pregnancy (WHO, 1986; WHO, 2000d). The most commonly used drug for chemoprophylaxis during pregnancy, which had evidence to reduce the rate of placental parasitaemia, and low birth weight was chloroquine (Cot *et al.*, 1995). Studies conducted showed reduction in the prevalence of placental malaria and LBW in women who took weekly chloroquine regularly (Denoeud *et al.,* 2007). This however had the challenge of weekly regimen throughout pregnancy, including the fears of adverse effects of the drugs during pregnancy (Shultz *et al*., 1994).

Health workers had concern that the use of the medicine for this purpose may deplete stocks needed for the treatment of acute infections that are widespread (MacCormack and Lwihula, 1983). Weekly prophylaxis with chloroquine soon proved unsuccessful because of logistical constraints (Spencer *et al.*, 1987), most importantly, the spread of high-grade CQ resistant parasites, pruritis (itching) and socio-behavioural barriers (Kaseje *et al.*, 1987; Sirima *et al*., 2003; Gregson and Plowe, 2005).

2.11 RESISTANCE OF PARASITE TO CHLOROQUINE (CQ)

Chloroquine, which was introduced in the 1940s, and for many decades served as a cheap and reliable drug, became ineffective against *P. falciparum* in most tropical areas (Rathod *et al*., 1997; Peters, 1998a; Warhurst, 2001; Patrick *et al*., 2003). Resistance to CQ developed in Southeast Asia and South America at the end of the 1950s and in Africa by the late 1970s (Warhurst, 2001). This led to limited use of the drug which could no longer eliminate *P. falciparum* infections and therefore could not be used for preventing malaria during pregnancy (Neequaye *et al*., 1986; Peters, 1998b; WHO, 2004; Enato, 2005). Consequently, national policies that continue to advocate CQ use for weekly prophylaxis had negligible programme effectiveness. This is due to the marginal efficacy of CQ, in addition to the aforementioned problems with compliance resulting from the need for frequent dosing.

Few African countries also had programmes that provided chemoprophylaxis to pregnant women. An example is a well-supported community health programme in western Kenya that used village health-care workers to provide weekly chemoprophylaxis with chloroquine to only 29% of pregnant women that are primigravidae (Spencer *et al.,* 1987). One problem that limited the coverage of pregnant women was acceptability of CQ chemoprophylaxis regimen. In Malawi, where more than 90% of pregnant women attend antenatal clinics, local taboos against ingesting bitter substances, such as CQ during pregnancy limited women's acceptance of chemoprophylaxis (Schultz *et al.*, 1994). A survey of seven regions in four African countries found that although 34% to 68% of pregnant women reported using an antimalarial medicine during pregnancy, only 1% to 18% reported using an

antimalarial medicine on a weekly basis at a dosage close to the World Health Organization recommendation (Steketee *et al.*, 1996).

Increasing resistance of *P. falciparum* malaria to CQ prompted many studies within the last two decades in different parts of Nigeria (Salako *et al.,* 1990; Sowunmi *et al*., 1990; Sowunmi and Salako, 1992; Sowunmi and Walker, 1993; Molta, 1995). Survellance network in the country from mid-1987 to 1990 revealed that chloroquine resistant *P. falciparum* (CRPF) was widespread (Ekanem, 1997). In the northern part of Nigeria, parasitological failures increased from 18.7% in 1988 to 24.5% in 1995 (Molta, 1995). Between 1987 and 1997, resistance ranging from 33% to 72% was also reported in the southern part of the country and 22% in Ibadan in 1997 (Ekanem, 1997; FMOH, 2005; Oyedeji *et al*., 2005). Drug therapeutic efficacy test (DTET) conducted with CQ and SP in the six geopolitical zone in Nigeria confirmed reduced efficacy of CQ in all the zones, while SP was still effective (FMOH, 2005).

2.12 INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY USING SULFADOXINE-PYRIMETHAMINE (IPTp-SP)

The negative impact of malaria in pregnancy has been extensively reported (Brabin 1983; McGregor *et al*., 1983; Steketee *et al*., 1996; Shulman *et al.* 1999; Rogerson *et al*., 2000; Shulman *et al*., 2001). Malaria studies conducted in different parts in Nigeria have shown that pregnant women particularly in their first pregnancy are at risk of malaria infection and that prevalence of *Plasmodium* parasitaemia is still very high (Idowu *et al*., 2005; Komolafe *et al.,* 2005; Dim and Onah, 2007; Bukar *et al*., 2008; Nnaji *et al*., 2009). Studies carried out in Southwest Nigeria reported malaria parasite prevalence of between 60% and 72% among pregnant women (Okwa *et al*.,

2003; Adefioye *et al*., 2007) and is still associated with complications particularly among primigravida (Nnaji and Ikechebelu, 2007; Nwagha *et al*., 2009; Nnaji *et al*., 2009).

Studies have shown that effective antimalaria drugs can have an impact on malaria, but several logistical and other constraints have hampered out-reach and compliance in many malaria endemic areas. In addition, the decreasing efficacy of several antimalarial drugs further complicates delivery (Menendez 1999; Yeung, 2004). In areas of stable malaria transmission, it is recommended that pregnant women receive at least two doses of IPT with SP (IPTp-SP) after quickening; sleep under ITNs and have prompt treatment of all malarial illnesses (WHO, 2004; Briand *et al*., 2007). Intermittent preventive treatment (IPT) (full therapeutic doses), given at defined intervals, has potential benefits, and is a promising strategy to control malaria during pregnancy (Newman *et al*., 2003; Greenwood, 2004). Studies conducted in Kenya, Malawi and elsewhere have shown that IPT with SP given twice during pregnancy can reduce malaria episodes, severe anaemia and improve birth weight (Schultz *et al*., 1995; Parise *et al*., 1998; Rogerson *et al*., 2000; van Eijk *et al*., 2004; Mubyazi *et al*., 2005).

In Kenya, Shulman *et al*., (1999) clearly demonstrated that IPTp-SP, given several times in pregnancy when women attend antenatal care (ANC), can reduce severe anaemia among primigravidae by 39%. In southern Ghana, studies showed that placental malaria and maternal anaemia declined substantially and birth weight increased after the implementation of IPTp-SP (Hommerich *et al*., 2007). However, the remnant prevalence of infection in women having taken three doses of IPTp-SP

suggests that additional antimalarial measures are needed to prevent malaria in pregnancy in this region (Hommerich *et al*., 2007).

Various studies in which pregnant women were given IPT have shown that placental malaria was reduced by 72% in primigravidae and secundigravidae (Schultz *et al*., 1994; Parise *et al*., 1998) and there was a beneficial effect on the haemoglobin with reduction in anaemia (Shulman *et al*., 1999; ter Kuile *et al.,* 2007).

Verhoeff *et al.,* (1998) reported that multiple doses of SP taken during pregnancy led to a highly significant reduction in the incidence of LBW infants born to primigravidae, even if the women had HIV infections. The reduction in parasite prevalence earlier in pregnancy, due to treatment with SP led to improved foetal growth. Shulman *et al.,* (1999) showed that SP conferred 85% protective efficacy on pregnant women who took SP compared to 39% protective efficacy for the placebo group. They reported that pregnant women who booked late and received only a dose of SP benefited significantly from the intervention. The effects were seen more in women who owned insecticide treated nets (Shulman *et al.,* 1999). Wolfe *et al*., (2001) suggested that in HIV prevalence of over 10% that it is more cost effective to treat all pregnant women with three-dose regimen of IPT with SP than to screen for HIV and provide this regimen to HIV-positive women only.

The studies conducted in Kenya, by van Eijk *et al.,* (2004), confirmed the effectiveness of IPT in reducing both placental malaria and the incidence of LBW. The study showed that the prevalence of placental malaria was 13.8% and 12.2% for LBW. Evaluation of the effectiveness of IPT using SP in pregnant women in Ekiti

State and Ibadan showed that IPTp-SP was able to prevent maternal and placental malaria as well as improve pregnancy outcomes (Falade *et al.,* 2007; Abasiattai *et al*., 2009). In multivariable analysis, one or more doses of IPTp-SP were associated with a reduction in placental malaria and LBW. The evaluation of IPT uptake in Ogun state showed the uptake to be 25% (Adeneye *et al*., 2007), while in the study in Ekiti state, only 27.3% had received a dose of SP during the index pregnancy (Falade *et al*., 2007). It has been suggested that awareness for IPT could be delivered through teachers which has been shown to be quite cost effective (Temperley *et al*., 2008) including surmounting misconceptions about IPTp-SP (Launiala and Honkasolo, 2007).

The increasing levels of parasite resistance with the IPTp-SP mean that the benefits of national IPTp-SP programmes may soon be seriously undermined in much of the region. Hence, there is an urgent need to develop alternative drug regimens for IPT in pregnancy. Review of published safety and efficacy data on various antimalarials proposed several candidate combination regimens for assessment in phase II/III clinical trials. There is some evidence that combining short-acting artemisinins with longer-acting agents is likely to delay the emergence of resistance to the slowly-eliminated component. Such antimalarials include dihydroartemisinin-piperaquine, artemether-lumefantrine and mefloquine in combination with artemether or artesunate to be administered in the second and third trimester of pregnancy (Vallely *et al.*, 2007).

2.13 SAFETY OF ANTIMALARIAL DRUG IN PREGNANCY

Reviews of the antimalarials drugs used in pregnancy have been conducted (Nosten *et al*., 2006). The antimalarials that can be used in pregnancy include: chloroquine, amodiaquine, quinine, azithromycin, sulfadoxine-pyrimethamine, mefloquine, dapsone-chlorproguanil, artemisinin derivatives, atovaquone-proguanil and lumefantrine. Antimalarial drugs that should not be used in pregnancy are: halofantrine, tetracycline/doxycycline, and primaquine. There are few studies in humans on the pharmacokinetics, safety and efficacy of antimalarials in pregnancy (Nosten *et al*., 2006). This is because pregnant women are systematically excluded from clinical trials. The absence of adequate safety data, especially in the first trimester, has been a major obstacle to developing treatment strategies. The pharmacokinetics of most antimalarial drugs are often times modified in pregnancy and dosages will need to be adapted. Other factors, including HIV status, drug interactions with antiretrovirals, the influence of haematinics and host genetic polymorphisms may influence safety and efficacy. For these reasons there is an urgent need to assess the safety and efficacy of antimalarial treatments in pregnancy, including artemisinin based combination therapies.

Inspite of the recommendation that pregnant women should use IPTp to reduce the burden of malaria during pregnancy in Africa, (WHO, 2004; Peters *et al*., 2007), implementation has not been optimal because of concerns of potential drug toxicities. Weekly SP prophylaxis is associated with rare but potentially fatal cutaneous reactions. Fortunately, SP use in IPTp programmes in Africa, with 2-4 treatment doses over 6 months, has been well tolerated in multiple IPTp trials (Peters *et al*., 2007). There has been no evidence that sulfa drugs of pyrimethamine cause abortion

or still births when administered in the second and third trimester (Morley, 1964; Sowunmi and Walker, 1993). Results from studies conducted by Gimning *et al*., (2006) showed no adverse effect to SP.

Sulfadoxine-pyrimethamine should not be administered concurrently with cotrimoxazole, because of drug interaction resulting in adverse drug reactions. In malaria endemic areas pregnant women who are already receiving cotrimoxazole prophylaxis should not also receive IPTp-SP. Although, the use of folate antagonist in the first trimester is associated with neural tube defects, large case-control studies have demonstrated that SP administered as IPTp (exclusively in the second and third trimesters and after organogenesis) does not result in an increased risk of teratogenesis. Folic acid supplementation is recommended for all pregnant women to reduce the rate of congenital abnormalities, though high doses of folic acid (5 mg/day) may interfere with the antimalarial efficacy of SP.

However, the recommended standard dose of folic acid supplementation (0.4 mg/day) does not affect antimalarial efficacy and may provide the optimal balance to prevent neural tube defects and maintain the effectiveness of IPTp-SP. There has been no report of clinical association between SP use and kernicterus. This is inspite of the extensive use of SP and related compounds to treat maternal malaria and congenital toxoplasmosis in near-term pregnant women and newborns. SP can be considered completely safe and has a favourable safety margin when delivered as IPTp–SP (Peters *et al*., 2007). Pharmacovigilance programmes throughout Africa are now needed to confirm its safety as access to IPTp-SP increases, (Talisuna *et al*., 2006).

Animal studies have been conducted by giving Pyrimethamine alone and in combination with Sulfadoxine to hamsters, rats and other animals. Complete embryo resorption and embrotoxicity in Wistar rats were observed when SP was given in early gestation (Phillips-Howard and Wood, 1996). An *in vivo* study was conducted by Otubanjo and Mosuru (2007), in which abnormal sperm induction was evaluated in mice using Sulfamethoxypyridazine-pyrimethamine. The study showed that 0.5X the human therapeutic dose of Sulfamethoxypyridazine-pyrimethamine (Metakelfin) gave a statistically significant increase over the negative control. The study however concluded that the medicine is probably not mutagenic as induction of sperm head abnormality was not dose dependent. Assessments of sperm abnormality have also been conducted by exposing mice to chemicals. This is followed by visual scoring of the percentage sperm with abnormal head forms and shapes in smears of sperm from epididymis according to the works of Wyrobek and Bruce (1975); Odeigah (1997).

It is known that during spermatogenesis DNA synthesis occurs before the pre-meiotic phase and no further synthesis occurs throughout the duration of spermatogenesis in the cell cycle. Thus, sperm-head morphological abnormalities may be as a consequence of a naturally occurring level of mistakes in the spermatozoon differentiating process and a chemical mutagen might increase the frequency of these mistakes according to the work of Bruce and Heddle (1979). The abnormalities may be as a result of the mistakes made in packaging the genetic material in the sperm head or perhaps as a result of an abnormal chromosome complement. However, it is probable that sperm with abnormal shapes would contain abnormal genetic material according to Wyrobek and Bruce (1978).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 THE STUDY AREA

The study area is Badagry Local Government Area, located in the Southwestern part of Nigeria (Figure 1). It covers an area of about 71,426 sq kms and a projected population of 167,762 as at 2002, (NPC, 1999). It lies to the South West of Lagos State having its boundary with the lagoon in the South, Ojo Local Government Area in the West, Benin Republic in the East and Ogun State in the North. It lies on latitude 6.25°N and longitude 2.53°E and situated on the narrow Southwestern plain of Nigeria with its characteristic network of creeks and marshy lowlands. The climatic condition of the area is tropical rain forest in nature and lies within the wet equatorial belt, characterized by high temperature, high humidity and heavy rainfall. There are twenty wards in the LGA out of which four wards are riverine.

The study was conducted in Ikoga ward which is one of the twenty wards in Badagry Local Government Area. Ikoga ward is made up of eight communities namely: Pota, Igborosun, Ikoga Zebbe, Javie, Iragan, Erekiti, Eretekiti Ajido and Iragbo, of which all these are on land. The local languages are Yoruba and Egun. Three communities (Ikoga-Zebbe, Igborosun and Pota were randomly selected for the study because of their proximity to Pota Health Facility which is the only Government Health Facility in Ikoga Ward. The choice of Badagry LGA was based on absence of documented study on malaria endemicity in the LGA; absence of parasite resistance to SP and because it is a peri-urban LGA. Badagry LGA is coastal LGA with defined boundaries and an identifiable community for easy follow up. Implementation of SP began in 2006. The occupation of the people includes farming, fishing, trading,

animal husbandry and civil servants. Agricultural products are maize, fish and vegetables.

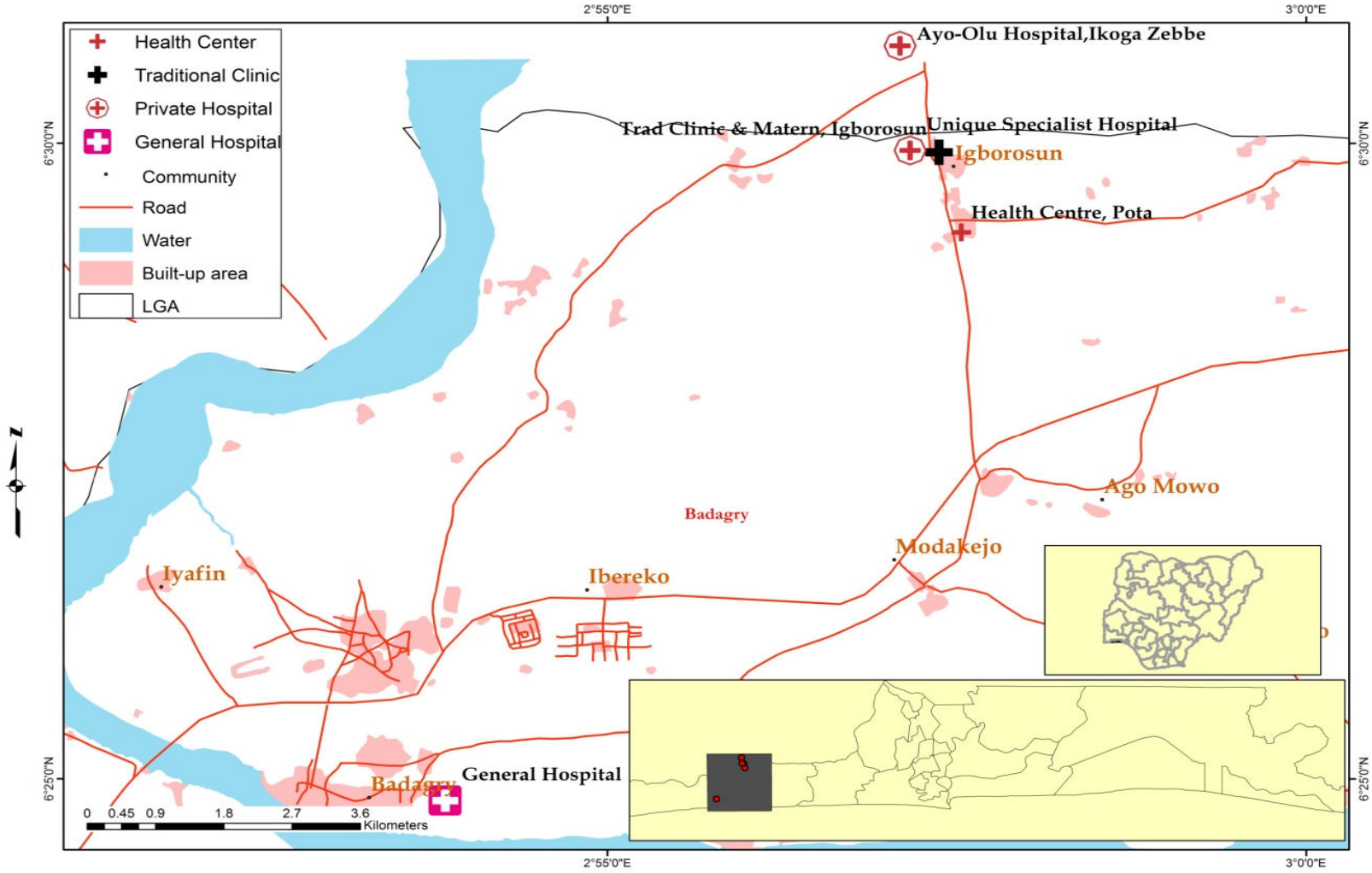

Figure 2: Map of Badagry Local Government Area showing the study site and Health Facilities

Study Population

The Health Facilities where the study was conducted were Pota Health Centre, Unique Private Hospital, Ayo-Olu Hospital, and Omowumi Traditional Birth Attendant Home, all in Ikoga ward. The Badagry General Hospital was included because all primigravidae are expected to attend ANC in a General Hospital, although it is not in Ikoga ward. The Traditional Birth Attendant (TBA) home was included because quite a good number of pregnant women patronize this TBA home and it is the only one in Ikoga ward. The TBA was also trained by United Nations Children Educational Funds (UNICEF) of which the certificate was well displayed. Private Health Facilities were included because some pregnant women have preference for such.

Study Design

Advocacy visit was made to the chairman of Badagry Local Government Area of Lagos and thereafter a meeting was held with the Counselor for health and the Medical Officer of Health. The essence of the study was presented and the support and consent of the LGA was solicited, of which permission was granted. A courtesy visit was made to the traditional rulers of the study communities in the company of Health Officials from Badagry Local Government Area. The study was designed to include studies on humans, vector and animal (mice).

Ethical Clearance for Studies on Humans

The study protocol was submitted to the Institutional Review Board (IRB) in the Nigeria Institute for Medical Research, Yaba, Lagos which subsequently gave the approval (Appendix 1). The purpose of the study was also explained to community

leaders and their consent and support solicited for the intended study. All the participants in the study gave their written consent.

Population and Sample Size Determination

The population of Badagry Local government area was given as 167,762 as at 2002, while the population of the study communities was estimated at 8,000, (NPC, 1999). The health facilities used in the study were mapped using Geopolitical Positioning System (GPS) and indicated on the map (Figure 2).

The study on pregnant women was a cross sectional survey, therefore the formula of Lemeshow *et al.*, (1990) was used in calculating the sample size for pregnant women that were recruited for the evaluation of Intermittent Preventive Treatment (IPT) using SP (Appendix 2).

3.2 STUDIES ON HUMANS

Studies conducted on humans included the administration of questionnaire to community members and pregnant women. Parasitological investigations were also carried out in the communities as well as pregnant women.

3.2.1 Questionnaire on Knowledge, Attitude and Practices on Malaria among Community Members in Ikoga Ward

The questionnaire of Otubanjo *et al*., (2000) was adapted, modified and used to collect information on knowledge, attitude and practices of community members in order to assess their knowledge, perception, attitude and practices on malaria prevention and treatment. The questionnaire was pilot tested and administered to

304 community members in the study sites who were heads of households and above 18 years of age (Appendix 3). The questionnaire was translated into the local language of the people in order to enhance the respondent's correct understanding of the contents of the instruments as intended, to obtain accurate response. The responses obtained from the questionnaires were documented and analyzed using Epi Info software version 6.04, 2002 (Centre for Disease Control and Prevention, Atlanta, Georgia, USA).

In addition, the KAP study was complemented with Focus Group Discussion (FGD), among individuals with similar social background (Agyepong *et al.*, 1995). The FGD comprised young women aged 15-40 years; older women aged over 40 years; young fathers aged below 40 years and older fathers over 40 years. There were 8 participants in each FDG and they were encouraged to participate in the group discussions. Verbal and informed consent of the participants were obtained prior to their recruitment.

3.2.2 Questionnaire on Knowledge of Malaria, Prevention Methods and Knowledge of Sulfadoxine- Pyrimethamine (SP) in Pregnancy

The questionnaire was administered to 450 pregnant women who accepted to participate in the study and who were attending antenatal clinic (ANC) in the four ANC clinics, including the Traditional Birth Attendant Home over the one year period of study (Appendix 4). History of illnesses, drug use and educational attainment and parity was obtained. They were also asked of preventive measures taken to prevent illness during pregnancy; their knowledge of SP and its use during pregnancy to prevent malaria. Information was also obtained on antimalarial

medication and the knowledge, ownership and use of treated nets. The percentage of pregnant women randomly interviewed in each health facility were as follows: 76% from General hospital, 10% from Pota health facility, 7% from Omowumi traditional birth attendant, 3.8% and 2.7% from Unique and Ayo Olu private hospitals respectively.

3.2.3 Collection, Processing and Microscopic Examination of Blood Smears

Finger prick blood samples were collected aseptically and blood smears prepared on a clean slide according to World Health Organization recommended standard procedures (WHO, 2000a). The thin blood smears were prepared on clean, grease free slides, fixed with methanol followed by staining of both the thin and thick films with 10% Giemsa for 30 minutes (Warhurst and Williams, 1996). The slides were rinsed in water, allowed to dry and examined under oil immersion microscopy for malaria parasites.

The thick blood film has the advantage that it concentrates the layers of red blood cells (RBC) on a small surface by a factor of 20 to 30 and is stained as an unfixed preparation using Giemsa stain. The thick blood film provides enhanced sensitivity of the blood film technique and is much better than the thin film for detection of low levels of parasitemia. Malaria parasitaemia was defined as asexual blood stage of any *Plasmodium* species detectable on a thick blood smear. A slide was considered positive by the identification of ring stage malaria parasites. The presence of gametocytes was also observed. A slide was considered negative if no asexual parasites were found in 100 fields of thick blood smear (WHO, 2000a). Placental smears were made from blood collected from the placenta; stained and examined

the same way as peripheral blood smears. All those who had signs and symptoms of malaria were treated with antimalarial medicine at the health facility by the health workers.

3.2.4 Assessment of Malaria Endemicity

3.2.4.1 Malaria Prevalence Rate among Community Members in Ikoga Ward

Blood samples were collected over a one year period from 3,978 community members who agreed to participate in the study. Verbal consent was obtained from the adults as well as for their children under 18 years. These slides were processed as described in 3.2.3. Information was also obtained on their age, educational background and socioeconomic factors. Prevalence rate was determined by dividing the number of positive slides by the total number of slides examined and multiplied by 100.

$$\text{Prevalence rate} = \frac{\text{Number of slides positive}}{\text{Number of slides examined}} \times 100$$

Prevalence rate is widely used to determine malaria endemicity level as described by Metselaar *et al.,* (1959).

3.2.4.2 Malaria Prevalence among Pregnant Women Attending Antenatal Clinics

Blood samples were collected from 483 pregnant women who were attending antenatal clinic in Pota Heath Centre, Badagry General hospital, Ayo Olu maternity home, Unique specialist hospital and Omowumi traditional birth attendant home and who gave their informed consent to take part in the study. At enrollment,

information was also obtained on the demographic characteristics, education and socioeconomic status, obstetrical history, illness and treatment during the current pregnancy according to the method of Meltzer *et al*., (2003). The blood samples were collected and processed as described in 3.2.3. There was a continuous enrolment into the study for twelve months; as many as consented were enrolled into the study.

3.2.4.3 Parasite Density Determination among Community Members in Ikoga Ward and Pregnant Women Attending Antenatal Clinic

Giemsa stained thick blood films from community members and the pregnant women were prepared and examined for malaria parasites under oil immersion microscope as described in 3.2.3. Malaria parasites were counted against 200 leukocytes and expressed per mm^3 assuming a leukocyte count of $8000/mm^3$. Slides were considered negative if no asexual parasites were found in 100 fields under the oil immersion microscopy (WHO, 2000b).

3.2.4.4 Determination of Anaemia by Haematocrit, Packed Cell Volume, (PCV) among Community Members and Pregnant Women Attending Antenatal Clinic

Packed Cell Volume (PCV) was used in the determination of anaemia in the community for adults and children as well as pregnant women who were attending antenatal clinic. Blood samples were collected monthly in heparinized micro-capillary tubes. The micro-capillary tubes were sealed at one end and then centrifuged in a micro-haematocrit centrifuge for 5 minutes at 10,000 rpm. The length of the column of red blood cells (RBC) relative to the length of the column of

the whole specimen was measured as described by World Health Organization (1989). The PCV which was expressed in percentage was read off from a chart and the readings recorded. PCV of less than 33% was considered as anaemia (WHO, 1989). The pregnant women were examined monthly for anaemia using the haematocrit until delivery. Haematocrit was also measured at 32 weeks in the pregnant women in order to determine anaemia according to the method of Parise *et al*., (1998) Typing of anaemia was carried out using World Health Organization criteria: mild anaemia was classified as PCV of 30-32.9%; moderate as 21-29.9% and severe as <21% (WHO, 1989).

3.2.4.5 Determination of Fever among Community Members in Ikoga Ward and Pregnant Women Attending Antenatal Clinic

Axillary temperature was measured monthly in all the community members and pregnant women using a digital thermometer in degree Celsius. The axilliary temperature of ≥37.5^{o}C is defined as indication of clinical malaria (Mabunda *et al*., 2009).

3.2.4.6 Spleen Rate in Children 2-9 Years in Ikoga Ward

Spleen rate which is also used used in determining malaria endemicity was calculated by examining the spleen of children 2 to 9 years in the community. The spleens of these children were palpated by medical personnel for spleen enlargement. The degree of enlargement was also determined and classified as well as the class of spleen enlargement; 0 indicated non-palpable spleen while the highest degree of spleen enlargement was represented by 5 (Bruce-Chwatt, 1993). The Averaged Enlarged Spleen (AES) index was calculated by multiplying the

number of individuals in each class of enlarged spleen by the class of spleen and dividing this figure by the total number of individuals with splenomegaly. AES is used to distinguish clearly between an endemic situation and a current or recent epidemic. This value is not expected to be more than 2 in an endemic area (Appendix 5). Spleen rate is therefore the total number of individuals in each class with splenomegaly divided with the total number of children palpated (Bruce-Chwatt, 1993).

3.2.4.7 Diagnosis of *Plasmodium* parasite using Optimal Diamed Rapid Diagnostic Test (RDT)

The OptiMAL Diamed rapid diagnostic technique dipstick manufactured by Diamed AG, Switzerland was evaluated in 194 blood samples collected from community members in Ikoga ward according to the manufacturer's instructions**.** A drop of buffer was placed inside the conjugate well which has been coated with an indicator tagged monoclonal (mouse hybridoma) antibody to pLDH produced by asexual and sexual stages of the parasite, while four drops were put inside the wash well (Makler and Hinrichs, 1993). This stood for one minute. The finger was aseptically cleaned, pricked with a lancet and a drop of blood placed inside the conjugate well containing the buffer and mixed thoroughly. The Diamed OptiMAL Dipstick was dipped into the mixture of blood and buffer and allowed to stand for 15 minutes, after which the dipstick was placed in the wash well containing the buffer.

The OptiMAL Diamed Dipstick has three antibodies dried onto it. These are goat anti-mouse antibody, (for the control); monoclonal anti-pLDH specific to the 4 *Plasmodium* species and monoclonal anti-pLDH specific to *Plasmodium*

falciparum. The pLDH present in the blood sample reacts with anti-pLDH conjugate and rises up the Dipstick where it is captured by one or both of the specific pLDH antibodies, which causes the appearance of a coloured band (Moody *et al*., 2000; Labbe *et al.*, 2001). A negative sample was indicated by only one line; the presence of *P. ovale* and *P. malariae* showed two positive lines while the presence of *P. falciparum* indicated 3 lines (Appendix 6).

3.3 EVALUATION OF SULFADOXINE-PYRIMETHAMINE (SP) IN PREGNANT WOMEN ATTENDING ANTENATAL CLINICS

3.3.1 Administration of Sulfadoxine-Pyrimethamine (SP) to Pregnant Women

Four hundred and eighty three (483) pregnant women who were between 16 and 34 weeks pregnant and who were attending antenatal clinics were enrolled in the study; however SP was evaluated in 426 pregnant women who were eligible. The biodata of pregnant women was collected and documented, followed by measurement of their weight, height and temperature. The gestational age was also determined. The pregnant women who met the inclusion criteria were given one dose of SP (3 tablets of SP totaling 1500mg/kg sulfadoxine and 75mg/kg pyrimethamine by Medreich) at enrollment at the Health Facility by trained health personnel. The second dose was given in the third trimester but not in the last month of pregnancy, totalling two doses as stipulated (WHO, 2004; FMOH, 2005); at least one month interval is recommended between the two doses. The number of SP doses administered depended on how old the pregnancy was at recruitment, 2 doses for those recruited early and one dose for those recruited late or those who reacted to the first dose of SP.

The pregnant women were given the SP as DOT (Directly Observed Treatment). The women gave written consent after thorough explanation of the study to them. Finger prick blood was collected monthly from the pregnant women and processed as in 3.2.3. Primary outcome measures were anaemia (PCV <33%) assessed at 32 weeks of pregnancy, malaria parasitaemia, low birth weight (<2.5kg) and preterm delivery at <37 weeks (Shulman *et al.*, 1999). Nutritional supplements such as B complex, folic acid multivitamins and iron tablets were given to the pregnant women routinely. These women were followed-up and were asked about malaria symptoms and the side effects of SP, if any. They were advised to report to the health facility in case of any adverse reaction to SP. Information on number of ANC visits with dates, gestational age as assessed by palpation, date of first day of last menstruation (LMP) if known were copied from the ANC card, which were confirmed with the client and including weight measurement (Parise *et al.*, 1998). The date the IPT was administered was also recorded. A nurse weighed the babies within 24 hours of delivery on a digital scale.

3.3.2 Effect of Sulfadoxine-Pyrimethamine (SP) on Prevalence of Malaria, Anaemia and Fever among Pregnant Women

Malaria prevalence was measured monthly among four hundred and eighty three pregnant women comprising four hundred and twenty six (426) pregnant women that took SP and fifty seven (57) that did not. The parasite density was measured by examining Giemsa stained thick blood smears as described in 3.2.3.

Haematocrit was used to measure anaemia in the pregnant women monthly and at 32 weeks of pregnancy in order to determine anaemia as described in 3.2.4.4.

The effect of IPT using SP on fever was determined by taking the temperature of the pregnant women who received either one or two doses of SP during monthly ANC visits. They were also asked the number of times they had malaria attack during the period of pregnancy, following the administration of SP.

3.3.3 Effect of Sulfadoxine-pyrimethamine on Placenta malaria

Blood smears were prepared from Placenta blood as described in 3.2.2. The blood was stained with Giemsa stain and examined under the microscope.

3.3.4 Determination of the Effect of Sulfadoxine-Pyrimethamine (SP) on Low Birth Weight (LBW) and Pre-Term Delivery

The effect of SP on LBW and pre-term delivery was determined by comparing the data obtained from pregnant women who were administered SP with pregnant women who did not receive SP. The data was also compared with previous data obtained from the health facilities prior to the introduction of SP. Data on LBW and pre-term delivery were obtained for one year (pre intervention period) from the medical records from the health facilities in Badagry LGA as a baseline (Appendix 7).

The data obtained from pregnant women who were not administered SP served as control. Comparison was also made between the data on LBW and preterm delivery obtained from the health facilities prior to IPT administration and therefore formed the basis for comparison (Falade *et al*., 2007).

3.4 ENTOMOLOGICAL STUDIES OF THE VECTOR

3.4.1 Pyrethrum Spray Collection (PSC)

Random sampling procedure was used to select houses in the study communities for collection of mosquitoes. Pyrethrum Spray Collection (PSC) method was conducted monthly between 6am and 10.00am in the 15 selected houses in each of the communities, as described by Service, (1993). The floors of the rooms in the selected houses where people slept the previous night were covered with spray sheets and the room sprayed thoroughly with Pyrethrum insecticide (a quick knocked down insecticide). The spray sheets were brought outside after ten minutes and the knocked down mosquitoes picked by the wings or legs using forceps and the mosquitoes identified morphologically (Appendix 8). The average *Anopheles* room density was calculated by dividing the number of mosquitoes collected by the number of rooms in which the collections were made (Appendix 9, 10, 11).

The data on annual rainfall pattern for the study area was obtained from the Nigerian Institute of Metereology, Oshodi for year 2002 (Appendix 12).

$$\text{Average room density} = \frac{\text{Total number of } \textit{Anopheles}}{\text{Number of room sampled}}$$

3.4.2 Morphological Identification of *Anopheles* Mosquitoes

Mosquitoes were identified on the field to species level using morphological criteria according to the identification keys (Gilles and Coetzee, 1987). All mosquitoes belonging to the *An. gambiae* complex were stored in individual tubes with silica gel for preservation. The identification of the adult *Anopheles* was based on the presence of scales in different densities, shapes, sizes and contrasting colours on the

body. The pattern of these scales on palps, the abdomen, the wings and the legs were used in identification of the collected *Anopheles* mosquito species. The identification key is presented in Appendix 8.

3.4.3 Sporozoite Rate Determination

The salivary glands of female *Anopheles* mosquitoes which were collected by Pyrethrum Spray Collection (PSC) were dissected out in physiological saline and examined under the microscope for sporozoites as described by (WHO, 1975; Manyi and Imandeh, 2008). Anopheles mosquitoes totalling 5,840 mosquitoes were collected over a one year period. The sporozoites were identified sporozoite rate calculated as indicated below:

$$\text{Sporozoite rate} = \frac{\text{Number of mosquitoes positive with sporozoite}}{\text{Number of mosquitoes dissected}} \times 100$$

3.4.4 Calculation of Entomological Inoculation Rate (EIR)

The entomological inoculation rate (EIR) is a more direct measure of transmission intensity than incidence, prevalence or other traditional epidemiological estimates (Kelly-Hope and McKenzie, 2009). EIR is a commonly used metric that estimates the number of infectious bites by mosquitoes per person per unit time (Birley and Charlwood, 1987; Smith *et al.*, 2004). It is a product of human biting rate (MBR) (the number of vectors biting an individual over a fixed period usually expressed per year) and the sporozoite rate (SP) (proportion of vectors with sporozoite in their salivary glands) (MacDonald, 1957; Githeko *et al.*, 1996; Beier *et al.*, 1999; The

EIR was calculated by multiplying the man-biting rate with the sporozoite rate (MacDonald, 1957; WHO, 1975), Appendices 9, 10 and 11.

The man-biting rate was calculated from the formula below:

$$MBR = \frac{\text{Number of occupants in the room}}{\text{Number of mosquitoes collected in the room}} \times 100$$

$$EIR = MBR \times \frac{\text{Sporozoite rate}}{100}$$

3.4.5 Molecular Identification of the Malaria Vectors using Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) which is a scientific technique in molecular biology was used in the identification of the *Anopheles gambiae* complex. The PCR amplifies a single or a few copies of a piece of DNA by enzymatic replication across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

3.4.5.1 Extraction of Deoxyribonucleic Acid (DNA) from *Anopheles gambiae sl*

DNA was extracted from the leg of each of the collected specimen as described by Scott *et al.*, (1993). The leg of the collected mosquitoes was placed inside a sterile eppendorf tubes and ground in 100µl extraction buffer, using pestle. These were incubated at 70^0C for 30 minutes in a dry bath (No. DB-006 Gemmy industrial Corp) and centrifuged using a Fresco Biofuge (Heraeus, Kendro Laboratory Products Germany).

3.4.5.2 Polymerase Chain Reaction (PCR) Amplification

One microlitre of the DNA extract from a single mosquito was added to the PCR mixture in a final reaction volume of 12.5µl that contains 10X PCR buffer (Takara Bio Inc. Japan), 2.5mM of each dNTPs, 25nM $MgCl_2$, species specific primers (Primer sequence 5' to 3') namely: 0.52 ng primer melas [TGA CCA ACC CAC TCC CTT GA], 0.53ng primer *gambiae* [CTG GTT TGG TCG GCA CGT TT], 0.57ng primer *arabiensis* [AAG TGT CCT TCT CCA TCC TA], 0.49ng primer *quadriannulatus* [CAG ACC AAG ATG GTT AGT AT], 0.47ng primer universal [GTG TGC CCC TTC CTC GAT GT], deionized water and 0.5µl DNA polymerase TakaRa Taq (enzyme) (Takara Bio Inc. Japan). A control tube was also prepared, but without mosquito. The DNA amplification was carried out in a thermal cycler (Primus 96 PCR-System MWG Genomic Technology), with an initial denaturation at 94 ^{o}C for 30 seconds, 46 ^{o}C for 30 seconds and 72 ^{o}C for 30 seconds (30 cycles). At 94 ^{o}C, there is denaturing of the DNA, followed by annealing at 46 ^{o}C and extension at 72 ^{o}C for 10 minutes. This is one cycle. The cycle was repeated 32 times bringing about amplification of the DNA (Appendix 14).

3.4.5.3 Identification of the *Anopheles gambiae sl*

The amplified PCR product was separated in 2.5% agarose gel which was prepared by adding 7.5 gm agarose to 350ml of TAE buffer. The gel was placed in the micro wave for 5 minutes on medium heat, cooled and ethidium bromide added to the agarose gel prior to its setting. The agarose mixture was poured into the gel tray that has been prepared with masking tape and gel comb. When the gel was set, the masking tape and the comb were removed and the gel placed into the electrophoresis tank with TAE buffer; 12.5 µl of the amplified DNA product was

taken from each reaction tube mixed with 2 µl of bromophenol loading dye. These were transferred into wells, starting with 5 micro liters of IKb Ladder marker. The negative control was also loaded and the tank switched on. Electrophoresis was carried out at 100 volts for 45 minutes, after which the gel was removed and viewed using an ultra violet light and photographed.

3.5 STUDIES ON ANIMALS

3.5.1 Administration of Sulfadoxine-Pyrimethamine (SP)

Three tablets of Malareich Sulfadoxine-pyrimethamine (SP) (1500mg Sulfadoxine and 75mg pyrimethamine) as a recommended adult dose were crushed and 100mg weighed in a weighing balance. This was dissolved in 20mls of water to give a concentration of 5mg/ml. The dose used was selected according to the human therapeutic dose (HTD). This was calculated based on average human weight of 65kg as stipulated in the manufacturer's information manual. The mice were weighed in a weighing scale and have an average weight of 20g. The dose of SP required by the mice was calculated based on the HTD, and was calculated to be 0.5mg/kg weight (Appendix 15).

3.5.2 Histopathological Studies to Determine Mutagenic Effect of Sulfadoxine-Pyrimethamine (SP) on the Foetus of Pregnant Albino Mice

Mutagenic effect of SP was studied, using 12 weeks old virgin Albino mice obtained from the animal house in the College of Medicine, Idi-Araba. The female mice were weighed and divided into four groups of 6 mice. The female mice in each group were put together with two male mice and allowed to mate during

eostrus of the female. Gestation was ascertained by detection of the presence of vaginal plug as well as presence of sperm in the vagina.

The pregnant mice received 0.1ml (0.5mg) of SP (based on their average weight of 20g) which represents the human therapeutic dose (HTD) for SP. Group one received 1X HTD of SP; group two 1½ X HTD, group three received 2X the HTD while the control group received only water. The first dose was given at gestational period of 5 days, while the second and third doses were given on the 10^{th} and 15^{th} day of gestation respectively. The days represented the first, second and third trimester of pregnancy respectively.

The mice were sacrificed on day 18 by cervical dislocation. At autopsy, each female was examined for total implants and any early foetal deaths. Histopathological examinations of the foetuses were undertaken to assess the impact of SP on the foetus.

3.5.3 Assessment of Sperm Head Abnormalities following Sulfadoxine-Pyrimethamine (SP) Administration in Male Albino Mice using Sperm Assay Test

Induction of sperm-head abnmormalities was tested according to Wyrobek *et al*., (1978). This test was conducted because fertilization of the female ova is by the spermatozoa of the male. Any abnormality in the spermatozoa is expected to affect the foetus and ultimately the offspring. The male mice were divided into four groups with five mice in each group. The mice were administered 0.5mg of SP orally representing the HTD. Three different dose level treatments were considered

for the SP corresponding to 1X, 1½X and 2X the HTD and each group was treated for each dose level and for each exposure period. These doses were administered orally to the male albino mice for five consecutive days (Wyrobek *et al*., 1976). Three different exposure periods were considered 5, 7 and 10 weeks from the first administration. One group of mice was treated with water only as a negative control. Spermatozoa were sampled from the cauda epididymes at 5, 7 and 10 weeks from first administration. Spermatogenesis in mice takes about 5 weeks to complete.

The mice were sacrificed by cervical dislocation. The epididymes were excised and minced with fine scissors in physiological saline. Smears were prepared on clean, grease free slides after staining the cells with a mixture of normal saline and 1% eosin-Y (9: 1) for 45 minutes. The slides were air-dried and coded for subsequent examination under oil immersion. Cytological evaluation for sperm-head abnormalities was carried out using a binocular microscope at 100 X magnification. Six separate slides were prepared for each mouse, which are three slides for each epididymes out of which four were randomly selected for scoring. The slides were read blind to treatment.

The sperms were assessed for morphological abnormalities of sperm head shape according to the criteria of Wyrobek and Bruce, (1976). For each animal, 600 sperms were assessed for morphological damage. Differences between the control and experimental groups were analysed by means of the Student's t-test. The test was considered positive when the frequency of abnormal sperm heads was at least double the negative control level, with $P< 0.05$ as the criterion of significance.

3.6 ANALYSIS OF DATA

Data collected were recorded into pre-coded case record forms. Thereafter, the data was entered using EPI-INFO 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and analysed. Descriptive statistics such as means and standard deviations were used to summarize quantitative variables, while categorical variables were summarized with proportions. Frequency tables and graphs were presented for relevant variables. The student t-test was used to compare two mean values, while the one way analysis of variance (ANOVA) was used to compare mean values in more than two groups. The Chi-square test was used to investigate associations between two categorical variables and also to compare proportions. For significant associations, the odds ratio (OR) and 95% confidence intervals (CI) were computed. A probability-value of less than 0.05 was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 KNOWLEDGE, ATTITUDE AND PRACTICES (KAP) OF COMMUNITY MEMBERS ON MALARIA

4.4.1 Background of Respondents

A total of 304 heads of households in Ikoga ward were interviewed comprising 153 (50.3%) males and 151 (49.7%) females. Fifty percent of the ethnic compositions were Egun and 37% Yoruba; Ibos constitute 4%, while Urhobo and Ilaje were 1.4% of the study population. The ages of members of the households ranged between one year and 95 years with a mean age of 33.4 years. Analysis showed that 11% of respondents were less than 19 years, 63% were between 20-39 years, 23.7% between 40-59 years while 3.3% were more than 60 years. Their economic activities included trading (55%), farming (13.4%), artisan (14%) and civil servants, (11%).

Their educational background revealed that 33% of respondent had primary education, 28% secondary, 6% post secondary and 34% had no formal education. Seventy nine percent were income earners while 21% had no income. Their monthly income ranged between ₦800 and ₦32,000.00. Majority of the respondents were Christians, (81%) while others were Moslems (17%) and 2% practice indigenous religion (Table 4.1). The marital status of the respondents showed that 79% were married, 19% were never married while 1% and 0.3% were divorced and separated respectively (Table 4.1). The number of children per family ranged between 1 and 10 with 19% of families having 4 children with a mean of 2 children. While 19% of the children were under 5 years old, 32% were between 5 and 9 years; 18% between 10 and 14 and 12% were between 15 and 19 years.

Table 4.1: Socio-demographic characteristics of respondents in the study communities

Variable	Number	Percentage
Age (range in years):		
<19	33	11
20-39	192	63
40-59	69	22.7
>60	10	3.3
Sex		
Males	153	50.3
Females	151	49.7
Religion:		
Christianity	245	81
Islam	52	17
Indigenous	7	2
Marital status:		
Single	58	19
Married	241	79
Separated	1	0.4
Divorced	3	1.2
Widow	1	0.4
Education:		
None	103	34
Primary	101	33
Secondary	86	28
Post secondary	14	5
Occupation:		
Trader	167	55
Artisan	41	13
Farmr	40	13
Student	30	10
Civil servants	16	5
Housewife	8	3
Chemist/nurse	2	1
Ethnicity:		
Egun	174	57
Yoruba	112	37
Igbo	13	4
Ilaje/Urhobo	5	2.

4.1.2 Perceived Health Problems in the Study Communities

The community members were probed on whether malaria was a perceived health problem, majority (88%) reported in the affirmative as observed elsewhere in malaria endemic areas while 12% said it was not a problem. Eighty seven percent of respondents mentioned fever as a health problem, while 7% mentioned other problems such as cough, headache, typhoid and hypertension. Another 6 % said there was no problem. 97% of respondents have had malaria at one point or the other and 81% associated malaria with mosquito bite, while 9% said it was the sun. Others (10%) mentioned eating of excessive oil, too much work and tiredness (Table 4.2).

Mosquito was perceived to be a problem in the study communities by 75% of respondents, while 25% disagreed. Eighty two percent of respondents associated the period of mosquito abundance with rainy season, while 12% associated it with the dry season. Mosquito abundance was attributed to bushes by 23% of respondents, standing water by 48%, filthy environment by 8% and blocked drains by 0.6% and there was no response in 21% of respondents. Seventy one percent of respondents claimed the mosquitoes to be a nuisance, while 29% had a contrary view (Table 4.2).

Table 4.2: Perceived Health Problems in the Community

Variable	Number	Percentage
Is Malaria a Problem?		
Yes	268	88
No	36	12
Have suffered malaria	295	97
Have not had malaria	9	3
Other Health Problems:		
Fever	264	87
Others	21	7
No problem	19	6
What Causes Malaria?		
Mosquito	246	81
Sun	27	9
Others	31	10
Is Mosquito a Problem?		
Yes	228	75
No	76	25
Period of mosquito abundance?		
Rainy Season	250	82
Dry Season	36	12
No Particular Time	9	3
Do Not Know	9	3
Why Abundance of Mosquitos?	280	48
Stagnant water	70	23
Bushes	64	8
Filthy environment	24	21
Do not know		

4.1.3 Signs and Symptoms of Malaria in Adults and Children in the Community

Thirty six percent of adult respondents mentioned bitter tongue as a sign of malaria; while 15% of respondents mentioned headache and another 15%, body pains, while 10% mentioned cold. Other signs stated were dizziness, weakness and sweating by 5% of respondents respectively, while 4% said it was yellow eyes.

Fever as a sign of malaria in children was mentioned by 56% of respondents, lack of apetite, (17%) and vomiting (9%). Other responses were yellow eyes (4%), persistent crying of the baby (3%) and sweating (2%), Table 4.3.

Table 4.3: Signs and symptoms of malaria in adults and children in the community

Variable	Number	Percentage
Signs of Malaria in Adult:		
Bitter tongue	109	36
Headache	46	15
Body pains	46	15
Cold	30	10
fever	15	5
Weakness	15	5
Dizziness	15	5
Sweating	15	5
Yellow eyes	12	4
Signs of Malaria in Children:		
Fever	170	56
Lack of appetite	52	17
Weakness	30	10
vomiting	27	9
yellow eyes	12	4
crying	9	3
sweating	4	1

4.1.4 Health Seeking Behaviour of Community Members

Table 4.4 presents the actions taken by community members when they perceive that they have malaria. Thirty two percent of respondents will go to the health facility, 24% visit the chemist and 27% will take herbs, 13% will use paracetamol and chloroquine without doctor's prescription. However, 4% of respondents do not know what to do. The action was taken by 76% of respondents in order to get cured of the malaria while 10% felt it was cheaper to take such action. Another 6% took the action based on traditional practices, 5% as a first aid, and 3% based on the advice of health workers. These actions were taken between 1 and 20 days. While 23% took the action after one day, 41% took action after the second day and 22% acted after three days. Only 7% of respondents took immediate action. Further actions taken if they did not recover included going to the health facility by 68% of respondents, 11% will visit the patent medicine vendor, while 19% will take herbs and 3.0% said they will continue to self-medicate.

The majority of the respondents, (49%) had malaria between one and six months ago, 22% said they had it recently (less than on month); while 27% of respondents had it between 6 months and one year. 1% of respondents claimed not to have had malaria (Table 4.4). The frequency of malaria attack was reported to be once a year by 26% of respondents, 29% have it twice a year, and 23% have it 3 times a year while 14% have it 4 times a year. Another 7% claimed to have it more than 5 times while 2% of respondents seldom have it.

Respondents were asked on what type of treatment they received at the health facility and whether their blood was tested. 21% of respondents had their blood tested while 73% did not, 1% of respondents could not remember.

Fansidar tablets was given to 31% of respondents for the treatment of malaria, 32%, received chloroquine tablets, 35% chloroquine injection, while 3% could not remember what they received. The medicines were obtained from the health facility by 77% of respondents while 16% obtained the medicines from the patent medicine vendors. The cost of treatment ranged between N20 and N5,000, with an average cost of N107 for malaria treatment. The distance to the nearest health facility was on average of 3 kilometres.

4.1.5 Utilisation of Health Facility

Health facility utilization among respondents varied from once a year to nine times a year. A larger number, 65% of respondents reported they use the health facility on average of two times a year (1-3 times), 24% visit on average of five times a year (4-6 times), while 2% visit on average nine times a year. 9% of respondents had not visited the health facility in the last one year. The reasons for not visiting the facility was attributed to the poor attitude of health workers; lack of money and preference for the patent medicine vendors (Table 4.5).

Table 4.4: Health seeking behaviour by members of the study communities

Variable	Number	Percentage
Actions taken when they have malaria:		
HF	96	36
chemist	72	24
Herbs	82	27
Self medication	41	13
Do not know	13	4
Why action was taken:	232	76
To get well (cured)	18	6
Traditional practices	30	10
Cheaper to take action	15	5
First aid	9	3
Advice by Health Worker		
How soon was the action taken:	21	7
Immediate	69	23
Within 1 day	124	41
Within 2 days	69	22
Within 3 days	21	7
>3 days		
Whether they do get well after taking the actions:	292	96
Yes	12	4
No		
Last time they had malaria:	67	22
Recently	150	49
Between 1 and 6 months	82	27
6 months to 1 year	3	1.3
Not had malaria	2	0.7
Did not remember		
Frequency of malaria attack per year:	5	2
Seldom	78	26
Once	88	29
Twice	69	23
Thrice	44	15
Four times	20	7
> 5 times		
Actions taken if not recovered:	205	68
Go to Health Facility	32	11
Go to chemist	58	19
Take herbs	9	3
Continue to self medicate		
Antimalarial given:	93	31
Fansidar	97	32
Chloroquine tablet	107	35
Chloroquine injection	7	5
Do not remember		

Table 4.5: Utilization of health facility by community members

Number of Times Health facility was utilized	Number	Percentage
2 times a year	198	65
5 times a year	72	24
>7 times a year	5	2
Did not visit in the last one year	29	9

4.1.6 Use of Herbs by Community Members

Herbs were used in the treatment of malaria by 66% of respondents, while 35% did not use it. Analysis of those who use herbs showed that 69% claimed it was effective in curing malaria, 13% said it was used for prevention, while 8% said it was affordable to use herbs. The use of herbs by 8% of respondents was to compliment other antimalarials given to them, while 3% of respondents said it was traditional to use herbs. Seventy five percent of respondents got the herbs from the bush; 17% from market; 4% from the herbalist and relatives accounted for 4%, (Table 4.6). These herbs consisted of orange leaves, mango leaves, lemon grass and guava leaves.

Table 4.6: Use of herbs by community members

Variable	Number	Percentage
Why use herbs?		
Effective	208	69
Prevention	39	13
Affordable	22	8
Traditional	9	3
Compliments treatment	24	8
What are the Source of Herbs?		
Bush	171	75
Market	38	17
Herbalist	9	4
Relatives	10	4

4.1.7 Preventive Measures against Malaria among Respondents in the Community

Actions taken to prevent malaria included taking of herbs by 30% of respondents, use of anti-malarial medicines by 27%, good water and eating good food (21%). Others (11%) use blood tonic, 0.3% take intra-muscular chloroquine injection while 9.7% of respondents did not take any preventive measures against malaria. Mosquito control by insecticide spray was practiced by 48% of respondents, 21% use mosquito coils, and 7.2% adopt bush clearing. Another 6.1% said they will clean the environment while 0.3% uses insect repellent. However, 4% had mosquito wire net on their doors and windows and 13.4% did nothing about the mosquitoes. 86% of respondents did not own mosquito net while 14% claim they have (Table 4.7).

Table 4.7: Measures taken to prevent malaria and mosquito bite in the communities

Variable	Frequency	Percentage
Actions taken to prevent malaria:		
Take herbs	91	30
Take antimalarials	83	28
Good water/food	63	21
Blood tonic	34	11
Intra muscular chloroquine injection	1	0.3
Did nothing	32	9.7
Actions to control mosquitoes		
Raid insecticide spray	145	48
Mosquito coil	64	21
Bush clearing	22	7.2
Clean environment	19	6.1
Repellants	1	0.3
Mosquito wire net	12	4
Did nothing	41	13.4

4.2 KNOWLEDGE, ATTITUDE AND PRACTICES (KAP) OF PREGNANT WOMEN ON MALARIA AND INTERMITTENT PREVENTIVE TREATMENT USING SULFADOXINE-PYRIMETHAMINE (SP)

4.2.1 Background of Pregnant Women

The ages of the pregnant women ranged between 16 years and 45 years with mean age of 29±4.84 years. Analysis of the KAP questionnaire of the 450 pregnant women attending Antenatal Clinics (ANC) showed that 62% were Yoruba, 22% Egun, 14% Igbo and 2% Hausa. Thirty six percent of the pregnant women were traders, 22% students, 12% fisher women and housewives respectively while 9% were civil servants. The others, 8% are unemployed while 0.7% are engaged in farming. Educationally, 15% of respondents had primary education, 41% had secondary education; 36% had post secondary education while 8.3% had no education. Seventy six percent of respondents are Christians while 24% are Moslems. Analysis showed that 96% of respondents are married, while 4% are single and 0.2% was separated (Table 4.8).

4.2.2 Gravida and number of pregnancies

The study revealed that 36% of pregnant women were primigravidae; 22.9% secondigravidae, while 41.1% were multigravida (had more than 3 pregnancies), Figure 4.8. The number of pregnancies they had ranged from 1 to 5, while the number of children they have was between 1 and 7, with a mean of 2 children. Analysis showed that 36% and 27% of pregnant women have one and 2 children respectively. Another 19% and 9% of pregnant women have three and four children, while 2% have five children. At the time of the interview, the ages of their pregnancies ranged between 8 weeks and 38 weeks with a mean of 28 weeks.

Table 4.8: Socio-demographic characteristics of respondents (pregnant women)

Variable	Frequency	Percentage
Age group in range:		
16-20	28	6
21-25	90	20
26-30	184	41
31-35	108	24
36-40	36	8
41-45	4	1
Marital status:		
Married	431	96
Single	18	3.8
Separated	1	0.2
Religion:		
Christian	345	77
Islam	105	23
Ethnicity:		
Yoruba	279	62
Egun	99	22
Igbo	63	14
Hausa	9	2
Education:		
Primary	68	15.1
Secondary	185	41.1
Tertiary	158	35.1
others	2	0.5
none	37	8.2
Occupation:		
Unemployed	42	8
Housewife	53	12
Farmer	3	0.6
Trader	160	35.4
Fishing	56	13
Civil servant	38	9
Student	98	22

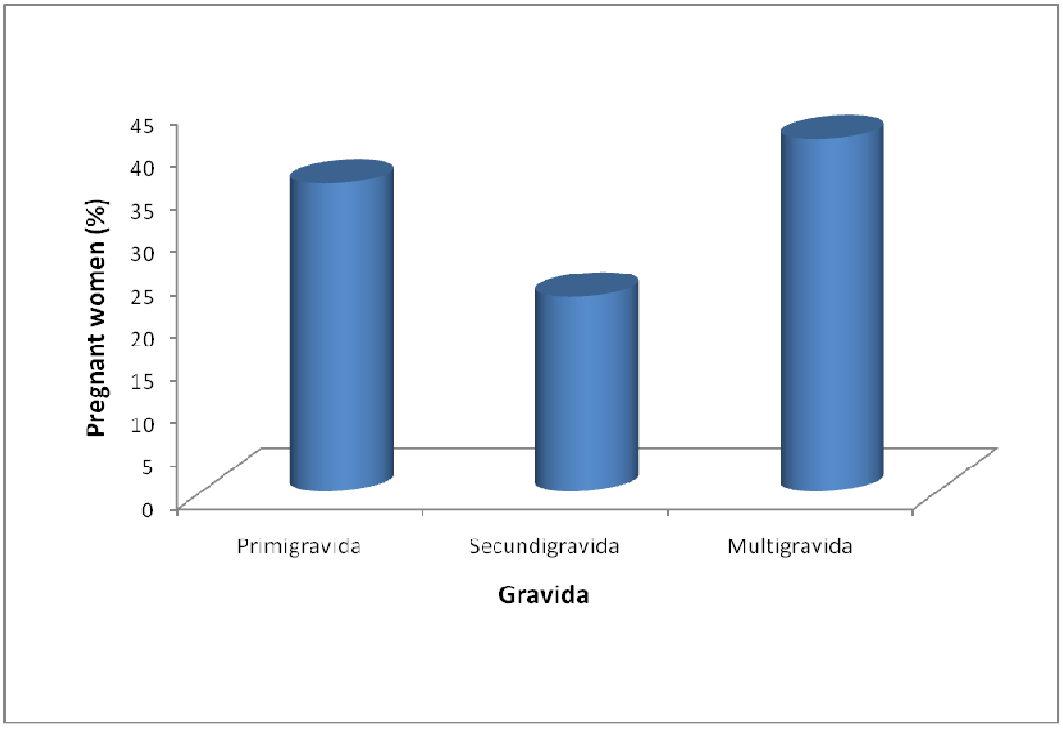

Figure 3: Frequency distribution of pregnant women in the study by gravida

4.2.3 Knowledge on Signs and Treatment of Malaria in Pregnant Woman

The pregnant women's understanding of malaria revealed 79% perceiving it as a serious illness; 3% of pregnant women believed that it was a parasitic infection but 1% associated malaria with mosquito bite, while 15% had no knowledge of malaria.

The signs of malaria listed by the pregnant women were headache (24.2%), weakness (17.1%), fever (17.1%), body pains and chills (10%) respectively. Other signs mentioned were lack of appetite and bitter tongue (4%) and (3.3%) respectively and yellow eyes by 3.1% of pregnant women (Table 4.9).

Majority of the pregnant women (58%) will seek treatment for malaria from a Health Facility. Home treatment of malaria using herbs was practiced by 11% of pregnant women, 17% will self-medicate; while 0.2% will visit the TBA or a nurse for treatment, 14% do nothing. The frequency of malaria attacks revealed that 36% of the pregnant women had malaria once during pregnancy while 18% had it twice, others, 6% had it three times a year while 5% had it four times and 35% claim not to suffer malaria attack (Table 4.9).

Table 4.9: Knowledge of malaria and health seeking behaviour of pregnant women attending antenatal Clinic.

Variable	Frequency	Percent
What they knew about malaria:		
Serious illness	355	79
Parasitic disease	13	3
Caused by mosquito bite	5	1
Did not know	68	15
Others	9	2
Signs of malaria:		
Headache	109	24.2
Fever	77	17.1
Weakness	77	17.1
Body pains	44	10
Chills	42	9.3
Lack of apetite	18	4
Bitter tongue	16	3.3
Vomiting	14	3.1
Yellow eyes	14	3.1
Dizziness	6	1.3
Others	27	6
Do not know	6	1.3
Frequency of malaria attack:		
Once	162	36
Twice	81	18
Thrice	27	6
Four times	22	5
Do not suffer from malaria	158	35
Treatment seeking for malaria:		
Go to hospital	174	58
Take herbs	32	11
Self medication	54	17
Did nothing	43	14

4.2.4 Utilization of Health Facilities by Pregnant Women Attending Health Facility

Analysis of result showed that 95% of pregnant women registered in the hospital when they are pregnant, while 5% said they did not. Fifty percent of those who did not register at the health facility said they prefer going to the TBAs, while the other 50% had no reason for not registering at the health facility. Registeration took place between 4 weeks and 36 weeks of pregnancy with a mean of 19 weeks.

Eighty six percent of pregnant women deliver in a government hospital, 12% deliver in a private hospital while 0.2% delivers at home. Those that deliver in the TBAs were 6% while less than 1% delivers in a religious house. The reasons given for delivering in government hospital included safety (78%), adequate care and attention (11%), husband's advice (5%) and 6% said it was due to proximity (Table 4.10).

Table 4.10: Utilization of Health Facility and health seeking behaviour of pregnant women attending health facilities

Variable	Frequency	Percentage
Use of health facilities:		
Registered in the hospital	426	95
Did not registered	24	5
Where do they deliver:		
Government hospital	367	81.5
Private hospital	53	11.5
Home	1	0.2
TBA	27	6.3
Religious house	2	0.5
Reason for delivering in Government hospital:		
Safety	352	78
Adequate care	48	11
Husband's advice	20	5
Proximity	28	6

4.2.5 Preventive Measures against Malaria by Pregnant Women

The preventive measures taken by pregnant women against malaria included the use of herbs (30%); chloroquine (27%) and daraprim (23%). Other measures taken were the use of blood tonic (12%), paracetamol (5%) and SP (4%). Measures taken to control mosquito included the use of insecticide spray (47.7%); mosquito coils (21%), bush clearing 6% and 4% put gauze on doors and windows. Environmental cleanliness was mentioned by 6% of pregnant women while use of repellants was by 0.3% and 13% did nothing. However 2% said they do not know. The respondents were further probed whether they have heard of ITNs, 79% claimed they had heard of ITN, while 21% had not and out of those who have heard, only 13% use ITNs; 87% do not (Table 4.11).

Table 4.11: Measures taken by pregnant women to prevent malaria

Variable	Frequency	Percentage
Actions taken to prevent malaria:		
Use herbs	134	30
Chloroquine	123	27
Daraprim	104	23
Blood clinic	51	11
Paracetamol	21	5
Sulfadoxine-pyrimethamine	17	4
Actions taken to control mosquitoes:		
Aerosol sprays	215	47.7
Mosquito coil	95	21
Bush clearing	27	6.0
Clean environment	27	6.0
Repellants	1	0.3
Gauze on doors/windows	18	4
Did nothing	58	13
Do not know	9	2
Heard of ITN?		
Yes	356	79
No	94	21
Use of ITN?		
Yes	59	13
No	391	87

4.2.6 Knowledge of Sulfadoxine-Pyrimethamine (SP) by Pregnant Women

The knowledge of SP was 77% among pregnant women, while 23% had no knowledge. Out of those that claimed to have knowledge, 84.4% claimed it was for treatment of malaria; 15.3% use it for prevention of malaria while 0.3% was of the opinion it is used for the treatment of typhoid.

The investigation revealed that 6% of pregnant women used SP monthly, while another 6% used it twice during pregnancy for the purpose of malaria prevention, 5% used it three times during pregnancy while 2% used it more than three times during pregnancy (Table 4.12).

Table 4.12: Knowledge of Sulfadoxine-pyrimethamine (SP) by pregnant women

Variable	Frequency	Percentage
Knowledge on SP:		
Had knowledge	345	77
Had no knowledge	105	23
Asked what it is used for:		
Treatment of malaria	380	84.4
Prevention	69	15.3
Typhoid treatment	1	0.3
Usage of SP during pregnancy:		
Once during pregnancy	337	75
Use whenever ill	29	6
Monthly	28	6
Twice during pregnancy	28	5
Thrice during pregnancy	20	6
>thrice during pregnancy	8	2
Trimester when SP was used:		
First trimester (up to 15 weeks)	65	15
Second trimester (up to 27 weeks	149	33
Third trimester (28 to 36 weeks)	61	14
Cannot remember	9	2
Did not use SP during pregnancy	166	37
Number of tablets of SP taken:		
Two tablets	37	8
Three tablets	413	92
Asked whether SP prevented malaria:		
Prevented malaria	435	97
Did not prevent malaria	15	3

4.2.7 Knowledge of Sulfadoxine-Pyrimethamine versus Parity

Analysis of the relationship between parity and knowledge of SP showed that knowledge of SP was highest among pregnant women (38%) that had one pregnancy compared to 24% of women that had two pregnancies. Knowledge among those that had 3 and 4 pregnancies was 16% and 13% respectively, while 9% of those that had five pregnancies had knowledge of SP. The result showed statistical significance between parity and knowledge of SP, X^2=14.0642, df=4, P=0.0075, (P<0.05) (Figure 4).

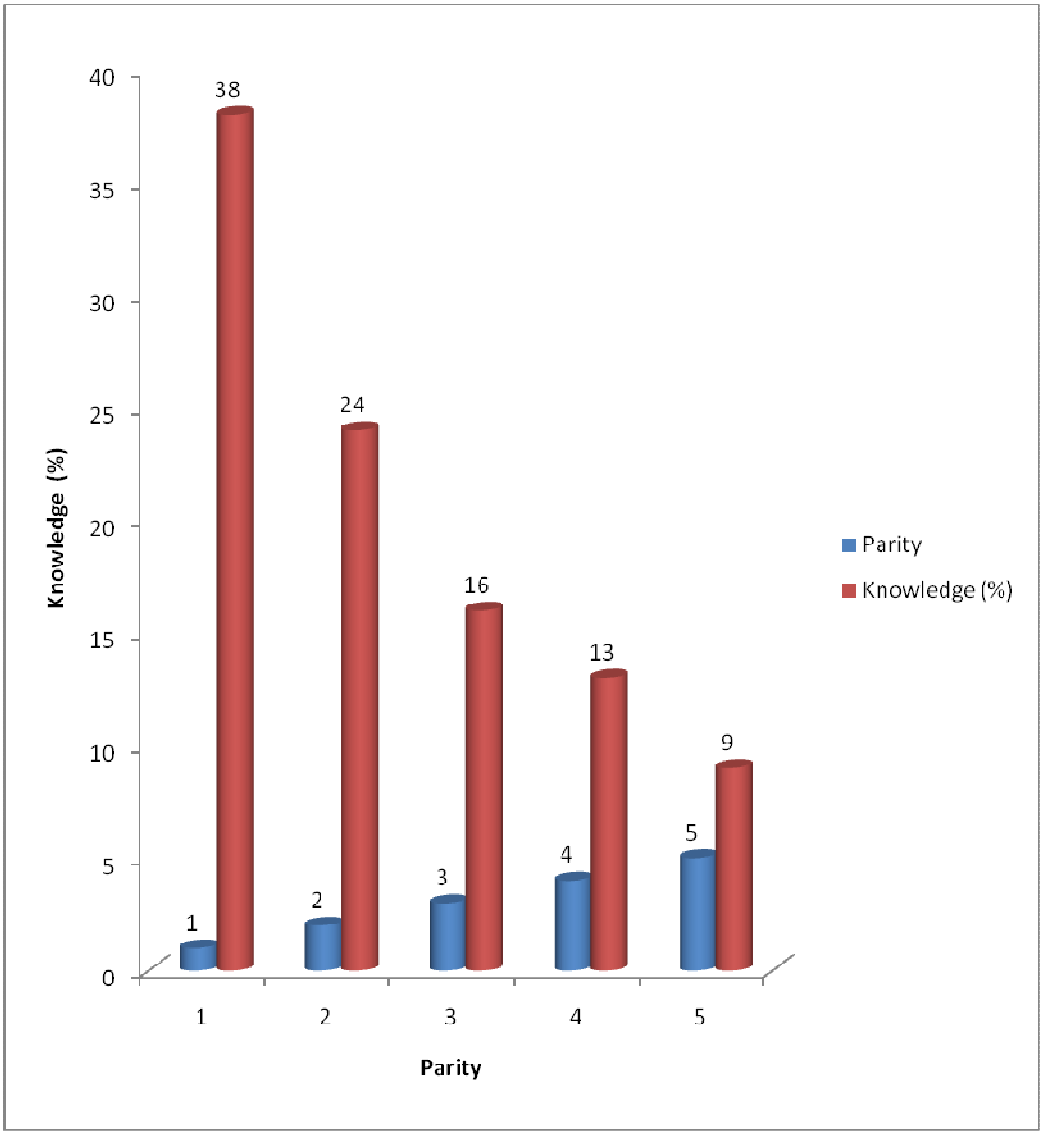

Figure 4: **Knowledge of Sulfadoxine-pyrimethamine by pregnant women of versus parity**

4.2.8 Knowledge of Sulfadoxine-Pyrimethamine (SP) among pregnant women with different educational backgrounds

A breakdown of the result showed that 43% of those with secondary education and 42% of those with post secondary education displayed good knowledge of Sulfadoxine-pyrimethamine. Further analysis revealed that while 10% of those with primary education were knowledgeable about SP, only 4% of illiterate women had knowledge of Sulfadoxine-pyrimethamine. The result showed a statistical significance between educational status and knowledge of SP, X^2= 69.2191, df=4, P=0.0001, (P<0.05) (Figure 5).

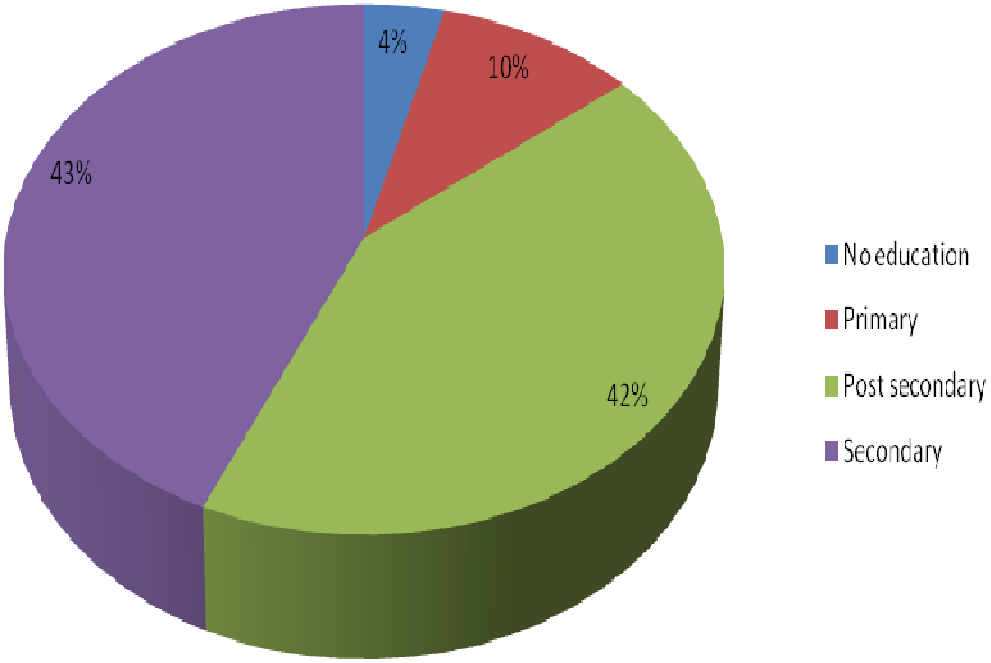

Figure 5: Knowledge of Sulfadoxine-pyrimethamine among women with various educational background

4.2.9 Knowledge of Sulfadoxine-Pyrimethamine (SP) among different age groups of pregnant women attending Antenatal Clinics

Figure 6 indicates the knowledge of SP among the pregnant women of different age groups. 43% of pregnant women in the age bracket of 16-20 years and 65% of age bracket 21-25 years have knowledge of SP. Eighty six percent and 75% from age groups 26-30 and 31-35 years respectively exhibited the highest knowledge of SP, (Figure 6). The data also showed that 71% and 25% in the age bracket of 36-40 years and 41-45 years respectively had knowledge of SP. The knowledge of SP was highest among the 26-30 years and shows statistical significance, $X^2=28.74$, df=5, $P<0.05$.

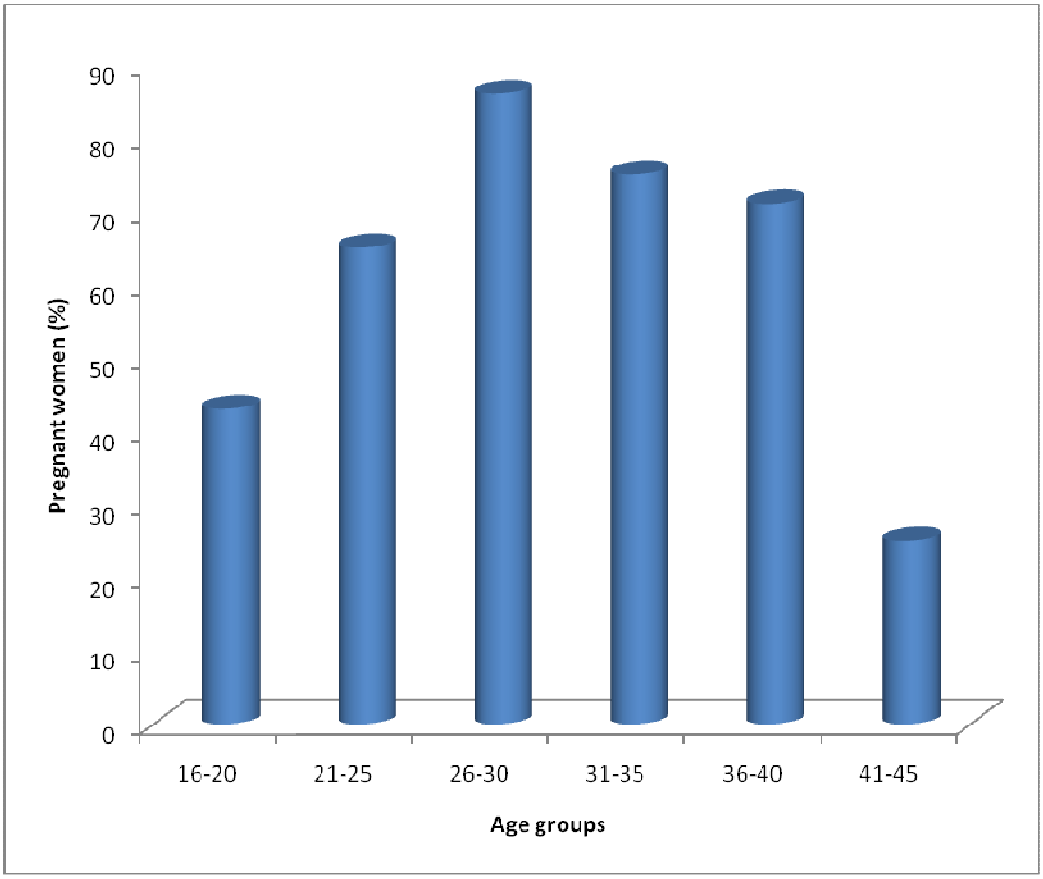

Figure 6: Knowledge of Sulfadoxine-pyrimethamine (SP) among pregnant women of different age groups attending antenatal Clinics

4.3 ASSESSMENT OF MALARIA ENDEMICITY

4.3.1 Characteristics of community members

The study site comprises three communities namely Igborosun, Ikoga Zebbe and Pota, all in Ikoga ward. The number sampled from Igborosun was 596 (55.1%) females and 486 (44.9%) males; for Ikoga Zebbe it was 982 (57.2%) females and 735 (42.8%) males and 611 (51.8%) females and 568 (48.2%) males in Pota community respectively (Table 4.13).

Table 4.13: Frequency distribution of community members by gender

COMMUNITY	SEX		Over all (%)
	Female (%)	Male (%)	
Igborosun	596 (55.1)	486 (44.9)	1082 (100)
Ikoga-Zebbe	982 (57.2)	735 (42.8)	1717 (100)
Pota	611(51.8)	568 (48.2)	1179 (100)

4.3.2 Characteristics of pregnant women

The ages of the pregnant women enrolled in the study ranged between 16- 45 years, with a mean of 29±4.48 years, out of which 6% were within the age group 16-20 years; 21% within 21-25 and 43% within 26-30 years age. The analysis further revealed that 24% of pregnant women were within 31-35 years, while 8% were between 36-40 years and 1% was between 41-45 years (Table 4.14). The percentage distribution of pregnant women was as follows: 2.5% were from Ayo-olu; 79% from general hospital; 9.8% from Pota; 5.2% from traditional birth attendant and 3.5% from Unique private hospital.

Table 4.14: Age group distribution of pregnant women enrolled in the study

Age group(years)	Frequency	Percentage
16-20	26	5
21-25	91	19
26-30	207	43
31-35	115	24
36-40	40	9
41-45	4	1

4.3.3 Age group distribution of pregnant women according to Health Facility

The result of the study showed the age group distribution of pregnant women according to health facility; women of age groups 26-30 and 31-35 attended health facilities more than other age groups. The result also showed that majority of pregnant women attended the Government Health Facilities (Table 4.15). There is significant association between the ages of the pregnant women and the health facility they attend, Chi-square=28.6467, df=16, P=0.0264, (P<0.05).

Table 4.15: Age group distribution of pregnant women according to Health Facility

Age group	Health Facility				
	Ayo Olu %	GH %	Pota %	TBA %	Unique %
16-20	4 (17)	4 (17)	9 (33)	7 (25)	2 (8)
21-25	4 (5)	15 (16)	39 (43)	24 (26)	9 (10)
26-30	19 (9)	48 (23)	79 (38)	44 (21)	19 (9)
31-35	14 (12)	46 (40)	51 (44)	5 (4)	0
36-40	0	10 (4)	2 (2)	1 (2)	0
41-45	0	8 (3)	1 (2)	0	0

Chi-square=28.6467, df=16, P=0.0264. There is a significant association between the ages of the pregnant women and the health facility they attend, $P<0.005$.

4.3.4: Monthly malaria prevalence in the Ikoga ward

Parasitological investigation showed malaria to be endemic in the three communities in Ikoga ward (Igborosun, Ikoga Zebbe and Pota). P. falciparum was the predominant species. The over all monthly malaria prevalence ranged between 12.3% and 38.5% (Table 4.16). There was a steady increase in malaria prevalence from the month of April to October coinciding with the rainy season and period of mosquito abundance (Figure 7). The malaria prevalence in these three communities that make up the study site shows statistical significance, $P<0.01$. Malaria prevalence among females and males in the three communities were 55.1% and 44.9% for Igborosun; 57.2% and 42.8% for Ikoga and 51.8% and 48.2% for Pota respectively. There is no statistical significance between malaria prevalence and gender in Ikoga ward (Table 4.17).

The presence of gametocytes was demonstrated during parasitological investigation in the majority of the examined stained blood smears. Gametocytes of *Plasmodium falciparum* were found present in the blood smears throughout the year. Figure 7 shows peaks for gametocytes in the months of March, June and October and also the monthly rainfall pattern including parasite rates in Ikoga ward (Figure 7).

Table 4.16: Monthly parasite rate in the three communities in Ikoga ward

Month	Parasite rate in the three Communities			Total	P-value
	Igborosun	Ikoga	Pota		
January	23 (13.5%)	33 (16.5%)	17 (11.3%)	73 (14%)	0.377
February	19 (11.2%)	36 (19.4%)	29 (19.3%)	84 (16.6%)	0.066
March	23 (20.9%)	52 (21.9%)	24 (21.6%)	99 (21.6%)	0.977
April	26 (26.0%)	39 (21.1%)	31 (20.7%)	96 (22.1%)	0.556
May	22 (22.0%)	23 (23.0%)	27 (27%)	72 (24.0%)	0.681
June	22 (28.6%)	54 (28.1%)	22 (23.7%)	98 (27.1%)	0.689
July	23 (28.8%)	24 (30.0%)	22 (31.0%)	69 (29.9%)	0.956
August	22 (36.7%)	29 (27.9%)	18 (31.2%)	69 (31.2%)	0.504
September	17 (42.5%)	32 (29.6%)	25 (33.3%)	74 (33.2%)	0.336
October	10 (25.0%)	52 (44.1)%	22 (36.7%)	84 (38.5%)	0.095
November	12 (21.8%)	41 (35.3%)	25 (25.8%)	78 (29.1%)	0.127
December	12 (15.0%)	10 (11.0%)	7 (10.8%)	29 (12.3%)	0.661
Total	231 (21.3%)	425 (24.8%)	269 (22.8%)	925 (23.3%)	0.106 (100%)

Table 4.17: Malaria prevalence in the three communities by gender in Ikoga ward

Malaria Prevalence	Females	Males	Total	Statistical analysis
IGBOROSUN				
Negative	482(56.6%)	370(43.46%)	852(78.7%)	X^2=3.59,
Positive	114(49.6%)	116(50.4%)	230(21.3%)	P=0.0579
				P>0.05
Total	596(55.1%)	486(44.9%)	1082	
IKOGA				
Negative	743(57.5%)	550(42.5%)	1293(75.3%)	X^2=0.16,
Positive	239(56.4)	185(43.6%)	424(24.7%)	P=0.6924
				P>0.05
Total	982(57.2%)	735(42.8%)	1717(100%)	
POTA				
Negative	481(52.9%)	429(47.1%)	910(77.2%)	X^2=1.71,
				P=0.1914
Positive	130(48.3%)	139(51.7%)	269(22.8%)	P>0.05
Total	611(51.8%)	568(48.2%)	1179(100%)	

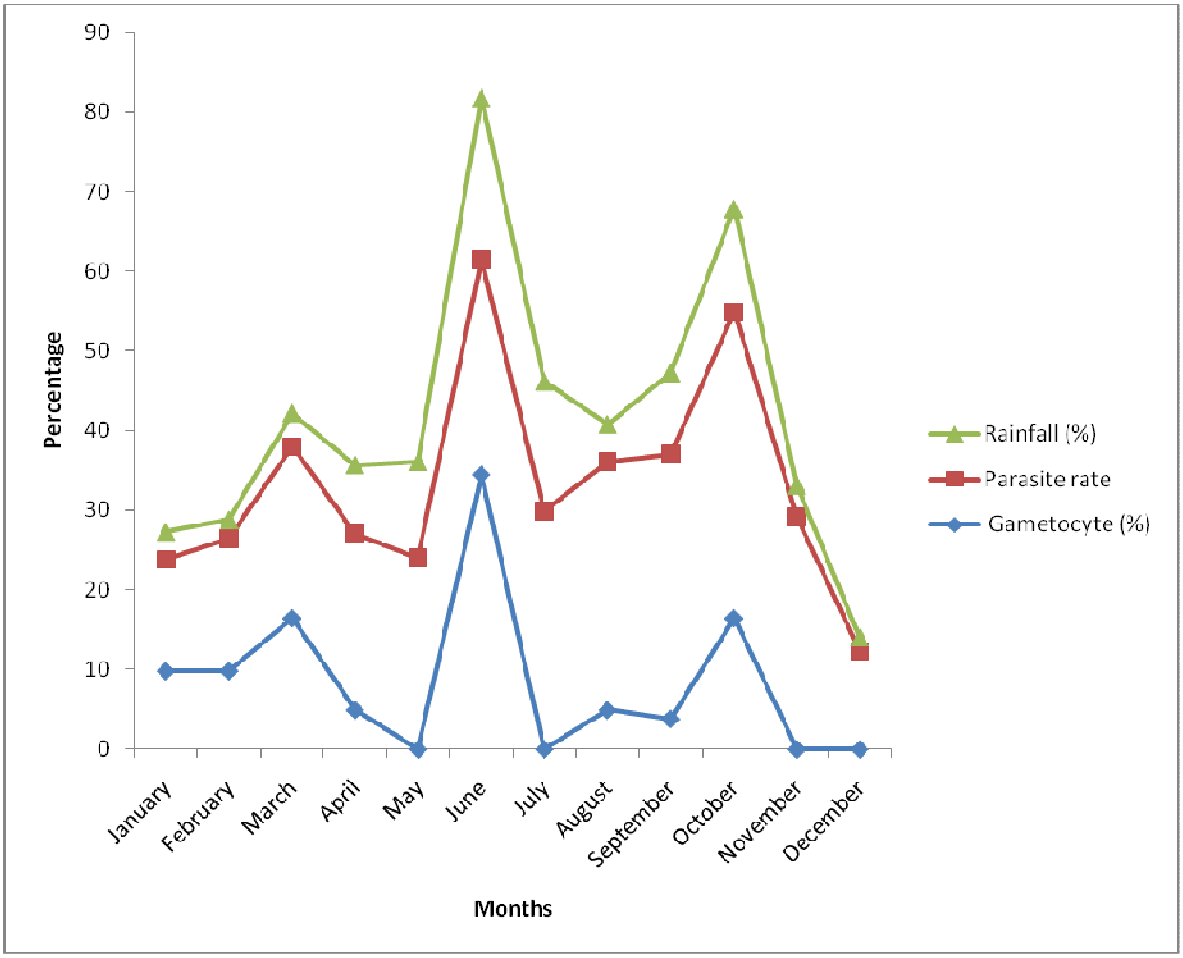

Figure 7: Comparison between parasite rate, gametocytes and rainfall

4.3.5 Prevalence of malaria in the different age groups in Ikoga ward

Malaria prevalence in the different age groups showed that malaria prevalence rate for children less than five years of age was 33.8%; 28.1% in 5-9 years, while in 10-14 years and 15 to 19 years it was 17.4% and 20.4% respectively. Malaria prevalence rate is higher in the lower age groups than the older age groups. There was a significant association between malaria prevalence and the different age groups, $P<0.001$ (Table 4.18).

Table 4.18: Malaria prevalence by age group in Ikoga ward

Age group (years)	Malaria Parasite		Total	Parasite rate
	Positive	Negative		
<5	298	583	881	33.8
5-9	317	812	1129	28.1
10-14	97	460	557	17.4
15-19	26	127	153	20.4
20-24	35	183	218	16.1
25-29	40	198	238	16.8
30-34	29	163	192	15.1
>35	81	529	610	13.3
Total	923	3055	3978	23.2

X^2 =135.20, df= 7, P=0.000000. There is statistical significance between age group and prevalence of malaria. P<0.001

4.3.6 Prevalence of malaria in different age groups among pregnant women

Figure 8 shows the monthly parasite rate among pregnant women attending antenatal Clinics.

Malaria prevalence rate among pregnant women of different age groups is shown in Table 4.19. Malaria prevalence in the 16-20 age group was 23.1% and 19.8% in the 21-25 age group. The rate amongst pregnant women between 26 and 30 years was 28.6% and 19.1% for 31-35 years old. Those who were 35 years and above had parasite rate of 10%. The over all malaria prevalence rate among pregnant women was 15.7% and showed no statistical significance between age group and parasite rate, $P<0.05$.

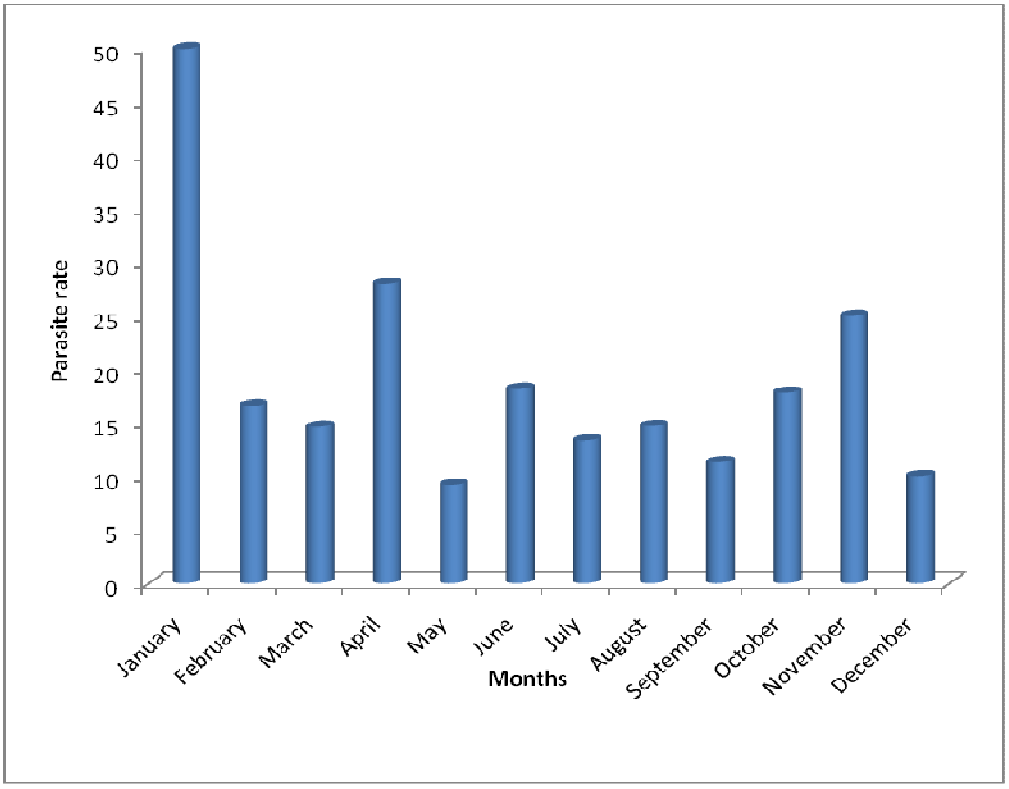

Figure 8: Monthly malaria prevalence rate among pregnant women

Table 4.19: Malaria prevalence in different age groups of pregnant women attending antenatal clinics

Age group	Frequency	No positive	Parasite rate
16-20	26	6	23.1
21-25	91	18	19.8
26-30	207	26	28.6
31-35	115	22	19.1
36-40	40	3	9
41-45	4	1	1
Total	483	76	15.7

X^2=8.6252, DF=4, P=0.0712. There is no association between prevalence of malaria and the different age groups of pregnant women P>0.05.

4.3.7 Measurement of Mean Parasite Densities in the Study Community

Table 4.20 shows the mean parasite densities for the three communities that make up the study sites and shows no statistical significance, $P<0.05$.

Table 4.20: Mean parasite density in the study community

Site	Mean Parasite density
Igborosun	**1782.991±2438.378**
Ikoga	**1999.396±6037.659**
Pota	**2345.398±3659.021**

X^2=3.205, df=2, P=0.201. There is no statistical association between the parasite density and the different communities, P>0.05.

4.3.8 Prevalence of Malaria and Mean Parasite Density among Different Gravida of pregnant women

The malaria prevalence among the different gravida ranged between 14.2% and 17.8%. The data revealed that 14.2% of primigravida and 17.7% of secondigravida as well as 15.7% of multigravida were positive for malaria parasite. There is no statistical significance between malaria prevalence rate and gravida. $X^2=0.9627$, df=2, P=0.6179, (P>0.05). The primigravida had the highest mean parasite density of 2236.2 parasite/µl of blood, followed by secundi, 2064.6 and 548.7 for multigravida (Figure 9).

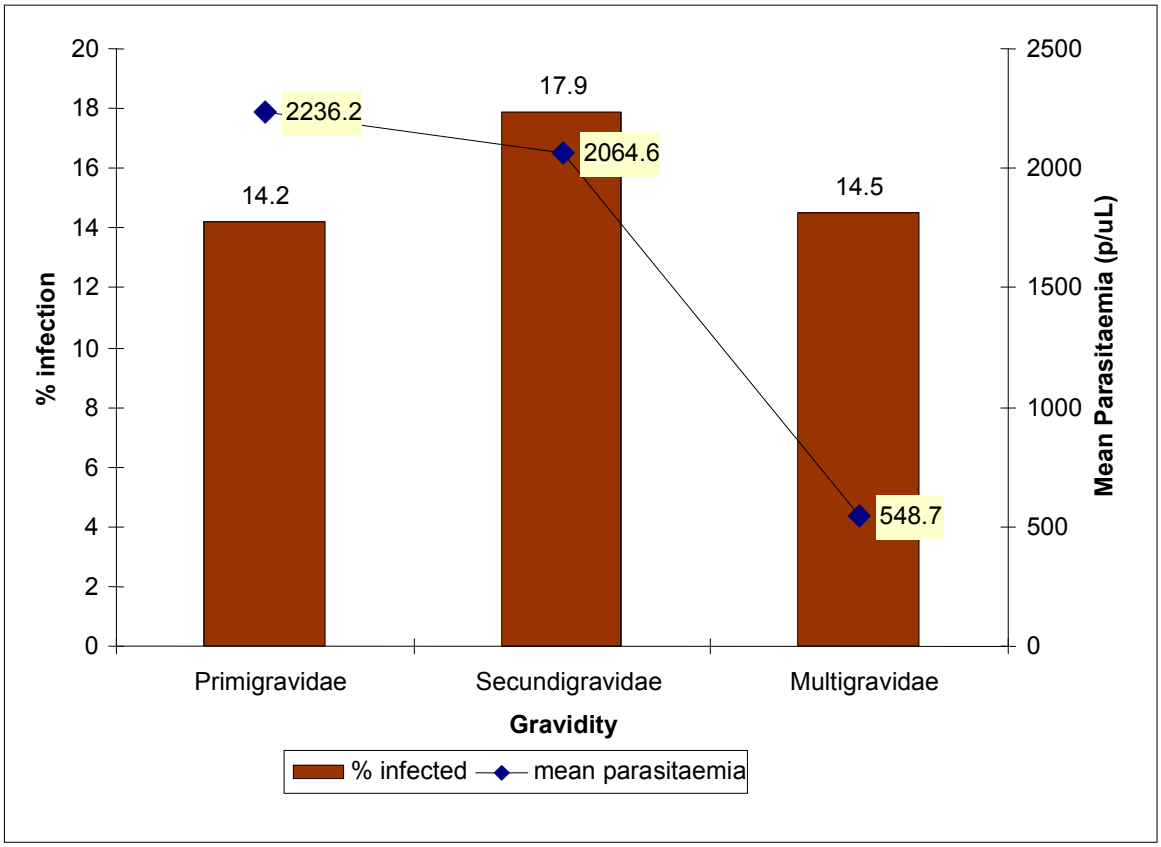

Figure 9: **Comparison of mean parasitaemia and proportion of infected pregnant women by gravidity**

4.3.9 Determination of Anaemia by Haematocrit (Packed Cell Volume) in the Community

Table 4.21 shows monthly measurement of anaemia in the community according to age group in Ikoga ward. Table 4.20 shows that 7.2% of the total persons sampled (285 out of 3978) were anaemic. The anaemia is moderate by classification and it was seen among the younger age group. There was no case of severe anaemia. There is a statistical significance between anaemia and age group, X^2=130.97 df=6, P<0.001, Table 4.22.

Table 4.21: Monthly distribution of anaemia among different age groups in Ikoga ward

Month	<5 years	5-9 years	10-14 years	>15 years	Total	P- value
January	14 (17.5%)	11 (7.4%)	3 (3%)	3 (1.6%)	31 (6%)	0.0000043
February	10 (8.7%)	14 (8%)	0	1 (0.6%)	25 (4.9%)	0.000968
March	10 (9.2%)	5 (3.9%)	2 (2.2%)	3 (2.3%)	20 (4.4%)	0.0376
April	2 (2.2%)	3 (3.0%)	1 (1.7%)	4 (2.2%)	10 (2.3%)	0.951
May	5 (7.2%)	4 (5.5%)	1 (2.0%)	1 (0.9%)	11 (3.7%)	0.1184
June	12 (9.0%	7 (8.2%)	2 (6.9%)	0	21 (5.8%)	0.01414
July	11 (13.6%)	5 (11.4%)	2 (6.9%)	3 (3.9%)	21 (9.1%)	0.18445
August	14 (35.9%)	11 (23%)	11 (23.9%)	7 (10%)	59 (26.8%)	0.000260
September	11 (25.6%)	1 (4.2%)	1 (4.2%)	2 (1.9%)	24 (10.8%)	0.000029
October	1 (4.8%)	1 (6.3%)	1 (1.2%)	1 (1.2%)	16 (7.3%)	0.01955
November	5 (14.3%)	1 (4.0%)	2 (2.0%)	2 (2.0%)	24 (9.0%)	0.00675
December	13 (20%)	2 (7.7%)	3 (3.2%)	3 (3.2%)	23 (9.7%)	0.006158
Total	108 (37.9)	65 (22.8%)	29 (10.2%)	30 (10.5%)	285	0.20604

X^2=249.16, df=3, P=0.00000.

Table 4.22: Distribution of anaemia in the various age groups in the community

Packed Cell Volume (PCV)	<5 years	5-9 years	10-14 years	>15 years	Total
Normal	776	1007	530	1380	3693
(>33%)	(87.7%)	(89.4%)	(95.2%)	(97.9%)	(92.8%)
Mild	0	0	3	1	4
(30-32.9%)	0	0	(0.5%)	(0.1%)	0.1%
Moderate	108	120	3	29	281
(21-29.9%)	(12.3%)	(10.6%)	(0.5%)	(2.1%)	(7.1%)
Total	881	1127	24	1410	3978
%	22.2	28.2	4.3	35.5	100

X^2=130.97 df=6 P=0.000000. There is statistical association between anaemia and age group in the community, P<0.001

4.3.10 Prevalence of anaemia among pregnant women according to Health Facility

The result showed that 41.7% and 42.4% of pregnant women from Ayo Olu and General hospital were anaemic respectively. Further analysis revealed that 55.3% and 29.4% of pregnant women from Pota and Unique health facilities were also anaemic. The highest number of pregnant women that were anaemic came from the Omowumi traditional birth home (Figure 10) Statistical analysis showed significant association between anaemia and health facility attended. X^2=12.1442, df=4, P=0.0163, (P<0.05)

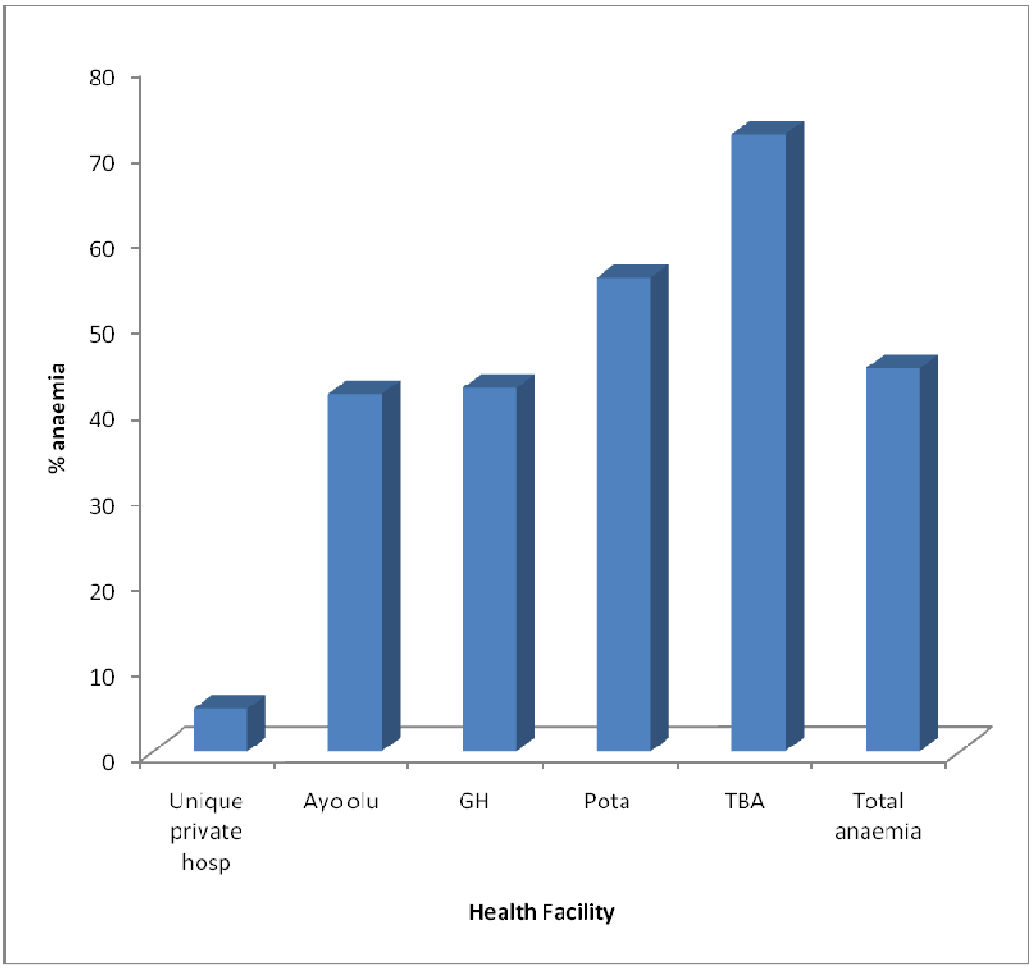

Figure 10: **Prevalence of anaemia among pregnant women according to Health Facility. X^2=12.1442, DF=4, P=0.0163. There is statistical significance between anaemia among pregnant women and the Health Facility attended, P<0.05.**

4.3.11 Distribution of anaemia types between gravida

The result showed that 25.7% of primigravida had mild anaemia, 12.6% moderate anaemia while 1.1% had severe anaemia. Among the secundigravida, 41% had mild anaemia, 12.7% had moderate anaemia while 0.7% had severe anaemia. Further analysis revealed that 27.7% of multigravida had mild anaemia; 13.9% had moderate anaemia while 1.2% had severe anaemia, (Table 4.23). There is no statistical significance between severity of anaemia and gravida, X^2=10.3340, df=6, P=0.1113, (P<0.05).

Table 4.23: Distribution of anaemia types among gravidas

Packed Cell Volume (PCV)	Primigravida N=183	Secundigravida N=165	Multigravida N=135	Total
Normal (>33%)	111 (60.7%)	61 (45.5%)	95 (57.2%)	267 55.3%
Mild (30-32.9%)	47 (25.7%)	56 (41%)	47 (27.7%)	148 (30.6%)
Moderate (21-29.9%)	23 (12.6%)	17 (12.7%)	23 (13.9%)	63 (13%)
Severe (<21%)	2 (1.1%)	1 (0.7%)	2 (1.2%)	5 (1%)
Total	183	135	165	483
%	(37%)	(28%)	34%	100

X^2=10.33340 df=6 P=0.1113. There is no statistical association between anaemia and gravid, (P>0.05)

4.3.12 Determination of fever by age group in Ikoga ward

Out of the 3,978 persons that had their temperature measured in Ikoga ward, 2.5% had temperature greater than 37.5^0C (which was taken as fever). The measured temperature ranged between 35^0C degrees and 38.8^0C degrees with a mean of 36.4^0C. According to age groups, 3.6% and 3.8% of children less than five years and 5 to 9 years had temperature greater than 37.5^0C respectively, 3% of 10-14 year olds also had temperature above 37.5^0C. There was statistical association between age group and fever, $X^2=24.64$, df=3, P=0.0001, ($P<0.05$) Table 4.24.

Table 4.25 shows that out of the 15.7% (76) pregnant women that had malaria, only 5.4% (4) had fever.

Table 4.24: Determination of fever cases by age group in the Ikoga ward

Age group	Temperature ^{0}C		Total
	<37.5	**>37.5**	
<5	849	32 (3.6%)	881
5-9	1086	43 (3.8%)	1129
10-14	550	7 (3.0%)	557
15-19	144	9 (0.66%)	153
20-24	213	5 (0.3%)	218
25-29	244	4 (0.1%)	238
30-34	192	0	192
>35	610	0	610
Total	3878 (97.5%)	100 (2.5%)	3978

X^2=24.64, df=7, P=0.0001. There is significant association between fever and age group, P<0.05.

Table 4.25: Parasite and Fever among pregnant women

Fever	Negative	Positive	Total
<37.5	405	72	477
	85	(15%)	98%
>37.5	2	4	6
	(0.5%)	(5.4%)	(1.2%)
Total	407	76	483
	(84.2%)	(15.75%)	(100%)

4.3.13 Measurement of Fever among Different Gravida

Analysis of fever cases by gravida showed that 1.2% of primigravida and 1.5% of secundigravida had fever. The percentage of multigravida that had fever was 1.2%. The overall proportion of pregnant women that have temperature was 1.2%.There is no statistical significance between fever and gravida, however there is a correlation between fever and primigravida (Table 4.26).

Table 4.26: Measurement of fever among different gravidas

Gravida	Frequency	No with fever	% fever
Primigravida	183	2	1.1
Secundigravida	135	2	1.5
Multigravida	165	2	1.2
Total	483	6	1.2

X^2=0.1036, df=2, P=0.9495. There was no statistical significance between fever and gravid, P>0.05.

4.3.14 Measurement of Spleen rate in Children, 2-9 Years in the community

The monthly spleen rate in the study community ranged between 12% and 42.6%. The overall spleen rate was 23.7%, Figure 11. Analysis of data revealed that 372 out of 1,491 (24.9%) of children 2-9 years old had enlarged spleen of various degree ranging from 1 to 5, with one being the lowest degree while 5 is the highest degree of enlargement. The analysis further revealed that malaria prevalence increased with increase in spleen enlargement. X^2=392.31, df=5, P=0.0001. There is significant association between malaria prevalence and spleen rate, P<0.05 (Table 4.27). The average enlarged spleen (degree of malaria endemicity) was calculated to be 2 (Appendix 5).

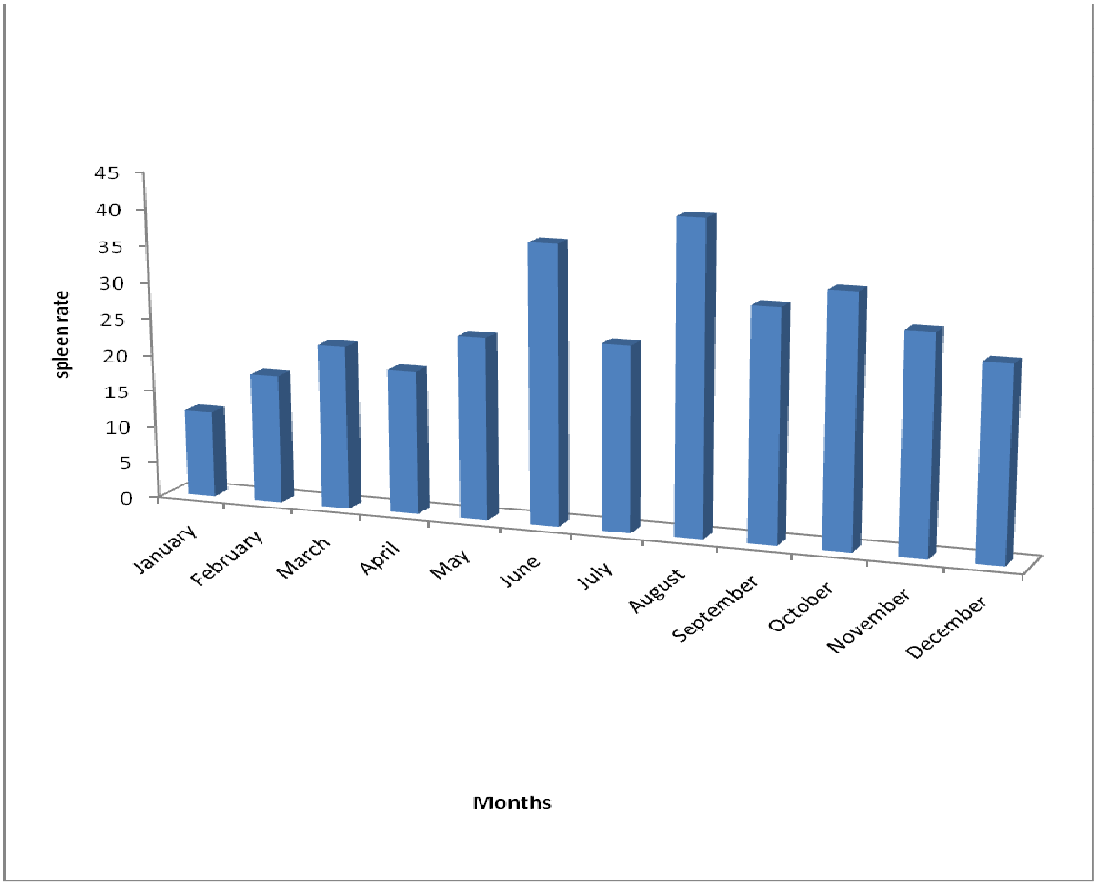

Figure 11: Monthly measurement of Spleen enlargement

Table 4.27: Association between Spleen enlargement and malaria prevalence

Class of spleen	Number of various classes of spleen	Average enlarged spleen (AES)	Malaria Parasite	
			Positive	Negative
0.0	1119	0	265 (22.2%	934
1.0	132	132	78 (59.5%)	54
2.0	117	254	95 (81.7%)	22
3.0	85	251	76 (90.4%)	9
4.0	34	136	31 (90.9%)	3
5.0	4	20	3 (75%)	1
Total	1491(372+ve)	793	548 (34.9%)	1023

X^2=392.31, DF=5, P=0.0001.

There was a significant association between malaria prevalence and spleen rate. As the degree of spleen enlargement increased, the prevalence of malaria also increased, P<0.05. The spleen rate was 24.9%.

4.3.15 Comparison between rarasite Rate, Spleen rate and fever

A comparison was made between the prevalence of malaria, spleen rate and fever cases in the community (Figure 12). The correlation (R) between malaria prevalence and spleen rate was found to be weak with R value of 0.711. The correlation between malaria prevalence and fever was weak with an R value of 0.4468 while the correlation between spleen rate and fever had an R value of 0.309. Strong correlation is indicated by R value of greater than 0.811.

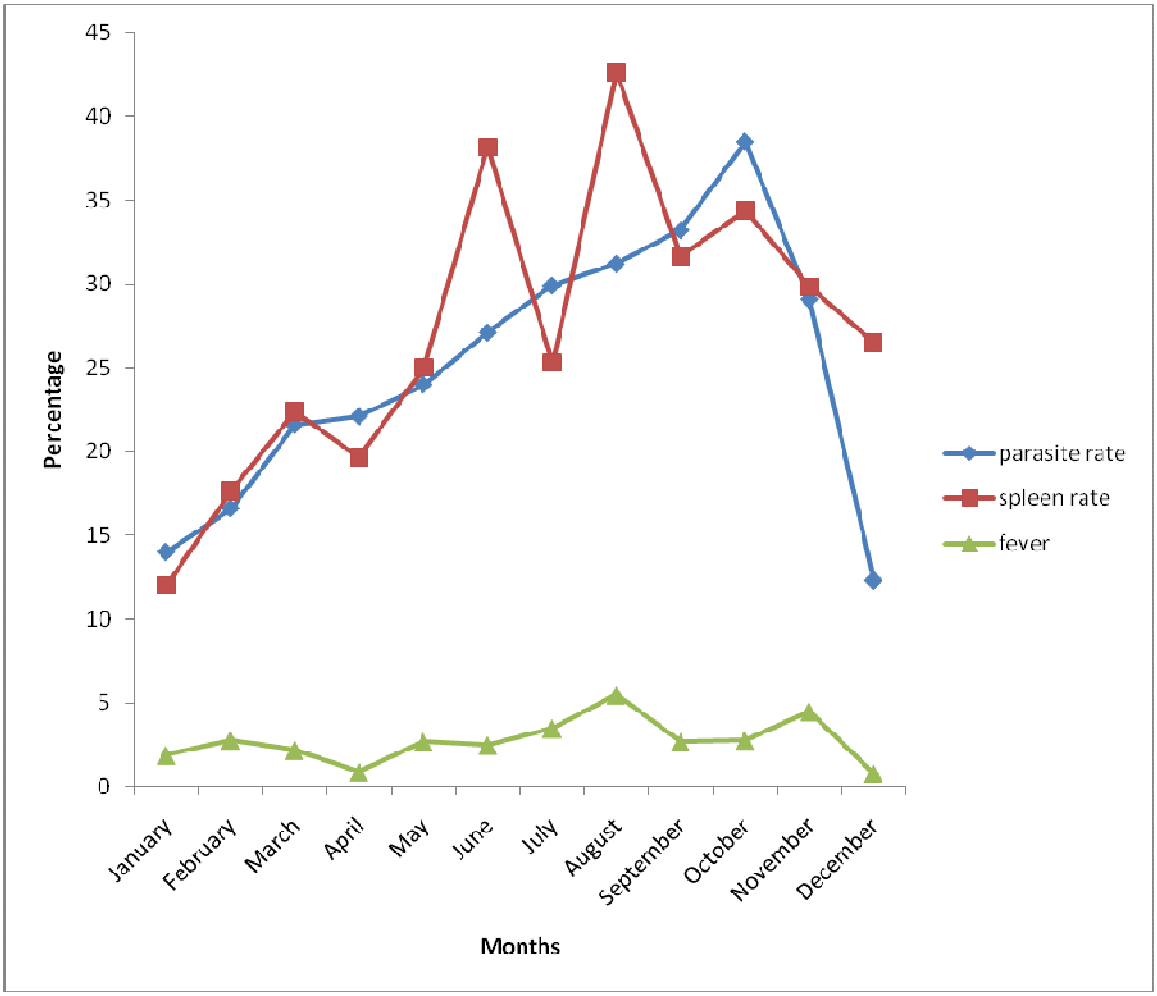

Figure 12: Comparison between parasite rate, spleen rate and fever

4.3.16 Gravida versus prevalence of malaria, anaemia and fever before Sulfadoxine-Pyrimethamine administration

A breakdown of the pregnant women by gravida showed that the malaria prevalence was 14.2% of primigravida, 17.8% of secundigravida and 14.2% of multigravida with over all malaria prevalence of 15.7%. Analysis showed also that 39.3% of the primigravidae were anaemic compared to 54.5% of secundigravida and 48.2% of multigravida. The overall percentage of pregnant women that were anaemic was 44.7% while 1.2% of pregnant women had fever (Table 4.28). There was no statistical significance between gravida and malaria prevalence, anaemia or fever, $P>0.05$.

Table 4.28: Gravida versus malaria prevalence, anaemia and fever

Gravida	Frequency	Prevalence of Malaria	Prevalence of anaemia	Prevalence of fever
Primigravida	183	26 (14.2%)	72 (39.3%)	2 (1.1%)
Secundigravida	135	24 (17.8%)	73 (54.5%)	2(1.5%)
Multigravida	165	26 (14.2%)	71 (48.2%)	2(1.2%)
Total	483	76 (15.7%)	216 (44.7%)	6(1.2%)
P value		P=0.6179	P=0.1113	P=0.287

4.4 COMPARATIVE EVALUATION OF DIAMED OPTIMAL (RAPID DIAGNOSTIC TECHNIQUE WITH MICROSCOPY

4.4.1 Diamed OptiMAL

The result of the rapid diagnostic test using DIAMED OptiMAL diagnostic test kit detected the presence of *P. falciparum* parasite in the tested blood samples. Analysis of the data revealed that out of a total of 194 blood samples tested in the community using thick film microscopy, 51.5% were positive for *P. falciparum* while OptiMAL DIAMED detected 19.6% *P. falciparum* malaria antigen (Table 4.29). A case of mixed infection with *P. falciparum and P. malariae* was detected by thick film microscopy but was not detected by Optimal DIAMED, (Table 4.30). The colour intensity produced by the antigen/antibody reaction with the OptiMAL DIAMED increased with the parasite density.

The sensitivity of OptiMAL increased with increase in parasitaemia. When parasitaemia was between 101 -500 parasites per µl of blood, the sensitivity of OptiMAL was 27.3% while at 1001 parasite per µl of blood and above, it was 42.4% (Table, 4.31).

Sixty two subjects were positive by TFM for malaria parasite when Optimal DIAMED was negative (Table 4.32). Analysis of the result showed the sensitity and specificity of OptiMAL to be 38% and 97.9% respectively, (Table 4.33**).**

Table 4.29: Comparison between Thick film microscopy and Rapid Diagnostic Technique

Diagnostic method	No. examined	No. +ve	%
Microscopy	194	100	51.5
OptiMAL DIAMED	194	38	19.6

Table 4.30: Species Specificity of DIAMED Optimal RDT

Species	Number positive by TFM	Number positive by DIAMED Optimal
Plasmodium falciparum	99	38
Plasmodium malariae		
Plasmodium ovale		
Mixed infection (*Pf* and *Pm*)	1	0
Total	100	38

Pf- Plasmodium. falciparum *Pm- Plasmodium malariae*

TFM-Thick film microscopy

Table 4.31: Sensitivity of DIAMED OptiMAL and TFM by level of parasitaemia in the study subjects

Parasite/ul of blood	TFM	OPTIMAL	Sensitivity
0	0	2	0
1-100	0	0	0
101-500	22	6	27.3
501-1000	10	2	20.0
1001 and above	66	28	42.4
Total	100	38	

Table 4.32: The sensitivity, specificity and positive predictive values of the two assays in the study subjects

		Thick film microscope		
		Positive	**Negative**	**Total**
DIAMED	Positive	38	2	40
OPTIMAL	Negative	62	92	154
	Total	100	94	194

Table 4.33: Analysis Optimal DIAMED

Variabe	Frequency	Percentage
Sensitivity	38/100	38
Specificity	92/94	97.9%
Positive predictive value	38/40	95
Negative predictive value	94/154	61

4.5 EVALUATION OF SULFADOXINE-PYRIMETHAMINE (SP) IN PREGNANT WOMEN

A break down of the result showed that out of the 483 pregnant women that enrolled in the study, 88.2% received SP while 11.8% did not. The 483 pregnant women comprised 183 primi-gravida, 135 secundi-gravidae and 165 multi-gravida. Among the primigravida, 33.3% received one dose while 58.5% received two doses and 8.2% did not receive any SP. Analysis of result showed that 32.6% of secundigravida received one dose while 57.8% received two doses and 9.6% did not receive. For the multi-gravida, 29.1% received one dose while 53.3% received two doses, however 17.6% did not receive. A break down of those who received SP showed that 56.5% (273 out of 483) received two doses, 31.7% (153 out of 483) received one dose while 11.8% (57 out of 483) did not (Table 4.34).

Table 4.34: Number of doses of Sulfadoxine-pyrimethamine (SP) versus gravida

SP dose	Primi	Secundi	Multi	Total
None	15(8.2%)	13(9.6%)	29(17.6%)	57(11.8%)
1	61(33.3%)	44(32.8%)	48(53%)	153(31.7%)
2	107(58.5 %)	78(58.2%)	88(32.2%)	273(56.5%)
Total	183(37.9%)	135(27.7%)	165(34.2%)	483(100%)

4.5.1 Effect of Sulfadoxine-Pyrimethamine (SP) on Monthly Prevalence of Malaria

The monthly malaria prevalence after SP administration ranged between 0 (January) and 50% (February), while before SP administration, it was between 9.2% (May) and 50% (January) (Figure 13). The result showed that the prevalence decreased after SP administration from 15.7% to 2.1%. There was a significant association between Sulfadoxine-pyrimethamine administration and malaria prevalence, $P<0.001$, Figure 14.

Comparison between prevalence of malaria between those who were administered SP and those not administered showed that 2.4% of pregnant women who were administered SP had malaria parasite compared with 17.5% of pregnant women who did not receive SP, (Figure 15). There was statistical significance between those who were administered SP and those who were not. $X^2=25.55$, df=1, P=0.001, ($P<0.05$).

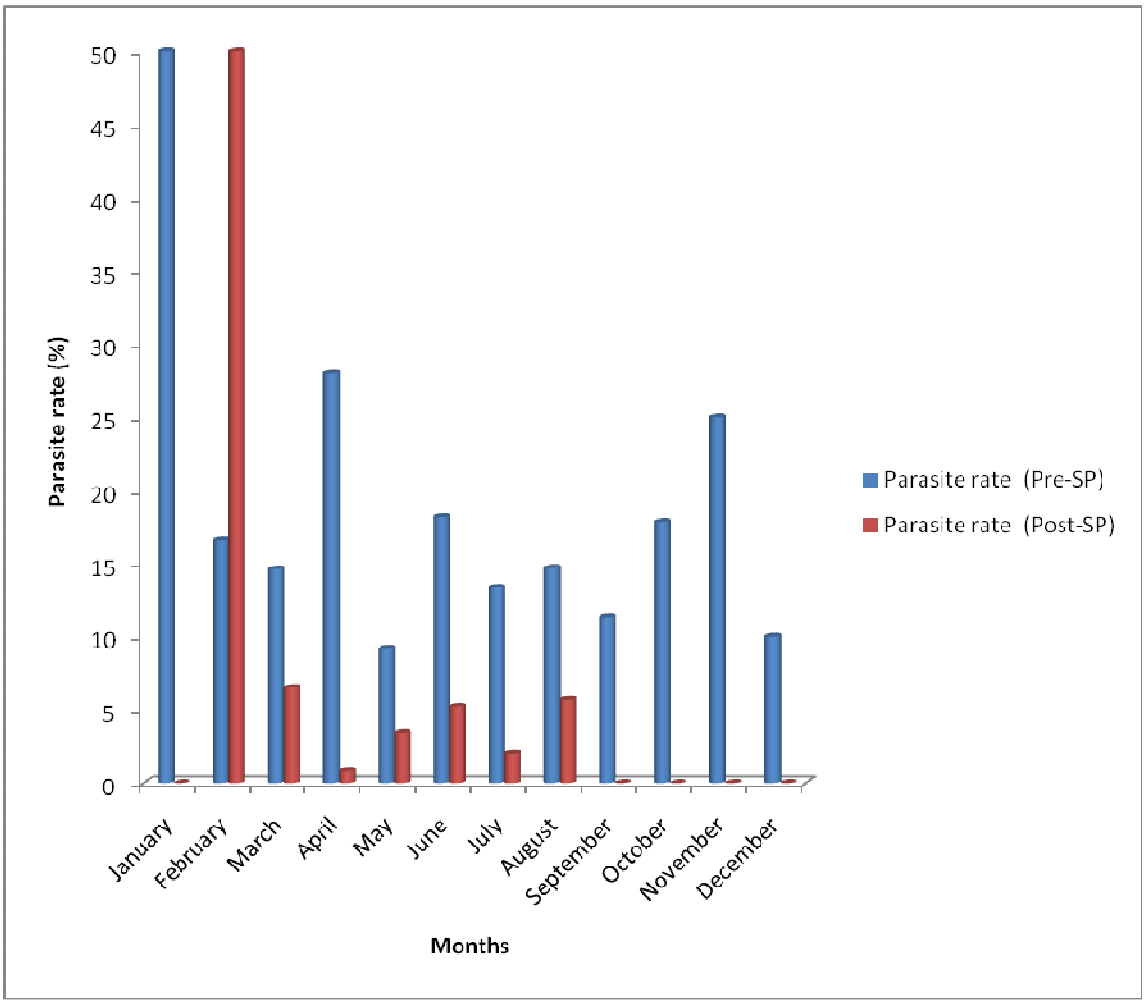

Figure 13: Malaria prevalence in pregnant women pre and post SP administration

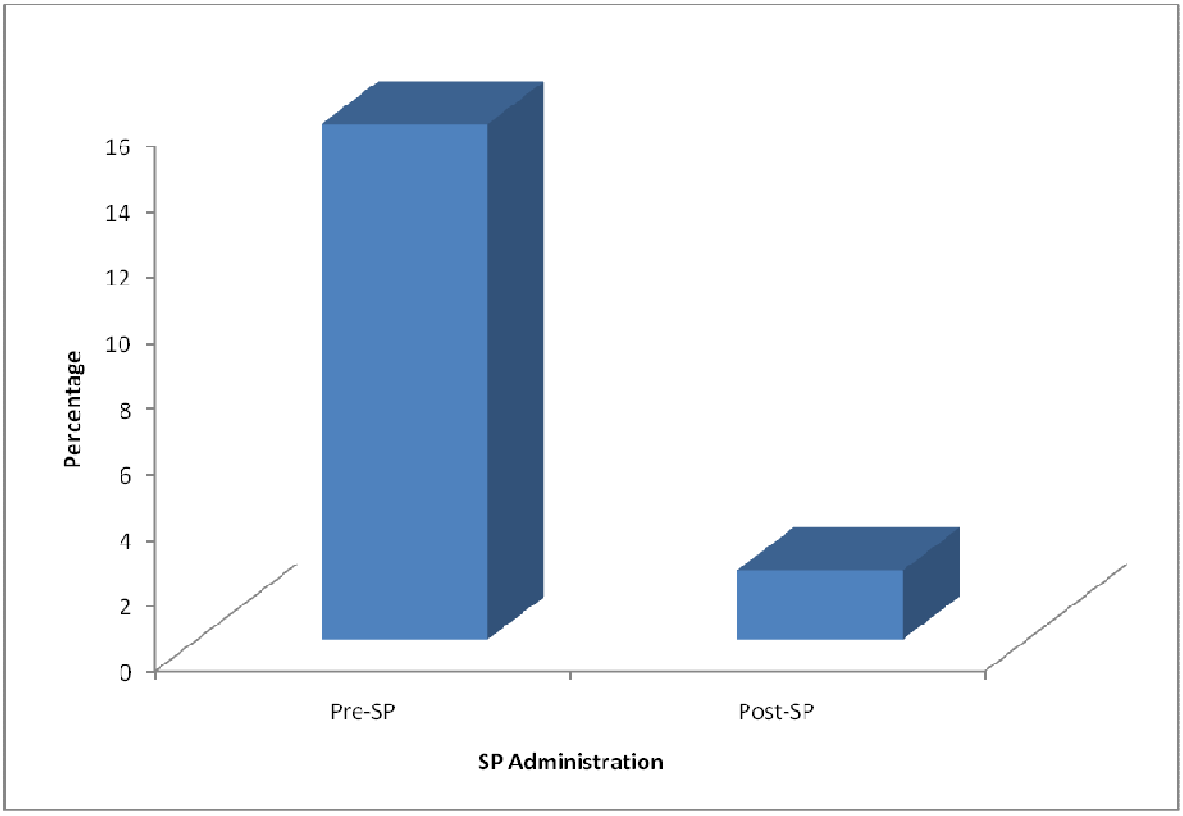

Figure 14: **Effect of Sulfadoxine-Pyrimethamine on prevalence of malaria among pregnant women that were administered Sulfadoxine-Pyrimethamine**

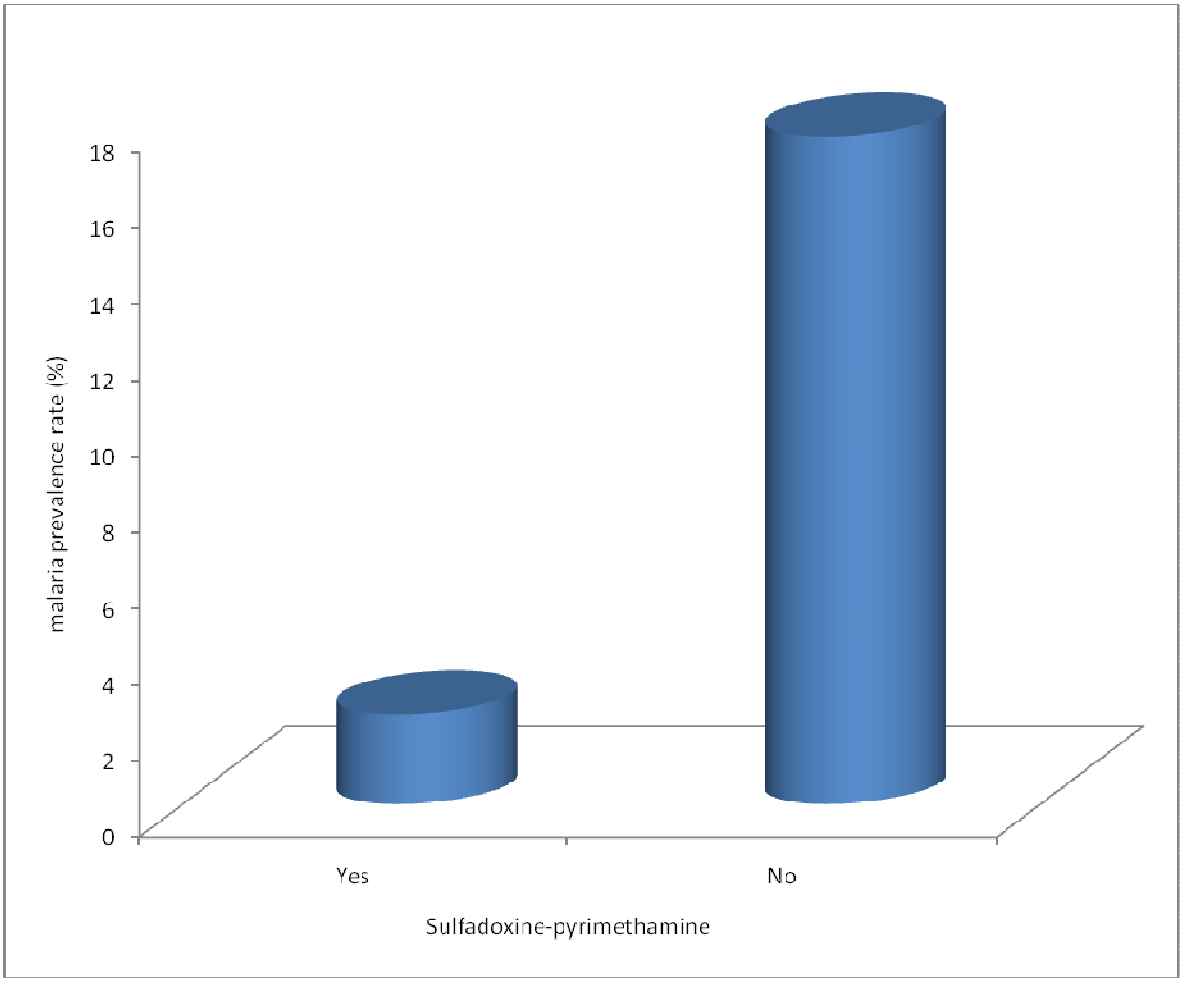

Figure 15: Effect of Sulfadoxine-pyrimethamine on malaria prevalence among pregnant women administered Sulfadoxine-pyrimethamine compared to those not administered.

4.5.2 Effect of Sulfadoxine-Pyrimethamine on Placenta Parasitaemia

3.1% of placentas of pregnant women that were examined for malaria parasite were positive. These included placentas from pregnant women who were administered SP and those who were not. The 3 positive placenta specimens were from both pregnant women who were administered SP (1 and 2 doses) and those who were not. Further analysis showed that 96.9% of placenta specimens examined were negative, and majority were from pregnant women who were administered SP (Figure 16). X^2=20.6414, df=2, P=0.0082. There was a statistical significance between SP administration and placenta parasitaemia, P<0.05

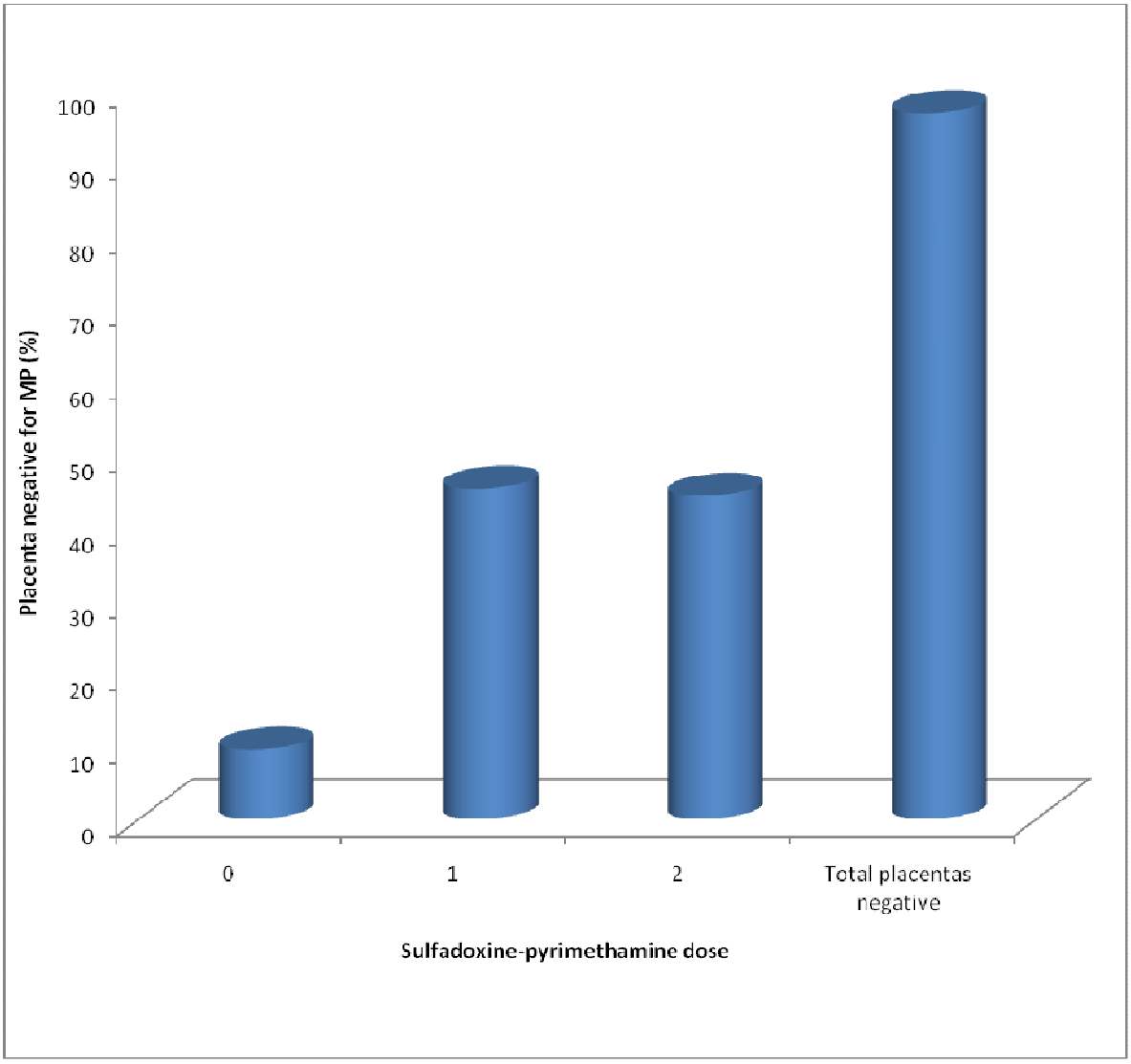

Figure 16: Effect of Sulfadoxine-pyrimethamine on Placenta malaria

4.5.3 Effect of Sulfadoxine-pyrimethamine on anaemia among different gravida and age groups of pregnant women

Sulfadoxine-pyrimethamine administration had no effect on anaemia among the different gravida and showed no statistical significance between SP administration and anaemia. X^2=7.5457 df=4 P=0.1097 (P>0.05) (Table 4.35)

SP administration also showed no statistical significance between anaemia and the different age groups (Table 4.36). X^2=12.9100 df=8 P=0.1150, (P>0.05).

Table 4.35: Effect of Sulfadoxine-pyrimethamine on anaemia among different gravida

PCV	Primigravida	Secundugravida	Multigravida	Total
≥33	104 (42.3%)	61 (24.8%)	81 (32.9%)	246 (57.3%)
30-32.9	45 (35.2%)	46 (35.9%)	37 (28.9%)	128 (29.8%)
21-29.9	18 (32.7%)	14 (25.5%)	23 (41.8%)	55 (12.8%)
Total	167 (38.9%)	121 (28.2%	141 (32.9%)	429 (100%)

X^2=7.5457 df=4 P=0.1097. Sulfadoxine-pyrimethamine showed no statistical significance between anaemia and the different gravida, P>0.05

Table 4.36: Effect of Sulfadoxine-pyrimethamine on prevalence of anaemia among different age groups of pregnant women

PCV	Age group (years)					Total
	16-20	21-25	26-30	31-35	<35	
>33	11	46	99	69	21	246
%	(4.5%)	(18.7%)	(40.2%)	(28%)	(8.5%)	(57.3%)
30-32.9	4	23	63	25	13	128
%	(3.1%)	(18%)	(49.2%)	(19.5%)	(10.2%)	(29.8%)
21-29.9	7	11	22	10	5	55
	(12.7%)	(20%)	(40%)	(18.2%)	(9.1%)	(12.8%)
Total	22	80	184	104	39	429
%	5.1	18.6	42.9	24.2	9.5	100%

X^2=12.9100 df=8 P=0.1150. There was no statistical significance between SP administration and anaemia among different age groups of pregnant women, (P>0.05).

4.5.4 Effect of Sulfadoxine-Pyrimethamine (SP) on Fever among Pregnant Women

After SP administration, 0.41% of pregnant women had temperature greater than 37.5^0C compared to 1.2% of pregnant women before the administration of SP. The other 98.8% had no fever, (Table 4.37) X^2=1.13, df=1, P=0.287. Although there was a reduction in the proportion of those that had fever, there was no statistical significance between SP administration and fever, P>0.05 (Table 4.37).

Table 4.37: Effect of Sulfadoxine-pyrimethamine (SP) on fever

Temperature	Pre-SP		Post SP	
	Frequency	Percentage	Frequency	Percentage
>37.5^{0}C	6	1.2	2	0.4
<37.5^{0}C	477	98.8	481	99.6
Total	483	100.0	483	100.0

X^2=1.13, df=1, P=0.287. Although there was a reduction in the proportion of those that had fever, there was no statistical significance between the administration of Sulfadoxine-pyrimethamine and fever, P>0.05

4.5.5 Effect of Sulfadoxine-Pyrimethamine on Malaria Prevalence, Anaemia and Fever among the Different Gravida

The study showed that the prevalence of malaria was 2.7% after SP administration among the primigravida; 1.5% among the secundigravida and 1.8% for the multigravida**.** Malaria prevalence was reduced with SP administration, but statistically, showed no significance between malaria prevalence and gravida, P>0.05 (Table 4.38).

The prevalence of anaemia after SP administration was 32.7% and 32.8% for primigravida and secundigravida while for multigravida, it was 32.8%. The observed reduction in prevalence of anaemia showed no statistical significance, P>0.05 (Table 4.38).

The prevalence of fever after SP administration was 0.2% for primigravida and multigravida respectively and showed no statistical significance, P>0.05, Table 4.38.

Table 4.39 shows comparison between prevalence of malaria, anaemia and fever before and after SP administration. SP significantly reduced the prevalence of malaria, and anaemia, P<0.05 among pregnant women. However for fever, it was not statistically significant, P>0.05.

Table 4.38: Effect of Sulfadoxine-pyrimethamine (SP) on prevalence of malaria, anaemia and fever among pregnant women of different gravida

Gravida	Prevalence of malaria	Prevalence of anaemia	Prevalence of fever
Primigravida	5 (2.7%)	63 (32.7%)	1 (0.2%)
Secundigravida	2 (1.5%)	60 (32.8%)	0
Multigravida	3 (1.8%)	60 (32.8%)	1 (0.2%)
Total	10(2.1%)	183 (37.9%)	2 (0.4%)
P value	P=0.7125	P=0.311	P=0.287

X^2=0.6781, df=2, P=0.7125. There was no statistical significance between gravida and malaria prevalence, anaemia and fever for those who took SP, P>0.05

There was a reduction in the percentage of pregnant women who were anaemic after SP administration, however there was no statistical significance between SP administration and anaemia amongst the different gravida after SP administration, X^2=2.3303, df=2, P=0.311, (P>0.05).

Table 4.39: Comparison Between Prevalence of Malaria, Anaemia and Fever before and after Sulfadoxine-pyrimethamine administration

Gravida	Prevalence Rate		
	Malaria	**Anaemia**	**Fever**
Primigravida N=183			
Pre SP	26 (14.2%)	72 (39.3%)	2 (1.1%)
Post SP	5 (2.7%)	63 (32.7%)	1 (0.2%)
Secundigravida			
N=135	24 (17.8%)	73 (54.5%)	(1.5%)
Pre SP	2 (1.5%)	60 (32.8%)	0
Post SP			
Multigravida N= 165			
Pre SP	26 (15.7%)	71 (48.2%)	2 (1.2%)
Post SP	3 (1.8%)	60 (32.8%)	2 (0.4%)
P value	0.00000	0.002766	0.5

SP reduced prevalence of malaria and anaemia and showed statistical significance, $P<0.05$. SP however reduced fever, but was not statistically significant, $P>0.05$.

4.5.6 Effect of Sulfadoxine-Pyrimethamine (SP) on Term of Delivery (Duration of Pregnancy)

Analysis of the results revealed that 88.3% of pregnant women who took SP had full term delivery compared to 11.0% that did not take sulfadoxine-pyrimethamine. The result further revealed that 0.7% of those who took SP had preterm delivery (Table 4.40).

Table 4.40: Effect of Sulfadoxine-pyrimethamine on Delivery Term (duration of pregnancy)

Term	No SP	SP	Total
Full term	52	420	472
%	(11.0%)	(88.3%)	(99.3%)
Preterm	0	3	3
%	0	(0.7%)	(0.7%)
Total	52	423	475
	(10.9%)	(89.1%)	(100%)

P = 0.706 (Fisher Exact Test) There was no significant association between delivery term and SP administration, P>0.05

4.5.7 Effect of Sulfadoxine-Pyrimethamine on Low Birth Weight among Different Gravida

The percentage of LBW in the group of pregnant women who did not take SP was 3.5% (comprising 1 primigravida and 1 multigravida). However, it was observed that 4% in pregnant women who took SP also had LBW. There was no significant association between taking SP and LBW in the different gravida, P=0.05, Table 4.41

Table 4.41: Effect of Sulfadoxine-pyrimethamine on LBW amongst the different gravida

SP	Primigravida		Secundigravida		Multigravida		Total	
	N	LBW	N	LBW	N	LBW	N	LBW
Yes	168	9	122	4	136	4	426	17
	%	(5.3%)		(3.3%)		(2.9%)		(4.0%)
No	15	1	13	0	29	1	57	2
	%	(6.6%)		0		(3.4%)		(3.5%)
Total	183	10	135	4	165	5	483	19
	%	(5.5%)		(2.9%)		(3%)		(4.0%)

N=normal, LBW=Low birth weight

X^2=0.03, df=2, P=0.852. There was no statistical significance between taking SP and LBW in the different gravida, P>0.05

4.6 ENTOMOLOGICAL STUDIES ON THE VECTOR

4.6.1 Collection and Identification of Malaria Vectors

The Pyrethrum spray collection carried out during entomological survey showed that *Anopheles gambiae sl* which is the vector of malaria parasite is present in Ikoga ward.

Anopheles funestus and *Anopheles melas* were very few. Analysis of the total number of Anopheles collected showed that mosquito density was higher in the rainy season compared to dry season (Figure 17), X^2=1346.26, df=1, P=0.001. There was a significant association between mosquito densities and the season of the year, P<0.005

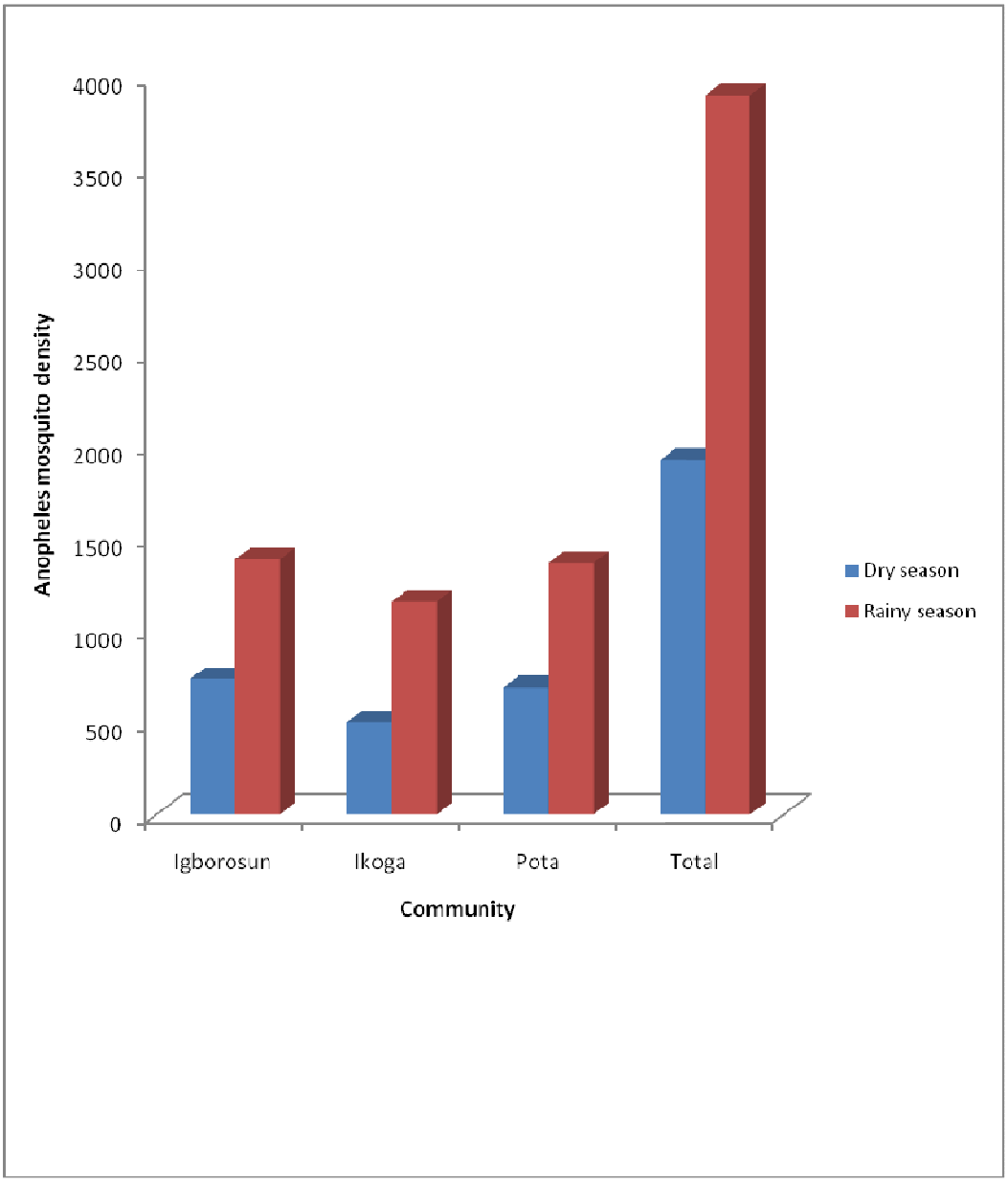

Figure 17: Anopheles mosquitoes density during dry and rainy season in Ikoga ward

4.6.2 Average Anopheles Room Density

The average Anopheles room density was between 3 and 22 mosquitoes per room per day. There was an increase in the density of mosquitoes between the months of May and October. This coincides with the rainy season.

4.6.3 The Entomological Inoculation Rate (EIR)

The entomological inoculation rate was calculated for each community by multiplying the man biting rates (MBR) and the sporozoites rates (SP) from the three communities. The annual entomological inoculation rates (EIR) for the Igborosun, Ikoga and Pota were 28.59, 17.44 and 12.0 mosquitoes per man per year (Appendix 13).

Annual EIR for the study community

Average EIR for Igborosun + Ikoga + Pota

=28.59+17.44 +12.0=58.3

$$= \frac{58.34}{3} = 19.34$$

Annual EIR=19.34 mosquitoes/man per year.

The results showed that the entomological inoculation rate for the study community was 19.34 (infective bites per man per year) mosquitoes biting man per year.

4.6.4 Identification of *Anopheles gambiae sl* by Polymerase Chain Reaction (PCR)

Analysis of the *Anopheles gambiae sl* using Polymerase Chain Reaction (PCR) revealed the presence of *Anopheles gambiae ss* and *An. arabiensis.* Out of the 5830

female Anopheles mosquito collected over the one year period, 67% were *Anopheles gambiae*, while 33% were *Anopheles arabiensis.*

The ratio of *Anopheles gambiae* ss to *A. Arabiensis* is 2:1. *Anopheles gambiae* has 390 kilo base pairs, *Anopheles arabiensis* 315 kilo base pairs while Anopheles melas has 464 kilo base pairs. *A. gambiae* is therefore between *A. arabiensis* and *A. Melas*, (Plate 4.1).

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

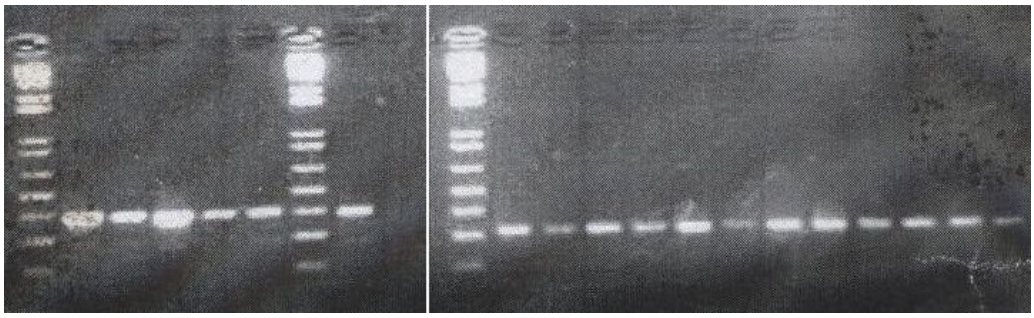

Plate 4.1: PCR Identification of members of the *Anopheles gambiae* group

Lane 1, 7 and 10, DNA Ladder

Lane 8 *A.gambiae s.s* positive control

Lane 9, Negative control

Lane 2-6 *Anopheles gambiae s.s*

Lane 11-21 *Arabiensis*

Lane 22 *Arabiensis* positive control

4.7 ASSESSMENT OF MUTAGENIC EFFECTS OF SULFADOXINE-PYRIMETHAMINE ON ANIMAL MODEL

4.7.1 Histopathological Evaluation of Sulfadoxine-Pyrimethamine on Foetuses of Female Albino Mice

Table 4.42 shows that the number of foetuses from each group of mice and their weight, which ranged between 1.6g and 2g with an average weight of 1.8g. The average weight of the control foetus was 1.8g. There was no difference between the weight of the control foetuses and test groups, $P>0.05$. The groups that received the 1X HTD and 1½X HTD had no dead foetus, neither did the control show any physical abnormality. However in the group that received 2X HTD, one mouse had IUGR with one dead foetus, giving a foetal death rate of 2% (Table 4.42). Histopathological investigation of the foetuses revealed the foetuses to be normal (Plates 4.2 to 4.7).

Table 4.42: Effect of sulfadoxine-pyrimethamine on foetuses of pregnant albino mice

No. of mice	Doses of SP	Average No. of foetus	Average weight of foetus (g)	No. of dead foetus	Percentage of dead foetus	P-value
5	**Control**	**10**	**1.8**	**0**	**0**	
5	**HTD**	**10**	**1.8**	**0**	**0**	**0.77**
5	**1½X**	**10**	**1.7**	**0**	**0**	**0.44**
5	**2X**	**10**	**1.7**	**1**	**2**	**0.41**

There was no statistical significance between the weight of the foetuses of control and test .mice, P>0.05.

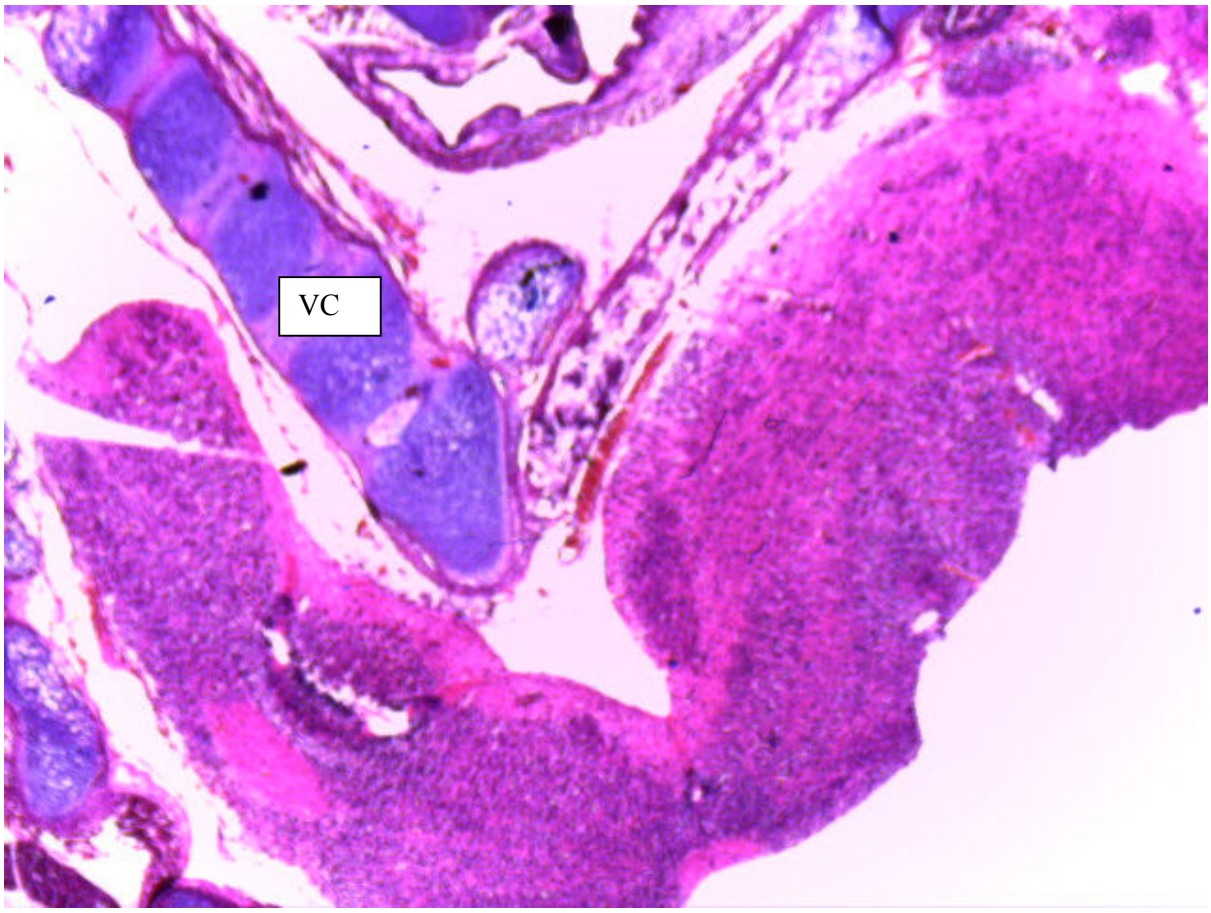

Plate 4.2: **Transverse section (TS) of foetus of the control female Albino mouse stained with Haemotoxylin and Eosin stains (H and E); shows normal Vertebral Column (VC), (X 100 magnification under oil immersion microscopy).**

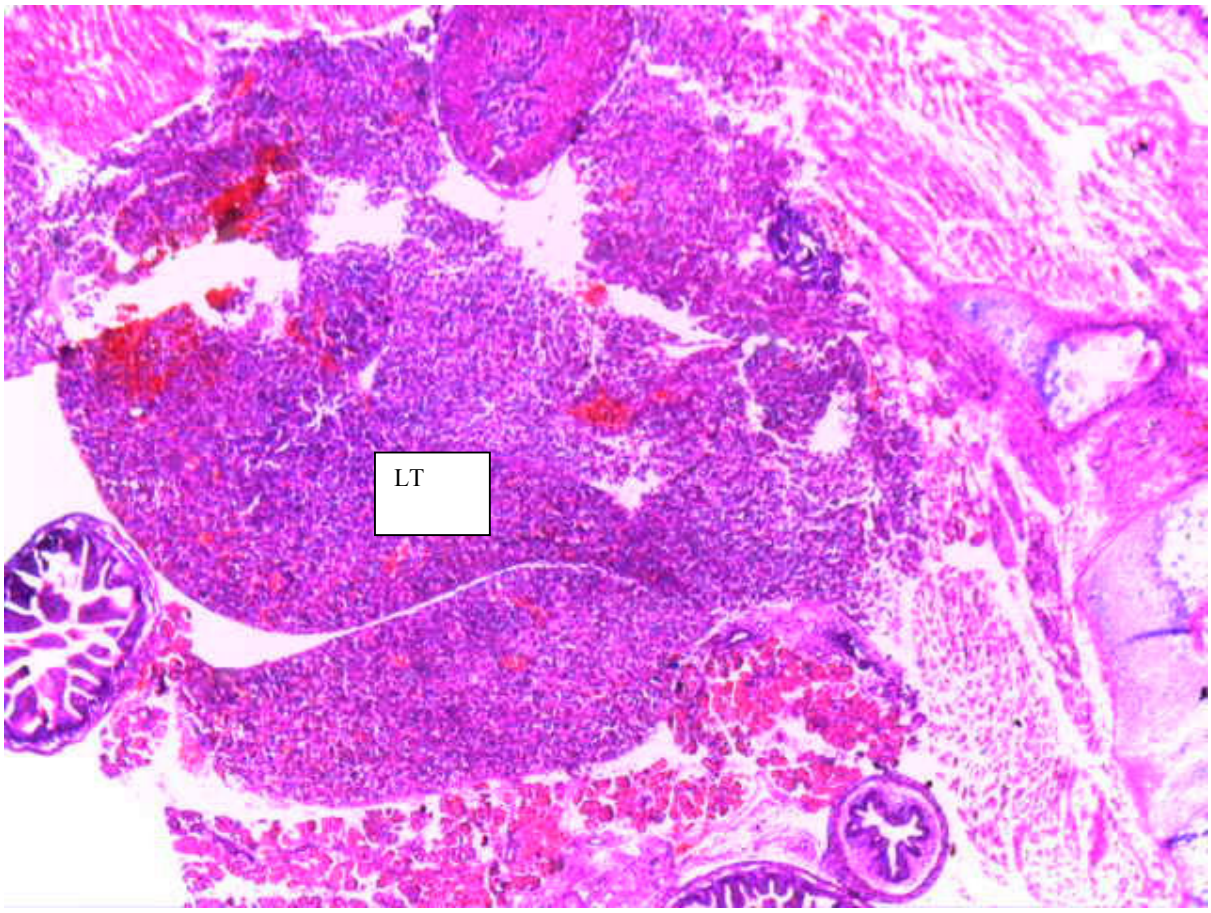

Plate 4.3: **Transverse section (TS) of the foetus of female Albino mouse administered 1X HTD of Sulfadoxine-pyrimethamine and stained with H and E stains; shows normal Liver Tissue (LT) without any neoplastic features, (X 100 magnification under oil immersion microscopy).**

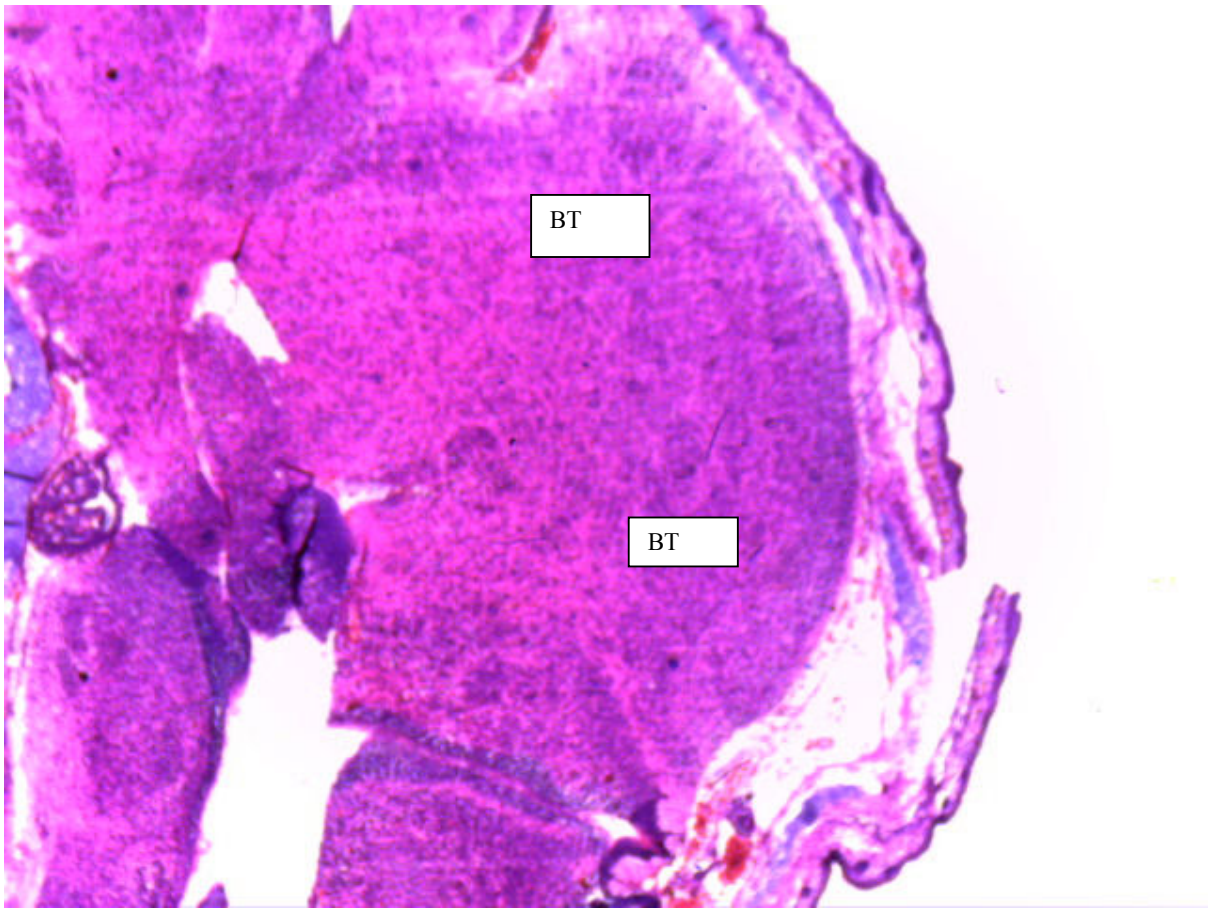

Plate 4.4: **Transverse section of Brain Tissue of foetus of female Albino mouse administered 1½ HTD of Sulfadoxine-pyrimethamine and stained with H and E stains; shows normal Brain Tissue (NBT), (X 100 magnification under oil immersion microscopy).**

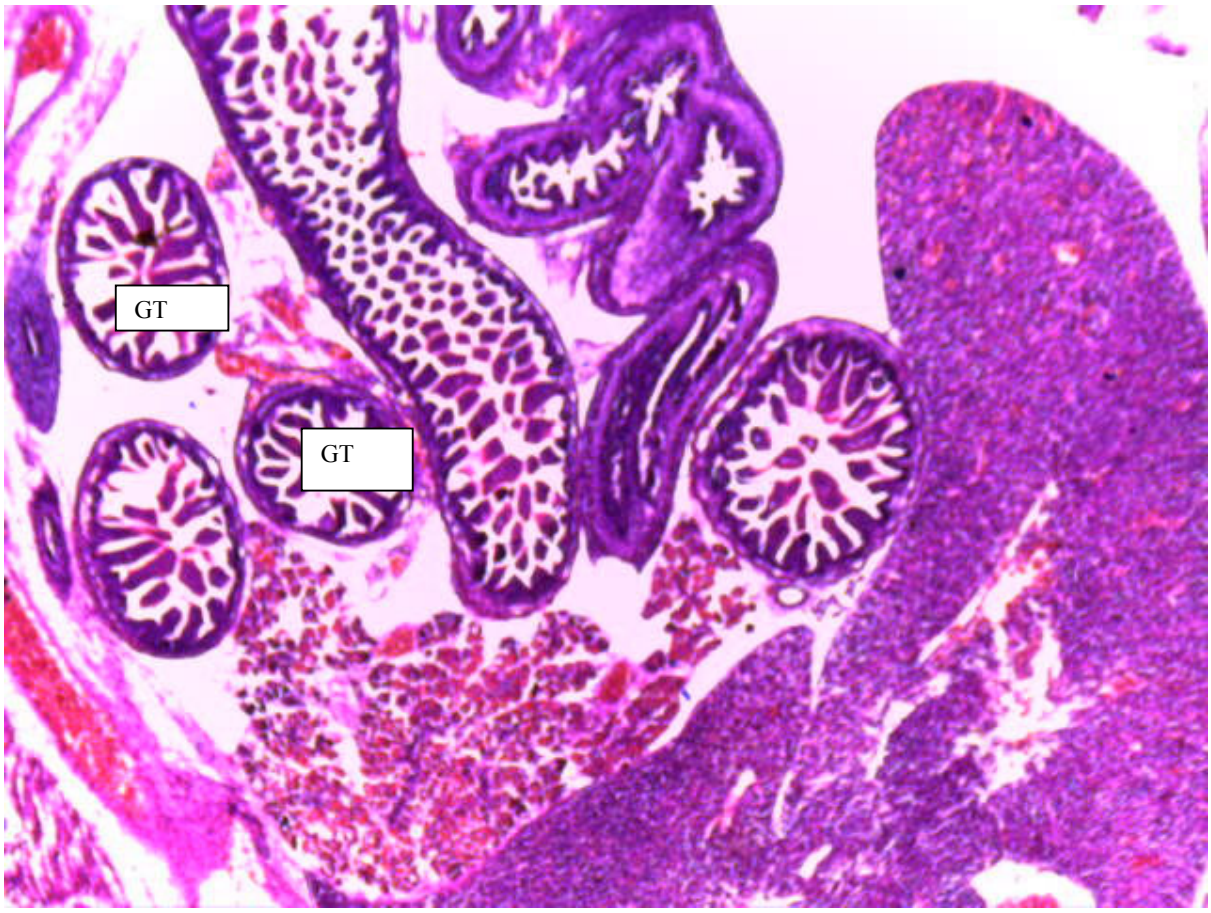

Plate 4.5: Transverse section of foetus of female Albino mouse administered 1½ X HTD of Sulfadoxine-pyrimethamine and stained with H and E stains; shows normal Glandular Tissue (GT), (X 100 magnification under oil immersion microscopy).

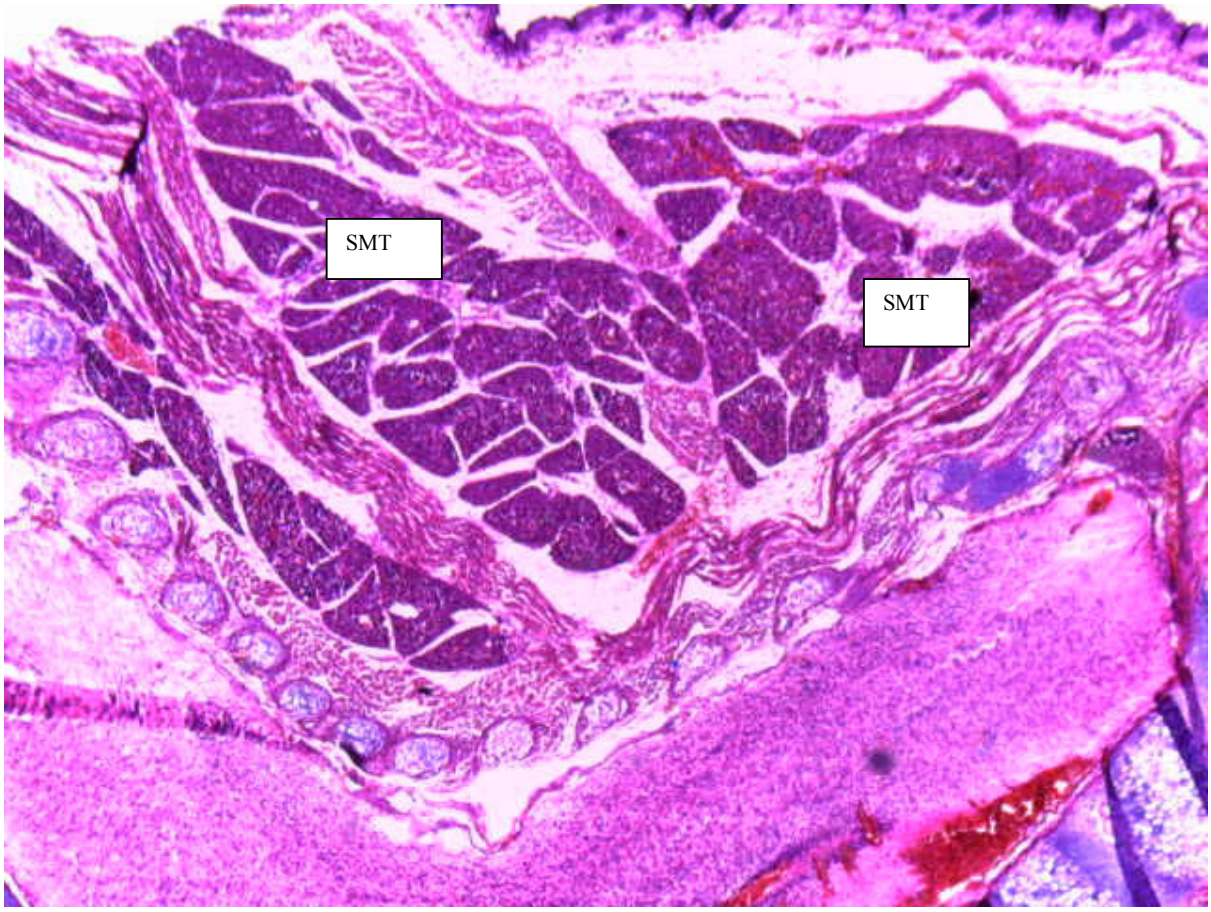

Plate 4.6: **Transverse section of foetus of female Albino mouse administered 2 X HTD of Sulfadoxine-Pyrimethamine and stained with H and E stains; shows normal Skeletal Muscle Tissue (SMT), (X 100 magnification under oil immersion microscopy).**

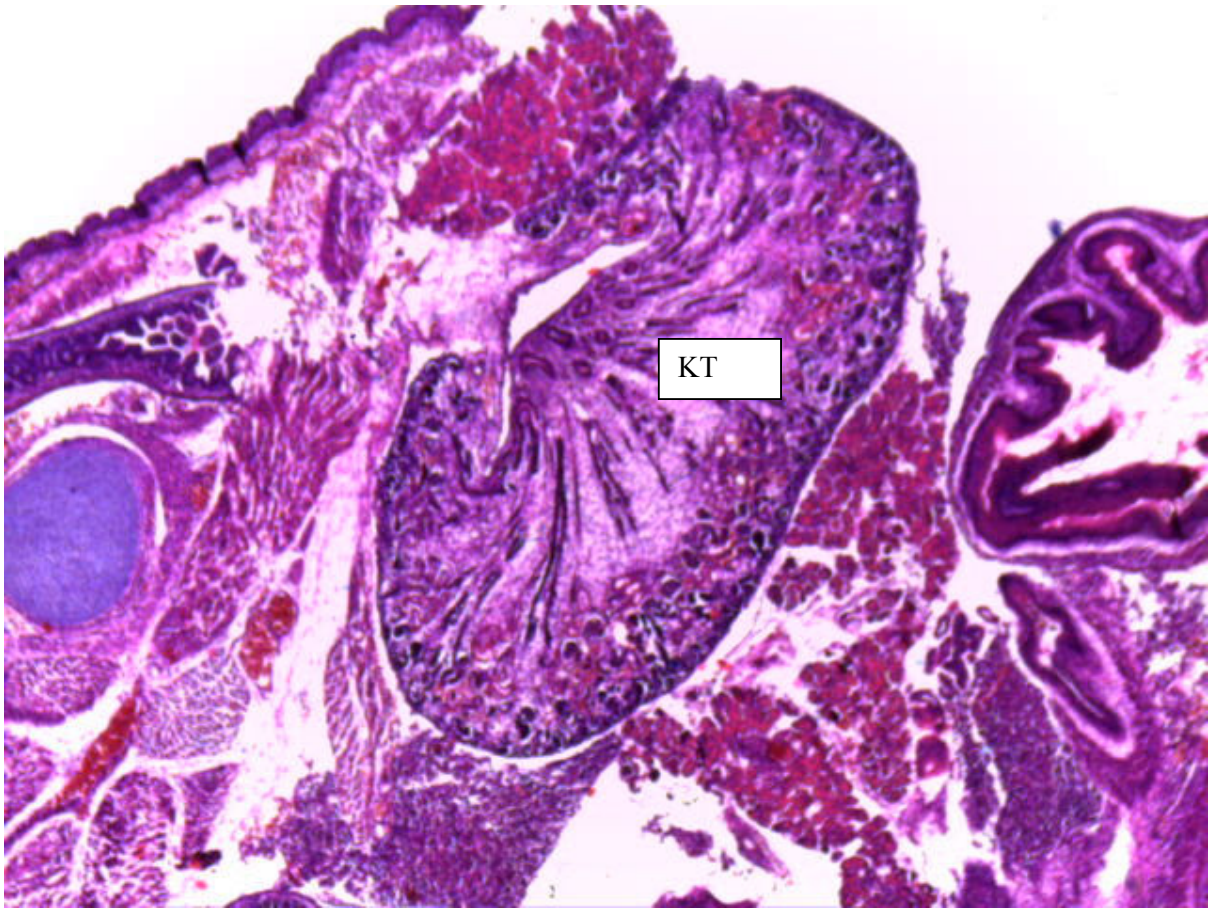

Plate 4.7: **Transverse section of foetus of female Albino mouse administered 2X HTD of Sulfadoxine-pyrimethamine and stained with H and E stains; shows normal Kidney Tissue (KT), (X 100 magnification under oil immersion microscopy).**

4.7.2 Sperm Assay test following Sulfadoxine-Pyrimethamine Administration

Spermatoazoa abnormalities were recorded in 5, 7 and 10 weeks following the exposure to the SP. Sperm observed at these times were presumably exposed to the SP while they were spermatocytes and spermatogonia. Plates 4.8 – 4.10 illustrate both normal and abnormal sperm cells. In the course of scoring the abnormalities, the various abnormalities found included heads with no hook, amorphous head, pin head bent tail and tail folded over head (Plates 4.8 – 4.10)., the pin head seemed to be more predominant followed by coiled tail. These abnormal cells occurred with different frequencies in both treated and control mice. Table 4.43 shows the effect of different dose levels of SP on sperm head abnormality after 5, 7 and 10 weeks exposure. The negative controls showed 5.3%, 2% and 2% abnormalities respectively compared with 5%, 7% and 3.3% with the 1X HTD. SP did not induce statistically significant increase in sperm abnormality at the 1X HTD over the control, P= 0.0822), nor at the other consecutive dose levels neither was it reproduceable at 7 and 10 week exposure periods, Table 4.43 and Table 4.44.

Table 4. 43: Sperm abnormalities in male albino mice after Sulfadoxine-pyrimethamine administration

Sperm Abnormalities					
Week 5					
Dosage	**AH**	**CT**	**PH**	**BT**	**Total Sperm abnormality**
Control	12	0	20	0	32 (5.3%)
1X	0	0	30	0	30 (5.0%)
1½X	12	0	20	0	32 (5.3%)
2X	12	0	10	0	32 (5.3%)
Week 7					
Control	10	0	0	0	10 (2%)
1X	0	0	20	20	40 (7.0%)
1½X	6	0	8	0	14 (2.3%)
2X	20	0	12	0	32 (5.3%)
Week 10					
Control	12	0	0	0	12 (2%)
1X	0	0	20	10	30 (3.3%)
1½	0	0	30	0	30 (5.0%)
2	12	0	0	0	12 (2.0%)

AH=Amorphous head; CT=Coiled tail; Pin head; BT=Bent tail

Table 4.44: Summary of Sperm Assay

HTD	Total Abnormality (%)				P value
	Exposure period in weeks			Average	
	5	7	10	%	
Control	32 (5.3%)	10 (2%)	12 (2%)	3.1	
1X	30 (5.3%)	40 (7.0%)	30 (3.3%)	5.2	0.0822
1½X	32 (5.3%)	14 (2.3%)	30 (5.0%)	4.1	0.356744
2X	32 (5.3%)	32 (5.3%)	12 (2.0%)	4.2	0.356744

The percentages are means for groups of six mice for each SP dose

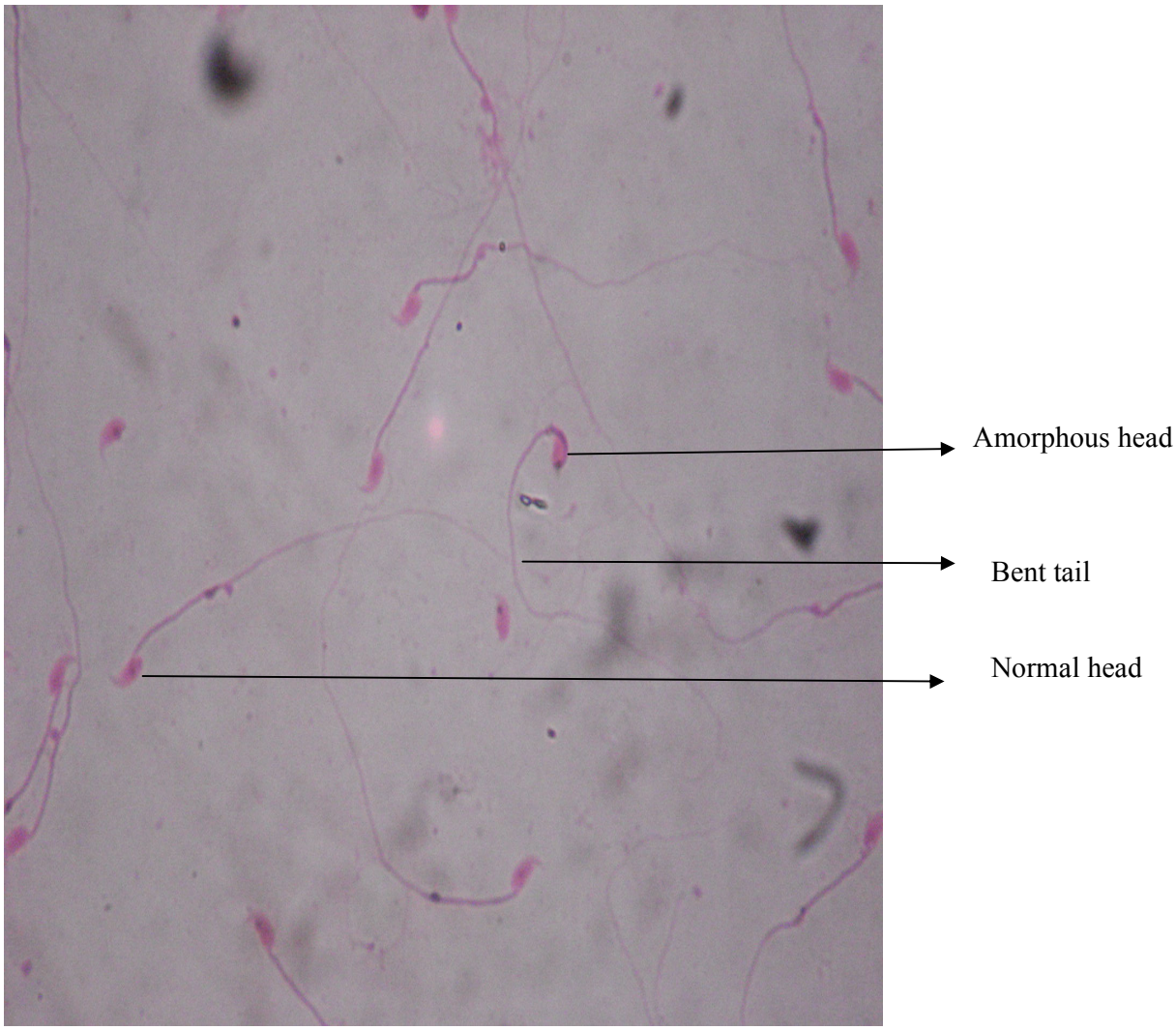

Plate 4.8: Control: Sperm of male Albino mouse shows normal sperm heads; bent tail and amorphous head; Stained with Eosin and examined under oil immersion (X 100 magnification).

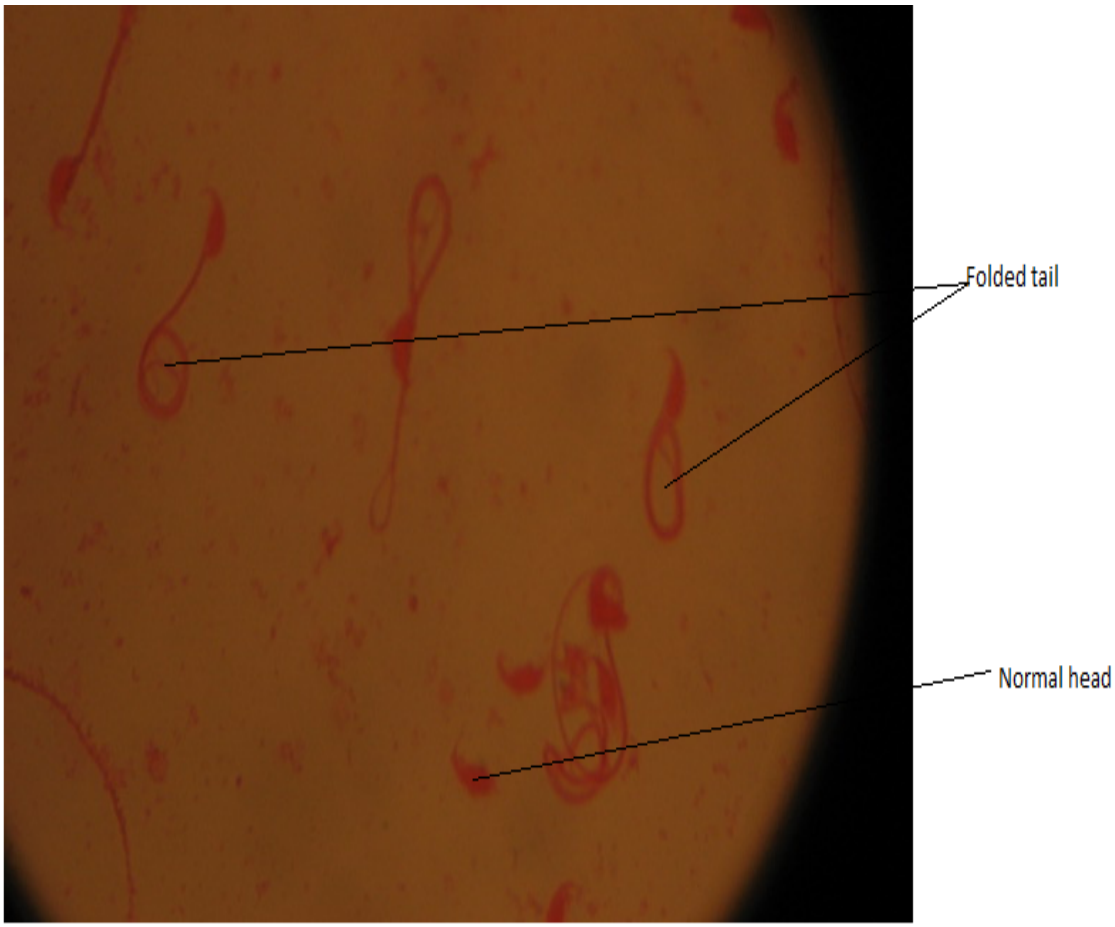

Plate 4.9: Male Albino mouse administered 1½X HTD of Sulfadoxine-pyrimethamine; Shows sperm cells with coiled tails and heads; Stained with Eosin and examined under oil immersion (X 100 magnification).

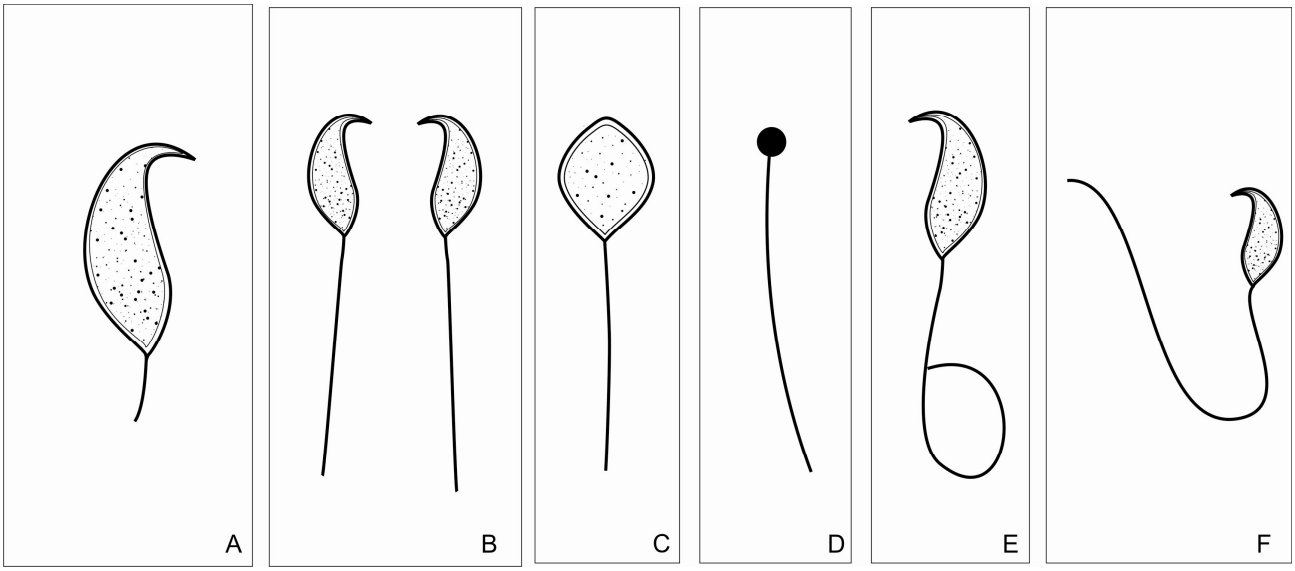

Plate 4.11: Sketch of different types of observed spermatozoa abnormalities. A=Normal head without tail; B=Normal heads; C=Amorphous head; D= Pin head; Coiled tail; F= Bent tail.

CHAPTER FIVE

5.0 DISCUSSION

5.1 ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICES OF COMMUNITY MEMBERS ON MALARIA

The community members in Badagry LGA demonstrated a good knowledge of malaria. Malaria was stated as a leading problem in the study community as corroborated by studies in other malaria endemic countries (Deressa and Ali, 2009; Okwa and Ibidapo, 2010). Majority (87%) were able to describe the signs and symptoms of malaria with fever being the most common. Malaria was identified mainly on the basis of the symptoms of fever/high temperature, general body weakness, bitter tongue and headache as previously reported (Dunyo *et al*., 2000; Booth *et al*., 2001; Deressa *et al*., 2003; Hlongwana *et al*., 2009). Oreagba *et al.,* (2004), however showed that urban dwellers are more knowledgeable than rural dwellers. Dike *et al*., (2006), in South east Nigeria have found a statistically significant association between higher knowledge of malaria among mothers who were educated, skilled or professionals than among the uneducated or unskilled category.

Despite the good knowledge of malaria by respondents in Badagry LGA, anaemia and convulsions were poorly associated with the disease by respondents. Their lack of clear knowledge on anaemia and convulsions which are associated with malaria in children could lead to delay in seeking appropriate care. Eighty one percent of respondents also associated malaria with mosquito bite as confirmed by various other studies conducted in Tanzania and Kenya (Mazigo *et al*., 2010; Imbahale *et*

al., 2010). This contrast with the findings of Okwa and Ibidapo, (2010) which reported that as high as 41.7% of respondents had misconception on malaria.

Health facilities were the most common sources of malaria treatment in the study population; 32% of respondents sought treatment in the health facility as corroborated by the study of Otubanjo *et al*., 2002; Nuwaha, 2002; Deressa *et al*., 2003; Hlongwana *et al.,* 2009; Okwa and Ibidapo, 2010. However treatment was sought by only 23% of respondents from the Badagry study within 24 hours. This is in disagreement with the findings obtained in Swaziland by Hlongwana *et al*., (2009) where 90% will seek treatment within 24 hours. The practice in Badagry LGA is far below the Abuja target which stipulates that 80% of those suffering from malaria seek and receive treatment within 24 hours of onset of the illness (WHO, 2005). Malaria self-treatment was also practiced by the participants in Badagry LGA as observed in other studies. Self treatment has been severally recorded in Uganda, (Kengeya-Kayondo, 1994), Western Kenya, up to 60% (Geissler *et al*., 2000), in Ethiopia (Deressa *et al*., 2003). Informal allopathic providers, such as drug vendors/stores and traditional healers were often consulted by respondents (Ahorlu *et al*., 1997).

Measures taken to prevent mosquito bite in the community were mainly the use of raid insecticide sprays and Tiger mosquito coils. Prevention of malaria included the use of herbs; taking of paracetamol and antimalarial medicines, such as CQ and SP as observed in other studies conducted in Swaziland (Hlongwana *et al*., 2009) and in Nigeria (Anumudu *et al*., 2006; Okwa and Ibidapo, 2010). The use of ITNs/LLNs was not mentioned as a means of malaria prevention in the Badagry study. This

observation was also made in another study conducted in Ethiopia (Deressa and Ali, 2009). The reason could be as a result of poor knowledge on how malaria is transmitted, lack of understanding about the relationship between mosquitoes and malaria, poor understanding about the role of mosquito net in malaria prevention, cultural beliefs and the generally low awareness of the people about disease prevention and control (Deressa and Ali, 2009).

5.2 ASSESSMENT OF KNOWLEDGE ATTITUDE AND PRACTICES OF PREGNANT WOMEN ON MALARIA AND SULFADOXINE-PYRIMETHAMINE FOR MALARIA PREVENTION

In this study, the majority (79%) of pregnant women perceived malaria as a serious illness, but very few (1%) could associate malaria with mosquito. In contrast, the study in Edo State, reported adequate knowledge on the causes of malaria by 69% of respondents (Wagbatsoma and Aigbe, 2010), which is in agreement with the study in Ethiopia (Deressa and Ali, 2009; Paulander *et al.*, 2009). Previous related studies in eastern and northern Nigeria reported low knowledge of malaria in pregnancy and management practice as well as poor maternal health care (Galadanci *et al*., 2007; Enato *et al.,* 2009). The knowledge of pregnant women on the use of SP for IPT for malaria prevention in pregnancy in Badagry LGA was low (6%), at the time of this study. Majority did not know that SP was for malaria prevention; 87% said it was for the treatment of malaria. The poor knowledge of SP for malaria prevention exhibited in this study in Badagry LGA corroborates earlier study conducted in Ekiti by Akinleye *et al*., (2009) but in disagreement with the study in Tanzania by Mubyazi *et al*., (2005) where pregnant women were generally aware of SP as the medicine recommended for IPT.

The poor knowledge of SP as the medicine recommended for IPT of malaria in pregnancy has implication for malaria control. It implies that pregnant women will be unable to demand for IPT during their ANC visits, and possibly Health workers might not have enough knowledge to administer IPT. The poor knowledge will also lead to lack of demand creation for IPT, thus making it impossible to meet the Abuja Target and hence the Millenium Development Goal (MDG) by the year 2015.

A survey conducted by team of experts showed that there is low coverage of IPTp and ITNs in high malaria transmission areas which contrasts with correspondingly high ANC attendance. This indicates that there are missed opportunities for coverage and the attainment and maintenance of high coverage of ITNs remains challenging (Van Eijk *et al*., 2011). The majority (90%) of pregnant women in the Badagry study displayed good knowledge on the correct dosage of SP (3 tablets) to be taken. This corroborates tthe study conducted by Akinleye *et al.,* (2009). Knowledge of SP was influenced by educational level. The pregnant women that had secondary and post secondary education were more knowledgeable on the correct dosing than those with none or primary education ($P<0.05$). Pregnant women of younger age group also had more knowledge of SP than the older ones.

Majority (58%) of the pregnant women said they patronize the Government Hospital because of the quality of care they receive and this showed statistical significance between the level of education and Health Facility attended ($P<0.05$). Malaria prevention by pregnant women in Badagry LGA, was mainly by the use of herbs (30%), CQ (27%) and pyrimethamine (23%), while mosquito control was by use of raid insecticide spray (47.7%) and mosquito coil (21%).

5.3 PREVALENCE OF MALARIA IN THE COMMUNITY AND PREGNANT WOMEN

P. falciparium was the predominant species in the Badagry study. This is similar to the results of Amajoh *et al.,* (2002); Awolola *et al.*, (2002); Nebe *et al*., (2002); Okwa, (2004); Umeaneto *et al.,* (2006). Malaria prevalence in the study community was 23.3% indicating mesoendemicity. The disease was higher in the younger age groups and shows statistical significance ($P<0.05$). The prevalence rate steadily increased from the month of May through to October coinciding with the period of mosquito abundance and period of increased malaria transmission. The mean parasite density did not show statistical significance in the three communities ($P>0.05$) nor between males and females in Ikoga and Pota communities ($P>0.05$). This agrees with the studies of Pelletier *et al.*, (1995) and Afolabi *et al.*, (1997) but contrasts with other studies conducted among coastal dwellers of Lagos State (Nebe *et al.,* (2002); Okwa and Ibidapo, (2010). The mean parasite density obtained in the study community in Badagry LGA was 1714.499±3199.572 which is at variance with the study in Enugu by Nnaji *et al*., (2009) which recorded mean parasite density of 776±1923 in non-pregnant women.

Gametocytes were observed in blood smears thoughout the year, with peaks in the months of March, June and October with June having the highest peak (34.4%). This coincides with the rainy season and peak period for malaria transmission. This period is marked proliferation of breeding which favours the multiplication of *Anopheles* mosquitoes, thus increasing their density. The

rainfall pattern during this period showed an increase between the months of April and October.

Malaria prevalence among pregnant women prior to SP administration was found to be 15.73%. Prevalence rate was higher amongst pregnant women of younger age groups, though not statistically significant, P>0.05. There was no difference in the malaria prevalence rate among the different gravida neither did ethnic or educational backgrounds show any statistical significance. Adults who are long-term residents of areas of moderate or high malaria transmission, including large parts of sub-Saharan Africa, usually have a high level of immunity to malaria. Infection is frequently asymptomatic and severe disease is uncommon. Pregnancy affects the immune system, thus making the pregnant women more susceptible to malaria (Bouyou-Akotet *et al*., 2003). Studies by various researchers have shown that pregnant women of younger age groups as well as primigravida are more vulnerable to malaria parasite infection because their immune system is compromised (Adefioye *et al*., 2007; Roger *et al*., 2007; Nnaji *et al*., 2009; Agan *et al.,* 2010). The impairment of humoral and cell mediated immunity results in increased susceptibility and increase in incidence of complications among primigravida and secundigravida when compared to non-pregnant women (Schulman and Dorman, 2003; Guyatt *et al*., 2004; Mubyazi *et al*., 2005).

The malaria prevalence rate among pregnant women obtained in the Badagry study is at variance with other studies that reported parasite rates as high as between 62% and 82% (Okwa *et al*., 2003; Adefioye *et al*., 2007; Uneke *et al*., 2008; Akinyele *et al*., 2009). Agomo *et al*., (2009) however recorded parasite rate of 7.7% in Lagos State. The mean parasite density among the gravida in the Badagry study were

2236.2 for primigravida, 2064.6 for secundigravida and 548.7 for multigravida and showed no statistical association (P<0.05). The mean parasite density obtained from this study (1594±1640) for age groups 16-20 years is in agreement with the study of Nnaji *et al*., (2009) who recorded mean parasite density of 1978±1532 in non-pregnant women. The variance in the high malaria prevalence rate reported by different workers was thought to be as a result of multiple factors. One factor is the method of diagnosis. Higher parasite rate may be obtained by the use of PCR (Mokuolo *et al*., 2009).

5.4 PREVALENCE OF ANAEMIA AMONG COMMUNITY MEMBERS AND PREGNANT WOMEN

The prevalence of anaemia among community members in Badagry was higher among the younger age group and was higher during the rainy season. The prevalence of anaemia among children that were less than 5 years was 37.9% followed by 22.9% for those that were between 5 and 9 years, while 10-14 years was 10.2% and 10.5% for those who were 15 years and above. There is a statistical significance between age group and anaemia (P<0.05). However, the prevalence rate is lower and in contrasts to findings from Ghana by Ronald *et al*., (2006), which recorded a prevalence rate of 67.8% among children less than 5 years in urban centre compared to Badagry LGA which is peri-urban.

Anaemia among pregnant women in Bagagry LGA ranged between 39.3% and 54.5% with 54.8% of the secundi-gravida being anaemic; 39.3% and 48.2% of primigravida and multigravidas were anaemic respectively. Analysis of data showed no statistical significance, between gravida and anaemia (P>0.05). This is

corroborated by the study of Agan *et al*., (2010) which found no ststistical significance between anaemia and gravida, but contrasts the reports of (Nagaraj, 2003; Dairo and Lawoyin, (2004). Thirukkanesh and Zahara, (2010) reported 35% prevalence rate of anaemia among pregnant Malaysian women. The study conducted by Komolafe *et al.,* (2005) in Ilesha indicated that 62% of pregnant women investigated were anaemic with women of higher parity having higher prevalence of anaemia during pregnancy (Nwonwu *et al*., 2009). Idowu *et al*., (2005), also recorded anaemia in as high as 80.6% of primigravidae with 1.9% of primigravida having severe anaemia.

In the Badagry LGA study, anaemia was found to be highest among the age group 16-20 years (30.8%), with 1.1% of primigravida and 0.7% of multigravida having severe anaemia, as corroborated by the study of Idowu *et al*., (2005). Ogbeide *et al*., (1994) and Thangaleela and Vijayalakshmi (1994) observed that antenatal bookings were late among primigravidas and this may have contributed to the high prevalence of anaemia recorded. Early antenatal care is expected to result in better monitoring and early detection of anaemia and its correction by appropriate supplementation. The report of the World Health Organization stated that anaemia is significantly higher in the 3rd trimester of pregnancy than the first two trimesters (WHO, 1992). The study of Idowu *et al.,* (2005) also showed that pregnant women attending TBA were more anaemic than those attending health facilities. This is corroborated by the study in Badagry where pregnant women attending the TBA were more anaemic than those attending other health facilities, and showed statisitical significance between health facility and anaemia ($P<0.05$).

5.5 PREVALENCE OF FEVER IN THE COMMUNITY AND PREGNANT WOMEN

The prevalence of fever in the community was age related, with the younger age group having higher percentage of fever cases. This is also related to the under development of their immune system. The percentage of fever cases were highest in July, followed by the month of October which represents the peak period of malaria transmission. However it was observed that not all that were parasitaemic had fever. In the age group of less than five years that had malaria prevalence of 33.8%, only 3.5% had fever indicating the possibility of remaining afebril even in the presence of malaria infection (Okwa and Ibidapo, 2010). Prevalence of fever among pregnant women was 1.2%. Fever did not seem to be a major problem among the pregnant women. Fever has not been found to be a good indicator of clinical malaria. Despite the fact that in endemic areas asymptomatic *P. falciparum* infections are frequent in adults, studies have shown that malaria was not the main aetiology of fever during pregnancy (Bouyou-Akotet *et al*., 2003).

5.6 SPLEEN RATE DETERMINATION IN CHILDREN (2-9 YEARS)

The monthly spleen rate increased with increase in malaria prevalence with an overall spleen rate of 24.9%. There is a significant association between spleen enlargement and malaria prevalence rate ($P<0.05$). The spleen rate obtained in Badagry study corroborates the study of Kaur, (2009) which recorded spleen rate of 23.7%. In the study in Badagry LGA, the average enlarged spleen was calculated to be 2 while 1.22 was reported from the study by Kaur, (2009) which is indicative of malaria endemic area. The AES has long been recognized as a good indicator of the

immunity of the population, since it has been shown that epidemics did not occur in areas where the spleen rate was consistently high, while a declining spleen rate was an indication of increasing epidemic risk. The Kampala conference of 1950 based its definition of endemicity on the spleen rate. Holoendemicity and hyperendemicity were defined as the condition that the spleen rates in children aged 2-9 years should at all times be greater than 75% for holoendemic and between 50% and 75% for hyperendemic malaria; 50% and 10% as mesoendemic and less than 10% as hypoenendemic (Hay *et al*., 2008). The endemicity in this study area was mesoendemic.

5.7 DIAGNOSIS USING RAPID DIAGNOSTIC TECHNIQUE (RDT)

Diagnosis of malaria parasite showed that microscopy is still the gold standard. When compared with OptiMAL RDT, microscopy gave a parasite rate of 51% compared to 19.6% by OptiMAL. The sensitivity and the negative predictive value for OptiMAL of 38% and 59.7% respectively obtained in the Badagry LGA study is in agreement with the result of Londono *et al.,* (2002) who obtained sensitivity of 40% and specificity of 98%. The result of Badagry study is however at variance with the study of Tagbor *et al.*, (2008) who found sensitivity and negative predictive values of 96.6% and 92.6% respectively. VanderJagt *et al*., (2005) found the OptiMAL RDT insensitive in a clinic in Lagos state. A negative RDT result therefore cannot at present be accepted at face value and will need to be confirmed by microscopic examination.

5.8 ADMINISTRATION OF SULFADOXINE-PYRIMETHAMINE (SP)

In this study, 64% of pregnant women received 2 doses of SP while 36% received one dose. This is contrary to what has been reported elsewhere (Anders *et al*., (2008) where it was estimated that 70% and 35% of pregnant women in Tanzania received one and two doses of SP respectively. In this study, administration of one or two doses of SP depended on when the pregnant women registered for antenatal care. Those that received one dose either reacted to SP or registered at late third trimester. The acceptability (64%) of SP in Badagry LGA seems good but would require more awareness creation to enroll all pregnant women.

5.8.1 Effects of Sulfadoxine-Pyrimethamine on Prevalence of Malaria, Anaemia, Fever, Low Birth Weight and Preterm Delivery

IPTp-SP was shown to reduce malaria prevalence rate in pregnant women in Badagry from 15.7% to 2.1%. This is an indication that IPT was able to reduce the rate of malaria infection among pregnant women and shows an association between SP administration and parasite rate ($P<0.05$). This agrees with various researches conducted in East and West Africa, which showed the protective effect of SP on prevalence of maternal parasitaemia, (Parise *et al*., 1998; Rogerson *et al*., 2000; Challis *et al*., 2004; van Eijk *et al*., 2004; Kayentao *et al*., 2005; Mbonye *et al*., 2006; Ramharter *et al*., 2007). The result is in agreement with the study of Ramharter *et al*., 2007; Falade *et al*., 2007) who observed decrease in prevalence of maternal *Plasmodium* parasitaemia after SP administration (risk ratio 0.16, $P < 0.001$). Comparison of prevalence rate between those who took SP and those who did not take SP showed statistical significance, $P<0.05$ and is corroborated by the study of Aziken *et al*., (2010).

SP admimistration was able to reduce placental malaria and showed statistical significance ($P<0.05$). Majority of pregnant women who took SP did not have malaria parasite in their placenta compared with those who did not take SP. This corroborates earlier studies conducted in East Africa and Mali which showed the protective effect of SP on prevalence of maternal and placental malaria (Kayentao *et al*., 2005). Various studies in which pregnant women were given IPT have shown reduction in placental malaria of 72% in Primigravidae and Secundigravidae (Shultz *et al*., 1994; Parise *et al*., 1998; van Ejik *et al*., 1998; Challis *et al*., 2004; van Eijk, 2004). In a study carried out in Ibadan, southwest Nigeria, IPTp-SP was found to be highly effective in preventing maternal and placental malaria among parturient women as well as in improving pregnancy outcomes such as delivery of bigger babies and lower prevalence of pre-term deliveries and maternal anaemia (Falade *et al*., 2007). In another study conducted in southern Ghana, placental *P. falciparum* infection was reduced by 43-57% ($P < 0.0001$) and median birth weight was 130g higher ($P = 0.02$), Hommerich *et al*., 2007).

However, placental *P. falciparum* infection was still observed in 11% of women (Hommerich *et al*., 2007). This suggests that the remnant prevalence of infection in women having taken three doses of IPTp-SP requires that additional antimalarial measures to prevent malaria in pregnancy in such region (Hommerich *et al*., 2007). Studies have shown that low EIRs during the dry season do not necessarily equate to low placental parasitaemia. Estimates of dry season prevalence are only slightly lower than those in the wet season (Brabin *et al*., 2008).

IPTp-SP during pregnancy was shown to reduce the prevalence of anaemia from 44.7% to 37.9%, in the study in Badagry LGA, and showed statistical significance ($P<0.05$). This is corroborated by the study of Kayentao *et al*., (2005) who demonstrated the protective effect of SP against maternal anaemia. The study showed that the number of pregnant women that had anaemia decreased with the number of SP doses taken and there was a significant association between the number of SP doses taken and anaemia ($P<0.05$). IPTp with SP has been shown to reduce the risk of maternal anaemia, and shows a beneficial effect on the haemoglobin (Schulman *et al*., 1999) and in Malawi (Rogerson *et al*., 2000). In Southern Ghana, SP was shown to reduce maternal anaemia by 33% and showed statistical significance, $P = 0.0009$, (Hommerich *et al*., 2007).

In Gabon, maternal anaemia was the second marker for maternal health in the study. Although a trend for a reduced prevalence of moderate and severe anemia was observed, this reduction did not reach the level of statistical significance. This finding is in contrast to a previous report from a controlled setting in Kenya where IPTp-SP had a protective efficacy of 39% against severe anaemia (Shulman *et al*., 2004). However, no beneficial effects on prevalence of maternal anemia have been reported for multigravida women in The Gambia (Mbaye *et al*., 2006). The implementation of IPT as a national program in Gabon was paralleled by a dramatic reduction of maternal *P. falciparum* prevalence (Ramharter *et al*., 2007**)**. Markers of neonatal health were affected beneficially, particularly in primi- and secundi-gravid women.

Another study conducted among pregnant women in Mali by Maiga *et al*., (2011) showed that adding a third dose of IPTp-SP halved the risk of placental malaria, LBW, and preterm births in all gravidas, compared with the standard 2-dose regimen, in that area of highly seasonal transmission with low levels of SP. There was however no significant reductions in maternal anaemia, which is in contrast to the result obtained from the Badagry study, where there is a significant association between IPTp-SP administration and anaemia. The etiology of maternal anemia is complex, and nutrient deficiencies, worm infestation, chronic inflammation, and HIV are important contributing factors and about half of pregnant women in sub-Saharan Africa have iron deficiency (van den Broek *et al*., 2000; van den Broek, 2001; ter Kuile *et al*., 2004). This means that even though pregnant women take IPT, there is the likelihood of anaemia being present.

IPTp-SP was able to reduce the proportion of pregnant women with fever from 1.2% to 0.4%, although not statistically significant, P=0.287. Fever has not been found to be a good indicator of clinical malaria. Despite the fact that in endemic areas asymptomatic *P. falciparum* infections are frequent in adults, studies have shown that malaria was not the main aetiology of fever during pregnancy. Other causes like urinary and genital infection could be the cause and should be treated to avoid subsequent obstetrical problems (Bouyou-Akotet, *et al.,* 2003). Reliance on reported febrile illness will not be adequate to identify parasitaemic pregnant women because many of those with heavy placental parasitisation may not report fever. This justifies the place of the IPT using SP in pregnant women living in malaria endemic areas such as sub-Saharan Africa (Nnaji and Ikechebelu, 2008).

The prevalence of LBW obtained from the study in Badagry LGA was 4 % which is far lower than the Nigerian national average of 14% (UNICEF, 2007). The prevalence of LBW in developed world are 5%, 6%, 8%, from Norway, Canada, and United Kingdom (UNICEF, 2007) respectively. The prevalence of LBW obtained from Badagry LGA is lower than the national average of other developing countries like 8.1% reported in Benin (Onyiriuka, 2006), Ghana (16%), Togo (18%), and Sierra Leone (23%) (Aitken, 1990; UNICEF, 2007). The prevalence of LBW among the pregnant women in the Badagry study was not reduced after SP administration. There was no difference in the birth weight of babies delivered by pregnant women who took SP (426) and those who did not take SP (57) ($P>0.05$). It could be because the sample size of those who did not take SP was small. A significant association was also observed between LBW and HF attended, $P=0.03$. Studies carried out in Ibadan, South west Nigeria showed that in addition to preventing maternal and placental malaria among parturient women, SP was able to improve the pregnancy outcomes, including the delivery of bigger babies (Falade *et al*., 2007). SP had no effect on delivery term and showed no statistical significance $P<0.05$), although Falade *et al.*, (2007) reported a lower prevalence of pre-term deliveries with SP administration.

5.9 VECTOR STUDIES

The monthly density variations of the mosquitoes observed in Badagry LGA is similar to those reported elsewhere in Nigeria (Hannay, 1960; Awolola *et al*., 2002; Onyido *et al*., 2009). Significantly higher densities of mosquitoes were collected in the rainy than dry season. A study in Kenya showed that the rainy season presents favourable environmental conditions that enhance mosquito breeding and survival,

through the proliferation of larval habitats and improved humidity, respectively (Minakaw *et al.*, 2002). The PCR showed that *Anopheles gambiae* complex, (the main vector of malaria parasite) comprised *Anopheles gambiae* and *Anopheles arabiensis* in the ratio of 2:1. The EIR in the three communities that make up the Ikoga ward was calculated to be 19.34 infective bites per person per year. The entomological inoculation rate in the three communities showed no statisitical significance (P>0.005). The annual entomological inoculation rate has association with malaria endemicity. The malaria endemicity in Badagry LGA was found to be mesoendemic and this is related to the low entomological inoculation rate of 19.34 infective bites per person per year (ib/p/year). In Kenya, the EIR value for *An. gambiae s.l.* was 29.2 ib/p/year (Shilulu *et al.,* 1998).

Areas with high malaria endemicity usually have high annual entomological inoculation rate. The study conducted in Uganda recorded EIR of between 5 and 1,500 ib/p/year (Hay *et al*., 2000). The study showed that the incidence of clinical *P. falciparum* malaria in children less than 18 months of age increased with increasing EIR (Smith *et al*., 1998) and furthermore, mortality in children < 1 year of age strongly increased with increase in EIR (Smith *et al*., 2001). The available data indicate that the diversity of the vectorial system might be affected by man-made environmental changes with relevant effects on ecoepidemiological stratification. Studies carried out in southern Nigeria showed that deforestation and urbanization favour the penetration of *Anopheles arabiensis* into rain forest zones originally occupied only by *Anopheles gambiae* monomorphic for the standard chromosome-2 arrangement (forest chromosome form) (Coluzzi *et al.*, 1985; and Kristan *et al.*, 2003). The human vector contact shows a remarkable stability and

flexibility, producing extremely high inoculation rates in a wide range of geographic and seasonal ecological conditions (Coluzzi, 1984). It is estimated that each person receives between 40- 140 infective bites each year (Moulineaux and Grammicia, 1980).

5.10 HISTOPATHOLOGICAL STUDIES TO DETERMINE MUTAGENIC EFFECT OF SP ON FOETUS OF PREGNANT ALBINO MICE

The histopathological studies showed no adverse effect of SP on the foetus of laboratory bred albino mice. Histopathological study on both the foetus from the test and control mice showed no significant difference in weight or abnormality of the cells. Studies carried out with SP on animals have shown the safety of SP (Peters *et al*., 2007). Although folate antagonist use in the first trimester is associated with neural tube defects, large case-control studies have demonstrated that sulfadoxine/pyrimethamine administered as IPTp (exclusively in the second and third trimesters and after organogenesis) does not result in an increased risk of teratogenesis (Peters *et al.*, 2007).

5.11 ASSESSMENT OF SPERM ABNORMALITY

Sperm abnormalities were observed in both test and control mice that were administered SP. There was no specific type of abnormality that was predominant as they all occurred with different frequencies in both treated and control mice. The number of pin head was highest in this study as observed in the study of Adoloju *et al.,* (2008), although chemical was used instead of medicine. The percentage abnormality in the control for week 5, 7and 10 were 5.3%, 2% and 2% respectively. SP induced statistically significant increase in sperm abnormality at the 1X HTD

over the control ($P<0.05$), but not at other consecutive dose levels and this was not reproduceable at 7 and 10 week exposure periods.

Thus the abnormality of sperm heads observed for these exposure periods may be due to induced point mutations in the early spermatocytes and spermatogonia at the pre-meiotic stages of spermatogenesis (Otubanjo and Mosuru, 2007). An *in vivo* evaluation of the induction of abnormal sperm by Sulphamethoxypyridazine-pyrimethamine (Metakelfin) showed that 0.5X the human therapeutic dose gave a statistically significant increase over the negative control value. The study recorded that a higher dose produced fewer abnormalities than the proceeding lower dose level. The study therefore concluded that Metakelfin is probably not mutagenic as induction of sperm head abnormality was not dose dependent (Otubanjo and Mosuru, 2007).

The occurrence of sperm head abnormalities have also been attributed to the chromosomal aberrations that occur during the packaging of genetic material in the sperm head or occurrence of point mutation in testicular DNA (Bruce and Heddle, 1979; Odeigah, 1997). Odeigah, (1997) reported that exposure to the chemicals could produce pituitary hypothalamic or sex hormonal deffects which in turn could affect spermatogenesis or exposure could cause abnormalities in seminal fluid resulting in functional or structural impairment of sperm.

Sperm abnormality may also arise as a consequence of naturally occurring level of mistakes in the spermatozoon differentiating process during spermatogenesis (Bakare *et al.,* 2005). During spermatogenesis, DNA synthesis occurs before the pre-meiotic phase and no further synthesis occurs throughout the duration of

spermatogenesis in the cell cycle. The inference from the Badagry study is that SP may not be adjudged to induce sperm head abnormalities and may not be mutagenic.

5.12 SAFETY OF SULFADOXINE-PYRIMETHAMINE IN PREGNANT WOMEN

Sulfadoxine-pyrimethamine (SP) was well tolerated in the pregnant women. In this study there was no recorded case of any adverse drug reaction attributable to SP during the study. Five pregnant women complained of headache, dizziness and weakness after administration of the first dose of SP. These all terminated within 30 minutes of taking SP. There were no congenital malformations or deaths (maternal or neonatal) among the study participants.

A single study conducted elsewhere found an increased risk of kernicterus in neonates treated with sulphonamides (White, 2005), but this requires further investigation.

5.13 CHALLENGES

The logistics involved in having access to a Polymerase Chain Reaction (PCR) machine was a challenge initially in Nigeria during the commencement of the study. The second challenge was difficulty in establishing pregnancy and its duration in female mice tha was used for histopathological studies. The third challenge was the misconception of the study community about the purported increase in mosquito density in the study communities after the commencement of the study. The fourth challenge was that five pregnant women delivered outside the hospital and were lost to follow up.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

Sulfadoxine-pyrimethamine was well tolerated in the pregnant women in this study. Adverse drug reaction attributable to SP was not recorded. Five people had few complaints like headache and weakness which terminated within 30 minutes of taking SP. There were no congenital malformations (maternal or neonatal) among the study participants.

IPT has been shown to reduce the prevalence of malaria among pregnant women; therefore pregnant women should be encouraged to take IPT during pregnancy to prevent malaria. However IPT should be used with another preventive measure for maximum benefit. There is also a need to create awareness on the use and benefits of IPTp-SP in Badagry LGA to increase acceptability and also achieve the target of Roll Back Malaria (RBM) and the Millennium Development Goal, number 6.

6.1 CONTRIBUTIONS TO KNOWLEDGE

(1) Prevalence data on malaria endemicity in Ikoga was documented.

(2) Microscopy was confirmed as the gold standard in malaria diagnosis in comparison with OptiMAL rapid diagnostic test (RDT).

(3) The acceptability and efficacy of IPTp-SP in the study area was established

(4) The study indicated that IPTp-SP requires additional supported preventive measures for maximum benefit.

(5) *An. gambiae ss* and *An arabiensis* were identified both morphologically and at molecular level as the major malaria vectors.

(6) Absence of foetal abnormality, mutagenicity and mortality in experimental mice established the safety of Sulfadoxine-pyrimethamine.

REFERENCES

Abasiattai, A.M., Etukumana, E.A. and Umoiyoho, A.J. (2009). Awareness and Practice of Malaria Prevention Strategies Among Pregnant Women In Uyo, SouthSouth Nigeria. *The Internet Journal of Gynecology and Obstetrics,* 2009 Vol. 11 no. 1.

Adams, I., Elhassan, E.M., Haggaz, A.E., Ali, A.A. and Adam, G.K. (2011). A perspective of the epidemiology of malaria and anaemia and their impact on maternal and perinatal outcomes in Sudan**.** *Journal of Infection in Developing Countries*, **5(2**):83-87.

Adefioye, O.A., Adeyeba, O.A., Hassan, W.O. and Oyeniran, O.A. (2007). Prevalence of Malaria Parasite Infection among Pregnant Women in Osogbo, Southwest, Nigeria. *American-Eurasian Journal of Scientific Research,* **2**:43-45.

Ademowo, O.G. (2000). Malaria: Africa's health challenge of the millennium. *Journal of Transfigural Mathematics,* **6**:29-38.

Adeneye, A.K., Jegede, A.S., Mafe M.A. and Nwokocha, E.E. (2007). A pilot study to evaluate malaria control strategies in Ogun state, Nigeria. *World Health and Population,* **9(2):**83-94.

Adinma, J.I.B., Ikechebelu, J.I., Onyejimbe, U.N., Amilo, G. and Adinma, E. (2002). Influence of antenatal care on the haematocrit value of pregnant Nigerian Igbo women. *Tropical Journal of Obstetrics and Gynaecology,* **19**: 68-70.

Aduloju, R.K., Otubanjo, O.A. and Odeigah, P.S.C. (2008). Chemical induction of sperm abnormalities in mice. An *In Vivo* Assay of the Mutagenic Potential of Praziquantel (PZQ) Using Sperm Head Abnormality Test. *Journal of Human Ecology,* **23**(1):59-63**.**

Afolabi, B.M., Sodeinde, O. and Audu R.U. (1997). Malaria in early infancy on the Atlantic coast of Lagos, Nigeria. *Nigeria Journal of Medical Research*, **1(2):**32-36.

Agan, T.U., Ekabua, J.E., Udoh, A.E. and Mgbekem, M.A. (2010). Prevalence of anaemia in women with asymptomatic malaria parasitemia at first antenatal care visit at the University of Calabar Teaching Hospital, Calabar, Nigeria. *International Journal of Women's Health*, **2010(2):** 229 – 233.

Agomo, P.U., Asianya, V., Akindele, S.K., Agomo, C.O., Akinyele, M.O., Adewole, T.A., Igbasi, U.T., Anyanwu, R.C. and Egbuna, K.N. (2003). Investigation of the efficacy of two rapid assessment techniques (OptiMAL 1 and SD-Bioline) for the diagnosis of malaria in rural areas of Nigeria. *African Journal of Clinical and Experimental Microbiology,* **4(1):**6-7.

Agomo, C.O, Oyibo, W.A., Anorlu, R.I. and Agomo, P.U. (2009). Prevalence of Malaria in Pregnant Women in Lagos**.** *African Journal of Reproductive Health,* **7:**77-83.

Ahorlu, C.K., Dunyo, S.K., Afari, E.A., Koram, K.A. and Nkrumah, F.K. (1997). Malaria-related beliefs and behaviour in southern Ghana: implications for treatment, prevention and control. *Tropical Medicine and International Health* **2(5):** 488–499.

Agyepong, I.A., Aryee, B., Dzikunu, H. and Manderson L. (1995). *The Malaria Manual*, UNDP/World Bank/World Health Organization, Special Programme for Research and Training in Tropical Diseases (TDR).

Aitken, I.W. (1990). Determinants of low birth weight among the Mendi of Sierra Leone: Implications for medical and socio-economic strategies. *International Journal of Obstetrics and Gynaecology,* **33:** 103-109.

Akinleye, S.O., Falade, C.O. and Ajayi, I.O. (2009). Knowledge and utilization of intermittent preventive treatment for malaria among pregnant women attending antenatal clinics in primary health care centers in rural southwest, Nigeria: a cross-sectional study. *British Medical Center, Pregnancy Childbirth,* **9**:28.

Amajoh, C.N, Odukoko, A.B. and Mosanya, M.E (2002). Preliminary investigations on malaria in the atlantic coastal margin of Ibeju-lekki, Lagos State, Nigeria. *Journal of Malaria in Africa and Tropics,* **1(1):** 17-19.

Anders, K., Marchanti, T., Chambo, P., Mapunda, P. and Reyburn, H. (2008). Timing of intermittent preventive treatment for malaria during pregnancy and the implications of current policy on early uptake in north-east Tanzania. *Malaria Journal,* **7:**79.

Anorlu, R.I., Odum, C.U. and Essien, E.E. (2001). Asymptomatic malaria parasitaemia in pregnant women at booking in a primary health care facility in a periurban community in Lagos, Nigeria. *Africa Journal Medical Science,* **30**: 39–41.

Anumudu, C.I., Adepoju, A., Adediran, M., Adeoye, O., Kassim, A. and Oyewole, I. (2006). Malaria prevention and treatment seeking behaviour of young Nigerian adults. *Annals of Africa Medicine,* **5(2):** 82-88.

Archibald, H.M. (1958). Influence of maternal malaria on newborn infants. *British Medical Journal,* ***ii***: 1512-1514.

Avery-Jones, S. (1958). Mass drug treatment with pyrimethamine. A study of resistance and cross resistance resulting from a field trial in the hyperendemic malarious area of Makueni, Kenya Sept 1952-Sept 1953. *Transactions of Royal Society of Tropical Medicine and Hygiene,* **52**: 547-561.

Awolola, T.S, Okwa, P., Hunt, R.H, Ogunrinade, A.F. and Coetzee, M. (2002). Dynamics of the malaria-vector populations in coastal Lagos, south-western Nigeria. *Annals of Tropical Medicine and Parasitology,* **96(1):** 75-82.

Awolola, T.S., Oduola, A.O., Obansa, J.B., Chukwurah, N.J. and Unyimade, J.P. (2007). Insecticide resistance in the *Anopheles gambiae* complex in Nigeria. *Journal of Vector Borne Disease*, **44**:241-244.

Aziken, M.E., Akubuo, K.K. and Gharoro, E.P. (2011). Efficacy of intermittent preventive treatment with sulfadoxine–pyrimethamine on placental parasitemia in pregnant women in midwestern Nigeria. *International Journal of Gynaecology and Obstetrics,* **112(1):** 30-33.

Baird, J.K. (2005). Effectiveness of antimalarial drugs. *New England Journal of Medicine,* **352**: 1565-1577.

Baker, J., Ho M.F, Pelecanos, A., Gatton, M., Chen, N., Abdullah, S., Albertini, A., Ariey, F., Barnwell, J., Bell, D., Cunningham, J., Djalle, D., Echeverry, D.F., Gamboa, D., Hii, J., Kyaw, M.P., Luchavez, J., Membi, C., Menard, D., Murillo, C., Nhem, S., Ogutu, B., Onyor, P., Oyibo, W., Wang, S.Q., McCarthy, J. and Cheng, Q. (2010). Global sequence variation in the histidine-rich proteins 2 and 3 of *Plasmodium falciparum*: implications for the performance of malaria rapid diagnostic tests. *Malaria Journal*, **9**:129.

Bakare, A.A., Mosuro, A.A. and Osibanjo, O. (2005). An *in vivo* evaluation of induction of abnormal sperm morphology in mice by landfill leachates. *Mutation Research,* **582**: 28–34.

Bako, B.G., Audu, B.M., Geidam, A.D., Kullima, A.A., Ashiru, G.M., Malah, M.B., Ngadda, H.A. and Musa, A.B. (2009). Prevalence, risk factors and effects of placental malaria in the UMTH, Maiduguri, North-eastern, Nigeria: a cross-sectional study. *Journal of Obstetrics and Gynaecology,* **29(4):** 307-310.

Beare, N.A.V., Taylor, T.E, Harding, S.P., Lewallen, S., Molyneux, M.E. (2006). "Malarial retinopathy: a newly established diagnostic sign in severe malaria". *American Journal Tropical Medicine Hygiene,* **75**(5): 790–797.

Beck, S., Mocknhaupt, F.P., Bienzle, U., Eggelte, T.A., Thompson, W.N. and Stark, K. (2001). Multiplicty of *Plasmodium falciparum* infection in pregnancy. *American Journal of Tropical Medicine and Hygiene,* **65**: 631-636.

Beier, J.C., Killeen, G.F. and Githure, J. (1999). A climate-based distribution model of malaria transmission in sub-Saharan Africa. *Parasitology Today,* **15**: 105-111.

Binka, F. and Akweongo, P. (2006). Prevention of malaria using ITNs: potential for achieving the millennium development goals. *Current Molecular Medicine,* **6:** 261-267.

Birley, M.H. and Charlewood, J.D. (1987). Sporozoite rate and malaria prevalence. *Parasitology Today,* **3**:231–232. doi: 10.1016/0169-4758(87)90145-1.

Bledsoe, G.H. (2005). Malaria primer for clinicians in the United States. *South Medical Journal,* **98**(12): 1197–204.

Bloland, P.B., Wirima, J.J., Steketee, R.W., Chilima, B., Hightower, A. and Berman, J.G. (1995). Material HIV infection and infant mortality in Malawi: evidence of increased mortality due to placental malaria infection. *AIDS*, **9**: 721-726.

Bloland, P. (2001). Drug resistance in malaria. WHO monograph WHO/CDS/CSR/DRS/2001.4. World Health Organization, Geneva, Switzerland.

Boivin, M.J. (2002). Effects of early cerebral malaria on cognitive ability in Senegalese children. *Journal Development Behaviour Pediatrics,* **23**(5): 353–264.

Booth, C.M. and MacLean, J.D. (2001). Knowledge, treatment-seeking, and socioeconomic impact of malaria on the Essequibo Coast of Guyana. *Medical Journal of Malaysia,* **6**:17–25.

Bouyou-Akotet, M.K.**,** Ionete-Collard, D.E., Manfoumbi, M.**,** Kendjo, E.**,** Matsiegui, P.B., Mavoungou, E and Kombila, M. (2003). Prevalence of

Plasmodium falciparum infection in pregnant women in Gabon. *Malaria Journal,* **2:** 18.

Brabin, B.J. (1983). An analysis of malaria in pregnancy in Africa. *Bulletin of World Health Organization,* **61**:1005-1016.

Brabin, B.J., Romagosa, C., Abdelgalil, S., Menendez, C., Verhoeff, F.H., McGready, R., Fletcher, K.A., Owens, S., D'Alessandro, U., Nosten, F., Fischer, P.R. and Ordi, J. (2004): The sick placenta-the role of malaria. *Placenta,* **25**:359-378.

Brabin, B.J, Warsame, M., Uddenfeldt-Wort, U., Dellicour, S., Hill, J., and Gies, S. (2008). Monitoring and evaluation of malaria in pregnancy – developing a rational basis for control. *Malaria Journal,* **7(1):**S6doi:10.1186/1475-2875-7-S1-S6.

Breman, J.G, Alilio, M.S, and Mills, A. (2004). Conquering the intolerable burden of malaria: whats needed: a summary *Annal. Journal of Tropical Medicine and Hygiene,* **71**:1-5.

Breman, J.G, Alilio, M.S. and White, N.J. (2007). Defining and Defeating the Intolerable Burden of Malaria III. Progress and Perspectives. *American Journal Tropical Medicine Hygiene,* **77(6)**: 6.

Breman, J.G. and Holloway, C.N. (2007). Malaria surveillance counts. *American Journal Tropical Medicine Hygiene,* **77 (Suppl 6**): 36–47.

Briand, V., Cottrell, G., Massougbodji, A. and Cot, M. (2007). Intermittent preventive treatment for the prevention of malaria during pregnancy in high transmission areas. *Malaria Journal,* **6:** 160.

Bruce-Chwatt, L.J. (1993). Essential Malariology, London Arnold, pp 141-147.

Bruce, W.R. and Heddle, J.A. (1979). The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella and sperm abnormality assays. *Canada Journal of Genetic Cytology,* **21**: 319–334

Bukar, M., Audu, B.M., Yahaya, U.R. and Melah, G.S. (2008). Anaemia in pregnancy at booking in Gombe, North-eastern Nigeria, *Journal of Obstetrics Gynaecology,* **28(8):** 775-778.

Cannon, D.S.H. (1958). Malaria and prematurity in the western region of Nigeria. *British Medical Journal,* ***ii*****:** 877-878.

Carrington, A. (2001). Harvard Health Policy Review. Malaria: Its Human Impact, Challenges and Control Strategies in Nigeria*, Fall*: vol 2, no. 2.

Challis, K., Osman, N.B., Cotiro, M., Nordahl, G., Dgedge, M. and Bergstrom, S. (2004). Impact of a double dose of sulphadoxine–pyrimethamine to reduce prevalence of pregnancy malaria in southern Mozambique. *Tropical Medical Inernational Health,* **9:** 1066–1073.

Chen, Q., Schlichtherle, M. and Wahlgren, M. (2000). "Molecular aspects of severe malaria". *Clinical Microbiology Reviews,* **13**(3): 439–450.

Chico, R.M. and Chandramohan, D. (2011). intermittent preventive treatment of malaria in pregnancy: at the crossroads of public health policy. *Tropical Medicine and International Health,* 2011 Apr 7. doi: 10.1111/j.1365-3156.2011.02765.x.

Chima, R.I., Goodman, C.A. and Mills, A. (2003). The economic impact of malaria in Africa: a.critical review of the evidence. *Health Policy,* **63**: 17–36.

Coatney, G.R., Myatt, A.V., Hernandez, T., Jeffery, G.M. and Cooper, W.C. (1952) Studies on the compound 50–63. *Transaction of Royal Society of Tropical Medicine and Hygiene,* **46:** 496-497.

Coetzee M., Craig, M. and Le Sueur, D. (2000). Distribution of African malaria mosquitoes belonging to the Anopheles gambiae complex. *Parasitology Today*, **16**: 74-77.

Cogswell, F.B. (1992). The hypnozoite and relapse in primate malaria. *Clinical Microbiological Review,* **5** (1): 26–35.

Coluzzi, M. (1984). Heterogenetics of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. *Bulletin World Health Organization,* **62 (suppl):** 107-113.

Coluzzi, M., Sabatini, A., Petrarca, V. and Di Deco, M.A. (1985). Chrosomomal differentiation and adaptation to human environment in the *Anopheles gambiae* complex. *Transaction of Royal Society of Tropical Medicine and Hygiene,* **73:** 483-497.

Cot, M., Breart, G. and Esveld, M. (1995). Increase of birthweight following chloroquine chemoprophylaxis during pregnancy: results of a randomized trial in Cameroon. *American Journal of Tropical Medicne and Hygiene,* **53**: 581-585.

Dairo, M.D. and Lawoyin, T.O. (2004). Socio-demographic determinants of anaemia in pregnancy at primary care level: a study in urban and rural Oyo State, Nigeria. *African Journal Medical Science*, **33(3):** 213-217.

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

Djènontin, A., Bio-Bangana, B., Moiroux, N., Henry., M.C., Bousari, O., Chabi, J., Ossè, R., Koudénoukpo, S., Corbel, V., Akogbéto, M. and Chandre, F. (2010) *Culicidae* diversity, malaria transmission and insecticide resistance alleles in malaria vectors in Ouidah-Kpomasse-Tori district from Benin (West Africa): A pre-intervention study. *Parasites & Vectors,* **3**:83doi:10.1186/1756-3305-3-83.

Deressa, W., Ahmed, A. and Enquoselassie, F. (2003). Knowledge, attitudes and practices about malaria, the mosquito and antimalarials drugs in a rural community. *Ethiopian Journal of Health Development,* **17**: 99–104.

Deressa, W. and Ahmed, A. (2009). Malaria-related perceptions and practices of women with children under the age of five years in rural Ethiopia. *BioMed Central Public Health,* **9:** 259.

Desai, M., ter Kuile, F.O., Nosten, F., McGready, R., Asamoa, K., Brabin, B. and Newman, R.D. (2007). Epidemiology and burden of malaria in pregnancy**.** *Lancet Infectious Dis*eases, **7(2**): 93-104.

Desowitz, R.S. and Alpres, M.P. (1992). Placental *Plasmodium falciparum* parasitaemia in East Sepik (Papua New Guinea) women of different parity: the apparent absence of acute effects of mother and foetus. *Annal of Tropical Medical Parasitology,* **86**: 95-102.

Diagne, N., Rogier, C., Cisse, B. and Trape, J.F. (1997). Incidence of clinical malaria in pregnant women exposed to intense perennial transmission. *Transaction of Royal Society of Tropical Medicine and Hygiene,* **91**: 166-170.

Dike, N., Onwujekwe, O., Ojukwu, J., Ikeme, A., Uzochukwu, B. and Shu, E. (2006). Influence of education and knowledge on perceptions and practices to control malaria in Southeast Nigeria. *Social Science Medicine,* **63**:103-106.

Dim, C.C. and Onah, H.E. (2007). The prevalence of anemia among pregnant women at booking in Enugu, South Eastern Nigeria. *Medscape General Medicine,* **9**(3): 11.

Disease Surveillance and Notification Report, Badagry LGA, Lagos State, 2000.

Dondorp, A.M., Pongponratn, E. and White, N.J. (2004). "Reduced microcirculatory flow in severe *falciparum* malaria: pathophysiology and electron-microscopic pathology". *Acta Tropica,* **89**(3): 309–317.

Dunyo, S.K., Afari, E.A., Koram, K.A., Ahorlu, C.K, Abubakar, I. and Nkrumah, F.K. (2000). Health centre versus home presumptive diagnosis of malaria in southern Ghana: implications for home-based care policy. *Transactions of Royal Society of Tropical Medicine and Hygiene,* **94**: 285–288.

Durand, R.., Eslahpazire, J., Jafari, S., Delabre, J.F., Marmorat-Khuong, A., di Piazza, J.P. and Le Bras, J. (2000). Use of Molecular Beacons To Detect an Antifolate Resistance-Associated Mutation in *Plasmodium falciparum. Antimicrobial Agents and Chemotherapy,* **44 (12):** 3461-3464.

Ekanem, O.J. (1997). Malaria-The ending Saga. In: Coping with Treatment failures-Proceedings of the Update Symposium on Rational use of anti-malaria drugs. Chris Obi (ed) May & Baker, Lagos pp 13-21.

Ekejindu, I.M., Udigwe, G.O. and Chijoke, I.R.C. (2006). Malaria and anaemia in pregnancy in Enugu, southeast Nigeria. *African Journal of Medical Science,* **35**: 1-3.

Elyazar, R.F., Hay, S.I. and Baird, J.K. (2011**).** Malaria distribution, prevalence, drug resistance and control in Indonesia. *Advances in Parasitology,* **74**: 41-175.

Enato, E.F.O. and Okhamafe, .A.O. (2005). *Plasmodium falciparum* malaria and antimalarial interventions in sub-Saharan Africa: Challenges and Opportunities. *African Journal of Biotechnology,* **4(13):** 1598-1605.

Enato, E.F.O., Mens, P.F., Okhamafe, A,O., Okpere, E.E., Pogoson, E. and Schallig, D.F.H. (2009). *Plasmodium falciparum* malaria in pregnancy: Prevalence of peripheral parasitaemia, anaemia and malaria care-seeking behaviour among pregnant women attending two antenatal clinics in Edo State, Nigeria. *Journal of Obstetric and Gynaecology*, **29**(**4**): 301 – 306.

Ezugwu, E.C., Onah, H.E., Odetunde, I.O. and Azubuike, J.C. (2006). Singleton Low Birth Weight Babies At A Tertiary Hospital In Enugu, South East Nigeria. *The Internet Journal of Gynaecology and Obstetrics* **14**:1.

Falade, C.O., Yusuf, B.O., Fadero, F.F., Mokolu, O.A., Hamer, D.H. and Salako, L.A. (2007). Intermittent preventive treatment with sulfadoxine-pyrimethamine is effective in preventing maternal and placental malaria in Ibadan, south-western Nigeria**.** *Malaria Journal,* **6:** 88.

Federal Ministry of Health, (2005). National guidelines and strategies for malaria prevention and control during pregnancy. *Federal Ministry of Health Nigeria,* 2005. pp. 1–50.

Fong, Y.L., Cadigan, F.C., Coatney, G.R. (1971). "A presumptive case of naturally occurring. *Plasmodium knowlesi* malaria in man in Malaysia". *Transactions of Royal Society of Tropical Medicine and Hygiene,* **65**(6): 839–840.

Forney, J.R., Wongsrichanalai, C., Magill, A.J., Craig, L.G., Sirichaisinthop, J., Bautista, C.T., Miller, R.S., Ockenhouse, C.F., Kester, K.E., Aronson, N.E., Andersen E.M., Quino-Ascurra, H.A., Vidal, C., Moran, K.A., Murray, C.K., DeWitt, C.C., Heppner, D.G., Kain K.C., Ballou, W.R. and Gasser, R.A. Jr. (2003). Devices for rapid diagnosis of malaria: evaluation of prototype assays that detect *Plasmodium falciparum* histidine-rich protein 2 and a *Plasmodium vivax*-specific antigen. *Journal Clinical Microbiology,* **41**: 2358–2366.

Gajida, A.U., Iliyasu, Z. and Zoakah, A.I. (2010). Malaria among antenatal clients attending primary health care facilities in Kano state, Nigeria. *Annals of African Medicine,* **9:** 188-193.

Galadanci, H.S., Ejembi, C.L., Illiyasu, Z., Alagh, B. and Umar, U.S. (2007). Maternal health in Northern Nigeria. A far cry from ideal. *BJOG*: *An International Journal of Obstetrics and Gynaecology*, **114:** 448-452.

Garrett-Jones, C., Boreham, P.F.L. and Paqnt, C.P. (1980). Feeding habits of anophelines (Diptera: Culicidae) in 1971-78, with reference to the human blood index: a review. *Bulletin of Entomological Research,* **70**: 165-185.

Gamble, C., Ekwaru, P.J., Garner, P., ter Kuile, F.O. (2007). Insecticide-treated nets for the prevention of malaria in pregnancy: a systematic review of randomised controlled trials. *Public Library of Science Medicine,* 2007, **4:**e107.

Geissler, P.W., Nokes, C., Prince, R.J., Achieng'Odhiambo, R., Aagaard-Hansen, J. and Ouma J.H. (2000). Children and medicines: self-treatment of common illnesses among Luo school children in western Kenya. *Social Science Medicine,* **51**:1771–1783.

Gikandi, P.W., Noor, A.M., Gitonga, C.W., Ajanga, A.A. and Snow, R.W. (2008). Access and barriers to measures targeted to prevent malaria in pregnancy in rural Kenya. *Tropical Medicine International Health,* **13(2):** 208–217.

Gillet, J.D. and Smith, J.G. (1972). Common African Mosquitoes and their Medical Importance. *Williams Heinemann Medical Books Limited*: London. pp.106.

Gilles, M.T. and Coetzee, M. (1987). *A supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region*). Johannesburg: South African Institute for Medical Research, publication, **55**:1-143.

Gimnig, J.E., MacArthur, J.R., M'bang'ombe, M., Kramer, M.H., Chizani, N., Stern, R.S., Mkandala, C., Newman, R.D., Stekette, R.W. and Campbell, C.H. (2006): Severe cutaneous reactions to sulphadoxine-pyrimethamine and trimethoprim-sulphamethoxazole in Blantyre District, Malawi**.** *American Journal of Tropical Medicine and Hygiene*, **74:** 738-743.

Githeko, A.K., Mbogo, V.N., Curtis, C.F., Lines, J. and Lengeler, C. (1996). Entomological Monitoring of large scale vector control interventions. *Parasitology Today,* **12**: 127-128.

Goodwin, L.G. (1952). Daraprim, clinical trials and pharmacology. *Transactions of Royal Society of Tropical Medicine and Hygiene,* **46:** 485-495.

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

Greenwood, B.M., Bojang, K., Whitty, C.J. and Targett, G.A. (2005). "Malaria". *Lancet,* **365** (9469): 1487–1498.

Gregson, A. and Plowe, C.V. (2005). Mechanisms of Resistance of Malaria Parasites to Antifolates. *Pharmacological Reviews,* **57(1):** 117-145.

Guyatt, H.L. and Snow, R.W. (2001). The epidemiology and the burden of *Plasmodium falciparum*-related anemia among pregnant woman in sub-Saharan Africa. *American Journal of Tropical Medicine and Hygiene,* **64** (1): 1-106.

Guyatt, H.L. and Snow, R.W. (2004). Impact of Malaria during Pregnancy on Low Birth Weight in Sub-Saharan Africa. *Clinical Microbiology Review*, **17**(4): 760–769.

Hannay, P.W. (1960). The mosquitoes of Zaria Province, northern Nigeria. *Bulletin of Entomological Research,* **51**: 145–171.

Hay, S.I., Rogers, D.J., Toomer, J.F. and Snow, R.W. (2000). Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, internet access and review. *Transaction of Royal Society of Tropical Medicine and Hygiene,* **94**: 113–127.

Hay, S.I, Smith, D. and Snow, W.R. (2008). Measuring malaria endemicity from intense to interrupted transmission. *Lancet Infectious Dis*ease, **8(6):** 369–378.

Hlongwana, K.W., Mabaso, M.L., Kunene, S., Govender, D. and Maharaj, R. (2009). Community knowledge, attitudes and practices (KAP) on malaria in Swaziland: a country earmarked for malaria elimination. *Malaria Journal,* **8**: 29.

Holding, P.A. and Snow, R.W. (2001). "Impact of *Plasmodium falciparum* malaria on performance and learning: review of the evidence". *America Journal Tropical Medicine Hygiene,* **64**(1–2Suppl): 68–75.

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

Hopkins, H., Oyibo, W., Luchavez, J., Mationg, M.L, Asiimwe, C., Albertini, A., González, I.J., Gatton, M.L. and Bell, D. (2011). Blood transfer devices for malaria rapid diagnostic tests: evaluation of accuracy, safety and ease of use. *Malaria Journal,* **10(1):** 30.

Hunt-Cooke, A.H., Chiodini, T., Docherty, A.H., Moody, J.R. and Pinder, M. (1999). Comparison of a parasite lactate dehydrogenase-based immunochromatographic antigen detection assay (OptiMAL®) with microscopy for the detection of malaria parasites in human blood samples. *American Journal of Tropical Medicine and Hygiene,* **60**: 20–23.

Hunt, R.H., Coetzee, M., and Fettene, M. (1998). The *Anopheles gambiae* complex. A new species from Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **92**: 231-235.

Hutton, G., Schellenberg D., Tediosi, F., Macete E., Kahigwa, E., Sigauque, B., Mas, X., Trapero, M., Tanner, M., Trilla, A., Alonso P. and Menendez, C. (2009). Cost-effectiveness of malaria intermittent preventive treatment in infants (IPTi) in Mozambique and the United Republic of Tanzania. *Bulletin of the World Health Organization,* **87**: 123-129.

Idowu, O.A., Mafiana, C.F. and Sotiloye, D. (2005). Anaemia in pregnancy: A survey of pregnant women in Abeokuta, Nigeria, *Africa Health Science,* **5 (4):** 295–299.

Imbahale, S.S., Fillinger, U., Githeko, A., Mukabana, W.R. and Takken, W. (2010). An exploratory survey of malaria prevalence and people's knowledge, attitudes and practices of mosquito larval source management for malaria control in western Kenya. *Acta Tropica*, **115(3):** 248-256.

Ishengoma, D.S., Lwitiho, S., Madebe, R.A., Nyagonde, N., Persson, O., Vestergaard, L.S., Bygbjerg, I.C., Lemnge, M.M and Alifrangis, M. (2011). Using rapid diagnostic tests as source of malaria parasite DNA for molecular analyses in the era of declining malaria prevalence. *Malaria Journal,* **10 (1):**6**.**

Iqbal, J.A, Siddique, M.J and Hira, P.R. (2004). Persistent histidine rich protein 2, parasite lactate dehydrogenase, and panmalarial antigen reactivity after clearance of *Plasmodium falciparum* monoinfection. *Journal Clinical Microbiology,* **42**: 4237–4241.

Jilly, P. (1969). Anaemia in parturient women, with special reference to malaria infection of the placenta. *Annal of Tropical Medicine and Parasitology,* **63**: 109-116.

Kagu, M.B., Kawuwa, M.B. and Gadzama, G.B. (2007). Anaemia in pregnancy: a cross-sectional study of pregnant women in a Sahelian tertiary hospital in Northeastern Nigeria. *Journal of Obstetrics and Gynecology,* **27:** 676-679.

Kapito-Tembo, A., Meshnick, S.R., Boele, M., van Hensbroek, M.B., Phiri, K., Fitzgerald, M. and Mwapasa, V (2011). Marked Reduction in Prevalence of Malaria Parasitemia and Anemia in HIV-Infected Pregnant Women Taking Cotrimoxazole With Or Without Sulfadoxine-Pyrimethamine Intermittent Preventive Therapy during Pregnancy in Malawi. *Journal Infectious Disease,* (2011) doi: 10.1093/infdis/jiq072.

Kaseje, D.C., Sempebwa, E.K. and Spencer, H.C. (1987). Malaria chemoprophylaxis to pregnant women provided by community health workers in Saradidi, Kenya. I. Reasons for non-acceptance. *Annals of Tropical Medicine and Parasitology,* **81(1):** 77-82.

Kaur, G. (2009). Malaria endemicity in an Orang Asli community in Pahang, Malaysia. *Tropical Biomedicine,* **26(1):** 57-66.

Kayentao, K., Kodio, M., Newman, RD., Maiga, H., Doumtabe, D., Ongoiba, A., Coulibaly, D., Keita, AS., Maiga, B., Mungai M., Parise, M. and Doumbo, O. (2005). Comparison of intermittent preventive treatment with chemoprophylaxis for the prevention of malaria during pregnancy in Mali. *Journal of Infectious Disease,* **191**:109–116.

Kelly-Hope, L.A. and McKenzie F.E. (2009). The multiplicity of malaria transmission: a review of entomological inoculation rate measurements and methods across sub-Saharan Africa. *Malaria Journal*, **8**:19

Kengeya-Kayondo, J.F., Seeley, J.A., Kajura-Bajenja, E., Kabunga, E., Mubiru, E., Sembajja, F. and Mulder, D.W. (1994): Recognition, treatment seeking behaviour and perception of cause of malaria among rural women in Uganda *Acta Tropica,* **58**: 267-273.

Kochar, D.K., Das, A., Kochar, S.K., Saxena, V., Sirohi, P., Garg, S., Kochar, A., Mahesh, P., Khatri, M.P. and Gupta, V. (2009). Severe *Plasmodium vivax* Malaria: A Report on Serial Cases from Bikaner in Northwestern India. *America Journal Tropical Medicine and Hygiene,* **80(2):** 194-198.

Komolafe, J.O., Kuti, O., Oni, O. and Egbewale, B.E. (2005). Sociodemographic characteristics of anaemic gravidae at booking: a preliminary study at Llesha, Western Nigeria. *Nigerian Journal of Medicine,* **14(2):** 151-154.

Konde-Lule, J.K., Musagara, M. and Sewankambo, N.K. (1991). Dynamics of spread of HIV-1 in a rural district of Uganda. *British Medical Journal,* **303**:1303-1306.

Kristan, M., Fleischmann, H., Della Torre, A., Stich, A. and Curtis, C.F. (2003). Pyrethroid resistance/susceptibility and differential urban/rural distribution of *Anopheles arabiensis* and *Anopheles gambiae* ss malaria vectors in Nigeria and Ghana, *Medical and Vertinary Entomology,* **17**: 1-17.

Kuti, O., Owolabi A.T., Makinde, O.M. (2006). Perception of malaria prophylaxis among pregnant Nigerian women at booking. *Tropical Journal of Obstetrics and Gynaecology*, **23(2):** 125-127.

Kyabayinze, D.J., Asiimwe, C., Nakanjako, D., Nabakooza, J., Counihan, H. and Tibenderana, J.K. (2010).Use of RDTs to improve malaria diagnosis and fever case management at primary health care facilities in Uganda. *Malaria Journal,* **9**: 200.

Labbe, A.C., Pillai, D.R., Leke, R.F., Djokam, R.R., Mbu, R., Leke, R.J., Fogako, J., Megnekou, R., Metenou, S., Sama, G., Zhou, Y., Cadigan, T., Parra, M. and Taylor, D.W. (1999). Detection of the *Plasmodium falciparum* antigen histidine-rich protein 2 in blood of pregnant woman: implications of diagnosing placental malaria. *Journal of Clinical Microbiology,* **37**: 2992-2996.

Launiala, A. and Honkasolo, M.L. (2007). Ethnographic study of factors influencing compliance to intermittent preventive treatment of malaria during pregnancy among Yao women in rural Malawi. *Transactions of Royal Society of Tropical Medicine and Hygiene,* **101**: 980-989.

Leke, R.F., Djokam, R.R., Mbu, R., Leke, R.J., Fogako, J., Megnekou, R., Metenou, S., Sama, G., Zhou, Y., Cadigan, T., Parra, M. and Taylor, D.W. (1999). Detection of the *Plasmodium falciparum* antigen histidine-rich protein 2 in blood of pregnant woman: implications of diagnosing placental malaria. *Journal Clinical Microbiology,* **37**: 2992-2996.

Lemeshow, S., Hosmer, D.W., Klar, J. and Lwanga, S.K. (1990). Adequacy of sample size in health studies. WHO Ed, pp 80.

Lindsay, S.W., Parson, L. and Thomas, C.J. (1998). Mapping the ranges and relative abundance of the two principal African malaria vectors, *Anopheles gambiae* sensu stricto and *An. arabiensis*, using climate data. *Proceedings of the Royal Society of London* B **265**: 847- 854.

Londono, B., Carmona, J. and Blair, S. (2002). Comparison between Optimal and the thick smear tests for malaria diagnosis in an endemic area during a non-epidemic period. *Biomedica* (*Bogota*), **22**: 466–475.

Lozovsky, E.R., Chookajorn, T., Brown, K.M., Imwong, M., Shaw, P.J., Kamchonwongpaisan, S., Neafsey, D.E.., Weinreich, D.M. and Hartl, D.L. (2009). Stepwise acquisition of pyrimethamine resistance in malaria parasite. *Proceedings of the National Academy of Sciences*, **106(29):** 12025-12030

Mabaso, M.L., Sharp, B. and Lengeler, C. (2004). Historical review of malarial control in southern Africa with emphasis on the use of indoor residual house-spraying. *Tropical Medicine and International Health,* **9:** 846-56.

Mabunda, S., Aponte, J.J., Tiago., A., and Alonso, P. (2009). A country-wide malaria survey in Mozambique. II. Malaria attributable proportion of fever and establishment of malaria case definition in children across different epidemiological settings. *Malaria Journal,* **8:** 74.

MacCormack, C.P. and Lwihula, G. (1983). Failure to participate in a malaria chemosuppression programme: North Mara, Tanzania. *Journal Tropical Medicine Hygiene,* **86**: 99–107.

MacDonald, G. (1957). *The Epidemiology and Control of Malaria*. London: Oxford University Press, London: 1957. Local features of malaria; pp. 63-99.

Maiga, O.M., Kayentao,K., Traore, B.T., Djimde, A., Traore, B., Traore, M., Ongoiba, A., Doumtabe, D., Doumbo,S., Traore, M.S., Dara, A., Guindo,O., Karim, D.M., Coulibaly, S., Bougoudogo, F., ter Kuile, F. O., Danis, M., and Doumbo, O.K. (2011). Superiority of 3 Over 2 Doses of Intermittent Preventive Treatment With Sulfadoxine-Pyrimethamine for the Prevention of Malaria During Pregnancy in Mali: A Randomized Controlled Trial. *Clinical Infectious Diseases,* **53(3):** 215–223.

Makler, M.T. and Hinrichs, D.J. (1993). Measurement of the lactate dehydrgenase activity of *Plasmodium falciparum* as an assessment of parasitaemia. *American Journal of Tropical Medicine and Hygiene,* **48**: 205-210.

Marchesini, P. and Crawley, J. (2004): Reducing the burden of malaria in pregnancy**.** *Mera 2004, Roll Back Malaria Department, WHO, Geneva.*

Marcucci, C., Madjdpour, C. and Spahn, D. (2004). "Allogeneic blood transfusions: benefit, risks and clinical indications in countries with a low or high human development index". *British Medical Bulletin,* **70**: 15–28.

Marsh, K. (1998). Malaria disaster in Africa. *Lancet,* **352**: 924.

Maude, R.J., Hassan, M.U. and Beare, N.A.V. (2009). "Severe retinal whitening in an adult with cerebral malaria". *American Journal of Tropical Medicine and Hygiene,* **80(6):** 881.

Mazigo, H.D., Obasy, E., Mauka, W., Manyiri P., Zinga, M., Kweka, E.J., Mnyone, L.L. and Heukelbach, J. (2010). Knowledge, Attitudes, and Practices about Malaria and Its Control in Rural Northwest Tanzania.

Research Article *Malaria Research and Treatment Volume 2010 (2010), Article ID 794261,* pp 9.

Manyi, M.M. and Imandeh, N.G. (2008). The infection rates of mosquitoes with Malaria and lymphatic filarial parasites in Makurdi, Benue state, Nigeria. *Journal of Pest, Disease and Vector Management,* **8**: 464-470.

Marielle, K.B.A., Denisa, E.I.C., Modeste, M.M., Eric, K., Pierre, B.M., Elie, M. and Maryvome, K. (2003): Prevalence of *Plasmodium falciparum* infection in Pregnant women in Gabon. *Malaria Journal,* **2**: 1-17.

Mbaye, A., Richardson, K., Balajo, B., Dunyo, S., Shulman, C., Milligan, P., Greenwood, B. and Walraven, G. (2006). A randomized, placebo-controlled trial of intermittent preventive treatment with sulphadoxine–pyrimethamine in Gambian multigravidae. *Tropical Medicine and International Health,* **11:** 992–1002.

Mbonye, A.K., Neema, S. and Magnussen, P. (2006). Perceptions on use of sulfadoxine-pyrimethamine in pregnancy and the policy implications for malaria control in Uganda. *Health Policy,* **77**: 279-289.

McGregor, I.A., Wilson, M.E. and Bilewicz, W.Z. (1983). Malaria infection of the placenta in The Gambia, West Africa, its incidence and relationship to birth weight and placenta weight. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **93**: 529–534.

McGregor, I.A. (1994). Epidemiology of malaria and pregnancy. *American Journal of Tropical Medicine and Hygiene,* **33**: 517-525.

Mckie, J.K., Douglas, K.T., Chan, C., Roser, S.A., Yates, R., Read, M., Hyde, J.E., Dascombe, M.J., Yuthavong, Y. and Sirawaraporn. (1998). Rational drug

design approach fot over coming drug resistance in malaria. *Journal of Medical Chemistry*, **41(9):** 1367-1370.

McCormick, M.C. (1985). The contribution of low birth weight to infant mortality and childhood mortality. *New England Journal Medicine,* **312:** 82-90.

Meltzer, M.I., Terlouw, D.J., Kolczak, M.S., Odhacha, A., ter Kuile, F.O., Vulule JM, Alaii, J.A, Nahlen, B., Hawley W.A and Philips-Howard, P.A (2003). The household-level economics of using permethrin-treated bed nets to prevent malaria in children less than five years of age. *American Journal of Tropical Medicine and Hygiene,* **68(4):** 149-160.

Mendis, K., Sina, B., Marchesini, P. and Carter, R. (2001). "The neglected burden of *Plasmodium vivax* malaria" (PDF). *American Journal Tropical Medicine Hygiene,* **64(1–2 Suppl):** 97–106.

Menendez, C. (1995). Malaria during pregnancy: a priority area of malaria research and control. *Parasitology Today,* **11**: 178–183.

Menendez, C. (1999). Priority areas for current research in Malaria during pregnancy. *Annals of Tropical Medicine Parasitology,* **1**: 571–574.

Menendez, C., Ordi, J., Ismail, M.R., Ventura, P.J., Aponte, J.J, Kahigwa, E., Font, F and Alonso. P.L. (2000). The impact of placental malaria on gestational age and birth weight. *Journal of Infectious Diseases,* **181**: 1740–1745.

Menendez, C., D'Alessandro, U. and ter Kuile, F.O. (2007). Reducing the burden of malaria in pregnancy by preventive strategies. *Lancet Infectious Diseases,* **7:** 126-135.

Metselaar, D.V. and Thiel, P.M. (1959). Classification of malaria. *Tropical Geography Medicine,* **11**: 157-161.

Minakaw, N., Sonye, G., Mogi, M., Githeko, A., and Yan, G. (2002). The effects of climate factors on the distribution and abundance of malaria vectors in Kenya. *Journal of Medical Entomology,* **39**: 833-841.

Mokuolu, O.A., Falade, C.O., Orogade, A.A., Okafor, H.U., Adedoyin, O.T., Tagbo, A., Oguonu, T.A., Dada-Adegbola, H.O., Oguntayo, O.A., Samuel, K., Ernest, S.K., Hamer, D.H. and Callahan, M.V. (2009). Malaria at Parturition in Nigeria: Current Status and Delivery Outcome. *Infectious Diseases in Obstetrics and Gynaecology* Volume 2009 (2009), Article ID 473971, 7 pages doi:10.1155/2009/473971.

Molineaux, L. and Gramiccia, G. (1980). The Garki Project. In: Research on the Epidemiology and control of Malaria in the Sudan savannah of West Africa. WHO Geneva, Switzerland, pp 311.

Molta, N.B. (1995). Susceptibility of *Plasmodium falciparum* to malarial drugs in North-eastern Nigeria. *Transaction of Royal Tropical Medicine and Hygiene,* **89**: 422-425.

Moody A, Hunt-Cooke A, Gabette E and Chiodini P (2000). Performance of the OptiMal malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the Hospital for Tropical Diseases, *London British Journal of Haematology,* **109**: 891-894.

Morley, D., Woodland, M., Cuthbertson, W.F.J. (1964). Controlled trial of pyrimethamine in pregnant women in an African Village. *British Medical Journal,* **1**: 667-668.

Mubyazi, G., Bloch, P., Kamugisha, M., Kitua, A., Ijumba, J. (2005). Intermittent preventive treatment of malaria during pregnancy: a qualitative study of knowledge, attitudes, and practices of district health managers, antenatal care staff and pregnant women in Korogwe District, North-Eastern Tanzania. *Malaria Journal,* **4**: 31-37.

Murphy, S.C., Breman, J.G. (2001). Gaps in the childhood malaria burden n Africa: Celebral malaria, neurologic sequelae, anaemia, respiratory distress, hypoglycaemia and complications of pregnancy. *American Journal Tropical Medicine Hygiene,* **64(Suppl 1-2):** 57-67.

Murray, C.K., Gasser, R.A., Magill, A.J. and Miller, R.S. (2008). Update on Rapid Diagnostic Testing for Malaria. *Clinical Microbiology Reviews,* **21(1)**: 97–110.

Mutabingwa, T.K. (2003). Treating malaria during pregnancy in Africa. *Postgraduate Doctor in Africa,* **25 (4):** 66-73.

Mwenesi, H., Harpham, T. and Snow, R.W. (1995). Child malaria treatment practices among mothers in kenya. *Social Science and medicine,* **40(9):** 1271-1277.

Nagaraj, K. (2003). Risk factors of severe Anaemia Among, Pregnant women attending a Government Maternity Hospital in Tirupato, India - A Multivariate Analysis. *Journal Human Ecolology,* **14(4):** 237–240.

Najera J. A. (1994). The control of tropical diseases and socioeconomic development (with special reference to malaria and its control). *Parassitologia,* **36**: 17-33.

Nahlen, B.L, Alakija, T., Ogunbode, O., Adetoro, O., Akintunde, A., Nguyen-Dinh, P., Edungbola, L.D. and Breman, J.G. (1989). Lack of pyrimethamine prophylaxis in pregnant Nigerian women. *Lancet,* **8667**: 830–834.

Nebe O.J, Adeoye G.O. and Agomo P.U. (2002). Prevalence and clinical profile of Malaria among the coastal dwellers of Lagos State, Nigeria. *Nigeria Journal of Parasitology*, **23**: 61-68.

Neequaye, J., Coe-Ene, J. and Taelman, H. (1986). *In vivo* chloroquine resistant *falciparum* malaria in West Africa. *Lancet,* **1**: 153-154.

Newman, R.D., Moran, A.C., Kayentao, K., Kayentao, K., Benga-De, E., Yameogo, M., Gaye, O., Faye, O., Lo, Y., Moreira, PM., Doumbo, O., Parise, ME. and Steketee RW. (2006). Prevention of malaria during pregnancy in West Africa: policy change and the power of subregional action. *Tropical Medicine and International Health,* **11:** 462–469.

Nnaji, G.A. and Ikechebelu, J.I. (2007). An evaluation of the use of reported febrile illness in predicting malaria in pregnancy. *Journal of Obstetrics and Gynaecology,* **27(8):** 791-794.

Nnaji, G.A. and Ikechebelu, J.I. (2008). An evaluation of the use of reported febrile illness in predicting malaria in pregnancy *Journal Obstetrics Gynaecology,* **28(4)**: 463.

Nnaji, G.A., Ikechebelu, J.I. and Okafor, C.I.A. (2009). Comparison of the prevalence of malaria parasitaemia in pregnant and non pregnant women. *Nigerian Journal of Medicine,* **18(1):** 47-51.

Nosten, F., McGready, R., d'Alessandro, U., Bonell, A., Verhoeff, F., Menendez, C., Mutabingwa, T. and Brabin, B. (2006). Antimalaial drugs in pregnancy: a review. *Current Drug Safety*, **1(1**): 1-15.

Nuwaha, F. (2002). People's perception of malaria in Mbarara, Uganda. *Tropical Medicine and Inernational Health,* **7:** 462–470.

Nwagha, U.I., Ugwu, V.O., Nwagha, T.U. and Anyaehie, B.U. (2009). Asymptomatic Plasmodium parasitaemia in pregnant Nigerian women: almost a decade after Roll Back Malaria. *Transactions of Royal Society of Tropical Medicine and Hygiene,* **103(1):** 16-20.

Nwonwu, E.U., Ibekwe, P.C., Ugwu, J.I., Obarezi, H.C. and Nwagbara, O.C. (2009). Prevalence of malaria parasitaemia and malaria related anaemia among pregnant women in Abakaliki, southeast Nigeria. *Nigeria Journal of Clinical Practice,* **12(2):** 182–186.

Odeigah, P.G.C. (1997). Sperm-head abnormalities and dominant lethal effects of formaldehyde in albino rats. *Mutation Research,* **389**: 141–148.

Oduola, A.M.G, Omitowoju, O., Sowunmi, A., Makler, M.T., Falade, C.O., Kyle, D.E., Fehintola, F.A., Ogundahunsi, O.A., Piper, R.C., Schuster, B.G., and Milhous, W.K. (1997). *Plasmodium falciparum*: evaluation of lactate dehydrogenase in monitoring therapeutic response to standard anti-malarial drugs in *Nigeria Experimental Parasitology,* **87:** 283–289.

Ogbeide. O., Wagbatsoma, V. and Orhue, A. (1994). Anaemia in Pregnancy. *East Africa Medical Journal,* **71(110):** 671–673.

Okoko, B.E., Enwere, E. and Ota, M.O. (2003).The epidemiology and consequences of maternal malaria: A review of immunological basis. *Acta Tropica,* **87**: 193-205.

Okonofua, P.E. (2004). Prevention of malaria in pregnancy, an important public health challenge. *A Peer Review Journal of Biomedical Sciences,* **3:** 15-6.

Okwa, O.O. (2003). The status of malaria among pregnant women: a study in Lagos, Nigeria. *Africa Journal Reproductive Health*. **7**:77–83.

Okwa, O.O. (2004). The status of malaria among pregnant women: a study in Lagos, Nigeria. *African Journal of Reproductive Health,* **7:** 77-83.

Okwa, O.O and Ibidapo, A.C. (2010). The Malaria situation, perception of cause and treatment in a Nigerian University. *Journal of Medicine and Medical Sciences,* **1(6):** 213-222.

Okwa, O.O., Bello, B.A and Olundegun, S. A. (2011). Social Aspects of Malaria among Students in Two Tertiary Institutions in Lagos, Nigeria. *Sierra Leone Journal of Biomedical Research,* **3(2):** 97-103.

Oladokun, A., Otegbayo, J.A. and Adeniyi, A.A. (2009). Maternal and foetal outcomes of jaundice in pregnancy at the University College Hospital, Ibadan. *Nigeria Journal of Clinical Practice*, **12(3):** 277-280.

Omo Aghoja, I.O., Aghoja, C.O., Oghagbon, K., Omo Aghoja, V.W. and Esume, C. (2008). Prevention and treatment of malaria in pregnancy in Nigeria: Obstetrician's knowledge of guidelines and policy changes – a call for action**.** *Journal of Chinese clinical Medicine,* **3:** 2.

Onyido, A., Deezia, N., Obiukwu, M. and Amadi, E. (2009). Ecology of Man-Biting Mosquitoes In The Development Site Of Nnamdi Azikiwe University Awka,

Anambra State Southeastern Nigeria. *The Internet Journal of Health* Vol. 9 No. 2.

Onyiriuka, A.N. (2006). Trends in incidence of delivery of low birth weight infants in Benin City, Southern Nigeria. *Nigeria Postgraduate Medical Journal,* **13(3)**: 189-194

Oreagba, A.I., Onajole, S.O., Olayemi, S.O. and Mabadeje, A.F.B (2004). Knowledge of malaria amongst caregivers of young children in rural and urban communities in Southwest, Nigeria. *Tropical Journal of Pharmaceutical Research,* **3(1):** 299-304.

Otubanjo, O.A, Mafe, M.A., Idowu, E.T. and Adeneye, A.K. (2000). Knowledge attitude and perception of malaria in Lagos State. *Nigeria Quarterly Journal of Hospital Medicine,* **10(1):** 73–77.

Otubanjo, O.A., and Mafe, M.A. (2002). Control of parasitic diseases of poverties: an overview of the Nigerian situation. *The Zoologist,* **1(1):** 1-24.

Otubanjo, O.A. and Mosuru, A.A. (2007). An *in vivo* evaluation of the induction of abnormal sperm morphology by Sulfamethoxypyridazine-pyrimethamine (Metakelfin). *Pakistan Journal of Biological Science,* **10(1**): 156-159.

Oyala, F.J., Escalante, A.A. and Rich, S.M. (1999). Evolution of *Plasmodium* and the recent origin of the world populations of *Plasmodium falciparum. Parasitologia,* **41**: 55-68.

Oyedeji, I.S., Bassi, P.U., Awobode, H.O. and Olumese, P.E. (2005). Comparative assessment of *Plasmodium falciparum* sensitivity to chloroquine and amodiaquine *in vitro. African Journal of Biotechnology,* **4(11)**: 1317-1320.

Oyibo, W.A, Ajuluchukwu, J., Adu, O.O., Okun, E.I., Adeniji, A., Chukwu, J., Otaigbe, I., Fagbenro-Beyioku, A.F., Bamiro, S.B., Agomo, C.O., Oladosu, O.O., Okangba, C.C., Ojuromi, O..T, Osibogun, A., Anorlu, R., Mezilliara, C. and Ogunleye, P. (2009). Evaluation of a merozoite surface protein antibody detection Malaria Pf/Pv Device for rapid diagnosis of malaria in Nigeria. *International Journal of Malaria and Tropical Diseases,* **5**: 94-98.

Ouma, P., van Eijk, A.M., Hamel, M.J., Parise, M., Ayisi, J.G., Otieno, K., Kager, P.A. and Slutsker, L. (2007). Malaria and anaemia among pregnant women at first antenatal clinic visit in Kisumu, western Kenya. *Tropical Medicine and International Health,* **12(12):** 1515-1523.

Paulander, J., Olsson, H., Lemma, H., Getachew, A. and Sebastian, M.S. (2009). Knowledge, attitudes and practice about malaria in rural Tigray, Ethiopia. *Global Health Action,* Vol 2 (2009).

Parise, E.M., Ayisi, G.J., Nahlen, L.B., Schultz, J.L., Roberts, M.J., Misore, A., Muga, R., Oloo, J.A. and Steketee, W.R. (1998). Efficacy of sulphadoxine-pyrimethamine for prevention of placental malaria in an area of Kenya with a high prevalence of malaria and human immunodeficiency virus infection. *American Journal of Tropical Medicine and Hygiene,* **59**: 813–822.

Patrick, O., Erah, P.O., Gertrude, A.G. and Okhamafe, A, O. (2003). *Plasmodium falciparum* malaria resistance to chloroquine in five communities in Southern Nigeria. *African Journal of Biotechnology,* **2(10):** 384-389.

Pelletier, D.L., Frongillo, E.A., Schroeder, D.G. and Habicht J.P. (1995). The effects of malnutrition on child mortality in developing countries. *Bulletin of World Health Organization,* **73(4):** 443-448.

Perrault, S.D, Hajek, J., Zhong, K., Owino, S.O., Sichangi, M., Smith, G., Shi, Y.P., Moore, J.M. and Kain, K.C. (2009). Human immunodeficiency virus co-infection increases placental parasite density and transplacental malaria transmission in Western Kenya. *American Journal of Tropical Medicine and Hygiene,* **80(1):** 119-125.

Peters, W. (1998a). Drug resistance in malaria parasites of animals and man. *Advance Parasitology,* **41**: 1-62.

Peters, W. (1998b). Varaitions in frequencies of drug resistance in *Plasmodium falciparum*. *Proceedings of the National Academic Science.* USA. 94: 9389-9393.

Peters, P.J, Thigpen, M.C., Parise, M.E. and Newman, R.D. (2007). Safety and toxicity of sulfadoxine/pyrimethamine: implications for malaria prevention in pregnancy using intermittent preventive treatment. *Drug Saf,* **30 (6):** 481-501.

Petersen, E. (1987) In vitro susceptibility of *Plasmodium falciparum* malaria to pyrimethamine, sulfadoxine, trimethoprim and sulfamethoxazole, singly and in combination. *Transaction of Royal Society of Tropical Medicine and Hygiene,* **81**: 238-241.

Pertrarca, V., Beier, J.C., Onyango, F., Koros, J., Asiago, C., Koech, D.K. and Roberts, C.R. (1991). Species composition of *Anopheles gambiae* complex (Diptera: Culicidae) at two sites in Western Kenya. *Journal of Medical Entomology,* **28(3):** 307-313.

Phillips-Howard, P.A. and Wood, D. (1996). The safety of antimalarial drug in pregnancy. *Drug Saf,* **14**(3): 131-45.

Piper, R., J. Lebras, L. Wentworth, A. Hunt- Cooke, S. Houze, P. Chiodini, and Makler, M**. (**1999). A capture diagnostic assay for malaria using *Plasmodium* lactate dehydrogenase (pLDH). *America Journal Tropical Medicine Hygiene,* **60:** 109–118.

Plowe, C.V., Djimde, A., Wel lems, T.E, Diop, S., Kouriba, B. and Doumbo, O.K. (1996). Community pyrimethamine-sulfadoxine use and prevalence of resistant *Plasmodium falciparum* genotypes in Mali: a model for deterring resistance. *American Journal Tropical Medicine Hygiene,* **55:** 467–471.

Raimi, O.G. and Kanu, C.P. (2010). The prevalence of malaria infection in pregnant women living in a suburb of Lagos, Nigeria. *African Journal of Biochemistry Research,* **4**(10): 243-245.

Ramharter, M., Schuster, K., Bouyou-Akotet, M.K., Adegnika, A.A. Kristen, Schmits, K., Mombo-Ngoma, G., Agnandji, S.T., Nemeth, J., Afène, S.N., Issifou, S., Onnas, I.N., Kombila, M. and Kremsner, P.G. (2007). Malaria in Pregnancy Before and After the Implementation of a National IPTp Program in Gabon. *American Journal Tropical Medicine Hygiene,* **77(3)**: 418-422

Rathod, P.K., McErlean, T. and Pei-Chieh, L. (1997). Varaitions in frequencies of drug resistance in *Plasmodium falciparum*. *Proceedings of National Academia Science,* USA. **94**: 9389-9393.

Recke, M., Bouma, M.J., Bamford, P., Gupta, S. and Dobson, A.D. (2009). Assessing the burden of pregnancy-associated malaria under changing transmission settings. *Malaria Journal,* **8**: 245.

Reinhardt, M.C, Ambroise-Thomas, P., Cavallo-Serra, R., Meylan, C., Gautier, R. (1978). Malaria at delivery at Abidjan. *Helv Paediatar Acta,* **3:** 65-84.

Rogerson, S.J, Chaluluka, E., Kanjala, M., Mkundika, P., Mhango, C. and Molyneux, M.E. (2000). Intermittent sulphadoxine-pyrimethamine in pregnancy: effectiveness against malaria morbidity in Blantyre, Malawi in 1997–99. *Transactions Royal Society Tropical Medicine and Hygiene,* **94**: 549–553.

Roger, S.J, Mwapasa, V. and Meshnick, S.R. (2007). Malaria in Pregnancy: Linking Immunity and Pathogenesis to Prevention. *American Journal Tropical Medicine and Hygiene,* **77(6)**: 14-22

Rogerson, S.J. (2007). New approaches to pathogenesis of malaria in pregnancy. *Parasitology,* **134**: 1883-1893.

Ronald, L.A**.,** Kenny, S.L**.,** Klinkenberg, E., Akoto, A.O., Isaac, Boakye, I., Barnish, G. and Donnelly, M.J. (2006). Malaria and anaemia among children in two communities of Kumasi, Ghana: a cross-sectional survey. *Malaria Journal,* **5**:105doi.

Rowe, J.A., Claessens, A., Corrigan, R.A. and Arman, M. (2009). Adhesion of *Plasmodium falciparum*-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. *Expert Reviews in Molecular Medicine* doi:10.1017/S1462399409001082; Vol. 11; e16; May 2009.

Sachs, J. and Malaney, P. (2002). The economic and social burden of malaria. *Nature,* **415:** 680–685.

Salako, L.A., Sowunmi, A. and Walker, O. (1990). Evaluation of the clinical efficacy and safety of halofantrin in *falciparum* malaria in Ibadan, Nigeria. *Transactions of Royal Society of Tropical Medicine and Hygiene,* **84**: 644-647.

Salako, L.A, Ajayi, F.O., Sowunmi, A. and Walker, O. (1990). Malaria in Nigeria, a resistance. *Annal Tropical Medical Parasitology,* **84(5):** 432-445.

Salako, L.K. (2006). Reflections malaria control and research in Nigeria. *Nigerian Journal of Clinical and Biomedical,* **1** (1): 19.

Sanh, N.H., Van Dung, N., Thanh, N.X., Trung, T.N., Van Co, T. and Cooper, R.D. (2008). Forest malaria in central Viet Nam. American. *Journal Tropical.Medicine Hygiene,* **79**: 652–654.

Savage, E., Msyamboza, K., Gies, S. and D'Alessandro, U. (2007). Indicator for monitoring malaria control in pregnancy in sub-Saharan Africa**.** *British Journal Obstetrics and Gynaecology,* **114:** 1222-1231.

Samane, A.K, Nahid H.Z., Saaed, S., Khazan, H., Ali, H., Ahmad, R., Hosein, E.G. and Abadi, Alireza. (2010). Comparision of microscopy and RDTs techniques for laboratory detection of malaria. *African Journal of Biotechnology,* **9(10)**: 1514-1516.

Schellenberg, D., Menendez, C., Kahigwa, E., Aponte, J., Vidal, J. and Tanner M., (2001). Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomized, placebo-controlled trial. *Lancet,* **357**: 1471-1477.

Schultz, L.J, Steketee, R.W, Chitsulo L, Macheso, A, Nyasulu, Y. and Ettling, M. (1994). Malaria and childbearing women in Malawi: knowledge, attitudes and practices. *Tropical Medical Parasitology,* **45**: 65–9.

Schultz, L.J., Steketee, R.W., Chitsulo, L. and Wirma, J.J. (1995). Antimalarials during pregnancy: a cost-effective analysis. *Bulletin of the World Health Organization,* **73**: 207-214.

Scott, J.A., Brogdon, W.G. and Collins, F.H. (1993). Identification of single specimens of the Anopheles complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene,* **49**: 520-529.

Seal, SL., Mukhopadhay, S. and Ganguly, R.P. (2010). Malaria in pregnancy. *Journal of the Indian Medical Assocociation,* **108(8):** 487-490.

Shililu, J.I., Maier, W.A., Seitz, H.M. and Orago, A.S. (1998). Seasonal density, sporozoite rates and entomological inoculation rates of *Anopheles gambiae* and *Anopheles funestus* in a high-altitude sugarcane growing zone in Western Kenya.*Tropical Medical International Health*, **3(9**): 706-710.

Shulman, C.E. (1999). Malaria in pregnancy: its relevance to safe motherhood programmes. *Annal Tropical Medical Parasitology,* **93** (1): S59-S66.

Shulman. C.E., Dorman, E.K., Cutts, F., Kawuondo, K., Bulmer, J.N., Peshu, N. and Marsh, K. (1999). Intermittent sulphadoxine–pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: a randomised placebo-controlled trial. *Lancet,* **353:** 632–636.

Shulman, C.E., Marshall, T., Dorman, E.K., Bulmer, J.N., Cutts, F., Peshu, N. and Marsh, K. (2001). Malaria in Pregnancy: Adverse effects on haemoglobin levels and birth weight in primigravidae and multigravidae. *Tropical Medical International Health,* **6:** 770-778.

Shulman, C.E., Dorman, E.K. and Bulmer, J.N. (2002). Malaria as a cause of severe anaemia in pregnancy. *Lancet* **360**: 494.

Shulman, C.E. and Dorman, E.K. (2003). "Importance and prevention of malaria in pregnancy." *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **97(1):** 30–35.

Singer, L.M., Newman, R.D., Diarra, A., Moran, A.C., Huber, C.S., Stennies, G., Sirima, S.B., Konate, A., Yameogo, M., Sawadogo, R., Barnwell, J.W. and Parise, M.E. (2004). Evaluation of a malaria rapid diagnostic test for assessing the burden of malaria during pregnancy. *American Journal of Tropical Medicine and Hygiene,* **70**: 481–485.

Singh, N. and Valecha, N. (2000). Evaluation of a rapid diagnostic test, 'determine malaria pf', in epidemic-prone, forest villages of central India (Madhya pradesh). *Annals Tropical Medicine and Parasitology,* **94**: 421–427.

Sirima,.S.B., Sawadogo, R., Moran, A.C., Konate, A., Diarra, A., Yameogo, M., Parise, M.E., and Newman, R.D. (2003). Failure of a Chloroquine Chemoprophylaxis Program to Adequately Prevent Malaria during Pregnancy in Koupela District, Burkina Faso. *Clinical Infectious Diseases,* **36**: 1374-1382.

Smith, T., Charlwood, J.D., Kitua, A.Y., Masanja, H., Mwankusye, S., Alonso, P.L. and Tanner, M. (1998). Relationships of malaria morbidity with exposure to *Plasmodium falciparum* in young children in a highly endemic area. *American Journal of Tropical Medicine Hygiene,* **59:** 252–257.

Smith, T.A., Leuenberger, R. and Lengeler, C. (2001). Child mortality and malaria transmission intensity in Africa. *Trends Parasitolology,* **17:** 145–149.

Smith, T.K, Lengelier, G. and Tanner, M. (2004). Relationships between the outcome of *Plasmodium falciparum* infection and the intensity of transmission in Africa. *American Journal of Tropical Medicine and Hygiene,* **71(2):** 80-86.

Sowunmi, A., Salako, L.A., Walker, O. and Ogundahunsi, O.A, (1990). Clinical efficacy of mefloquine in children suffering from CRPF in Nigeria. *Transactions Royal Society of Tropical Medicine and Hygiene,* **84**: 761-764.

Sowunmi, A. and Salako, L.A. (1992). Evaluation of the relative efficacy of various antimalarial drugs in Nigerian children *Medical Parasitology,* **86**: 1-8.

Sowunmi, A. and Walker, J.A. (1993). Presumptive diagnosis of malaria in infants. *Transaction Royal Society of Tropical Medicine and Hygiene,* **87(4):** 422.

Spencer, H.C, Kaseje DC, Sempebwa E.K, Huong, A.Y and Roberts, J.M. (**1987).** Malaria chemoprophylaxis to pregnant women provided by community health workers in Saradidi, Kenya. II. Effect on parasitaemia and haemoglobin levels. *Annals Tropical Medical Parasitology,* **81(Suppl 1**): 83–89.

Steketee, R.W., Wirima J.J., Boland P.B., Chilima, B., Mermin, J.H, Chitsulo, L. and Breman, J.G. (1996). Impairment of a pregnant woman's acquired ability to limit *Plasmodium falciparum* by infection with human immunodeficiency virus type-1. *American Journal Tropical Medicine and Hygiene,* **55 (1):** 42-49.

Steketee, R.W., Wirima J.J. Slutsker, L., Hamann, D.L. and Breman, J.G. (1996). The problem of malaria and malaria control in pregnancy in sub-Saharan Africa. *American Journal Tropical Medicine and Hygiene,* **55**(1): 2-7.

Steketee, R., Nahlen, B., Parise, M. and Menendez, C. (2001). The burden of malaria in pregnancy in malaria-endemic areas**.** *American Journal of Tropical Medicine and Hygiene,* **64:** 28-35.

Sullivan, A.D., Nyirenda, T. and Cullinan, T. (1999). Malaria infection during pregnancy: intrauterine growth retardation and preterm delivery in Malawi. *Journal Infectious Diseases,* **179**:1580–1583.

Tagbor, H., Bruce, J., Browne, E., Greenwood, B. and Chandramohan, D. (2008). Performance of the OptiMAL® dipstick in the diagnosis of malaria infection in pregnancy. *Therapeutic Clinical Risk Management,* **4(3):** 631–636.

Talisuna, A.O., Staedke S.G. and D'Alessandro, G. (2006). Pharmacovigilance of antimalarial treatment in Africa: is it possible? *Malaria Journal,* **5:**50.

Tarimo, D.S., Minjas, J.N. and Bygbjerg, I.C. (2001). Malaria diagnosis and treatment under the strategy of the integrated management of childhood illness (imci):Relevance of laboratory support from the Rapid Immunochromatographic Tests of ICT malaria *Plasmodium falciparum/Plasmodium vivax* and OptiMAL. *Annals Tropical Medicine and Parasitology,* **95**:437–444.

Temperley, M., Mueller, D.H., Njagi, J.K., Akhwale, W., Clarke, S.E., Jukes, M.C., Estambale, B.B. and Brooker, S. (2008). Costs and cost-effectiveness of delivering intermittent preventive treatment through schools in western Kenya. *Malaria Journal*, **30(7)**: 196.

ter Kuile, F.O., Terlouw, D.J., Penelope, A., Phillips-Howard, W. and Haawley, A. (2003). Reduction of malaria during pregnancy by permethrin-treated bed nets in an area of intense perennial malaria transmission in western kenya. *American Journal of Tropical Medicine and Hygiene,* **681:(4):** 50-60.

ter Kuile, F, Parise, M.E., Verhoeff, F.H., Udhayakumar, V., Newman, R.D., van Eijk, A.M., Rogerson, S.J. and Steketee, R.W. (2004). The burden of co-infection with human immunodeficiency virus type 1 and malaria in

pregnant women in sub-saharan Africa. *American Journal of Tropical Medicine and Hygiene,* **71**: 41–45.

ter Kuile, F.O., van Ejik, A.M. and Filler, S.J. (2007). Effect of Sulfadoxine-Pyrimethamine Resistance on the Efficacy of Intermittent Preventive Therapy for Malaria Control during Pregnancy. *Journal of the American Medical Association,* **297 (23):** 2603-2616.

Thangaleela, T, and Vijayalakshmi, P. (1994). Prevalence of Anaemia in Pregnancy. *The Indian Journal of Nutrition and Dietetic,* **31(2):** 26–29.

Thévenon, A.D., Zhou, J.A., Megnekou, R., Ako, S., Leke, R.G.F. and Taylor, D.W. (2010). Elevated Levels of Soluble TNF Receptors 1 and 2 Correlate with *Plasmodium falciparum* Parasitemia in Pregnant Women: Potential Markers for Malaria-Associated Inflammation. *Journal of Immunology,* **185(11):** 7115-7122.

Thirukkanesh, S. and Zahara, A.M. (2010). Compliance to Vitamin and Mineral Supplementation among Pregnant Women in Urban and Rural Areas in Malaysia. *Pakistan Journal of Nutrition,* **9 (8):** 744-750.

Trampuz, A, Jereb, M., Muzlovicm, I. and Prabhu, R. (2003). Clinical review: Severe malaria.. *Critical Care,* **7(4):** 315–323.

Umeaneto P.U., Ekejindu, I.M. and Ifeanyichukwu, M.O. (2006) Prevalence and intensity of malaria in blood donors at NAUTH Nnewi, Anambra State, Nigeria. *Nigeria Journal of Parasitology*, **27**: 11-15.

Uneke, C.J. (2007). Impact of Placental *Plasmodium falciparum* Malaria on Pregnancy and Perinatal Outcome in Sub-Saharan Africa. *Yale Journal of Biology and Medicine,* **80(2):** 39-50.

Uneke, C.J. (2008). Impact of Placental *Plasmodium falciparum* Malaria on Pregnancy and Perinatal Outcome in Sub-Saharan Africa. Part III: Placental Malaria, Maternal Health, and Public Health. *Yale Journal of Biology and Medicine,* **81(1):** 1–7.

UNICEF, (2007). Definition of indicators, In; State of the world's children 2007 by UNICEF, pp 109.

Uzochukwu, B.S.C., Onwujekwe, E., Ezuma, N.N., Ezeoke, O.P., Ajuba, M.O. and Sibeudu, F.T. (2011). Improving Rational Treatment of Malaria: Perceptions and Influence of RDTs on Prescribing Behaviour of Health Workers in Southeast Nigeria. *Public Library of Science One,* **6(1)**: e14627.

Vallely, A., Vallely, L., Changalucha, J., Greenwood, B., and Chandramohan, D. (2007). Intermittent preventive treatment for malaria in pregnancy in Africa: What's new, what's needed? *Malaria Journal,* **6**:16.

van den Broek, N. (1996). The Cytology of Anaemia in Pregnancy in West Africa. *Tropical Doctor*, **26:** 5-7.

van den Broek, N.R. and Letsky, E.A. (2000). Etiology of anemia in pregnancy in south Malawi. *American Journal of Clinical Nutrition,* **72**: 247S–256S.

van den Broek, N. (2001). Anaemia in pregnancy in sub-Saharan countries. *European Journal of Obstetrics Gynecology and Reproductive Biology,* **96**: 4–6.

van den Broek, N. (2003). Anaemia and micronutrient deficiencies. *British Medical Bulletin,* **67**: 149-160.

VanderJagt, T.A., Ikeh, E.I., Ujah, I.O., Belmonte, J., Glew, R.H. and Vanderjagt, D.J. (2005). Comparison of the Optimal® rapid test and microscopy for detection of malaria in pregnant women in Nigeria. *Tropical Medicine International Health,* **10**:39–41.

van Eijk, A.M., Ayisi, J.G., ter Kuile, F.O., Otieno, J.A., Misore, A.O., Odondi, J.O., Rosen, D.H., Kager, P.A., Steketee, R.W. and Nahlen, B.L. (2004). Effectiveness of intermittent preventive treatment with Sulphadoxine-pyrimethamine for control of malaria in pregnancy in Western Kenya: a hospital-based based study. *Tropical Medical International Health,* **9**:351–360.

van Eijk, A.M, Hill, J., Alegana, V.A., Kirui, V., Gething, P.W., ter Kuile, F.O., Snow, R.W. (2011). Coverage of malaria protection in pregnant women in sub-Saharan Africa: a synthesis and analysis of national survey data**.** *The Lancet Infectious Diseases,* **2011; DOI**: 10.1016/S1473-3099(10)70295-4.

Van Geertruyden, J.P., Thomas, F., Erhart, A. and D'Alessandro, U. (2004). The contribution of malaria in pregnancy to perinatal mortality. *American Journal Tropical Medicine Hygiene,* **71**: 35–40.

Verhoeff, F.H., Brabin, B.J., Chimsuku, L., Kazembe, P., Russell, W. and Broadhead, R.L. (1998.) An evaluation of the effects of intermittent sulfadoxine-pyrimethamine treatment in pregnancy on parasite clearance and risk of low birth weight in rural Malawi. *Annals of Topical Medicine and Parasitology,* **92**(2): 141-150.

Wagbatsoma, V.A. and Aigbe, E.E. (2010). ITN utilization among pregnant women attending ANC in Etsako West Lga, Edo State, Nigeria. ,*Nigerian Journal of Clinical Practice,* **13**(2): 144-148.

Walter P.R, Garin., Y and Bolt, P. (1982). Placental pathologic changes in malaria. A histologic and ultrastructural study. *American Journal Pathology,* **109**: 330-342.

Warhurst, D.C and Williams, J.E. (1996). Laboratory diagnosis of malaria. *Journal of Clininical Pathology,* **49:** 533–538.

Warhurst, D.C. (2001). A molecular marker for chloroquine-resistant *falciparum* malaria (editorial). *New England Journal of Medicine,* **344**: 299-302.

Wernsdorfer, W.H. (1994). Epidemiology of drug resistance in malaria. *Acta Tropica,* **56:**143-156.

White, B. (1974). *Anopheles gambiae* complex and disease transmission in Africa. *Transactions of the Royal Society for Tropical Medicine and Hygiene,* **68**: 278-298.

White, N.J. (1999). Delaying antimalarial drug resistance with combination chemotherapy. *Parassitologia,* **41:** 301-308.

White, N. (2005). Intermittent presumptive treatment for malaria**.** *Public Library of Science Medicine,* 2005, **2:**e3.

Wilson, T. and Edeson, J.F.B. (1953). Acute malaria and pyrimethamine. *British Medical Journal,* **1**: 253-255.

Wolfe, E.B., Parise, M.E. and Haddix, A.C. (2001). Cost effectiveness of sulfadoxine-pyrimethamine for the prevention of malaria-associated low

birth weight. *American Journal of Tropical Medicine and Hygiene,* **64**: 178-186.

World Health Organization (1951). WHO Report on the malaria conference in equatorial Africa World Health Organ Tech Rep Ser. 1951, **38**: 72.

World Health Organization (1975). Manual on practical entomology in malaria. Part II. Methods and Techniques. World Health Organisation Offset Publication 1975. Geneva 13, pp 175.

World Health Organization (1986). WHO Expert Committee on Malaria 18th Report. *World Health Organization Technical Report Series,* **735**: 57-59.

World SHealth Organization (1989). Preventing and Controlling Iron Deficiency Anaemia through Primary Health Care. WHO Publications, August, (1989), pp 58.

World Health Organization (1992). The Prevalence of Anaemia in Women: A tabulation of available information 2nd edition. Geneva: WHO, 1992, pp 40.

World Health Organization (1993a). A rapid Dipstick Antigen Capture Assay for the Diagnosis of *falciparum* malaria. Report of a WHO expert committee (WHO/MAL/9S, 1075). World Health Organization, Geneva, pp 8.

World Health Organization (1993b). A global strategy for malaria control. Report of WHO Expert Committee, World Health Organization, Geneva, 1993, pp 14.

World Health Organization (1993c). Implementation of the global Malaria Control Strategy. WHO Tech. Rep. Ser No. 839. World Health Organization, Geneva, pp 62.

World Health Organization (2000a). Bench Aids for the diagnosis of malaria infections, second edition, World Health Organization, Geneva, pp 24.

World Health Organization (2000b). New Perspectives: Malaria Diagnosis. Report of a joint WHO/USAID informal consultation 25-27 October 1999. Geneva, *World Health Organization*, pp 8.

World Health Organization (2000c). The Abuja Declaration on Roll Back Malaria in Africa. African Heads of States and Governments, 25 April 2000, Abuja, Nigeria.

World Health Organization (2000d). The use of Antimalarial drugs: Report of an Informal Consultation, WHO, Geneva, 13-17 November, 2000, pp 141.

World Health Organization (2003). Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated *falciparum* malaria. WHO, Geneva, 2003, pp 67.

World Health Organization (2005). Protecting vulnerable groups in malaria-endemic areas in Africa through accelerated deployment of insecticide-treated nets, WHO Geneva, 2005, pp 2.

World Health Organization (2004**).** A strategic framework for malaria prevention and control during pregnancy in the Africa Region. In *AFR/MAL/04/01*. World Health Organization, Geneva, 2004, pp 38.

World Health Organization, United States Agency for International Development (USAID), the Special Program for Research and Training in Tropical Diseases (TDR) and the Australian Agency for International Development (AusAID) (2006). How to use a Rapid Diagnostic Test, pp 48.

World Health Organization (2009). *World malaria report* 2009. Geneva, 2009. (WHO/HTM/GMP/2009.1), pp 66.

World Health Organization (2010). World malaria report 2010. Geneva, 2010, pp 203.

Wyrobek, A.J. and Bruce, W.R. (1975). Chemical induction of sperm abnormalities in mice. *Proceeding Sciences of United States of America,* of the National Academy, **72(11):** 4425-4429.

Wyrobek, A.J. and Bruce, W.R. (1976). The induction of sperm shaped abnormalities in mice and humans. In a Hollanmder and FJ de Serress (Eds). *Chemical Mutagens,* Vol 5, Prenum Press, New York, pp. 257-285.

Wyrobek, A.J. and Bruce, W. R. (1978). The induction of sperm-shape abnormalities in mice and humans, In: *Chemical Mutagens.* A. Hollander and F.J. de Serres (Eds.). Plenum Press, New York (1978), Volume 5, pp. 257-285.

Yamada, M, Steketee, R, Abramowsky, C. and Kida, M. (1989). *Plasmodium falciparum* associated placental pathology: a light and electron microscopic and immunohistologic study. *American Journal Tropical Medicine Hygiene,* **41**:161-168.

Yartey, I.E. (2006). Malaria in pregnancy: Access to effective interventions in Africa. *International Journal of Obstetrics and Gynaecology,* **94:** 364-373.

Yeka, A., Gasasira, A., Mpimbaza, A., Achan, J., Nankabirwa, J., Nsobya, S., Staedke, S.G., Donnelly, M.J., Wabwire-Mangen, F., Talisuna, A., Dorsey, G., Kamya, M.R. and Rosenthal, P.J. (2011). Malaria in Uganda: efforts**,** *Acta Tropica* 2011; **121:**184-195.

Yeung, S., Pongtavornpinyo W., Hastings, I.M., Mills A.J. and Whiten, N.J. (2004). Antimalaria drug resistance, Artemisinin-based combination therapy (ACT),

and the contribution of modeling to elucidating policy choices. *American Journal of Tropical Medicine and Hygiene* **71(2 suppl):** 179-186.

ELECTRONIC REFERENCES

Sachs, J.D. (2001) Macroeconomics and health: investing in health for economic development. Report of the commission on Macroeconomics and Health. Geneva. World Health Organization December 2001 (http//www3.who.int/whosis/cmh/cmh.health/e/pdf/001-004pdf).World Health Organization (1993d) A Global Strategy for Malaria Control. WHO, Geneva. http://whqlibdoc.who.int/publications/ 9241561610.pdf.

World Health Organization (2007). *Technical expert group meeting on intermittent preventive treatment in pregnancy (IPTp)*. Geneva, World Health Organization,2007.http://www.who.int/malaria/publications/atoz/9789241596640/en/index.html

APPENDICES

Appendix 1: Nigerian Institute of Medical Research Review Board Approval Letter

NIGERIAN INSTITUTE OF MEDICAL RESEARCH

FEDERAL MINISTRY OF HEALTH

Cable MEDRESCON, LAGOS

Telephone: 234-01-7744723
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Research for National Health

6, Edmond Crescent,
(Off Murtala Muhammed Way),
P.M.B. 2013, Yaba,
Lagos, Nigeria.

Our Ref:

Date: 24th July, 2006

INSTITUTIONAL REVIEW BOARD APPROVAL LETTER.

PROJECT TITLE: INTERMITTENT PREVENTIVE TREATMENT (IPT) OF MALARIA IN PREGNANCY (MIP) WITH SULFADOXINE-PYRIMETHAMINE (SP) IN BADAGRY LOCAL GOVERNMENT AREA OF LAGOS STATE .

APPROVAL

The above named proposal has been adequately reviewed; the protocol and safety guidelines satisfy the conditions of NIMR IRB, policies regarding experiments that use human subjects.

Therefore the study under its reviewed state is hereby approved by Institutional Review Board, NIMR.

DR. P. U. AGOMO
Name of vice IRB chairman

24/07/06
Signature & Date of IRB vice Chairman

DR. M .A MAFE

Name of IRB Member

28/07/06
Signature & Date of IRB Member

This approval is given with the investigator's Declaration as stated below;
By signing below I agree/certify that:

1. I have reviewed this protocol submission in its entirety and that I am fully cognizant of, and in agreement with, all submitted statements.
2. I will conduct this research study in strict accordance with all submitted statements except where a change may be necessary to eliminate an apparent immediate hazard to a given research subject.
   - I will notify the IRB promptly of any change in the research procedures necessitated in the interest of the safety of a given research subject.
   - I will request and obtain IRB approval of any proposed modification to the research protocol or informed consent document(s) prior to implementing such modifications.
3. I will ensure that all co-investigators and other personnel assisting in the conduct of this research study have been provided a copy of the entire current version of the

research protocol and are fully informed of the current (a) study procedures (including procedure modifications); (b) informed consent requirements and process; (c) potential risks associated with the study participation and the steps to be taken to prevent or minimize these potential risks; (d) adverse event reporting requirements; (e) data and record-keeping; and (f) the current IRB approval status of the research study.

4. I will respond promptly to all requests for information or materials solicited by the IRB or IRB Office.

5. I will submit the research study in a timely manner for IRB renewal approval.

6. I will not enroll any individual into this research study until such time that I obtain his/her written informed consent, or, if applicable, the written informed consent of his /her authorized representative (i.e., unless the IRB has granted a waiver of the requirement to obtain written informed consent).

7. I will employ and oversee an informed consent process that ensures that potential research subjects understand fully the purpose of the research study, the nature of the research procedures they are being asked to undergo, the potential risks of these research procedures, and their rights as a research study volunteer.

8. I will ensure that research subjects are kept fully informed of any new information that may affect their willingness to continue to participate in the research study.

9. I will maintain adequate, current, and accurate records of research data, outcomes, and adverse events to permit an ongoing assessment of the risks/benefit ratio of research study participation.

10. I am cognizant of, and will comply with, current federal regulations and IRB requirements governing human subject research including adverse event reporting requirements.

11. I will make a reasonable effort to ensure that subjects who have suffered an adverse event associated with research participation receive adequate care to correct or alleviate the consequences of the adverse event to the extent possible.

12. I will ensure that the conduct of this research study adheres to Good Clinical Practice guidelines.

N J CHUKWURAH
Principal Investigator Name

26/7/06
Principal Investigator signature and Date

Appendix 2: Calculation of sample size for pregnant women for the IPT using Sulfadoxine-pyrimethamine intervention.

$$N = \frac{Z^2 p (1-p)}{d^2}$$

Where p= prevalence =15.8%

The malaria prevalence obtained from the malariometric survey in the community was 15.8%.

z = k (constant= 1.96)

d =precision (5%).

$$\text{The sample size } N = \frac{(1.96)^2 p (1-0.15)}{(5/100)^2}$$

$$= \frac{(3.8416) \text{ X } 0.158 \text{ X } 0.8425}{0.0025}$$

$$= \frac{0.50976}{0.0025}$$

$$= 204$$

The calculation showed that a minimum sample size of 204 pregnant women was required to be enrolled for the study on IPT administration.

Appendix 3: KAP of Community Members on Malaria Treatment and Prevention in Ikoga ward in Badagry Local Government Area of Lagos State

LGA: _____2. Community/Village: ---------- 3. Date ----------

4. Name of interviewer: _____

5. Name of Respondent: ----------

6. Address _____

7. Name of head of household:

_____

SECTION A: Background Characteristics of Respondent:

8. Sex: 9. Age 10. Tribe/ language

11. Occupation: ---------- 12. Educational status: none, primary, secondary, post secondary----------

13. Religion: (i) Christianity (ii) Islam (iii) Indigenous

(iv) Others (please specify)

_____

14. Marital ostatus: (1) Single (2) Married (3) Separated (4) Divorced (5) Widowed

15. Number of children: _____ 16. List ages: _____

17. Earnings per month----------

SECTION B: Knowledge on Perception of health problems

18. What are the common health problems here? ----------

!9. Is malaria a problem here? Y/N: 20. What causes malaria: --------

21. What are the signs of malaria in a child: ---------- 22. What are the signs of malaria in an adult?

23. Have you had malaria before? ---------- 24. What is the usual first action when you have malaria? ----------

25. Why did you choose this action? ----------
--
26. If you did not recover what would you do next? ----------
---

SECTION C: Health Seeking Behaviour

27. When was the last time you had malaria? ----------
28. How frequent do you have malaria? (No. of times a year) ----------
----
29. How did you know it was malaria? ----------

30. What did you do? ----------
31. Where did you go for treatment----------?
32. If not a health facility, why did you not go there? ----------
33. What is the distance from your home to the nearest health facility? ----------
34. How many days or part of the day after illness started was this action taken? ----
----
35. Was your blood tested at the health facility? Y/N
36. What treatment was prescribed? ----------
37. If anti-malarial, what was the dosage? ----------
38. Where was the medicine obtained from? Y/N
39. Did you get well after the treatment? Y/N
40. What was the total cost of drugs and fees charged? ----------
41. What was the total cost of the transport to the HF? Y/N
42. Do you react to the antimalarial? Y/N
43. If yes, what is the reaction? ----------
44. Do you normally complete the prescribed drug?
45. If no, what are your reasons? Y/N
46. Do you take any other drugs with the prescribed drugs? Y/N
47. If yes, what other drugs do you take? ----------
48. How many times a year do you go to the health facility for treatment? ----------
--
49. Do you use herbs for malaria treatment? Y/N
50. If yes why do you use herbs? ----------

51. Where do you obtain the herbs from? ----------

52. What are the names of the herbs? ----------

53. How do you take the herbs? ----------

54. Do you experience any side effects after taking the herbs? ----------

55. What are the side effects? ----------

SECTION D: Malaria Prevention

58. How do you prevent malaria? ----------

59. Do you have wire gauze on your doors and windows? Y/N

60. Do you use bednets? Y/N

61. Does everybody in the house sleep under a bed net? Y/N?

62. How many of your bed have treated nets? ----------

63. How were the nets acquired? ----------

64. Do you think there are a lot of mosquitoes around here Y/N?

65. At what time of the year do you have them? ----------

66. Are they a nuisance? Y/N

67. Why do you think there are a lot of mosquitoes around here? ----------
--

68. What do you do to keep the mosquitoes away? ----------

Thank you

Appendix 4: KAP on SP Utilization in Pregnant Women Attending Antenatal Clinics in Badagry Local Government Area of Lagos State

QNO: _____ Health Facility:

_____

Name of Interviewer:

_____

Name of Respondents (Optional):

_____

Date: _____ Address:

_____

SECTION A: Background Characteristics of Respondent:

1. Age (in years): _____
2. Ethnicity: (1) Hausa (2) Igbo (3) Yoruba (4) Others (please specify)_____
3. Occupation: (1) unemployed (2) Housewife (3) Farmer (4) Trader (5) Fishing (6) civil servant (7) Student (8) Other (please specify)
4. Educational Status: (1) None (2) Primary (3) Secondary (4) Tertiary (5) Others (please specify)_____
5. Religion: (1) Christianity (2) Islam (3) Indigenous (4) Other (please specify)_____
6. Marital status: (1) Single (2) Married (3) Separated (4) Divorced (5) Widowed
7. Number of children_____: 8. List their ages:

_____

9. How old is your pregnancy now? _____
10. Parity: (1) First (2) Second (3) Third (4) Fourth (5) More than four

SECTION B: Health Seeking Behaviour of Respondent during Pregnancy

11. Do you normally register in the hospital when you are pregnant? (1) Yes (2) No

12. If no, why?

13. If Q11 is no, where do you go? (1) Traditional birth attendant's home? (2) Others (please specify)

14. At what month do you register?

15a. Where do you usually deliver your pregnancy?

(1) Govt. hospital (2) Private hospital (3) At home (4) TBA's home.

15b. Why?

SECTION C: Knowledge and Perception of Malaria and SP Utilisation in Malaria Treatment

16. What do you know about malaria?

_____

_____

__

17. What are the signs/symptoms of malaria?

_____

_____

__

18a. What preventive measures do you usually take against malaria when pregnant?

_____

__

_____

__

18b. Do your actions really prevent illness? (1) Yes (2) No

19. If insecticides treated bednet (ITN) is not mentioned in response to Q18, probe by asking: have you ever heard of ITN? (1) Yes (2) No

20. If Q19 is yes, do you use it? (1) Yes (2) No

21. If Q20 is no, why?

_____

_____

__

22. How do you treat malaria when you are pregnant?_____

_____

__

23. How many attacks do you usually have during pregnancy? (State the actual number)_____

24. Do you know what Sulfadoxine-pyrimethamine is? (1) Yes (2) No

25. What is it used for?

_____

_____

__

26. Have you ever used it for malaria prevention before? (1) Yes (2) No

27. If Q26 is yes, how did you use it? (1) Weekly (2) Monthly
(3) Twice a month (4) Twice during pregnancy
(5) Other (please specify_____

28. At what stage of pregnancy did you use it?

_____

29. How many tablets did you take?

_____

30. Did it prevent malaria during pregnancy?

_____

31. Did you have a better pregnancy outcome? (1) Yes (2) No

31a. If Q31a is no, what happened?

_____

_____

__

32. Was there any recorded side effects to the SP? (1) Yes (2) No

33. If Q32 is yes, please state the side-effects recorded_____

_____

_

Appendix 5: Calculation of average enlarged spleen (AES)

Class of spleen	**Number of various classes**	**AES**
0	1199	0
1	132	132
2	117	254
3	85	251
4	34	136
5	4	20
Total	1571	793

$$\text{Spleen rate} = \frac{\text{Number positive}}{\text{Total number examined}}$$

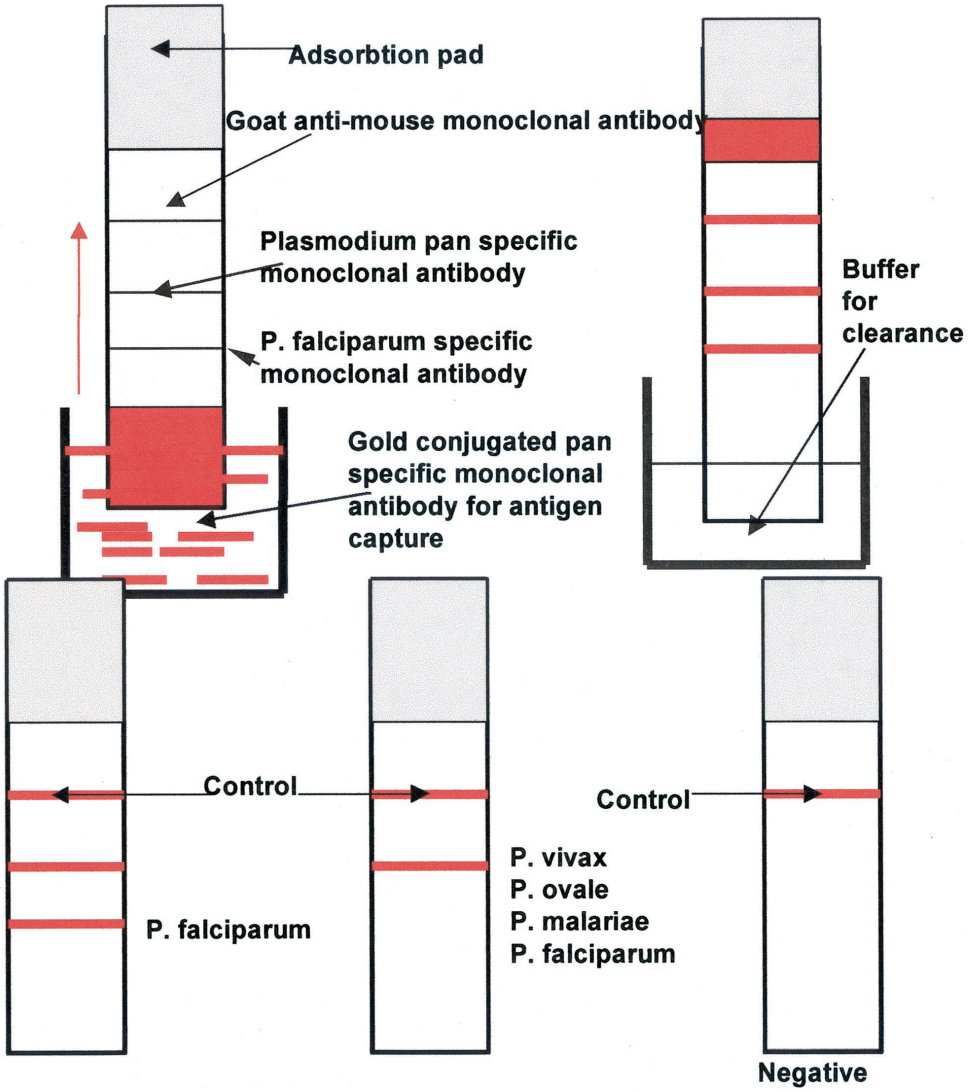
$$= \frac{372}{1{,}571}$$

$$= 0.2368 = 23.68\%$$

The Average Enlarged Spleen (AES) = 132+234+255+136+20=793

$$= \frac{793}{372} = 2$$

Appendix 6: Positive *P. falciparum* (left) and negative strip (right)



Appendix 7: Monthly data on low birth weight (L) and pre-term delivery (P) for 2005 from Badagry General Hospital.

Year	2005			Total recorded deliveries
Month	Male L/P	Female L/P	Total L/P	
January	2/0	6/1	8/1	50
February	2/0	2/0	4/0	54
March	4/0	6/0	10/0	52
April	7/0	6/1	1/1	49
May	3/0	9/0	12/0	54
June	11/0	9/0	20/0`	55
July	7/1	5/0	12/1	57
August	3/0	4/0	7/0	52
September	3/0	4/0	7/0	55
October	2/0	1/0	3/ 4	52
November	3/1	1/1	4/2	52
December	1/0	1/0	2/0	48
Total	39/2	54/3	93/9	645
Average			14/1.4	54

Appendix 8: *Anopheles mosquitoes* – Quick Identification Key

ANOPHELES MOSQUITOES - QUICK IDENTIFICATION KEY

FEMALES

HIND LEGS

White feet, black legs	OR	White feet + white speckles on legs	OR	Black legs with white/yellow speckles	OR	Black legs, or white on apical joints of legs
An. coustani *An. rufipes* *An. theileri*		*An. maculipalpis* *An. pretoriensis* *An. natalensis* (v. rare)		*An. squamosus* *An. pharoensis* *An. ardensis* (v. rare) *An. gambiae* gr.		*An. funestus* gr. *An. rhodesiensis* *An. cinereus* *An. marshallii* gr. *An. demeilloni* *An. longipalpis* *An. listeri*

PALPS

1 band on tip of palps	3-banded palps	4-banded palps	Black tipped palps
An. nili	All the others + *An. gambiae* gr. (in part)	*An. cinereus* *An. ardensis* (v. rare) *An. natalensis* (v. rare) *An. gambiae* gr. (in part)	*An. listeri*

3-banded + speckles

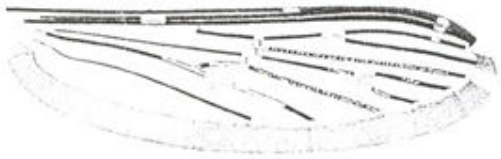
An. maculipalpis
An. squamosus (bushy)
An. pharoensis (bushy)

ABDOMEN

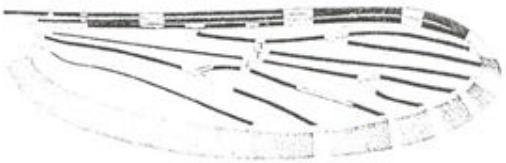
With laterally projecting tufts of scales

An. squamosus / pharoensis

WINGS →



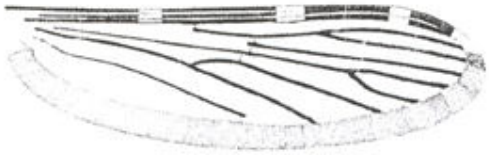
An. coustani gr. large, black



An. rufipes white feet, black legs
An. pretoriensis white feet, speckled legs
An. maculipalpis white feet, speckled legs & palps

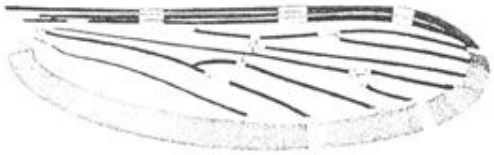
An. theileri white feet, yellow

An. gambiae gr. speckled legs, brownish
An. marshallii gr. black legs, white joints
An. squamosus speckled legs, black, bushy palps



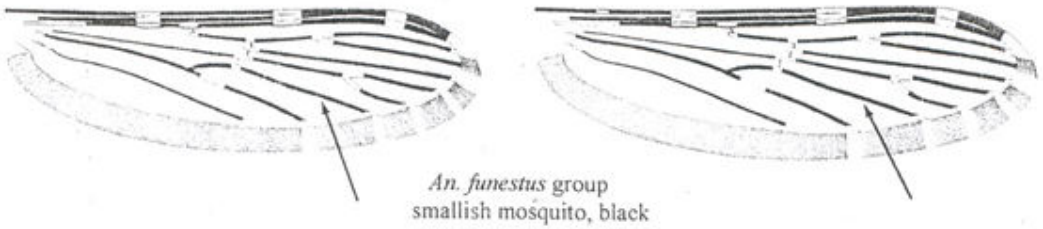
An. rhodesiensis black

An. demeilloni black legs
An. cinereus large, 4-banded palps



An. nili 1 pale band on tip of palps
An. longipalpis 3-banded palps

An. listeri black tip on palps



An. funestus group
smallish mosquito, black

Appendix 9: Number of mosquitoes collected versus number of occupants in the room in Igborosun community and average room density.

S/N	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
1	0/2	3/2	2/2	1/2	¼	6/2	10/2	8/2	10/2	4/2	2/2	3/2
2	2/4	2/4	6/4	2/4	¾	16/4	20/4	18/4	15/4	10/4	20/4	¾
3	4/4	5/4	17/4	10/4	14/4	18/4	16/4	18/4	12/4	18/4	20/4	4/4
4	2/3	3/7	7/3	5/3	6/3	10/3	7/3	8/3	18/3	5/3	12/3	3/3
5	7/4	10/4	5/4	0/4	5/4	15/4	16/4	18/4	10/4	10/4	8/4	8/4
6	4/2	3/2	4/2	10/2	15/2	20/2	30/2	26/2	32/2	20/2	10/2	3/2
7	¾	2/4	¾	12/4	15/4	20/4	26/4	30/4	24/4	19/4	10/4	2/4
8	4/2	3/2	10/2	5/2	5/2	10/2	15/2	18/2	16/2	15/2	12/2	2/2
9	5/1	4/1	10/1	7/1	10/1	20/1	18/1	20/1	16/1	10/1	8/1	3/1
10	4/2	3/2	15/2	10/2	20/2	25/2	25/2	20/2	22/2	15/2	12/2	2/2
11	2/1	0/1	10/1	16/1	10/1	15/1	16/1	20/1	20/1	10/1	12/1	3/1
12	3/2	6/2	7/2	10/2	12/2	20/2	21/2	25/2	22/2	22/2	10/2	4/2
13	4/1	2/1	8/1	16/2	20/2	25/2	30/2	35/2	28/2	20/2	18/2	2/2
14	0/1	4/1	5/1	6/1	4/1	10/1	15/1	16/1	14/1	12/1	16/1	9/1
15	½	5/2	15/2	10/2	10/2	10/2	15/2	20/2	16/2	20/2	10/2	3/2
Total	35	55	130	120	150	240	270	300	275	210	180	50
Average Room density	4	4	10	10	10	16	18	20	15	14	12	4

Appendix 10: Number of female Anopheles mosquitoes collected versus number of occupants in the room in Ikoga and average room density.

R/N	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
1	¾	4/4	12/4	2/4	5/4	10/4	8/4	15/4	11/4	10/4	12/4	0/4
2	4/4	6/4	10/4	3/4	8/4	10/4	11/4	16/4	8/4	11/4	0/4	15/4
3	3/2	0/2	½	1/2	0/2	5/2	10/2	25/2	12/2	12/2	½	½
4	6/5	5/5	2/5	3/5	0/2	7/2	15/2	16/2	13/2	15/5	5/5	4/5
5	7/4	0/4	7/4	6/4	11/4	6/4	16/4	20/4	14/4	15/4	5/5	4/5
6	4/2	6/3	7/3	10/3	10/4	15/4	14/4	15/4	16/4	12/4	5/3	11/3
7	6/3	5/3	6/3	2/3	8/3	8/3	10/3	15/3	11/3	11/3	2/3	2/3
8	7/4	6/4	10/4	13/4	10/4	12/4	14/4	20/4	13/4	13/4	4/4	7/4
9	5/3	6/3	9/3	10/3	15/3	10/3	13/3	20/3	14/3	12/3	7/3	5/3
10	8/2	5/2	6/2	5/2	10/2	15/2	20/2	16/2	19/2	14/2	6/3	12/2
11	6/3	3/3	7/3	6/3	12/3	13/3	17/3	19/3	19/3	18/3	6/3	12/3
12	0/4	¼	10/4	5/4	6/4	7/4	20/4	30/4	18/4	16/4	8/4	¾
13	1/5	1/5	3/5	10/5	16/5	20/5	27/5	40/5	29/5	12/5	7/5	6/5
14	4/4	6/4	5/4	6/4	25/4	21/4	20/4	16/4	18/4	13/4	5/4	¾
15	0/3	6/3	10/3	5/3	8/3	6/3	10/3	18/3	10/3	11/3	5/3	6/3
Tot	75	60	105	85	150	165	225	300	225	195	75	90
Average room density	5	4	7	6	10	11	15	20	15	13	5	6

Appendix 11: Number of mosquitoes collected versus number of occupants in the room in Pota Community and Average room density.

R/N	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
1	0/3	0/3	1/3	0/3	3/3	10/3	15/3	5/3	10/3	15/3	10/3	2/3
2	1/3	2/3	4/3	8/3	20/3	5/3	15/3	10/3	20/3	10/3	5/3	5/3
3	2/3	3/3	4/3	3/3	11/3	25/3	20/3	15/3	5/3	30/3	5/3	8/3
4	2/3	3/3	2/3	2/3	20/3	10/3	15/3	10/3	5/3	20/3	10/3	10/3
5	¼	2/4	2/4	1/4	10/4	15/4	10/4	10/4	10/4	10/4	5/4	10/4
6	¼	2/4	2/4	1/4	8/4	10/4	35/4	15/4	30/4	15/4	10/4	5/4
7	2/4	¾	¾	6/4	9/4	12/4	20/4	30/4	30/4	20/4	15/4	5/4
8	10/5	11/5	6/5	9/5	10/5	13/5	15/5	10/5	20/5	15/5	5/5	10/5
9	9/3	9/3	15/3	5/3	15/3	14/3	40/3	10/3	30/4	15/4	10/4	5/4
10	12/4	5/4	6/4	7/4	20/4	15/4	30/4	50/4	30/4	10/4	8/4	2/4
11	¼	5/4	6/4	4/4	15/4	30/4	40/4	30/4	50/4	20/4	9/4	¾
12	¼	5/2	4/2	5/2	16/2	5/4	30/4	40/4	20/4	10/4	8/4	2/4
13	¼	5/4	¾	6/4	4/4	20/4	40/4	30/4	40/4	15/4	10/4	4/4
14	2/4	4/4	6/4	5/4	5/4	20/4	20/4	30/4	20,4	15/4	10/4	¼
15	0/3	1/3	¼	2/4	5/4	10/4	20/4	30/4	10/4	10/4	5/4	¼
Tot	45	60	60	60	150	225	300	330	300	225	120	75
Average Room density	3	4	4	4	5	15	20	22	20	15	8	5

Appendix 12: Monthly rainfall measurement in the study community for 2002

Monthly Rainfall (mm^3)	**Anopheles room density**
64.6	4
44	4
76.5	7
159.5	7
221.9	8
372.1	14
296	18
85.3	20
189.1	17
245.8	14
30.4	8
49.2	5

Curtesy: Institute of Metereology, Oshodi

Appendix 13: Calculation of Man biting Rate and Sporozoite rate and EIR in Igborosun, Ikoga and Pota communities

Month	Igborosun			Ikoga			Pota		
	MBR	SR	EIR/year	MBR	SR	EIR/year	MBR	SR	EIR/year
Jan	44.33	0	0	35.65	1.66	7.08	20.77	0	0
Feb	47.88	0	0	29.68	0	0	27.26	0	0
Mar	114.7	0.77	10.56	53.63	0	0	28.52	0	0
April	111.0	0.77	20.52	46.2	0	0	32.4	0	0
May	124	0.70	10.32	77.5	1.66	15.48	81.84	0	0
June	94.2	0.90	32.4	86.4	2.0	20.64	122.83	0.92	16.02
July	212.7	1.25	33.48	131.1	0.9	14.16	163.7	1.07	21.0
Aug	221.3	1.20	27.36	149.1	0.8	14.28	187.2	0.93	20.88
Sept	223	0.76	25.2	115.5	1.5	20.64	162.6	0.69	13.44
Oct	177	0.5	14.88	107.26	1.11	14.28	122.8	0.48	7.08
Nov	142.6	0.63	11.16	35.65	1.66	6.84	56.7	0	0
Dec	35.3	0	0	41.85	0	0	36.6	0	0
Average			28.59			17.44			12

Appendix 14: Protocol for DNA extraction

Step 1: Switch on heating block (dry bath) to reach at least 70 degrees

Step 2: Place mosquito leg in eppendorf tube

Step 3: Homogenize mosquito leg in 100ul of grinding buffer

Step 4: Incubate in the dry bath for 30 minutes

Step 5: Add 28ul 8M Kac and mix gently by tapping with the finger. Incubate on ice for 30 minutes

Step 6: Centrifuge for 10-15 minutes at 16000rpm with the hinge of eppie facing out

Step 7: Pipette off all liquid without disturbing the pellet. Place liquid in new eppendorf and discard old eppendorf with pellet. Add 400ul of 100% ethanol (ice cold, from -20^0 C freezer), mix by inverting tube. Incubate at room temperature for 5 minutes or overnight in -20^0 C freezer

Step 8: Centrifuge for 15 minutes at 16,000rpm

Step 9: Pipette off 70% ethanol. Air dry on bench with tops open, either overnight or until the pellet is dry

Step 12: Resuspend the pellet in 200ul of 1x TE making sure the pellet dissolves.

Store in the refridgerator for 1 month or freezer.

Use 0.5ul or 1ul

Appendix 15: *An. gambiae* species-specific polymerase chain reaction assay

Master Mix

Reagent	Quantity
PCR buffer (x10)	1.25
DNTPs	1.25
GA	1.0
AA	1.0
ME	1.0
QD	0.5
UN	1.0
$MgCl_2$	0.5
Water	4.9
RTaq	0.1

Procedure

Mix thoroughly and add 12.5ul of PCR master mix to each eppendorf tube in the PCR machine and choose programme

Add 1ul of DNA to each tube

Load each tube in the PCR machine and choose programme

Prepare 1.5% agarose gel with TAE buffer and 10 ul of ethidium bromide

Load 12.5ul of PCR product with 1ul of loading buffer into each well

Load 10ul of standard marker per gel

Run PCR product at 100 volts

Remove and photograph the gel

Appendix 16: Calculation of SP dose to be administered to the mice for histopathological studies

A.

1. 3 tablets of SP =1500 (sulfadoxine) +75 (pyrimethamine)

   =1575mg recommended for adult.

2. Average weight of an adult

   =65kg

3. Average weight of the mice

   =20g

4. Required dose for 20g mouse

   65kg = 1575mg.

   20 = ?

   $\frac{31500}{65000}$

   =0.5

The Human Therapeutic Dose (HTD) for the mice is 0.5mg of SP.

B. **To obtain the volume of SP that will correspond to 0.5mg SP**

100mg of SP was dissolved in 20mls distilled water

100mg = 20mls

0.5mg = ?

$\frac{0.5 \text{ X } 20}{100}$

=0.1ml

Therefore for HTD, 0.5mg of SP is equivalent to 0.1ml of diluted SP administered

to the mice.

Appendix 17: Slide preparation protocol for Histology

H&E stain is a popular staining method in histology. It is the most widely used stain in medical diagnosis; for example when a pathologist looks at a biopsy of a suspected cancer, the histological section is likely to be stained with H&E and termed H&E *section*, H+E section, or HE section.

The staining method involves application of hemalum, which is a complex formed from aluminium ions and oxidized hematoxylin. This colour the nuclei of cells (and a few other objects, such as keratohyalin granules) blue. Materials coloured blue by hemalum are often said to be basophilic, but this is an incorrect use of the word. The nuclear staining is followed by counterstaining with an aqueous alcoholic solution of eosin Y, which colours eosinophilic structures in various shades of red, pink and orange.

For histology, the tissue pieces were fixed in a suitable fixative, typically formalin, and embedded in melted paraffin wax. The wax block was then cut on a microtome to yield a thin slice of paraffin containing the tissue. The specimen slice was then applied to a microscopic slide, air dried, and heated to cause the specimen to adhere to the glass slide. Residual paraffin is then dissolved with a suitable solvent, typically xylene, toluene, or others. These so-called deparaffinizing solvents were then removed with a washing-dehydrating type reagent prior to staining. Alternatively, slices may be prepared from frozen specimens, fixed briefly in 10% formalin, then infused with dehydrating reagent. Consequently, a common step for both cytology and histology specimens is the removal of the dehydrating reagent prior to staining with an aqueous stain.

(a) Removing dehydrating reagent from the specimen affixed to a microscope slide

and hydrating the specimen by soaking in water;
(b) Applying hematoxylin for staining the cell nuclei in the specimen;
(c) Removing excess hematoxylin by rinsing with water [For a regressive hematoxylin stain, the water rinse is usually followed by rinsing with an acid-alcohol followed by rinsing with water to remove the acid-alcohol];
(d) Contacting the slide with a concentrated solution having a pH above 5.0 to turn the hematoxylin blue (bluing solution);
(e) Removing the bluing solution by rinsing with water;
(f) Staining other cytoplasmic elements with an alcoholic solution of eosin Y, a red stain, and light green or fast green.
(g) Removing excess stain and water by a series of sequential washes in a dehydrating reagent;
(h) Contacting the slide with a chemical-clearing agent (toluene, xylene, or t-butanol) to remove residual dehydrating reagent remaining from the washing step;
(i) Applying a cover-slip mountant and a cover-slip after first removing the slide from the chemical-clearing agent. The clearing agent evaporates and the mountant hardens leaving a stained and mounted slide.

Histology

Tissues of about 5 mm thickness were obtained from the (organs) and fixed in 10% neutral buffered formalin. These tissues were processed for histopathological examination using a routine paraffin-wax embedding method. Sections of 5 μm thickness were stained with Haematoxylin and Eosin.

Histopathological assessment and photomicrography of the prepared slides was done by a pathologist, using an Olympus light Microscope with attached Kodak digital camera.