KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY



# Molecular Epidemiology of *Plasmodium falciparum* in Ashanti Region, Ghana in a Setting of Artesunate-Amodiaquine Use

by

**Denis Dekugmen Yar**

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# Molecular Epidemiology of *Plasmodium falciparum* in Ashanti Region, Ghana in a Setting of Artesunate-Amodiaquine Use

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**Denis Dekugmen Yar**

**A Thesis submitted to the Department of Theoretical and Applied Biology,**

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**in fulfilment of the requirements for the award**

**of**

**DOCTOR OF PHILOSOPHY IN BIOLOGICAL SCIENCES**

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**June 2009**

## DECLARATION

This thesis is submitted to the Kwame Nkrumah University of Science and Technology, School of Graduate Studies through the College of Science, Department of Theoretical and Applied Biology. I hereby declare that this thesis has been composed by myself and has not been accepted in any previous application for a degree here or elsewhere. This thesis presents results of original research undertaken by me personally. Information taken from other works has been specially and duly acknowledged.



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(Head of Department)

## DEDICATION

I dedicate this thesis to God for His grace and my dear Mother, Mrs. Pobeyel Yar for her support and encouragement throughout the period of my education.



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## DEFINITION OF TERMS

1. **Caregivers** -Women who have and or are taking care of children less than five years old

2. **Drug resistance of malaria parasites**: is the ability of a parasite strain to multiply or to survive in the presence of concentrations of a drug that normally destroy parasites of the same species or prevent their multiplication

3. **Exons:** the segment of DNA or RNA that contain information for polypeptide or RNA synthesis.

4. **Gene:** a linear sequence of nucleotides coding for a polypeptide or RNA molecule; the unit of inheritance.

5. **Genome:** the entire complement of the genetic information carried by an organism.

6. **Introns:** intervening sequences in DNA or RNA that do not contain information needed for either peptide or RNA synthesis.

7. **Molecular Epidemiology**: it is a science that deals with the contribution of potential genetic and environmental risk factors identified at the molecular level, to the aetiology, distribution and control of disease in groups of relatives and populations.

8. **Mutation:** any change in the nucleotide sequence of the genome, whether DNA or RNA

9. **Point mutation:** a change in one base in DNA or RNA.

10. **Polymerase chase reaction:** the procedure for amplifying DNA.

11. **Polymorphism:** the condition in which a gene appears in more than one form in a population

12. **Recrudescence:** this is a level of drug resistance, R1 in which a patient's clinical response to treatment is satisfactory following normal full course of antimalarial drug and there is clearance of parasiteamia (trophozoites) within 7 days, but within three weeks or more the illness recurs.

13. **Treatment arm:** This is the study drugs / test antimalarial drugs used to treat a group of the study children in the study area

## ACRONYMS AND ABBREVIATIONS

ACTs: - Artemisinin-based Combination Therapies
AIDS: - Acquired Immunodeficiency Syndrome
AMQ: - Amodiaquine
ART: - Artesunate
CDD: - Community Drugs Distributor
CHOs: - Community Health Officers
CHPS: - Community Health Planning and Services
CQ: - Chloroquine
CTs: - Combination Therapies
DHMTs: - District Health Management Teams
DHS: - Demographic and Health Surveys
DNA: - Deoxyribonucleic Acid
GDHS: - Ghana Demographic and Health Survey
GDP: - Gross Domestic Product
GHS: - Ghana Health Service
GHAT: - Ghana Health Assessment Project Team
GIT: - Gastro Intestinal Tract
GLURP: - Glutamine-Rich Protein
GSS: - Ghana Statistical Services
HB: - Haemoglobin
HMM: - Home Management of Malaria
IEC: - Information, Education and Communication
IPT: - Intermittent Preventive Treatment
ITN: - Insecticide-Treated Net
KNUST: - Kwame Nkrumah University of Science and Technology
MOH: - Ministry of Health
MOI: - Multiplicity of Infection
MSP: - Merozoite Surface Protein
MQ: - Mefloquine
NGO: - Non-governmental Organization
NMCP: - National Malaria Control Programme
OPD: - Out Patient Department
ORS: - Oral Rehydration Salt
RBM: - Roll Back Malaria
PCR: - Polymerase Chain Reaction
PCV: - Packed Cell Volume
SD: - Standard Deviation
SMS: -School of Medical Sciences
SP: - Sulfadoxine-Pyrimethamine
TDR: -Tropical Disease Research Programme
UNDP: - United Nations Development Programme
UNICEF: - United Nations Children's Fund
WHO: - World Health Organization

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## ABSTRACT

The approach to making antimalarial drugs available in the community and ensuring that they are used appropriately is now well established through WHO/TDR-supported multi-country studies using Chloroquine. The use of ART-AMQ combination treatment for home management of childhood malaria is a new area of research and programme implementation. Natural populations of *P. falciparum* are heterogeneous mixtures of individuals with different, genetically determined degrees of drug response during treatments. The first part of this study was, therefore, aimed at determining the effectiveness of treatments and molecular epidemiology of *P. falciparum* in a setting of ART-AMQ use in the home setting in a rural district in Ghana. Community Drug Distributors (CDDs) in the study communities in the Ejisu-Juaben Municipal District were trained to examine children presenting with fevers and those suspected to be due to malaria, were treated presumptively with unit-dose prepacked ART-AMQ over three days. CDDs from these communities were also trained to take finger-prick blood samples prior to treatment for polymerase chain reaction (PCR) analysis and parasitological confirmation and those confirmed positive were enrolled into the study. Febrile children treated were followed up and had their blood samples taken on days 14 and 28 after the start of treatment to assess the effect of treatment on peripheral parasitaemia. Of the 836 blood samples collected, 71% of the presumptive treatments were confirmed microscopically to have malaria parasites whilst 29% were without the parasites. Parasitological failure by day 28 in children treated at home was 27.8% whilst parasitological failure corrected for re-infections using merozoite surface protein-2 (*msp2)* and glutamine-rich protein (*glurp*) markers was 11.6%. Over 50% of the febrile children treated at home had multiple infections using both *glurp and msp2* markers. In *msp2* marker, over 80% of the children had *P. falciparum* clones belonging to the IC/3D7 allelic family with only 2% having both FC27 and IC/3D7 allelic families. Twenty different IC/3D7 family alleles and four different FC27 families were detected. The accuracy of the CDDs and caregivers was quiet high implying that febrile children had early and appropriate home care of malaria although not all of them had malaria parasites. However, the parasitological failure rate of 11.6% for ART-AMQ use at home, calls for search for alternative treatment regimen. The high level of polymorphism, antigenic variation, multiplicity of infections and the frequency of infections had contributed to the treatment outcomes. The second part of the study was to compare in-vivo field trial of ACTs with Artesunate-Amodiaquine in the treatment of uncomplicated malaria in school pupils. The failure of Chloroquine has necessitated the re-examination of the potential of combinations of existing products and the development of new combination drugs. Hence, school pupils were screened microscopically in two surveys and those that were positive were enrolled and followed up for 28 days. In all, 56.8% (476) pupils were positive and were subsequently randomized into seven different treatment groups of ACTs. School pupils were followed up for 28 days period and had their blood samples taken on days 14 and 28 after the start of treatment to assess the effect of treatment on peripheral parasitaemia and on haemoglobin concentrations. Parasitological failure by day 28 in pupils treated with various ACTs was 28.4%, 18.2%, 12.0%, 38.1%, 30.8%, 33.3% and 7.1% for Artesunate-Sulfadoxine-Pyrimethamine, Artesunate-Amodiaquine, Artesunate-Mefloquine, Artesunate-Chlorproguanil-Dapsone, Arthemeter-Lumefantrine, Artesunate-Chloroquine and AMOTEX (co-formulation of ART-AMQ) treatment groups respectively. The change in mean haemoglobin over the baseline haemoglobin by days 14 and 28 were 0.7g/dl and 0.9g/dl respectively ($p<0.001$). The burden of malaria and anaemia among school children is high and warrants investment to reduce these levels. In this study, AMOTEX had the least failure rate (7.1%) by day 28, but was the least tolerated among the pupils. However, Artesunate-Mefloquine combination has demonstrated a high potential alternative option to ART-AMQ since it was well tolerated and has high parasite clearance among study pupils.

# CHAPTER ONE - INTRODUCTION

# CHAPTER ONE - INTRODUCTION

## 1.0 Background to the Study

Malaria remains the world's most devastating human parasitic infection affecting over 40% of the world's population. The world malaria situation is worsening, with the geographic spread of resistance widening to previously unaffected areas and a remorseless increase both in the prevalence and degree of drug resistance (Shunmay *et al*., 2004). Success in developing new antimalarial drugs has been short-lived because *Plasmodium* parasites continue to develop resistance to broad classes of antimalarial drugs. The evolution of drug resistance in *Plasmodium* is not fully understood although the molecular basis for resistance is becoming clearer (Cattamanchi *et al*., 2003).

The frequency of genetic diversity in *P. falciparum* determines the number of functionally and antigenically different parasites that can be generated. Variability at certain loci could lead to parasite strains that differ in their ability to escape recognition by the host immune system and are resistant to certain antimalarial drugs (Ron *et al*., 2005). The genetic variability of *P. falciparum* can be estimated by the extent of polymorphism in the genes coding for the merozoite surface proteins 1 and 2 (*msp-1 & msp-2*) and glutamate-rich protein (*glurp*). Variability in these genes can also be used to determine the multiplicity of individual infections. Multiplicity of infection will affect both the prevalence of parasite genetic markers such as those involved in resistance to antimalarial drugs (Jelinek *et al*., 1999; Schneider *et al*., 2002) and the risk of clinical disease.

In endemic areas, the number of clones of malaria parasites co-infecting a single host can be a useful indicator of the level of transmission and/or the immune status of the host. Natural populations of *P. falciparum* are heterogeneous mixtures of individuals with different, genetically

determined degrees of drug response (Shunmay *et al*., 2004). The extent of frequency of multiplicity of infections and genetic diversity of *P. falciparum* will have implications for the introduction of new antimalarial regimens and for malaria control programmes in Ghana.

## 1.2 Problem Statement

The Ghana Health Service (GHS, 2004a) has changed its policy on the first line drug for malaria case management from Chloroquine (CQ) to Artesunate-Amodiaquine (ART-AMQ). This took effect from January 1st, 2005. The decision to change policy was based on local evidence of increasing spread of Chloroquine-resistant *Plasmodium falciparum* (CRPF) malaria, ranging between 6% and 25% of treatment failure (GHS, 2004b) and WHO recommendation on artemisinin combination therapy (ACTs). However, of most of the ACTs currently in use or being evaluated, the partner drugs such as Amodiaquine, mefloquine etc. are eliminated slowly. These partner drugs are therefore unprotected when the artemisinins have been eliminated from the body. They are then in subclinical doses and when exposed to new infections, might lead to drugs resistance. All antimicrobial agents in general exert selection pressure on the microorganisms they act upon. This may result in the development of resistant strains and reduction in effectiveness of the drug. Little is, however, known about the genetic diversity profile of *P. falciparum* in the forest belt of Ghana where the transmission of malaria is severest (Ahmed, 1989; GHS, 2001).

## 1.3 Rationale of the study

Since 2000, both clinical and other evidence in Ghana and parts of Africa and beyond have shown conclusively that Chloroquine is no longer useful as a first line drug in the treatment of uncomplicated malaria due to increasing resistance of the malaria parasite to the drug (GHS, 2002a, b). The failure of Chloroquine has necessitated the re-examination of the potential of

combinations of existing products and the development of new combination drugs. This, thus, calls for concerted effort to re-examine the few antimalarial drugs in combination with Artesunate to determine the most effective, affordable and readily available combination which is safe and efficacious for the management of malaria in Ghana.

The choice of Artesunate in combination with Amodiaquine (ART-AMQ) as first line drug in Ghana was based largely on studies from parts of Africa since very limited local studies have been done prior to its introduction as first line drug. The selection of drugs and treatment protocols must, however, be based on reliable clinical and epidemiological assessments of efficacy and safety. Hence a randomized in-vivo comparative trials study using various antimalarial drugs in the country was needful to give credence and basis for the choice of ART-AMQ as the first line drug for the management of uncomplicated malaria in Ghana. Any drug therapy strategy should be designed to minimize the threat of resistant parasites (Shunmay *et al*., 2004). Specific strategies however, must be tailored to the patient, the community and the region in which they are employed.

The increasing knowledge of the parasite's extreme diversity and the recognition that an individual is exposed to a variety of constantly changing infection require that the genetic diversity profile of *P. falciparum* be determined with the introduction of ACTs. The proposed study therefore sought to determine the effectiveness of Artesunate-Amodiaquine and some antimalarial drugs in combination with Artesunate under the given environmental and sociological conditions in order to determine alternative treatment regimens for the management of uncomplicated malaria. The findings would also serve as a guide to health policy makers in the design of antimalarial drug policy in a bid to combating malaria in the region and beyond. The study also sought to determine the frequency of genetic diversity of *P. falciparum*, its multiplicity

of infections in individuals and their susceptibility to the combination therapy of ART-AMQ, the result of which would enhance the diagnosis and treatment of malaria.

## 1.4 Conceptual Framework

The ever-increasing resistance of malaria parasites to drugs is a matter of great concern. In developing or revising an antimalarial treatment policy, the efficacy of alternative regimens should also be taken into consideration as should the distribution of strains of *P. falciparum.* Very little of such studies have been done in the forest belt of rural Ghana where the incidence of malaria is severest. **Figure 1.1** below is the plan of a randomized in-vivo comparative study of different antimalarial drugs in combination with Artesunate to assess their effectiveness and the diversity of *P. falciparum.* Two different experimental designs were employed in the study. A longitudinal study was carried out to assess the molecular epidemiology of *P.falciparum* in the setting of ART-AMQ as first line drug for the management of uncomplicated malaria. Study participants were followed up for at least six months, prior to the presence of malarial fevers, for sample collection and medication with ART-AMQ. Children treated were followed up for days 14 and 28 samples respectively. An in-vivo comparative trial of ACTs also was carried out in two separate studies in dry and wet seasons to determine their effectiveness. Study pupils were also followed up for days 14 and 28 samples.

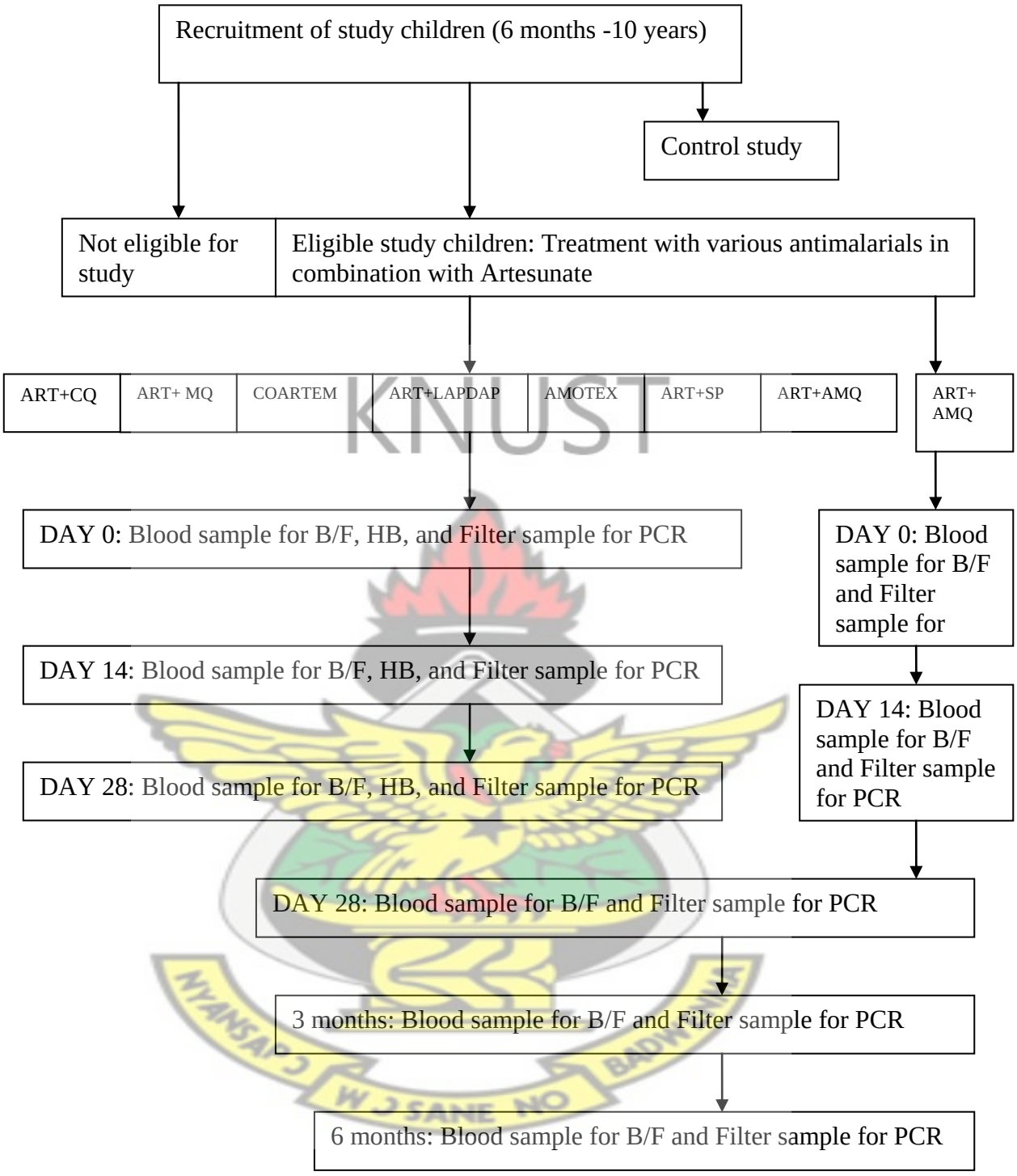


**Figure 1.1: Conceptual Framework**

## 1.5 Research Hypotheses

1. Antimalarial drugs exert selection pressure on parasites resulting in evolution of drug resistance over time.
2. The emergence of antimalarial drug resistance is dependent on the occurrence of a spontaneous genetic change (mutation or gene amplification) in a malaria parasite, which interferes with that parasite's susceptibility to a drug.
3. There is an association between the extent of parasite genetic diversity and malaria transmission.
4. Failure to adhere to drug regimens and compliance, the misuse of, and substandard drugs are major contributory factors to resistance development of *Plasmodium falciparum* strains.



## 1.6 Research Questions

It is worth finding out whether

1. there is any association between the extent of genetic diversity and malaria transmission
2. the parasite populations carry different gene types and how these gene types may be distributed in the host population
3. in the context of multi-drugs use and ACTs, there occurs an increase in genetic diversity
4. resistance to drugs is developed by some strains only and
5. certain factors may predispose to the development of resistance.

## 1.7 Aims and Objectives

The main aim of the study, therefore, was to determine the genetic diversity of *P.falciparum* and assess the effectiveness of Artesunate in combination with other antimalarials for the management of uncomplicated malaria.

### *1.7.1 Specific Objectives*

The specific objectives were to:

1. determine the prevalence of peripheral parasitaemia, parasite density and gametocyte rate in study children at day 0.
2. determine the effect of different ACTs used on the prevalence of peripheral parasitaemia, parasite densities and gametocyte rates on days 14 and 28 post treatment.
3. compare the effect of different ACTs used on haemoglobin levels of study children on days 14 and 28 post treatment.
4. compare the incidence of adverse effects in the different ACTs used.
5. determine *P.falciparum* genetic diversity in the study population.
6. determine the multiplicity of *P.falciparum* strain infections in individuals.
7. compare *P.falciparum* strains in different episodes of malaria in individual patients.
8. determine resistant strains of *P.falciparum* in children treated with ART-AMQ.



## 1.8 Scope and Limitation of the Study

Molecular epidemiology is based on general epidemiology and utilizes molecular biology to define the distribution of the disease and its etiologic determinants. It is a science that deals with the contribution of potential genetic and environmental risk factors identified at the molecular level, to the aetiology, distribution and control of disease in groups of relatives and populations. The objectives of molecular epidemiology are quite broad and include:

1. Descriptive and analytical studies to evaluate host/environmental interactions in disease
2. The development of prevention strategies for the control of bacterial, parasitic and viral disorders through molecular diagnosis
3. The prevention of non-communicable diseases and genetic disorders by assessing risk and identifying susceptible individuals through genetic screening.



The molecular epidemiology part of this study, however, was limited only to molecular genotyping of *P.falciparum* on blood spotted filter samples collected from study children. Only a representative sample could be used for PCR analysis for genotyping as a result of constraints and difficulty in acquiring materials and reagents to run the entire samples. This study did not include DNA sequencing of the parasites and restriction fragment length polymorphism (RFLP). The study was limited to only children aged 6 months to 10 years, caregivers and CDDs. Assessing drugs adverse effects demanded recall of post treatment health status and signs of adverse effects. There could therefore be the introduction of recall biases as some caregivers could fail to recall adequately.

## 1.9 Organization of the Thesis

The thesis is divided into the following chapters:-

**Chapter One Introduction to the thesis**

This chapter gives the background, problem statement, rationale, hypotheses and objectives of the study and provides a general overview of the thesis organization.

**Chapter Two Literature review**

This chapter presents a brief review of literature on the biology of malaria parasite, drug resistance development, global malaria situation, the epidemiology of malaria and its control and prevention in Ghana. This is followed by a summary of the properties of the study drugs including known adverse effects and finally a brief overview of drug treatment policy change.

**Chapter Three Materials and Methods**

This chapter outlines the study design and gives a description of the study area and population. This is followed by a description of recruitment procedures and training of field workers, general procedures, ethical issues and the overall activities during the study.



**Chapter Four Results**

This chapter is subdivided into three segments:

**Recruitment, screening and enrolment**

This segment outlines screening processes leading to enrolment of eligible children into to the study and randomized groups. It also includes description of the demographic and social characteristics of caregivers and the assessment of children's temperature and weight including baseline parasitological and haematological indices.

**Treatment outcomes, safety and tolerance**

This segment presents the parasitological and haematological outcomes after the initial treatment with the test drugs during a 28-day follow up. The proportions of treatment (parasitological) failures at days 14 and 28 of the different ACTs were compared to ART-AMQ. The proportion of the mean changes in haemoglobin concentrations were also compared within treatment groups and to the baseline mean. This also included the level of change in the mean haemoglobin at days 14

and 28 of the various ACTs. The last part of this segment presents the adverse outcomes after treatments with the different ACTs for a follow up period of one week following initial treatment.

**Genetic diversity, multiplicity of infection and resistant strains**

This last segment presents the results of the parasite diversity and multiplicity of infections of *P.falciparum* infection. The first part of this segment outlines the PCR genotyping outcome and describes the proportion of recrudescence and re-infection at day 28 follow up at the start of treatment. This is followed by the level of diversity, multiplicity and the change in strains in different episodes of malaria.



**Chapter Five Discussions**

This chapter of the thesis presents the discussion of the key findings of the study and the inherent limitations within the study of molecular epidemiology. It further discusses the epidemiology of malaria in children, the home management of malaria and the impact of treatment outcomes on peripheral parasitaemia and anaemia in school pupils.

**Chapter Six Conclusions, Recommendations and Reflection for Future Research**

This is the last chapter of the thesis and presents the summary and conclusions of the key findings. It further discusses the implications of the findings for future research and the implication for policy direction in malaria control in Ghana.

# CHAPTER TWO - LITERATURE REVIEW

# CHAPTER TWO - LITERATURE REVIEW

## 2.0 Malaria: the Parasite and Drug Resistance

### 2.1 Malaria Parasitology

Malaria parasites have been with us since the dawn of time. They probably originated from Africa (along with mankind) and fossils of mosquitoes up to 30 million years old showed that the vector for malaria was present well before the earliest history. The *Plasmodium* parasites are highly specific, with man as the main vertebrate host and *Anopheles* mosquitoes as the vectors. Malaria is generally endemic in the tropics with extensions into subtropics. Distribution varies from country to country and within countries.



#### *2.1.1 Biology of Plasmodium Parasites*

Malaria parasites are protozoan parasites belonging to the phylum *Apicomplexa*, subclass *Coccidia* and the family *Plasmodiidae*. The genus of this protozoal parasite is *Plasmodium* with four main species. *P.falciparum* and *P. vivax* are the widespread species whilst *P. malariae* and *P.ovale* are less widespread. They have a life cycle that splits between a vertebrate host (intermediate host) and an insect vector (definitive host). The *Plasmodium* species with the exception of *P.malariae* (which may infect other higher primates) are exclusively parasites of man (Cheesborough, 1998).

#### *2.1.2 Life Cycle of Plasmodium parasites*

The life cycle begins when an infected female *Anopheles* mosquito takes a blood meal and sporozoites from the infected mosquito's salivary gland are injected into the human. The mosquito injects anticoagulant saliva to ensure an even flowing meal. The sporozoites are

elongated bodies measuring about 11µm long and 1µm across with a central nucleus (**Figure 2.1:1**). Like many protozoa, plasmodia pass through a number of stages in the course of their two-host life cycle. The stage infective to man is the uninucleate, lancet-shaped sporozoite. The sporozoites are produced by sexual reproduction in the mid-gut of the vector anopheline mosquito and migrate to the salivary gland. The sporozoites are thought to enter the liver parenchyma cells/hepatocytes via the Kupffer cells within 45 minutes of inoculation (Cheesborough, 1998) when an infested female Anopheline mosquito bites the human host.



In the liver cells, the parasite develops into a spherical, multinucleate liver stage schizont **(Figure 2.1:3)**, which contains 2,000 to 40,000 uninucleate merozoites (www.cdc.gov/). This process of multiple amplifications is called exoerythrocytic / pre-erythrocytic schizogony (**Figure 2.1: A)**. This liver phase of the disease usually takes 5 to 21 days depending on the species of *Plasmodium*. However, in *P. ovale* and *P. vivax* infections, maturation of the liver stage schizonts may be delayed for as long as 1 to 2 years. At this quiescent liver-phase, the parasites are called hypnozoites (Cheesborough, 1998).

Regardless of the time required for development, the mature schizonts rupture releasing thousands of uninucleate merozoites **(Figure 2.1:2)** into the blood stream (www.cdc.gov/). Each merozoite can enter the red blood cell through the sinusoids of the liver. A proportion is phagocytosed and destroyed. Within the red cell the merozoite develops first into a stage referred to as the trophozoite (**Figure 2.1: B-d)**, which feeds on the haemoglobin by ingesting a small amount of the red cell cytoplasm. The trophozoite grows and becomes globular in form, Maurer's clefts form and the nucleus begins to divide, followed by a division of the cytoplasm into an erythrocytic stage schizont (erythrocytic schizogony) (**Figure 2.1: B)**. The mature erythrocytic schizont contains 8 to 36 merozoites, each up to 5 µm to 10 µm long that are released into the

blood stream when the schizont ruptures (www.malariasite.com). These merozoites proceed to infect another generation of erythrocytes. The time required for erythrocytic schizogony determines the interval between peaks of successive classic periodicity of malaria fever. Within the erythrocyte, schizogony occurs to produce more merozoites and in *P.falciparum* erythrocytic schizogony takes 48 hours (www.cdc.gov/). After several erythrocytic cycles, some of the merozoites enter red blood cells and instead of developing into schizonts, they follow a sexual development and by gametocytosis (**Figure 2.1:B)** become spherical or banana shaped uninucleate macrogametocytes and microgametocytes (**Figure 2.1:7)**. In *P.falciparum*, gametocytosis takes 10-12 days (Cheesborough, 1998).



When another mosquito feeds on an infected individual's blood, it may suck up these gametocytes into its gut. The macrogametocytes increase in volume while the microgametocytes undergo exflagellation (**Figure 2.1:8)** after undergoing three mitotic divisions, and are expelled explosively. The macrogametocyte undergoes no further change until fertilization where the plasmalemmas of the male and the female fuse and the nucleus of the microgamete enter the female cytoplasm (www.malariasite.com). After fertilization, the zygote is a motionless globular cell, but after 18 to 24 hours it becomes elongated and motile, containing micronemes and pellicle, forming the ookinette (**Figure 2.1:10)**. This cell invades the microvillus border through the midgut cells and lies beneath the basement membrane. The ookinette then becomes a static oocyst (**Figure 2.1:11)**, between the basal lamina and the basement membrane. The chief source of nutrients is the heamolymph where the oocyst develops. Sporoplast forms and sporozoites bud off (**Figure 2.1:C)**. After the cyst ruptures, the sporozoites escape into the haemocoele and migrate to penetrate salivary gland cells, where they lie in vacuole for the maturation. The sporozoites develop and become up to 100 times more infective than when in oocyst (www.cdc.gov/). Another life cycle begins when an infected female *Anopheles* mosquito takes a blood meal and these sporozoites from the infected mosquito salivary gland are injected into man.

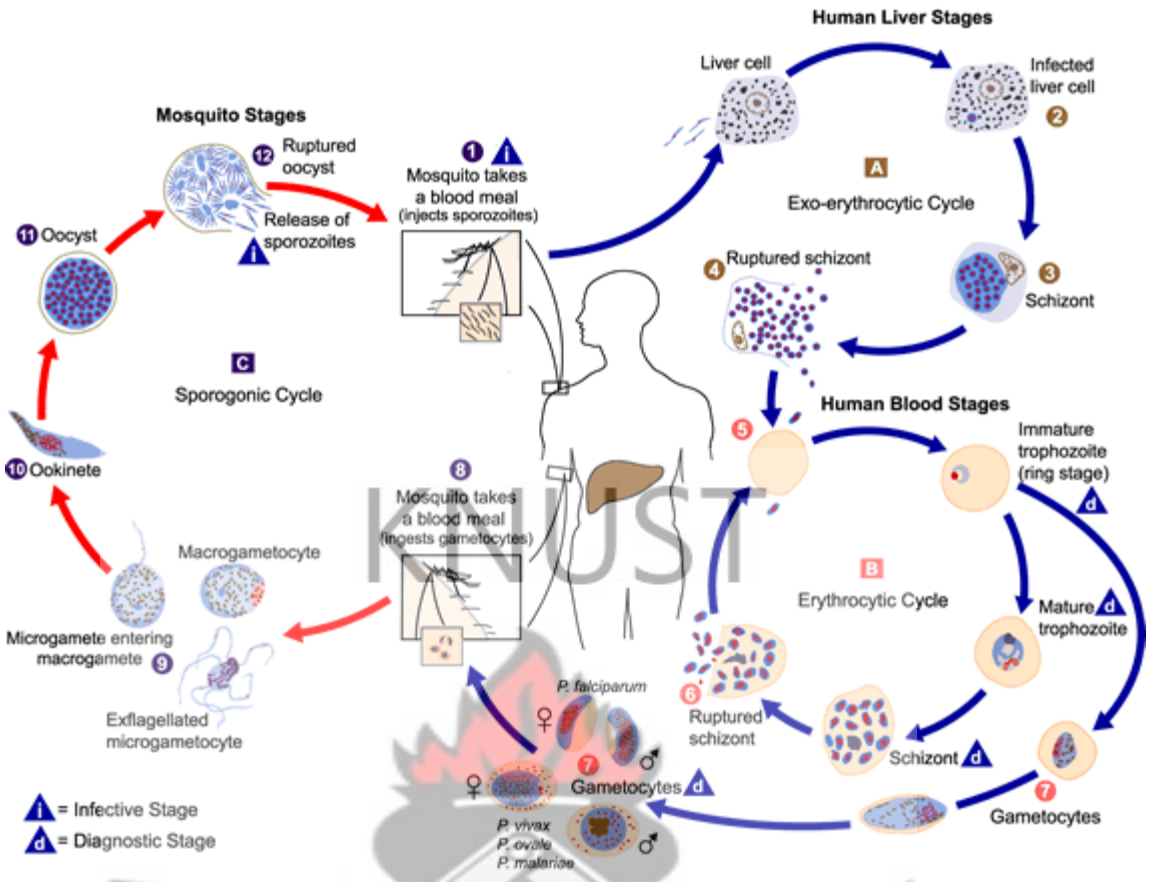


**Figure 2.1: Life cycle of the Malaria Parasite**

**(Source: Centres for Disease Control and Prevention, USA)**

### *2.1.3 Anopheline Vector*

The mosquito is always the vector of malaria and it is always an anopheline mosquito, although out of the 434 species known, only 60 can transmit malaria (Kettle, 1995). Only female anopheline mosquitoes are involved as the males do not feed on blood. Mosquitoes belong to the phylum *Arthropoda*, class *Ins*ecta and order *Diptera*. The life span of a mosquito is generally 3-4 weeks. The principal vectors of the malaria parasites are the *Anopheles gambiae complex* and *Anopheles funestus*. Other secondary vectors of malaria are *A. hargreavesi* Evans and *A. nili* Theobald. *A. gambiae* and *A. funestus* usually account for 95 percent of total "knock down" catches. *A.gambiae*

s.s of the complex predominates and transcends across the country (GHS, 2001). *Anopheles arabiensis* and *Anopheles melas* also exist but in small proportions.

### *2.1.4 Malaria Transmission and Infectivity*

Transmission intensity of malaria depends on the density and infectivity of the anopheline vector and on variation in parasite rate in the human host (Smith *et al*., 1993; Peyerl-Hoffmann *et al.*, 2001; Koram & Molyneux, 2007). The effects of human behaviour on malaria transmission are place-specific. Anthropogenic changes such as deforestation, road-construction and agricultural development generally increase the intensity of malaria transmission. But the specific effects of such ecological disturbances are often unpredictable, due to geographical diversity in the biology of the mosquitoes that transmit the disease.

No single biological, economic or political reason can be adduced for the observed patterns and trends in malaria transmission. No single intervention, therefore, is appropriate in all contexts. Interventions should be adapted to specific local ecological, epidemiological, economic, and social conditions. The goals of malaria interventions should be place-specific (WHO, 2003b; Snow *et al*., 2005).

Infection with *P. falciparum* has a wide spectrum of manifestations that are roughly classified into three clinical groups: asymptomatic infection, mild malaria, and severe malaria. In malaria-endemic areas, a significant proportion of children harbour parasites without presenting signs of clinical malaria and are considered asymptomatic cases (Greenwood, 1987; Siâne *et al*., 1998). Of those presenting signs of clinical malaria, the bulk of morbidity and mortality are focused on pre-school children (1-5 years old), and most research has naturally focused on this high-risk group (WHO, 2005b).

### *2.1.5 Immunology of malaria in humans*

In areas of intense transmission, children gradually acquire immunity that protects them from severe malaria attack and death (Snow *et al*., 1999). Because of differing transmission intensities, semi-immunity at a younger age develops faster in a holo- or hyperendemic area than in an area where transmission is less intense (Mayxay *et al*., 2001; Baird, 1995; Smith *et al*., 1999 a, b). Variant-specific immunity is one of the key components to explain the low-grade infection during extended periods without clinical symptoms (Staalsoe *et al*., 1998; Peyerl-Hoffmann *et al*., 2001).



Humans living in malaria-endemic regions acquire semi-immunity to *Plasmodium falciparum* as a result of natural exposure to multiple infections over many years. It is usually assumed that immunity to *P. falciparum* has two components: an antidisease immunity, which is believed to develop rapidly, and an antiparasite immunity, which is acquired slowly and leads to a marked decrease in parasite densities (Trape *et al*., 1994; Ntoumi *et al*., 1995). Among the host genetic factors other than red blood cell-related defects, genetic variants of mannose binding lectin (MBL), tumor necrosis factor (TNF), and inducible nitric oxide synthase 2 (NOS2) loci have been associated with resistance/susceptibility status to clinical malaria (Luty *et al*., 1998; McGuire *et al*., 1999; Landry-Erik *et al*., 2003).

### *2.1.6 Human- Plasmodium Interactions*

In regions where malaria is endemic the immune response to *Plasmodium* infection is poor (Marsh, 1998). A number of factors may contribute to the low levels of immune responsiveness to *Plasmodium*. The maturational changes from sporozoite to merozoite to gametocyte allow the organism to keep changing its surface molecule resulting in continual changes in the antigen seen by the immune system. The intracellular phase of the life cycle in the liver and erythrocytes also reduce the degree of immune activation generated by the pathogen and allow the organism to

multiply while it is shielded from the attacking immune system. Furthermore the most accessible stage, the sporozoite, circulates in the bloodstream for about 45 minutes before it infects liver hepatocytes; it is unlikely that much immune activation can occur in such a short period of time. Moreover, when an antibody response does develop for sporozoites, the *Plasmodium* evolves a way of overcoming that by sloughing off the surface circumsporozoite antigen coat, thus rendering the antibody ineffective (Ntoumi *et al.*, 2007).



Immunity against malaria can be classified into natural or innate immunity and acquired or adaptive immunity. The innate immunity is naturally present in the host and is not dependent on any previous infection. Alterations in the structure of hemoglobin or in certain enzymes have been found to confer protection against either the infection or its severe manifestations and these traits are often found in areas of high malaria transmission (Carter & Mendis, 2002; Doolan *et al*., 2009). Duffy negativity in red cells protects against *P. vivax* infection. It is found to be widely prevalent in Africa and this may be responsible for the virtual elimination of this parasite from the continent (Carter & Mendis, 2002). Certain thalassemias (50% reduction in infection), homozygte hemoglobin C (90% reduction), hemoglobin E, and ovalocytosis carrier status hav been reported to confer protection against *P. falciparum* or *P. Vivax* (Carter & Mendis, 2002; Doolan *et al*., 2009). Glucose 6 phosphate dehydrogenase deficiency (50% protection) and sickle cell hemoglobin (90% protection) confer protection against severe malaria and related mortality (Doolan *et al*., 2009).

Acute malarial infection also induces immediate, non-specific immune response that tends to limit the progression of disease (Mannoor *et al*., 2001). The humoral and cellular mechanisms of this 'nonspecific' defense are poorly defined. Natural killer (NK) cells are found in blood, in secondary lymphoid organs as well as in peripheral non-lymphoid tissues. NK cells in peripheral

blood produce Interferon-gamma in response to *Plasmodium* infected erythrocytes, leading to parasiticidal macrophage activation, (Doolan *et al*., 2009) and this may be of greater importance for innate malaria immunity than their potential to lyse infected host erythrocytes. These cells are also important in the initiation and development of adaptive immune responses. NK cells induce the production of the proinflammatory chemokine Interleukin-8, that in turn plays its role in the recruitment and the activation of other cells during malaria infection (Mannoor *et al*., 2001).



### *2.1.7 The Plasmodium Genome*

The genome of *Plasmodium* species is estimated to be about 30Mb and is distributed among 14 chromosomes, which range in size from 650 kb to 3.5 Mb with estimated 5000–7000 genes (Pollack, 1982). *Plasmodium falciparum* and several other *Plasmodium* species are unusual in that their genomes have an extraordinary bias towards two nucleotides: adenine (A) and thymine (T). In regions that code for proteins, the A-T bias is greater than 76%, whereas in intergenic regions (regions between genes) and in introns (regions within genes that are removed before final transcription), the A-T content can approach 100% (Greenwood, 2002). This extreme A-T bias is thought to be responsible for the observed difficulty in cloning and maintaining large segments (greater than several kb) of *P. falciparum* DNA in *Escherichia coli* (Greenwood, 2002). This instability has been problematic because there are, as yet, no bacterial libraries available that can accept large inserts of *P. falciparum* DNA.

The DNA bears the genes, carriers of the genetic information which are used in the synthesis of a functional gene products which are often proteins. Some of these gene products on the parasites surface include the merozoite surface antigen/proteins (called MSP-1, MSP2, MSP-3 etc) are used for molecular study of the malaria parasites.The primary structure of merozoite surface protein- 2 (MSP-2) is characterized by highly conserved N- and C-terminal domains and a variable central domain that gives rise to large size polymorphisms. The central region is further characterized by

the presence of one of two unrelated dimorphic allelic families, designated FC27- and 3D7-types, which refer to the sequences of the corresponding *P. falciparum* reference clones (Basco *et al*., 2001; Ntoumi *et al*., 2007).

The merozoite surface protein-1 (MSP-1) locus of *Plasmodium falciparum* codes for a major asexual blood-stage antigen currently proposed as a major malaria vaccine candidate. The protein, however, shows extensive polymorphism, which may compromise its use in sub-unit vaccines (Hoffman & Miller, 1996). Comparisons of nucleotide sequences led to the identification of seven variable blocks in gene, which are interspersed with five conserved and five semi-conserved blocks. There are essentially two versions of each block, named after the representative isolates MAD20 and k1 (Snounou *et al*., 1999). There is, however, a third version originally described in the isolate RO33 (Snounou *et al*., 1999). Most allelic diversity is generated by intragenic recombination between these representative sequences at the 5' end of the gene, within blocks 3, 4 and 5. Minor differences also exist between homologous versions of the same variable block, and substitutions occur in the conserved and the semi-conserved blocks (Basco *et al*., 2004).

## 2.2 Global Malaria Epidemiology

Malaria remains the world's most devastating human parasitic infection affecting over 40% of the world's population (WHO, 2005a). The World Health Organization (WHO, 2005a) estimated that over 3.2 billion people live under the threat of malaria and an estimated 350-500 million clinical malaria episodes occur annually. Most infections are caused by *P. falciparum* and *P vivax*, 90% of them occurring in sub-Saharan Africa, while two thirds of the remaining cases occur in six countries (India, Brazil, Sri Lanka, Vietnam, Colombia and Solomon Islands). In endemic African countries, malaria accounts for 25%-35% of all outpatients' visits, 20%-45% of hospital admissions and 15%-35% of hospital deaths, impose a great burden on already fragile health care

systems (WHO, 2005a). In certain regions of Africa, more than 70% of the citizens are chronically infected with *Plasmodium falciparum* (Breman *et al*., 2004 & 2007).

### *2.2.1 Global burden of malaria in children*

Malaria continues to be one of the public health problems of mankind in spite of the tremendous efforts put in to eradicate or at least control it. Now exactly 100 years since Ronald Ross discovered the mosquito's role in malaria transmission, malaria kills one person, mostly children under five years of age every 30 seconds (Ross, 1911; WHO, 2003b). Each year, approximately 300 to 500 million malaria infections lead to over one million deaths, of which over 75% occur in African children under-5 years (WHO, 2005a; Snow *et al*., 1999). Malaria accounts for one in five of all childhood deaths in Africa (WHO, 2005a; Murphy *et al*., 2001).

Anaemia, low birth-weight, epilepsy, and neurological problems, all of which are frequent consequences of malaria, compromise the health and development of millions of children throughout the tropical world (Holding *et al*., 1999). *Plasmodium falciparum* causes the vast majority of infections in this region and about 18% of deaths in children under-five years of age (WHO, 2005b). Of malaria cases reported in outpatient visits in public health facilities, 36%-40% are typically in children under five years of age and nearly 25% of all deaths in children under-five years in Africa are due to malaria (WHO, 2005a).

### *2.2.2 Antimalarial drug resistance development*

Despite considerable efforts made to eradicate or control malaria, its control and treatment have been complicated by the emergence of resistance to widely used antimalarial drugs such as Chloroquine (Neequaye, 1986; WHO, 2001a). Resistance to antimalarial drugs can be defined as

the ability of the parasite strain to survive and or multiply despite the administration and absorption of a drug given in doses equal to or higher than, those usually recommended, but within the limit of tolerance of the subject. Furthermore, the active form of the drug must be able to gain access to the parasite or the infected red blood cell for duration of time necessary for its normal action (WHO, 1996).

Antimalarial drug resistance is now generally acknowledged to be one of the greatest threats to our ability to "Roll Back Malaria" (White *et al*., 1999a, b; Shunmay *et al*., 2004). However, the emergence and rapid spread of *P. falciparum* resistance to commonly used antimalaria drugs poses a serious challenge to the effectiveness of early diagnosis and prompt treatment as a priority strategy within current malaria control efforts (Marsh, 1998). Resistance to affordable drugs in Africa, which carries an estimated 90% of the burden of malaria, has reached critical levels. The continent was faced with the crucial issue of which drug regimen to switch to and when to make a switch. There are various factors relating to drug, parasite and human host interactions which contribute to the development and spread of drug resistance. The molecular mechanism of drug action is a critical element in the speed at which resistance develops (Shunmay *et al*., 2004).

#### *2.2.2.1 Parasites and drugs*

Parasite factors associated with resistance include the *Plasmodium* species concerned and the intensity of transmission. Natural populations of *P. falciparum* are heterogeneous mixtures of individuals with different, genetically determined degrees of drug response. The efficacy of medication will depend on the concentration of the drug in relation to the parasite's sensitivity and the time over which concentrations above this threshold are maintained (Shunmay *et al*., 2004). A small fraction of the original parasite population might always survive the drug but it will be eventually removed by the immune system. However, the infection will not be cleared if the

surviving fraction is too large due either to reduced sensitivity or to sub-critical drug concentrations. Selection of resistant strains could occur when a particular drug is misused (Wernsdorfer and Noedl, 2003). The transmission of such parasites might also be enhanced by an increased production of gametocytes (Robert *et al*., 1996; Drakeley *et al*., 2004).

#### *2.2.2.2 Type of antimalarial drugs*

Readily absorbed drugs with a long half-life, like mefloquine and sulfadoxine-pyrimethamine (SP), can permit effective single dose treatment of malaria and the following chemoprophylactic period prevents infection for several weeks and may be important in recovery from anaemia (Takechi *et al*., 2001; WHO, 2001a). However, these drugs are likely to exert undesirable drug pressure for a long time once their concentrations drop below the critical threshold and might select resistant parasites (Hastings *et al*., 2002). This has been shown in Kenya where a potent selective pressure for resistance operates even under conditions of supervised drug administration and optimal dosage (Watkins *et al*., 2002). In addition, drugs with a long terminal elimination half-life enhance the development of resistance, particularly in areas of high transmission (Watkins & Mosobo, 1993; Shunmay *et al*., 2004). Similarly, increased drug pressure is a significant contributor to drug resistance (Brockman *et al*., 2000). As increased amounts of a drug are used, the likelihood that parasites will be exposed to inadequate drug levels rises and resistant mutants are more readily selected (Watkins & Mosobo, 1993; Hastings *et al*., 2002; Shunmay *et al*., 2004).

#### *2.2.2.3 Humans and drugs*

Human host factors include the widespread and /or irrational use of antimalarial drugs and possibly the level of host immunity. The role of host immunity in propagating resistance is unclear

(Buckling & Read, 2001; Taylor-Robinson, 2002). However, immunity acts synergistically with chemotherapy and can enhance therapeutic effects and even parasite clearance of drug-resistant infections (Watkins & Mosobo, 1993; Shunmay *et al*., 2004).

#### *2.2.2.4 Vector and parasite*

Vectors may be more receptive to resistant strains and may produce more parasites compared to sensitive strains. Enhanced drug pressure and uninhibited transmission might produce a fast selection and spread of resistant parasites (Wernsdorfer & Noedl, 2003).



### *2.2.3 Molecular mechanism for drug resistance development*

The emergence of antimalarial drug resistance is dependent on the occurrence of a spontaneous genetic change (mutation or gene amplification) in a malaria parasite, which interferes with that parasite's susceptibility to a drug. A single mutation may be sufficient to confer almost complete resistance to some drugs (e.g., Atovaquone) or more usually there is a series of mutations that confer increasing tolerance of the parasite to increasing drug concentrations, as in the cases of Pyrimethamine and Chloroquine (Wellems & Plowe, 2001; Peters *et al*., 2002). However, for resistance to spread, the spontaneous occurrence of a mutation in itself is not sufficient. In the absence of the drug to which it is potentially resistant, a parasite with the resistant mutation does not have a survival advantage and therefore does not reproduce faster than the non-mutants. There may even be a survival disadvantage, a so-called fitness cost to having the mutation (Peters *et al*., 2002; Drakeley *et al*., 2004). In the presence of the particular drug, the multiplication of the sensitive parasites is inhibited allowing the drug-resistant mutants to survive and multiply (i.e., selection), increasing the likelihood of transmission to the next host and therefore the spread of resistance.

**Chloroquine** - Drug resistance in *P. falciparum* to Chloroquine had been attributed to two mutations in key parasite genes, a threonine (T) encoded by codon 76 of *Plasmodium falciparum* resistance transporter (pfcrt) and a tyrosine (Y) encoded by codon 86 of *Plasmodium falciparum* multidrug resistance 1 (pfmdr1) are strongly associated with CQ treatment failure in these populations (Hallett *et al*., 2004). These amino acid substitutions result from single nucleotide substitutions that were detected by a sequence-specific oligonucleotide probing assay (Alloueche *et al*., 2000).



**Sulfadoxine-Pyrimethamine (SP)** - The point mutations linked to SP resistance have been observed in the parasite genes encoding for dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*), the targets for Pyrimethamine and Sulfadoxine respectively (Wensdorfer & Noedl, 2003). A serine in position 108 of the *dhfr* gene is linked to in-vitro sensitivity to both Pyrimethamine and Cycloguanil. A mutation to asparigine at position 108 seems to be the key mutation for conferring in-vitro Pyrimethamine resistance (Wensdorfer & Noedl, 2003).

### *2.2.4 Molecular epidemiology and techniques*

Conventional microscopy allows the species of malaria parasites present in the blood sample to be determined and its density to be estimated but the amount of epidemiological information that the microscopy can provide is limited (Greenwood, 2002). Molecular techniques now offer new possibilities to get a deeper understanding of host-parasite interactions and the biology of the parasite population. The usefulness of the polymerase chain reaction (PCR) technique together with the minimal requirement of infected samples have led to a multitude of typing studies combining various *P. falciparum* polymorphic markers such as *msp1* and *msp2* (Walliker, 1994; Basco *et al*., 2004). Typing of *P. falciparum* in human hosts has been used in work on the

diversity of parasite populations (Babiker *et al*., 1999; Magesa *et al*., 2001), the search for markers of parasite virulence (Greenwood, 2002), in researching the importance of multiplicity of infection (Hoffmann *et al*., 2001; Contamin *et al*., 1995; Beck *et al*., 1997) and the geographical distribution of the various alleles of these polymorphic genes of the parasite (Hoffmann *et al*., 2001; Babiker *et al*., 1997; Felger *et al*., 1999).

### *2.2.5 Genetic markers of parasite*



Merozoite surface antigen/protein-2 (*msa/p-2*) is one of the four integral membrane proteins characterized to date that is present on the surface of *Plasmodium falciparum* merozoites. Among genetic markers used to assess parasite diversity in past studies, including *msp-1*, circumsporozoite protein (*CSP*) and glutamine-rich protein (*glurp*), *msp-2* was found to be the most discriminatory for field isolates (Felger *et al*., 1999; Snounou *et al*., 1999; Basco *et al*., 2000). In areas with low transmission of malaria, *msp-2* size polymorphism has been shown to be limited. These polymorphic features of *msp-2* may be exploited for genotyping naturally-occurring parasites (Basco *et al*., 2004).

Two related aspects of *msp-2* highlight its importance for malaria research. First, it is a candidate target for a subunit vaccine against the asexual erythrocytic stage of *P. falciparum*. Second, as a highly polymorphic protein, it may serve as a tool to analyze the complexity of parasite population structure in the field. Studies on the extent of antigenic diversity of a target protein are also a necessary step before field evaluation of vaccine candidates (Basco *et al*., 2001). Reports from Senegal, Papua New Guinea and Malawi linked an association of *msp1* and *msp2* genotypes with severity of disease (Greenwood, 2002). The severity of falciparum malaria varies from place to place. Mortality is consistently higher in tropical Africa than parts of South-East Asia, probably

due to socio-cultural and economic factors. There are unequivocal genetic markers for virulence of falciparum parasites among the wild parasite population (Greenwood, 2002).

## 2.3 Development of Drug Resistance

Antimalarial drug resistance is the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended, but within the limits of tolerance of the subject (WHO, 2005a). Resistance to antimalarial drugs arises as a result of spontaneously-occurring mutations that affect the structure and activity at the molecular level of the drug target in the malaria parasite or affect the access of the drug to that target (Peters, 1987). Mutant parasites are selected if antimalarial drug concentrations are sufficient to inhibit multiplication of susceptible parasites but are inadequate to inhibit the mutants, a phenomenon known as "drug selection" (Peters, 1990; White, 1998b). This selection is thought to be enhanced by sub-therapeutic plasma drug levels and by a flat dose-response curve to the drug.

The increase in Chloroquine resistance in East Africa has led to a rise in malaria mortality (Marsh, 1998). Similarly, a significant rise in malaria mortality in children under 5 years of age has been observed in Senegal in West Africa, coinciding with the emergence of Chloroquine resistance in the area (Trape *et al.*, 1998). The incidence of severe malaria has risen with increasing Chloroquine resistance in Africa (WHO, 2003b). Antimalarial drug resistance has also been implicated in the increasing frequency and severity of epidemics (Bloland *et al*., 1999). The incidence of severe malaria has risen with increasing Chloroquine resistance (Su *et al*., 1997).

There are various factors relating to drug, parasite and human host interactions which contribute to the development and spread of drug resistance. The molecular mechanism of drug action is a

critical element in the speed at which resistance develops. In addition, drugs with a long terminal elimination half-life enhance the development of resistance, particularly in areas of high transmission (Watkins & Mosobo, 1993). Similarly, increased drug pressure is a significant contributor to drug resistance. As increased amounts of a drug are used, the likelihood that parasites will be exposed to inadequate drug levels rises and resistant mutants are more readily selected (Watkins & Mosobo, 1993).



Parasite factors associated with resistance include the *Plasmodium* species concerned (Ron *et al*., 2005) and the intensity of transmission (Ron *et al*., 2005). Human host factors include the widespread and/or irrational use of antimalarial drugs and possibly the level of host immunity (Ntoumi *et al*., 2007). The role of host immunity in propagating resistance is unclear. However, immunity acts synergistically with chemotherapy and can enhance therapeutic effects and even parasite clearance of drug-resistant infections (Watkins & Mosobo, 1993; Mayxay *et al.*, 2001).

As a result of the continued increase of resistance to antimalarial drugs in many regions of the world, with the resultant effect on morbidity and mortality (Trape *et al*., 1993 & 1998), it is essential to ensure rational deployment of the few remaining effective drugs, to maximize their useful therapeutic life while still ensuring that safe, effective and affordable treatment is accessible to those at risk. This requirement has resulted in a re-examination of the potential of combinations of existing products and the development of new combination drugs.

### *2.3.1 Molecular Methods for Drugs resistance Surveillance*

Molecular diagnostic methods for detecting resistant parasites have been proposed for monitoring the level and spread of resistance (Plowe *et al*., 1996; Wensdorfer & Noedl, 2003). The methods are suited for use on large numbers of samples in a laboratory in a malaria-endemic country and

have major advantages over in-vitro tests that require parasite cultivation and take days to perform (WHO, 2003b). Studies carried out by Wensdorfer & Noedl (2003) on the molecular epidemiology of *Plasmodium falciparum* strongly suggest that it is a useful tool for drug resistance surveillance on population mechanics and dynamics. These molecular tools are based on the detection by PCR of point mutations in the parasite genes responsible for in-vitro resistance.

The characterization of two polymorphic merozoite surface antigens, *msp1* and *msp2* has also been used to establish whether a parasitaemia observed after treatment is caused by a recrudescence of drug-resistant parasites or by a new infection (Wellems & Plowe, 2001). This could be important information when carrying out in-vivo tests, particularly in areas with a considerable amount of transmission where, after a certain time, it is impossible to distinguish between recrudescence and new infection. Decisions on the national drug policy will continue to be based on the results of in-vivo tests as these reflect more closely the therapeutic efficacy of a given drug.



### *2.3.2 Drugs Resistance Surveillance*

One of the major questions is whether the first-line drug is still `working'. Unfortunately, the criteria on which this decision can be based are not clear (Shunmay *et al*., 2004). The standard methods to assess the efficacy of a given drug can be divided in two broad groups: ***in vivo*** and ***in vitro*** tests.

**In vivo test -** During the in-vivo test the recommended dose of an antimalarial drug is administered to infected subjects and the parasite's response in the host is assessed. The test could be done on symptomatic or asymptomatic people (WHO, 2003a) with a 7-day follow-up (WHO, 2001a). It assesses only the initial parasitological response and, to a limited extent, clinical response to therapy during the follow-up. However, it does not address the implications and

manifestations of persistent parasitaemia occurring after poor response, for example its impact on other condition such as anaemia and malnutrition (Shunmay *et al*., 2004).

The 14-days in-vivo test proposed by WHO (2003a) tries to address this problem by looking mainly at treatment failure and change in the haematological parameters (Hb/PCV) at day 0 and 14. It is a more clinical test, carried out on sick children (fever + parasitaemia) aged 6 months-5 years (WHO, 2003a). This test does not permit a quantitative assessment of the drug sensitivity of individual parasite populations, that may occasionally be influenced by the abnormal fate of the drug in individual patients and it is influenced by the immunological host response to the parasite. However, it gives information that is closer to real-life situation and therefore essential in deciding drug policy changes. With the advent of PCR, a 28 days in-vivo test has been proposed as more appropriate and cost effective (WHO, 2005b).

**In vitro test** - The in-vitro test consists in measuring the inhibition of schizont maturation by increasing doses of a given antimalarial drug. It allows the quantitative measurement of drug response, permits to test several drugs at once and imitate the non-immune state. However, it is generally held that the in-vitro tests do not reflect the degree of in-vivo resistance, since the latter is substantially determined by factors related to the host's response (WHO, 2003a).

Furthermore, it is estimated that the technical capacity to conduct the necessary assays is difficult to develop and maintain in national malaria control programmes. Therefore, in-vitro testing cannot substitute in-vivo observations of malaria therapy and is inappropriate for making policy decision on drug use. Nevertheless, in-vitro tests may provide an early warning of impending resistance before this becomes clinically apparent. The optimal deployment of in-vitro tests should be to define specific issues related to temporal and geographical trends of parasite's response to drugs. Such issues include the longitudinal follow-up of parasite drug susceptibility, monitoring the

patterns of parasite cross-resistance to different drugs and the establishment of baseline data on the susceptibility of local parasites to new drugs (WHO, 2003a).

## 2.4 Ghana: the People, Health System and Malaria Situation

### *2.4.1 Demographic, socioeconomic and cultural features*

Ghana is a multi-cultural and multi-ethnic country with a total population for 2007 estimated to be at 23 million approximately 50/50 rural-urban split (CIA world factbook, 2009). The population is relatively young – about 60% are below the age of 25 years, and about 5% above 65 years – fueling a deep sense of the role of the youth in fostering national development (GSS/NMIMR/ORC, 2004).

The growth rate is estimated at 2.5% per annum and a population density of about 78 persons /sq.km. Twenty percent of the population are children aged 5 years and below, with infants forming 4%. There is a trend of increase in the population density in a north-south direction towards the coast, where the bulk of the nation's resources are concentrated in the forest and coastal regions, which also have most of the urban centres. This resulted in the exodus of the northern-rural to the forest and coastal regions of Ghana (Ghana Statistical Services, GSS 2002; 2007). Ghana is a republican nation governed by a democratically elected president under a multi-party system of governance. It is divided into 10 administrative regions that are subdivided into administrative districts.

The country is typically tropical rainforest in the middle belt, coastal savannah in the lower belt and northern savannah in the northern part. The total land area is 238,533 sq. km. It has a tropical climate with temperatures ranging from 18ºC to 40ºC with two main seasons – dry and wet. The

per capita GDP is estimated at 600 US dollars per annum (GSS/NMIMR/ORC, 2004). The major exports are cocoa, gold, timber, bauxite and non-traditional exports. The country is currently an oasis of peace, stability and good governance in conflict-ridden West Africa (GSS 2002; GSS/NMIMR/ORC, 2004).

In the 2003 GDHS, infant mortality rate was 64 per 1000 live births and child mortality rate was 50 per 100 live births (GSS/NMIMR/ORC, 2004). However, under-5 mortality ranged from 75 per 1000 live births in Greater Accra to 208 per 1000 live births in the Upper West Region (GSS/NMIMR/ORC, 2004). The major causes of disease, deaths and disability are malaria, acute respiratory infections, diarrhoeal diseases, complications of pregnancy and accidents. In the adult working population, hypertension, diabetes mellitus and cancers are emerging as important causes of morbidity and premature mortality.



### *2.4.2 The Health System in Ghana*

The health system is based on decentralized Primary Health Care with the District Administration as the focus of implementation. Since 1988, health sector reforms have significantly improved performance at district level with further decentralization to the sub-district level and greater involvement of the private sector in public health programmes. The Ministry of Health (MOH) has developed a medium-term strategic framework for health development with further strengthening of health districts featuring prominently as part of strengthening the reform process (MOH, 1995). All health districts are being further strengthened and receive direct budgetary allocation from MOH Headquarters with the Regional level only playing a supervisory and monitoring role.

In 1995, the Parliament of Ghana passed a law establishing the Ghana Health Service (GHS) and Teaching Hospitals Board. Under this new law, the GHS becomes responsible for all health service

delivery in the public sector, whilst the Ministry of Health, Ghana retains responsibility for policy formulation and strategy development, resource mobilization, development of human resources for health, research co-ordination, monitoring and evaluation. In 2003, the Parliament of Ghana passed the National Health Insurance Law to replace "cash and carry" (cost-recovery) with health insurance using District Mutual Health Insurance Schemes. Currently, there is significant improvement to clinical care services due to the District Mutual Health Insurance Schemes implemented across the country.



### *2.4.3 The Malaria Situation in Ghana*

Malaria is hyper-endemic and the leading cause of all reported illnesses at the outpatient department (OPD). It accounts for 40 – 45% of all OPD visits to health facilities and contributes significantly to severe anaemia whilst accounting for 25% of premature childhood death (GHS, 2004b). However, in 2007, malaria was responsible for 38.6% of outpatient attendances compared to 43.7% in 2006. It was the highest cause of mortality, accounting for over 18% of deaths reported at health facilities It is the second most common cause of death (after anaemia), especially in children under-five years (GHS, 2001; 2007). In Ghana, malaria continues to be the most important public health problem (GHS, 2004a) with its' main effect on children and pregnant women especially primigravidae (Browne, 1996). The malaria situation in Ghana is hyper-endemic although up-to-date information is lacking. For control purposes the country has been stratified into five zones, namely:

1. Tropical Rain Forest
2. Coastal Savannah, including Lagoons and Mangrove Swamps
3. Northern Savannah
4. Urban
5. Development-related malaria.

The burden of malaria is heaviest in the forest belt of rural Ghana (Ahmed, 1989; GHS, 2001). Malaria is the single most important cause of morbidity in Ghana (GHAT, 1981). In 1975, malaria accounted for 26% of admissions and 30% of total deaths in the paediatric ward in Komfo Anokye Hospital in Kumasi in the forest belt of Ghana (Asafo-Agyei, 1978). Binka and colleagues (1994) estimated that malaria may account for over 25% of under-5 mortality in Northern Ghana. Afari and colleagues (1992), working in the Gomoa district in the Central Region, also estimated that malaria may account for at least 10% of early childhood deaths in the coastal regions of Ghana. Definitive studies were carried out on malaria in Ghana in the past 4 decades in 3 eco-epidemiological zones - peri-urban Accra (coastal savannah), Bomfa in the present-day Ejisu-Juaben district (forest) and Yorugu (northern savannah) by Colbourne and Wright (1955a, b). A malariometric update conducted in the same area in 1999 showed that the situation remained unchanged (Browne *et al*., 2000).



### *2.4.4 Malaria Control in Ghana*

Though malaria control activities in Ghana started in the pre-independence era just before the Second World War, historically malaria control has been the purview of the Ministry of Health sometime to the exclusion of other stakeholders. Furthermore, various groups and organizations have been working independently of each other on issues relevant to malaria control. Before 1993, malaria control was restricted to the urban areas with emphasis on source reduction and chemoprophylaxis with Quinine, Pyrimethamine or Proguanil. A 5-year [1993-1997] national malaria action plan (MOH, 1991) has been implemented with focus on case management. An Accelerated Malaria Control Programme, which started in 30 selected districts with WHO/AFRO support, with the focus on case management and training of prescribers and chemical sellers was extended nation-wide. Since 1999, Ghana has been undertaking integrated malaria control activities

in the context of the Roll Back Malaria (RBM) initiative. It is aimed at providing at least 60 percent of those at risk access to insecticide-treated bed nets, prompt and effective treatment and intermittent preventive treatment (IPT) for at least 60 percent of pregnant women. However, net use nationally is very low. Ghana's Roll Back Malaria Initiative /Program has the following four main strategic components.

1. Improved malaria case management;
2. Multiple prevention;
3. Focused research; and
4. Improved partnerships



According to the Minister of Health (2009), Ghana was expected to spend an estimated 772 million Ghana Cedis on malaria treatment.

### *2.4.5 Malaria Research in Ghana*

Malaria control efforts have been fragmented, uncoordinated, and in some cases antagonistic rather than synergistic (Ghana's Rollback Malaria Strategy, 2001-2010). There is now extensive research that is done and ongoing, however most of these are not coordinated and centralized for purposes of sharing relevant knowledge and requisite skills to enhance their work. Funding for research work is woefully inadequate and sometimes non-existent from Ministry of Health and Ghana Government.

## 2.5 Antimalarial Drugs

### *2.5.1 Artesunate*

Artesunate is an antimalarial agent. It is a water-soluble hemisuccinate derivative of artemisinin. Artemisinin is a sesquiterpene lactone isolated from *Artemisia annua*, herb that has traditionally

been used in China for the treatment of malaria. Artesunate tablets are white, flat, round and scored. *Excipients*: Lactose, microcrystalline cellulose, croscaramellose, anhydrous colloidal silica and magnesium stearate (Dafra Pharma, 2004).

**Pharmacokinetics**

Artemisinins and its semi-synthetic derivatives such as Artesunate are typical blood schizonticides in all forms of malaria. Artesunate has a peroxide bond which breaks up inside the parasite, forming singlet oxygen as well as free radicals (Dafra Pharma, 2004; WHO, 1998).



Following oral administration, Artesunate is rapidly absorbed and reaches maximum blood levels maximum concentration (Cmax) within 45 to 90 minutes. The product is metabolized in plasma and in the liver by hydrolysis of the succinic acid ester bond, giving rise to the generation of dihydroartemisinin which is also effective against malaria by the same mechanism of action. Elimination half-life is approximately 1 to 2 hours (Dafra Pharma, 2004). Protein binding varies with the species organism being studied (rat, guinea pigs etc.), but tends to be about 50% in man. The oral formulation is probably hydrolyzed completely before entering the systemic circulation. Peak serum levels occur within one hour of an oral dose of Artesunate and persist for up to 4 hours. Dihydroartemisinin has a plasma elimination half-life of less than 2 hours, which may slow the development of resistance to Artesunate (Dafra Pharma, 2004; McIntosh & Olliaro, 1998).

***Mechanism of Action***

Artesunate and its active metabolite dihydroartemisinin is a potent blood schizonticide agent for *P. falciparum*. Artesunate binds tightly to parasitized erythrocyte membranes. The functional group responsible for antimalarial activity of Artesunate is the endoperoxide bond. Release of an active oxygen species from this bond kills the parasite if accumulated in the erythrocytic cells. It

also suppresses the production or activity of antioxidant enzymes in the erythrocytes, causing lysis of the parasitic cell due to the highly reactive free oxygen radicals. It reduces gametocyte carriage rate (White, 1997; McIntosh & Olliaro, 1998). Aremisinim derived free radicals are thought to chemically modify and inhibit a variety of parasite molecules, resulting in parasite death (Robert *et al*., 2001). It is also thought to cause an irreversible inhibition on the malarial calcium-dependent ATPase (Pf ATP6) mechanism (Krishna *et al*., 2004) and the inhibition of multidrug receptor in the parasite.



### *2.5.2 Amodiaquine*

Amodiaquine is a 4-aminoquinoline with schizonticidal activity. Amodiaquine tablets are yellowish, flat, round and scored. Excipients: Cornstarch, lactose, sodium starch glycolate, magnesium stearate, anhydrous colloidal silica, talc and parabens (Dafra Pharma, 2004).

**Pharmacokinetics**

It is likely that the metabolite is rather responsible for most of the observed antimalarial activity, and that the toxic effects of Amodiaquine after oral administration may, in part, be due to its metabolite. After oral intake the absorption of Amodiaquine is very fast and it is very extensively metabolized or biotransformed. Amodiaquine hydrochloride is rapidly absorbed and undergoes rapid and extensive metabolism to desethylamodiaquine which concentrates in blood cells. This process is very fast (5 to 10 minutes). The peak plasma concentrations (Cmax) of the metabolites are reached after a mean of 3.5 hours (Dafra Pharma, 2004).

Amodiaquine and desethylamodiaquine accumulates in red blood cells and are about 90 % protein bound. All are eliminated by renal excretion. Desethylamodiaquine has a longer elimination half live than Amodiaquine with values ranging from 9 till 31 days. The plasma elimination half-life of

desethylamodiaquine has varied from 1 to 10 days or more. About 5% of the total administered dose is recovered in urine while the rest is metabolized in the body (Dafra Pharma, 2004; White, 1997; McIntosh & Olliaro, 1998).

***Mechanism of Action***

Amodiaquine is an antimalarial with schizonticidal activity. It is effective against the erythrocytic stages of all 4 species of *P. falciparum*. (Dafra Pharma, 2004). Amodiaquine accumulates in the lysosomes and brings about loss of lysosomal function. The parasite is unable to digest haemoglobin on which it depends for its energy (Dafra Pharma, 2004).



### *2.5.3 Chloroquine*

Chloroquine is a 4-aminoquinoline compound. It is an antimalarial and amebicidal drug.

**Pharmacokinetics**

Chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract (GIT) following oral administration and peak plasma concentrations of the drug are generally attained within 1-2 hours (White, 1997; McIntosh & Olliaro, 1998). Approximately 55% of the drug in the plasma is bound to plasma proteins. The plasma half life of Chloroquine in healthy individuals is generally reported to be 72-120 hours. Chloroquine is eliminated by renal route; 42 to 47% of Chloroquine is excreted unchanged whilst 7 to 12% is excreted in urine as desethylChloroquine (White, 1997; McIntosh & Olliaro, 1998).

***Mechanism of Action***

Chloroquine has been found to be active against the asexual erythrocytic forms of all species of malaria parasites. It is a rapid acting blood schizonticide with some gametocytocidal activity against *P. ovale, P. vivax, P. malariae* and immature gametocytes of *P. falciparum* (White, 1997).

Chloroquine also is taken up into the acidic food vacuoles of the parasite in the erythrocyte. It increases the pH of the acid vesicles, interfering with vesicle functions and possibly inhibiting haemoglobin metabolism (White, 1997; McIntosh & Olliaro, 1998).

### *2.5.4 Mefloquine*

It is a 4-quinolinemethanol derivative with the specific chemical name of (R*, S*)-(+)-α-2-piperidinyl-2, 8-bis (trifluoromethyl)-4-quinolinemethanol hydrochloride. It is a 2-aryl substituted chemical structural analog of quinine.



**Pharmacokinetics**

Mefloquine is slowly absorbed from the GIT and appears to undergo little, if any first pass elimination. The presence of food significantly enhances the rate and extent of absorption, leading to about a 40% increase in bioavailability. Mefloquine may accumulate in parasitized erythrocytes. Protein binding is about 98%. The mean elimination half life of mefloquine varied between 2 and 4 weeks, with an average of about 3 weeks. Mefloquine is excreted mainly in the bile and faeces (Karbwang & White, 1990; Houston *et al*., 1998).

***Mechanism of Action***

Mefloquine is an antimalarial agent that acts as a blood schizonticide. It is active against the erythrocytic stages of *Plasmodium* species. Similar to Chloroquine and quinine, mefloquine appears to interfere with the parasite's ability to metabolize and utilize erythrocyte haemoglobin (Karbwang & White, 1990). The antimalarial activity of Mefloquine may depend on the ability of the drug to form hydrogen bonds with cellular constituents. Mefloquine may exert its antimalarial action by disrupting the membrane trafficking events involved in the uptake of phospholipids (Karbwang & White, 1990; Houston *et al*., 1998).

### *2.5.5 Sulphadoxine-Pyrimethamine*

Sulphadoxine-Pyrimethamine is a combination of N1-(5, 6-dimethoxypyrimidin-4-yl)-sulphanilamide (Sulphadoxine) and 5-(4-chlorophenyl)-6-ethylpyrimidine-4-diyldiamine (Pyrimethamine). Pyrimethamine, an aminopyrimidine derivative, is an antimalarial agent that is structurally related to trimethoprim.

**Pharmacokinetics**



Both Sulphadoxine and Pyrimethamine are well absorbed from the GIT. Like other sulphonamides, Sulphadoxine is widely distributed in the body. Plasma protein binding is about 90% for both Pyrimethamine and Sulphadoxine (White, 1997; Watkins & Mosobo, 1993). About 5% of Sulphadoxine appears in the blood as acetylated metabolite, about 2-3% as the glucuronide. Both Sulphadoxine and Pyrimethamine are excreted mainly by the kidneys. The apparent elimination half life of Sulphadoxine ranged from 100 to 231 hours with a mean of 169 hours, whereas Pyrimethamine half lives ranged from 54 to 148 hours with a mean of 111 hours (White, 1997; Watkins & Mosobo, 1993).

***Mechanism of Action***

Sulphadoxine and Pyrimethamine combination is an antimalarial agent, which acts by reciprocal potentiation of its two components, achieved by a sequential blockade of two enzymes involved in the biosynthesis of folinic acid within the parasites. They are blood schizonticidal agents and are active against the asexual erythrocytic forms of susceptible plasmodia (White, 1997; Watkins & Mosobo, 1993). The pre-erythrocytic stages are also affected, and the gametocytes are rendered non-infective in the mosquito. Pyrimethamine is a folic acid antagonist and has a mechanism of action similar to that of trimethoprim. By binding to and reversibly inhibiting dihydrofolate

reductase, Pyrimethamine inhibits the reduction of dihydrofolic acid to tetrahydrofolic acid (folinic acid) (Watkins & Mosobo, 1993).

### *2.5.6 Chlorproguanil-Dapsone (LAPDAP)*

LAPDAP is a combination of chlorproguanil hydrochloride and dapsone.

**Pharmacokinetics**



After oral administration peak concentrations of Chlorproguanil (CPG) and Dapsone (DDS) are achieved by 8 hours. Approximately 50-80% of dapsone in the circulation is bound to plasma proteins while chlorproguanil is about 28-63% bound to plasma proteins. Both drugs are widely distributed throughout the body (Phillips-Howard & West, 1990). Dapsone is predominantly metabolized in the liver and up to 15% is excreted unchanged. Chlorproguanil is also metabolized into its active form chlocycloguanil. The elimination half-lives of CPG and DDS are 32 hours and 33 hours respectively. Both are eliminated predominantly via metabolism (Anabwani *et al*., 1999; Phillips-Howard & West, 1990).

***Mechanism of Action***

Chlorproguanil (CPG)/dapsone (DDS) is schizonticidal and exert itself on the erythrocytic stage of the life cycle of the parasite (trophozoite and schizont) (Anabwani *et al*., 1999). Chlorcycloguanil (CCG) the active metabolite of CPG is a selective inhibitor of plasmodial dihydrofolate reductase (DHFR) thereby blocking the formation of tetrahydrofolic acid and so preventing nuclear cell division. DDS inhibits plasmodial dihydropteroate synthetase (DHPS) and hence blocks the conversion of para-aminobenzoic acid to hydrofolic acid. They act sequentially blocking the two sites of the folate pathway (Anabwani *et al*., 1999; Phillips-Howard & West, 1990).

### *2.5.7 Arthemeter-Lumefantrine (COARTEM)®*

Arthemeter is a sesquiterpene laptone derived from the naturally occurring substance artemisinin. Lumefantrine is a synthetic racemic fluiorene mixture. Coartem® comprises a fixed ratio of 1:6 of artemeter and lumefantrine.

**Pharmacokinetics**

Artemeter is absorbed fairly rapidly with peak plasma concentrations reached about 2 hours after dosing. Absorption of lumefantrine, a highly lipophilic compound, starts after a lag time of 2 hours and peaked plasma concentrations about 6-8 hours after dosing (Novartis Pharma, 1997a & 1998). Food (fat meal) enhances the absorption of both drugs. Artemeter is metabolized in the liver to dihydroartemisinin. Artemeter and dihydroartemisinin are rapidly cleared from the body with an elimination half-life of about 2 hours. Lumefantrine is eliminated slowly with a terminal half-life of 2-3 days (Novartis Pharma, 1997a & 1998).

***Mechanism of Action***

The site of antiparasitic action of both components is the food vacuole of the malaria parasite where they are thought to interfere with conversion of haem, toxic intermediate produced during the breakdown of haemoglobin to the non-toxic haemozoin, malaria pigment (Novartis Pharma, 1997b &1998). Lumefantrine is thought to interfere with the polymerization process while artemeter generate reactive metabolites as a result of the interaction between its peroxide bridge and the haem iron. Both have a secondary action involving the inhibition of nucleic acid and protein synthesis in the malaria parasite (Novartis Pharma, 1997b & 1998).

### *2.5.8 AMOTEX (co-formulation of ART and AMQ)*

This is a co-formulation of ART and AMQ, a combination of sesquiterpene lactone and 4-aminoquinoline. Amotex is a schizonticidal drug with each component with independent modes of action and different biochemical targets in the parasite (Kinapharma Ltd, 2005). Its pharmacokinetics and mode of action are similar to that of Artesunate and Amodiaquine which are the constituents.



## 2.6 Antimalarial drugs Resistance development in Ghana

In Ghana, Chloroquine resistance was confirmed in Accra in 1986 and has since been observed nationwide (Neequaye *et al*,, 1987). Ghana is estimated to have reached 25% of Chloroquine resistance (GHS / NMCP, 2004). National Malaria Control Programme (NMCP) of Ghana Health Service in collaboration with the Epidemiology Unit of the Noguchi Memorial Institute for Medical Research has been monitoring Chloroquine resistance across Ghana in six sentinel sites: Hohoe, Sunyani, Navrongo, Yendi, Tarkwa and La-Accra.

Adequate clinical response to Chloroquine ranged between 65.7% and 87%; early treatment failure ranged between 4% and 18% whilst late treatment failure ranged between 4% and 14%. Overall, adequate clinical response was 58%; early treatment failure was 9% whilst late treatment failure was 8%. The highest clinical response of 87% was recorded in the capital, Accra, whilst the lowest was in Sunyani (tropical rainforest zone). Sensitivity ranged between 65.7% (Sunyani) and 87% (Accra). Three levels of resistance (R) as defined by WHO were recorded: RI resistance was between 14% and 28%, the highest occurred in Navrongo whilst the lowest was recorded in Accra. RII resistance was between 4% and 8% which were recorded in Sunyani whilst RIII was between 4% and 14%; the highest was recorded at Yendi and the lowest in Sunyani. Overall, sensitivity to

Chloroquine was only 58%, RI resistance was 20%, and RII was 7%, whilst RIII was 8% (GHS, 2001).

## 2.7 Antimalarial Drugs Policy in Ghana

In April 2004, a national stakeholders' sensitization meeting was held for briefing them on the antimalaria drug policy change with a proposed take off date of 1st January 2005. The first line drug recommended was Artesunate-Amodiaquine. The Ghana Health Service has accordingly revised its antimalaria drug policy (GHS/NMCP, 2004). The choice of ART-AMQ was based on WHO recommendation of artemisinin combination therapy (ACTs) and studies done in the African sub-region as there was limited local community-based evidence. There is, therefore, the urgent need to study the use of ACTs, the safety, efficacy, side effects and consequences on molecular epidemiology of Plasmodium falciparum in rural Ghana. There is also the need to closely monitor drug efficacy and possible evolution of resistance to ACTs for case management of malaria, especially those used for home management of childhood malaria. The patterns of incidence of malaria, and the costs associated with it, are different in different contexts and therefore, no magic bullet can be applied universally.



## 2.8 Combination Therapy (CT)

It has been accepted that the ideal treatment of malaria is to use two or more combinations of antimalarial drugs, preferably with an artemisinin derivative as one of the partner drugs (White & Olliaro, 1998; Sutherland *et al*., 2002a, b; Drakeley *et al*., 2004). Combination therapy with antimalarial drugs (CTs) is the simultaneous use of two or more blood schizonticidal drugs with independent modes of action and different biochemical targets in the parasite.

Combination therapies can be either fixed-combination medicinal products, in which the components are co-formulated in the same tablet or capsule, or multiple-drug therapy, in which the components are co-administered in separate tablets or capsules. Fixed-combination and multiple-drug therapies are used to exploit the synergistic and additive potential of individual drugs. The aim is to improve efficacy and to retard the development of resistance to the individual components of the combination. This concept has been realized in multiple-drug therapy for leprosy, tuberculosis and cancer and, more recently, in antiretroviral treatments (Shunmay *et al*., 2004). It has also already been realized to some extent in the field of malaria with the development of such drugs as Sulfadoxine-Pyrimethamine, atovaquone-proguanil and mefloquine-sulfadoxine-pyrimethamine (Shunmay *et al*., 2004).



The rationale for the impact of CTs on drug resistance is based on the assumption that drug resistance essentially depends on mutation. Provided that the constituent drugs administered in the combination have independent modes of action, the probability that a mutant will arise that is simultaneously resistant to both drugs is the product of the respective mutation rates, multiplied by the number of parasite cells exposed to the drugs (White, 1999a; Shunmay *et al*., 2004). For example, if two drugs are used, and for each one a single mutational event confers complete resistance and such events occur with a frequency of $1{:}10^{10}$ nuclear divisions, then the probability of a mutation resistant to both drugs is $1{:}10^{20}$ ($10^{10} \times 10^{10}$) . The number of asexual parasites (parasite biomass) during an acute malaria infection is usually between $10^{9}$ and $10^{14}$ (White, 1996a). This has led to calls for the widespread introduction of effective combination therapy for falciparum malaria as a matter of urgency (WHO, 2001b). It has been established that there are therapeutic benefits and the gains that will be enjoyed if combination therapy also brings about a significant reduction in parasite transmission to *Anopheles* mosquitoes, particularly parasites carrying genes conferring drug resistance (Drakeley *et al*., 2004). This requires that the carriage of

gametocytes, the parasite's transmissible stage, be minimized in treated patients (Butcher *et al*., 2000; Shunmay *et al*., 2004).

### *2.8.1 Artemisinin-based combination therapies (ACTs)*

Artemisinin-based combination therapy (ACTs) is antimalarial combination therapy with an artemisinin derivative as one component of the combination. There is a growing interest in using antimalarial combinations containing an artemisinin derivative as first-line treatment. The aim is to provide efficacious and safe antimalarial drug treatment while probably delaying the onset and spread of resistance to both drugs in the combination. This interest results from experience with the combination of Artesunate and Mefloquine on the Thai-Myanmar border (WHO, 1998). Artemisinins are a particularly effective partner drug because they are more active than any other antimalarial, reducing the number of parasites by approximately $10^4$ per asexual cycle (White, 1999b; Sutherland *et al*., 2005) and therefore reducing the number of parasites that are exposed to the partner drug alone.

Artesunate is known to act against immature gametocytes during the period of sequestration (7 days) that precedes emergence into the peripheral circulation as mature infectious gametocytes and can thus be used to reduce gametocyte carriage (Butcher *et al*., 2000). In addition, artemisinins have broad stage specificity and can be used to treat severe as well as uncomplicated malaria. They inhibit the production of gametocytes and therefore have a potential to reduce transmission (Hallett *et al*., 2004) and finally, to date, there has been no evidence of stable resistance either in therapeutic use or in experimental systems. Because of the short half-life of artemisinin derivatives, their use as monotherapy requires daily doses over a period of 7 days. Combination of one of these drugs with a longer half-life partner antimalarial drug allows a reduction in the duration of antimalarial treatment while at the same time enhancing efficacy and reducing the

likelihood of resistance development (Butcher *et al*., 2000). The major immediate effect of the artemisinin component is to reduce the parasite biomass. The residual biomass is exposed to maximum concentrations of the partner drug, well above its minimum inhibitory concentration, resulting in a lesser likelihood of resistant mutations breaking through (Butcher *et al*., 2000; Shunmay *et al*., 2004).

### *2.8.2 The argument against using ACTs*



There are a number of concerns about the use and widespread deployment of ACTs (Bloland *et al*., 2000) the chief one being cost. In addition, concerns about the practical difficulties in implementing any change in policy and the uncertainties about future costs, risks, and benefits, all make the decision of whether to switch, when to switch, and what drug regimen to switch to, a complex one (Goodman *et al*., 1999; Shunmay *et al*., 2004 ). In order for national governments, donor countries, and international institutions to make rational decisions on drug policy, there is a need to clarify how much of a burden antimalarial resistance causes currently, how much it is likely to cause in the future under different control strategies, and how much these strategies will cost and save (Shunmay *et al*., 2004). Not only does each change require a major investment of scarce human and financial resources, but frequent policy change is likely to lead to confusion among the public and a loss of credibility of the policy makers.

Currently there is only one registered co-formulated ACTs that is produced to internationally recognized good manufacturing practice standards; Artemether-Lumefantrine (Co-Artem®). Fixed Artesunate-Mefloquine and Artesunate-Amodiaquine co-formulated drugs are also being developed. Artemisinin-based combination used today, such as Artesunate and Mefloquine or Artesunate and SP, Artesunate and Amodiaquine, are given as two separate types of tablets and there is a risk that patients will take only the artemisinin derivative responsible for rapid symptom

resolution and this will only be taken for a few days. Not only will this result in treatment failures, but also it will theoretically increase the risk of drug resistance emerging in the future (Shunmay *et al*., 2004).

## 2.9 Assessment of Antimalarial Drugs Susceptibility

Drug efficacy is determined by the drug sensitivity of the *Plasmodium* species concerned, pharmacokinetics and the development of resistance as a function of time (influenced by the drug's half-life) (WHO, 2000a, b & 2005b). In developing or revising an antimalarial treatment policy, the efficacy of alternative regimens should also be taken into account. Parasite susceptibility to antimalarial drugs can be assessed by *in vitro* or *in vivo* techniques. *In vitro* techniques rely on the collection of parasitized blood from patients and the testing of parasite susceptibility to drugs in culture or by the use of molecular techniques such as PCR. *In vivo* techniques rely on monitoring of the symptoms associated with malaria, such as fever, and parasitaemia. *In vivo* can also be carried out among asymptomatic patients who will involve monitoring of parasitaemia (WHO, 2003a & 2005b).

A major purpose of assessing the therapeutic efficacy of antimalarial drugs in confirmed malaria patients is to monitor efficacy over time, especially in vulnerable groups in highly endemic areas, and to guide treatment policy. Antimalarial drug responses are assessed clinically from rates of symptom resolution e.g. fever clearance, coma recovery, or parasitologically from parasite clearance and overall cure rates. Follow-up of treated patients for evidence of recurrence of parasitaemia may continue for 7, 14 or 28 days, depending on the investigators' interest in detecting lower levels of resistance and on budgetary limitations (Bruce-Chwatt, 1986).

### *2.10 WHO guidance on treatment policy change*

The WHO has recommended the following in considering the suitability of a first line drug for a significant public health problem like malaria (WHO, 1999):

I. **Alert phase**- period when treatment failure rate with first line drug is 6-15%. During this phase the mechanism is set in motion for change.

II. **Action phase-** Treatment failure rate of 16-25%. During this period, implementation for change should have commenced.

III. **Change phase**- Over 25% treatment failure rate.



WHO recommends that an effective malarial treatment policy must achieve the following goals:

1. Reduce morbidity
2. Arrest progression of uncomplicated malaria to severe and fatal disease, thereby reducing mortality
3. Reduce the impact of placental malaria infection and malaria-associated anaemia through chemoprophylaxis or intermittent preventive treatment
4. Minimize antimalarial drug resistance development

#### *2.10.1 Policy Options*

##### *2.10.1.1 Uncomplicated malaria*

Drug options for first line drug for the management and treatment of uncomplicated malaria where malaria is endemic and home management is common should have the following properties:

- Good efficacy and safety profile
- High degree of acceptability and ease of administration
- Capable of being used by special groups (e.g. Infant and pregnant women)

- Cost-effectiveness
- Little or no reported resistance or cross-resistance
- Useful therapeutic life

Since resistance to Chloroquine was deemed to be unacceptably high, CQ was thus ruled out as an option for the drugs to be considered.

### *2.10.2 Current management of uncomplicated malaria: Amodiaquine plus* **Artesunate**

The Ghana Health Service (GHS, 2004b) has since changed its policy on first line drug for malaria case management from Chloroquine to Artesunate-Amodiaquine. The 3-day regimen of both components is currently co-administered although co-formulation is feasible. Amodiaquine has a greater efficacy than Chloroquine in Africa (Mutabingwa *et al*., 2005). However, the rate of development and spread of Amodiaquine resistance is unknown and cross-resistance with Chloroquine may be a limiting factor for long-term efficacy. In addition, Amodiaquine toxicity following repeated doses requires further evaluation.

#### *2.10.2.1 Option appraisal for the choice of antimalarials for uncomplicated malaria*

The choice of alternatives to CQ was guided by the understanding of the characteristic of the different possible substitutes. The key characteristics considered were efficacy levels, compliance, route of administration, side effects, cost effectives, impact on local industry and demographic variables such as the appropriateness for treating children under-five and in pregnancy (GHS, 2004b). Because of WHO recommendation of the use of ACTs, various antimalarial drugs in combination with ART were considered. MQ was not recommended because of its high cost of treatment, possible reactivity with CQ and could hardly be made available. Artemether-lumefantrine is very expensive and was not feasible to be produced in Ghana and could not be

recommended as well. SP with ART could not be considered because SP is currently being used for IPT in pregnancy.

### *2.10.2.2 Choice of ART-AMQ versus other alternatives*

Ghana Health Service (2004b) has recommended ART-AMQ as the first line drug based on these premises. That it has an efficacious combination with low side effects and studies from various parts of the world showed ART-AMQ to have high parasite clearance and cure rate with adequate treatment duration (GHS, 2004b). ART-AMQ could be produced and packaged locally in Ghana. There was little evidence of cross-resistance and the cost for treatment was moderate (GHS, 2004b). Hence ART-AMQ was, therefore, considered as the most cost-effective drug compared to all other alternatives.

There was, however, very limited local evidence on the ART-AQM efficacy, acceptability and adverse events and safety before the introduction of ACTs in Ghana. The selection of drugs and treatment protocols must be based on reliable clinical and epidemiological assessments of efficacy (WHO, 2003b). The adoption and use of ART-AMQ in the treatment of uncomplicated malaria in Ghana was rather based largely on evidence from clinical and epidemiological studies from the sub- regions of West and Central Africa. Local data, however, were lacking until Koram *et al*. (2005) demonstrated that ACTs were superior to monotherapies and the replacement of CQ with ART-AMQ in the management of uncomplicated malaria in Ghana was in the right direction.

# CHAPTER THREE - MATERIALS AND METHODS

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## 3.0 Study Design

The study had both analytical and experimental designs. Two study types were conducted:

1. A longitudinal sub-study was undertaken to determine the molecular epidemiology of *P. falciparum* in a setting of Artesunate-Amodiaquine use in a main home management of malaria study (Ajayi *et al*., 2008a). The success or failure of any public health program is largely determined by the effective use of the services offered to the public.

2. There was also a seven-arm single blind randomized in-vivo comparative trial of the efficacy and safety of ACTs in the treatment of school pupils with uncomplicated malaria and /or parasitaemia. This study sought to compare the parasitaemia clearance and haemoglobin concentrations among school pupils, before and after ACTs administration.

### *3.0.1 Qualitative research*

Focus group discussions, in-depth interviews, case studies and observations were used to explore the perceptions, attitude, acceptance and compliance with the new regimen of the malaria case management amongst caregivers, health staff, community leaders and opinion leaders. This was done at the communities to serve as a tool for face-to-face education of caregivers so as to promote complete compliance with the new intervention and also participation in the study.

## 3.1 Study Area

The study was carried out in the Ejisu-Juaben Municipal district which is one of the 23 political districts of the Ashanti Region of Ghana. The district capital, Ejisu is 20 km from Kumasi the regional capital. It is located in the south-eastern part of the region and shares boundaries with

Kwabre, Afigya-Sekyere, Sekyere East and West districts to the north, Asante Akim North and South districts to the east, Bosomtwe-Atwima-Kwanwoma district to the south and Kumasi to the west. It has a total land area of 1,637.2 sq km. This district shares similar ecological characteristics with other districts in the region. The climate is tropical; temperature variation is $20^{o}$C - $36^{o}$C with monthly rainfall varying from 2.0 mm in February to 400 mm in July. Its' 2006 population is estimated to be 151,761**,** with a growth rate of 3.1%. The population aged below one year is 4% whilst pre-school children 20% of the population (Ejisu-Juaben- DHMT, 2003). It is a predominantly rural district, with the main occupation of the people being subsistence farming.



The district is divided into five sub-districts: Achiase, Bomfa, Ejisu, Juaben and Kwaso. There are 23 health facilities including five hospitals, five health centres, three public clinics and maternity homes, four mission clinics and six private maternity homes. It has 81 communities with 39 of them having functional village health committees. The district health system is based on a 3-tier Primary Health Care. These are the district, the sub-district, and the community. The activities at the district level are headed by the DHMT led by a District Director of Health Services (DDHS) while the Sub-District Health Team (SDHT) oversees health activities in the sub-district led by a Medical Officer or Medical Assistant. The Village Health Committee (VHC) manages the community level. There are 31 community-based agents who have been trained in home management of malaria using prepacked Chloroquine, acute respiratory infections (ARI) and diarrhoea case management using ORS, operating in 16 communities. There is a network of community-based agents (CBAs), who provide home management of malaria using pre-packed Chloroquine. There are also community volunteers, who assist in outreach clinics, national immunization programmes, community surveillance and community health education. Malaria is the leading cause of outpatient visits and accounts for 44.3% of outpatient department visits.

Malaria is hyperendemic (Browne *et al*., 2000) and rated 42.9% of the total admissions of the top five diseases in the district (Ejisu-Juaben- DHMT, 2003).

This district was the site for the Home Management of Malaria (HMM) study funded by WHO/TDR conducted by the Department of Community Health, School of Medical Sciences KNUST. A total of 37 rural farming communities in the Juaben and Bomfa sub-districts were used as sites for the HMM study. This study was carried out in some of the 37 communities where HMM programme was undertaken. Abetinim, Atia, Dumakwai, Nkyerepoaso, Odoyefe, Ofoase, New Koforidua and Krofofrom were selected for blood sample collection for the molecular epidemiology of *P. falciparum* in a setting of Artesunate-Amodiaquine use. A randomized comparative in-vivo study was conducted in public schools in these communities: Abesewase, Abetinim, Atia, Nkyerepoaso, Odoyefe, Ofoase and Nobewam.

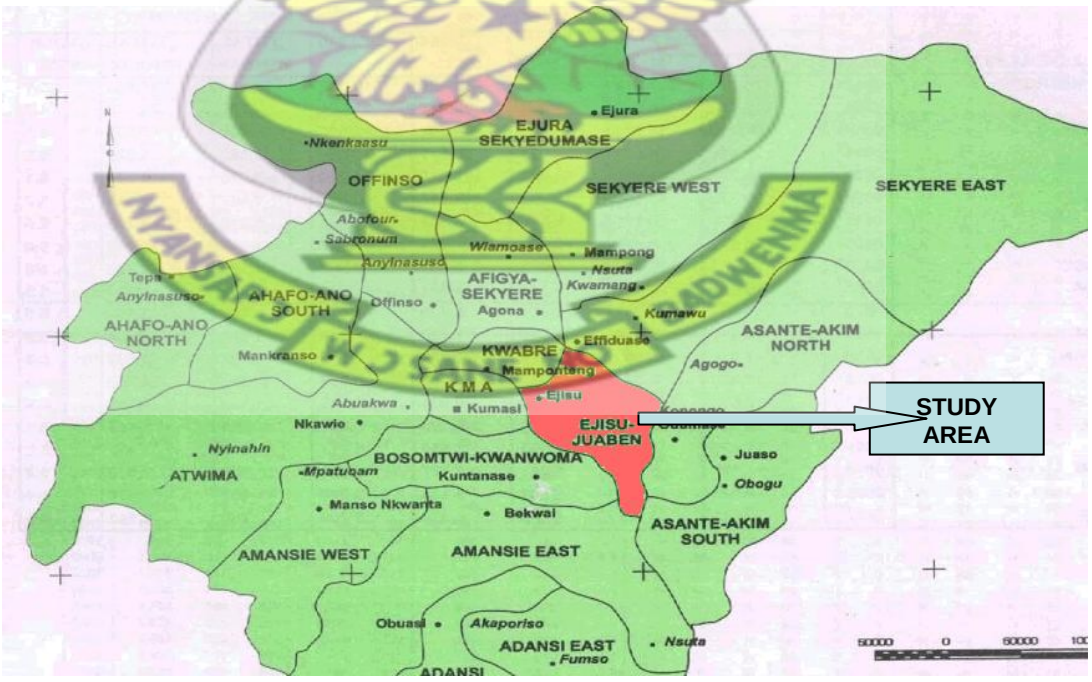
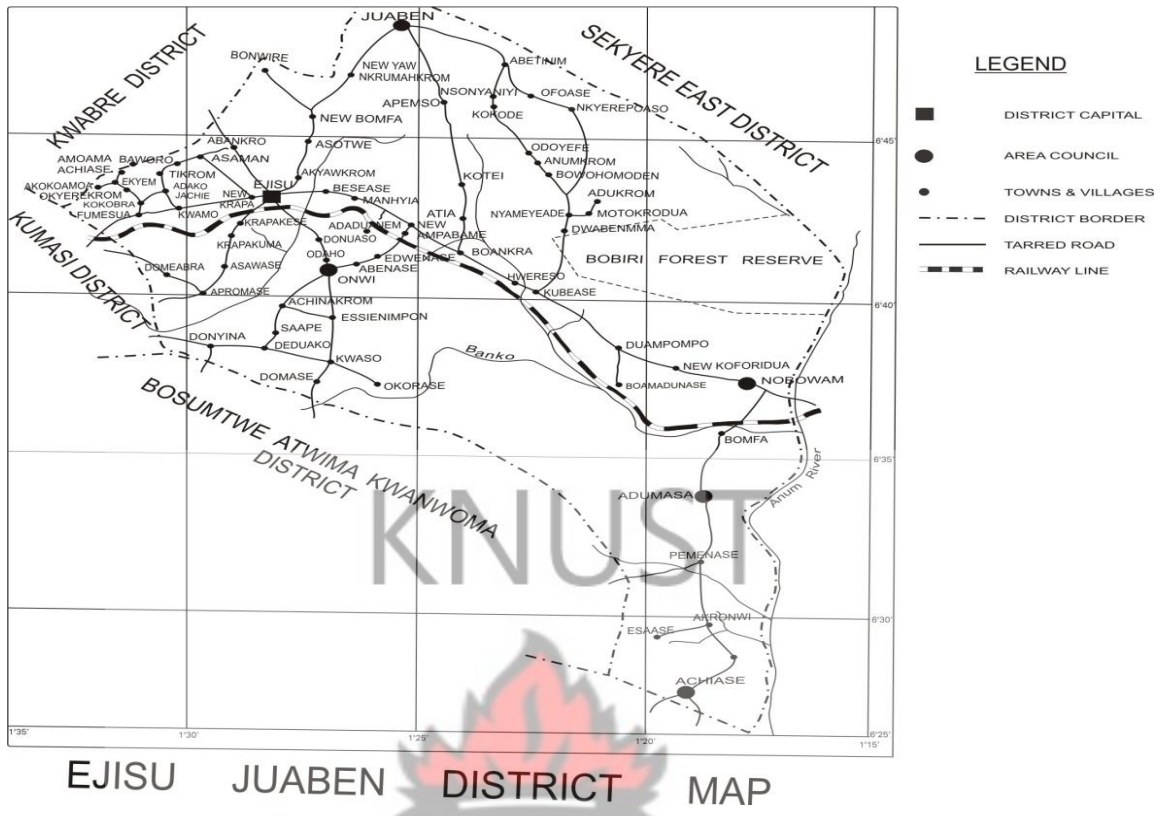



**Figure 3.1: Map of Ashanti Region showing Ejisu-Juaben Municipal District**




EJISU JUABEN DISTRICT MAP

**Figure 3.2: Map of Ejisu-Juaben Municipal District (Study Area)**



## 3.2 Study Population

The study population comprised of all children aged 6 months–59 months and school pupils between 3 to 10 years in the selected communities in the district. Caregivers, Head teachers, Health staff and Community Drug Distributors were also included from August 2004 to December 2006.

## 3.3 Recruitment of Study Children

Two study types were conducted: thus two sets of criteria were employed in the inclusion and exclusion criteria.

### *3.3.1 Inclusion criteria*

For the molecular epidemiology of *P. falciparum* in a setting of Artesunate -Amodiaquine use, a child was eligible for inclusion in the study if:-

1. He/she was between the ages of 6 months to 59 months old.
2. He/she had fever and other symptoms of malaria (≥37.5 $^{o}$C).
3. He/she had *P.falciparum* parasitaemia with fever as the key symptom.
4. He/she and main caregiver were permanent residents in the study site.
5. He/she was not under treatment with any antimalarial drugs.
6. He/she had no severe malaria and any chronic illness
7. The main caregiver was willing and gave consent to allow the child to partake in the study and to complete all tests scheduled.

The home management of malaria in children is usually targeted at children under-five years that are usually most vulnerable to malaria, hence, children > 59 months were excluded.

For ACTs study, a pupil was included into the study if the

1. pupil was between the ages of 3-10 years old
2. pupil had *P. falciparum* parasitaemia with density ≥ 500 parasites/µL of blood with or without symptoms
3. pupil was resident in the community
4. pupil was not under antimalarial treatment
5. main caretaker was willing and gave consent for pupil to partake in the study.

Children from 3-10 years were included in this study because they were in school, vulnerable and during and after treatment could adequately report on any side effects of the drugs.

### *3.3.2 Exclusion criteria*

For the molecular epidemiology of *P. falciparum* in a setting of Artesunate -Amodiaquine use, a child was ineligible for inclusion in the study if the

1. child was < 6 months old and > 59 months old
2. child had no *P.falciparum* parasitaemia and fever as the key symptom
3. child has been treated with antimalarial drug
4. child any sign of respiratory distress, convulsion or severe malaria
5. caregiver was not resident in the study community, and if
6. caregiver gave no consent.



For ACTs study, a pupil was ineligible for inclusion into the study if the

1. pupil was < 3 years old and > 10 years old
2. pupil had *P. falciparum* parasitaemia with density < 500 parasites/µL of blood
3. pupil weighed less than 10kg
4. pupil was domicile outside the study community
5. there was any evidence of chronic disease or acute infection other than malaria and signs of severe malaria and if the
6. main caregiver or pupil refused to give consent.

## 3.4 Recruitment and Training of Field Staff

The research required the support of health staff and head teachers in the various communities. Therefore they were taken through the aims and objectives of the study and their role in the study. The research team (Principal Investigator, Research Assistants, laboratory technicians, data

manager and entry clerks and other senior hospital staff) underwent training on the research methodology. They were trained in specific tasks such as:

- Compilation and maintenance of registers
- Qualitative research methods
- Survey methods
- IEC campaigns
- Record-keeping, data analysis and report-writing
- Stock management and control
- Supervision
- Home management of malaria using prepacks
- Completion of questionnaires
- Basic computing skills
- Data entry skills

CDDs and chemical sellers training concentrated on:

- Diagnosis, treatment and follow-up of childhood malaria cases
- Referrals
- IEC (mothers/caregivers face-to-face, house-to-house, community, campaigns)
- Record-keeping, data analysis and report-writing
- Stock management and control
- Maintenance of fever registers

Training sessions for research team and field staff took place at the Ejisu-Juaben District Health Service Office and Juaben Hospital in the afternoons from 1.00 pm when the OPD was less busy. There were monthly updates for all categories of research staff at the hospital.

### *3.4.1 Selection and training of Field Supervisors*

Two (2) field supervisors drawn from the DHMT or sub-district team were trained to supervise 10 communities each. They were given training on riding motorcycles safely. They were expected to visit each CDD at least once every fortnight and complete a supervision checklist. CDDs and Field Supervisors met with the research team and the DHMT once or twice a month for regular updates and reviews. They were trained in laboratory methods to provide additional support to laboratory staff in supervising CDDs in blood sample collection and completion of data forms. Field staff had monthly updates after initial training. CDDs, field supervisors and laboratory staff were trained to maintain registers on study children in these selected communities. Field supervisors extract the relevant data from registers during field visits.



### *3.4.2 Training of network of Community Drug Distributors (CDDs)*

A network of CBAs, who were trained as part of the first Ghana study of HMM using Chloroquine, was retrained to prescribe and dispense Artesunate-Amodiaquine for home management of malaria. An average of two CDDs was retrained for each community. First dose of the treatment was given under the supervision of the CDD to demonstrate how mother/caregiver should administer treatment. All CDDs were given a toolbox with essential items for their work. Ten CDDs, two per community from five communities were initially recruited for laboratory training. One week training was held at Juaben Government Hospital. Three training sections were held:

i) The overview of the project, theory on malaria, completion of data collection forms and aseptic techniques.

ii) Practice on the preparation of both thin and thick films for microscopy

iii) Practice on how to spot filter paper sample for PCR analysis

Practical sections were held at Nkyerepoaso and Ofoase where Child Welfare Clinics were held. The CDDs learned at firsthand how to take blood samples from children under observation and this was satisfactory though some needed more time to cope with the procedures. In their communities CDDs also held educational campaigns on the need for blood sample collection. They were trained on aseptic techniques, to use one lancet per child and taking blood from a one finger- prick for both blood films and filter papers (Whatmann 3MM).



## 3.5 Data Collection

Mainly primary data were collected using data collection tools from children and /caregivers, with the help of research assistants recruited from the communities.

### *3.5.1 Data collection tools*

Cross-Sectional Survey Form, Laboratory Forms (day 0, 14, 28, 3 months and 6 months) and Drug Adverse Effect Form and were tools used in soliciting for information from respondents.

### *3.5.2 Sampling techniques*

Two sub-districts were purposefully selected from a total of five sub-districts in the district. Eight villages were randomly selected out of the villages in the two sub-districts. Both purposive and convenient sampling techniques were also employed to sample health providers, community leaders and caregivers. For the selection of the schools, seven communities were randomly selected and where there was more than one public school in a community, one school was randomly selected.

### *3.5.3 Sample size*

Two different study types were conducted and hence two different sample sizes were used. A sample size of 400 malaria positive children has been estimated to determine the molecular epidemiology of *P. falciparum* in a setting of Artesunate -Amodiaquine use. An assumption of parasitaemia prevalence of 70% (based on prevalence in the study area) was used, with 5% significance and 5% precision, giving a sample of 323 positive children. However, 400 were estimated to increase the power of the study. Sample size calculation was based on this formula: $n = Zá^2 p (1-p) d / i^2$ (Smith & Morrow, 1996) where n = sample size required, Zá = risk (0.05) (1.96), p = proportion of event of interest, d = design effect, i= precision of the confidence interval.

For the single blind randomized in-vivo comparative trial of the efficacy and safety of ACTs, a sample size of 420 pupils was estimated; 60 per each arm of ACTs. Sample size calculation was based on expected proportion (P) of treatment failures of 15%, desired confidence interval (CI) of 95% and precision (d) of 10%. For this to be representative, a minimum of 50 patients per arm was estimated. However, loss due to follow-up and withdrawals was factored into the sample size calculation to give 60 per arm using the formula: $n = (1 + l\ \%) \times N$, where N=initial total: $n = Zá^2 p (1-p) d / i^2$ (Smith & Morrow, 1996). Where n = sample size required, Zá = risk (0.05) (1.96), p = proportion of event of interest, d = design effect, i= precision of the confidence interval.

### *3.5.4 Screening and enrolment procedures*

All study children were recruited from the study communities over a 17-month period, August 2005 to December 2006. All study children were given an 8 digit identification number identifying the villages / communities, schools and the individuals' and their demographic data. For the

molecular epidemiology of *P. falciparum* in a setting of Artesunate-Amodiaquine use, study children were recruited by CDDs in the communities. Standard operation procedures employed by the CDDs were as follows:

1. Screening of all children for the key symptoms of malaria, fever and an axillary temperature of > 37.5˚C.
2. Obtaining informed consent from the main caregiver if child was 'diagnosed' to have malarial fever.
3. Preparation of thick and thin blood films by CDDs from a finger prick blood sample for quantification and identification of malaria parasites respectively.
4. Preparation of blood spotted filter samples from the same finger prick blood.
5. Treatment of all children "diagnosed" to have malaria with ART- AMQ prepack (25mg ART/75mg AMQ and 50mg ART/150mg AMQ for children below 12 months and those aged 12 - 59 months respectively).
6. Collection of blood films and spotted filters samples of all recruited children by field supervisors from CDDs for subsequent analysis within 48 hours and
7. Following up of microscopically positive study children on days 3, 7, 14, 28 and instructing caregivers to see CDDs in the event of any febrile episode.

School children of the seven communities were initially screened in their various schools to be recruited into the ACTs trial. Standard operation procedures employed in the screening process were as follows:

1. Physical and clinical examination and taking of axillary temperatures.
2. Weighing pupils whose caregivers gave consent for their inclusion into the study.

3. Collection and preparation of thin and thick blood films by laboratory technician together with PI from a finger prick blood sample for quantification and identification of malaria parasites respectively.
4. Preparation of blood spotted filter samples and also determination haemoglobin from the same finger prick blood.
5. Staining and microscopic examination of blood films within 12 hours and
6. Randomization of microscopic positive pupils into the various different trial arms.




**Figure 3.3: Pupil being weighed during screening**

### *3.5.5 Cross- sectional surveys*

A single blind randomized in-vivo comparative trial of ACTs was conducted among schools. Two cross-sectional surveys were conducted in January to March 2006 and May to August 2006

coinciding with the dry and wet seasons respectively. At each survey, the following were determined:

- Axillary temperature
- Weight
- Haemoglobin levels using HemoCue®.
- Thick and thin blood films and blood spotted filter samples preparation.




**Figure 3.4: Blood sampling and haemoglobin determination**

### *3.5.6 Blood sample collection*

The longitudinal follow-up study of children aged 6–59 months in the selected villages in the Ejisu-Juaben district began with caregivers reporting to CDDs with a complaint of fever and other symptoms of malaria. Study children who were clinically diagnosed to have "malaria" by CDDs in homes had their blood samples and data taken and treated with Artesunate- Amodiaquine and enrolled into the study. Samples taken at enrolment were labelled day zero (0). The details of the laboratory follow up are summarized as follows: Blood films and blood spotted filter samples: Day 0, Day 14, Day 28 and subsequent episodes of malaria fever.



## 3.6 Laboratory Methods

Laboratory investigations conducted included:

1. Microscopic examination of thick and thin blood films
2. Haemoglobin determination
3. Polymerase chain reaction (PCR) assay and genotyping

### *3.6.1 Microscopic blood examination*

#### *3.6.1.1 Giemsa staining technique*

Giemsa stain is an alcohol-based Romanowsky stain (azure dyes and eosin) that requires a dilution in pH 7.1-7.2 buffered water before use. Freshly prepared 3% working Giemsa solution was used in staining blood films for 30-45 minutes.

### *3.6.1.2 Microscopic reading of slides*

Thin and thick blood smears were prepared from a finger-prick sample. A small drop (about 2-5mm) of blood was used to prepare thin films whilst a larger drop (about 10-15mm) was used to prepare thick films on the same slide. All slides were then labeled with their respective identification numbers using markers and pencils. These slides were kept in a netted tray to air-dry. All thin films were immediately fixed with an absolute methanol. These slides were then stained for 30-45 minutes with 3% Giemsa stain. These films were examined microscopically, thin smears to establish *Plasmodium* species and thick smear to quantify asexual parasitaemia (per µL), and detect gametocytes. A slide was declared negative after examining 200 high power fields. Thick blood smears were read twice and parasitaemia quantified against 200 white blood cells (Marsh *et al*, 1999; Cheesborough, 1998). Discrepant slides were read again by a third slide reader and the majority opinion taken. Parasite density was calculated per µL of blood using the formula:

$$\text{Parasite Density} = \frac{\text{Number of parasites counted} \times 8000 \text{ white blood cells (WBCs)}}{\text{Number of WBCs counted}}$$

When the number of asexual parasites counted was less than 10 against 200 WBCs, counting was done against 500 WBCs. This technique was used for all slides read during the study.

### *3.6.1.3 Quality assurance*

Two microscopists read the slides independently and all discrepant slides were read again by a third reader. A 10% sample of all blood films were read again for quality control. There were only minor differences in parasite densities found during the reading of the slides.

### *3.6.2 Haemoglobin determination*

Haemoglobin measurement was done using capillary blood from a finger-prick sample using HemoCue haemoglobinometer (HemoCue AB, Angelholm, Sweden). Capillary blood was collected from a finger-prick sample into a mircocuvette containing sodiumdesoxycholate which hemolyses the erythrocyte and haemoglobin is released. Sodiumnitrite in the cuvette converts the haemoglobin to methemoglobin which together with sodiumazide, gives azidemethemoglobin. The absorbance of this is measured at two wave-lengths (570 and 880nm) in order to compensate for turbidity in the sample.



### *3.6.3 Preparation and storage of blood filter paper blots*

Blood filter spots were made for all children on enrolment however, only those confirmed by microscopic examination were followed for the corresponding days 14 and 28 visits samples. Whatmann 3MM filter papers were used for the preparations. Spotting was done from a figure-pricked blood, the first drop was wiped away with cotton, and then the next 2-3 drops of blood were put on the filter paper at three sites. Enough blood was spotted to completely saturate the paper through both sides. Spotting was done without touching the patient's finger with the filter paper. Blood spotted filter papers were air-dried in a netted tray for at least three hours. These samples were then kept in well labelled self-lock sachets, containing silica desiccant pouches and stored at room temperature until they were used for genotyping. For each enrolled patient, blood spotted filter samples were collected on days 0, 14, and 28 and any other subsequent visits.

### *3.6.4 Molecular genotyping*

To compare the rate of parasite recrudescence in children treated with ART-AMQ samples from children who were parasite positive at days 0 and 28 were differentiated into recrudescent or new

infections by PCR genotyping. Re-infections and recrudescences were distinguished by comparing the pre-treatment and post-treatment genotypes of the *P. falciparum* gene loci coding for the *msp*-2 and the *glurp* (Happi *et al*., 2004; Snounou & Singh 2002; MMV /WHO, 2007; Mugittu *et al*., 2006& 2007).

### *3.6.4.1 DNA extraction and PCR amplification*

Extraction and amplification were done at Ibadan Biomedical Research laboratory and Kintampo Medical research Centre. Parasite genomic DNA was extracted from blood samples collected on filter paper using the chelex extraction method (Cattamanchi *et al*., 2003). Nested PCR amplifications of the highly polymorphic merozoite surface protein 2 (*msp2)* and glutamate rich protein (*glurp)* loci of *P. falciparum* were done using oligonucleotide primers, reaction conditions and procedures described previously (Cattamanchi *et al*., 2003; Happi *et al.,* 2004 England Biolabs, Beverly, MA).

### *3.6.4.2 PCR determination of P. falciparum isolates population structure in patients*

Genotyping of parasite populations were performed in each sample collected from patients with microscopically confirmed *P. falciparum* infections at enrolment, and during follow-up if the patient was parasitaemic at any point during the follow-up period of 28 days as required by the protocol of the study. A nested PCR approach (Cattamanchi *et al*., 2003; Happi *et al.*, 2004) was used to determine parasite population structure in isolates of *P. falciparum* obtained at enrolment and at re-occurrence of parasitaemia during the follow-up period of 28 days.

Analysis of genetic polymorphisms was performed on paired primary and post-treatment parasite samples obtained from ART-AMQ treated patients. Paired primary and post-treatment parasites were analyzed using parasite loci that exhibit repeated numbers of polymorphisms in order to

distinguish true treatment failures from new infections. Block 3 of *msp-2* (merozoite surface protein-2) and region II of *glurp* were amplified by two rounds of PCR using primers and amplification conditions described previously (Cattamanchi *et al*., 2003; MMV /WHO, 2007; Mugittu *et al.,* 2006 & 2007; Happi *et al.,* 2004).

Ten microliters of each nested PCR product was resolved by electrophoresis on a 2% agarose gel stained with ethidium bromide and sized against a 100-base pair molecular weight marker (New England Biolabs, Beverly, MA). The banding pattern of the post-treatment parasites was compared with matched primary parasites in each of the patients who had parasitaemia after treatment with ART-AMQ. Post-treatment and pre-treatment parasites samples showing identical bands were considered as true treatment failure, while non-identical in banding patterns were considered as newly acquired infections. A lack of allelic identity in at least one locus in matched primary and post-treatment samples indicated newly acquired infections. An un-determinant ("unresolved") result was recorded when the parasite DNA could not be amplified either in the pre-treatment or post-treatment samples and these samples were excluded from the analysis (MMV /WHO, 2007; Mugittu *et al.,* 2006& 2007; Happi *et al*., 2004).



#### *3.6.4.3 Determination of P. falciparum clonal profiles in days 0 and 28 infections*

Each *P. falciparum* infection was characterized on the basis of fragment sizes of PCR products for each locus and determination of sizes of *msp-2* and *glurp* alleles. Infections were defined as polyclonal if parasites in matched primary and post-treatment samples from the same patient showed more than one allele from one or more genes. If an isolate had one allele at each of the loci, the clone number was taken to be one. Post-treatment and pre-treatment parasites samples showing bands were also used to determine the multiplicity of infections in patients.

### 3.7 Trial of Antimalarial Drugs Used for the Study

The drugs that were used for the study were Artesunate – Amodiaquine (ARSUCAM) for molecular epidemiology of *P. falciparum.* The ARSUCAM prepack was manufactured by a Moroccan company, MAPHAR and procured by WHO for the HMM project. Lariam® (Mefloquine) produced by F.Hoffmann-La Roche Ltd, Basel Switzerland, Coartem® (Arthemeter-Lumefantrine) manufactured by Beijing Novartis Pharma Ltd, Beijing China, LapdaP™ (Chlorproguanil/ Dapsone) produced by Wűfing Pharma GmbH, Gronau, Germany, Amodiaquine and Chloroquine manufactured by Phyto-Riker (GIHOC) Pharmaceuticals Ltd, Ghana, Adamsunate® (Artesunate) made by Danpong-Adams Pharmaceutical Industry, Ghana, AMOTEX® (co-formulation of Artesunate-Amodiaquine) and Malafan™ (Sulfadoxine-Pyrimethamine [SP) produced by Kinapharma Ltd, Ghana were purchased in pharmacy shops for the randomized in-vivo comparative trials of ACTs.



#### *3.7.1 Allocation and randomization of ACTs (antimalarial drugs)*

On the basis of the revised anti-malaria drug policy of the Ghana Health Service (GHS/NMCP, 2004b), Artesunate-Amodiaquine was the drug of choice for home management of malaria in the study. ART-AMQ intervention was provided in 2 treatment doses: The drugs were packaged into two treatment doses; preschool (children aged 12-60 months) with 50mg Artesunate and 153mg Amodiaquine base daily for three (3) days as a full dose treatment. The research team, however, repacked some of these drugs for infants aged 6 to 11 months doses. The infant prepack were 25mg Artesunate and 76.5mg Amodiaquine base daily for three (3) days as a full dose treatment, which is exactly half of the preschool dose, was labelled with the logo of a crawling child for easy identification. The ART-AMQ prepacks were used only for the treatment of febrile children within the targeted age ranges. That overweight or underweight children might receive slightly lower or a

higher dose was recognized, however, the practical and logistical advantage and overall benefit favoured the use of prepacks.

**Table 3:1: Treatment of uncomplicated malaria using ART-AMQ**

| **Weight** | **Age** | **Artesunate tablets** | | | **Amodiaquine tablets** | | |
|---|---|---|---|---|---|---|---|
| **(kg)** | **(years)** | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 |
| 5 – 10 | Infants | 25mg | 25mg | 25mg | 76.5mg | 76.5mg | 76.5mg |
| 11 – 24 | 1 – 6 | 50mg | 50mg | 50mg | 153mg | 153mg | 153mg |

For the single blind randomized in-vivo comparative trial of ACTs, children recruited into the study were randomly allocated into 7 treatment arms using a computer assisted statistical soft ware Stata. Chloroquine (CQ), Mefloquine (MQ), Amodiaquine (AMQ), Sulfadoxine-Pyrimethamine (SP) and chlorproguanil-dapsone (LAPDAP) were co-administered with Artesunate (ART). Children received 50mg/ ART kg body weight over a period of 3 days. Arthemeter-Lumefantrine (Coartem) and Amotex were already co-formulated artemisinins-based combination. The treatment arms were designated with Arabic numerals as ART/SP (1), ART/AMQ (2), ART/MQ (3), ART/LAPDAP (4), Coartem (5), ART/CQ (6), and Amotex (7). A list of study ID numbers were also generated which corresponded with these treatment arms at least 60 per treatment arm. At each recruitment session, pupils determined their treatment arm by picking from a batch of study IDs with treatment arms and were treated according to the Ghana Health Service (2004b) and WHO (2006c) Guidelines for the treatment of malaria for a period of 3 days.

**Table 3:2: Schedule treatment dosage for the test drugs according to age & weight**

| Study drug | Age (yrs) | Weight (kg) | Dosage-Size-Base-mg) |
|---|---|---|---|
| **CQ** | 3-10 | 15-35 | 1-2.5 (tabs) x3 (150mg base) |
| **SP** | 3-10 | 15-35 | 1-2 (tabs) x1 (500mg/25mg) |
| **AMOTEX** | 3-10 | 15-35 | 1 (tab) x3 (50mgART +200mg AMQ) |
| **AMQ** | 3-10 | 15-35 | 1-2 (tabs) x3 (200mg) |
| **Coartem** | 3-10 | 15-35 | 2-3 (tabs) x2x3(60mgA+360mgL) |
| **MQ** | 3-10 | 15-35 | 1.5-2 (tabs) x2 (25mg) |
| **LapDap** | 3-10 | 15-35 | 1 (tab) x3(2mg chlorproguanil+2.5mg dapsone) |



The drugs were administered by the research team on the first day of treatment after a meal and continued by the caregivers (teachers/ parents) on the second and third days. Each child was observed and any child that vomited within 30 minutes was given full dosage and half dosage after an hour. Drug adverse events forms were given to CDDs/ teachers to record possible adverse effects after treatment. Patient's follow-up visits were scheduled for days 14 and 28 respectively from the day of treatment. At these visits, thick and thin blood films and blood spotted filter samples were taken, and haemoglobin determination. The children's health was also assessed at these visits in addition to the treatment days (0, 1, and 2).


**Figure 3.5: Pupils eating and getting ready for medication**


**Figure 3.6: Pupil taking medication**

### *3.7.2 Endurance of drug compliance*

The first dose of treatment was directly observed by the research team and subsequent doses were given by caregivers and teachers with the help of CDDs on the second and third days. A compliance check was also performed to determine the level of adherence to treatment regimens.

### 3.8 Follow-up Procedures

The study children and pupils were followed up at the following times: days 3, 7, 14 and 28. Drug adverse events forms were given to CDDs and teachers to record possible adverse effects after treatment on day 3. On days 14 and 28 follow-up visits, all laboratory indices required according to the study design were taken. Drugs adverse effects were also assessed as well as the child's health.



#### *3.8.1 Follow up visit on day 3*

Field worker/CDDs followed up caregivers and their children in the communities to assess the conditions of the children after treatment. They asked caregiver about any signs of adverse events and treatment outcomes. They also physically observed for conditions such as weakness, skin rash, jaundice or pallor. In addition, study children who fell sick were referred to the Hospitals for further treatment. Reminder notices for scheduled visits were given to caregivers for days 14 and 28 follow up. For study pupils, teachers together with CDDs observed them whist they were in school. Those pupils absent were followed up in their homes.

#### *3.8.2 Follow up on days 14 and 28*

All study children and pupils who completed their treatment regimens were visited on days 14 and 28. Blood films and blood spotted filter paper samples were taken and haemoglobin determined. CDDs collected samples of children in their homes whilst pupils' samples were taken by the research team together with CDDs in schools.

### 3.9 Escape of Medication

All study children and pupils that had difficulty taking medication orally manifested by vomiting were considered to have escaped medication. Children with signs of deteriorating clinical condition such as a rise in body temperature and children whose caregivers refused to continue medication as result of adverse events were classified as escape medication.

### 3.10 Monitoring of Drug Adverse Effects



All study children and pupils were assessed clinically for the outcome of treatment and the presence of adverse events. An adverse event was defined as the presence of any outward medical or clinical occurrence in the study children which may or may not be related to the study drugs within seven days of treatment. These occurrences might have developed or increased in severity following medication. Mother/caregivers were asked about the clinical outcome after the drugs administration and signs and symptoms of adverse occurrences. These events were classified as mild and non-life threatening and serious and life threatening based on the information provided by the caregivers and CDDs and a judgment was made based on the protocol guidelines.

#### *3.10.1 Mild and non-life threatening Events*

An event was classified as mild if it did not interfere with the child's normal way of daily living and had no need for intervention. An event was considered as moderate if it did interfere with the child's normal way of daily living and may demand some kind of intervention.

#### *3.10.2 Serious and life threatening events*

Any event resulting that required intense care or hospitalization was typed as severe. It was classified as serious if following the ingestion of a study drug the study subject died or

experienced life-threatening conditions. The following medical conditions known to be related to the study drugs were classified as serious and thus monitored within seven days after treatment.

1. dystonia and extrapiramidal reactions
2. "restlessness"
3. syncope,
4. spasticity,
5. convulsions and involuntary movements



### *3.10.3 Adverse effects and causality*

In order to attribute the event to the study drug after ingestion, the following conditions had to be defined:

1. If the event is clearly related to other factors such as different clinical conditions or other medication, then this was classified as unlikely to have been caused by the study drug.
2. If the event followed closely in sequence after treatment with the study drug, but could result from conditions other than the study drug, it was considered likely.
3. If the event followed a reasonable sequence after ingestion of the study drug, but is unlikely to be caused by other conditions, it was classified as probable.

### *3.10.4 Referrals*

A child was referred to Juaben Government Hospital or the nearest health centre by the CDD if the child's condition did not improve after taking and completing medication. Those study children with serious adverse events were either stopped from continuing with the treatment and were referred to the hospital for supervised care and attention. No action was taken in the event of

mild and moderate adverse reactions; normal regimens of treatment, however, were followed to the end.

### 3.11 Withdrawal from the Study

A study subject was withdrawn from the study due to:

1. Failure to comply with treatment regimes
2. Withdrawal of informed consent
3. Self medication with other antimalarials
4. Developing serious adverse events
5. Emergence of other serious medical conditions or severe malaria.



### 3.12 Data Handling and Analysis

#### *3.12.1 Sorting*

The Principal Investigator (PI), together with research assistants, were responsible for compilation of all relevant information and data collection using agreed standard procedures. The questionnaires were numbered according to the variables and data sorted into groups. Data from hospital records and community/village visits were cleaned through running programmes on legal values and consistency checks. Data entry and preliminary data analysis were done in MS Access and more detailed data analysis done using Stata 8.0 for Windows.

#### *3.12.2 Statistical Methods*

Analysis of data was done by the PI, with the assistance of a data manager and a biostatistician. Descriptive statistics were used to summarize and display data in graphs and charts. Continuous

variables were compared using student t-test/ANOVA and discrete variables analyzed using $\chi^2$ in r X n tables and non-parametric tests. For continuous variables, the estimates were for difference in means with 95% confidence intervals and for binary data, the estimate was for relative risks or odds ratio with 95% confidence levels. Qualitative data obtained were described, summarized and interpreted.

### 3.13 Ethical Issues



Ethical clearance was obtained from the School of Medical Sciences Ethics Committee and the Ethics Committee of the Health Research Unit/GHS. Permission was obtained from the Ejisi-Juaben Health and Education Directorates, head teachers and community leaders. Individual consent was obtained from caregivers of study children before enrolment into the study.

# CHAPTER FOUR - RESULTS

# CHAPTER FOUR - RESULTS

This chapter of the thesis is divided into three segments:

1.) Screening, enrolment and recruitment, (2.) Treatment outcomes, safety and tolerance and (3.) molecular epidemiology.



## 4.1 Screening, Enrolment and Recruitment

This segment of the thesis shows the processes leading to the screening and enrolment of study pupils into randomized treatment arms of ACTs and the recruitment of study children and their caregivers into the longitudinal study of ART-AMQ use at homes. The first section describes the screening processes that led to the randomization of eligible children into the ACTs trial study and the recruitment of children into ART-AMQ use at homes. This is followed by the baseline temperature, weight, parasitological and haematological indices of the study pupils and also the demographic and social characteristics of caregivers and CDDs.

### *4.1.1 Screening, enrolment and recruitment*

A total of 836 febrile children were recruited into the longitudinal study of ART-AMQ use at homes. Some of these children had as many as nine febrile episodes and were treated with ART-AMQ within 12 months. A total of 812 pupils were enrolled into the ACTs trial study, 58.5% (475) were microscopically positive and 41.5% (337) were negative. All the study pupils who were positive were randomized into seven treatment arms of ACTs. For control of the study, children in an urban setting were screened microscopically for malaria parasites. Out of a total of 1126 children whose blood samples were examined, 29.2% (329) were positive whilst 70.8%

(797) were negative. A flow diagram summarizing the enrolment, randomization and recruitment of study children is shown in **Figure 4.1.**

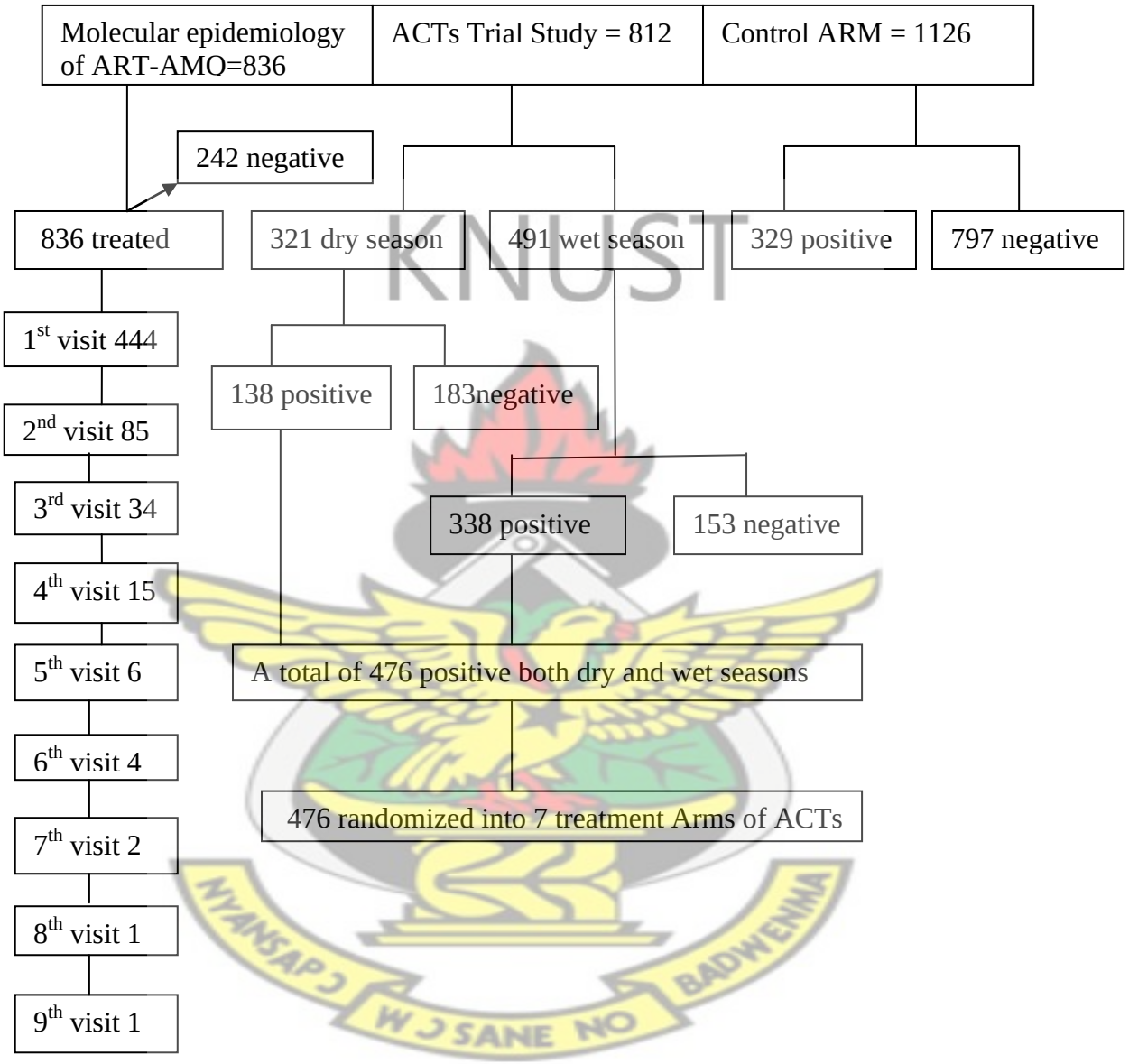



**Figure 4.1: Flow diagram of screening and enrolment processes of study children**

### *4.1.2 Prevalence of peripheral parasitaemia in the study population*

Recruitment of children into the longitudinal study of ART-AMQ use at homes began on August 31, 2005 and continued until August 30th, 2006. During the period under study, out of a total of 836 febrile children seeking treatment from CDDs, 71% (594) of them were positive by microscopy whilst 29% were without the parasites (**Table 4.1**). A total of 71.8% of them had parasites during the wet season and 68.8% in the dry season (**Table 4.1**). There was no significant difference in the parasite rates between the different age groups and between the seasons (**Table 4.1**). Three human plasmodia species were detected during the microscopic examination; *P. falciparum* (96.2%), *P. malariae* (2.7%) and *P. ovale* (1.1%). Most infections due to *P. malariae* and *P. ovale* were mixed infections with *P. falciparum.* Overall, only 2.3% of the microscopic positive cases had gametocytes. Parasite density ranged from a minimum value of 40/µl to a maximum value of 194880/µl with a geometric mean of 4912.62/µl (95% confidence interval). Over 4% had ≥100,000 parasites/µl of blood.



**Table 4.1: Parasitaemia prevalence in febrile children seeking treatment from CDDs**

| Febrile episodes | Positive | | Negative | | p-value |
|---|---|---|---|---|---|
| | n | (%) | n | (%) | |
| **Total episodes** | 594 | 71 | 242 | 29 | - |
| **< 12 months** | 117 | 70.5 | 49 | 29.5 | 0.562 |
| **≥ 12 months** | 476 | 71.3 | 192 | 28.7 | |
| ***Rainy season** | 464 | 71.8 | 182 | 28.2 | 0.491 |
| ***Dry season** | 139 | 68.8 | 63 | 31.2 | |

**** Rainy season (Apr – Nov) & Dry season (Dec – Mar) p-value ≤ 0.005 at 95% CI***

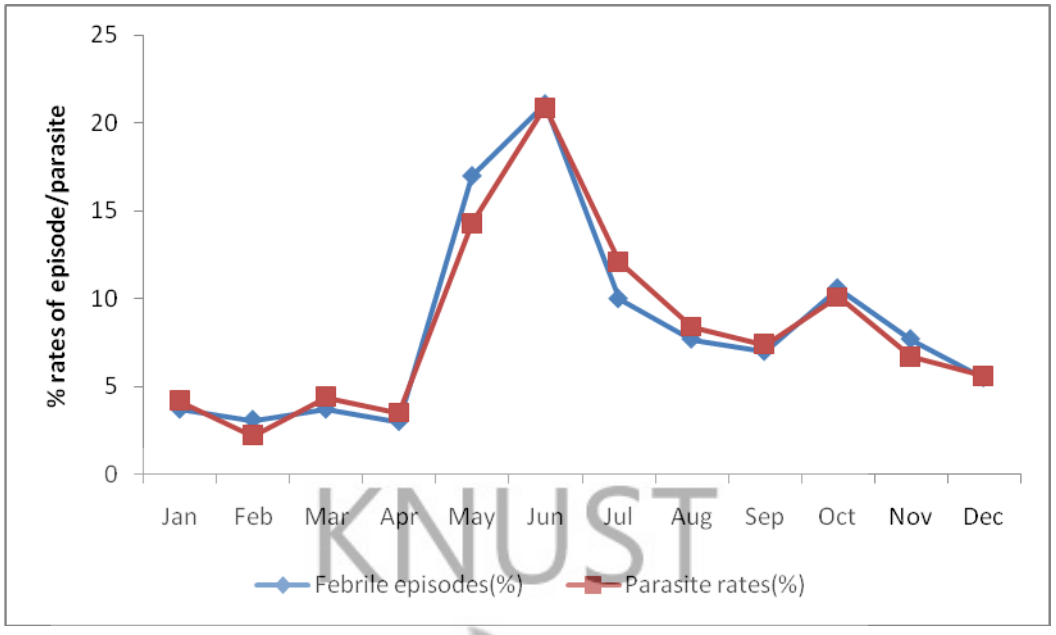
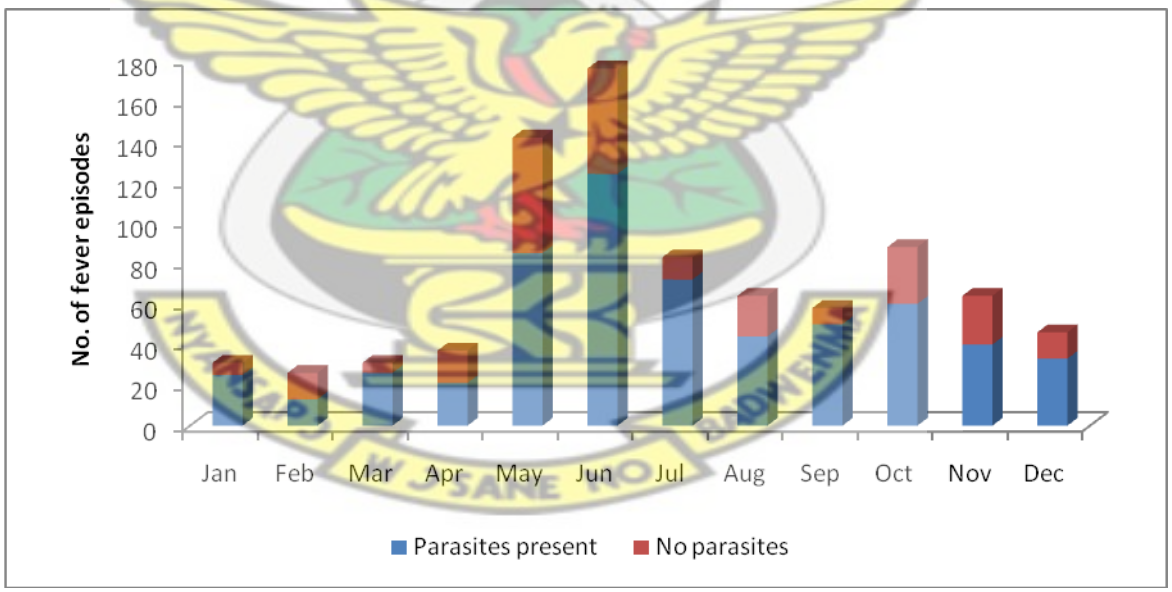



**Figure 4.2: Febrile episodes and parasite rates in children seeking treatment from CDDs**




*($X^2 = 79.1917$, $p < 0.001$ and $n = 836$)*

**Figure 4.3: Monthly febrile episodes in children seeking treatment from CDDs**

#### *4.1.2.1 Monthly febrile episodes and parasitaemia rates*

Febrile episodes and parasitaemia rates varied from month to month. About 21.1% of the overall febrile episodes occurred in June which also recorded the highest parasite rate of 20.9% **(Figures 4.2 & 4.3)** whilst the least episode occurred in April (3%). However, the least parasite rate of 2.2% was recorded in February coinciding with the peak of the dry season (**Figure 4.2**). The parasite rates showed a significant increase ($p < 0.001$) starting from May (14.3%) and peaked in June. There was a significant drop both in the parasite and febrile episode rates in July (12.1%) through to August (8.4%) and to September (7.4%). The parasite rate, however, increased marginally in October (10.1%) and started declining until it reached the lowest in February (2.2%). The trend of the monthly parasite rates followed very closely the pattern of febrile episodes (**Figure 4.2**).



**Table 4.2: Febrile episodes and parasitaemia rates in children seeking treatment at home**

| No. of visits | No. of children | | Positive | | Negative | |
|---|---|---|---|---|---|---|
| | n | % | n | % | N | % |
| **1** | 631 | 75.7 | 444 | 70.4 | 187 | 29.6 |
| **2** | 115 | 13.8 | 85 | 73.9 | 30 | 26.1 |
| **3** | 45 | 5.4 | 34 | 75.6 | 11 | 24.4 |
| **4** | 18 | 2.3 | 15 | 83.3 | 3 | 16.7 |
| **5** | 12 | 1.4 | 6 | 50.0 | 6 | 50.0 |
| **6** | 8 | 1.0 | 4 | 50.0 | 4 | 50.0 |
| **7** | 3 | 0.4 | 2 | 66.7 | 1 | 33.3 |
| **8** | 1 | 0.1 | 1 | 100 | 0 | 0.0 |
| **9** | 1 | 0.1 | 1 | 100 | 0 | 0.0 |

In the 836 children blood samples collected for microscopic examination to confirm the "diagnosis" of the CDDs and / or caretakers, about 76% of the children had their first febrile episode and medication. However, 70.4% of these were confirmed microscopically positive with 29.6% confirmed negative (**Table 4.2**). The number of febrile episodes per visit decreases (from 75.7% for the first visit to 0.1% in the eighth and ninth visits) with increasing number of visits

(**Table 4.2**). Parasite rates per visit ranged from 50% as recorded in the fifth and sixth visits to 100% in the eighth and ninth visits (**Table 4. 2**).

#### *4.1.2.2 Prevalence of peripheral parasitaemia in pupils*

A total of 812 school pupils were screened in two cross-sections; 321 in the dry season (January to march 2006) and 491 in the wet season (May to August 2006). In the dry season, about 43% were positive by microscopy and 69% during wet season (**Table 4.3**) with an overall parasitaemia prevalence of 58.6%. Species of *Plasmodium* detected were *falciparum (*97.4%), 1.6% *malariae* and 1% were *ovale* with a gametocyte rate of 5.3%.



**Table 4.3: Parasitaemia prevalence in pupils enrolled into the ACTs trial study**

| Parasites | positive | | Negative | | p-value | Total |
|---|---|---|---|---|---|---|
| | n | (%) | n | (%) | | N |
| Dry season | 138 | (42.9) | 183 | (57.1) | < 0.0001 | 321 |
| Wet season | 338 | (68.8) | 153 | (31.2) | < 0.0001 | 491 |
| Total | 426 | (58.6) | 337 | (41.1) | | 812 |

Parasitaemia prevalence varied from community to community ranging from 49.7% in Abetenim to 71% in Nkyerepoase as seen in **Figure 4.4** below. There was a significant difference in the parasitaemia prevalence among the communities (p =0.002), however, parasitaemia prevalence among the ages varied only slightly (**Figure 4.5**).

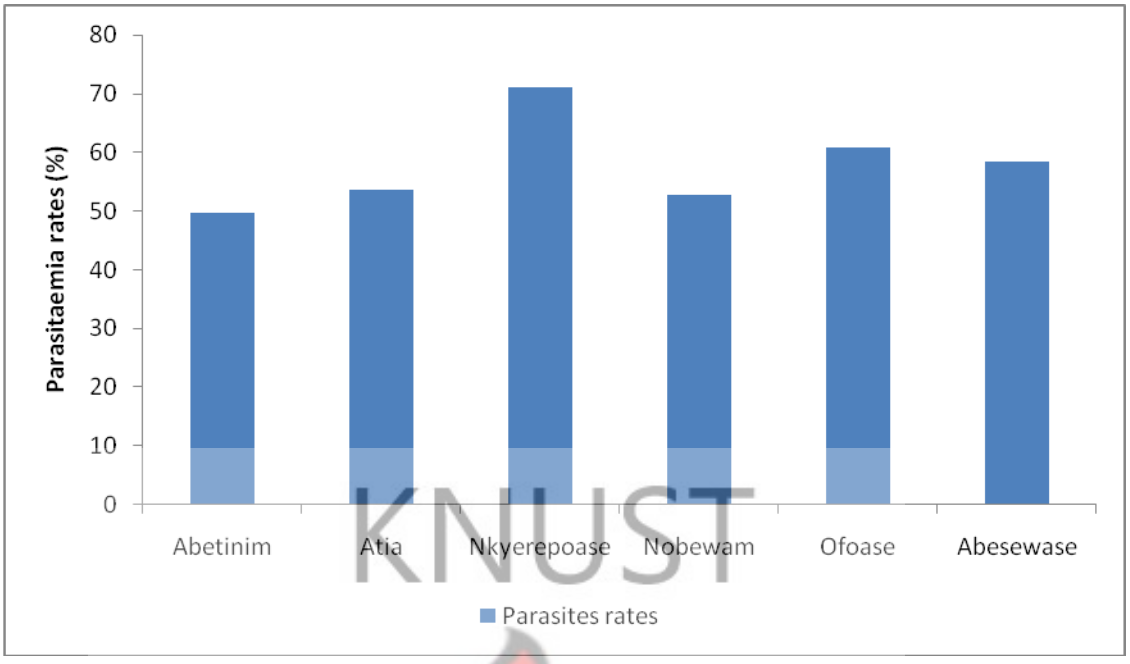



**Figure 4.4: Parasitaemia rates in pupils per study community**

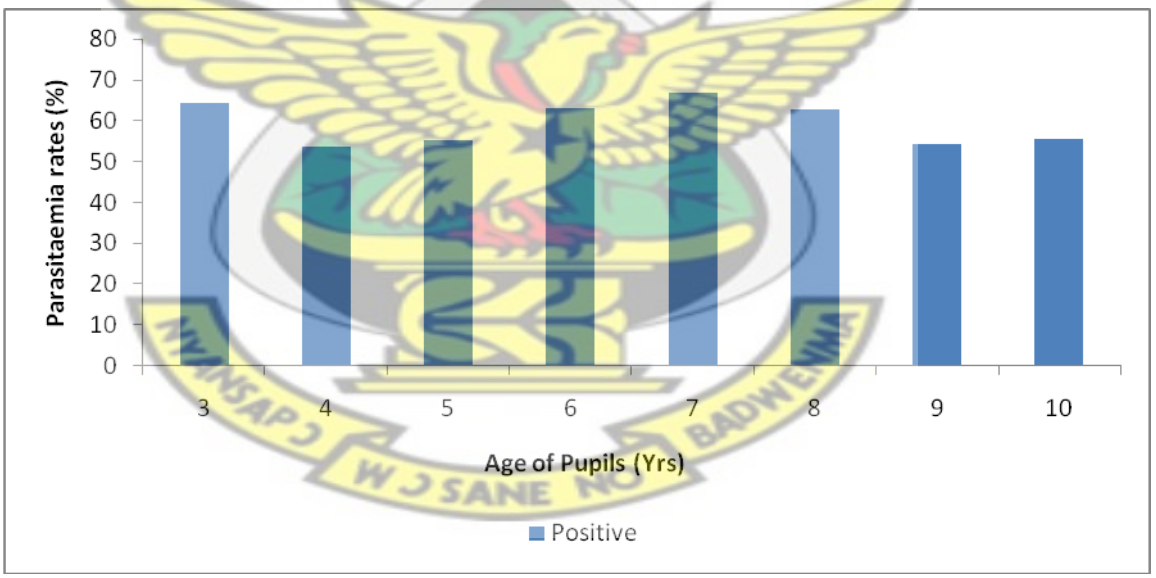



**Figure 4.5: Parasitaemia rates in pupils per age**

#### *4.1.2.3 Baseline temperature, weight, and haematological indices of pupils*

Of the school pupils screened, the weights ranged from a minimum value of 11.0kg to a maximum value of 35.0kg with a mean value of 18.3kg (SD ±3.7). Haemoglobin levels ranged from a minimum value of 8.0g/dl to a maximum value of 14.6g/dl with a mean of 10.8g/dl (SD ±1.2). Pupils with peripheral parasitaemia had haemoglobin levels ranging from a minimum value of 8.0g/dl to a maximum value of 14.0g/dl with a mean of 10.8g/dl (SD ±1.2). Those without parasites had haemoglobin levels ranging from a minimum to maximum values of 8.0g/dl to 14.6g/dl respectively with a mean of 11.1g/dl (SD ±1.2). Over 53% of children had haemoglobin (Hb) concentrations between 8.0 to 10.9g/dl (moderately anaemic) and those with below 8.0g/dl (14%) were withdrawn from the study. Temperatures were ranging from a minimum value of 34.5ºC to a maximum value of 39.5ºC with a mean of 36.5ºC (SD ±0.6). The baseline weights, temperatures and haematological indices among the study communities varied only marginally as shown in **Table 4.4** below.



**Table 4.4: Baseline weight, temperature and haematological indices of pupils**

| Variable | Total | Mean Wgt (Kg) | SD ± | Mean Hb (g/dl) | SD ± | Mean Temp (ºC) | SD ± |
|---|---|---|---|---|---|---|---|
| Abetenim | 147 | 19.5 | 3.9 | 11.0 | 1.1 | 36.5 | 0.5 |
| Atia | 164 | 17.3 | 2.5 | 10.2 | 1.0 | 36.4 | 0.9 |
| Ofoase | 179 | 18.5 | 4.0 | 11.3 | 1.5 | 36.5 | 0.8 |
| Abesewase | 53 | 16.4 | 3.5 | 10.6 | 1.3 | 36.6 | 0.9 |
| Nkyerepoase | 176 | 19.8 | 3.7 | 10.9 | 1.0 | 36.4 | 0.7 |
| Nobewam | 93 | 17.0 | 2.3 | 10.7 | 1.4 | 36.6 | 1.1 |
| Odoyefe | 18 | 18.7 | 2.3 | 10.1 | 1.6 | 36.6 | 0.9 |

#### *4.1.2.4 Parasitaemia prevalence in an urban setting*

Out of the 1126 children examined as a control arm of the entire study in one survey between March to April 2006 in ten peri-urban and ten urban communities, overall parasitaemia rate was 29.2% (**Table 4.5**). The mean parasite rate of urban communities was 20.1% whilst that of peri-urban was 39.1%. There was a significant difference between the peri-urban parasite rate and that of urban rate ($p < 0.001$).



**Table 4.5: Parasitaemia prevalence in urban and peri-urban settings**

| **Parasite rates** | **Positive** | | **Negative** | | **P-value** | **Total** |
|---|---|---|---|---|---|---|
| | n | (%) | n | (%) | | N |
| **Total Mean** | 329 | 29.22 | 797 | 70.78 | | 1126 |
| **Urban Mean** | 120 | 20.1 | 477 | 79.9 | 0.001 | 597 |
| **Peri-urban Mean** | 207 | 39.13 | 322 | 60.87 | 0.001 | 529 |

The lowest parasite rates ranging from 6.3% to 14.8% were recorded in urban communities whilst the highest parasite rates of 47.2% to 54.2% were in the peri-urban communities (**Figure 4.6**). Parasite rates recorded in the peri-urban communities ranged from a lowest rate of 22.2% to a highest value of 54.2%. Compared with urban communities, parasite rates ranged from a lowest value of 6.3% to a highest value of 35.2% (**Figure 4.6**). There was significant difference between the peri-urban parasite rates and urban communities ($p < 0.001$). Over 96% of *Plasmodium* species detected were *falciparum*, 2.1% *malariae* and 1.2% *ovale* with gametocyte rate of 7.9%.

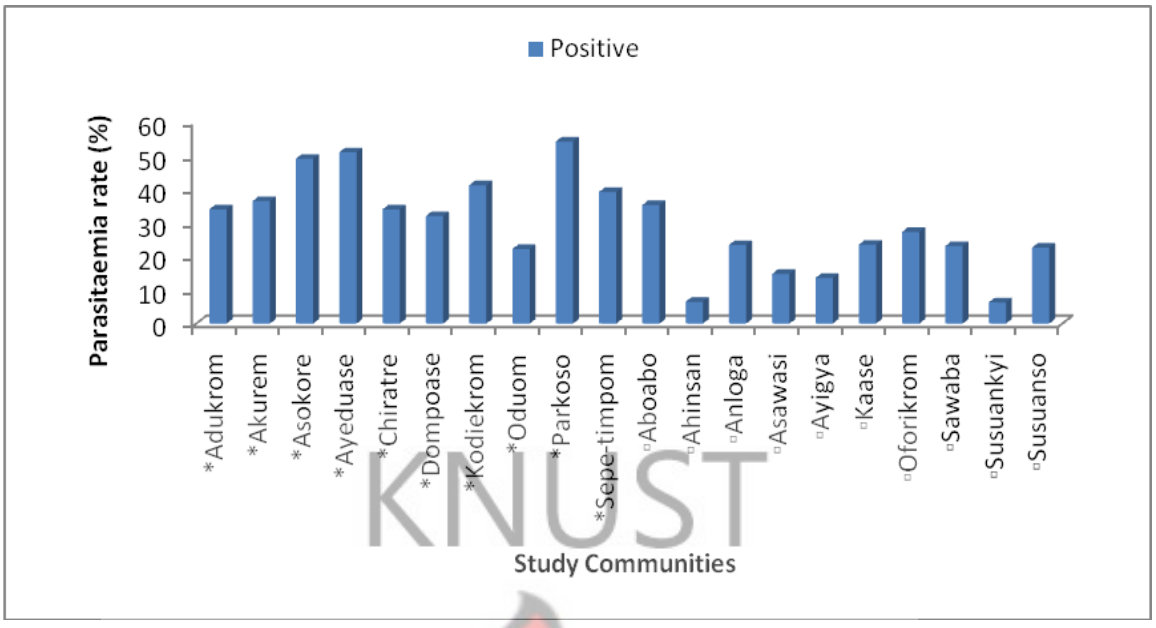



**Figure 4.6: Parasitaemia rates in *peri-urban and ▫urban communities**

### *4.1.3 Baseline parasite density of ACTs trial study, Control Arm and ART-AMQ Use at Home*

On the screening day (day 0), pupils recruited into the ACTs trial study, parasite density were between 40 – 230200/µL with geometric mean of 2231.64/µL. Over 62% of the children screened had parasite density ≤ 999/µL with only 0.2% with parasite density ≥100,000 /µL (**Figure 4.7**). The parasite density of the control arm of the study in an urban setting was ranging from a minimum value of 40/µL to a maximum value of 124880/µl with a geometric mean of 2011.12/µL. The proportions of children with parasite density ≤ 999/µL was 61.7% whilst only 1.2% had ≥100,000 parasites/µL **(Figure 4.7**).

For febrile children seeking treatment from CDDs (ART-AMQ use at home), parasite density ranged from a minimum value of 40/µL to a maximum value of 194880/µL with a geometric mean of 4912.62/µl. More than 41% had between 10000_99999 parasites/µL of blood with as many as

4.2% with parasite density ≥100,000 /µL, whilst about 27% had parasite density ≤ 999/ µL (**Figure 4.7**).

Baseline gametocyte rates for ACTs trial study, control arm in urban setting and ART-AMQ use in homes were 5.3%, 7.9% and 2.3% respectively.

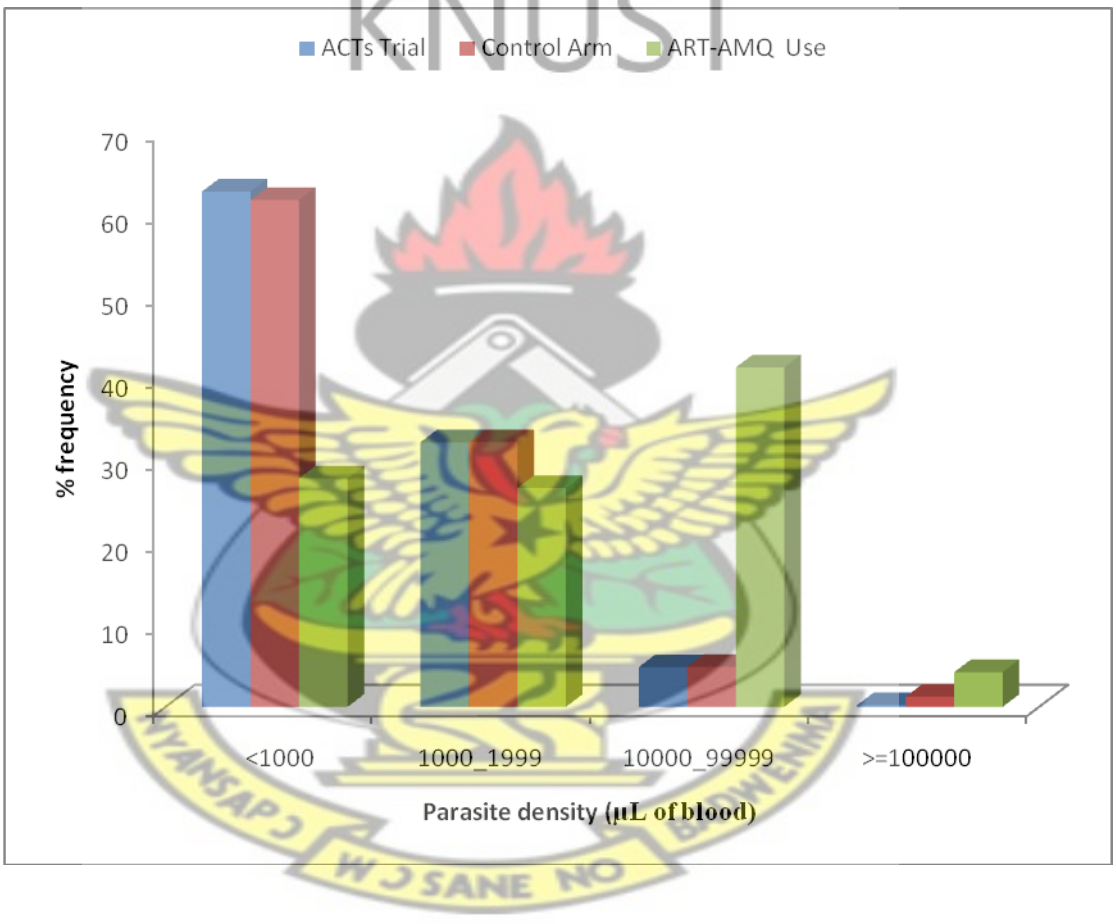



**Figure 4.7: Baseline parasite density**

**Table 4.6: Demographic characteristics of caregivers and CDDs**

| Characteristics | N | (%) |
|---|---|---|
| **Caregivers** | | |
| **Age** | | |
| **<20** | 53 | 4.8 |
| **20-29** | 497 | 45.2 |
| **30-39** | 376 | 34.2 |
| **>39** | 173 | 15.7 |
| **Occupation** | | |
| **Unemployed** | 101 | 9.2 |
| **Farming** | 580 | 52.8 |
| **Artisan** | 81 | 7.3 |
| **Trading** | 270 | 24.6 |
| **Civil Servant** | 12 | 1.1 |
| **Apprenticeship** | 36 | 3.3 |
| **Housewife** | 6 | 0.5 |
| **Other** | 13 | 1.2 |
| **Educational status** | | |
| **Primary** | 241 | 21.9 |
| **JSS /Mid** | 581 | 52.9 |
| **SSS/Sec** | 30 | 2.7 |
| **Tech/Comm./Voc** | 9 | 0.8 |
| **Univ.** | 2 | 0.2 |
| **Non-formal education.** | 4 | 0.4 |
| **None** | 227 | 20.7 |
| **Marital Status** | | |
| **Single** | 90 | 8.2 |
| **Married** | 962 | 87.5 |
| **Separated** | 12 | 1.1 |
| **Widowed** | 14 | 1.3 |
| **Divorced** | 21 | 1.9 |
| **Religion** | | |
| **Orthodox** | 424 | 38.6 |
| **Spiritual** | 131 | 11.9 |
| **Pentecostal** | 351 | 31.9 |
| **Traditional** | 9 | 0.8 |
| **Islam** | 116 | 10.6 |
| **Other** | 68 | 6.2 |
| **CDDS** | | |
| **Educational status** | | |
| **Secondary school/Higher** | 42 | 77.8 |
| **Below Secondary school** | 12 | 22 |
| **Primary Occupation** | | |
| **Farming** | 47 | 87 |
| **Trading** | 4 | 7 |
| **Others** | 3 | 6 |

### *4.1.4 Demographic and social characteristics of caregivers and CDDs*

Majority of caregivers (45%) were between 20-29 years with an overall mean age of 30.7 (SD±8.8). Teenager caregivers were less than 5% whilst caregivers >39 years were about 16% (**Table 4.6**). The age of caregivers ranged from 16 to 70 years. As in most rural communities in Ghana, the main occupation of caregivers (53%) was farming, whilst 25% of them were involved in petty trading with only 1% in civil service. Fifty-three percent of caregiver had education up to JSS/Middle school, 22% primary education, whilst 21% had no formal education. Only 3% had up to secondary level of education (**Table 4.6**). Most caregivers were married (88%) whilst 8% were single parents. About 71% of them belong to the Christian religion, 11% Moslems, with 18% belonging to other religions.

A total of 54 CDDs from 34 communities were trained to provide ART-AMQ to febrile children at homes. However, only 24 were trained to collect blood samples from febrile children. More than 77% had secondary education with 22% below secondary level (**Table 4.6**). Most CDDs were engaged in farming with 13% of them involved in petty trading or other vocations.

## 4.2 Treatment Outcomes, Safety and Tolerance

This segment presents the parasitological and haematological findings during the days 14 and 28 follow up after the start of treatment. The proportion of parasite density at days 0, 14 and 28 are compared as well as parasitological failure rates at days 14 and 28 follow up of the various treatment arms of ACTs. The changes in mean haemoglobin concentrations at days 14 and 28 follow up are compared with enrolment haemoglobin levels. Drugs tolerance and safety are also described as well as compliance with ART-AMQ use at homes.

### 4.2.1 Follow up of study children during the days 14 and 28 visit schedules

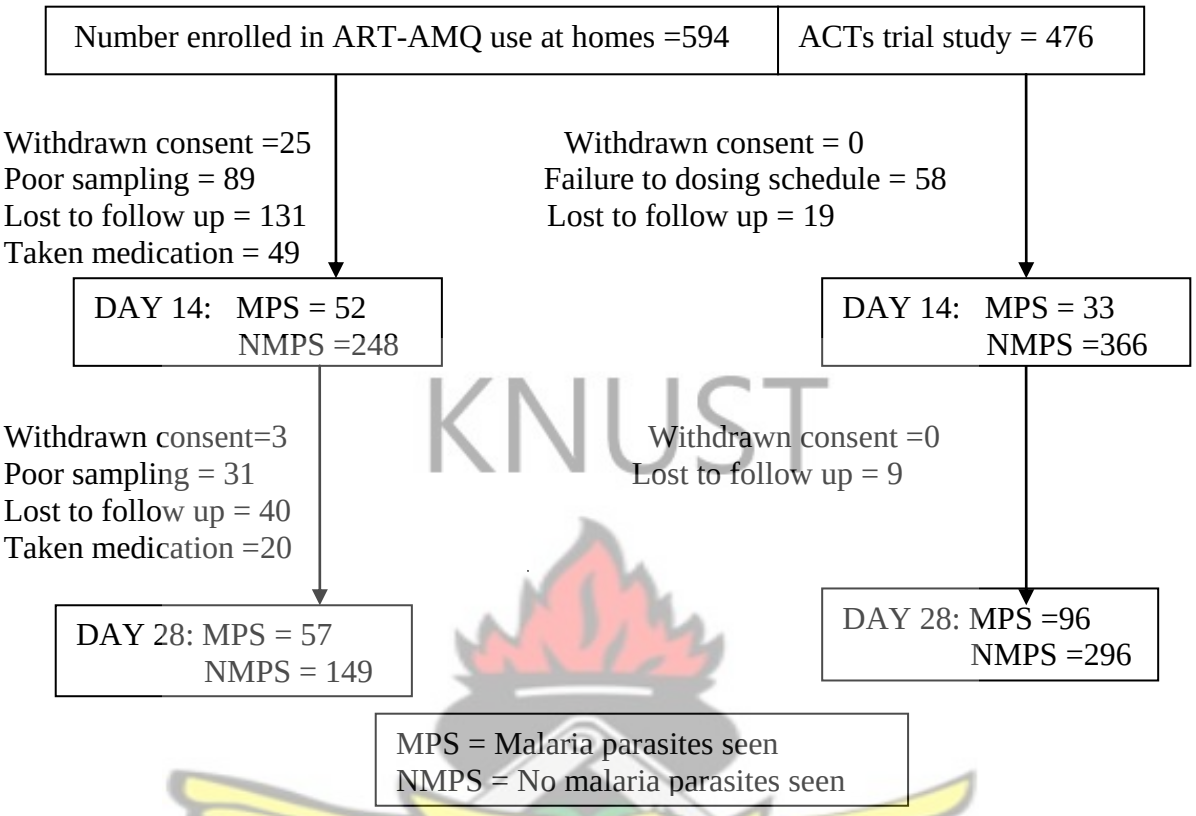



**Figure 4.8: Flow diagram of study children at days 14 & 28 follow up visits**

A child was said to be withdrawn following withdrawal of consent by the caregivers. A child was said to be lost to follow up when he / she was absent for sample collection on days 14 and 28 follow up of the day of sample collection and treatment.

A total of 594 children recruited into the home based study of ART-AMQ, were subsequently followed up for 28 days period. At day 14 follow up, 25 caregivers withdrew their consent, 49 were given some medication and 131 children were lost to follow up. However, 89 of the day 14 blood films collected failed to meet the standard protocol and were subsequently taken out of the total day 14 samples. Three hundred children were successfully followed up and 52 of them had

parasitological failure whilst 248 did not have parasites. At day 28 follow up, 3 withdrew consent, 40 were lost to follow up whilst 20 were withdrawn for taking medication. Thirty one samples collected did not meet the standard protocol and were withdrawn from the total samples collected. A total of 206 children were followed up successfully out of which 57 had parasitological failure and 149 did not have parasites. Out of the 476 pupils recruited into the ACTs trial study, at day 14, 19 of them were lost to follow up and 58 failed to meet the treatment schedules and were thus excluded from the study. Thirty three had parasitological failure whilst 366 were without the parasites. At day 28 follow up, 7 were lost during follow up and 96 out of the followed up had parasitological failure whilst 296 were without parasites. Detail of the individual treatment groups are shown in **Table 4.7** below.



**Table 4.7: Follow up outcomes at days 14 and 28 of pupils in ACTs trial study**

| Day | ART+SP | ART+AMQ | ART+MQ | ART+LAPDAP | COARTEM | ART+CQ | AMOTEX |
|---|---|---|---|---|---|---|---|
| Day 0 | 77 | 82 | 55 | 57 | 61 | 87 | 57 |
| Day 14 | 72 | 81 | 26 | 41 | 39 | 84 | 56 |
| Lost to follow up | 4 | 1 | 8 | 2 | 1 | 3 | 1 |
| Failure to schedule medication | 1 | 0 | 21 | 14 | 21 | 0 | 0 |
| Day 28 | 67 | 77 | 25 | 42 | 39 | 84 | 56 |
| Lost to follow up | 5 | 4 | 1 | - | 0 | 0 | 0 |

A total of 476 pupils were randomized into the various study groups of the ACTs trial study and were subsequently followed for 28 days period. The initial day 0 numbers for each treatment arm were based on the randomization process and differed accordingly (**see 3.7.1**). Thus, 77 were assigned to ART+SP, 82 to ART+AMQ, 55 to ART+MQ and 57 to ART+LAPDAP. Also, 61 were assigned to COARTEM, 87 to ART+CQ and 57 to AMOTEX. Pupils were excluded from

the study if they failed to adhere to treatment schedules within the three days period. At day 14 follow up, 1, 21, 14 and 21pupils were withdrawn from ART+SP, ART+MQ, ART+LAPDAP and COARTEM treatment groups respectively for failing to meet the treatment schedules.

### *4.2.2 Parasitological outcomes after initial treatment with ART+AMQ at homes*

This is the crude parasitological failure incidence by days 14 and 28 after initial treatment of ART+AMQ at home-based study summarized and shown in **Table 4.8.**



**Table 4.8: Parasitological failure by days 14 & 28 after treatment of children with ART-AMQ**

| | Uncorrected parasitological response after treatment with ART-AMQ | | | | | |
|---|---|---|---|---|---|---|
| | Failure | | Success | | Total | |
| | n | % | n | % | N | % |
| DAY 14 | 52 | 17.3 | 248 | 82.70 | **300** | |
| **Wet season** | 46 | 18.7 | 200 | 81.3 | 246 | **82** |
| **<12 months** | 7 | 30.4 | 16 | 69.8 | 23 | 9.3 |
| **≥12 months** | 39 | 17.5 | 184 | 82.5 | 223 | 90.7 |
| **Dry season** | 6 | 11.1 | 48 | 88.9 | 54 | **18** |
| **<12 months** | 3 | 27.3 | 8 | 72.7 | 11 | 20.4 |
| **≥12 months** | 3 | 7.0 | 40 | 93) | 43 | 79.6 |
| DAY 28 | 57 | 27.8 | 149 | 72.2 | **206** | |
| **Wet season** | 50 | 31.5 | 109 | 68.5 | 159 | **77.2** |
| **<12 months** | 7 | 36.8 | 12 | 63.2 | 19 | 9.3 |
| **≥ 12months** | 43 | 30.7 | 97 | 69.3 | 140 | 90.7 |
| **Dry season** | 7 | 16.7 | 35 | 83.3 | 42 | **22.8** |
| **<12 months** | 1 | 25.1 | 3 | 75 | 4 | 20.4 |
| **≥12 months** | 6 | 16.2 | 31 | 83.8 | 37 | 79.6 |

As shown in **Table 4.8**, by day 14, 17.3% had crude parasitological failure with 18.7% occurring in the wet season whilst 11.1% during the dry season. Also, 30.4% and 17.5% crude parasitological failures occurred in children under 12 months and children ≥12 months in the wet season respectively. Crude parasitological failures recorded during the dry season were 27.3% and

7% for children below 12 months and those ≥12 months respectively. By day 28, crude parasitological failure was 27.8% with 31.5% and 16.7% occurring in the wet and dry season respectively. Crude parasitological failures recorded in the children under-12 months and children ≥12 months during the wet and dry seasons were 36.8%, 30.7%, 25.1% and 16.2% respectively. All the day 28 crude parasitological failures were genotyped to determine recrudescence (true failures) and re-infections using *msp2* and *glurp* markers. A total of 7 samples were classified as indeterminate with one failure and were excluded from the final analysis. These samples either failed to amplify in the day 0, 28 or both by the PCR genotyping. PCR adjusted failure rate was 11.6% (23/198). Thus, the true parasitological failure was 11.6% for ART+AMQ use for the home management of malaria.



#### *4.2.2.1 Comparison of parasite, gametocyte rates and parasite densities at days 0, 14 and 28*

After the initial treatment, the parasitological failures by days 14 and 28 were compared with the PCR-corrected day 28 failure. By day 14, crude parasitological failure was 17.3%, 27.8% by day 28 and PCR-corrected failure was 11.6%. At day 0, parasite and gametocyte rates were 71% and 2.3%, by days 14 and 28, parasite and gametocyte rates were 17.3%, 27.8%, 9.6 % and 3.5 % respectively. Parasite densities at days 0, 14 and 28 are shown in **Figure 4.9.**

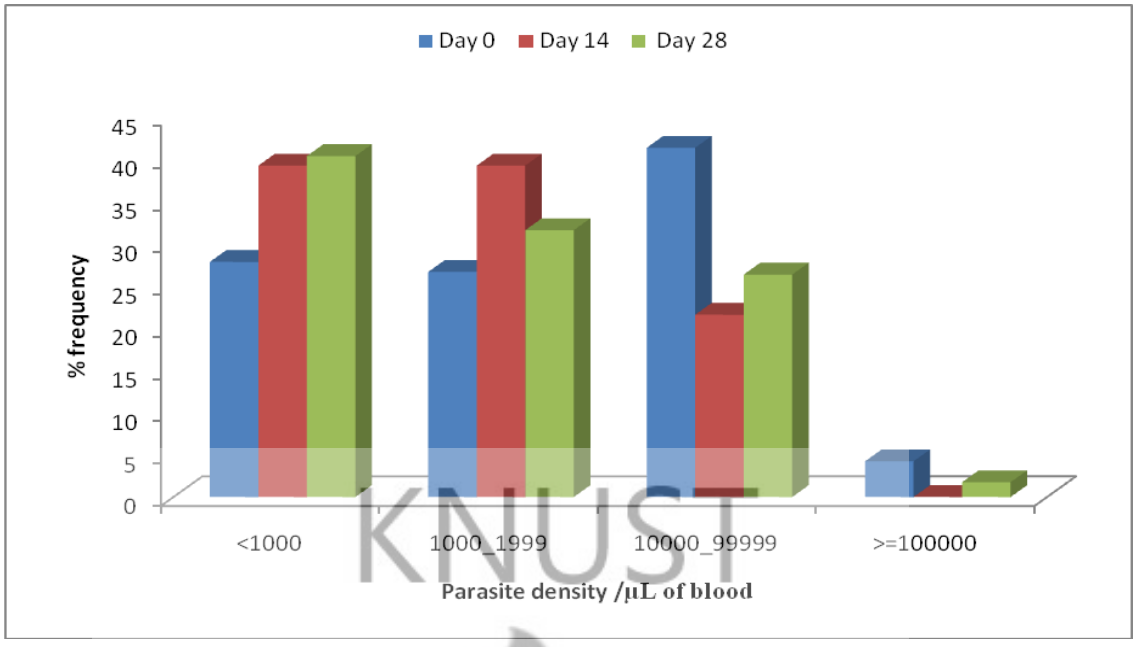



**Figure 4.9: Comparison of parasite densities at days 0, 14 & 28 in children treated with ART-AMQ at home**

### *4.2.2.2 Adherence of caregivers to treatment schedules for home management study*

Compliance to instructions was an important variable monitored by the research team. A total of 211 caregivers were interviewed to determine the adherence to treatment schedules. Of the total interviewed, however, 72.5% (153) caregivers had febrile children treated with ART+ AMQ prepackeds at home. Ninety-seven percent (149) were alleged to have correctly treated children according to schedules (dose and duration) with only 3% failing to adhere to the treatment schedules.

### *4.2.3 Parasitological outcomes after initial treatment of pupils with ACTs*

The incidence of parasitological failure by days 14 and 28 after treatment with ACTs are summarized and shown in **Table 4.9.**

**Table 4.9: Parasitological failure by days 14 & 28 after initial treatment of pupils with ACTs**

| | Uncorrected parasitological response | | | | | |
|---|---|---|---|---|---|---|
| | Failure | | Success | | Total | |
| | n | (%) | n | (%) | N | (%) |
| **Day 14** | | | | | | |
| ART/SP | 4 | 5.6 | 68 | 94.4 | **72** | 18.1 |
| ART/AMQ | 6 | 7.4 | 75 | 92.6 | **81** | 20.3 |
| ART/MQ | 0 | 0.0 | 26 | 100.0 | **26** | 6.5 |
| ART/LAPDAP | 8 | 19.5 | 33 | 80.5 | **41** | 10.3 |
| Coartem | 3 | 7.7 | 36 | 92.3 | **39** | 9.8 |
| ART/CQ | 8 | 9.5 | 76 | 90.5 | **84** | 21.1 |
| Amotex | 4 | 7.1 | 52 | 92.9 | **56** | 14.0 |
| Total | 33 | 8.3 | 366 | 91.7 | **399** | 100 |
| **Day 28** | | | | | | |
| ART/SP | 19 | 28.4 | 48 | 71.6 | **67** | 17.2 |
| ART/AMQ | 14 | 18.2 | 63 | 81.8 | **77** | 19.7 |
| ART/MQ | 3 | 12.0 | 22 | 88.0 | **25** | 6.4 |
| ART/LAPDAP | 16 | 38.1 | 26 | 61.9 | **42** | 10.8 |
| Coartem | 12 | 30.8 | 27 | 69.2 | **39** | 10.0 |
| ART/CQ | 28 | 33.3 | 56 | 66.7 | **84** | 21.5 |
| Amotex | 4 | 7.1 | 52 | 92.9 | **56** | 14.4 |
| **Total** | **96** | 24.5 | **296** | 75.5 | **392** | 100 |



The analyses were done with reference to ART+AMQ as the baseline treatment for uncomplicated malaria in Ghana. Here, the outcome of each treatment arm was compared with ART+AMQ arm. The incidence of crude parasitological failures by day 14 for ART+SP, ART+AMQ, ART+MQ, ART+LAPDAP, COARTEM, ART+CQ and AMOTEX were 5.6% , 7.4%, 0.0%, 19.5%, 7.7%, 9.5%, 7.1% and 8.3% respectively. By day 28 the incidence of crude parasitological failures were 28.4% ,18.2%, 12.0%, 38.1%, 30.8%, 33.3% and 7.1% for ART+SP, ART+AMQ, ART+MQ, ART+LAPDAP, COARTEM, ART+CQ and AMOTEX respectively.

In the present study, only microscopy was used to determine the parasitological outcomes after treatment. Thus, no PCR genotyping was done to correct for re-infections because of logistical difficulties and also because this was not the core subject matter. The prevalence of the parasite failure rates by days 14 and 28 of the various treatment arms and parasite densities at days 0, 14

and 28 are shown in **Figures 4.10** and **4.11** respectively. There was no significant difference ($p > 0.05$) of the parasitological failures by day14 among the different ACTs used, but was significant ($p < 0.001$**)** by day 28 after treatment.

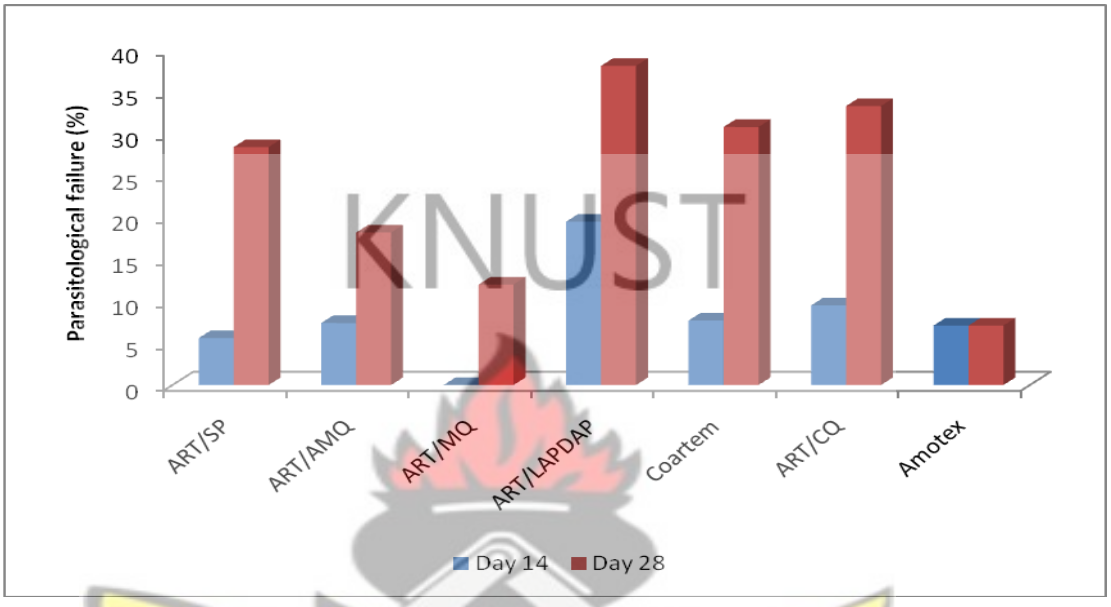



**Figure 4.10: Crude parasitological failure at days 14 & 28 of pupils treated with ACTs**

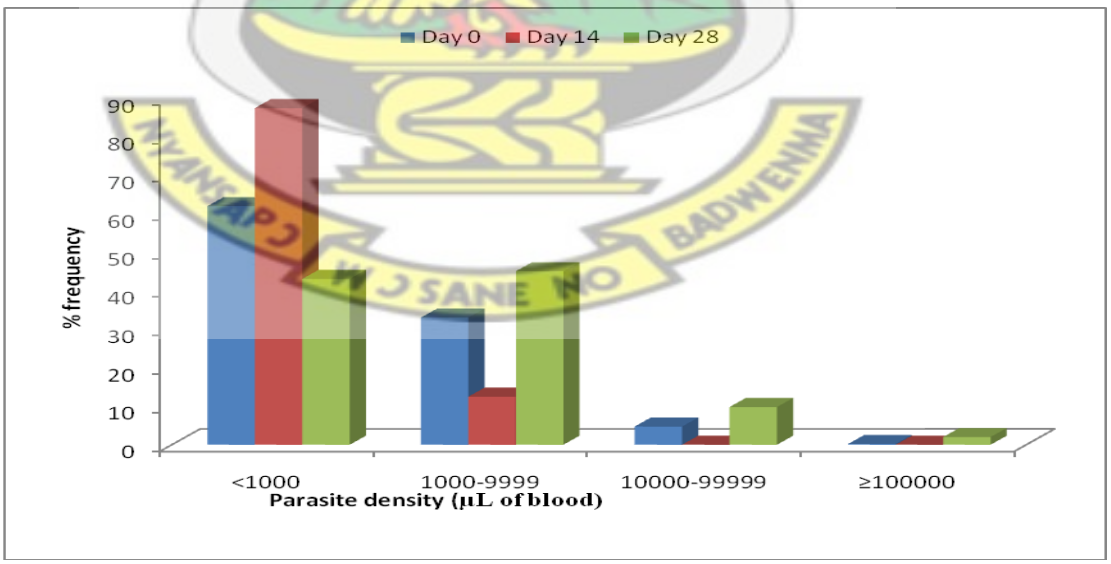



**Figure 4.11: Comparison of parasite densities at days 0, 14 & 28 in pupils treated with ACTs**

The odds ratio of parasitological failure in the treatment groups compared to the ART+QMQ by days 14 and 28 are shown in **Table 4.10.**

**Table 4.10: The odd of parasitological failure at days 14 & 28 in pupils treated with ACTs**

| | Unadjusted Odds ratio (95% CI) | | **P-value** |
|---|---|---|---|
| Day 14 | | | |
| ART/SP | 0.6 | (0.1 to 2.3) | 0.4 |
| ART/AMQ | 1.0 | | |
| ART/MQ | - | - | 0.2 |
| ART/LAPDAP | 0.3 | (0.1 to 1.0) | 0.04 |
| Coartem | 1.4 | (0.3 to 7.2) | 0.7 |
| ART/CQ | 0.9 | (0.3 to 2.7) | 0.8 |
| Amotex | 1.0 | (0.3 to 3.8) | 0.9 |
| Day 28 | | | |
| ART/SP | 1.8 | (0.8 to 4.0) | 0.1 |
| ART/AMQ | 1.0 | | |
| ART/MQ | 1.6 | (0.4 to 6.0) | 0.1 |
| ART/LAPDAP | 0.4 | (0.1 to 0.8) | 0.01 |
| Coartem | 0.5 | (0.2 to 1.3) | 0.1 |
| ART/CQ | 0.4 | (0.2 to 0.9) | 0.02 |
| Amotex | 2.8 | (0.9 to 9.3) | 0.07 |



ART+SP compared to ART+AMQ decreased the risk of crude parasitological failure on day 14 (OR=0.6; 95% CI 0.1 to 2.3) and by day 28 increased the risk of crude parasitological failure (OR=1.8; 95% CI 0.8 to 4.0). These decrease and increase risk of crude parasitological failure on days 14 and 28 were not significant. ART+MQ compared to ART+AMQ increased the risk of crude parasitological failure on days 14 and 28 (OR=1.6; 95% CI 0.4 to 6.0). The increased risk of parasite failure by days 14 and 28 were not significant. ART+LAPDAP compared to ART+AMQ decreased the risk of crude parasitological failure on days 14 (OR=0.3; 95% CI 0.1 to 0.1) and 28 (OR=0.4; 95% CI 0.1 to 0.8) and the decreased risks of parasite failure were significant (p =0.04 and 0.01 respectively).

COARTEM compared to ART+AMQ increased the risk of crude parasitological failure on day 14 (OR=1.4; 95% CI 0.3 to 7.2) and by day 28 decreased the risk of crude parasitological failure (OR=0.5; 95% CI 0.2 to 1.3). The increase and decrease were not significant. ART+CQ compared to ART+AMQ decreased the risk of crude parasitological failure on days 14 (OR=0.9; 95% CI 0.3 to 2.7) and 28 (OR=0.4; 95% CI 0.2 to 0.9). The decrease in the risk of crude parasitological failure on day 14 was not significant but, significant on day 28 (p =0.02).



AMOTEX compared to ART+AMQ show no risk of crude parasitological failure on day 14 (OR=1.0; 95% CI 0.3 to 3.8) and by day 28 increased the risk of crude parasitological failure (OR=2.8; 95% CI 0.9 to 9.3) and this increased risk was not significant (p=0.07). Assumptions were made for baseline parasite density, Hb, age, weight etc prior to treatment as being similar in all respect, thus, these were not adjusted for in calculating for the odds ratio.

### *4.2.4 Haematological outcomes after initial with ACTs*

The effect of treatment with ACTs on haemoglobin outcome was assessed by determining the absolute change from the baseline haemoglobin (day 0) over 28 days follow up period using the One-way Analysis of Variance (ANOVA) and regression analysis. The mean haemoglobin of each treatment group was compared with ART+AMQ and the proportion of children with two categories of haemoglobin concentrations at days 14 and 28 after initial treatment with ACTs are shown in **Tables 4.11 and 4.12.**

**Table 4.11: Haemoglobin outcomes at day 14 after start of treatment of pupils with ACTs**

| HB (g/dl) | 8 – 10.9 | | ≥ 11 | | Mean | SD | Min | Max | Total |
|---|---|---|---|---|---|---|---|---|---|
| Day 14 | n | % | n | % | | | | | |
| **ART/SP** | 23 | 31.9 | 49 | 68.1 | 11.5 | 1.1 | 8.9 | 13.5 | 72 |
| **ART/AMQ** | 28 | 34.6 | 52 | 65.4 | 11.4 | 1.2 | 8.7 | 15.6 | 81 |
| **ART/MQ** | 7 | 26.9 | 19 | 73.1 | 11.6 | 1.2 | 9.4 | 13.9 | 26 |
| **ART/LAPDAP** | 11 | 26.8 | 30 | 73.2 | 11.7 | 1.2 | 8.9 | 14.3 | 41 |
| **Coartem** | 12 | 30.8 | 27 | 69.2 | 11.4 | 1.1 | 9.3 | 13.8 | 39 |
| **ART/CQ** | 28 | 33.3 | 56 | 66.7 | 11.6 | 1.1 | 8.9 | 14.6 | 84 |
| **Amotex** | 21 | 37.5 | 35 | 62.5 | 11.3 | 1.1 | 8.9 | 14.6 | 56 |
| Total | **130** | **32.6** | **269** | **67.4** | **11.5** | **1.2** | **8.7** | **15.6** | **399** |



In **Table 4.11**, by day 14, the overall proportion of children with haemoglobin (Hb) concentrations between 8.0 to 10.9g/dl was 32.6% whereas children with Hb concentrations ≥11.0g/dl was 67.4% with an overall mean Hb concentration of 11.5g/dl. Within the treatment groups, the proportion of children with Hb concentrations between 8.0 to 10.9g/dl ranged from minimum of 26.8% to a maximum of 37.5% with mean Hb concentrations ranging from 11.3 to 11.7g/dl.

**Table 4.12: Haemoglobin outcomes at day 28 after start of treatment of pupils with ACTs**

| HB (g/dl) | 8 – 10.9 | | ≥ 11 | | Mean | SD | Min | Max | Total |
|---|---|---|---|---|---|---|---|---|---|
| Day 28 | n | % | n | % | | | | | |
| **ART/SP** | 20 | 29.9 | 47 | 70.1 | 11.5 | 1.0 | 9.5 | 14.6 | **67** |
| **ART/AMQ** | 17 | 22.1 | 60 | 77.9 | 11.7 | 1.1 | 8.9 | 14.6 | **77** |
| **ART/MQ** | 7 | 28 | 18 | 72 | 11.7 | 1.2 | 9.8 | 14.3 | **25** |
| **ART/LAPDAP** | 9 | 21.4 | 33 | 78.6 | 11.8 | 1.1 | 9.6 | 14.7 | **42** |
| **Coartem** | 12 | 30.8 | 27 | 69.2 | 11.5 | 1.2 | 8.9 | 13.4 | **39** |
| **ART/CQ** | 22 | 26.2 | 62 | 73.8 | 11.7 | 1.0 | 8.8 | 14.2 | **84** |
| **Amotex** | 12 | 21.4 | 44 | 78.6 | 12.0 | 1.2 | 9.7 | 15.5 | **56** |
| **Total** | **99** | **25.3** | **293** | **74.7** | **11.7** | **1.1** | **8.8** | **15.5** | **392** |

As shown in **Table 4.12**, by day 28, the overall proportion of children with haemoglobin (Hb) concentrations between 8.0 to 10.9g/dl was 25.3% whereas children with Hb concentrations

≥11.0g/dl was 74.7 % with an overall mean Hb concentration of 11.7g/dl, an increase of 0.2g/dl over the day 14 mean Hb (p =0.4). The proportion of children with Hb between 8.0 to 10.9g/dl has reduced by 7.3% whilst children with Hb concentrations ≥11.0g/dl increased by 7.3% from the day 14 Hb concentrations. Within the treatment groups, the proportion of children with Hb concentrations between 8.0 to 10.9g/dl ranged from minimum of 21.4% to a maximum of 30.8% with mean Hb concentrations ranging from 11.5 to 12.0g/dl. The change in mean Hb concentrations by days 14 and 28 within and between the treatment groups are compared as shown in **Table 4.13** and **Figure 4.12** and the increase of the mean Hb concentrations by days 14 and 28 over the day 0 mean Hb concentration are compared and shown in **Figure 4.13**.



**Table 4.13: Comparison of the mean haemoglobin changes by ACTs with ART-AMQ**

| | Mean change | SD | Mean difference compared to ART/AMQ (95% CI) | P-value |
|---|---|---|---|---|
| **Day 14** | | | | |
| **ART/SP** | 0.7 | 1.1 | 0.2 (-0.2 to 0.5) | 0.2 |
| **ART/AMQ** | 0.9 | 1.2 | | |
| **ART/MQ** | 0.9 | 1.2 | -0.2 (-0.8 to 0.4) | 0.7 |
| **ART/LAPDAP** | 1 | 1.2 | -0.4 (-0.9 to 0.3) | 0.9 |
| **Coartem** | 0.7 | 1.1 | 0.0 (-0.4 to 0.4) | 0.5 |
| **ART/CQ** | 0.9 | 1.1 | -0.1 (-0.5 to 0.3) | 0.7 |
| **Amotex** | 1.2 | 1.1 | 0.0 (-0.4 to 0.5) | 0.4 |
| **Day 28** | | | | |
| **ART/SP** | 0.7 | 1.0 | -0.2 (-0.6 to 0.1) | 0.8 |
| **ART/AMQ** | 0.6 | 1.1 | | |
| **ART/MQ** | 0.8 | 1.2 | 0.1 (-0.5 to 0.6) | 0.4 |
| **ART/LAPDAP** | 0.9 | 1.1 | -0.1 (-0.5 to 0.3) | 0.7 |
| **Coartem** | 0.6 | 1.2 | 0.3 (-0.2 to 0.7) | 0.1 |
| **ART/CQ** | 0.8 | 1.0 | 0.1 (-0.2 to 0.4) | 0.3 |
| **Amotex** | 0.5 | 1.2 | -0.2 (-0.6 to 0.2) | 0.9 |

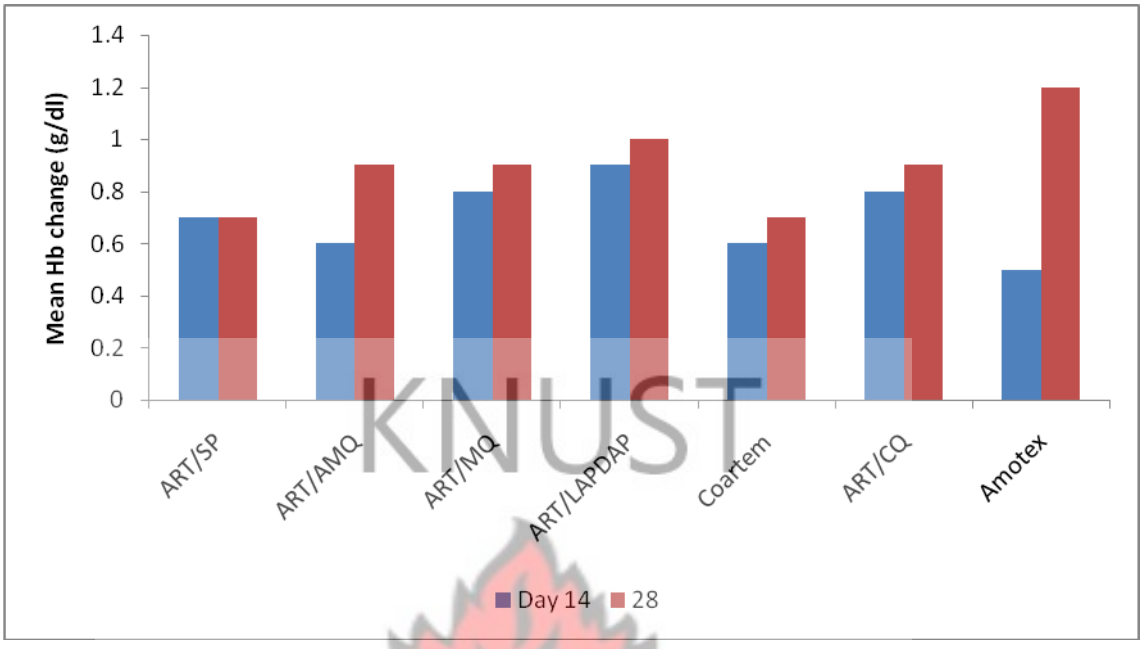



**Figure 4.12: Changes in mean haemoglobin concentrations at days 14 & 28 after initial treatment of pupils with ACTs**

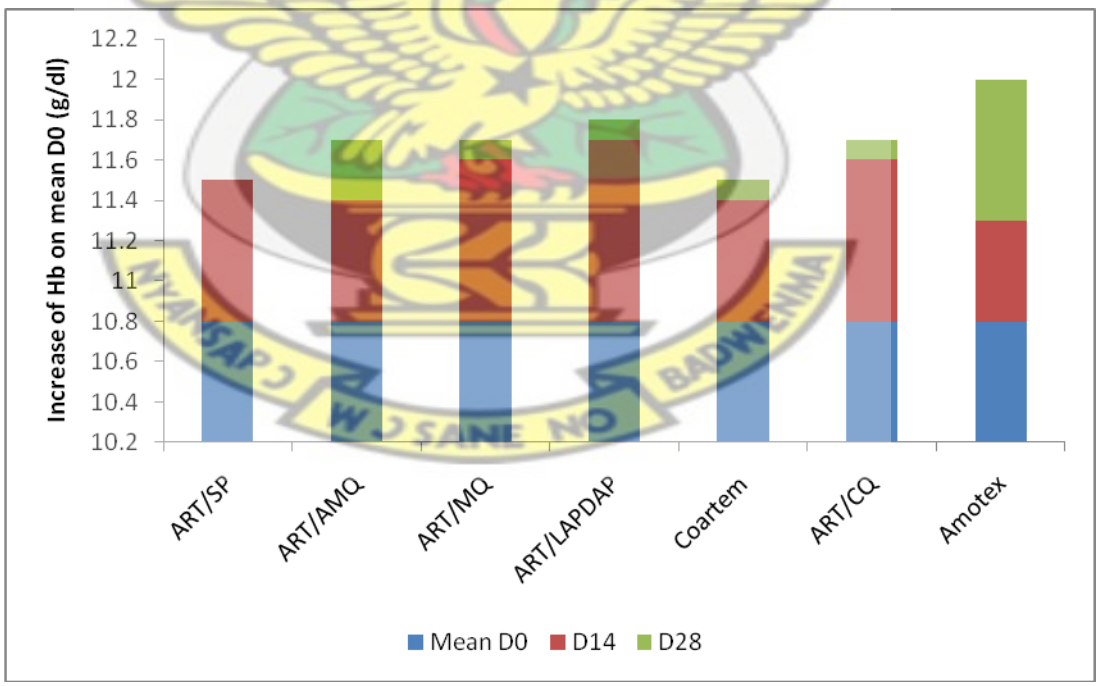



**Figure 4.13: Improvement of mean haemoglobin concentrations after initial treatment of pupils with ACTs**

By the days 14 and 28 after treatment, there was an overall mean increase of 0.7g/dl and 0.9g/dl respectively over the day 0 baseline Hb concentration ($p<0.001$). The change in mean Hb concentrations over the baseline Hb by day 14 were 0.7, 0.6, 0.8, 0.9, 0.6, 0.8 and 0.5g/dl for ART+SP, ART+AMQ, ART+MQ, ART+LAPDAP, COARTEM, ART+CQ and AMOTEX respectively. However, the change in mean Hb concentrations between treatment groups were not significant ($p = 0.1$). By day 28, change in mean Hb concentrations over the baseline Hb were 0.7, 0.9, 0.9, 1, 0.7, 0.9 and 1.2g/dl for ART+SP, ART+AMQ, ART+MQ, ART+LAPDAP, COARTEM, ART+CQ and AMOTEX respectively. However, the changes in mean Hb concentrations between treatment groups were significant ($p < 0.001$).

The change in mean Hb concentrations over the day14 Hb concentration by day 28 were 0.0, 0.3, 0.1, 0.1, 0.1, 0.1 and 0.7g/dl for ART+SP, ART+AMQ, ART+MQ, ART+LAPDAP, COARTEM, ART+CQ and AMOTEX respectively ($p > 0.05$). The mean difference of Hb concentrations of the various treatment groups compared with the mean Hb concentration of ART+AMQ at days14 and 28 respectively were not significant ($p>0.05$) as shown in **Table 4.13.**

### *4.2.5 Incidence of drug adverse effects*

Children treated both at home and at schools were followed up after treatment dosing schedules were concluded (day 3) for a maximum of 7 days at the start of treatment.

#### *4.2.5.1 Adverse effects associated with the use of ART+AMQ prepackeds in homes*

Adverse effects recorded were itching (28.6%), vomiting (38.1%), weakness (22.2%) and others (11.1%) with an overall incidence of 11.0%. These were mild and non-life threatening events.

#### *4.2.5.2 Adverse effects associated with the use of ACTs in pupils*

Adverse effects reported were itching, vomiting, dizziness, general weakness, nausea, headache and interference with routine daily activities ranging from 4% in interference with routine daily activities to 21.7% in dizziness as seen in **Figure 4.14.** AMOTEX recorded the highest adverse effects; dizziness, general weakness, nausea, headache and interference with routine daily activities and it was the only treatment arm that interfered with pupils routine activities and some events were very serious and life threatening. ART+AMQ and ART+CQ recorded the highest cases of itching whist the highest vomiting cases were reported in COARTEM group. An overall adverse effect reported after the seven days follow up was 34.9%. Adverse effects reported among pupils in the different treatment arms were 8.4%, 19.9%, 9.6%, 9.6%, 7.2% 12.0% and 33.1% for ART+SP, ART+AMQ, ART+MQ, ART+LAPDAP, COARTEM, ART+CQ and AMOTEX respectively. Adverse events reported per treatment groups ranged from 18.2% in ART+SP to as high as 96.5% in AMOTEX as seen in **Figure 4.15**

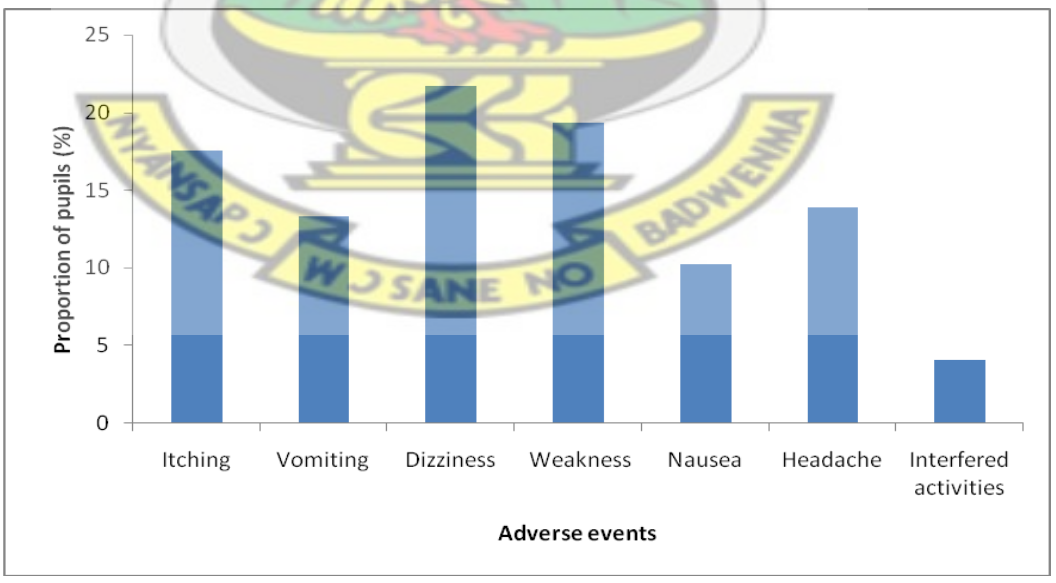



**Figure 4.14: Incidence of adverse effects reported by pupils treated with ACTs**

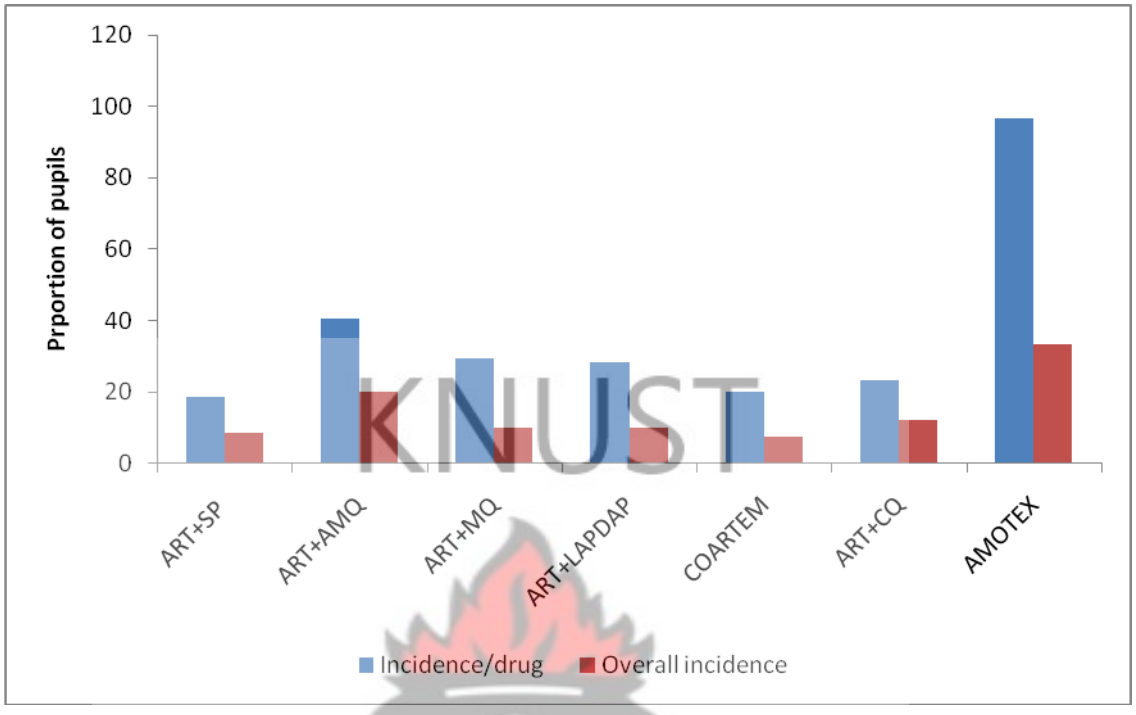



**Figure 4.15: Proportions of adverse effects reported by pupils in the treatment arms**

### 4.3 Molecular Epidemiology

All children samples collected by day 28 follow-up that were positive by microscopy were analyzed with their corresponding day 0 samples by polymerase chain reaction (PCR) amplification using allelic polymorphisms of *msp-2* and *glurp* genes for allelic diversity in the study population and also to determine recrudescence or re-infections after treatment with ART+AMQ at home. Size variations in the different lengths of IC /3D7 and FC27 were observed on agarose gels of *msp-2* gene and *glurp* gene.

### *4.3.1 Parasite DNA amplification in the study population*

A total of 57 days 0 and 28 samples were genotyped by polymerase chain reaction (PCR) using *msp-2 gene* marker. In the *msp-2* nested gene PCR amplification, 87.7% successfully amplified, but 12.3% failed to amplify. However, 11.7% failed to amplify at both days 0 and 28 for both allelic families and 11.7% amplified either in one allelic family or both at day 0 samples, but failed in day 28 and vice versa. All 57 days 0 and 28 samples were also genotyped by PCR using glutamate-rich protein (*glurp*) gene and 94.7% amplified at both days 0 and 28 whilst 5.3% failed to amplify at both days 0 and 28 samples. Comparisons were then made between day 0 and day 28 samples. Where the bands on both days were similar, it was classed as recrudescent infection (R), and where new alleles showed on day 28, it was classed as a new or re-infection (NI). Where day 28 alleles consisted of both new and day 0 alleles, were classed as a mixed infection (MI). Any sample which showed alleles on day 0 but not on day 28 and vice versa, was classed as undetermined (I), while any sample which showed no alleles on both days was considered failure to amplify (F).

Both *msp2* and *glurp* clones were used to determine whether the amplifications at day 28 were recrudescence or re-infections. A combination of *msp*2 and *glurp* gave 46% (23) as recrudescences and 54% as re-infections. However, 11.7% were classified as indeterminate with only 1.7% as failure. Polymorphisms in the merozoite surface protein 2 (*msp-2*) genes and the glutamate-rich protein (*glurp*) gene of *Plasmodium falciparum* in patient isolates are shown in **Figures 4.16 and 4.17** respectively.

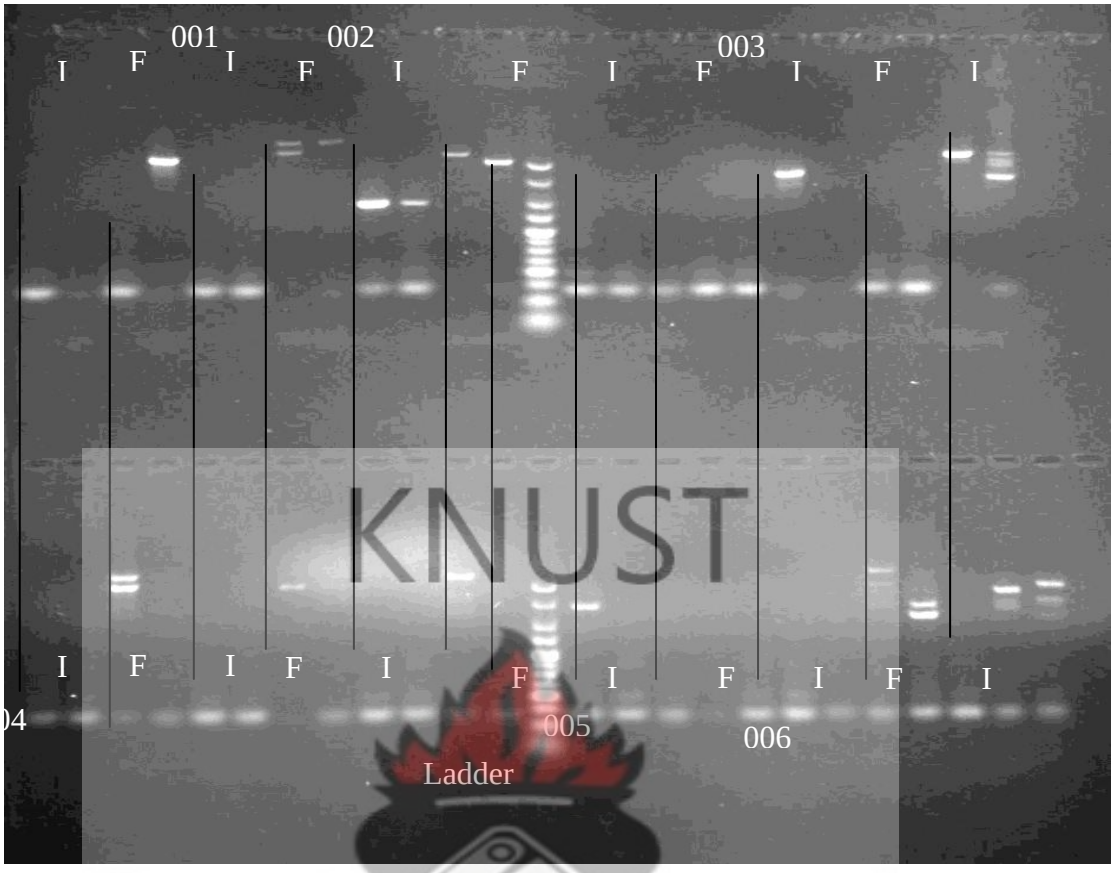



**Figure 4.16: Msp2 gene nested PCR amplification products using FC27- specific primer (F) & IC/3D7-specific primers (I)**

**Key:**

**F: FC27-specific primers**
**I: IC/3D7- specific primers**
**Ladder: molecular mass marker**
**001: recrudescence at I**
**002: recrudescence at F & re-infection at I**
**003: indeterminate**
**004: F failed to amplify whilst I amplified**
**005: F amplified whilst I failed to amplify**
**006: multiplicity of infections at I**

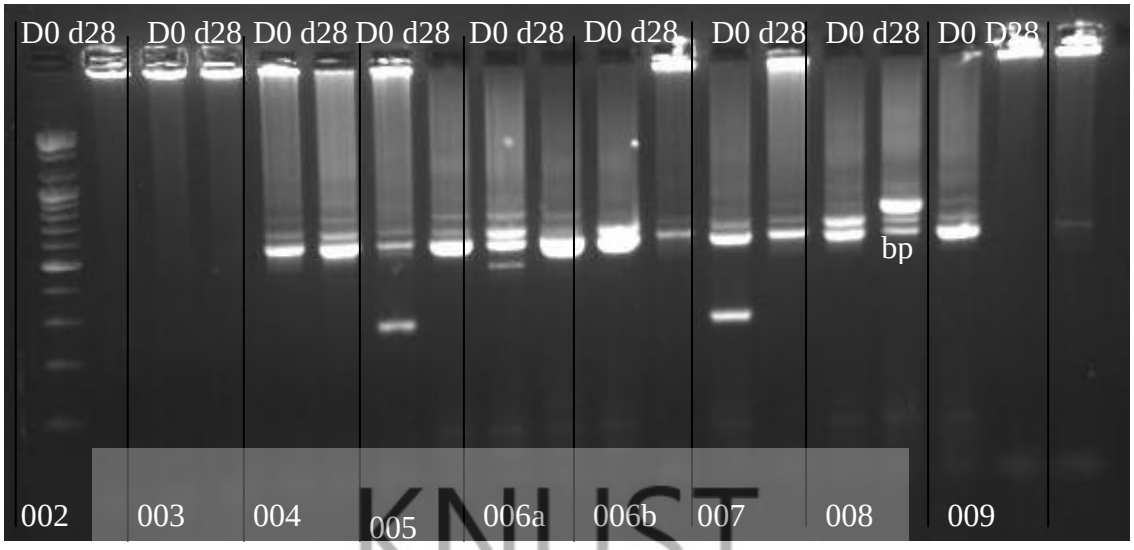




**Figure 4.17: Glurp gene PCR amplification products showing alleles with 2 repeats (006 A &B)**

**Key:**

**001: molecular mass marker**
**002: both days 0 and 28 failed to amplify**
**003: indeterminate**
**004: recrudescence**
**005: recrudescence and multiplicity of infections**
**006a& b: alleles with 2 repeats**
**007: recrudescence**
**008: multiplicity of infections**
**009: failed implication**

#### *4.3.1.1 Parasite genetic diversity in the study population*

A total of 100 *P. falciparum* clones were detected; 18% belonged to the FC27 allelic sub-family while 82% belonged to the IC/3D7 allelic family. Two percent of the patients had both FC27 and IC/3D7 sub-families at the days 0 and 28 samples. Allelic diversity in the *msp2* gene detected in this study was high with 20 different IC/3D7 family alleles and 4 different FC27 family alleles observed. This indicates that, at the *msp2* gene, IC/3D7 alleles were five times distributed (ratio = 20/4) than alleles of FC27 family in the study population whilst in the *glurp* gene, 18 strains were detected in the population.

### *4.3.2 Multiplicity of infections of P. falciparum*

In this context, multiplicity of infection (MOI) was defined as the number of genotypes per infection and was calculated as the highest number of genotypes at the *msp2* and *glurp* locus of the parasite DNA and was the basis for establishment of parasite diversity .The number of infecting genotypes in an isolate (MOI) was determined without the usual Restriction Fragment Length Polymorphism (RFLP) analysis of the *msp2* gene and *glurp* gene on agarose gel. Parasite multiplicity of infections of *P. falciparum* was thus determined in both *msp*2 and *glurp* by counting the number of bands per isolate although this has some serious limitation in accuracy.



MOIs were ranging between 1- 6 infections per blood sample and 1 - 4 per blood sample in *msp*2 and *glurp* respectively as shown in **Figure 4.18** with overall means of 2.8 and 2.1 respectively per sample. Single FC27 infections were found in 10% of patients and IC/3D7 single infections were found in 26% of patients. Double infections were detected in 5% patients for FC27 and 36% for IC/3D7 sub-families. However, multiplicity in FC27 was only up to 2 infections per sample whilst in IC/3D7, it was up to 6 infections per sample. MOIs for *msp2* isolates were 32.9%, 50%, 19.7%, 7.9%, 1.3% and 1.3% for 1, 2, 3, 4, 5 and 6 respectively whiles *glurp* isolates were 48.6%, 29.2%, 18.1% and 4.2% for 1, 2, 3 and 4 respectively.

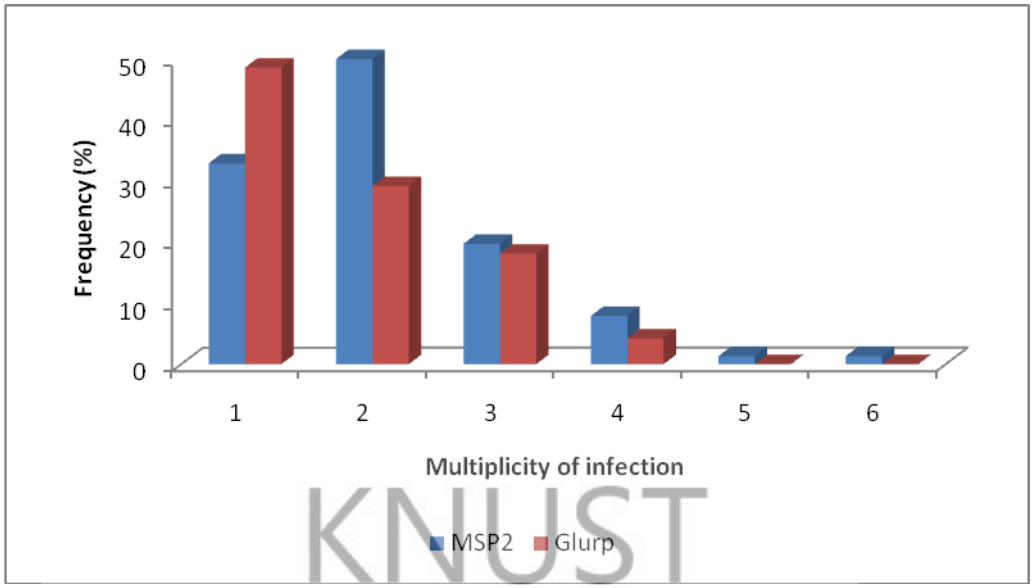



**Figure 4.18: Multiplicity of infections in *P. falciparum* using *msp*2 and *glurp* markers**

#### *4.3.2.1 Multiplicity of infections of P. falciparum and recrudescent development*

The numbers of infecting genotypes in an isolate (MOIs) were determined in day 0 samples and this was used to determine the likelihood of recrudescent development in the day 28 follow-up samples given that the corresponding day 0 samples were either a single infection or multiple infections (> 1). The odd ratio (OR) or relative risk (RR) of developing recrudescent in multiplicity of infections were determined in both *msp2* and *glurp* genotypes. The odd of multiple infections developing recrudescent in *msp2* and *glurp* were 1.5 and 5 respectively. This implies that the risk of multiple infections developing recrudescence in *msp2* and *glurp* genes by day 28 was about 2 times and 5 times likely than in a single infection respectively. Different multiple infections and recrudescent development in both the *glurp* and *msp2* are shown in **Figures 4.19 and 4.20** respectively.

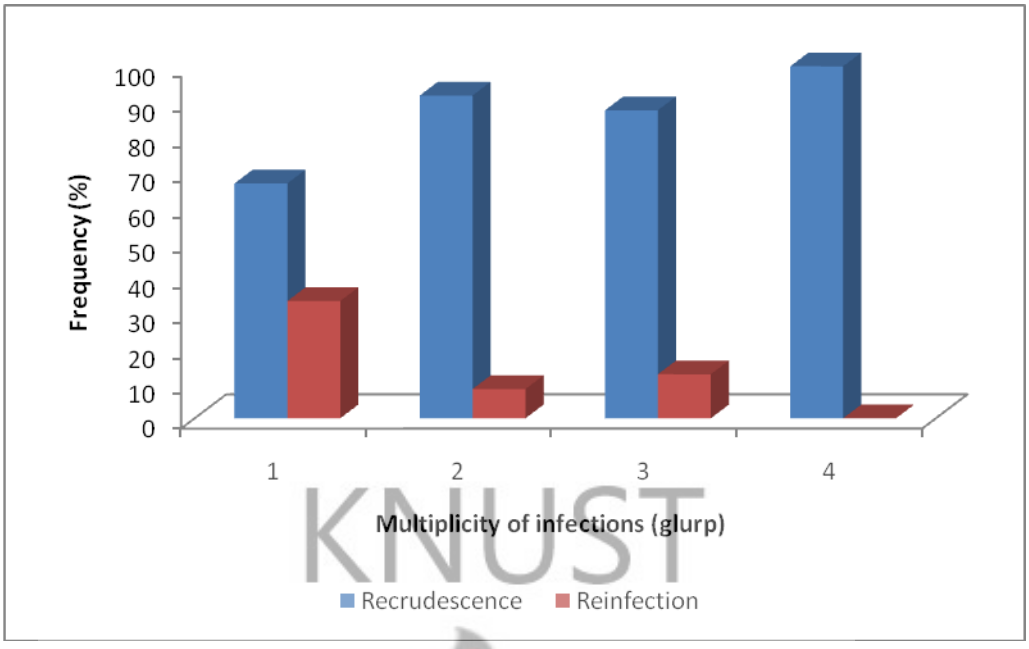



**Figure 4.19: Recrudescence development of *P. falciparum* MOIs using *glurp* marker**

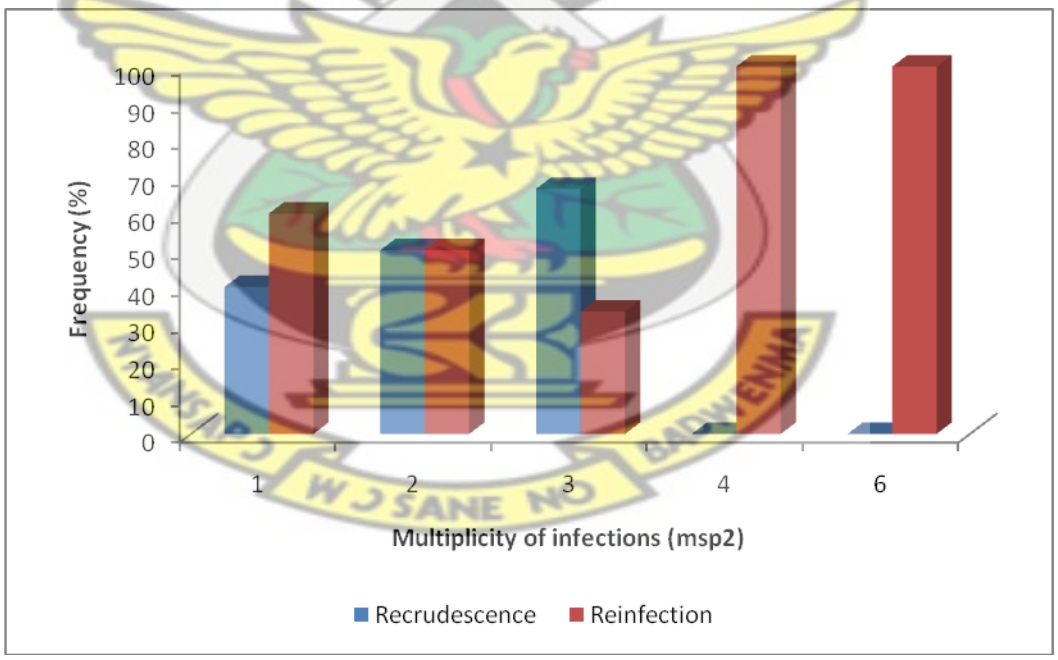



**Figure 4.20: Recrudescence development of *P.falciparum* MOIs using *msp2* marker**

### *4.3.3 Base pair size variation*

Amplification of the polymorphic part of the *glurp and msp2* genes of parasites from the patients showed varied size (base pairs) variation. Polymerase chain reaction products approximately ranging from 200 to 900 base pairs for *glurp* and 100 to 1200 base pairs for *msp2* were observed as shown in **Figure 4.21.** Over 61% of *glurp* isolates had base pairs from 500 to 599, 87.7% had base pairs from 400 to 700, 5.5% from100 to 300 and 4.9% from 800 to 1200 as shown in **Figure 4.22.** Over 37% of *msp2* isolates had base pairs from 500 to 599, 69.4% had base pairs from 400 to 700, 22.7% from 100 to 300 and 5.4% from 800 to 1200 (**Figure 4.22).**



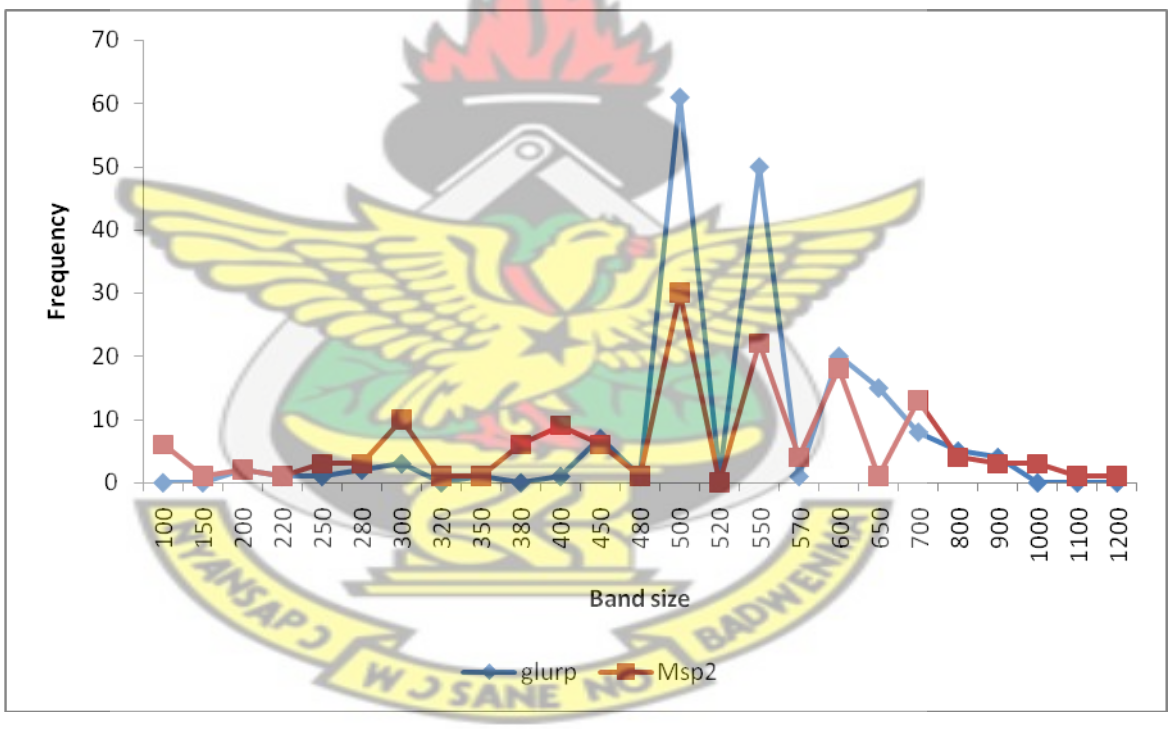



**Figure 4.21: PCR amplification products base pairs size variations using *glurp* & *msp*2 markers**

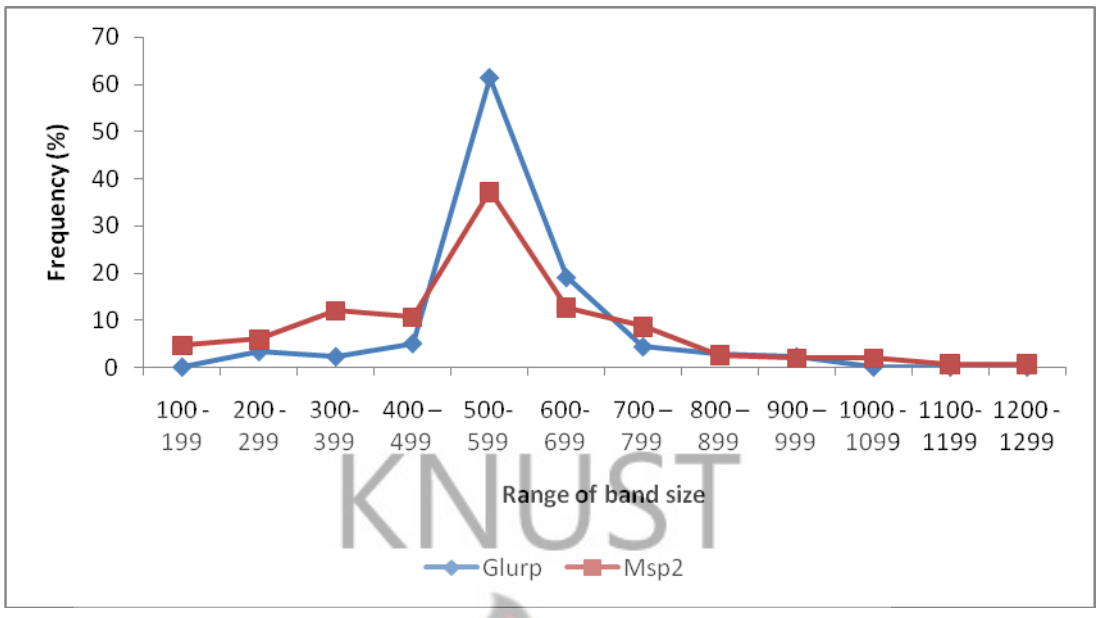



**Figure 4.22: Range of PCR amplification products size variations using *glurp* and *msp2* genes**

### *4.3.4 Comparison of P.falciparum strains in different episodes of malaria in individual patients*

Patients who had their blood samples taken after 3 months that were positive by microscopy had their days 0 and 3-month samples also genotyped by PCR using *msp2* and *glurp* genes. The amplification products at both days 0 and 3-month samples bands size were compared to determine whether both were same or not. In *msp2* gene, only 3D7 allelic family occurred in both day 0 and 3 months samples.

For *msp2* marker, the first patient at the first visit (P1v1) had triple infections at day 0 (B1, B2 and B3) but double infections during the 3-month visit (v2) and the bands were different from day 0 sample. P2v1 had quadruple infections (D0), but double infections at month- 3 (P2v2) and were all different from day 0 bands. P3v1 and P3v2 had triple infections at both days 0 and 3-month visits, however, only B2 in both P3v1 and P3v2 had similar patterns / bands. P4v1 had double

infections (D0) and single infection at P4v2 (3-month) and the bands sizes were not similar as shown in **Figure 4.23.**

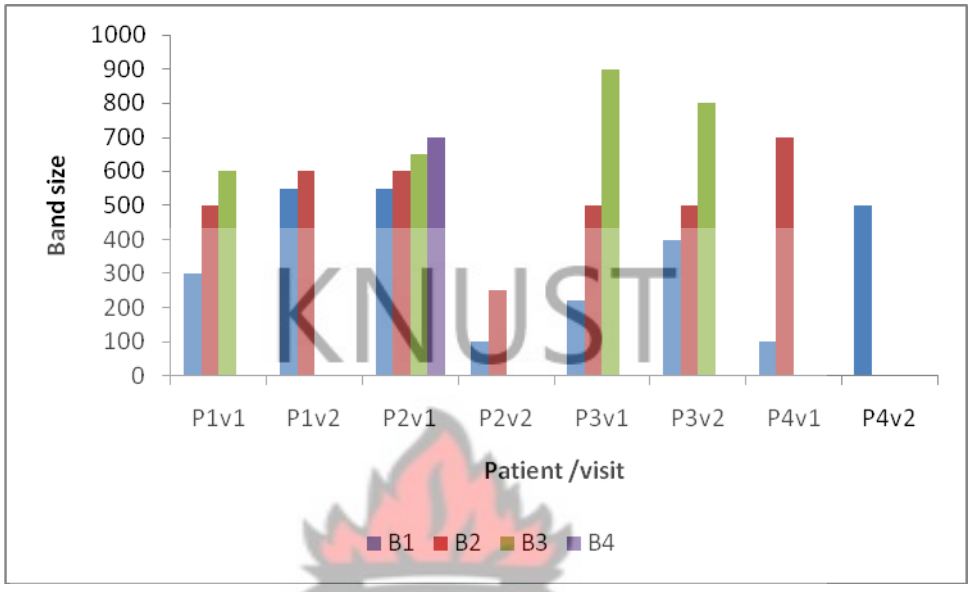



**Figure 4.23: Comparison of *P.falciparum* isolates in different episodes of malaria in individuals patients using *msp*2 gene**

Six patients' day 0 and 3-month samples were compared using the *glurp* gene amplification products as shown in **Figure 4.24.** P1v1 had single infection (D0) but P1v2 had double infections and were not similar to D0 band pattern. P2v1 and P2v2 had double infections but pattern of bands were not similar whilst P3v1 and P3v2 had single infection with different band patterns. P4v1and P4v2 had triple infections and double infections respectively with band patterns, B1 & B2 similar in both P4v1and P4v2. P5v1 and P5v2 had double and quadruple infections respectively and had similar B2 bands pattern. P6v1 and P6v2 had triple and quadruple infections respectively. However, banding patterns in B1 and B2 in P6v1 were similar to B2 and B3 in P6v2 respectively.

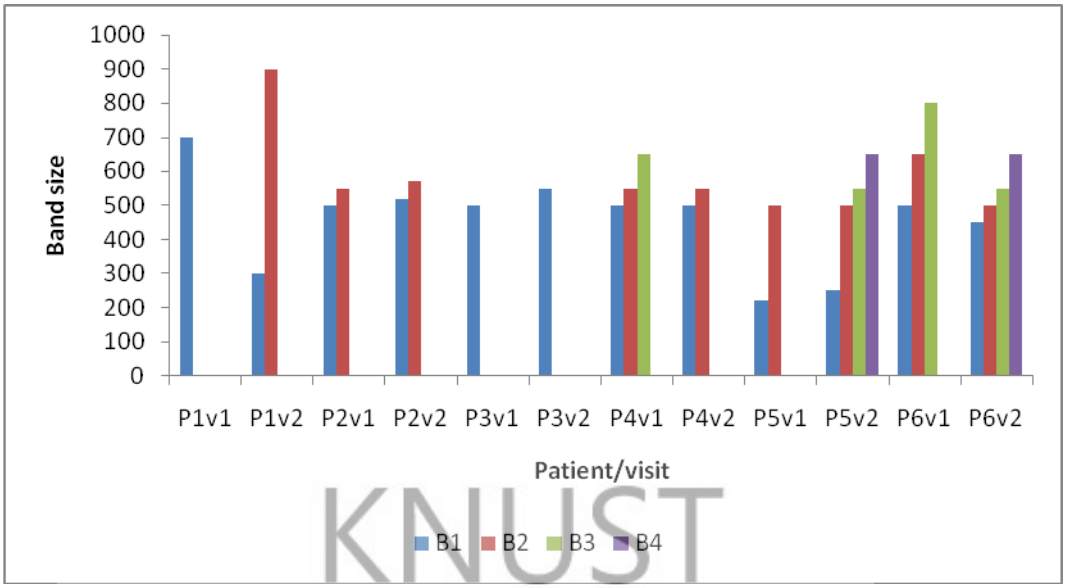



**Figure 4.24: Comparison of *P.falciparum* isolates in different episodes of malaria in individuals patients using *glurp* marker**

### *4.3.5 Malaria parasite resistant strains*

In the FC27 allelic family that amplified, 50% had recrudescent whilst 50% were re-infections. For IC/3D7 allelic family, 45.5% were recrudescent and 54.5% were re-infections. There were five different resistant strains detected in the msp2 gene with only one belonging to the FC27 family with base pairs size approximately 380. The other four stains belonging to the IC/3D7 allelic family had approximate base pairs size ranging from 500 to 600. In the *glurp* gene, 81% had recrudescent with 19% as re-infection. Four resistant strains were observed with approximate base pairs size ranging from 500 to 650.

# CHAPTER FIVE - DISCUSSIONS

# CHAPTER FIVE - DISCUSSIONS

## 5.1 Introduction

This chapter discusses the major findings on the trend of malaria epidemiology in children, parasitaemia prevalence, drug efficacy, safety and tolerance and the molecular epidemiology of *P. falciparum*.

## 5.2 Local malaria epidemiology in children

Malaria is a polymorphous disease, and its burden varies among different regions and populations. The mean parasitaemia prevalence in children in this study varied from 29.2% in an urban setting, 58.6% among school pupils in rural schools and to 71% in children in rural homes. A malariometric update study conducted by Browne *et al*. (2000) in the same area in 1999 showed that the malaria situation remained unchanged.

### *5.2.1 Malaria epidemiology in an urban setting*

The epidemiology of many diseases is ever changing with the increasing human population of urban areas and this could pose challenges for the prevention and control of these diseases (Keiser *et al*., 2004; Hay *et al*., 2005). Malaria in urban areas shows marked heterogeneity and complexity from those of rural environments. The effects of human behaviour on malaria transmission are place-specific.

In the present study, the mean parasitaemia prevalence in children under-five years in urban centre was 20.1% whilst that of peri-urban was 39.1% and this difference was significant. There was a general trend of increase in parasitaemia prevalence from the centre of the urban area towards the periphery with values ranging from 6.3% to 35.2% and 22.2% to 54.2% respectively.

Anthropogenic changes such as deforestation, road-construction and agricultural development generally increase the intensity of malaria transmission. However, the specific effects of such ecological disturbances are often unpredictable, due to geographical diversity in the biology of the mosquitoes that transmit the disease and these could influence the trend of malaria parasitaemia rates observed in this study. Malaria is a poverty-related disease and in the urban setting, the prevalence rates were community-related.



These findings are similar to those of a study conducted by Klinkenberg *et al*. (2005) in Accra with mean parasite prevalence of 14.9%, with rates ranging from 6% to 22% and Lisa *et al*. (2006) which reported malaria parasite prevalence of 37.8% in Moshie Zongo (a peri-urban area) and 12.8% in Manhyia (an urban area), both in Kumasi metropolis. Kazadi *et al*. (2000) reported parasite rate in children ranging from 14% in central-urban to 65% in peri-urban areas in Kinshasa, Democratic Republic of the Congo. The epidemiology of many diseases is changing with the changing population in urban centres and so it is with malaria (Keiser *et al*. 2004; Hay *et al*., 2005). The parasite rates observed though relatively stable showed marked heterogeneities which might vary significantly with time. These rates were significantly higher than those recorded in studies conducted over a decade ago in other African cities (Trape, 1987; Watts *et al*., 1990; Lindsay *et al*., 1990; Trape *et al*., 1993).

These high rates could be directly related to the study areas and human activities. In this present study, six urban communities had parasitaemia rates greater than 20%. Aboabo, Kaase, and Sawaba are densely populated urban areas of the metropolis with poor housing and low socio-economic status and relatively far from health facilities. Anloga, Oforikrom and Susuanso have poor housing and are also situated in marshy and water logged areas. The intensity of the malaria risk is often heterogeneous over small distances, depending on the degree of urbanization and the

proximity to possible vector breeding sites. Variations were either associated with the vicinity of vector breeding sites or differences in the vulnerability of communities and individuals.

Peri-urban areas often lacked infrastructure, including health facilities. Lack of potable water supply and good sanitation, also provide an ideal environment for vector breeding, hence these areas recorded high parasite rates. Some peri-urban communities, however, had parasite rates lower than some urban communities. This is either due to their proximity to health facilities or high socioeconomic status or both. Much of malaria is man-made; because most of the breeding habitats of *Anopheles* vector are created by human activity, such as construction works, agricultural activities and poor management of the environment. Transmission is dependent upon a number of factors relating to the parasite, the human host, the vector's ecology and the social environment.



### *5.2.2 Malaria burden amongst school pupils*

In this study, parasitaemia prevalence was place-specific with significant seasonal variations. The predominant species of malaria was *P.falciparum* with gametocyte rate of 5.3%. Parasitaemia prevalence ranged from 49.7% to 71% in the communities and these values were closely related to the level of vegetation cover, streams and the level of human activities in the community. School children are often thought of as naturally healthy, however, studies have shown that in Africa, more than half of the school children are stunted in height and are anaemic (Partnership for Child Development, 2005; WHO, 1997).

The effects of human behaviour on malaria transmission are place-specific. Transmission intensity of malaria depends on the density and infectivity of the anopheline vector and on variation in parasite rate in the human host (Smith *et al.*, 1993; Peyerl-Hoffmann *et al.*, 2003; Koram *et al.*,

2007). Communities that were more rural with large vegetation cover and suitable breeding sites had significantly high prevalence rates in this study. The parasite prevalence was significantly higher during the wet season (69%) than in the dry season (43%). This implies that, malaria transmission occurred throughout the year and showed marked seasonal influences. Thus, rain fall and other climatic factors could have contributed to the high parasitaemia prevalence observed during the rainy season.



In malaria-endemic areas, a significant proportion of children harbour parasites without presenting signs of clinical malaria and are considered asymptomatic cases (Landry-Erik *et al*., 2003; Greenwood, 1987). Asymptomatic malaria can affect the individuals who carry the parasites and are cryptic carrier reservoirs for the community (Osorio *et al.*, 2004). A relatively large proportion of the school pupils were chronically infected with the malaria parasites. Over 5% of them were carrying gametocytes and were therefore cryptic carrier reservoirs for the communities (Osorio *et al.*, 2004).

The high gametocyte rate would also ensure continuous circular of the parasite in these communities and could have contributed to the high prevalence rates observed in the study site. These findings affirmed the fact that in certain regions of Africa, more than 70% of the citizens are chronically infected with *Plasmodium falciparum* (Breman *et al*., 2004 & 2007). These findings are also consistent with those in studies conducted in holoendemic western Kenya**,** in Cotonou, Benin and in Ouagadougou, Burkina Faso in school children (Siân *et al*., 2004; Wang *et al*., 2004).

The high prevalence of parasitaemia and the gametocyte rates would result in repeated episodes and sastained transmission of malaria in these communities. The impact of repeated malarial episodes is thought to have detrimental effects on the development of the child, particularly his/her mental and cognitive function (Fernando *et al*., 2003a, b; Siân *et al*., 2004). Although the school

performance of a child depends on multiple factors, repeated absenteeism from school due to malaria significantly affects his/her performance (Jukes, 2005; Sternberg *et al*., 2001& 2002).

In malaria-endemic areas, asymptomatic cases result due to development of variant-specific immunity and explain the low-grade infection during extended periods without clinical symptoms (Staalsoe *et al*. 2004). This could explain the decrease in parasite rates amongst pupils with increasing age since the older pupils would have had several episodes and could thus have acquired some levels of immunity against the parasites although this was not found to be significant. However, in this study parasitaemia prevalence was age independent.



### *5.2.3 Anaemia burden in school pupils*

In this study, a remarkable percentage (60%) of the asymptomatic children were anaemic on the screening day however, 14% of them were severely anaemic (Hb < 8.0g/dl). Similarly, Kurtzhals *et al*. (1999) reported 55% anaemic amongst school pupils. In this study, however, anaemia prevalence was higher than those reported in GHS report (2007). Anaemia is thought have negative consequence on childhood development and cognisance function. It is the leading cause of death in under-5 children in the Ejisu-Juaben District. This highlights the need for urgent and prompt intervention for malaria control.

The cause of anaemia is multi-factorial including nutritional deficiencies, hookworm infection, HIV and haemoglobinopathies, however, in endemic countries; malaria is one of the most important factors (Murphy *et al.,* 2001). More than 50% of the study pupils were infected with malaria parasites and this group is usually unidentifiable by most malaria control programmes (Osorio *et al.*, 2004).

The high parasitaemia prevalence observed in the school pupils could have contributed to the high anaemia in these pupils. Studies have shown that long-term asymptomatic malaria could lead to anaemia (Gendrel *et al*., 1992). Malaria is known to be a major cause of anaemia in children and over 40% of the world's children live in malaria-endemic countries (WHO, 2005a). In Ghanaian children under-five years, anaemia is mostly reported to be due to malaria and nutritional disorders (GHS, 2007). Schools, however, can offer a potential delivery mechanism for interventions for malaria and anaemia.



***5.2.4 Malaria epidemiology in the rural setting of Ejisu-Juaben***

The prevalence of malaria in febrile children seeking treatment from CDDs detected by microscopy was 71% with a significant monthly variation. Owusu-Agyei *et al*. (2002) reported 70% parasitaemia prevalence during malaria transmission season in Northern Ghana and Dunyo *et al*. (2000) reported 64.7% and 62.6% in Southern Ghana. *P. falciparum* was found to be the main species responsible for malaria in these children. A gametocyte rate of 2.3% ensures a continuous transmission and circulation of the parasite in the community. The circulation of the parasites is probably enhanced by the high transmission rate by the vector, the female Anophele mosquito.

In Ghana, annual biting rates and annual entomological inoculation rates are estimated to range from 6-27 infection/man/night to 100-1000 infective bites/man/annum respectively (Appawu *et al*., 2001). This high entomologica inoculation rates could have accounted for the more than 4% of children in the study site experiencing four or more febrile episodes within a year though they were treated with ART-AMQ during each malaria bout. Malaria episodes among African children are estimated to be between 1.6 and 5.4 million each year. This, however, varies according to

geographical and epidemiological circumstances (Murphy *et al*., 2001. Intensities of transmission in this study site are enhanced by changes in climate variables and with a large vegetation cover.

The epidemiology of malaria in Ghanaian children has not changed; however, its dynamics differed from region to region and is severe in the forest belt in the rural areas (Browne *et al*., 2000; Ahmed, 1989; GHS, 2001). These features are consistent with malaria episodes in sub-Saharan Africa, where falciparum is pervasive and the major killer of children under five (Breman *et al*., 2007). In endemic African countries, malaria accounts for 25%-35% of all outpatients' visits, 20%-45% of hospital admissions and 15%-35% of hospital deaths (WHO, 2005a). In Ghana, malaria accounted for 40 – 45% of all OPD visits in 2004, 43.7% in 2006, 38.6% in 2007and accounted for over 18% of deaths reported at health facilities in 2007 (GHS, 2004a; 2007). This suggests that the morbidity of malaria in Ghana is evolving and will require novel and multi-faceted approach to its prevention and control.



### *5.2.5 The impact of genetic variations on the local epidemiology of malaria*

A total of 100 *P. falciparum* clones were detected with MOIs ranging between 2- 6 infections per blood sample and fragment band sizes ranged from 100 to 1200 base pairs. Most allelic diversity is generated by intragenic recombination between representative sequences at the 5' end of the gene, within blocks 3, 4 and 5 (Basco *et al*., 2004).These variations in the parasite enhance intense malaria transmission and infections in the study site. Variability in genes and multiplicity of infection affect both the prevalence of parasite, genetic markers such as those involved in resistance to antimalarial drugs (Jelinek *et al*., 1999; Schneider *et al*., 2002) and the risk of clinical disease.

The epidemiology of malaria in children in Ghana has not changed despite considerable efforts made to eradicate or control malaria. Its control and treatment have been complicated by the

emergence of resistance to widely used antimalarial drugs such as Chloroquine (Neequaye, 1986; WHO, 2001a). Natural populations of *P. falciparum* are heterogeneous mixtures of individuals with different, genetically determined degrees of drug response during treatments (Shunmay *et al*., 2004). This present study demonstrated a high level of polymorphism, genetic variability and multiplicity of infection thus contributing to the burden of malaria in children.

### 5.3 Home management of malaria in children

The approach to making antimalaria drugs available in the community and ensuring that they are used appropriately is now well established through WHO/TDR-supported multi-country studies using Chloroquine. This is however, the first time in the Home Management of Malaria (HMM) Programme in Ghana that CDDs were taught how to take finger-prick blood samples for malaria microscopy. This was done to determine the accuracy of their presumptive treatment in order to assess the extent to which non-malarial fevers were treated with ACTs. This was also done to determine the parasitaemia status of febrile children seeking care from CDDs under the HMM studies in Ghana. In all previous HMM studies conducted in Ghana, CDDs have relied on caregivers' narration of history of fever and decide whether to treat for malaria or refer cases to a health facility (Yeboah-Antwi *et al*., 2001a, b; Browne *et al*., 2002).

This study has demonstrated conclusively that CDDs can be trained to take blood samples for malaria microscopy in order to ascertain the accuracy of their treatment decisions. CDDs, however, did not have the capacity to read these blood films on the site prior to initiation of treatment. Rather, blood films were collected for trained Laboratory Technicians who read them after 24 hours at KNUST, Kumasi.

### *5.3.1 Justification for the Home Management of Malaria*

The current proposition is that all cases of suspected malaria with the possible exception of children in high-prevalence areas and certain other situations should be parasite-based diagnosis (WHO, 2005a & 2006a) as a result of high-cost of ACTs (WHO, 2008). This, thus, calls for reassessment of current diagnostic practices in order to determine the most cost-effective method. The current diagnostic methods are microscopic examination of Giemsa-stained blood smears and the newly developed rapid diagnostic tests (RDTs) for malaria (Breman *et al*., 2007). CDDs in this study have shown that they are trainable; however, they lack the technical capacity to read blood films on the site prior to initiation of treatment. Since microscopy is generally limited to larger clinics and required technical ability, rapid diagnostic test (RDT) for malaria could be considered for most patients in endemic regions if this is found to be cost effective (Ajayi *et al*., 2008a; Shillcutt *et al*., 2008). If and when the use of RDTs becomes a national policy, CDDs can be trained and supported to provide this care and services at the community level. However, a cost-effective analysis study by Lubell and others (2007) on ACTs trial using RDTs concluded that it was expensive and may not be affordable to many countries.

About 71% of the febrile children treated with ACTs by CDDs were confirmed microscopically to have malaria. This suggests that caregivers' ability to recognize malaria at home and the presumptive treatments of CDDs were highly accurate. Dunyo *et al.* (2000) in a comparative study showed that, caregivers' recognized febrile episodes slightly better compared to health centre report of fevers although parasitaemia rates were similar.

In most typical health facilities in Ghana, and especially in the rural and peripheral areas, clinical diagnosis of malaria is the most widely used approach (Dunyo *et al*., 2000) and in many situations the only feasible one. Clinical diagnosis or presumptive treatment is inexpensive (Pagnoni *et al*.,

1997); however, the symptoms of malaria are very non-specific and overlap with those of other febrile illnesses. A diagnosis of malaria based on clinical ground alone is therefore unreliable. Notwithstanding this, in most cases in Ghana, it is the only option and the grand rule for antimalarial therapy. That 71% of their presumptive treatment were actually confirmed microscopically, suggests that 71% of these children had early and appropriate treatment.

The 29% of the children treated with ART-AMQ were without malaria parasites and this could be other childhood fevers. Koram and Molyneux (2007) affirm that the lack of diagnostic acumen could result in erroneous diagnoses and treatment, and dissipation of resources. In this study, however, more than 4% of the children experienced four or more febrile episodes per annum making it necessary for antimalaria drug treatment in the absence of precise diagnostic tool (Breman *et al*., 2007). At very high malaria prevalence, the probability that non-malarial febrile infection is bacterial is irrelevant (Shillcutt *et al*., 2008).

The success or failure of any public health program depends largely on the public effective use of the services offered. The 71% of febrile children confirmed to have malaria suggests that caregivers' ability to recognize malaria at home were highly accurate. However, merely making an efficacious treatment available is not enough to reduce malaria mortality; the treatment also must be used optimally.



### 5.4 The impact of ACTs treatment on haematological outcomes

Children with severe anaemia (Hb < 8.0g/dl) on the screening day were excluded from the study; however, over 53% of the children were mildly anaemic (Hb< 11g/dl). Pupils with peripheral parasitaemia had mean Hb of 10.8g/dl compared with 11.1g/dl of those without parasites and this

difference was not significant. It has been assumed that parasite density, age, weight, community, helminthes etc. could influence the outcomes of Hb concentrations.

After pupils with malaria parasites were treated with ACTs, there were remarkable reduction of parasite prevalence by days 14 and 28 with a corresponding increase in the mean Hb values. This suggests that the treatment and hence parasite clearance has contributed to the rise in the post treatment Hb concentrations. This implied treatment was important in recovery from anaemia (Takechi *et al*., 2001; WHO, 2001a) and that malaria parasites were mainly responsible for the low values of Hbs observed among the school pupils. It is known that in malaria-endemic areas, a significant proportion of children harbour parasites without presenting signs of clinical malaria (Landry-Erik *et al*., 2003) and these children could have low to moderate anaemia which would go unnoticed. This, therefore, would have negative consequences on their health and their school performance (Snow *et al*., 1999).

There was a general improvement of the Hb concentrations during follow up with a corresponding decrease in the proportion of pupils with mild-to-moderate anaemia. This is similar to improvement of Hb concentrations following treatment with ACTs (Koram *et al*., 2005). By day 14, the overall proportion of children with Hb < 11g/dl was 32.6% and by day 28, the proportion was 25.3% with mean Hbs of 11.5g/dl and 11.7g/dl respectively which were significant over the day 0 Hb concentrations. The mean Hb difference of the different treatment groups compared with ART-AMQ were not significant, however, the changes in mean Hb concentrations between treatment groups were significant.

Malaria is associated with anaemia (Ezzati *et al*., 2002) and its insidious nature of presentation means that mild-to-moderate degrees of anaemia frequently remain undetected and untreated by

health care workers and in the community (Phillips-Howard *et al*., 2003; Schellenberg *et al*., 2003). Many other studies, however, associated anaemia with other factors such as iron, folate, and vitamin deficiencies, worm infestation, HIV/AIDS and haemoglobinopathies (Lozoff *et al*., 2000; WHO, 2001c). Thus, although malaria plays a key aetiological role in anaemia in endemic countries, it is clear that other factors make important additional contributions. However, studies have shown that long-term asymptomatic malaria could lead to anaemia (Gendrel *et al*., 1992).



In the present study, in spite of all other contributors, malaria is the major contributor to anaemia in the pupils since treatment impacts very heavily on haemoglobin improvements following peripheral parasites clearance. Malaria, therefore, could be the lead and significant contributor to anaemia in children in malaria endemic areas such as Ghana. It is therefore commendable that intermittent preventive treatment of pupils in schools especially those in the rural settings be a national policy. This could improve their health and school performance. The Government of Ghana through the Ministry of Education is providing capitation grant and school feeding programme to boost education at the basic levels. However, the role of poor health as an intervening factor in education has not been fully recognized. The economic effects of the neglect of children's health and nutrition will inevitably appear in the long run: a poorly educated and unhealthy population will act as a drag on economic growth of the nation.

### 5.5 Efficacy of treatment drugs

The standard methods to assess the efficacy of a given drug are based on two broad tests: ***in-vivo*** and ***in-vitro*** tests. The treatment efficacy in ACTs trial in this study is based mainly on treatment failure and change in the haematological parameter (Hb) at day 0 and 14 after start of treatment and was assessed based on 14-days in-vivo test proposed by WHO (WHO, 2003a) on asymptomatic pupils (WHO, 2003a). PCR 28 days' in-vivo test has been proposed as more

appropriate and cost effective (WHO, 2005b), however, only children treated with ART-AMQ prepackeds in home management of malaria had their day 0 and 28 samples analyzed to correct for re-infection. The emphasis of treatment outcome in this study is on parasitological response to the study drugs.

In ACTs trial study, except children treated with COARTEM, who have to take 8-hour dose schedule on first day at home, all other pupils took all treatment doses at school supervised by teachers and CDDs. Those who did follow the treatment protocol (83.8%) were included in the days14 and 28 follow up, however, some treatment groups fell short of the minimum sample size. Adherence to treatment protocol by the pupils followed up was nearly 100%. The effectiveness / efficacy of the various treatments groups of ACTs were compared with ART-AMQ as the drug of choice for treating uncomplicated malaria in Ghana. By days 14 and 28 follow up visits, only 50.5% and 68.7% respectively of the children were successfully followed up with over 95% adherence to treatment.

### *5.5.1 Parasitological outcomes of ACTs in-vivo field trial*

The parasitological responses were based only on microscopy detection (crude parasitological failure) without the PCR analysis proposed as more appropriate and cost effective (WHO, 2005b) for ACTs trial study. This is due to logistical and financial constraints to run the PCR analysis for the ACTs trial study. The proportion of treatment failure by day 14 was generally low, except for ART-LAPDAP group that was about 20%. ART-MQ showed a better parasite clearance (100%) than the other treatment arms (treatment groups) by day 14. Artesunate (ART) in combination with SP had a better parasite clearance than with Amodiaquine (AMQ) and the other drugs; however, ART-AMQ had similar parasite clearance with fixed combination of ART and AMQ,

AMOTEX and COARTEM, but better than ART in combination with Chloroquine (CQ). Readily absorbed drugs with a long half-life, like Mefloquine (MQ) and Sulfadoxine-Pyrimethamine (SP), can permit effective single dose treatment of malaria and prevents infection for several weeks (Takechi *et al*., 2001; WHO, 2001b). This could explain why MQ and SP in combination with ART were superior to the other treatment groups at parasite clearance by day 14. CQ in combination with ART was inferior in parasite clearance by day 14, confirming its failure to clear parasites (GHS, 2002). Koram and colleagues (2005) conducted similar study in Ghanaian children using ART-AMQ and COARTEM and had 100% parasite clearance by day 14 whilst Oduro *et al*. (2005) confirmed that ART in combination with SP and AMQ were better at parasite clearance than monotherapies.



By day 28, AMOTEX had better parasite clearance than the others, closely followed by ART in combination with MQ, AMQ, SP and then COARTEM with values similar to studies conducted in Ghana (Koram *et al*., 2005; Oduro *et al*., 2005). LAPDAP in combination with ART had rather low parasite clearance at both days 14 and 28 compared with ART-AMQ. AMOTEX and MQ in combination with ART were superior in parasite clearance both at day 14 and 28 than AMQ in combination with ART, but AMQ- ART was superior to the others with only marginal differences than COARTEM and ART- SP.

The parasite clearance observed by days 14 and 28 could be due to treatment failure or new infections. It is known that below a certain critical threshold optimal dosage of drugs might result in treatment failure (Hastings *et al*., 2002). This could arise from counterfeit drugs or failure to adhere to treatment schedule. Parasitaemia observed could also be new infections after treatment particularly in areas of high transmission (Shunmay *et al*., 2004) such as Ghana. These two scenarios could be resolved by PCR analysis to establish whether parasitaemia observed after

treatment is caused by a recrudescence of drug-resistant parasites or by a new infection (Wellems & Plowe, 2001). These findings although not conclusive, are useful policy options for the treatment of uncomplicated malaria in children and possibly the adult population. This also makes it necessary for periodic surveillance and reviews of antimalarials drugs use and policy in the country.

### *5.5.2 Parasitological outcomes of ATR-AMQ under home management*



Children treated with ART-AMQ prepacked under home management had a relatively high proportion of treatment failures by days 14 (17.3%) and 28 (27.8%) compared to the ACTs trial study in school pupils. Only the day 28 parasitological failures were genotyped to correct for re-infections using *msp2* and *glurp* markers. A total of 7 samples were classified as indeterminate with one failure and were excluded from the final analysis. These samples either failed to amplify in the day 0, 28 or both by the PCR genotyping. This failure to amplify could be due to varied reasons including failure in species identification during microscopy, poor storage of filter samples, ineffective DNA extraction or the absence of the alleles in the parasite genotyped.

The treatment failure by day 28 corrected for re-infections by PCR was11.6% (Ajayi *et al.*, 2008b). This is contrary to Koram and colleagues (2005) with 100% parasite clearance after correcting for re-infection whilst Dorsey *et al*. (2007) in Ugandan children had 4.6% treatment failure. This failure rate recorded is higher than those reported in controlled Coartem and ART-AMQ efficacy studies (Dorsey *et al*., 2007; Koram *et al*., 2005). However, the cure rate is above the threshold below which WHO recommends changing antimalarial drug policy (WHO, 2001a). The high failure rate could be attributed to several factors including high baseline parasite densities (1000/µL) and sub-critical drug concentrations due to failure to complete treatment schedule (Shunmay *et al*., 2004).

#### *5.5.2.1 Parasitological outcomes and methodology limitations*

In interpreting these results, it is important to consider some limitations of the study methods. The protocol used to determine the parasitological efficacy of ACT is simply based on collection of blood samples done only at baseline and after 28 days of treatment for children treated at home (Ajayi *et al.*, 2008b). This has limited the capacity to detect the timing at which treatment failures occurred and to analyze the relative contribution of recrudescence and re-infection over time. Also, the follow-up procedure of children with malaria positive microscopy at baseline was mainly based on spontaneous reporting, which may have introduced a selection bias in the group of children that were tested at day 28.



Finally, adherence was measured by questionnaires administered to caregivers, providing potential for recall bias, and no determination of drug in blood levels was done. Most of these limitations derive from the fact that the study was carried out as part of a larger study to determine feasibility and acceptability of ACT use within HMM, including caregiver adherence and treatment coverage (Ajayi *et al.*, 2008a). In this context there was a need to minimize interference with spontaneous behaviours and real life conditions of use of antimalarials. A strict follow up protocol would have influenced the behaviour of the caregivers and thus limited the findings of the main study.

The community effective use of antimalarial treatment is known to be influenced by a variety of factors beyond parasitological efficacy. These include access to effective treatment, quality of prescription and caregiver adherence (Ajayi *et al*., 2008a). However, measuring the parasitological cure rate that can be obtained using ACT in the context of HMM is of fundamental importance, given the current call for widespread use of ACT at community level to move towards malaria elimination (WHO, 2006b). While further studies, carried out under more controlled conditions,

are necessary to establish the cure rate of ACT with a higher degree of precision, this study provides initial information and reassurance with regard to the effectiveness of ACT used at the community level under real life conditions of use. In this sense, findings of this study provide further evidence to support scaling-up implementation of HMM with ACTs.

#### *5.5.2.2 Adherence under home management*

Adherence by caregivers to the correct treatment was assessed in febrile children across the study communities. Adherence was measured in terms of caregiver's report of the number of doses administered to the child, the number of days over which the treatment was given and the promptness of treatment after onset of symptoms. Overall, 97% (C.I. 95%–99%) of children were treated correctly in terms of drug dose, duration of administration and level of adherence (Ajayi *et al*., 2008a). The level of adherence to treatment schedules and promptness to treatment directly affect the parasitological outcomes of treatment. The level of compliance in this study, however, is much better than the compliance with the national policy of ACTs use. According to report by the GHS (2007), just a little over 50% of all malaria cases were placed on ACT whilst in the Ashanti Region the proportion is less than 30%. It has been reported that some clinicians are using monotherapy which carries the risk of early emergence of resistance of the malaria parasite to the drugs so used (Butcher *et al*., 2000; Shunmay *et al*., 2004) whilst others are still using Chloroquine (GHS, 2007) which carries a significant risk of treatment failure.



### 5.6 Safety and tolerability of the study Drugs

The safety and tolerability of the study drugs are based on adverse outcomes following treatment of a given regimen of the study drugs. The reports on adverse events based on symptoms and signs only as reported by the child, caregivers or school teachers and CDDs within seven days are discussed in this part of the chapter.

### *5.6.1 Adverse manifestations reported at homes*

Adverse events recorded were itching, vomiting, and weakness in children treated at home using ART-AMQ and these were not life-threatening (Ajayi *et al*., 2008a). It is important to note that similar adverse events were also recorded in the earlier home management study using Chloroquine. It is possible that underweight children might have received higher doses but the practical and logistical advantage and overall benefit favoured the use of prepacks.



There have been reports on adverse events however, associated with the use of ART-AMQ in Ghana. For instance, the review of 55 adverse events reports submitted in Ghana (2005) to the National Pharmacovigillance Centre has identified 12 reports of dystonia and extrapiramidal reactions, 11 reports of "restlessness" and 24 reports of common adverse events, such as gastrointestinal upset and general weakness/fatigue. Six (6) brands were associated with these reactions, making a fault with a single brand unlikely. The review of these reactions pointed out that once daily exposure to ART 200mg/AMQ 600mg was common to all events, making it plausible that this dose might have been excessive in the Ghanaian subjects. In this study, ART50mg/ AMQ153mg daily dose and half of each were given to study children between 1 to 5 five years and those under one year respectively and this appeared to be effective and safe for the study children.

### *5.6.2 Adverse manifestations reported among school children*

About 35% of pupils reported adverse effects in the course of the follow up days. Adverse manifestations including itching, vomiting, dizziness, general weakness, nausea, headache and interference with routine daily activities were reported among school pupils.

All the study regimes (treatment dosages of drugs) except AMOTEX are safe and generally well tolerated and were similar to studies in Africa (Dorsey *et al*., 2007; Sowunmi *et al*., 2005; Abacassamo *et al*., 2004). AMOTEX recorded the highest cases of dizziness, general weakness, nausea, and headache and interfered with the routine daily activities compared to the other treatment groups and it was the only treatment arm that interfered with pupils routine activities. Some effects were very serious but not life threatening, however, no child was withdrawn because of drug intolerance. The co-formulation of ART and AMQ made it plausible that this dose might have been excessive in the subjects (the age category).



Most of the adverse effects were directly associated with the treatment drugs as these were not found in pupils who were not treated with the drugs. Four percent of the pupils who had serious adverse effects that interfered with their daily activities such as failure to attend school was only associated with the AMOTEX group. Dizziness was the highest adverse effects reported among the study pupils and ART-SP was the most tolerated, followed by COARTEM. These were however, not significant with respect to ART-AMQ group, but with AMOTEX group.

### 5.7 Molecular Epidemiology

Microscopy has been the method of choice for the diagnosis of malaria in endemic areas and for identification of species of *Plasmodium* causing human malaria. This has been the method employed in epidemiological studies of malaria sometimes with its resultant incorrect speciation in mixed infections, sub-species and of low levels parasitaemia may be missed. Thus, to overcome the limitations of microscopy for detection of malaria in epidemiological studies, molecular techniques have been employed such as polymerase chain reaction (PCR) based assays have been developed for detection of malaria parasites, species and sub-species in epidemiological studies, thus, molecular epidemiology. The biological technique, PCR was used to determine the parasite

genotypes in pre- and post- treatment samples of patients classified as failures by microscopy, genetic diversity, multi-clonality and resistant strains. Typing of *P. falciparum* in human hosts has been used to study the diversity of parasite populations (Magesa *et al*., 2001), parasite virulence (Greenwood, 2002), multiplicity of infection (Hoffmann *et al*., 2001) and the geographical distribution of the various alleles of these polymorphic genes of the parasite (Hoffmann *et al*., 2001).



### *5.7.1 Multiplicity of infections*

Multiplicity of infection (MOI) is a measure of the number of different *P.falciparum* strains simultaneously infecting an individual and is assessed by genotyping of polymorphic markers of genes. Most studies on the MOIs used *msp2* or *msp1* markers (Kobbe *et al*., 2006; Owusu-Agyei *et al*., 2002; Mbugi *et al*., 2006). From this study, MOIs were assessed by both *msp2* and *glurp* markers. MOIs were slightly higher with *msp2* marker of the IC/3D7 sub-family than with *glurp* and could be a better marker for detection of MOI study (Happi *et al*., 2004).

Molecular determination of parasites demonstrates that parasite multiplicity is very common in this region with over 50% of patients having multiple infections using both markers. However, in contrast to this study, MOIs in patients were nearly 85 % and 95% obtained by Owusu-Agyei *et al*. (2002) and Kobbe *et al*. (2006) in the Northern Ghana and the Middle/ forest belt of Ghana respectively. In many areas, the parasite population changes with time and season (Mbugi *et al*., 2006). Epidemiological data from studies from sites in West Africa attributed MOIs to be closely related to the intensity of malaria transmission (Owusu-Agyei *et al*., 2002; Happi *et al*., 2004) and also the epidemiology of drug resistant strains development of *P. falciparum* in treatment outcomes in patients (Happi *et al*., 2004).

MOIs reported in the present study are similar to reports in children in holoedemic areas in Tanzania (Mbugi *et al*., 2006) and Burkina Faso (Paganotti *et al*., 2004) but slightly less than reports in Northern and Middle belt of Ghana ( Kobbe *et al*., 2006; Owusu-Agyei *et al*., 2002). Other studies reported similar findings; however, differences in MOIs could be due to the limitations resulting from varying genotyping protocols of different laboratories and the category of study population (Kobbe *et al*., 2006; Owusu-Agyei *et al*., 2002; Ntoumi *et al*., 1995).The limitation resulting from this study on the MOIs could result from the genotyping protocol; the failure to apply Restriction Fragment Length Polymorphism (RFLP) to completely delimit close bands. The MOIs in an individual also depends on the gene marker and the level of endemicity (Greenwood, 2002; Franks *et al*., 2001). MOIs are found to be closely associated with drug resistance development especially with the *glurp* marker and this finding agrees with reports by Shunmay *et al*. ( 2004) and Ron *et al*., (2005).



### *5.7.2 Parasite genetic diversity*

Of the *msp2* gene allelic families detected, over 80% of the children had *P. falciparum* clones belonging to the IC/3D7 allelic family with only 2% having both FC27 and IC/3D7 sub-families. Allelic diversity in the *msp2* gene detected in this study was high with twenty (20) different IC/3D7 family alleles and four different FC27 family alleles. This shows that, of the two polymorphic *msp2* gene allelic families, the IC/3D7 was more prevalent in the study population than the FC27. IC/3D7 alleles were five times more distributed than alleles of FC27 family in the study population whilst in the *glurp* gene; eighteen (18) strains were detected in the population. The composition of *msp2* gene alleles in each allelic family in the present study was however, different from reports from similar studies in Ghana (Kobbe *et al*., 2006; Owusu-Agyei *et al*., 2002) and in Tanzanian children (Mbugi *et al*., 2006). These studies reported nearly 50% composition of IC/3D7 family alleles and FC27 family alleles contrary to the present study. The

differences could be due to differences in the geographical distribution of the various alleles of these polymorphic genes of the parasite (Hoffmann *et al*., 2001). The geographical distribution of the various alleles of these polymorphic genes of the parasite has implication on malaria control, treatment and virulence across the country.

The level of polymorphism and antigenic variation are survival strategies of the malaria parasites in human populations (Felger *et al*., 1999). In endemic areas, individuals experience numerous episodes of infection with varying symptoms and outcome as a result of a combination of factors both from the parasite and the host and have implications on interventions (Hoffman *et al*., 2001). The parasite surface proteins are polymorphic and antigenic and have integral role to the survival of the parasite (Aubouy *et al*., 2003; Mbugi *et al*., 2006).



### *5.7.3 Genotypic outcomes of different episodes of malaria in individual patients*

It has been observed in this study that over 69% and 87% of *msp2* and *glurp* had varying band fragment sizes ranging from 400 base pairs (bp) to 700bp respectively with most fragments ranging between 500bp and 650bp suggesting that these are predominant genotypes circulating in the study area. Longitudinal monitoring of *P. falciparum* strains was carried out only in children who had their first clinical episodes during the study period and were treated with ART-AMQ and followed up for up to at least three months until they developed another episode. Blood samples taken at the first episode (day 0) and second episode (3-month) were then genotyped and compared.

The band size fragments of the first episode (day 0) and the second episode (3-month) were compared to determine whether these were the same or different alleles in both episodes. In many outcomes, different alleles were detected in successive samples (3-month) from individual

children. This is probably due to new infections with different alleles (Contamin *et al*., 1996) and most samples for both episodes have MOIs. This could be explained as a result of the children acquiring variant-specific immunity to those strains of parasites on the first exposure (first episodes) and would not strive in the presence of their corresponding antibodies (Trape & Rogier, 1996; Snow & Marsh, 2002).

There were, however, outcomes where successive episodes (3-month samples) from individual children have one or more similar alleles with the first episode (day 0 samples). But these always occurred together with other different alleles in multiple infections. These alleles could be the same genotypes from new infections which are predominant in the study area (Smith *et al*., 1999b) or late recrudescent infections emerging from the liver (Sutherland *et al*., 2002 a, b & 2005). A previous chronically infected individual may develop clinical symptoms following the introduction of a new parasite with different genotype from the original one (Contamin *et al*., 1996) and this could explain the presence of genotypes in both episodes (day 0 and 3-month samples) from single individuals.

New infections from the second episode (3-month) emerging from the liver or from mosquito's bites with similar alleles to the first episode genotype, on its or their own could not elicit clinical symptoms and would require the presence of different genotypes from it or them (Contamin *et al*., 1996) to elicit clinical disease. This phenomenon is due to the presence of variant-specific immunity and could explain the low-grade infection during extended periods without clinical symptoms (Staalsoe *et al*., 1998). This has implication on malaria vaccine development since vaccine must contain a range of variant genotypes in a given area.

When red cells are infected by a parasite, the parasite inserts into the infected red blood cell membrane surface a protein, known as *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which is considered to be a key adhesive ligand mediating sequestration. In a process known as antigenic variation, clonal *P. falciparum* parasites can vary the type of PfEMP1 molecule they express, so as to avoid antibody-mediated clearance (Cheng *et al*., 1998; Kyes *et al*., 1999). This could explain the outcomes of the different clinical episodes in individual patients with different alleles. *P. falciparum* infections are persistent, and chronicity is promoted by antigenic variation at the infected red blood cell surface. Proteins of the repetitive interspersed family (rifins) are also expressed at the surface of infected red blood cells, and, like PfEMP1, these undergo antigenic variation (Cheng *et al*., 1998; Kyes *et al*., 1999). All the different antigenic variations generated from different proteins on the surface of the parasite have implication on malaria vaccine development especially using the erythrocytic stage of the parasite.



### *5.7.4 Resistant strains*

Recrudescent strains in *msp*2 were detected in both IC/3D7 and FC27 allele's sub-families. There were five different resistant strains detected in the *msp*2 gene with only one belonging to the FC27 family which had an approximate base pairs size of 380. The other four strains belonging to the IC/3D7 allelic family have approximate base pairs size ranging from 500 to 600. This allelic family was predominant in the study area and were exposed to sub-critical drug concentrations due to failure to adhere to treatment doses (Shunmay *et al*., 2004).

IC/3D7 allelic family generated different alleles with high frequency of antigenic diversity. Variability at certain loci could lead to parasite strains that differ in their ability to escape recognition by the host immune system and are resistant to certain antimalarial drugs (Ron *et al*., 2005). Multiplicity of infection also enhance resistance development to antimalarial drugs (Jelinek

*et al*., 1999; Schneider *et al*., 2002) and the risk of clinical disease. The high frequency of resistant strain in IC/3D7 allelic family resulted from its variability and multiplicity of infections in the study population.

In the *glurp* gene, four resistant strains were observed with approximate base pairs size ranging between 500 and 650. The high presence of resistant strains could be attributed to the high MOIs (Happi *et al*., 2004) with different parasite population with different drug response profiles selecting for the resistant strains. It has been observed in the present study that resistant strains in both *msp2* and *glurp* genes had band size fragments ranging from 500 to 650 base pairs. Over 69% of *msp2* and *glurp* have most band fragments ranging between 500bp and 650bp. Parasite genotypes with these band sizes are therefore predominant in the study area and some of the recrudescent / resistant strains could be new infections circulating in the study area (Smith *et al*., 1999b). However, the molecular basis for drug resistance in *Plasmodium* can not fully be explained since this type of study has its inherent limitations (Cattamanchi *et al*., 2003).


# CHAPTER SIX - CONCLUSIONS AND RECOMMENDATIONS

# CHAPTER SIX - CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Introduction

This chapter of the thesis summarizes the key findings and conclusions, recommendations to stakeholders and reflections for future research.

## 6.2 Summary of key findings and conclusions



The epidemiology of malaria in Ashanti Region has shown marked heterogeneities in malariometric indices in both urban and in rural settings. The parasitaemia prevalence of malaria in urban and peri-urban communities was 20.1% and 39.1% respectively. High parasitaemia incidence in urban communities is associated with vulnerable groups in low level of socioeconomic status. However, parasitaemia prevalence was 71% in febrile children seeking treatment from CDDs in a rural setting. The number of febrile episodes experienced per child seeking treatment from CDDs ranged from one to nine within 12 months of the study with a mean of 4.5 per year making malaria burden severe in this Region.

Parasitaemia prevalence rate amongst school children in a rural setting was as high as 58.6% with values ranging from 49.7% to 71% whilst 14% and 53% of pupils have severe anaemia and mild anaemia respectively. The results of this study demonstrate conclusively that the burden of malaria and anaemia amongst school pupils are sufficiently high and warrant intervention.

This is the first time in Ghana that CDDs were taught how to collect finger-prick blood samples in order to assess their own presumptive treatments in an HMM setting. This study has demonstrated conclusively that CDDs are trainable to make accurate decisions on treatment. The accuracy of

the CDDs and caregivers was quite high (71%). In the absence of diagnostic acumen and at the rate of febrile episodes experienced by children per year, it is imperative to employ the HMM strategy to prevent fatal febrile episodes in children.

The rates of treatment success by day 14 for the different ACTs ranged from 80.5% (ART-LAPDAP) to 100% (ART-MQ) and by day 28, 61.9% (ART-LAPDAP) and 92.9% (AMOTEX). Using the day-14 in-vivo method in the absence of PCR analysis, ART-MQ is superior in parasite clearance whilst ART-LAPDAP is inferior at parasite clearance and has failed to meet the threshold of 90% recommended by WHO (2006a). However, the cure rates by day 14 of the rest of ACTs were above the threshold below which WHO recommends changing antimalarial drug policy. The use of ART-AMQ in home management of malaria in rural setting was, however, found to be at the alert phase as recommended by WHO (2006a, b) after correcting for re-infections.

After pupils with malaria parasites were treated with ACTs, there were remarkable reduction of parasite rates and a significant corresponding improvement in the Hb concentrations over the baseline Hb concentrations. The change in mean Hb concentrations over the baseline Hb by day 14 ranged from 0.5g/dl (AMOTEX) to 0.9g/dl (ART-LAPDAP) and by day 28, ranged from 0.7g/dl (ART-SP) to 1.2g/dl (AMOTEX). The change in the mean Hb between groups were not conclusive, however, ART-LAPDAP was superior in Hb recovery by day 14 and AMOTEX by day 28. Malaria parasites appeared to be mainly responsible for the low values of Hbs observed among the school pupils.

AMOTEX recorded the highest cases (33.1%) of adverse effects compared to the other treatment groups and it was the only treatment arm that interfered with pupils routine activities whilst COARTEM has the least of adverse effects (7.2%). All the treatment groups were well tolerated except AMOTEX and it is therefore not recommended for use by children.

Of *P. falciparum* clones detected using *msp*2 marker, 18% were of FC27 allelic sub-family whereas 82% belonged to the IC/3D7 allelic family. Of the allelic diversity in the *msp*2 gene, twenty were IC/3D7 alleles and four FC27 alleles making IC/3D7 alleles five times more distributed in the study population whilst in the *glurp* gene, 18 strains were detected in the population. Amplification products of the polymorphic part of the *glurp* and *msp*2 genes of *P. falciparum* showed band fragment size variations with over 87% and 69% amplification product sizes ranging from 400bp to 700bp respectively. Multiplicity of infections observed were between 2- 6 infections per blood sample and 2 - 4 per blood sample in *msp*2 and *glurp* with overall means of 2.8 and 2.1 respectively. The risk of multiple infections developing recrudescence in *msp*2 and *glurp* genes by day 28 was 2 times and 5 times likely than in a single infection respectively.

The *P.falciparum* strains amplification products in different episodes of malaria per individual patient (days 0 and 3-month samples) had band sizes that differed from each another. However, where amplification products were similar in both episodes, this usually occurred in multiple infections and this was frequent with *glurp* marker. Five different resistant strains of *P.falciparum* were detected using *msp*2 gene with only one belonging to the FC27 family which had an approximate base pairs size of 380bp. The other four strains belonging to the IC/3D7 allelic family had approximate base pairs sizes ranging between 500 -600bp. Four resistant strains were observed in *glurp* with approximate base pairs sizes ranging between 500 -650bp.

### *6.2.1 Concluding remarks*

The prevalence of malaria is high and has shown marked heterogeneities in malariometric indices between neighbouring urban communities and rural communities in the study area. The burden of malaria and anaemia in school children in this area is sufficiently high and warrant investment for its control and management.

This is the first time in Ghana that CDDs were taught how to collect finger-prick blood samples and have shown that they are trainable and should be trained and supported to provide HMM and other health services at the community level.

At the current level of parasitological cure rate of less than 90% for Artesunate-Amodiaquine use in the Home Management of Malaria, this calls for a search for alternative treatment regimen. In the present study, Artesunate-Mefloquine combination was the most efficacious, safe and well tolerated alternative treatment regimen for uncomplicated malaria in children compared to the first line drug. It has demonstrated good parasite clearance although corrections for re-infections could not be determined.

The levels of polymorphism, antigenic variation, MOIs and the frequency of infections have implications on the treatment outcome. The choice of a first line drug therefore needs careful consideration of the genetic diversity and the frequency of infections of *P.falciparum* in the study area in addition to other epidemiological factors.

### 6.3 Recommendations

i. The Ministry of Health, National Malaria Control Programme and other stakeholders should prioritize when designing malaria control measures to target the most vulnerable groups, the urban and rural poor and should be place specific.

ii. National Malaria Control Programme should include schools in its malaria control programmes since schools can offer a potential delivery mechanism for interventions.



iii. The Government and the Ministry of Education should design school-based health programmes for malaria and this could be amongst the most cost-effective of public health interventions. This could act as catalyst, promoting learning and simultaneously reducing repetition and absenteeism. It can also be used as leverage for existing investments in schools and teachers.

iv. The Ministry of Health, the Ghana Health Service, Ghana Roll Back Malaria and National Malaria Control Programme with support from WHO/UNICEF should scale up HMM programme in the country to provide quality, appropriate and prompt treatment for malaria fevers in children.

v. Stakeholders should involve community leaders in the selection and training of community medicine distributors to speed up the scale-up process for the HMM programme. However, stakeholders should task research based institutions to monitor and review the HMM programme regularly to ensure that ACTs are used optimally.

**vi.** International Health Organisations such as the UNICEF, WHO/TDR and the Ministry of Health should support research to evaluate the cost-effective study on RDTs by trained CDDs at the community level before initiation of ACTs treatment to febrile children reporting to them. This could further eliminate and avoid treating non-malarial fevers with ACTs.

**vii.** Anti-Malaria Drug Review Task Force (AMDRTF), the National Pharmacovigillance together with research institutions should design a programme for regular malaria drug surveillance and further research need to be conducted on ACTs quality and usage in Ghana. The molecular epidemiology of malaria parasites in the different eco-zones and its impact on treatment outcomes also need further research.



## 6.4 Reflections for future research

A number of issues of importance remain unresolved such as the impact of intermittent preventive treatment of pupils (IPT) on their academic performance, effectiveness of ACTs use at home and *P.falciparum* diversity across different population and eco-zones.

### a) Malaria in school pupils

Individuals chronically infected with malaria parasites are usually unidentifiable by most malaria control programmes. This present study demonstrated that the burden of malaria amongst school children is sufficiently high and most of them were mildly anaemic and warrant intervention. The impact of repeated malarial episodes has detrimental effects on the development of the child, particularly their mental and cognitive function (Fernando *et al*., 2003a; Siân *et al*., 2004). When pupils with malaria parasites were treated with ACTs, there was a general improvement of the

haemoglobin levels during follow up and this has resulted in a decrease in the proportion of pupils with mild to moderate anaemia.

It is common knowledge that most schools in the rural areas in Ghana perform poorly at the basic and junior secondary school examinations and the later potential development is therefore hampered. Although the school performances of a child are multi-factorial, repeated absenteeism from school due to malaria plays a significant role in this regard.



In the light of these situations, the following questions need careful answers:

i. What are the consequences of malaria and anaemia on the cognitive function of pupils?
ii. What is the role of poor health as an intervening factor on pupils' education?
iii. Would IPT of pupils with ACTs improve their academic performance?
iv. Would IPT and school feeding programme improve academic performance of pupils?

The above questions could be addressed by undertaking a study with four arms:

a. Screening for malaria and anaemia and its impact on the cognitive function of pupils
b. Determining the impact of general poor health on pupils' academic performance
c. Using IPT alone and
d. Using IPT together with school feeding programme to determine their impact on pupils school performance

The goal of this study would be to compare the impact of IPT alone and IPT together with the school feeding programme on pupils academic performance and cognitive function.

**b) ACTs trials**

The proportion of treatment failure of the ACTs trial study by day 14 was generally low, except for ART-LAPDAP group that was about 20%. AMOTEX although had better parasite clearance than the others by day 28 was however, poorly tolerated among the study pupils. ART in combination with MQ showed a better potential for parasite clearance and was well tolerated. ART in combination with AMQ also gave good parasite clearance at both days 14 and 28 visits.



The current national policy for the management of uncomplicated malaria is ART plus AMQ which has been shown be to be efficacious, safe and tolerable and most favourable in terms of cost and local production (GHS, 2004a). Febrile children seeking treatment from CDDs in home setting were treated with ART in combination with AMQ. After day 28 follow up, the effectiveness of parasite clearance after adjusting for re-infection was 88.4% success. True parasitological failure of 11.6% for ART-AMQ use for the home management of malaria is in the alert period according to WHO guidelines on antimalarial drug policy.

If this failure rate should pertain in the general population, there will be the need for alternative treatments. However, ART-AMQ combination is poorly tolerated among the adult population (Ghana National Pharmacovigillance report, 2005) although this was well tolerated among children under-five years. It is alleged that many prescribers and patients are resorting to the use of ART as a monotherapy with the potential attendance of generating resistant strains of malaria parasites. In the event of these developments, ART in combination with MQ and COARTEM could be alternatives to the first line drugs if these would be well tolerated among the adult population. The sample sizes for MQ -ART and COARTEM were much smaller and corrections for re-infections could not be determined due to financial constraint making the findings inconclusive.

In the light of this, the following questions are worth asking:

i. How efficacious are these alternative treatment groups among febrile children?

ii. What is the level of usage, tolerance and acceptance of ART-AMQ as the first line drug for malaria case management among the adult population?

These questions can be addressed by carrying out a study on:

a. Antimalarial trial study using large sample size of febrile children and correcting for re-infection as end point.



b. The current usage, tolerance, acceptance and compliance with ART-AMQ use as the first line drug among the adult population.

The objective would be to determine alternative treatment regimes to ART -AMQ and the level of its current usage among the adult population.

**c) Presumptive treatment of malaria by caregivers and CDDs**

Although 71% of febrile children were positive and received early and appropriate treatment, 29% were without malaria parasites and were treated for malaria with ACTs. The current parasite-based diagnostic methods are microscopic examination of Giemsa-stained blood smears and the newly developed rapid diagnostic tests (RDTs) for malaria (Breman *et al*., 2007). A cost-effective analysis study on ACTs trial using RDTs is needed especially in a rural setting and under the HMM to treat only febrile children with parasites. CDDs in the present study have shown that they are trainable and can be trained and supported to provide this care and services at the community level. This requires the need to address the issues of cost, affordability, sustainability and usage of RDTs at the community levels in the context of HMM.

i. What would be the consequences of treating only RDT positive febrile children seeking treatment from CDDs?

ii. Would the use of RDTs be cost effective under HMM using ACTs?

These questions can be addressed by undertaking a study on:

a. Comparative cost effective analysis study on ACTs using RDTs and presumptive diagnosis

b. Cost effective analysis study on ACTs using RDTs under HMM



The objective of this would be to determine a cost effective diagnostic method for malaria in a rural setting for effective management of febrile cases in children.

**d) Genetic diversity**

The composition of *msp*2 gene alleles in each family and *glurp* gene in the present study was limited to children below five years. If the allelic diversity was studied across the general population, the composition of the diversity could be different. The level of polymorphism and antigenic variation are survival strategies of the malaria parasites in human populations (Felger *et al.*, 1999).

In endemic areas, individuals experience numerous episodes of infection with varying symptoms and outcome as a result of a combination of factors from both the parasite and host and this has implications on the interventions (Hoffman *et al*., 2001). The level of polymorphism, antigenic variation and the number of episodes individuals experienced would have implication on malaria vaccine development. Molecular determination of parasites using *msp*2 and *glurp* markers differed markedly from similar studies in Northern Ghana and the Middle/ forest belt of Ghana (Owusu-Agyei *et al*., 2002; Kobbe *et al*., 2006). Differences could be due to varying genotyping protocols

and study population. This thus, highlighted the need to address the issues of protocols and the category of study population. Under these circumstances, the following questions are worth asking:

i. Does the genetic diversity of *P. falciparum* vary among different populations?

ii. Does the number of episodes of infections affect the MOIs in an individual?

The above questions can be addressed by undertaking a study on:

a) Genetic diversity of *P. falciparum* in different populations using different markers

b) Longitudinal follow up study on the MOIs in individuals and in different populations

The aim would be to determine the genetic profile and antigenic variations of *P. falciparum* in order to determine its biological survival strategies.

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## REFERENCES


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# APPENDICES

## APPENDICES

### *APPENDIX 1 CONSENT FORM*

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY, COLEGE OF HEALTH SCIENCES/
DEPARTMENT OF THEORETICAL & APPLIED BIOLOGY, FALCUTY OF BIOSCIENCES OF COLLEGE OF SCIENCE

Molecular Epidemiology of *Plasmodium falciparum* in Ashanti Region,
Ghana in a Setting of Artesunate –Amodiaquine Use.

Certificate of Consent for Mothers/Caregivers

My child and I have been invited to take part in the research to assess the medium and long term safety, efficacy and side effects of Artesunate-Amodiaquine use for case management of childhood malaria in Ghana. I have been told the purpose of this research study is to improve the case management of childhood malaria in Ghana using Artesunate-Amodiaquine. The procedures will involve asking some questions, examination of my child, and collection of blood sample from my child for laboratory investigation at defined periods, treatment of my child, and follow visits to the clinic. I have been assured that if I am uncomfortable with any procedures or treatment, I have every right to withdraw from the study. I have also been assured that I am free to refuse to participate in or withdraw when I choose to. I have been assured that all information and answers I provide will be confidential and used solely for research purposes.

I consent voluntarily to allow my child to be a participant in this study and understand that I have the right to withdraw my child at any time without in any way affecting my medical care.

| | |
|---|---|
| Name of Subject | Date and Signature/Thumbprint of Subject |
| _____ | _____ |
| | ___/___/___ (*dd/mm/yy*) |
| Name of Independent Witness | Date and Signature of Witness |
| _____ | _____ |
| | ___/___/___ (*dd/mm/yy*) |
| Name of Researcher/Moderator | Date and Signature of Researcher/Moderator |
| _____ | _____ |
| | ___/___/___ (*dd/mm/yy*) |

## APPENDIX 2 DATA COLLECTION TOOLS

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY, COLLEGE OF HEALTH SCIENCES/
DEPARTMENT OF THEORITICAL &APPLIED BIOLOGY OF FALCULTY OF BIOSCIENCES, COLLEGE OF SCIENCE

Molecular Epidemiology of Plasmodium falciparum in Ashanti Region, Ghana in a Setting of Artesunate -Amodiaquine Use.

**[ME- ACTS STUDY]**

**SCREENING FORM**

**ROUND……../…….** **FORM 01**

**Study Number** [ ][ ][ ][ ] **IDENTITY No.** [ ][ ][ ][ ][ ][ ]



Date: OPD [ ][ ][ ][ ][ ][ ][ ][ ]

***Demographics***

| | |
|---|---|
| Childs name | |
| Childs Mother's name | |
| Childs Father's name | |
| Address: | |
| Locality: | |
| Village | |
| Date of Birth OPD (Dd/mm/yyyy)<br>[ ][ ] / [ ][ ] / [ ] [ ] [ ] [ ] | Sex of Child [ ]<br>1=Male 2=Female |

Why did you send the child to the health facility the past two weeks? ……[ ] [ ]

1 Need to consult since child is sick
2 Return for further treatment (illness worse, new illness)
3 Return after weekend consultation
4 Immunization (the child is also ill)
5 Immunization (the child is not ill)
6 Child was referred
7 Other …….……,

What are the main symptoms of this illness and for how many days?

| Symptoms | days |
|---|---|
| OPDSYM1 …….. | [ ][ ] |
| OPDSYM2 …….. | [ ][ ] |

Prompt in addition: did the child have: 1=Y 2= N days

| | | | |
|---|---|---|---|
| Rash OPD | | MRSHD | |
| Cough OPD | | MCGHD | |
| Difficult breathing OPD | | MBTHD | |
| Loss of weight OPD | | MWGTD | |
| Vomiting OPD | | MVOMD | |
| Diarrhoea OPD | | MDRHD | |
| Fever OPD | | MFEVD | |
| Swelling OPD | | MSWLD | |
| Unable to feed OPD | | MFDD | |
| Stiff neck OPD | | MNCKD | |
| Convulsions/unconscious OPD | | MCOND | |



If the child has had fever, has he/she had any of following?

| | | | |
|---|---|---|---|
| Hot body/joint pains, yellow body | | MHOTD | |
| Serious type of malaria | | MMALD | |
| Seriously ill: fever, vomit, fits | | MTALD | |

If the child has had diarrhoea, has he/she had any of the following

| | | | |
|---|---|---|---|
| Yellow/greenish diarrhoea | | MORI | |
| Bloody diarrhoea | | MATUD | |
| Foamy diarrhoea, skin colour change | | DFMD | |

Did the sick child visit anyone for health care before coming here? HCB [ ]

1=Y 2=N 8=D/K 9=N/A

If yes which (probe) [ ] [ ]

1= family/friend OPD

2= Traditional healer OPD

3= Community H/worker OPD

4= Spiritualist OPD

5= Homeopathic doctor OPD

6= Health Centre OPD

7= Shop/Kiosk OPD

10=Chemist OPD

11=Private clinic OPD

12= OPD Hospital OPD

Was the child referred to this health facility from one of the above services?
OPDREF1 1st [ ] [ ] 1=Y 2=N 8=D/K 9=N/A
If yes, which of the above services referred the child to come here?
OPDREF2 2nd [ ] [ ]

What illness do you think the child has now?
OPDMILL1 …….
1=Y 2=N 8=D/K 9=N/A
Did you treat the child for this illness before coming to the health facility? OPD [ ]

If yes, do you know the name of the treatment? OPD [ ]

If yes what is/are the name(s) of the medicine?

[ ] Traditional medicines/herbs OPD [ ] Chloroquine OPD

[ ] Analgesics (i.e. panadol) OPD [ ] Amodiaquine (Camoquine) OPD

[ ] Fansider, Oraphan, Metakelfin OPD [ ] Quinine OPD

[ ] Septrin (Cotrimoxazole) OPD [ ] Metronidazole (Flagyl) OPD

[ ] Tetracycline OPD [ ] Amoxycillin OPD

[ ] Nalidixic acid OPD [ ] Ciprofloxacin OPD

[ ] Gentamycin OPD [ ] Pen V OPD

[ ] Artesunate OPD [ ] Other OPD (Specify)……
Do you have any bednets in your house? OPD [ ] 1=Y 2=N 8=D/K 9=N/A
Does the child sleep under a bednet OPD [ ] 1=Y 2=N 8=D/K 9=N/A

***Diagnosis***:
[ ] Malaria OPD [ ] Conjunctivitis OPD

[ ] Pneumonia OPD [ ] Gastroenteritis OPD

[ ] Bronchitis OPD [ ] Diarrhoea OPD

[ ] URTI OPD [ ] Dysentery OPD

[ ] Measles OPD [ ] Intestinal worms OPD

[ ] Anaemia OPD [ ] Septic wound/ulcers OPD

[ ] Malnutrition OPD [ ] Meningitis OPD

[ ] Other OPD (Specify)……

***Treatment***:

[ ] Analgesics (i.e. panadol) OPD [ ] Amoxicillin OPD

[ ] Chloroquine OPD [ ] Nalidixic acid OPD

[ ] Amodiaquine (Camoquine) OPD [ ] Ciprofloxacin OPD

[ ] Fansidar, Oraphan, Metakelfin OPD [ ] Gentamycin OPD

[ ] Quinine OPD [ ] Valium OPD

[ ] Septrin (Cotrimoxazole) OPD [ ] Tetracycline OPDTTET

[ ] Pen V OPD [ ] Metronidazole (Flagyl) OPD

[ ] Other OPD (Specify)……



Was the child referred to seek hospital care? OPDHOSP [ ] 1=Y 2=N 8=D/K 9=N/A

CHECKED & CERTIFIED CORRECT BY …../……./…..... DATE

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY, COLLEGE OF HEALTH SCIENCES/

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Molecular Epidemiology of *Plasmodium falciparum* in Ashanti Region,

Ghana in a Setting of Artesunate -Amodiaquine Use.

**[ME- ACTS STUDY]**

**ROUND……../……GENERAL**

## LABORATORY - FORM 02A

**IDENTITY No.** | | | | | | |  **STUDY No.** | | | | |

Name of Child

…….....

Date of Birth | | | | | | | | |

Address

.....……..…….....

Date of Visit/Exam | | | | | | |

Follow Up Visit Number | | |

Axillary Temp $^0$C | | | . | |

Laboratory No. | | | | |

Staff code | | |

Name of Recorder .....

**BLOOD FILM EXAMINATION**

Date | | | | | | |

Malaria Parasites seen | 1. Yes | 2. No |

Parasite count per 200 wbcs | | | | | | |

P. Falciparum (ASEXUAL) | | | | | | |

P. Falciparum (GAMETOCYTES) | | |

P. Malariae | | |

P. Ovale | | |

P. Vivax | | |

**HEMOCUE RESULTS**

Date | | | | | | |

Haemoglobin (g/l) | | |

CERTIFIED CORRECT BY DATE……/……/……/ | | CCB

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY, COLLEGE OF HEALTH SCIENCES/
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**[ME- ACTS STUDY]** **DAY: [0] [14] [28]**

**VISIT:...../..... In-vivo Study: (CQ, SP, ART, AQM, COAR, MQ & AP)**

## LABORATORY - FORM 02C: IN-VIVO

**IDENTITY No.** | | | | | | | **STUDY No.** | | | | |

Name of Child
.....

Date of Birth | | | | | | | | |

Address
.....

Date of Visit/Exam | | | | | | |

Follow Up Visit Number | | |

Axillary Temp $^0$C | | | . | |

Laboratory No. | | | | |

Staff code | | |

Name of Recorder .....

**BLOOD FILM EXAMINATION**

Date | | | | | | |

Malaria Parasites seen | 1. Yes | 2. No |

Parasite count per 200 wbcs | | | | | | |

P. Falciparum (ASEXUAL) | | | | | | |

P. Falciparum (GAMETOCYTES) | | | |

P. Malariae | | | |

P. Ovale | | | |

P. Vivax | | | |

**HEMOCUE RESULTS**

Date | | | | | | |

Haemoglobin (g/l) | | | |

| | | | |
|---|---|---|---|
| WEIGHT (kg) | | | |
| HEIGHT (cm) | | | |

CERTIFIED CORRECT BY DATE...../...../...../ | | | CCB

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY, COLLEGE OF HEALTH SCIENCES/
DEPARTMENT OF THEORETICAL & APPLIED BIOLOGY OF FALCULTY OF BIOSCIENCES, COLLEGE OF SCIENCE

Molecular Epidemiology of Plasmodium falciparum in Ashanti Region,

Ghana in a Setting of Artesunate -Amodiaquine Use.

**[ME-ACTS STUDY]**

**ROUND……../…….** **FORM 03: ADVERSE EVENT** **Date**:……/……./…….

**Study Number** | | | | | **IDENTITY No.** | | | | | | |

Date of Birth | | | | | | | | | Sex 1. Male 2. Female



Name of Child ……. Name of Mother……..…

Locality:…… Date of Visit ……/……/……/……/……/…….

Name of Recorder…… Source…….

Has your child been sick for the past two weeks? 1. Yes 2. No

If yes, did you give any medicine? 1. Yes 2. No

If yes, describe any problems after the child taking the drug. (Tick appropriate side effects)

| **EFFECTS** | **Yes (S)** | **Yes (P)** | **No (P)** |
|---|---|---|---|
| Felt Alright | | | |
| Headache | | | |
| Nausea | | | |
| Vomiting | | | |
| Dizziness | | | |
| Weakness | | | |
| Skin Reaction | | | |
| Fits | | | |
| Wheezing/Asthma | | | |
| Others | | | |

**[(S) -SPONTANEOUS (P) - PROBED]**

CHECKED & CERTIFIED CORRECT BY DATE …../……./……..

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**[ME-ACTS STUDY]**

**ROUND**……/……. **Hospital Referral/ Surveillance: FORM 04**

STUDY No. | | | | | | IDENTITY No. | | | | | | |

Recorder: …….. Date: | | | | | | | | |

***Health facility*** …….. | | | | | | | |



***Demographics***

| | |
|---|---|
| Childs name | |
| Childs Mother's name | |
| Childs Father's name | |
| Address: | |
| Locality: | |
| Village | |
| Date of Birth (Dd/mm/yyyy)<br>[ ][ ] / [ ][ ] / [ ][ ][ ][ ] | Sex of Child: 1.Male 2.Female [ ] |

Date of admission: (dd/mm/yyyy) | | | | | | | | |
Time of admission (GMT) | | | | | | |
Date of examination/interview: | | | | | | | | |

Why did you bring the child to the hospital today?
1. Need to consult since child is sick
2. return for further treatment (illness worse, new illness)
3. Child was referred from health centre
4. Other _____

What are the main symptoms of this illness and for how many days?

| | Symptoms | | | | days | |
|---|---|---|---|---|---|---|
| IPDSYM1 …… | | | | IPDSYM1D | | |
| IPDSYM2 …… | | | | IPDSYM2D | | |
| IPDSYM3 …… | | | | IPDSYM3D | | |
| IPDSYM3 ……. | | | | IPDSYM4D | | |

Prompt in addition: did the child have 1=Y 2=N days

| | | | |
|---|---|---|---|
| Rash | | IPDMRSHD | |
| Cough | | IPDMCGHD | |
| Difficult breathing | | IPDMBTHD | |
| Loss of weight I | | IPDMWGTD | |
| Vomiting | | IPDMVOMD | |
| Diarrhoea | | IPDMDRHD | |
| Fever | | IPDMFEVD | |
| Swelling | | IPDMSWLD | |
| Unable to feed | | IPDMFDD | |
| Stiff neck I | | IPDMNCKD | |
| Convulsions/unconscious | | IPDMCOND | |



If the child has had fever has he/she had any of following?

1=Y 2=N 8=D/K 9=N/A

| | | | days |
|---|---|---|---|
| Hot body/joint pain, yellow body | | IPDMHOTD | |
| Serious type of malaria | | IPDMMALD | |
| Serious ill: fever, vomit, fits | | IPDMTALD | |

Did the sick child visit anyone for health care before coming here? IPDHCB ……... [ ]

If yes which (probe) 1=Y 2=N 8=D/K 9=N/A

| | | | |
|---|---|---|---|
| 1 = Family/friend IPDVFF | | 2 = Traditional healer IPDVTH | |
| 3 = Community Health Worker IPDVCHW | | 6 = Health Centre IPDVHC | |
| 7 = Shop IPDVSH | | 10 = Chemist IPDVCH | |
| 11 = Private Clinic IPDVPC | | 12 = OPD Hospital IPDVHOS | |
| 13= Spiritualist IPDSP | | 14 = Other _____ | |

Was the child referred to this health facility from one of the above services? IPDREF …… [ ]

If yes, which of the above services referred the child to come here? OPDWREF1…... ……. [ ] [ ]

What illness do you think the child has now?

| | | | | |
|---|---|---|---|---|
| IPDMILL1 | | | | |
| IPDMILL2 | | | | |
| IPDMILL3 | | | | |

1=y 2=N 8=D/ 9=N/A

Did you treat the child for this illness before coming to the health facility? IPDMTRB…..…[ ]

If yes, do you know the name of the treatment? IPDMTRN ……..[ ]

If yes what is the name(s) of the medicine?

| | | | |
|---|---|---|---|
| Traditional medicines/herbs IPDMTTM | | Chloroquine IPDMTCQ | |
| Analgesics (i.e. asprin, panadol) IPDMTAN | | Amodiaquine (Camoquine) IPDMTAM | |
| SP(Fansidar, Oraphan, Metakelfin) IPDMTSP | | Quinine IPDMTQU | |
| Septrin (Cotrimoxazole) IPDMTCO | | Metronidazole (Flagyl) IPDMTMET | |
| Tetracycline IPDMTTET | | Ampicillin IPDMTAMP | |
| Nalidixic acid IPDMTNAL | | Ciprofloxacin IPDMTCIP | |
| Gentamycin IPDMTGEN | | Pen V IPDMTPEN | |
| Valium IPDMTVAL | | Other IPDMTOTH (Specify below | |
| Artesunate IPDART | | | |
| | | IPDDRG ……. | |



**Admission evaluation**

1=Y 2=N 8=cannot evaluate

Physical examination

Respiratory Rate (in 30 secs) IPD [ ][ ] Nasal flaring IPD [ ]

Pulling at ribs IPD [ ] Grunting IPD [ ]

Temperature ($^0$C) IPD [ ][ ] [ ]

Palpable liver present IPD [ ] Splenomegaly IPD [ ]

Weight (kg) [ ][ ] [ ] Height (cm) IPD [ ][ ][ ]

**Laboratory investigations**

Blood Examination: Was a blood slide taken? IPD …… [ ]

Date blood taken (dd/mm/yyyy) IPD [ ][ ][ ][ ][ ][ ][ ][ ]

Was hospital treatment started before the blood slide was taken IPD …….. [ ]

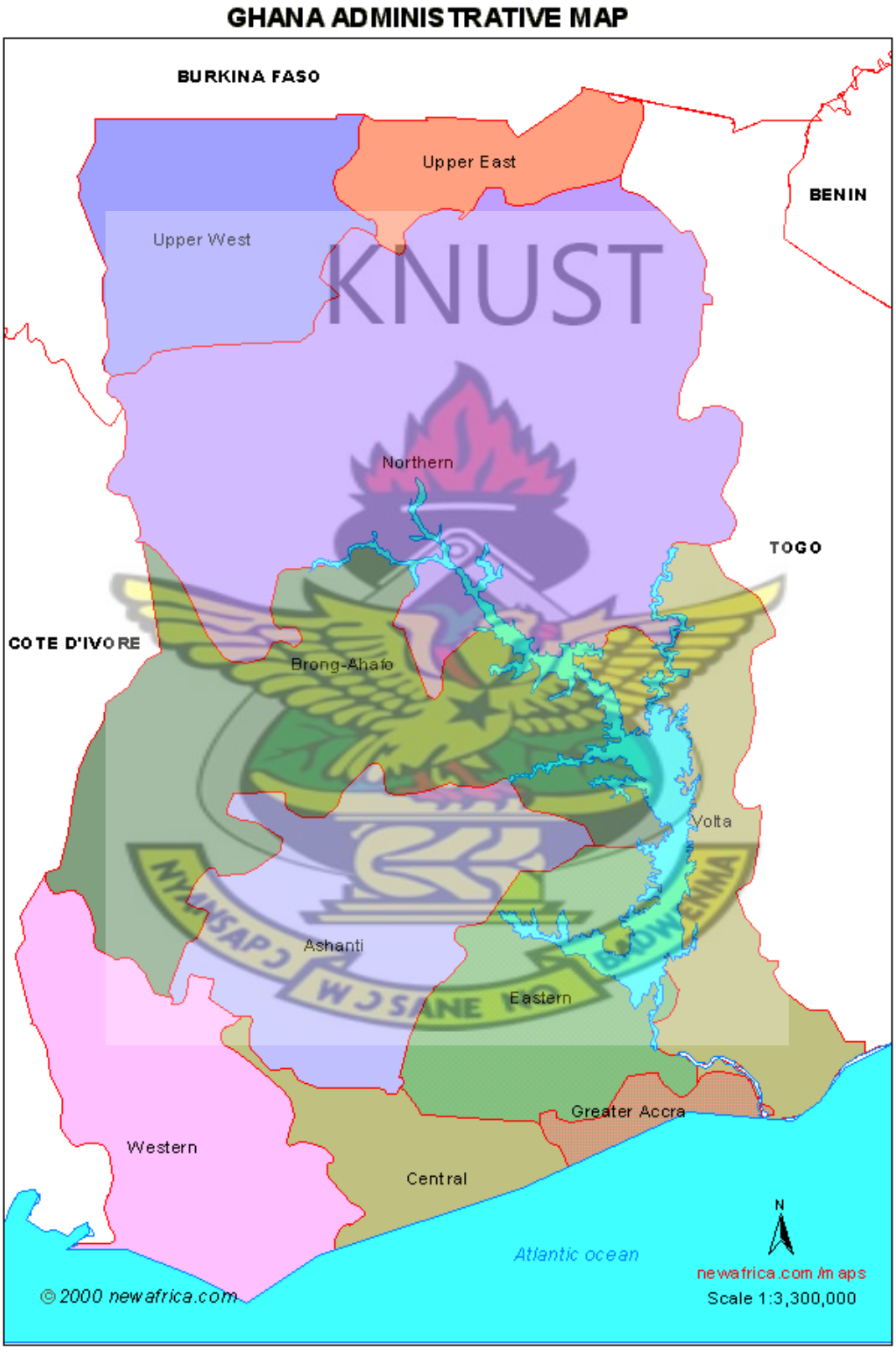
Smear results: IPDLBSPEC1 Species 1 [ ]
IPDLBSPEC2 Species 2 [ ]

0 No parasites found
1 P.falciparum
2 P. falciparum gametocyte
3 P. malariae
4 P. Ovale
5 No second species
8 Smear taken; no results
9 Smear not taken

Parasite density IPD [ ][ ][ ][ ][ ] Hct (%) IPD [ ][ ][ ][ ]

Haemoglobin (g/dl) IPD [ ][ ][ ][ ][ ] WBC IPD [ ][ ][ ]

## APPENDIX 3 MAP OF GHANA



**APPENDIX 4** RESULTS OF TREATMENT FAILURE AND DATA ON DRUG ADVERSE EFFECTS

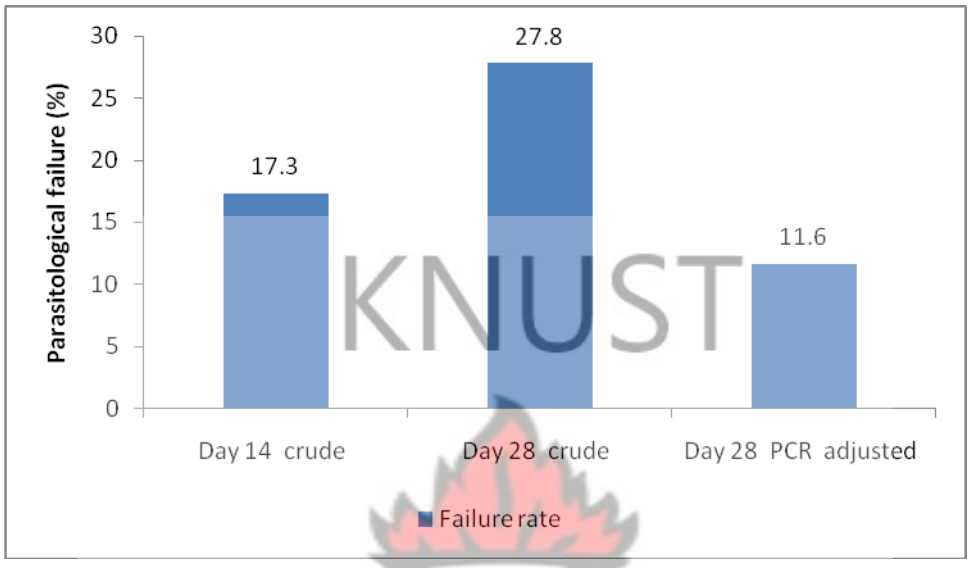



**Figure 1: Parasitological failure by days 14 and 28 for home base use of ART+AMQ**

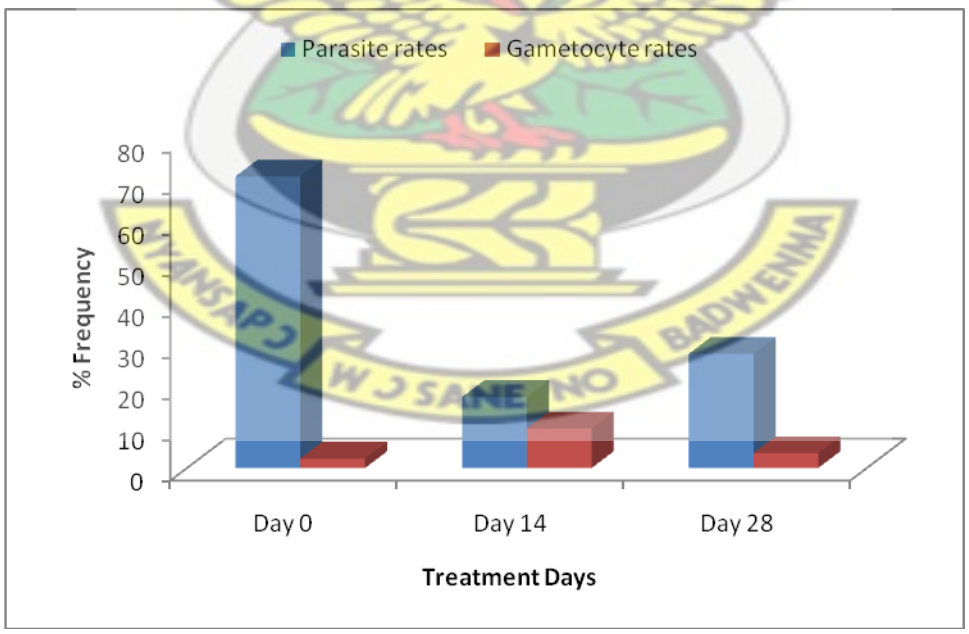



**Figure 3: Comparison of parasite and gametocyte rates at days 0, 14 and 28**

**Table: 1 Data on drug adverse effects in school pupils**

| Adverse Effects | ART+SP | ART+AMQ | ART+MQ | ART+LAPDAP | COARTEM | ART+CQ | AMOTEX | Total |
|---|---|---|---|---|---|---|---|---|
| Itching | 4 | 7 | 1 | 3 | 1 | 7 | 6 | 29 |
| Vomiting | 2 | 4 | 4 | 2 | 5 | 2 | 3 | 22 |
| Dizziness | 3 | 9 | 4 | 3 | 2 | 2 | 13 | 36 |
| General Weakness | 2 | 7 | 3 | 2 | 1 | 4 | 13 | 32 |
| Nausea | 1 | 3 | 2 | 4 | 2 | 2 | 3 | 17 |
| Headache | 2 | 3 | 2 | 2 | 1 | 3 | 10 | 23 |
| Routine activities interfered | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 7 |
| Total | 14 | 33 | 16 | 16 | 12 | 20 | 55 | |

**APPENDIX 5 TREATMENT PROTOCOL FOR THE INVIVO STUDY**

| DRUG | AGE (yrs) RANGE | WEIGHT (Kg) | DOSAGE (Tablets) | | | |
|---|---|---|---|---|---|---|
| | | | D1 | D2 | D3 | COMMENT |
| Chloroquine-150mg/kg | 5-7 | 19-24 | 1.5 | 1.5 | 1 | |
| | 8-10 | 25-35 | 2.5 | 2.5 | 1 | |
| Amodiaquine-200mg/kg | 5-7 | 19-24 | 1.5 | 1 | 1 | |
| | 8-10 | 25-35 | 2 | 2 | 1.5 | |
| AMQ-153mg/kg | 5-7 | 19-24 | 1.5 | 1.5 | 1.5 | |
| | 8-10 | 25-35 | 2.5 | 2.5 | 2 | |
| SP | 3-5 | 14.1-20 | 1 | - | - | |
| | 6-8 | 20.1-30 | 1.5 | - | - | |
| | 9-11 | 30.1-40 | 2 | - | - | |
| Mefloquine-15mg/kg | 5-7 | 17-24 | 1.5 | - | - | |
| | 8-10 | 25-35 | 2.0 | - | - | |
| MQ 25mg/kg | 5-7 | 17-24 | 1.5 | 1.0 | - | |
| | 8-10 | 25-35 | 2.0 | 1.5 | - | |
| COARTEM-40mgA+240mgL | 4-7 | 15-24 | 0hr, 8hr, 24hr, 36hr, 48hr & 60hr (start 12noon, 8pm,12noons | | | |
| 60mgA+360mgL | 8-10 | 25-35 | 0hr, 8hr, 24hr, 36hr, 48hr& 60hr (start 12noon, 8pm,12noons | | | |
| Artesunate 50mg | 4-7 | 19-24 | 1.5 -2 | 1.5- 2 | 1.5 - 2 | |
| | 8-10 | 25-35 | 2- 2.5 | 2- 2.5 | 2- 2.5 | |
| Lapdap | | | 1-2 | 1-2 | 1-2 | |
| | | | | | | |
| Amotex | 4-10 | 19- 35 | 1 | 1 | 1 | |
| | | | | | | |