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**Efficacy, safety, tolerability of Dihydroartemisinin
Piperaquine and Sulfadoxine-Pyrimethamine plus
Amodiaquine for Seasonal Malaria Chemoprevention
(SMC) in children in Burkina Faso**



Issaka Zongo

**Thesis submitted to the University of London in fulfilment
of the requirements for the Doctorate of Philosophy**

2014

**Infectious Disease Epidemiology Department
Faculty of Epidemiology and Population Health
London School of Hygiene and Tropical Medicine
Keppel Street, London WC1E 7HT**

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Statement of the author's role

I declare that this thesis is my own work, completed under the supervision of Dr. Paul Milligan. I acknowledge the following assistance and collaboration: The analysis of antimalarials in blood samples described in chapters 5 and 7 was carried out by Dr. Joel Tarning (Thailand) and Dr. Harparkash Kaur from the London School of Hygiene and Tropical Medicine. Laboratory analysis of molecular markers from samples from asymptomatic at baseline and at the end of the transmission season, presented in chapter 6 on drug resistance, was done at the Department of Medicine of San Francisco General Hospital by Mr Some Fabrice. Where results from the published or unpublished work of other people have been used in the thesis I have cited the appropriate references.

The trial in Burkina Faso was sponsored by Holley Cotec through a grant to Prof François Nosten and Jean Bosco Ouedraogo, in collaboration with Prof Phil Rosenthal. Initially a study of the use of DHA-PQ for prophylaxis in infants, compared to SP, had been planned. This plan was subsequently thought to be unsuitable because of the seasonality of malaria transmission in Burkina, the high burden in older children, and because by the time of the trial, SP+AQ had been established as the optimum regimen for seasonal prophylaxis. Therefore, following discussion with my supervisor, and with agreement from Prof François Nosten and Prof Jean Bosco Ouedraogo, I planned a study of seasonal Intermittent Preventive Treatment (now called Seasonal Malaria Chemoprevention) in children under 5 years of age, comparing DHA-PQ with SP+AQ in a non-inferiority study. I had primary responsibility for writing the protocol, preparing the Standard Operating Procedures and questionnaires, the ethics submissions to LSHTM and Burkina committees, registration of the trial, selecting the field sites, training the staff, organising the field work, supervising the enrollment and follow-up of study subjects, supervising data entry, writing the analysis plan and the statistical analysis, with guidance from Dr Milligan and advice from my advisory panel (Prof Greenwood, Prof Chandramohan, Dr Sutherland, later to be joined by Dr Matt Cairns). I prepared the first draft of a manuscript reporting the trial findings for submission for publication.

Signed:..... Date:.....

Issaka Zongo, February 2014

Abstract

Children in areas of highly seasonal malaria transmission in the Sahel should receive SMC with sulfadoxine-pyrimethamine plus amodiaquine (SPAQ). These drugs retain their efficacy in the areas where SMC is recommended, but alternative regimens are needed if SMC is used in other areas or if these drugs start to lose efficacy. The aim of this study was to investigate the suitability of dihydroartemisinin-piperaquine (DHAPQ) for SMC, using a non-inferiority trial design. 1500 children randomized to receive SPAQ or DHAPQ monthly from August to October, and a cohort of untreated children outside the trial, were followed-up for malaria. SPAQ was more efficacious than DHAPQ, but the difference was within the margin set for non-inferiority. Both regimens gave a very high level of protection lasting 4 weeks. Protection was related to dosage. Both regimens were well tolerated, incidence of mild adverse events decreased in successive months, consistent with toleration to study drugs. In malaria cases, the frequency of the CVIET haplotype of *pfprt*, the 86Y polymorphism of *pfmdr1*, and *pfdhfr59* and *dhps437* mutations, was greater among children who received SPAQ than in untreated children. However the number of cases, and the prevalence of parasitaemia, was much lower in treated children, reducing the scope for SMC to select for resistance. The frequency of the CVIET haplotype of *PfCRT*, thought to be associated with resistance to PQ, was not increased in children treated with DHAPQ. There was an enormous burden of malaria in the untreated children. SMC with SPAQ should be introduced for children in Burkina Faso without delay. DHAPQ is a potential alternative regimen in areas where SPAQ cannot be used but there are some drawbacks associated with its use. There is a need to develop alternative long-acting drugs with simple regimens that can be used for chemoprevention of malaria.

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Acronyms and abbreviations used in the thesis

ACT	Artemisinin-based Combination Therapy
AL	Artemether-Lumefantrine
AQ	Amodiaquine
AS+AQ	Artesunate+Amodiaquine
AS+MQ	Artesunate+Mefloquine
ASME	Advanced Statistical Methods in Epidemiology
ATP	According To Protocol
AUC	Area Under the Curve
C _{max}	Maximum Concentration
CQ	Chloroquine
CV8	Antimalarial drug combination
CVIET	PfCRT resistant halpotype
CVMNT	PfCRT sensitive halpotype
CYP450	Cytochrome 450
cyt b	Cytochrome b
DDT	DichloroDiphenylTrichloroethane
DHAPQ	Dihydroartemisinin-piperaquine
DNA	Desoxyribo-Nucleic Acid
Dpalt	Dihydroartemisinin-piperaquine alternatif
DPm	Dihydroartemisinin-piperaquine monthly
DSS	Data Surveillance System
EDTA	EthyleneDiamine Tetraacetic Acid
EIR	Entomological Innoculation Rate
EPI	Expanded Programme of Immunisation
GMP	Good Manufacturing Practice
HIV	Human Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography
HR	Hazard Ratio
IAEA	International Agency Energy Atomic
ICH	International Conference of Harmonisation
IPD	Intestinal Parasitic Disease
IPT _c	Intermittent Preventive Treatment in Children
IPT _i	Intermittent Preventive Treatment in infant
IPT _p	Intermittent Preventive Treatment in Pregnancy
IQR	Inter Quartile Range
IRS	Indoor Residual Spraying
IRSS	Institut de Recherche en Science de la Sante
ITN	Insecticide Treated Bednet
ITT	Intention To Treat
LLIN	Long Lasting Insecticide Treated Net
LLoQ	Lower Limit of Quantification

LoD	Lower limit of Detection
LoQ	Lower Limit of Quantification
LSHTM	London School of Hygiene and Tropical Medicine
MDA	Mass Drug Administration
MQ-SP	Mefloquine+Sulfadoxine-pyrimethamine
NMCP	National Malaria Control Program
NGO	Non-Governmental Organisation
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
<i>pf</i>	plasmodium falciparum
<i>pfprt</i>	plasmodium falciparum Chloroquine Resistant Transporter
<i>pfmdr-1</i>	Plasmodium falciparum Multidrug resistance
<i>dhfr</i>	Plasmodium falciparum Dihydrofolate Reductase
<i>dhps</i>	Plasmodium falciparum Dihydropteroate synthase
PhD	Doctor in Philosophy
PK	Pharmacokinetic
PQ	Piperaquine
qPCR	Real Time Polymerase Chain Reaction
RCH	Reproductive and Child Health
RDT	Rapid Diagnostic Test
RGPH	Recensement General de la Population et de l'Habitat
RMD	Rising Multiple Doses
RSD	Rising Single Dose
SD	Standard Deviation
sIPTc	Seasonal Intermittent Preventive Treatment in children
SMC	Seasonal Malaria Chemoprevention
SME	Statistical Methods in Epidemiology
SP+AS	Sulfadoxine-pyrimethamine+Astesunate
SP+PQ	Sulfadoxine-pyrimethamine+Piperaquine
SPAQ	Sulfadoxine-pyrimethamine-Amodiaquine
STD	Sexually Transmitted Disease
T _{1/2}	Half life
T _{max}	Maximum Concentration Time
UCSF	University of California, San Francisco
UNDP	United Nation Development Program
UNICEF	United Nation Children's Fund
VHW	Village Health Worker
WHO	World Health Organization

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“Bless the LORD, O My soul and all that is within me, bless his holy name” (Psalms 103:1)
“...Till now the Lord has helped us” (1 Samuel 7:12)

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Chapter 1 Introduction

Seasonal Malaria Chemoprevention (SMC) is now recommended by WHO, in areas of seasonal transmission in the Sahel children should receive sulfadoxine-pyrimethamine plus amodiaquine (SPAQ) each month, to protect them from malaria. These drugs remain effective in the areas where SMC is recommended, but other regimens may be required, both for use for SMC in other parts of Africa with seasonal malaria but where there is resistance to SP, and in the Sahel if SPAQ starts to lose efficacy due to resistance. The relatively long half-life of piperazine makes dihydroartemisinin-piperazine (DHAPQ), a potentially good choice for SMC. The purpose of this study was to investigate the suitability of DHAPQ for SMC.

1.1 Malaria in Sub Saharan Africa

Despite more than 100 years of research since the discovery of the malaria parasite in human blood by Charles Laveran in 1880 and the establishment of the mosquito's role in the transmission of malaria by Sir Ronald Ross in 1898, malaria continues to be a threat for the Sub-Saharan Africa population (Raghavendra et al., 2011). A precise estimation of the burden of the disease cannot be made but according to the world malaria report for 2012 there were 216 million (95% credible interval 149-274) cases of clinical malaria in 2011 of whom 81% or 174 million cases occurred in the African region. Approximately 655 000 malaria deaths occurred in 2011 of which 91% or 596 050 were in the African region; deaths in children less than 5 years old accounted for 86% of the estimated deaths. To face this unacceptably high burden of malaria in the world and mainly in Sub Saharan Africa, more attention has been paid to the disease in the 21st century than previously, with much more political and social support. Different approaches have been proposed and the best strategy has been subject to debate. It has been argued that malaria could be defeated through progressive and integrative activities including health infrastructure strengthening, training of qualified staff, improved access to diagnosis and treatment, environmental management, and improvement of economic conditions. Those opposed to this approach argue that it is too slow and that larger-scale more aggressive and formal programmes are required in order to quickly prevent and eradicate malaria from the world.

1.2 The historical malaria eradication program

Eradication is defined as the permanent reduction to zero of the worldwide incidence of an infectious organism as a result of deliberate efforts (Enayati and Hemingway, 2010). The

commitment seen in the 20th century nourished the hope that malaria could be eradicated through a comprehensive program. This program relied heavily on the use of DichloroDiphenylTrichloroethane (DDT) for Indoor Residual Spraying (IRS) between 1957 and 1969, and Mass Drug Administration (MDA). Substantial progress was made with IRS: a reduction by 50% of the total population at risk by 1975 and a dramatic decrease in the mortality rate from 19.4 per 10 000 in 1900 to 1.61 per 10 000 in 1975. Despite these initial successes, serious technical, financial and logistic issues led to the failure of this strategy, and prevented it ever reaching tropical Africa.

MDA is defined as the administration of a full therapeutic dose of an antimalarial regimen to an entire population or well defined sub-population at the same time. The strategy was initiated in the 1930's and endorsed by the World Health Organization in the 1950's as an additional tool to replace failing strategies (WHO, 1951). MDA in conjunction with other control measures was successful in a number of situations: MDA with sulfalene-pyrimethamine combined with IRS achieved high level of efficacy in the Garki project in the Northern Nigeria in 1969 (Molineaux, 1980), but did not interrupt transmission. MDA works through the reduction of the prevalence of peripheral parasitaemia and the reduction of transmission via the inhibition of the liver or asexual intra erythrocytic stages of the parasite, the direct action on the gametocytes or a sporonticidal effect and the inhibition of the sporogonic cycle in the mosquitoes. Considering the short-lasting benefit of MDA one modification has been to repeat drug administration, the precursor to the development of Intermittent Preventive Therapy. More than four decades later, malaria eradication is back on the global health agenda, and was discussed at the 2007 Malaria Forum. Recently large and rapid scale-up of effective malaria control interventions has led to a consequent reduction in the number of malaria cases and deaths in some areas. It is hoped that with this encouraging evidence, with currently available tools, malaria can be better controlled, and even eliminated at least in some countries and regions. However, a broad consensus also exists that new tools focused specifically on the interruption of transmission need to be developed if malaria eradication is to be eventually achieved. While investigating new tools and exploring new strategies, in areas where the disease is still of high burden the most realistic approach probably relies on programs that aim to control rather than eliminate malaria.

1.3 Malaria control programmes

Malaria control has three main components: vector control, which aims to prevent contact with mosquitoes; chemotherapy if infectious contact happens and results in a clinical case;

and finally chemoprevention to prevent new cases and to contain current infection from further development to a clinical case.

1.3.1 Vector control

Vector control is a key part of the strategies to prevent and reduce the burden of malaria in endemic countries, by reducing transmission. It has been endorsed by WHO and the RBM partnership. The WHO Study Group on Vector Control for Malaria and other Mosquito-borne Diseases defined selective vector control as the application of targeted, site-specific control activities that are cost-effective (WHO, 1995). Vector control measures aim to prevent physical contact with the vectors and include Insecticide Treated Nets (ITNs) or Long Lasting Insecticide-Treated Nets (LLINs), and Indoor Residual Spraying (IRS).

1.3.1.1 Insecticide Treated Bed Nets (ITNs)

The Roll Back Malaria strategy relies mainly on the use of the ITNs which has shown to reduce all causes mortality in children less than 5 years old by a mean of 17% in the first year of their implementation (Reyburn et al., 2005). Studies on the efficacy of ITNs have showed a positive impact on entomological parameters (Lengeler, 2000). Report from key studies (Bradley et al., 1986) in the Gambia and in 1991 by Alonso and colleagues (Alonso et al., 1991) showing a negative association of ITN use with mortality placed a real hope in the use the insecticide treated bed nets. Despite this proven efficacy, the achievement of widespread use has been difficult because of sociologic or financial barriers or insufficiency of the provision. Recent considerable effort has been made to make the ITNs – LLINs available to those who need it: The ITNs were distributed free of charge to pregnant women and children less than 5 years old during the antenatal care and during the EPI vaccination and large campaign of distribution free of charge by the national malaria control program or at a subsidized price by other donors like NGOs has permitted a high level of ITNs ownership (more than 80% of household in 2011) in Burkina Faso according the world malaria report in 2011. The treated bed nets are effective on the female mosquitoes attracted by the human seeking for blood while those resting on the walls are better captured through the treatment of these surfaces.

1.3.1.2 Indoor Residual Spraying (IRS)

IRS is one of the operational interventions which are effective in reducing the transmission of malaria. A stable formulation of insecticide is applied to the insides of houses to kill the

female resting adult mosquitoes (Enayati and Hemingway, 2010). IRS with DDT was used in the 1940's by several national malaria control programmes during the global malaria elimination period from 1957 to 1969 (Erlanger et al., 2004). IRS with propoxur was used effectively in the Nigerian Garki project, complemented by MDA (Molineaux, 1980). IRS requires well-known epidemiology of malaria in the area, requires good logistics and a well trained staff, rendering this strategy unlikely to achieve sustainable results on a large scale. Other innovative vector control strategies are therefore needed to complement the ITNs use and the IRS. Innovative vector control strategies include the development of transgenic mosquitoes those are refractory to malaria infection, the use of *Wolbachia* strains to shorten the life span of mosquitoes, and the use of repellents and attractants.

1.3.2 Chemotherapy

1.3.2.1 Treatment of clinical malaria

The prompt diagnosis and rapid treatment of clinical malaria is a keystone of malaria control strategies. Different classes of drug have been synthesised. For decades, chemotherapy and prevention of malaria relied on chloroquine (CQ) which as the other amino-4-quinoleines targets the food vacuole of the parasite and blocks the heme metabolism. Chloroquine has the advantage of easy use and is well accepted despite the bitter taste of the tablets. However, resistant strains which appeared spread quickly rendering chloroquine no longer effective to treat malaria (Sirima et al., 2003, Tinto et al., 2002, Guiguemde et al., 1994, SO:, 2000, Gansané A, 2005); Amodiaquine was considered as a replacement for CQ in cases of poor tolerability to CQ (cutaneous side effects were less common), but seems to be less well-tolerated. At the same time, sulfadoxine-pyrimethamine the second line treatment of uncomplicated falciparum malaria remains effective but its routine use as monotherapy has accelerated the spread of resistance. The global consequence is the widespread of the resistance to these “old” well-tolerated drugs (Sirima et al., 2003, Tinto et al., 2002, Guiguemde et al., 1994, SO:, 2000, Gansané A, 2005 , Korenromp et al., 2003, EANMAT, 2003). In the face of the failing drugs, the malaria community sought viable alternatives. The idea of combination therapy has emerged arguing the same rationale behind the treatment of conditions like the tuberculosis and the HIV aids (Attaran et al., 2004). In the 1990's combination therapy using artemisinin derivatives was advocated by the World Health Organization, which pushed national malaria control programmes to adopt these new combinations in their treatment policy (Attaran et al., 2004). The management of clinical malaria should be based on one of the recommended artemisinin combinations: Artemether-

Lumefantrine, Artesunate-Amodiaquine, Artesunate-Mefloquine, Artesunate-Sulfadoxine-Pyrimethamine and more recently Dihydroartemisinin-Piperaquine (WHO, http://www.who.int/malaria/publications/atoz/who_appt_position.pdf (accessed 11 Jan 2013)). These drug combinations have been extensively evaluated in Africa (Abdulla et al., 2008, Adjuik et al., 2004, Agomo et al., 2008, Arinaitwe et al., 2009, Ashley et al., 2005, Bassat et al., 2009, Bukirwa et al., 2006, Zongo et al., 2007b, Karema et al., 2006, Faye et al., 2012, Abuaku et al., 2012) and Asia (Karunajeewa et al., 2004, De la Hoz Restrepo et al., 2012, Myint et al., 2007, Karunajeewa et al., 2008b, Hasugian et al., 2007) where they achieved a high level of efficacy with a good tolerability profile. However the hope born with this new class of drugs for the control of malaria is waning with the detection of foci in the Thai-Cambodian borders where parasites have become tolerant and less sensitive and even resistant to the artemisinin derivatives (Lim et al., 2009, Na-Bangchang et al., 2010, Noedl et al., 2010, Yeung et al., 2009). The way forward that has been proposed is the containment of the foci of resistance while investigating additional strategies including innovative vector control strategies, the development of a malaria vaccine, and chemoprevention.

1.3.3 Chemoprevention of malaria

Anti-malarial drugs have been used in different ways to prevent the occurrence of malaria in the populations living in the endemic countries for nearly 100 years. The objective of the use of drugs for prevention was originally to interrupt transmission, this has failed but the administration of anti-malarial drugs has often resulted in a marked reduction in the prevalence of malaria and the incidence of clinical attacks (Greenwood, 2004). As a result of lessons learnt about the difficulties of sustainability, strong drug pressure, impairment of natural immunity, the cost and safety issues of the use of drugs for prevention, the strategy has evolved from the MDA, targeting a whole population at one point in time, to chemoprophylaxis, and then to Intermittent Preventive Treatment protect vulnerable groups from clinical malaria. Intermittent Preventive Treatment (IPT) of malaria involves administration of full therapeutic doses of drug at specified points in time to persons at risk irrespective of their parasitological status. As with chemoprophylaxis, IPT was initially investigated in pregnant women (IPTp), and subsequently in infants (IPTi) and in older children.

1.3.3.1 Intermittent preventive treatment in pregnancy (IPTp)

The difficulty of sustaining chemoprophylaxis in pregnant women led Schultz and colleagues to initiate the first Intermittent Preventive Treatment trial in pregnant women in Malawi (Schultz et al., 1994). This study compared the efficacy of weekly chemoprophylaxis with chloroquine with intermittent administration of sulfadoxine-pyrimethamine, given twice during the pregnancy. They reported a reduced proportion of low birth weight babies in the group who received the SP twice during the pregnancy, results which were subsequently confirmed in large trials in Malawi (Rogerson et al., 2000) and Kenya (Shulman et al., 1999). Based on this somewhat limited evidence, the World Health Organization recommended the use of two doses of SP during pregnancy (one dose after quickening and one dose in the third trimester). Further studies demonstrated the efficacy of SP twice during pregnancy in reducing the incidence of malaria episodes, the incidence of malaria-related anaemia and the frequency of low birth weight (Kayentao et al., 2005, Diakite et al., 2011). Subsequently the concept has been applied to infancy who like pregnancy women represent a particularly vulnerable group that bears a high burden of malaria clinical episodes and mortality especially in areas where the transmission of malaria is intense and year-round.

1.3.3.2 Intermittent preventive treatment in infant (IPTi)

The first of these studies were undertaken in Tanzania where full doses of sulfadoxine-pyrimethamine (Schellenberg et al., 2001) or amodiaquine (Massaga et al., 2003) were given during the first year of life. These studies reported a reduction in the incidence of clinical attacks of malaria and of severe anaemia, and no rebound effect was seen in the infants who received the SP the year following the discontinuation of the intervention. The findings of these preliminary studies were consolidated by trials in Gabon (Grobusch et al., 2007), Ghana (Chandramohan et al., 2005, Mockenhaupt et al., 2007, Kobbe et al., 2007a, Kobbe et al., 2007b) and Mozambique (Macete et al., 2006). A consortium was established to address the outstanding issues surrounding the use of Intermittent Preventive Treatment of malaria in infants and to advocate for its recommendation by the World Health Organisation as a tool for malaria control in endemic areas where SP is still effective. IPTi eventually became a WHO recommendation (WHO, 2010), although it has yet to be implemented on a large scale. The delivery of IPTi is achieved through the Expanded Program of Immunisation (EPI), when children come for their routine vaccinations, a consequence of this is that the interval between successive treatments is not consistent with the known half-life (28 days) of sulfadoxine-pyrimethamine, so protection lapses. To optimize the protective benefit a different method of

delivery is required. This strategy targeting infants is justified in areas of high and year-round transmission, where the burden of malaria in term of incidence of clinical malaria, proportion of deaths and severe anaemia, is borne by this age group. In areas where the epidemiology of malaria is different, the picture is not similar. Epidemiological data support that in areas of short intense seasonal transmission of malaria, the weight of the disease is borne by age groups extending beyond infancy (Becher et al., 2008), IPTi in these settings can make only a limited impact on the overall burden of children. Extending the age range up to 5 years to cover all those at high risk during the childhood in these areas, termed as Intermittent Preventive Treatment of malaria in children (IPTc), was subsequently proposed, and its restriction to the high transmission period in areas of seasonal transmission led to the term Seasonal Intermittent Preventive Treatment of malaria in children, sIPTc.

1.3.3.3 Seasonal Intermittent preventive treatment of malaria in children sIPTc

A randomized trial was conducted in Senegal to explore if the IPTi concept could be applied to older children (Cisse et al., 2006). This preliminary study and subsequent studies will be reviewed in the first part of the literature review section in chapter 2. sIPTc was developed specifically for the epidemiology characteristics of malaria transmission in the countries of the Sahel and Sub-Sahel located mainly in West Africa. sIPTc was later renamed Seasonal Chemoprevention of Malaria (SMC), in order to make a clear distinction between the use of drugs for prevention and for treatment, the latter being always based on a positive parasitological diagnosis. Burkina Faso is one of the countries of the Sahel suited to the SMC strategy.

1.4 Burkina Faso and the study site

1.4.1 Geography

Burkina Faso is a Sahelian country located in the heart of Western Africa and covering a surface of 274400 km². The country is located between 3° longitude East, 6° longitude West, the parallel 9° and 15° of latitude North. Burkina Faso shares borders in the North and West with Mali, in the North East with Niger, in the South East with Benin and the South with Ghana, Ivory Coast and Togo. The capital city is Ouagadougou.

1.4.2 Demography

The latest census of the population of Burkina Faso reported 14 017 262 inhabitants in 2006 (RGPH 2006). One third of the population in Burkina Faso is less than 10 years old and almost half of the population is 15 years old or less (figure 1.1).

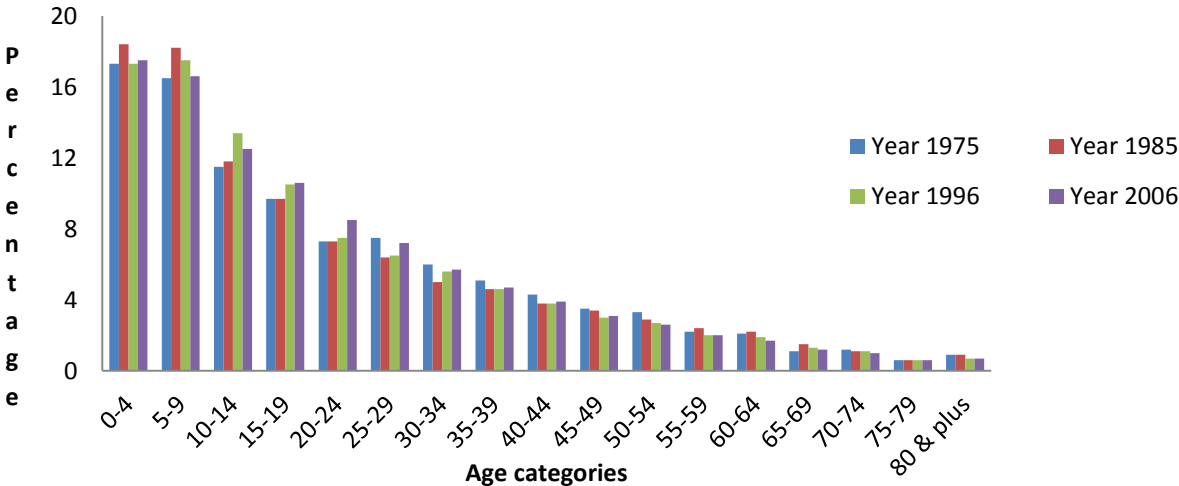


Figure 1-1: Age structure of the population in Burkina Faso from 1975 to 2006

1.4.3 Climate

The country is characterised by a tropical climate, Sahelian in the North and Sudanese in the South. There are two distinct seasons: The rainy season lasts 5-6 months (May to October) and the dry season over 6 months from November to April. Rainfall varies from 1200 mm per year in the South to 600 mm per year in the North. The highest temperatures (40°-45°) are recorded between March and June while the lowest are seen in December and January. Based on these factors, the country is approximately divided into three different zones:

- The Sudanese zone in the South, the rainy season lasts 6 months
- The Sudano – Sahelian zone in the central region with 4-5 months of rain
- And the Sahel zone where the rainy season lasts only 2 months.

1.4.4 Economy

The economy of Burkina Faso relies on farming and agriculture; more than 85% of the population lives on cultivation and livestock farming. Cotton is the main crop. According to the latest report of the UNDP (for the year 2012), Burkina Faso is classified as the 183 less developed country over 187 countries.

1.4.5 Malaria in Burkina Faso

1.4.5.1 Epidemiology and the burden of malaria in Burkina Faso

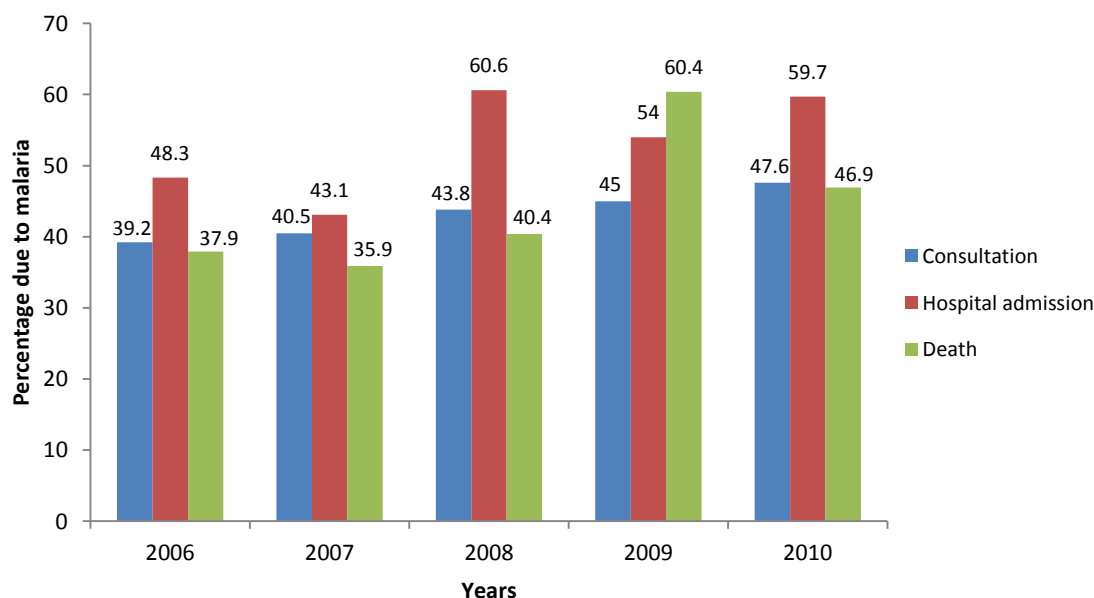


Figure 1.2 Percentage of consultations, hospital admissions and hospital deaths attributed to malaria in Burkina Faso 2006-2010.

Over the five years 2006-2010, reported malaria remained a significant cause of consultation in the health districts. In 2010 reported malaria accounted for almost half of the reasons for care seeking in health centres. The same trend was observed for hospital admissions; more than one admission in two was attributed to malaria between 2008 and 2010 (figure 1.2). More recently, in 2012, 46% of causes of care seeking were attributed to malaria with 52% of hospital admissions and 37% of deaths.

1.4.5.2 Malaria transmission in Burkina Faso

Burkina Faso, as many countries of the Sahel, is characterised by high seasonality of malaria transmission (figure 1.3); the conjunction of the climate and temperature conditions determine different durations of the transmission season through the country. Thompson and colleagues developed a tool which estimates the suitability for malaria transmission based on climatic variables (temperature and humidity and rainfall), and using 50 years of climatic data determines the probability that transmission could occur in each calendar month. Using this tool, maps of the country based on the seasonal climatological suitability for malaria transmission in Burkina Faso were produced (Fig 1.3).

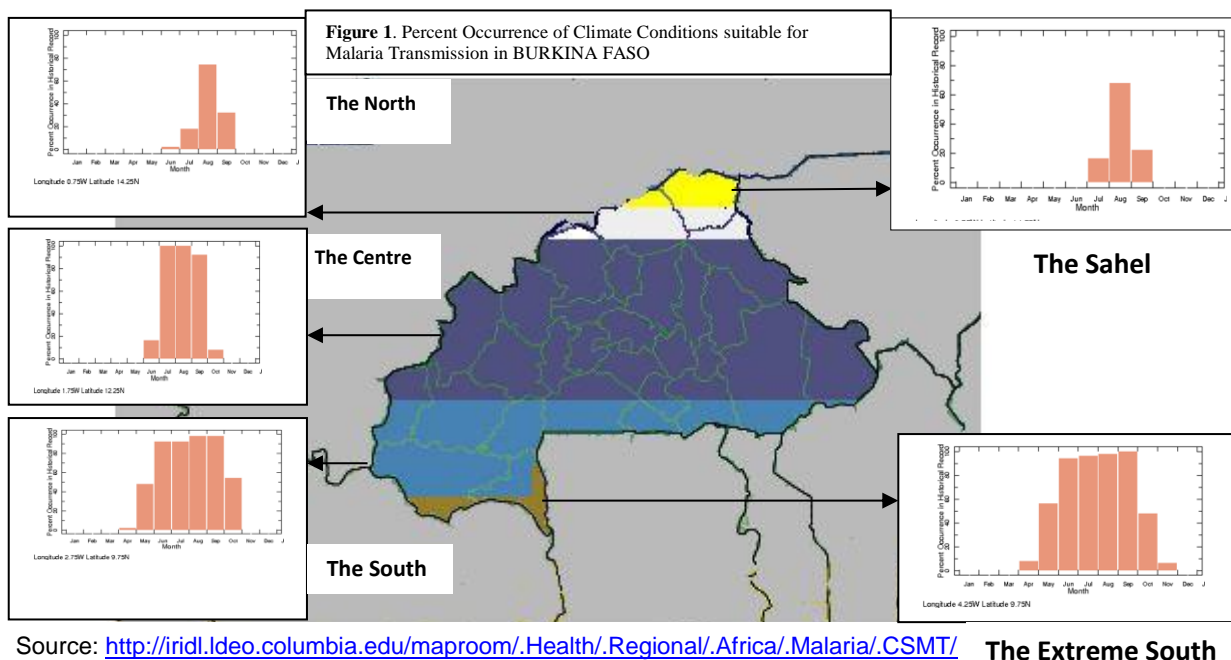


Figure 1.3: Percent occurrence of climate conditions suitable for malaria transmission in Burkina Faso

1.4.5.3 Malaria species, vectors and entomological inoculation rate

The main malaria species are *Plasmodium falciparum*, responsible for over 90% of malaria cases, *Plasmodium ovalae* (0.5-2%) and *Plasmodium malariae* (3-8%). *Anopheles gambiae s.l.* and *An. funestus* are the common malaria transmission vectors. An exophilic abundant subgroup of *Anopheles gambiae* highly susceptible to infection with wild *Plasmodium falciparum* has been recently described (Riehle et al., 2011). Estimates of the annual entomological inoculation rate range from 7.7 infective bites per person per year in Ouagadougou (the capital city) and 113 in the rural area in 1984, to up to 697 infective bites per man per year in Bama in 1999 (Baldet et al., 2003). A more sensitive method was used in 1999 but the rainfall and practices favourable to the vector in Bama area helped to explain the large difference in the estimates.

1.4.5.4 Malaria control in Burkina Faso

Early diagnosis and prompt treatment of malaria with effective antimalarial drugs is the main strategy for malaria control in Burkina Faso. Since 2005, artemether-lumefantrine and artesunate-amodiaquine over three days have been the first-line therapies for uncomplicated

malaria; severe cases are treated with either intravenous quinine or arthemether. Other strategies include insecticide treated bed-nets distributed free of charge to pregnant women alongside antenatal care, and Intermittent Preventive Treatment of malaria in pregnancy with two doses of SP in the second and third trimester. IPTi has been adopted as policy but is not yet implemented.

1.4.5.5 Use of antimalarial drugs and antimalarial drug resistance in Burkina Faso

Chloroquine (CQ) was introduced for malaria chemoprophylaxis in the 1970s for children under 5 years old and pregnant women. In 1979, chloroquine was used at 10 mg per kg body weight to treat presumptive cases of malaria (NMCP, 2011). The detection of the first cases of resistance prompted the National Malaria Control Program (NMCP) to increase the dose of chloroquine for malaria management to 25 mg per kg. Following the detection of the first case of CQ resistant *Plasmodium falciparum*, sulfadoxine-pyrimethamine (SP) was introduced as an alternative to CQ, and amodiaquine (AQ) was used in cases of poor tolerability to CQ. Despite its bitter taste, CQ was universally used while the use of SP remained limited. The prevalence of resistance to CQ increased to 16% in-vivo in 1990. Surprisingly this resistance remained low at 14% in the region of Bobo-Dioulasso between 1988 and 1991 and only two cases of in-vivo resistance to SP were recorded (Guiguemde et al., 1994). Subsequent monitoring data from the NMCP have reported a significant increase in the CQ failure rate from 27% to 63% in 2003 and that of SP to 10% (NMCP, 2011). Clinical trial data reported 9.1% failure with SP and 18% with AQ in 2005 from Bobo-Dioulasso (Zongo et al., 2005). At a consensus meeting held in February 2005, mono therapies with CQ, AQ and SP were removed from clinical malaria treatment and replaced with the more effective ACT drugs (NMCP, 2005). This meeting adopted IPTp with two doses of SP in pregnancy. Despite the implementation of several strategies (diagnosis and treatment, use of insecticide treated bed-nets), the prevalence and incidence of malaria remains high according to the latest statistics reported from the Ministry of Health (Ministère de la Santé, 2012). In this context, it is anticipated that the new strategy of SMC could have a substantial impact. An estimation of the population likely to benefit and the magnitude of the reduction of the cases (clinical cases, deaths, severe anaemia) was made in order to contribute to the evidence considered by policy makers that SMC is relevant for malaria burden reduction among Burkina Faso's population.

1.4.5.6 Areas where SMC might be of benefit: Population at risk

The estimate of the population in Burkina Faso by the National Demographic and Statistics Institute in the middle of 2009 and reported in the annual health statistics for 2009 released by the Ministry of Health was 15,224,780 inhabitants of whom 19% were children under five years old (Ministry of Health, 2010). Based on these statistics and the approximate delimitation of the country into three transmission zones, we estimated that 16% of the under 5 years population live in the short transmission zone, 61% in the moderate long transmission zone and 23% in long transmission area (rural and urban areas combined). This gives an indication of the number of children that might benefit from IPTc (table 1.1).

Table 1.1: Population at risk in different malaria transmission zones

Malaria transmission pattern	< 5 years old	General population
Short transmission season (< 3 months)	470,733	2,321,619
Moderate length transmission season (3 – 4 months)	1,786,641	9,330,125
Long transmission season (> 6 months)	656,981	3,573,036
Total	2,914,355	15,224,780

1.4.5.7 Potential impact of SMC

In the statistics provided by the Ministry of Health in 2011 malaria (uncomplicated and severe malaria) was the leading cause of morbidity and mortality, accounting for 48% of all outpatient attendances, 63% of hospital admissions and 71% of hospital deaths in children under five years old (Ministère de la Santé, 2010). However, almost all malaria cases are unconfirmed despite recent improvements in diagnostic services. The only reliable information about the malaria burden comes from research studies. The incidence of malaria in the control group of the IPTc trial in Bousse, in the Sahelian zone (Konate et al., 2011) was 1232 episodes (of which 982 episodes were with a parasite density of 5000/ μ L or more) among 1500 children under 5 years of age who were followed for one transmission season and all of whom were using an insecticide treated bed-net (ITN). With a total population of about 3 million children under 5yrs in the country, if only 50% of these are exposed to this level of malaria risk, this would be a total of 982,000 cases with high parasite density and 1,232,000 cases with any parasitaemia each year among children under 5 yrs. If even 60% coverage of seasonal IPT could be achieved at each monthly round, with an efficacy of 85% for one month after each dose, then half of these cases ($0.6 \times 0.85 = 0.51$) could be prevented. Burkina Faso appears therefore to be suitable for a seasonally targeted form of malaria control.

Chapter 2 Literature review

The concept of seasonal chemoprevention of malaria (SMC) was developed to retain the advantages of chemoprophylaxis while minimising some potential drawbacks. It is limited to a short period of transmission each year, thereby limiting intake of drugs, and is restricted to a particular age range (children under 5 years of age), which may limit the scope for selection for drug resistance. The first section of this review focuses on the efficacy and clinical tolerability of the drugs used, next evidence relating to safety and tolerability is considered, followed by pharmacokinetic data and the duration of protection with different regimens based on data are from therapeutic studies (treatment of clinical cases), which give an indication of the duration of protection when these drugs are used in the context of the SMC. Finally the key known molecular markers mediating the resistance to the anti-folate drugs and amodiaquine are reviewed. At the end of each section, a brief summary of the research questions that this thesis aims to address is presented.

2.1 Overall efficacy and tolerability of SMC

A review and meta-analysis (Wilson, 2011) from a series of studies evaluating the efficacy, safety and tolerability of different drug regimens for SMC showed that the strategy is safe and has the potential to avert a substantial proportion of incident cases of malaria and of severe anaemia and death: the pooled estimate of the efficacy of monthly SMC against uncomplicated malaria was 80%. Evidence for an effect on mortality is less clear, the studies were not designed to estimate such effects, but the results did not rule out a substantial benefit. Subsequently a WHO GRADES assessment found that monthly SMC can avert 75% of malaria cases both uncomplicated and severe, and found that a reduction in the risk of death would be consistent with the high quality evidence of a reduction in severe malaria but there were too few deaths in these trials to evaluate effects of SMC on mortality.

The first of the trials was conducted in Mali (Dicko et al., 2008). Two hundred and sixty two children 6 months to 10 years were randomized in Kambila to receive SP (monotherapy twice) or a placebo eight weeks apart in the 2002 malaria transmission season and followed up for 12 months and the subsequent transmission season of 2003. Over 12 months follow up, the age-adjusted protective efficacy was 43%; [95% CI 29% to 54%] and 68%; [95% CI 55%-77%] when the follow up was restricted to 16 weeks. In the subsequent year (2003 transmission season), when no intervention was given, the incidence of malaria was similar in the two groups. The intervention was safe and there was no evidence of a rebound effect after

one year of intervention. The benefit was moderate and the contribution of this intervention to the growth of SP related molecular markers (which could wane the clinical benefit) was not assessed; the protective efficacy could potentially be improved by a more effective drug regimen given on more frequent basis, it would also be important to assess the impact on drug resistance assessed at the end of the transmission season. To address these issues, in Senegal in 2002 one dose of artesunate plus one dose of SP or two placebos were given monthly to 1136 children aged 2 to 59 months over three months, the children were then followed up for 13 weeks. The primary outcome was the incidence of malaria in the cohorts (Cisse et al., 2006). The intervention achieved a very high level of protection, 86% [95% CI 80%-90%] and the treatment was well tolerated. This study reported evidence of a high degree of selection of resistant parasites, carrying mutations associated with resistance to SP, although the authors stated the study was not designed to assess this effect. At the end of the intervention, there was a significant increase in the prevalence of *pfdhfr* mutations +20% [95% CI 10%-30%] and in the *pfdhps* mutations +42% [95% CI 25%-58%] among children who were parasitaemic at the end of the transmission season. This was a worrying finding and may reflect the fact that the combination of SP+AS exposes parasites to monotherapy with SP as AS is eliminated within a few hours. While the authors stated that firm conclusions could not be drawn, the trend of selection for resistance if confirmed would make SP alone or SP combined with artesunate not suitable for SMC and more suitable regimens should be investigated.

In 2005 Kweku et al. (Kweku et al., 2008) compared the efficacy in Ghanaian children of SMC with either artesunate+amodiaquine monthly or bimonthly, or SP bimonthly, with a placebo. The incidence of clinical malaria and anaemia were the main endpoints. A total of 2451 children 3-59 months from 30 villages were recruited and followed up for a 6 months period. The protective efficacy was low, 17% [95% CI 6.3%-27%] and 24% [95% CI 14%-33%] in the bimonthly AS+AQ and bimonthly SP groups respectively and up to 69% [95% CI 63%-74%] in the monthly AS+AQ group. The protection from malaria anaemia was significantly better in the monthly AS+AQ group 82% [95% CI 54%-93%]. Adverse drug reactions were similar in the intervention groups. In this report, no formal assessment was done for the selection of resistant parasite which would be particularly relevant in view of the low protective efficacy. Furthermore, the limited efficacy in the bimonthly and short-acting combination regimens might be improved by using more frequent or longer-acting drug regimens.

Following the lessons learnt from the first study experience, scientists from Senegal repeated in 2008 a similar design of trial with more drug regimen options (Sokhna et al., 2008). One dose of SP was associated with either one dose of artesunate (1AS+SP), three doses of artesunate (3AS+SP), three doses of amodiaquine (3AQ+SP) or one combination without SP, (3AQ+3AS). A total of 2102 children were randomly allocated to one of these four combinations, and treated for three consecutive months over one transmission season. The primary outcome was the incidence of clinical malaria. Three daily doses of artesunate combined with SP were more effective than one dose of artesunate combined with SP, incidence of clinical malaria was 10% with 1AS+SP and 9% with 3AS+SP, but combining three doses of amodiaquine to three doses of artesunate was less effective (11%) in preventing clinical malaria and in reducing the prevalence of parasitemia at the end of the transmission season. The combination of three doses of amodiaquine with SP outperformed the three other regimens, with only 5% of incidence of clinical malaria, hazard ratio 0.50 [95% CI 0.30-0.81]. The outstanding clinical efficacy of SPAQ was offset by two other findings:

- The report of adverse events. A substantial number of children experienced at least one adverse event, 32% [95% CI 28-36%], more than for the other regimens; over 10% of these children vomited or presented with fever. Tolerability is of particular relevance in the context of chemoprevention where drugs will be given to healthy children, poor tolerability may limit adherence and such regimens may be poorly accepted by mothers and the community.
- The prevalence of molecular markers associated with resistance to sulfadoxine and pyrimethamine among parasitaemic children was higher at the end of the transmission season than at the beginning. It is important to consider this finding in relation to the overall prevalence of parasitaemia. The prevalence of parasitaemia at the end of the transmission season was reduced by SMC with SPAQ, so that although all the positive children in the SPAQ group carried the triple mutation associated with resistance to SP, the overall prevalence of resistant parasites was lower in children who received SPAQ than in the placebo group. Nevertheless, in terms of transmission of the resistant parasites, even though fewer children carried resistant strains, the probability of recombination with sensitive strains is reduced by the elimination of sensitive strains within infected children.

Two further studies were conducted in 2007 in Senegal and in The Gambia. The design was different in the two studies, individually randomized in the Gambia and cluster randomized in Senegal. 1893 children 3-59 months were enrolled in Senegal (Cisse et al., 2009) and 1008

children 6-59 months in the Gambia (Bojang et al., 2010). The children were treated with either SPAQ, or SP+piperazine (SP+PQ), or dihydroartemisinin-piperazine (DHAPQ). Three courses of treatment were given over three months and the follow up lasted for one transmission season. All regimens were highly effective. In The Gambia, the overall incidence of malaria was 0.22 per child per year [95% CI 0.15-0.28] and no child experienced more than one episode. The incidence rate of malaria (any parasitemia) was respectively 0.79 [95% CI 0.57-1.07], 0.06 [95% CI 0.02-0.15], 0.10 [95% CI 0.04-0.21] and 0.06 [95% CI 0.02-0.15] in the control, the SPAQ, the DHAPQ and the SP+PQ groups. The overall tolerability profile was excellent. In Senegal the incidence rate of malaria (intention to treat) of any parasitemia was 5.4%, 5.3% and 3.4% respectively for SPAQ, DHAPQ and SP+PQ treatment regimens. No serious adverse event was reported, but vomiting was more common in the children treated with SPAQ. While the report in The Gambia study did not assess the carriage of drug-resistant parasites (due to the very low prevalence of parasitemia), Cisse et al. reported 4.4% of positive slides at the December survey. Among children with parasitaemia, 53% of the genotyped samples were positive for the triple mutation in the *pfdhfr* gene and 38% for the *pfdhps* gene. The studies in the Gambia and Senegal were carried out in areas with very low incidence of malaria and were certainly under powered to assess the effect of the treatment on the selection of the resistant parasite, and were underpowered for demonstrating non-inferiority of DHAPQ compared to SPAQ. An unexpected increase in the prevalence of anaemia was found in the DHAPQ treated group and it was unclear what the reason was.

Further studies exploring the impact of chemoprevention on the incidence of clinical malaria were undertaken in school children in Uganda and in adult population in South East Asia. In Uganda, school children were recruited, given SP alone or SPAQ or DHAPQ and followed up for 42 days to assess their efficacy safety and tolerability in the context of prevention malaria (Nankabirwa et al., 2010). By the end of the follow up 98.6% of the participants were assigned an outcome. DHAPQ was the most effective; the risk of parasitaemia was significantly lower in DHAPQ treated children 12% [95% CI 7.9%-17%] than that in the SPAQ 44% [95% CI 38%-52%] and the SP 80% [95% CI 74%-85%]. The risk in the placebo group was 85% [95% CI 79%-89%], $p=0.022$. No serious adverse event was recorded but vomiting was more frequent in the SPAQ group. An efficacy trial design does not seem adequate to investigate the suitability of a drug regimen to be used for the prevention of malaria even if a post-therapeutic prophylactic effect may be demonstrated. The safety profile

in repeated courses may differ from a single course; furthermore, the age group studied in this area of intense transmission is less likely to be the most vulnerable group.

At the Northwest border of Thailand, healthy volunteers male were randomised to receive bimonthly or monthly DHAPQ to prevent clinical malaria. They received three days treatment and were followed up weekly for 9 months. The protective efficacy was 98% [95% CI 96%-99%] in the monthly DHAPQ treatment and 86% [95% CI 81%-90%] in the bimonthly treatment arm. In Africa the target of chemoprevention strategy is pregnant women and children up to 5 or 10 years old; the figure is different in this part of Asia where the occupation of the adults puts them at high risk of acquiring malaria. The report from Thailand (Lwin et al., 2012) showed a high protective efficacy in adults; however the study is unique of its kind and no previous data is available to allow any comparison. It is also an indication that ultimately, chemoprevention could target adults in Africa when effective strategies will tend to contain the infection in children and when the adult population becomes the most potent reservoir.

Considering the use of seasonal chemoprevention of malaria as a tool for malaria control, an important question is whether SMC may add benefit to the other interventions. Previous trials had been done in areas with low ITN coverage. To determine whether there is an additional benefit of SMC in children using ITNs, two studies were completed, in Burkina Faso (Konate et al., 2011) and in Mali (Dicko et al., 2011). The two studies have the same design with a similar primary objective. In Burkina Faso, 3014 children aged 3-59 months were given long lasting insecticide-treated bednets and randomized to receive either three days treatment of SPAQ (1509 children) or a placebo (1,505 children). The incidence rate (per person years at risk) of clinical malaria (parasitemia >5000 per μl) was 2.88 [95% CI 2.70-3.06] per child during the intervention period in the control arm against 0.87 [95% CI 0.78-0.97] in the intervention arm, yielding a protective efficacy of 70% [95% CI 66%-74%] ($p < 0.001$). There was a 69% [95% CI 6%-90%] reduction in incidence of severe malaria ($p = 0.04$); the reduction in the incidence of all-cause hospital admissions was 46% [95% CI 7%-69%] ($p = 0.03$). In Mali, 1,508 children were enrolled in the control and 1,509 in the intervention arm, all of them received a LLIN. In both trials, during the intervention period, the reported number of children sleeping under a LLIN was very high, over 99% in Burkina Faso and in Mali. In Mali, the incidence rate of malaria in episodes per person year (parasitemia >5000 per μl) in the control group was 1.90 [95% CI 1.76-2.05] against 0.34 [95% CI 0.29-0.41] in

those receiving SPAQ in addition to the bed-nets indicating a protective effect of 82% [95% CI 78%-85%] ($p < 0.001$). The protective efficacy against episodes of severe malaria 87% [95% CI 42%-99%] ($p = 0.001$). In both trials the intervention was safe and well tolerated.

Unlike IPTi which uses the EPI as the delivery channel, there is no established delivery mechanism for SMC; new routes of delivery were needed to ensure high coverage and good adherence. Several studies investigated SMC delivery through community participation, involving training of community health workers or volunteers, and engagement of the local communities through community sensitization, based on positive experiences with community case management of childhood illnesses, and home management of malaria (Haines et al., 2007).

In The Gambia in 2006 (Bojang et al., 2011) two strategies of delivery were compared through a cluster randomised clinical trial. Twenty-six reproductive and child health (RCH) trekking clinics each serving a population that included about 400 to 500 children aged up to 6 years of age, were randomly allocated to receive monthly chemoprevention of malaria either from the trained Village Health Workers (VHWs) or from the RCH trekking teams. In The Gambia the RCH trekking teams provide most of the health care to children less than 5 years old in rural areas. VHWs are trained by the Ministry of Health to recognise and treat common illness including malaria. The study reported two important conclusions: delivery of the chemoprevention of malaria was feasible outside the formal health facilities, and delivery through the VHW was more effective, at a much reduced cost. The VHWs achieved a high coverage compared to the RCH trekking teams over three courses of treatment, 74% versus 48%, risk difference of 27% [95% CI 16%-38%]. The VHWs channel was in place before the onset of the trial and just needed a short period of training to be operational; the VHWs interacted with the local population with whom they may be already more familiar, so the level of coverage (or adherence to the VHWs) could be different in settings where this system did not exist before.

A further study to explore the use of the community to deliver SMC was carried out in the Gambia to evaluate the potential benefits of combining the seasonal chemoprevention of malaria with the home management of malaria (Sesay et al., 2011) and delivered through the VHWs. In the 2008 malaria transmission season, 1277 Gambian children aged 3-59 months living within the rural Farafenni demographic surveillance system were randomized to

receive monthly SMC with either SPAQ or placebo. The intervention was given by VHWs on three occasions in September, October and November 2008 in a double blinded manner. The VHWs were previously trained to manage the clinical cases of malaria with artemether-lumefantrine (Coartem®). Ninety-four percent of children received all three treatments during the intervention period confirming the ability of the community to successfully deliver SMC in combination with Home Management of Malaria. Similar results were obtained in Ghana (Kweku et al., 2009): twelve villages were randomised to deliver the SMC through either the community based volunteers or through health workers. In this study four rounds of treatment were administered in May, June, September and October 2006. The district management supervised delivery by the community volunteers. The results showed that the proportion of children who received at least the first of three or more courses of SMC was slightly higher in the community-based arm (90.5% versus 86.6% $p=0.059$; however for the completion of three doses, the proportion was similar in the two groups. This study also demonstrated the feasibility of the community delivery channel; a high coverage level can be achieved through the communities after a short period of training.

Seasonal chemoprevention of malaria in children using SP+AQ is highly effective in these areas of the Sahel. However, there is little evidence about the best alternative regimen that could be used in areas where amodiaquine is already in use combined with the artesunate as first line treatment of uncomplicated malaria in settings like Burkina Faso, and regimens that could be used if resistance to SP and AQ starts to limit the effectiveness of SMC with these drugs, or in areas where there is already resistance to SP in parts of south-east Africa where transmission is seasonal and SMC could potentially be used.

Summary of the research questions

- 1) What is the safety, tolerability and efficacy of DHAPQ compared with SPAQ when used for SMC?
- 2) What is the incidence of malaria episodes in untreated children in the study area and hence what is the potential burden preventable by SMC?
- 3) What is the pharmacokinetic profile of PQ and of SPAQ when used for SMC in children? (in order to check if dosing is optimal and to use this data to interpret the duration of protection and understand the drug levels required for clinical protection)
- 4) What is the effect of SMC in selecting for drug-resistant parasites?

2.2 Safety and tolerability of DHAPQ and SPAQ

The safety and tolerability of drugs used for SMC has been referred to above, in this section further data on safety and tolerability of DHAPQ and SPAQ from malaria treatment studies is reviewed to have a more complete picture of possible adverse event with the use of drugs studied in this project.

2.2.1 Dihydroartemisinin-piperaquine:

Artemisinin derivatives have now been extensively studied, and they are remarkable for a lack of serious toxicity when used for the treatment of malaria (Meshnick et al., 1996). In addition to formal studies, artemisinins have now been widely used, with millions of treatments administered, mostly of artesunate, in Southeast Asia and Africa. The artemisinin derivatives are remarkably well-tolerated in humans. In a clinical safety review, no serious adverse events or significant toxicity was reported (Ribeiro and Olliaro, 1998, McIntosh and Olliaro, 2000).

Dihydroartemisinin-piperaquine is an artemisinin-containing fixed-combination drug developed in China. Recent randomized efficacy clinical trials in Cambodia, Vietnam, and Thailand indicated excellent tolerability and high cure rates against multi-drug resistant falciparum malaria. Piperaquine has been widely used in China, and has been a standard antimalarial drug in China since the 1970s. The main side effects are: mild headache, listlessness, nausea, and dizziness (Chen et al., 1982). DHAPQ is now in routine use in Vietnam with no reports of serious adverse events although limited resources are available for pharmacovigilance. In recent efficacy trials from all parts of Africa, DHAPQ has shown a good tolerance but cardio toxicity has to be evaluated. The regimen was well tolerated with mild to moderate side effects including headache, diarrhea, dizziness, abdominal pain. Five serious side effects were seen with 5 patients over 215 who were treated (Yeka et al., 2005) but were unlikely to have been drug-related. An SMC trial comparing DHAPQ with other regimens has been completed in Senegal in 2008 and reported a good tolerability profile of the drug although there was a slight but statistically significant increase in the prevalence of anemia in children who received DHAPQ and it was unclear if this was a true effect of the drug.

2.2.2 Amodiaquine (AQ):

AQ has been described as “very well tolerated” for routine use, (Luzzi and Peto, 1993) and it was widely used for chemoprophylaxis against malaria in the past. However, prophylactic use was discontinued due to rare instances of agranulocytosis, aplastic anemia, and hepatotoxicity, principally associated with use for malarial chemoprophylaxis in travellers (Luzzi and Peto, 1993, Olliario et al., 1996). In Uganda and Burkina Faso, no serious toxicities were observed with AQ monotherapy (Staedke et al., 2001, Zongo et al., 2005). In SMC trials, the most common adverse event noted were cough, diarrhoea, with the vomiting being more frequent in the treatment with SPAQ (these studies used tablets) (Sokhna et al., 2008, Cisse et al., 2009, Bojang et al., 2010).

2.2.3 Sulfadoxine-pyrimethamine (SP):

SP has generally been the preferred replacement for CQ for the treatment of uncomplicated malaria in Africa. SP is no longer recommended for chemoprophylaxis due to rare, but serious toxicity. Adverse reactions listed on the SP package insert (Roche, USA) are blood dyscrasias (agranulocytosis, aplastic anaemia, thrombocytopenia), allergic reactions (erythema multiforme and other dermatological conditions), gastrointestinal reactions (glossitis, stomatitis, nausea, emesis, abdominal pain, hepatitis, diarrhoea), central nervous system reactions (headache, peripheral neuritis, convulsions, ataxia, hallucinations), respiratory reactions (pulmonary infiltrates), and miscellaneous reactions (fever, chills, nephrosis); based on widespread experience with the drug, all of these reactions appear to be uncommon or rare with short-term therapeutic use.

2.2.4 Sulfadoxine-pyrimethamine plus Amodiaquine (SPAQ):

A systematic review of 3 older studies that included SPAQ reported no serious adverse events (McIntosh, 2000). In five published studies from Kampala, Uganda, and Bobo-Dioulasso Burkina Faso a total of 1441 SPAQ treatments were administered without the occurrence of severe adverse events (Gasasira et al., 2003, Staedke et al., 2001, Zongo et al., 2005, Zongo et al., 2007a, Zongo et al., 2007b). SPAQ was also studied in 59 children with malaria in Tanzania and adverse events other than those expected for acute malaria were not identified (Schellenberg et al., 2002). In a study from Kampala, which compared three antimalarial combination therapies (Staedke et al., 2004), rigorous surveillance for adverse events, including laboratory testing, was done to evaluate drug safety and tolerability. Of the patients treated with SPAQ, six (6) experienced serious adverse events (defined as events that resulted

in hospitalization, required medical intervention, or were life-threatening). Four of the events were attributable to severe malaria and included convulsions (3 patients) and vomiting (1 patient). Three hematologic events that were not associated with severe malaria or other illnesses occurred in a single patient. Further laboratory results reported in the SPAQ group included transient asymptomatic neutropenia (1 patient), and elevation of ALT associated with clinical hepatitis (1 patient), which resolved spontaneously by Day 28. The combination SPAQ was used in the SMC studies in The Gambia (Bojang et al., 2010), Senegal (Cisse et al., 2009) and Burkina Faso (Konate et al., 2011).

This review shows that the clinical tolerability of the DHAPQ and SPAQ deserves a further investigation; the reviewed studies used the drug as tablets; in the present project, a paediatric formulation (syrup) was used, and it is unclear whether the adverse effect seen with the tablets will occur in similar proportion.

2.3 Pharmacokinetics of piperazine and sulfadoxine-pyrimethamine

In SMC the prophylactic effect after the treatment is a key to the success of the strategy, we aimed to understand how this is achieved and the investigation of relevant pharmacokinetic (PK) parameters will be the most appropriate approach. We will first define these key parameters: population versus rich pharmacokinetic approach, the steady state, the bioavailability of the drug after single or multiple administration and the time taken to eliminate the drug (the terminal half-life, $t_{1/2}$) and the area under the curve; then we will explore in the relevant pharmacokinetics studies the information gathered about these parameters.

2.3.1 Definition of the key pharmacokinetics parameters

The rich PK studies involve an intensive sampling (high number of samples) of an individual at different point in time (possibly with a short interval of time between samples); the analysis of such data is done on an individual basis.

The population sparse PK studies are an approach where individuals are less frequently sampled and each individual will contribute with a limited number of samples; the analysis is done on pooled data of all participants.

The steady state of a drug: As repeated doses of a drug are administered its plasma concentration builds up and reaches what is known as a steady state. This is when the amount of drug in the plasma has built up to a concentration level that is therapeutically effective and as long as regular doses are administered to balance the amount of drug being cleared the

drug will continue to be active. The time taken to reach the steady state is about five times the half-life of a drug. Sometimes a loading dose may be administered so that a steady state is reached more quickly.

The bioavailability is the proportion of the administered dose that reaches the systemic circulation.

The terminal half-life $t_{1/2}$: this is the time required to halve the plasma concentration after the point of pseudo-equilibrium has been reached.

The area under the curve (AUC), the area under the plot of plasma concentration of drug (not logarithm of the concentration) against time after drug administration is conveniently determined by the “trapezoidal rule”: the data points are connected by straight line segments, perpendiculars are erected from the abscissa to each data point, and the sum of the areas of the triangles and trapezoids so constructed is computed. When the last measured concentration (C_n , at time t_n) is not zero, the AUC from t_n to infinite time is estimated by C_n/k_{el} . The AUC is of particular use in estimating bioavailability of drugs, and in estimating total clearance of drugs (CLT).

2.3.2 Pharmacokinetics of piperazine studies

4-aminoquinoline piperazine 1,3-bis-[4-(7-chloroquinoly-1-yl)-4-piperazinyl-1]-propane is an anti-malarial drug synthesized in China and in Rhone Poulenc in the 1960s (Hung et al., 2003). The development of the resistance to the “old” drugs and the recent advocacy of combination therapy with artemisinin derivatives (Nosten and White, 2007, White, 2006) has placed PQ in the centre of malaria treatment reorientation, piperazine (PQ) is therefore referred to as a resurgent antimalarial (Davis et al., 2005). For its second “life” in combination of an artemisinin derivative, dihydroartemisinin, close attention has been paid to how to maximize the efficacy of the combination while protecting the drugs from the development of resistance. Extensive efficacy and tolerability evaluations of the regimen have been performed in South East Asia (Ashley et al., 2004, Denis et al., 2002, Song et al., 2011, Tjitra et al., 2012) and in Africa (Arinaitwe et al., 2009, Bassat et al., 2009, Nambozi et al., 2011, Yavo et al., 2011, Zongo et al., 2007a); all studies reported high cure rate for the treatment of uncomplicated falciparum malaria (Keating, 2012).

Despite extensive clinical evaluation, few studies have been published on the pharmacokinetics of PQ in malaria patients (Annerberg et al., 2011, Karunajeewa et al., 2008a, Nguyen et al., 2009, Tarning et al., 2008, Tarning et al., 2012, Hung et al., 2004) and

in healthy volunteers (Roshammar et al., 2006, Ahmed et al., 2008, Nguyen et al., 2008, Chinh et al., 2009, Sim et al., 2005).

2.3.2.1 Pharmacokinetic of Piperaquine in healthy volunteers

Earlier studies on the pharmacokinetics of piperaquine were published in 2006 by Roshammar et al. (Roshammar et al., 2006). CV8®, a co-formulation of 320 mg of piperaquine, 32 mg of dihydroartemisinin, 5 mg of primaquine and 90 mg of trimetoprim was given to 12 fasted Vietnamese healthy volunteers over three days and followed up for 29 days. An intensive approach was used to obtain 468 piperaquine concentration-time points. Piperaquine was characterised by multiple peaks during the absorption and the elimination phases. This study was seen as a pilot; however the drug content including primaquine, trimethoprim, and a different dosage of dihydroartemisinin than currently used (40 mg), may have affected the pharmacological properties of the piperaquine and this study is therefore less relevant for the purpose of this report. Further trials were conducted in Asia to better evaluate the pharmacokinetic profile of the piperaquine. In 2008, seventy-two male Caucasian volunteers (40 aged 20-45 y and 32 aged 18-45 y) were enrolled in a complex pharmacokinetic study. Rising single oral (RSD) and rising multiple doses (RMD) in adults were administered in order to determine the safety, tolerability and pharmacokinetics of piperaquine alone. In the RSD study, 5 escalating single oral doses (500, 750, 1000, 1250 and 1500 mg) of PQ were given to 40 volunteers. In the RMD, 4 escalating doses (500, 750, 1000 and 1500 mg) of PQ were given to 32 volunteers. All volunteers underwent intensive blood sampling and were followed up to 60 days. In the RSD cohort, the reported maximum concentration C_{max} ranged from 41.6 ± 29.5 ng/ml with 500 mg of PQ to 147 ± 110 ng/ml with 1500 mg of PQ, a maximum time T_{max} to reach the maximum concentration from 4.0 h to 2.5h (500mg-1500mg) and a terminal half-life from 11.2 days to 12.7 days. In this report the escalating of the doses improves the C_{max} and the T_{max} decreased but the terminal half-life remained constant. In the RMD, the T_{max} was shorter with the 1500 mg of PQ 3 h and maximal with 750 mg. This approach indicated that a single rising dose of PQ (even high dosage) has no benefit for SMC as it does not improve the half-life of piperaquine.

Chinh et al. measured the pharmacokinetic profile of piperaquine in 24 healthy Vietnamese adults taking three tablets of Arterakin® or Artekin®, all of them containing piperaquine 320 mg and 40mg of dihydroartemisinin but manufactured respectively in Vietnam (Central Pharmaceutical Factory No.1 Hanoi) and China (Holleykin Pharmaceutical Co. Ltd, Guangdong). After a single dose (tablets containing 120 mg Dihydroartemisinine, 960 mg

piperavaquine), the participants were followed up for 28 days (Chinh et al., 2009). The C_{max} was higher in the drug manufactured locally compared to the Artekin® (232 ng/ml versus 204 ng/ml) and they had similar time to reach the maximum concentration (3 hours). The terminal half-life of 25 days was similar in the two groups. This result from a single dose of three tablets doses could be an interesting option for SMC in term of adherence to the strategy in a long term perspective. However, clinical tolerability and biological safety should be carefully evaluated after repeated doses (over three to four months) in these adult populations and especially in children (Hung et al., 2004).

2.3.2.2 Pharmacokinetic of Piperaquine in patients with uncomplicated malaria

Eighty-five Cambodian patients (38 adults and 47 children 2-10 years) presenting with clinical malaria due to *P. falciparum* or *P. vivax* were given Artekin orally at 0, 6, 24 and 32 hours with a total piperavaquine dose of 32-35 mg base per kg and followed up to 35 days for efficacy. The adults received Artekin® tablets (320 mg piperavaquine 40 mg dihydroartemisinin) while children received either tablets or granules (120 mg piperavaquine 15 mg dihydroartemisinin) over three days. Participants underwent rich and sparse sampling schemes and plasma PQ concentration was measured using a validated high-performance liquid chromatographic assay with ultra violet detection at 340 nm (Hung et al., 2003). The findings with regard to the main pharmacokinetic parameters showed a bigger volume of distribution in children 614l/kg, 95% CI [332-1205] compared to adults, 574l/kg, 95% CI [371-711]. The oral clearance time was longer in the children 1.8 liters per hour per kg (1.29-2.3 liters per hour per kg) with a shorter terminal half-life 14 days (10-18 days); the treatment success rate was 98% over 28 days follow up. In this study, mixing the sampling approach and the drug formulation may have ignored variability in bioavailability between the two formulations. Subsequently, studies with more sensitive approaches with a much lower limit of quantification (Tarning et al., 2005, Ahmed et al., 2008) indicated that the terminal half-life had been under-estimated. More recently in 2012, a population approach was used to characterise the pharmacokinetic profile of PQ in African children 2-10 years old in Burkina Faso presenting with clinical malaria (Tarning et al., 2012). This is the largest study in term of sample size ever published on the clinical pharmacokinetics properties of Piperaquine in sick children from Africa. Standard doses of DHAPQ were given to 236 children who were followed up for 42 days in Bobo-Dioulasso, Burkina Faso. In this study, the time to maximum concentration was 3.84 hours (1.52-12.4) and the terminal half-life was 23.2 days (14.8-31.3). The mean day-7 capillary concentration was 64.0 (16.8-130ng/ml). Younger

children (2-5 y) had lower day-7 plasma concentrations compared to the older even though they received higher normalized per-body-weight doses of the drug. In this study, day-7 plasma concentration of piperaquine predicted treatment outcome at the end of the 42 d-follow up. Children whose day 7 piperaquine concentrations on capillary sample were below 57ng/ml were more likely to fail the treatment; the cut-off for venous blood sampling was 30ng/ml. This study contributed to the accumulating data on PK of PQ in children but does not address the issue of the pharmacokinetic characteristics of piperaquine when given to healthy children.

The population pharmacokinetics profile was studied in Thailand in a randomised study aiming at preventing malaria in adult. Healthy male volunteers whose occupation puts them at risk in the Northwest border of Thailand were randomized to a 3-day treatment dose of dihydroartemisinin-piperaquine monthly (DPm) or every 2 months (DPalt) or an identical placebo with or without fat (6.4 g/dose) over a 9-month period (Lwin et al., 2012). All volunteers were monitored weekly. They reported 114 episodes of malaria (49 *P. falciparum*, 63 *P. vivax*, and 2 *P. ovale*). The protective efficacy against all malaria at 36 weeks was 98% (95% CI, 96% to 99%) in the DHAPQ monthly group and 86% (95% CI, 81% to 90%) in the DHAPQ two monthly groups (for both, $P < 0.0001$ compared to the placebo group). All regimens were well tolerated. There was no case of malaria in volunteers with plasma concentration above 31ng/ml. The administration of fat did not influence the main results.

The lower limit of detection (LoD) is the lowest specimen concentration which is likely to be reliably distinguished from the Lower Blank (LoB) and at which detection is possible. The LoB is the highest *apparent* specimen concentration expected to be found when replicates of a blank sample containing no specimen are tested.

The lower limit of Quantification (LoQ) is the lowest concentration at which the specimen can be reliably detected and at which some predefined goals for bias and imprecision are met. The LoQ may be equivalent to the LoD or it could be at a much higher concentration.

In general pharmacokinetic evaluations are done in adult volunteers and only recently in children with uncomplicated falciparum malaria; most of these results are from Asia. Clinical pharmacological evaluation of the drugs has been limited in the African continent and no pharmacokinetic data are available on healthy children.

Population pharmacokinetic studies, possible due to the development of more efficient techniques when analysing the data, are preferred to intense studies due to the difficulty of sampling more often in young participants, furthermore, the parameters reported are dependent on the sensitivity of the experiment. There is substantial inter-individual variability and the terminal half-life is a function of the duration of follow up (appears to be longer in long follow up trials). In sick children, the terminal half-life is longer but the steady state maximum concentration in the plasma is much lower in these children.

2.3.2 Pharmacokinetics of sulfadoxine-pyrimethamine plus Amodiaquine

SPAQ is the most effective regimen for SMC in children, but there is little information about the pharmacokinetics of these drugs in children. Although this combination has been used for many years for treatment of clinical malaria there have been no studies of the pharmacokinetics of this combination. A recent study described the pharmacokinetics of amodiaquine when used in combination with artesunate to treat children with clinical malaria (Stepniewska et al., 2009), and another recent study described the pharmacokinetics of SP in children with malaria (Barnes et al., 2006), and a further study of SP in children with uncomplicated malaria demonstrated a low plasma level of the drug in children who failed the treatment (Obua et al., 2008), but there are no studies of these drugs given together or in healthy children.

This section has reviewed the key pharmacokinetics parameters sustaining the efficacy of the DHAPQ and SPAQ: the maximum concentration C_{max} , the terminal half-life $T_{1/2}$, the time taken to reach the C_{max} . Except in the preventive study conducted in Thai adults, all the reviewed studies were done either in healthy adults or in patients with acute malaria; therefore some important aspects remain unexplored.

- What is the relationship between PQ dose in mg/kg and the PQ plasma concentration, and the relationship between the plasma concentration and the incidence of clinical malaria, and are these associations modified by age?
- Does the duration of protection reported in this trial support the current monthly interval for the drug administration?

These research questions will be investigated in chapter 5.

2.4 Selection of molecular markers

2.4.1 Molecular basis of de novo selection of drug resistance

The molecular basis of drug resistance has been well established for chloroquine (CQ), the anti-folate fixed combination antimalarial sulfadoxine-pyrimethamine (SP) and the cytochrome b inhibitor atovaquone (Djimde et al., 2001, Schwobel et al., 2003, Korsinczky et al., 2000, Wellems, 1991, Wellems and Plowe, 2001, Basco et al., 1998, Brooks et al., 1994, Hyde, 1990, Triglia and Cowman, 1994). Following the large-scale deployment of ACTs in the 1990s, molecular studies have reported a link between the PfMDR1 gene and the most advocated ACT, artemether-lumefantrine (Sisowath et al., 2005, Humphreys et al., 2007, Sisowath et al., 2007, Gadalla et al., 2011, Sidhu et al., 2005).

2.4.2 Emergence of drug resistance

The genetic events leading to drug resistance are spontaneous and rare; these are thought to be independent of the drug used; the event may be a mutation or a change in the copy number of genes encoding for the parasite target (White, 2004). A single mutation or change of amino acids may lead to the establishment of resistance (as it is the case with the *cyt b* for atovaquone) or a gradual increase (mefloquine resistance) or finally in a stepwise process (resistance to pyrimethamine) (Hastings et al., 2002).

2.4.2.1 Spread of drug resistance

The spread of drug resistance depends on the transmission of resistant gametocytes; in mathematical terms the ratio (gametocyte density of resistant strain/gametocytes density of the sensitive strain) drives the spread of resistance. Resistant strains have survival advantages as they are more likely to recrudescence and show a slow response to antimalarial treatments (White, 2004), and thus also are more likely to produce infective gametocytes (Hallett et al., 2006, Sutherland CJ, 2002). Factors which are likely to limit or increase the spread of resistant parasites include (i) host immunity and pressure, (ii) intensity of transmission, (iii) terminal half-life and (iv) population movement and transmission of resistant parasites.

2.4.2.1.1 Host immunity and treatment pressure

Host immunity plays an important role in the spread of drug resistance (Hastings et al., 2002). In high transmission settings, host immunity is continuously boosted by repeated exposure to infective bites, thus while children are susceptible to severe infections, adults have varying levels of acquired immunity and are often asymptomatic, and thus remain untreated. In low

transmission settings, individuals are less frequently exposed to malaria infection, and consequently a higher proportion of infected individuals become sick and seek treatment with antimalarial drugs (Hastings et al., 2002, White, 2004). This difference in drug pressure pattern may contribute to the spread of antimalarial drug resistance. Host immunity has the ability to eliminate parasites resistant to antimalarial drugs and there may contribute to limiting the spread of these strains (Diakite et al., 2010).

2.4.2.1.2 Intensity of transmission

As well its role in determining the community level of acquired immunity, transmission intensity also plays a role in the spread of resistant parasite as a result of its relationship with clone multiplicity. In settings where the entomological inoculation rate (EIR) is low, monoclonal infections are common (Hill et al., 1995). The phenomenon of self-fertilization within the same parasite will more often occur, resulting in resistant parasites being more likely to be transmitted to the next generation of parasites, particularly under sustained drug pressure (Curtis and Otoo, 1986). In high transmission settings, the frequent rate of new entomological inoculation could result in a multiplicity of clones of parasites infecting people and this may facilitate the colonisation of the space by the resistant parasite after an effective treatment which killed the susceptible ones (Hastings, 1997). This selection of particular sub-populations has recently been shown for submicroscopic parasites in western Kenya (Beshir et al., 2013).

2.4.2.1.3 Drug elimination parameter – Terminal half-life

There is renewing interest in the role of drug pharmacokinetics in the spread of resistance (Barnes et al., 2007) and completed studies in Africa have indicated that the use of ACTs can lead to the selection of resistance to the partner drug (Holmgren et al., 2006, Djimde et al., 2008, Martensson et al., 2005). In malaria endemic settings, where the intensity of malaria transmission is very high, several genotypes of *P. falciparum* may infect a subject although certain of these may remain undetected. If an antimalarial drug was given to a subject harbouring such a genetically complex population of parasites, it will encounter not only the circulating parasites but also any recently inoculated sub-populations emerging from the liver. This may occur during the drug's elimination phase (expressed as the terminal half-life). Particularly when the new parasites emerge during this time window when the drug may be at sub-therapeutic concentration, there is a higher probability that partially resistant parasites may be selected (Hastings et al., 2002, Watkins and Mosobo, 1993).

2.4.2.1.4 Population movement and transmission of resistant parasites

Population movement has contributed to the spread of infectious disease (Prothero, 1977). The migration of an infected person (e.g. worker or trader) from a malaria endemic country to a region where the disease has been eradicated can lead to the resurgence of malaria transmission in the latter area (Martens and Hall, 2000). Populations migrating from an area of low transmission to a high transmission area are at high risk of infection and evidence from large resettlement of populations in Ethiopia, Indonesia and Brazil showed a significant increase in morbidity and mortality in all age groups in migrants from low to high transmission settings. In the same way, displaced human populations are likely to introduce resistant parasites from their place of origin into new places. Indeed intercontinental travel may have played a major role in the widespread pattern of antimalarial drug resistance (Roper et al., 2004) and explained the introduction of chloroquine and sulfadoxine-pyrimethamine resistance in Africa through South East Asia between the 1970s and 1990s (Anderson and Roper, 2005, Lynch C, 2011).

2.4.3 Molecular markers of drug resistance.

2.4.3.1 *P. falciparum* chloroquine –resistance transporter (PfCRT)

Chloroquine acts by interfering with heme metabolism in the digestive vacuole of *Plasmodium falciparum*; in resistant parasites, the accumulation of chloroquine in the food vacuole is diminished (Bray et al., 1998, Fitch, 1970, Krogstad et al., 1985, Sullivan et al., 1996, Yayon et al., 1984). The *pfcr*t is a gene of 13 exons located on the chromosome 7 (Fidock et al., 2000b). This gene encodes for a transmembrane protein in the food vacuole of the parasite. Among several point mutations in *pfcr*t associated with resistance to CQ *in vitro* in laboratory lines of *P. falciparum* from Africa, South America and South East Asia, the substitution of threonine (T76) for lysine (K76) at position 76 (K76T), was present in all resistant isolates and absent from all sensitive isolates tested *in vitro* and pointed out the key role of this gene in *in vitro* resistance to CQ. The K76T change mostly confers resistance to CQ in association with other mutations in the same gene (Carlton et al., 2001, Djimde et al., 2001, Bray et al., 2005). These data along with direct genetic evidence from studies of transgenic *P. falciparum* (Fidock et al., 2000a, Fidock et al., 2000b) strongly support the role of *pfcr*t in CQ treatment failures, and this has been subsequently confirmed in further clinical studies (Abruquah et al., 2010, Mockenhaupt et al., 2005, Sarr et al., 2005, Talisuna et al., 2002). The prevalence of treatment failures to CQ had significantly increased since it was

first reported in the 1980-90s (Guiguemde et al., 1994, Sirima et al., 2003, NMCP, 2011, Tinto et al., 2006) and CQ is no longer considered useful for treatment of *P. falciparum* infections in Africa.

Clinical resistance to piperazine emerged in China following extensive use in mono therapy (Chen, 1982, Davis et al., 2005). There is an active search for molecular markers determining the resistance to piperazine a resurgent antimalarial in combination with dihydroartemisinin. The structural similarities of piperazine and chloroquine suggests possible cross-resistance between these drugs, so that *pfcr* can be seen as a possible marker of piperazine resistance. Using genetically modified *pfcr* and *pfmdr-1*, San et al. reported the first evidence of evidence that resistance to piperazine is conferred by mutations in *pfcr* that are most commonly associated with resistance to CQ (Muangnoicharoen et al., 2009). However a recent study reported another independent relationship between the IC₅₀ of piperazine and dihydroartemisinin and mutations in *pfcr* and *pfmdr-1* (Briolant et al., 2010).

Nonetheless in this section we will investigate possible association of *pfcr* CVIET and *pfmdr-1* mutations with clinical resistance to piperazine. Any other comparison of selection by piperazine (for the antifolate resistance marker) is of limited interest.

2.4.3.2 The *P. falciparum* multidrug resistance gene (PfMDR-1)

This gene encodes an ortholog of the P-glycoprotein found in mammals mediating the multidrug resistance to cancer cells. The protein located on the food digestive vacuole plays a key role in the traffic regulation across the membrane (Valderramos and Fidock, 2006, Rohrbach et al., 2006); N86Y modulates but is not essential in chloroquine resistance and the presence of the SNP Y86 in clinical studies was not associated with chloroquine failure (Duah et al., 2007). The simultaneous presence of *pfcr* (T76) and PfMDR mutation in the position 86 (Y86) was associated with *in vitro* resistance to chloroquine but the relationship disappears when the analysis was adjusted for the presence of the *pfcr* (Tinto et al., 2003); this confirm that *pfmdr-1* is essential but not sufficient on its own to determine the resistance of CQ; however these two mutations together can be used to monitor the resistance to amodiaquine (AQ), another amino-4-quinolone and sharing some structural similarities with chloroquine (Tinto et al., 2008). The mutations in Y86 of the PfMDR was also linked to different susceptibility to other antimalarial drugs, for example the mutant 86Y was linked to mefloquine hypersensitivity while the N86 was found after treatment with artemether-

lumefantrine (Sidhu et al., 2005, Sisowath et al., 2005, Sidhu et al., 2006, Sisowath et al., 2007, Baliraine and Rosenthal, 2011). *In vitro* studies have reported a correlation between IC₅₀ estimates for CQ and AQ (Ochong et al., 2003), which is suggestive of cross resistance *in vitro* (Childs et al., 1989, Basco and Le Bras, 1993). However, cross resistance between CQ and AQ has not been confirmed *in vivo* (Bloland and Ruebush, 1996, Sowunmi et al., 2001, Schellenberg et al., 2002).

2.4.3.3 *P. falciparum* Dihydrofolate reductase (PfdHFR) and dihydropteroate synthetase (PfdHPS)

The synthesis of folate is essential for parasite *P. falciparum* survival; antifolate drugs prevent the completion of this process through the inhibition of dihydrofolate reductase (*dhfr*) (by pyrimethamine and proguanil), and inhibition of dihydropteroate synthetase (*dhps*) (by sulfadoxine) (Biswas et al., 2000, Bzik et al., 1987, Triglia and Cowman, 1994). Although the molecular basis of pyrimethamine *in vitro* resistance is well established (Aubouy et al., 2003, Foote et al., 1990, Peterson et al., 1990), the relationship between the identified markers and clinical failure is not linear. *dhfr* and *dhps* mutations which appear in a stepwise process, have been associated with an enhanced transmission of gametocytes to mosquitoes despite a low level of *in vivo* treatment failure (Hallett et al., 2006, Méndez F., 2002). The presence of the I164L mutation in *pf dhfr* signified an established high level resistance to pyrimethamine in SE Asia (Plowe, 1998), but this mutation is extremely rare in Africa (Nzila A, 2005, Ochong et al., 2008). *P. falciparum* with different levels of sensitivity to sulfadoxine have sequence variation in the *dhps* gene (Brooks et al., 1994, Triglia and Cowman, 1994), and the *pf dhps* mutations A437G, K540E and A581G are particularly associated with established sulfadoxine-pyrimethamine failure *in vivo* (Dunyo et al., 2006, Hallett et al., 2006). Of these, only the A437G mutation has been reported in Burkina Faso and in most other parts of West Africa.

In East Africa sulfadoxine-pyrimethamine clinical failure is common (Talisuna et al., 2004, Iriemenam et al., 2012, Karema et al., 2010, Eriksen et al., 2008) and is consistent with high frequency of the quintuple mutations in *dhfr* (51I/59R/108N) and the double mutation in *dhps* (S437G /K540E) along with the emergence of A581G mutation in Tanzania (Gesase et al., 2009), in Kenya (Iriemenam et al., 2012) and in Uganda (Sendagire et al., 2005). The use of sulfadoxine-pyrimethamine for Intermittent Preventive Treatment of malaria in pregnancy and the recent recommendation by the World Health Organization for SMC in the Sahel and

sub-Saharan regions warrant a continued monitoring of *pfdhfr* and *pfdhps* for the emergence of mutations which might signify the establishment of high-level SP resistance in West Africa.

The aim of our study is to identify any evidence of selection by SMC on known markers of resistance for SP and AQ. The following objectives will be evaluated successively for the baseline and end of transmission season surveys, as well as in incident episodes over the period of follow up.

The specific objectives were:

- (i) Determine the prevalence of mutations in *pfdhfr* and *pfdhps*.
- (iii) Determine the prevalence of the triple mutations in Dhfr (51/59/108).
- (iv) Determine the prevalence of the quadruple mutations.
- (v) Test whether early episodes (week 1) following SMC have different marker profiles
- (vi) Perform exploratory analysis on the association of combination of mutations, and haplotypes of interest, and the role of some markers seen elsewhere but recently identified in the study area.

Detailed findings addressing these specific objectives will be presented in the chapter 6.

Summary

This chapter has presented a review of four main aspects: the efficacy and clinical tolerability of the DHAPQ and SPAQ; clinical tolerability and biological safety in efficacy studies; the key pharmacokinetics parameters of Piperaquine, Sulfadoxine-Pyrimethamine and Amodiaquine summarised in population PK studies; selection of drug resistance and SMC. Each section has a brief summary of the research question (or presented as objectives). The following chapter presents the methods used in the clinical trials.

Chapter 3: Methods

3.1 Overall design

An individually opened randomised trial of seasonal chemoprevention of malaria was conducted during the peak of the transmission in the 2009 transmission season with dihydroartemisinin-piperaquine or sulfadoxine-pyrimethamine plus Amodiaquine given on three occasions to children 3 to 59 months of age. The treatment was administered monthly at the beginning of August, September and October. An untreated group to serve as a comparator was recruited one month after the first cohort in the middle of September 2009.

A randomized placebo group was not included because at the time of the study, there was evidence of the benefits of SMC, rendering unethical the recruitment of proper randomised group of untreated children. All cohorts were monitored for adverse events after each round at home and for malaria morbidity at the health facilities. A sub-study was undertaken within the dihydroartemisinin-piperaquine cohort to characterise the pharmacokinetic profile of piperaquine; full pharmacokinetic analysis of these data was outside the scope of this thesis, for the purpose of this thesis, day-7 concentration of piperaquine was used to determine the relationship between piperaquine concentration and the duration of protection provided by the SMC. Another subset of children within the two cohorts was randomised to have additional sampling for biological safety evaluation. A cross-sectional survey took place at the end of transmission season in November for the treated children and December for the untreated cohort (figure 3.1). Blood samples were taken at the indicated periods for assessment of parasitemia, haemoglobin concentration and for molecular studies.

A separate study was planned during the low transmission period in 2011 in Lamarame, Senegal, in which a cohort of children aged 3-59 months was enrolled and treated with sulfadoxine-pyrimethamine plus amodiaquine, and followed for 28 days in order to characterise the pharmacokinetic profile of sulfadoxine-pyrimethamine and amodiaquine (desethylamodiaquine). Blood samples were collected onto filter paper to measure the level of sulfadoxine-pyrimethamine and desethylamodiaquine. These studies in Burkina Faso and Senegal are successively presented in the sections 3.2 and 3.3 of this chapter.

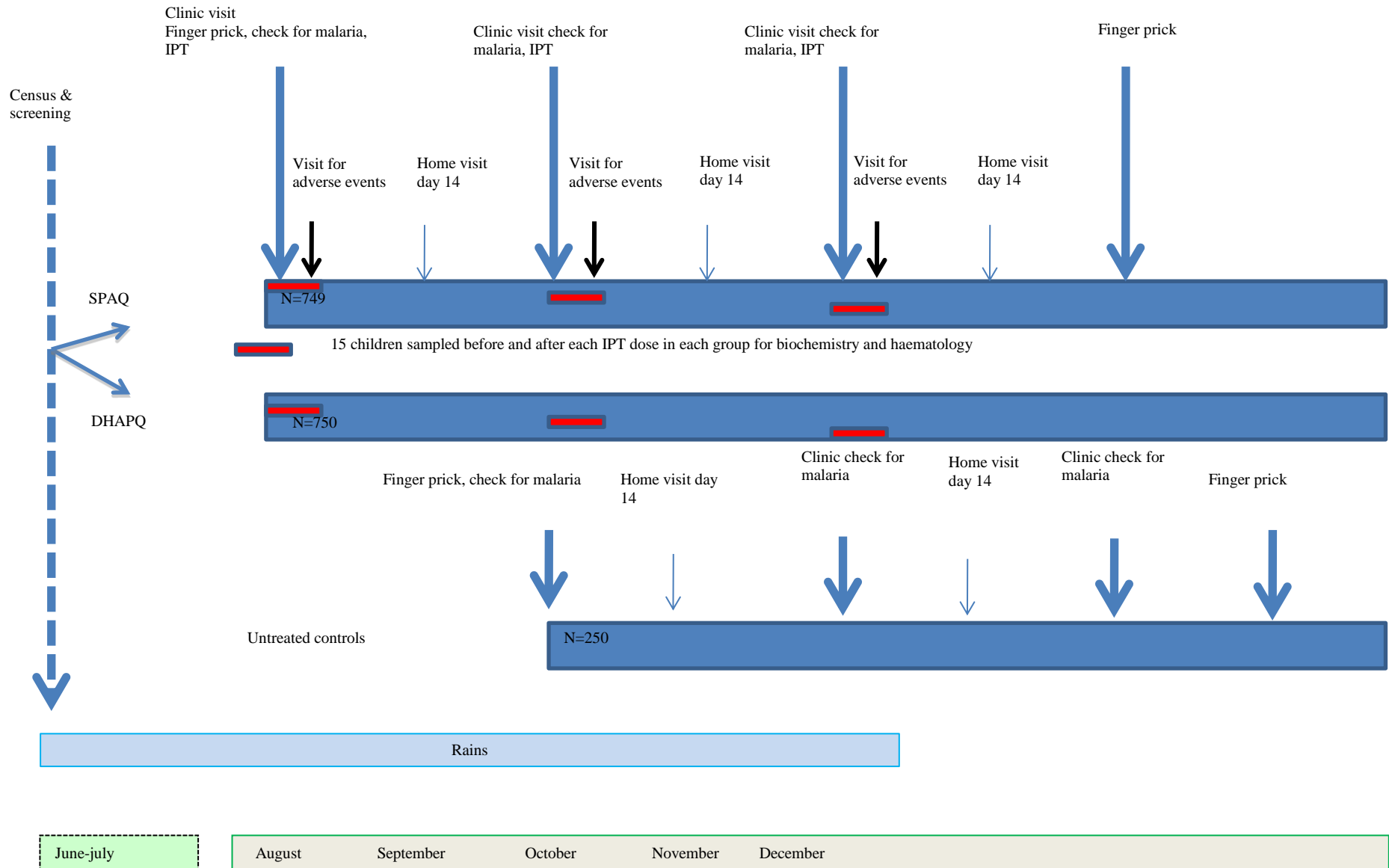


Figure 3.1 Study plan

3. 2 Randomised clinical trial in Burkina Faso

3.2.1 Study sites

The study was conducted in region to the west of Bobo-Dioulasso, the main capital city of the region. The study sites were three peripheral health facilities of one of the six health districts of the region, the health district of Lena:

- Satiri (CSPS), the largest centre with 3540 inhabitants of whom 701 were under 5 years old.
- Balla, distant of 2 miles from the centre of Satiri had 3028 inhabitants of whom 748 were children under 5 years old.

These two centres are regular government health centres and staffed by two trained nurses, a midwife or an assistant mid-wife and a local pharmacist to manage the essential generic drug shop.

- Kadomba, the third centre did not have a public health centre, the study was the first to provide direct care to the population in this site; the estimated population was 2831 inhabitants and 560 children under 5 years old. Each of the sites was surrounded by a small dam which remains a factor for malaria transmission.

In the study areas, the diagnosis of malaria is mainly presumptive (rapid diagnostic tests were provided by the National Malaria Control Program for a short period between 2008 and 2009).

The infrastructure was basic comprising an outpatient department, a small hospitalisation room with 3 to 4 beds, and the essential drug shop. The national hospital at Souro Sanou in Bobo-Dioulasso served as the reference hospital in case of any serious condition (severe or complicated malaria) to be treated in emergency.

3. 2.2 Preliminary census and recruitment of participants

Before the start of the study, community meetings were held in the villages to explain the objectives, the procedures and the duration of the project; these meetings targeted the mothers, the local community leaders and broadly all community members. These meetings facilitated by local community workers provided village members with information about the study. An enumeration of children less than five years old was then conducted, at the beginning of August, then screening and enrolment took place after an individual informed consent was given by the children's parents or legal guardians (Appendix 1).

3. 2. 3 Inclusion and exclusion criteria

3. 2. 3 .1 Inclusion criteria

Children 3-59 months were screened (appendix 2) and enrolled in the cohort if they fulfilled the following criteria (appendix 3):

- Ability to respect the follow-up schedule.
- No intention to move outside the study site during the trial.
- Residency within the peripheral health facility catchment area.
- Agreement to come to the study clinic for any febrile episode or other illness and avoid medications given outside the study protocol.
- Acceptance of home visits and
- The provision of informed consent by the parent/guardian.

In this study, the presence of a clinical attack of malaria at the time of enrolment was not an exclusion criteria; the patient was randomized to a treatment at entry but received the treatment for acute malaria with artemether-lumefantrine (Coartem® tablet 20mg artemether 120 mg lumefantrine) over three days and then received the intervention drug at the subsequent round of SMC.

3. 2. 3.2 Exclusion criteria

Children were not enrolled if one of the following criteria was found:

- History of allergy or sensitivity to SP, AQ, or DHAPQ
- Active medical problem requiring in-patient evaluation at the time of screening
- Intention to move outside the three health facilities vicinity during the follow-up.
- Chronic medical condition, i.e. malignancy requiring frequent medical attention
- Known HIV infected infant and children or children from HIV infected mother. We did not formally test the children for HIV.

3.2 4 Procedure of randomization

All children who fulfilled the inclusion criteria were referred to the study nurse responsible for randomization. The randomization list was computer-generated at LSHTM by a person not involved in the day to day management of the study, and ID numbers including one check-digit number to control for transcription errors. This list and sealed opaque envelopes, bearing the study number and containing a card with the treatment allocation, were prepared at the London School of Hygiene and Tropical Medicine (LSHTM) and sent to the study site in Bobo-Dioulasso, Burkina Faso. Each enrolled child was assigned the next available envelope as the

randomisation progressed; the child’s identity was then recorded next to his/her randomisation number in a register. A small number of randomization errors occurred. Children who consistently switched from a regimen to another over all rounds were considered as valid at the end of follow up and therefore included in the intention to treat analysis; those who mixed the treatment from a round to another were excluded from the according to protocol analysis.

3.2.5 Description of the intervention

3.2.5 .1 Dihydroartemisinin-Piperaquine: DHAPQ

Dihydroartemisinin-Piperaquine was provided by Holley Cotec Beijing Limited Ltd China as (Duocotexcin®) tablets of 320 mg of piperaquine phosphate and 40 mg dihydroartemisinin in package of 8 tablets. The drug was provided with a quality test certificate and was not subsequently tested during the course of the study (table 4).

3.2.5.2 Amodiaquine: AQ

This drug was bought from the local market; at the start of the study, the tablet presentation (Flavoquin® tablet 200 mg containing 153 mg base) was chosen, but an earlier shortage of this presentation prompted the team to use the syrup formulation for all rounds of the study; therefore the drug was in bottles of 60 ml (Camoquine® syrup 5ml/mg) (table 3.1).

3.2.5.3 Sulfadoxine-pyrimethamine: SP

Sulfadoxine-pyrimethamine manufactured by Roche (Fansidar® tablet) was bought from the local market and presented as tablet of 500 mg sulfadoxine and 1.25 mg pyrimethamine and contained in boxes of 3 tablets.

Likewise the dihydroartemisinin-piperaquine, the amodiaquine and sulfadoxine-pyrimethamine did not undergo a quality testing during the study. We relied only on the certificate of GMP provided by the local pharmacist.

Table 3.1: Summary of the intervention drugs

Study drug	Manufacturer	Drug formulation	Dosing schedule per month
Sulfadoxine-pyrimethamine (Fansidar®)	Roche	Tablet 500 mg + 25 mg	Single dose
Amodiaquine (Camoquin®)	Pfizer	Syrup 5ml/mg	Daily for 3 days
Amodiaquine (Flavoquine®)	Rhone Poulenc	Tablet 200 mg	Daily for 3 days
Dihydroartemisinin-piperaquine (Duocotexcin®)	Holley Cotec	Tablet 40 mg + 320 mg	Daily for 3 days

3.2.6 Drug administration

The study drugs were dispensed by the nurse in charge of the randomization; all drugs were administered according to the child's weight at enrolment and the same dosage was used for the subsequent administrations in September and October (appendix 7). The first dose was given at the clinic by the study nurse and all subsequent doses were given to the mothers for home administration directly supervised by trained field workers during home visits. In case a child vomited a dose within 30 minutes following the drug administration, a half dose was given again; if vomiting occurred again, no further replacement was administered. Children were weighted at entry and the dose of drug given accordingly. At home or at the clinics, the time of drug administration was recorded and the time of repeated dose administration in case a child vomited the first dose.

The tablets were rounded to the nearest quarter and the syrup to the nearest 5 ml.

3.2.7 Additional medication

3.2.7.1 Treatment of clinical episode of malaria: Artemether-Lumefantrine (Coartem®)

Artemether-Lumefantrine (Coartem ®) is one of the first line treatments of uncomplicated falciparum malaria in Burkina Faso, and was the treatment for all clinical cases of malaria occurring during the trial period. The drug was presented as tablet 20 mg artemether and 120 mg of lumefantrine, this regimen was chosen to avoid using a regimen containing amodiaquine (artesunate-amodiaquine is also adopted as the first line therapy of malaria in Burkina Faso). Coartem® was given as per the National Malaria Control Program guidelines over three days without fat. The drug was also bought from local pharmacists.

3.2.7.2 Antipyretics: Paracetamol syrup 120 mg

The management of clinical cases of malaria included the prescription of oral antipyretics, paracetamol syrup over three days or up to the relief of any axillary temperature over 38.0°C

3.2.7.3 Antibiotics: Amoxicillin syrup 250 mg

Generic amoxicillin from the national generic drug manufacturer was purchased; this is a broad-spectrum antibiotic used for all febrile cases where the malaria is not the cause of the fever or in case of suspected associated bacterial infection, prescribed without any laboratory confirmation of infection. The drug was given over 7 days based on 25 mg/kilogram body weight.

3.2.7.4 Metopimazine (Vogalene®)

This dopamine D₂ receptor antagonist was used in the study to treat vomiting arising after children entered the study, whether or not they have recently received the study drug. It was used to treat any vomiting suspected to be drug-related.

3.2.7.5 Diosmectite (SMECTA®, powder for oral suspension 1g)

This was used to treat diarrhoea occurring after drug administration; the total quantity per day did not exceed 2 grams.

3.2.8 Schedule for the intervention administration

The most studied schedule for SMC was three rounds of treatment corresponding in general to the highest peak of malaria transmission in the area. In the present study, the intervention was given over three months from August to October 2009. The first round in August commenced from 11 to 18 August, then the second from 10 to 17 September and finally the third round from 10 to 17 October 2009. This represented approximately seven days per round with an intensive workload examining all recruited patients, administering the treatments, supervising the home doses, while preparing for the day 4 visits to ask about adverse reactions. Meanwhile, passive surveillance was maintained, children in need of care were received at the clinic by the study physicians. Additional staff were employed during this peak period in each SMC round.

In the untreated cohort, the children were followed in the same fashion; after their recruitment from 17 to 19 September, they were asked to return to the clinic for clinical check-up every month up to November 2009.

3.2.9 End of transmission season epidemiological survey

An epidemiological survey was conducted one month after the last dose of the intervention from 10 to 17 November 2009 in each of the three sites for the intervention group and 17-19 December 2009 for the untreated group. At this survey all children were evaluated by the study physicians; children were asked to provide finger-prick blood sample for blood smear and haemoglobin measurement. The randomized cohorts were sampled at the same time for the final survey, but the untreated group was sampled later, this may have led to some differences in the parameters measured. In this report, in order to compare malaria incidence between the SMC and untreated groups, this comparison period was limited to the period starting from 17 to 20 September and ended during the epidemiological survey in November. For the comparison of the

two randomized groups, the time at risk started at randomization and ended at the November survey in November.

3.2.10 Extended follow up

The formal plan of the study was to implement three months of SMC followed by an end of transmission season survey. However, as the control group was recruited later than the main cohorts, follow-up of this group was continued, the team was kept on site to mid December 2009. Passive follow up of all cohorts was maintained with passive detection of malaria for all subjects visiting the study clinics, until January 16, 2010.

3.2.11 Active surveillance

Upon enrolment and after the first dose children received at the clinic, a total of 21 well trained field workers visited them on a fixed time per month using active case detection forms (appendix 5).

3.2.11.1 On days 1 & 2 (day 0 being the first dose of the treatment)

The field workers working in pairs went to the child's home to supervise the administration of the daily treatment dose. All children from a given household were grouped in the same place to ease the supervision of the drug uptake and each child was observed after the drug administration for at least 30 minutes; a replacement dose was given in case of vomiting; in this case the time of the vomiting was recorded along with the time of the regular administration; then the field worker team could move to another household.

3.2.11.2 On day 4 one day after the last dose of the treatment

The field workers went back to the child's home to check whether the child had any unusual symptom since the start of the treatment (Appendix 5); any signs or symptoms reported by the mothers or the guardians were carefully recorded and transmitted to the study physician.

These records were part of the treatment regimen tolerability assessment tools (Appendix 4).

3.2.11.3 On day 14 after the intervention

The field-worker always worked in pairs to minimise errors, returned to enquire about any abnormality noted by the parents. At all visits, fieldworkers were urged to encourage the mother to bring back to the clinic any child who was unwell. Study personnel were available 24hours a week to take care of any presenting child. To ensure the best level of care for the children in the cohorts, for any symptom or sign found during the home visit, the field worker was instructed to

refer the child to the study clinic, the consequence being that no blood smear or rapid diagnostic test was done in the field. All referred children received prompt and effective care at the clinic but their records were not kept separately to build a passive case detection record which would allow a separate active surveillance analysis.

3.2.12 Passive surveillance

Beside the active follow up at the participant's home, the study clinic was opened all day to receive any mother with a sick child. A full health check was done and the appropriate treatment given free of charge; a blood smear was always done when a fever (axillary temperature above 37.5°C) or history of fever was associated with the complaints.

3.2.13 Malaria diagnostics

Unwell children who returned to the clinics were questioned (the parents or the guardians) and a full clinical examination was done. In case there was history of fever or a documented axillary temperature over 37.5°C a finger prick was undertaken, thick and thin blood smears were collected, dried and stained with 2% Giemsa for 30 minutes. The blood smears were double read by two independent senior laboratory technicians (none was aware of each other's result). If the difference between the two readers was less than 30%, the final parasitemia was the mean of the two individual readings. In case the difference exceeded 30%, a third reader assessed the slides. The final parasitemia was calculated between the readers who had a difference less than 30%. Parasite densities was calculated by counting the number of asexual parasites per 200 leukocytes or per 500 leukocytes, if the count was <10 asexual parasites/200 leukocytes, assuming a leukocyte count of 8,000/µl. A blood smear was considered negative when the examination of 100 high power fields does not reveal asexual parasites. Gametocytemia was also determined from thick smears by counting the number of gametocytes per 1000 leukocytes. The study was not blinded but microscopists were not aware of the study participants' treatment assignments.

3.2.14 Management of the periods at risk

Any episodes of malaria occurring within 7 days of a first episode were considered to be part of the initial episode and were not counted as separate episodes. All clinical episodes of malaria were treated according to the National Malaria Control Program policy using Artemether-Lumefantrine (Coartem®) over three days. Children who were within 7 days of starting treatment with Coartem® at the time of the scheduled SMC round did not receive the SMC.

3.2.15 Sub-studies: laboratory procedures

3.2.15.1 Samples on filter paper for parasite genotyping

Two or three drops of blood were saved onto a filter paper at each presentation of a child for a clinical attack of malaria. These filter papers were air dried and packed into small plastic bags with a desiccant. Samples for the confirmed cases of malaria were brought to London (LSHTM), and samples from the cross-sectional surveys sent to San Francisco (UCSF), for the drug resistance genotyping.

3.2.15.1.1 Baseline and end of the transmission season survey filter papers

A random sample of 120 children's filter papers was selected from the baseline and end of transmission sample to check the status of the key mutations prior and after the intervention. Mutations studied included the anti folates genes (*dhfr* 51/59/108, *dhps*436/437/613), the *P.f* multidrug resistant genes (*Pfmdr1* 86Y, 184F, 1246Y) and the *P.f* chloroquine resistant transporter (*pfprt* CVIET) using protocols developed in the hosting laboratory of the Department of Medicine of the San Francisco General Hospital part of the University of California, San Francisco. The samples were processed by Mr Fabrice Somé (PhD student).

3.2.15.1.2 Incident cases of clinical malaria during the follow up period

Samples from the incident cases of clinical malaria were brought to reference laboratory of Dr Colin Sutherland at the London School of Hygiene and Tropical Medicine (LSHTM) for processing. We aimed at the beginning to genotype all incident clinical cases; however time and resources constraints restricted the genotyped samples to 720. The protocol for the genotyping and sequencing, and for the real time qPCR, was as previously described by Gadalla et al., 2010.

3.2.15.2 Venous blood sampling for biological safety evaluation

Ninety children were selected at randomization, forty five children per treatment regimen, for venous sampling before and after SMC doses, with 15 children sampled at each round.

At each round, two milliliters of venous blood was collected from each child on day 0 (pre-treatment sample) and again on day 7 (post-treatment sample) for the selected children. The samples were collected into an EDTA tube and brought to the Centre Muraz Haematology and Biochemistry Laboratory for processing using an automated machine; then the results were printed out and recorded into a laboratory book and a copy of the print out was given to the study team.

3.2.15.3 Capillary blood collection for measurement of piperazine concentration

For the population pharmacokinetic study, a sub-sample of the children in the dihydroartemisinin-piperazine arm of the main clinical trial was selected. The full analysis of these data is being done in Bangkok and for the purpose of our work day-7 concentration is to be analysed in relation to the incidence rate and the duration of protection from clinical attacks of malaria. The anticipated sample size was 210 children aged 3-59 months of age at the time of enrolment. The sample size was equally split into three groups; each group being sampled once either in August, September or October. Each child participant in this study was asked to give finger-prick blood samples collected into three capillary tubes for a total whole blood volume of 200 µl and a 2.5 ml of venous blood. The blood collected was centrifuged and the plasma collected into 0.5 ml cryo-tube and stored immediately at -20 °C up to the end of the study and then sent to Bangkok. Three windows of sampling were selected: Between day 0 and day 6, a fixed sampling on day 7 and a third window between day 8 and day 30; the day 30 corresponding to the beginning of the next round of the intervention. Within these windows, the exact time of sampling was generated randomly by collaborators in Thailand (Dr Joel Tarning). The objective was to get a higher density of the samples during the critical phases of the profile. Being a subset of the main trial, parents signed a separate informed consent form for this particular study. All samplings were made by well trained personnel. Because of the necessity to be sure the drug was given and at what time, particular attention was given to these patients; the field worker assisted to the drug administration and reported the time and waited for 30 minutes in case vomiting occurred. Adherence to the indicated time of sampling was facilitated by asking the parents to come back with the children 30 minutes before the time set for the sampling and in a few cases, the field worker had to pick the child and bring him/her to the clinic. Parents who were reluctant after the first sample was taken were visited to explained again about the study, and those who wished to leave the cohort were allowed to do so. After each blood collection, the child received a sweet and the mother received re-imburement of cost of travel to the health clinic. The pharmacokinetic assays were done at the laboratory of clinical pharmacology of the University of Mahidol in Thailand. Piperazine concentrations were determined using solid-phase extraction and liquid chromatography with mass spectroscopy detection (Lindegardh et al., 2008). The low limit of quantification was 1.5 ng/ml and the lower limit of detection was 0.375 ng/ml. For the purpose of this thesis, we evaluated how the day 7 concentrations related to the dose given in mg/kg, the age of the children and the incidence of clinical malaria expressed as the duration of protection following the administration of piperazine combined with dihydroartemisinin-piperazine.

3.2.16 Definition of the outcomes for the evaluation of the efficacy of SMC

3.2.16.1 Clinical malaria

The cumulative incidence of clinical malaria was the primary endpoint and defined as the presence of history of fever in the past 24 hours or an axillary temperature equal or greater than 37.5°C associated with a parasitemia \geq 3000 parasites per μ L

3.2.16.2 Anaemia

Anaemia in this study was defined as children with haemoglobin level less than 10g/dL. Children were assessed for the anaemia at the final survey, one month following the last round of the Intermittent Preventive Treatment of malaria. All children with haemoglobin less than 10 g/dL were then treated with iron folate and Mebendazole.

3.2.16.3 Adverse events

The assessment of adverse events was one of the main endpoints of the study and made using the forms in Appendix 4. An adverse event was defined as "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment" (ICH Guidelines E2A). A serious adverse event was defined as an experience that results in any of the following outcomes: 1) death during the period of study follow-up, 2) life-threatening experience (one that puts a patient at immediate risk of death at the time of the event), 3) inpatient hospitalization during the period of study follow-up, 4) persistent or significant disability or incapacity, or 5) specific medical or surgical intervention to prevent one of the other serious outcomes listed in the definition. All recorded signs were coded according to a pre-specified guide (Appendix 6).

3.2.17 Participant withdrawal

Participants were withdrawn if they withdrew their informed consent or if they left the study area for good.

3.2.18 Ethical approval

The study conducted in Burkina Faso was submitted and approved by the London School of Hygiene and Tropical Medicine Ethics Committee, the Institutional Ethics Committee of Centre Muraz.

3.2.19 Data Safety and Monitoring Board

A Data Safety and Monitoring Board was appointed to oversee the safety aspects of the trial. It comprised a statistician (Dr Neal Alexander, LSHTM, chair), a paediatrician (Dr Hassane Tamboura, National Hospital Sourou Sanou, Burkina Faso) and an epidemiologist (Dr Corinne Merle, LSHTM).

3.2.20 External quality control: the trial monitoring

An external Clinical Research Organization led by Dr Raouf Ousseini monitored the study to check adherence to the protocol and to Good Clinical Practice guidelines and local regulatory requirements.

3.2.21 Timing of field activities

The study activities started late in July 2009. Formal contacts had been made with the local authorities to introduce the institutions in charge of the conduct of the study, and the study team. Local field workers were recruited and trained locally; just after the training sessions, a census of all children less than 5 years old present in the three sites targeted by the intervention was conducted. Meanwhile, the full team was set up and ready to enrol participants, and the first child was enrolled on 11 August 2009. The last follow up was the 16 January 2010.

3.2.22 Statistical methods

The primary endpoint was incidence of clinical malaria defined as fever or history of fever in the last 24 hours and *P.falciparum* density of at least 3000 parasites per microlitre. Secondary endpoints included: incidence of clinical malaria of any parasitemia, the prevalence of asexual parasitaemia and of gametocyte carriage, and of anaemia, at the end of the transmission season; the prevalence of molecular markers of resistance to study drugs among patients diagnosed with clinical malaria during the trial and in subjects with parasitaemia at the end of the transmission season. Sample size was chosen to give adequate power to demonstrate that SMC with DHAPQ was as effective as with SPAQ. SPAQ is highly effective for SMC in Burkina Faso (Konate et al., 2011) and in Mali (Dicko et al., 2011). Other regimens will be needed if SPAQ starts to lose its efficacy due to resistance. The aim of the study was to determine whether DHAPQ is as effective in preventing malaria as SPAQ, a non-inferiority design was therefore used. Since the aim of SMC is to completely protect children, the cumulative incidence (the proportion of children who had one or more attacks of malaria) was chosen as the primary endpoint.

To motivate the choice of non-inferiority margin we considered the cumulative incidence or risk (the proportion of children with malaria) by the end of the transmission period, if this was 7% in the SPAQ group, a difference not exceeding 4% would be required for non-inferiority of DHAPQ. This difference is equivalent to an odds ratio of 1.64. The odds ratio was used in order to define a fixed margin independent of the level of incidence in the comparison group, such that the trial would have approximately the same power regardless of the incidence in the comparator group. The size of the margin in relation to risks in the active and untreated groups is shown in the figure below, to show in perspective the size of the margin. The proportion of children with malaria, rather than the incidence rate, was chosen as the primary measure since the aim of SMC is to completely protect children. A sample size of 1500 children was needed for 80% power using a one-sided 2.5% significance level (corresponding to the use of 95% confidence intervals), allowing for 10% reduction in the number of subjects included in the according-to-protocol analysis due to loss to follow-up or non-adherence to the protocol. The sample size required in each group is given by:

$$n_1 = n_2 = \frac{2\pi(1-\pi)(z_{1-\alpha} + z_\beta)^2}{d^2}$$

where π is the proportion (risk) in each group, assumed to be the same under the alternative hypothesis, and d is the risk difference. The $z_{1-\alpha}$ is used instead of $z_{1-\alpha/2}$ because the test is one-sided, so for a 5% significance level the value 1.64 is used, and for a significance level of 2.5%, a z value of 1.96 should be used. z_β is 0.84 for 80% power and 1.28 for 90% power. This formula is used by taking a value for π , computing d assuming an odds ratio of 1.64, for example if $\pi=0.07$, the odd is $0.07/(1-0.07)=0.075269$, the odds in the comparator group is $1.64 \times 0.075269=1.23441$, which is a risk of $1.23441/(1+1.23441)=0.109877$, so the risk difference is $d=0.109877-0.07=0.039877$. Substituting $\pi=0.07$ and $d=0.04$ into the formula gives a sample size of 642 for 80% power and a 2.5% significance level. With 10% loss to follow up this comes to $642/(1-0.1)=713$. For 90% power and 5% significance we have 698:

Table 3.2 Sample size calculation

% significance (% confidence)	$z_{1-\alpha}$	Power	z_β	$(z_{1-\alpha} + z_\beta)^2$	n	n/0.9
2.5% (95%)	1.96	90%	1.28	10.4976	859.4991	955
5% (90%)	1.64	80%	0.84	6.1504	503.5687	560
2.5% (95%)	1.96	80%	0.84	7.84	641.906	713
5% (90%)	1.64	90%	1.28	8.5264	698.1055	776

This approach is conservative in that the power will be greater if the baseline risk is greater than is used in the calculation, but is an improvement on using a fixed margin, which could lead to much reduced power if the baseline risk is greater than was used in the sample size calculation.

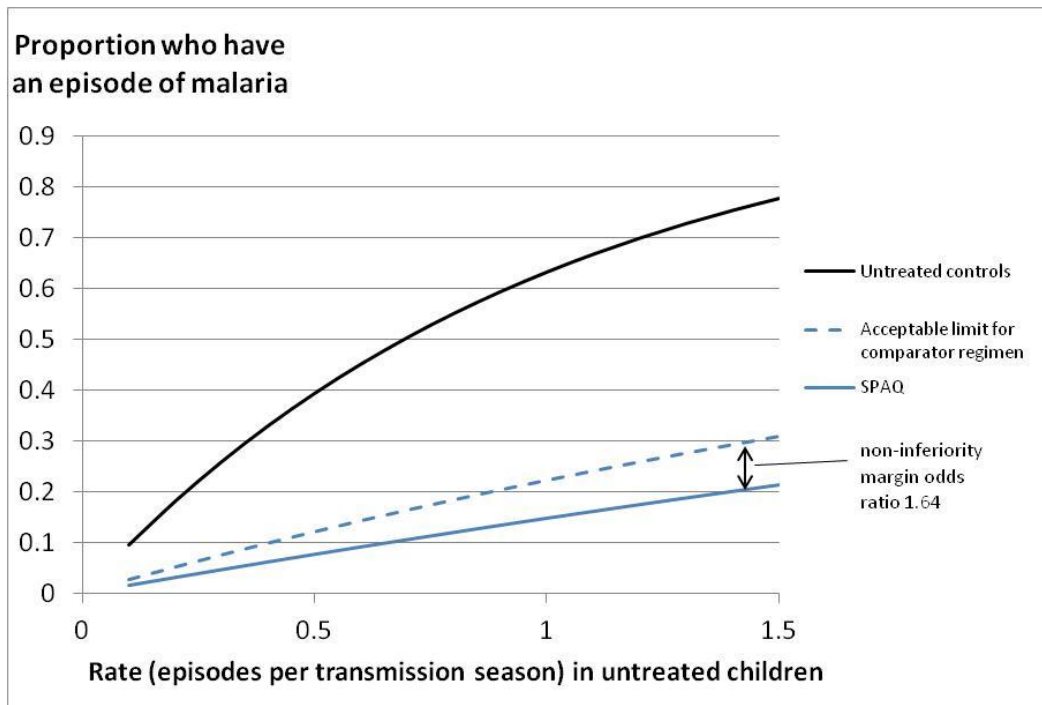


Figure 3.2 Illustration of size of the margin in relation to the risks in untreated and treated children. The expected proportion with malaria (y-axis) corresponding to a given incidence rate of malaria (x-axis) is shown. For a rate λ per child per season, the proportion in the untreated group was calculated assuming a Poisson distribution, as $1 - \exp(-\lambda)$, and the proportion in the SPAQ group calculated assuming an efficacy of 84% (the efficacy of SPAQ in this study), as $1 - \exp(-\lambda \times 0.16)$.

The purpose of the untreated cohort was to estimate the incidence of the primary endpoint in untreated persons. So the sample size was based on the degree of precision we need on this estimate. The 95% confidence interval for the rate was $(r/n) \times EF$ where EF is the error factor $\exp(1.96 \times \sqrt{1/r})$, r was the number of events and n the number of child years. We estimated that with a rate of 1 episode per child year at risk, we needed 250 children with 95% [0.88 to 1.13]. Data was entered using Access 2000 and analysed with Stata 11.0 following an analysis plan written before the end of the trial. For efficacy endpoints, both intention-to-treat and according-to-protocol analyses were done. The intention to treat population included all randomized children, analysed in the group to which they were assigned at randomization. For the ATP analysis, we excluded children who did not attend for an SMC treatment round. Children who attended, but did not receive SMC due to malaria, were included in the ATP. The WHO 2006 growth standard was used to determine nutritional status of the children. Malaria was defined as the presence of fever with an axillary temperature $>37.5^\circ\text{C}$, or history of fever in

the last 24 hours, with a parasitemia of at least 3000 per μl of blood. Two episodes of malaria occurring at least seven days apart were considered as two different episodes. For the primary analysis, time at risk was assumed to start on the date the first child received SMC, and end one month after the last SMC round. For children who died or were known to have left the study areas, observations were censored on the date of death or emigration. Analysis of non-inferiority was based on the 95% confidence interval on the odds ratio for malaria, obtained from the Kaplan-Meier estimate of the proportion of children who had malaria and its standard error, using the delta method. This gives the standard error of the log odds ratio as

$s = \sqrt{\{V(p_1)/[p_1(1-p_1)]^2 + V(p_0)/[p_0(1-p_0)]^2\}}$ where p_1 and p_0 are the Kaplan meier failure estimates and $V(p_1)$, $V(p_0)$ their variances estimated by Greenwood's formula, and the odds ratio is $OR = [p_1/(1-p_1)]/[p_0/(1-p_0)]$ and confidence limits $OR \cdot \exp(1.96xs)$ to $OR/\exp(1.96s)$ (P Milligan personal communication).

The cumulative hazard function, (an estimate of the average number of malaria episodes per child), was estimated using the Nelson-Aalen method including repeated events. Efficacy of SMC compared to the untreated group was defined as the percentage reduction in the number of episodes, which was estimated as $100 \times (1-R)$ where R is the hazard ratio from Cox regression, with confidence intervals calculated using a robust estimate of the standard error to account for repeated malaria episodes in the same child. For comparisons with the control group, time at risk started on the date of SMC round 2, or the date of enrolment for the untreated cohort, and ended one month after SMC round 3. Adjustment for covariates was critical because this was a non-randomized comparison. The effects of age (age at entry in whole years), ITN use at baseline, village, and nutritional status at entry, were assessed by including these variables in the regression model, variables which did not contribute (no evidence of confounding and not associated with outcome) were removed from the model. Follow-up was continued until 16 January 2010 and in a further analysis of duration of protection, time at risk was assumed to continue until this date, smoothed estimate of the hazard ratio was obtained using regression splines using the method of Lambert and Royston (Lambert, 2009), the efficacy (1-hazard ratio) with 95% confidence band was plotted against time since the final round of SMC. The logrank test for trend, stratified by month, was used to assess association of piperazine concentration with protection. Analysis of covariance was used to estimate the difference between groups in the mean of each biochemical and haematological parameter, with the day 0 value included as a covariate, pooled across months. In the table below the link between the research question, the outcome and the statistical method used for its analysis is presented.

Table 3.2 a Link between research question, outcome and analysis method

Research questions	Study endpoints	Statistical method
1. What is the safety, tolerability and efficacy of DHAPQ compared with SPAQ when used for SMC?	Protective efficacy of SMC with AQSP and DHAPQ	Cox regression
2. What is the incidence of malaria episodes?	Incidence rate of malaria Number of episodes per child	- Kaplan-Meier estimate - Nelson-Aalen method including repeated events
3. What is the pharmacokinetic profile of PQ and of SPAQ when used for SMC in children? (in order to check if dosing is optimal and to use this data to interpret the duration of protection and understand the drug levels required for clinical protection)	- Duration of protection - Correlation between day 7 concentration of piperaquine and protection against clinical malaria	- Regression splines using the method of Lambert and Royston - The logrank test for trend
4. What is the effect of SMC in selecting for drug-resistant parasites?	Selection of key molecular markers by SMC	Chi square test

3.3 Pharmacokinetics of SPAQ during the SMC in Lamarame, Senegal

3.3.1 Study site

The study was conducted in Lamarame, NDoffane District, Senegal. After holding community meetings to explain the study, boys and girls who did not have history of allergy to study drugs and whose parents agreed for them to participate were enrolled at the clinic.

3.3.2 Screening and enrolment

During the study organization phase, meetings were held at the health facilities, we explained, in local language, the aims of the study, the recruitment and follow up procedures, the total sample

size needed, and the duration of follow up as well as the detailed schedule for blood sampling. Subsequently we visited families at home to explain the study procedures and invite people to come to the clinic. Mothers or guardians were asked to bring the child to the clinic where, after signing the indicated consent. The child was weighed and had a clinical examination, a finger prick blood sample into a microtainer, from which 70 μ L of blood the laboratory technician pipetted onto filter paper.

3.3.3 Drug administration

After the first blood samples were collected, the children were given one course of treatment with sulfadoxine-pyrimethamine, and amodiaquine over three days, in April 2011, and were followed-up for one month. Each child was weighed, treated on day 1 with SP (25mg/kg sulfadoxine 1.25mg/kg pyrimethamine) and one dose of amodiaquine (10mg/kg amodiaquine base). Drugs were given according to the child's weight and he was kept under observation for 30 minutes before being allowed to return home. The remaining doses of amodiaquine (days 1 and 2) was given to a field worker who visited the child at home to supervise drug administration. The exact date and time of the administration was recorded. Children who vomited a dose were given a repeat dose and the vomiting carefully recorded. Children with repeated vomiting were kept out of the PK cohort but followed for adverse event purpose and if necessary referred for appropriate care.

3.3.4 Blood sampling for pharmacokinetics analysis

Children were asked to provide a finger prick blood sample on three further occasions between day 1 and day 28, determined according to a pre-defined schedule. All enrolled children were asked to give blood before the first dose of the treatment; then they were randomly allocated in one of three different windows for the subsequent sampling, the timing of the samples within the indicated window was randomly allocated, with an even spread within the window. This scheme reflects the need to estimate PK parameters for two different drugs with different half-lives.

Table 3.3 PK sampling windows per group

	Sample 2	Sample 3	Sample 4
Group 1:	Day 1-5	Day 6-14	Day 15-28
Group 2:	Day 1-3	Day 4-10	Day 11-28
Group 3:	Day 1-3	Day 4-6	Day 7-28

Finger-prick blood was collected into a microtainer containing EDTA or heparin, and then an exact amount of 70 µL of blood taken and stored onto a filter paper labelled with the child's identification number, the time, date and day the sample was collected. The filter paper was dried at room temperature taking care that the blood spot does not have any contact with any surface while it was drying.

3.3.5 Follow up and home visits

Trained field staff visited each child on day 4, to ask about any adverse reactions to the drugs, and on day 14 and 28 to check the child was well. Children with signs of severe malaria, or any other severe illness were referred immediately to the health post. If a child had axillary temperature $\geq 37.5^{\circ}\text{C}$ or a history of fever in the last 48 hours a finger prick blood sample was taken for malaria diagnosis by rapid test and blood film. Children with a positive test were treated with Coartem.

3.3.6 Pharmacokinetic experiment protocol

The measurement of drug concentrations for pharmacokinetics parameters was performed at the London School of Hygiene and Tropical Medicine by Dr Kaur Harparkash between the end of July and August 2011. Individual filter paper was transferred into a small tube, then 50µL of Internal Standard added to each tube, followed by 250µL of CH₃OH/CH₃CHO in HCL, Somike for 2 minutes, then transfer the solution into the HPLC machine for procession.

3.3.7 Statistical methods

The sample size determination of 150 children aged 3-59 months was based on experience from other PK studies.

3.3.8 Ethical approval

The study was approved by the LSHTM Ethics Committee and the Conseil National de Recherche en Santé of the Senegalese Ministry of Health.

3.3.9 Data safety and monitoring board

A data safety and monitoring board was appointed within the University of Dakar to assess the safety aspect of the trial. They convened of regular meeting during the course of the trial and a final meeting upon the completion of the study.

3.3.10 Timeline of the field activities

The study took place in Lamaramé between May and July 2011

3.3.11 Quality control

The study benefited from an external clinical monitoring during the course of the trial and the tablets (SP & AQ) quality testing was undertaken in LSHTM, showing the drugs were of food quality.

3.3.12 Pharmacokinetic modelling

The full data analysis is specialised involving non-linear fitting, this was being done by Dr Neal Alexander, statistician at the London School of Hygiene and Tropical Medicine, London. For the purpose of the thesis, I performed simple descriptive analyses of the concentrations.

Chapter 4: Impact of SMC on malaria morbidity, clinical tolerability and biological safety

4.1 Overview

The preceding chapter 3 described the methods used in the clinical trial in Burkina Faso and in the pharmacokinetic study in Senegal which led to key findings presented in this chapter: the trial profile, the impact of SMC on malaria related morbidity, clinical tolerability and biological safety. The pharmacokinetic parameters resulted from the study in Senegal will be presented in the next chapter 5.

4.2 The trial profile

A total of 1519 children were screened in August 2009, 19 were not included due to recent use of antimalarial drugs (7), outside the age range (6), severe malnutrition (3) and other reasons (3). 1499 children were randomized, 750 to DHAPQ and 749 to SPAQ. 12 allocation errors occurred, 7 children randomized to SPAQ received DHAPQ in error, 2 randomized to SPAQ received mixed treatments, and 3 randomized to DHAPQ received mixed treatments, leaving 754 who received DHAPQ and 740 SPAQ, in the ATP analysis. 97% (1454/1499) of randomized children were seen at the survey at the end of the transmission season

The distribution of the randomized and untreated children is summarised in figure 4.1.

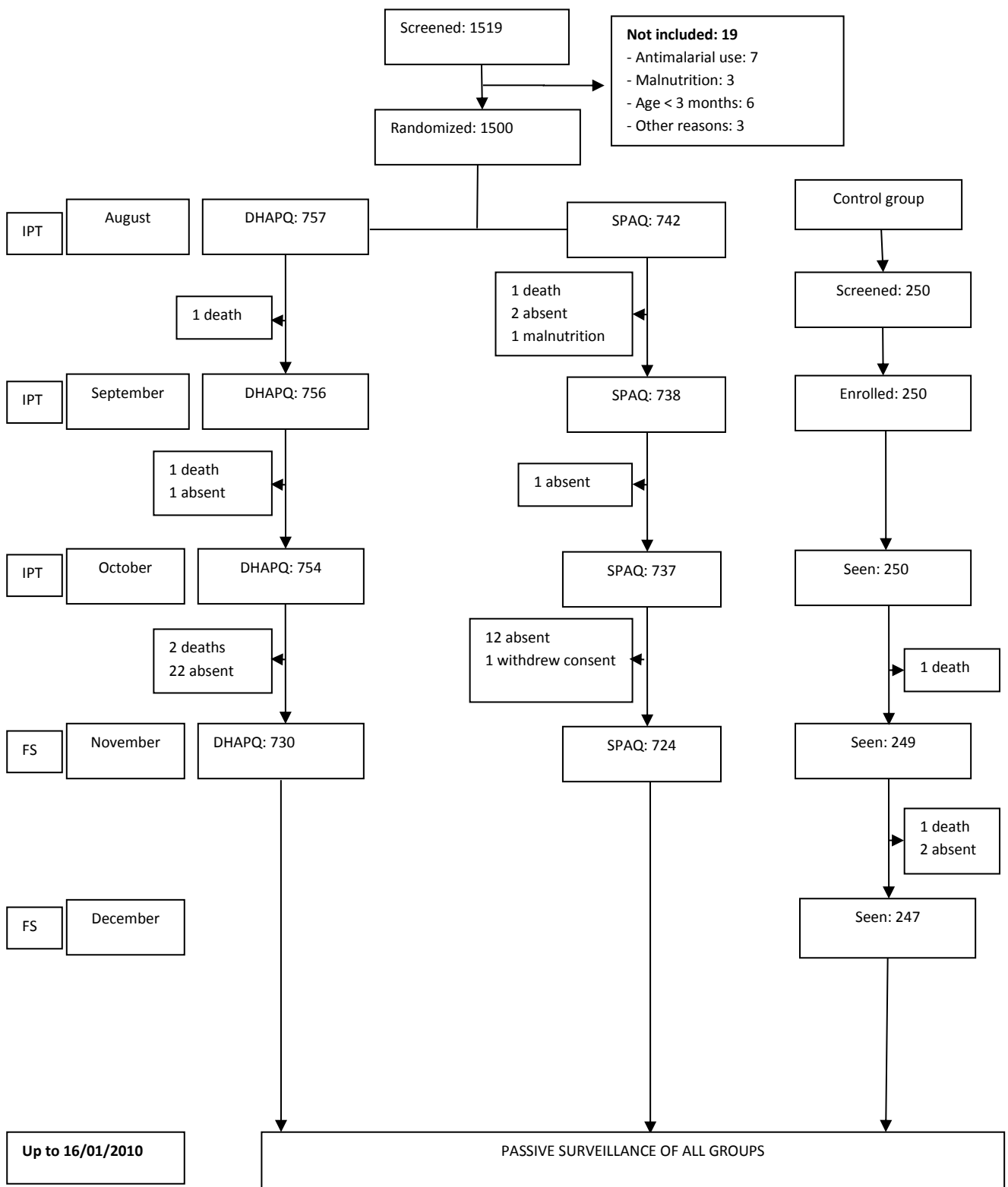


Figure 4.1 Trial profile

4.3 Compliance with treatment doses

During all rounds, the first dose of the drug was given and directly observed at the clinic by the study nurses. Subsequent doses were released to the mothers and the administration was done in the presence of the field worker who recorded the time of the drug uptake and the occurrence of any vomiting; a replacement dose was provided once if the child vomitted.

Compliance with daily doses: One patient did not receive the third dose of amodiaquine in August 2009. Two patients did not receive the second and third dose of AQ in August. Two patients missed the second and third dose of DHAPQ in August and one patient missed his doses in October.

Compliance with monthly doses: At enrolment, 9.5% (72/757) and 9.4% (70/742) respectively in the DHAPQ and SPAQ groups did not receive the SMC treatment due to clinical malaria. These proportions were respectively 8.1% (61/756), 7.5% (55/738) in September and 8.8% (66/754) and 8.3% (61/737) in October.

4.4 Baseline characteristics of the participants

Baseline characteristics of the three cohorts are shown in Table 4.1. The untreated cohort was not randomized, and was recruited later in the season.

4.4.1 Age distribution

Age distribution was similar across the groups, the mean age in month was 27.3 ± 15.8 months in the DHAPQ treatment group, 28.5 ± 15.8 months in children treated with SPAQ and 27.4 ± 15.5 in the comparator group. When the age was categorised, the proportion of the children in each age group was comparable across the cohorts.

4.4.2 Gender

The proportion of females was the same in the SPAQ and the comparator group (49%) and 51% in the DHAPQ treatment group.

4.4.3 The study villages

The children were recruited from three villages: Satiri, Kadomba and Balla. They were approximately equidistant (3 miles) from each other. The comparator group was selected in the village of Balla.

4.4.4 Prevalence of clinical malaria

The proportion of children with clinical malaria at enrolment was similar across the two groups 16% in the DHAPQ and 15% in the SPAQ group. This proportion was higher in the comparator group 64%.

4.4.5 Bed nets and ITNs use

The ownership of bednets was very low and similar across the study area; the percentage of children who slept under a bed net the night preceding the survey was also low.

4.4.6 Nutritional status

The proportion of children who were stunted (height for age Z-score less than -2 SD), wasted (weight for height Z-score less than -2 SD) and underweight (weight for age Z-score less than -2 SD) was similar in the two randomised groups. The comparator group recruited one month later had a higher proportion of wasted participants (42%).

4.4.7 Prevalence of fever at enrolment

Fever was defined as the measured axillary temperature equal to or greater than 37.5°C, the proportion of children with fever was exactly the same in the two SMC groups; a higher proportion of children in the comparator (untreated) group was febrile reflecting the timing of enrolment later in the malaria season.

Table 4.1: Baseline characteristics of the enrolled cohort

Variable	SMC randomized groups		Untreated cohort (n=250)
	SPAQ (n=742)	DHAPQ (n=757)	
Date enrolled	11-20 Aug	11-20 Aug	17-19 Sep
Study site			
Kadomba	323	325	0
Balla	151	150	250
Satiri	268	282	0
Gender %Male:%Female	49%:51%	50%:50%	49%:51%
Age group			
<12months	129 (17%)	153 (20%)	47 (19%)
12-23months	155 (21%)	158 (22%)	63 (25%)
24-35months	155 (21%)	152 (20%)	56 (22%)
36-47months	147 (20%)	138 (18%)	45 (18%)
48-59months	156 (21%)	156 (21%)	39 (16%)
Mean weight kg (SD)	10.9 (3.22)	10.7 (3.13)	10.1 (2.72)
Underweight	26%	25%	38%
Stunting	25%	24%	14%
Wasting	22%	22%	42%
Use of bednets (%)	36% (267)	36% (273)	32% (79)
Slept under ITN the night before (%)	27% (204)	25% (186)	31% (77)
Geometric mean parasite density/ μ L	7893	8027	15068
Number with fever*(%)	29% (213)	29% (216)	72% (179)
Number with malaria** (%)	96 (13%)	98 (13%)	81 (32%)
Number with parasites (%)	336 (45%)	323 (43%)	152 (61%)
Number of gametocytes (%)	80 (11%)	80 (11%)	75 (30%)

* Axillary temperature $>37.5^{\circ}\text{C}$ or history of fever in the past 24 hours and ** Fever with any parasitaemia, measured in August for the randomized groups and a month later, in September, for the untreated cohort.

The children in the control group came from only one of the three villages. The age distribution was similar to the randomized groups, the proportion under-weight was greater, this may be because the children in the control group were assessed later in the transmission season, but there were also village differences. The prevalence and density of parasitaemia was greater, also reflecting that this cohort was recruited later. The two randomized groups were similar to each other in all the baseline characteristics.

4.5 SMC and malaria-related morbidity parameters

4.5.1 Overall impact of SMC on the incidence of malaria

The primary outcome was the incidence of clinical malaria defined as fever with at least 3000 parasites per microlitre; this incidence was respectively 119 and 161 cases in the SPAQ and

DHAPQ group yielding an incidence rate of 54.7 episodes per 1000 per month in the SPAQ group and 72.2 in the DHAPQ group (table 4.2) in the according-to-protocol analysis. The number of incident cases of malaria was similar in the intention-to-treat analysis (table 4.3).

Table 4.2: According To Protocol analysis of the incidence rate over three months of SMC

Drug	N	Cases	Person months	Rate /1000 /month
Fever with any parasitaemia				
SPAQ	740	156	2175.4	71.7
DHAPQ	754	200	2228.9	89.7
Fever with parasitemia $\geq 3000/\mu\text{L}$				
SPAQ	740	119	2175.4	54.7
DHAPQ	754	161	2228.9	72.2

Table 4.3: Intention To Treat analysis of the incidence rate over three months of SMC

Drug	N	Cases	Person months	Rate /1000 /month
Fever with any parasitaemia				
SPAQ	749	161	2202.5	73.1
DHAPQ	750	199	2216.6	89.8
Fever with parasitemia $\geq 3000/\mu\text{L}$				
SPAQ	749	122	2202.5	56.1
DHAPQ	750	159	2216.6	71.3

4.5.2 Analysis of non-inferiority

142 children (70 in the SPAQ group and 72 in the DHAPQ group) had malaria prior SMC administration, and received artemether-lumefantrine (Coartem®) instead of SMC drugs during this round. These children were included but the cases on day 0 were not counted in the analyses of SMC efficacy. During three months from the first dose of SMC the Kaplan-Meier estimate of the proportion of children with an episode of malaria ($\geq 3000/\mu\text{L}$) was 0.15 in SPAQ group and 0.19 in DHAPQ group, the odds ratio was 1.36 (95% CI 1.04,1.76; 90% CI 1.09,1.69). The cumulative hazard function (the mean number of malaria episodes per child) at three months was 0.16 in the SPAQ arm and 0.21 in the DHAPQ arm, hazard ratio 1.31 (95% CI 0.99, 1.74, two-sided P-value 0.059). When malaria with parasitemia at any density was considered, the odds ratio was 1.29 (1.02, 1.64) (Table 4.4). Similar results were obtained by ITT analysis (Table 4.5). The Kaplan Meier estimates (Figure 6) of the cumulative incidence of malaria of any parasitemia showed an increase in the incident cases around the next round (before the intervention was given).

Table 4.4: According To Protocol (ATP) analysis of malaria incidence over 3 months from the first round of SMC

	N	Cases	Person Mths	Rate /1000 /month	Proportion with malaria (K-M estimate)	se	OR (95% CI)	Cum. Hazard	se	HR (95% CI)	P-value
Fever with any parasitaemia											
SPAQ	740	156	2175.4	71.7	0.193	0.014	1	0.213	0.017	1	-
DHAPQ	754	200	2228.9	89.7	0.236	0.015	1.29 (1.02,1.64)	0.266	0.019	1.25 (0.97,1.62)	0.090
Fever with parasitemia $\geq 3000/\mu\text{L}$											
SPAQ	740	119	2175.4	54.7	0.149	0.013	1	0.161	0.015	1	-
DHAPQ	754	161	2228.9	72.2	0.192	0.014	1.36 (1.04,1.76)	0.212	0.017	1.31 (0.99,1.74)	0.072

Table 4.5: Intention To Treat (ITT) analysis of malaria incidence over 3 months from the first round of SMC

	N	Cases	Person months	Rate /1000 /month	Proportion with malaria (K-M estimate)	se	OR (95% CI)	Cum. hazard	se	HR (95% CI)	P value
Fever with any parasitaemia											
SPAQ	749	161	2202.5	73.1	0.195	0.014	1	0.215	0.017	1	-
DHAPQ	750	199	2216.6	89.8	0.234	0.015	1.26 (1.00, 1.59)	0.264	0.019	1.22 (0.95,1.58)	0.122
Fever with parasitemia $\geq 3000/\mu\text{L}$											
SPAQ	749	122	2202.5	56.1	0.151	0.013	1	0.163	0.015		-
DHAPQ	750	159	2216.6	71.3	0.191	0.014	1.33 (1.02,1.72)	0.210	0.017	1.29 (0.97,1.71)	0.075

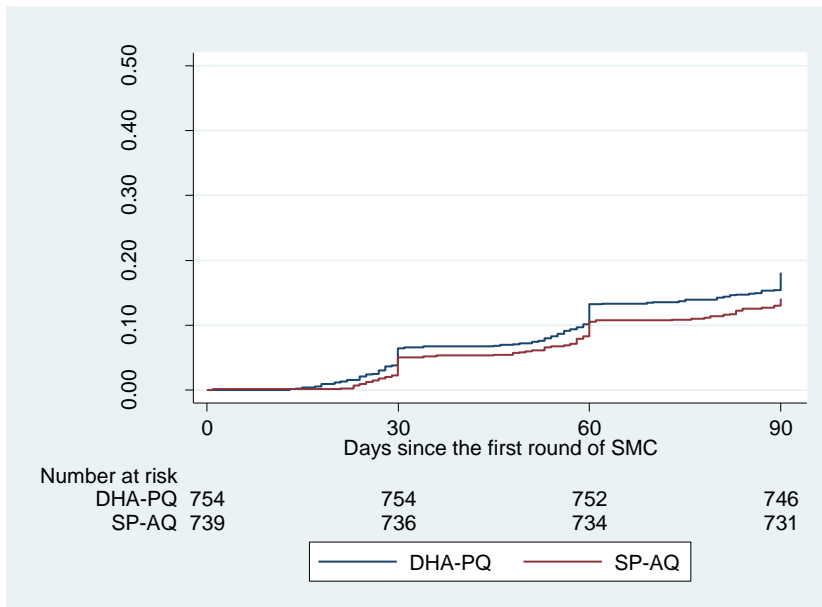


Figure 4.2a: Kaplan-Meier estimates of the proportion of children with an episode of malaria

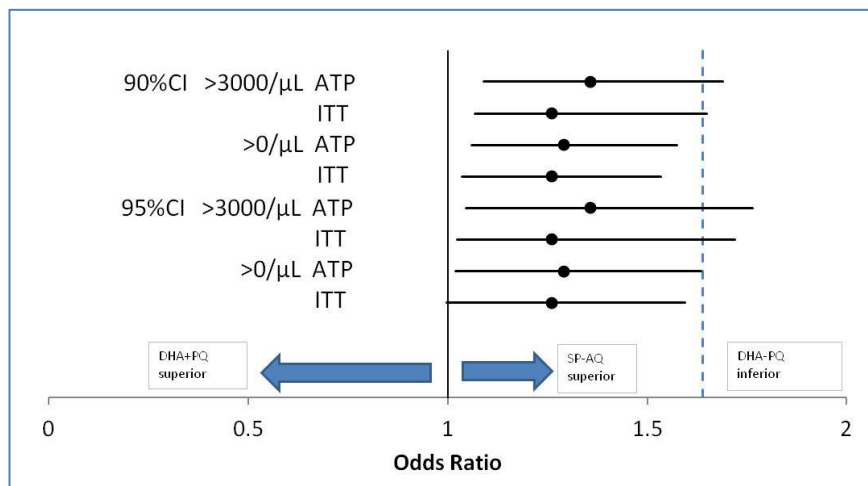


Figure 4.2b: Interpretation of the odds ratios and confidence intervals. The diagram shows the 90% and 95% confidence intervals for the odds ratios for ATP and ITT analyses for the primary endpoint (malaria with parasitaemia above 3000/ μL) and for the secondary endpoint (malaria with parasitaemia at any density). An odds ratio of 1.64 was specified as the non-inferiority margin. SMC with SPAQ is highly efficacious reducing the risk of malaria by about 80% compared to placebo. The non-inferiority margin is the amount of this protection that we would be prepared to give up in order to use an alternative drug regimen. DHA-PQ would be regarded as not inferior to SPAQ if it could be concluded that the odds of having malaria was not more than 1.64 times as great in children who received DHA-PQ, as in children who received SPAQ. All the 95% confidence intervals for the odds ratio lie entirely above 1, indicating that SPAQ is superior to DHAPQ. For the primary endpoint, ATP and ITT 90% intervals just cross the non-inferiority margin, but for malaria defined as fever with parasitaemia at any density, the margin is not crossed by the 90% or by the 95% confidence intervals. The fact that the margin is not crossed for this endpoint, and just crossed for the other endpoint, suggests the difference is small and may not be regarded as a very important difference. In summary, the 90% and 95%CI's do cross the margin for some analyses, but are entirely above 1, so we are confident SPAQ is superior to DHAPQ, but we are somewhat less confident in our conclusion that the DHAPQ is not inferior to SPAQ.

4.5.3 Comparison of the incidence of malaria in SMC and untreated groups

Two hundred and fifty children 3-59 months were recruited in September when the second round of SMC was being administered to children in the trial. To estimate the efficacy of SMC, the incidence of malaria was compared between the SMC groups and the untreated group over 2 months following the second round of SMC, adjusting for site, age group and insecticide treated bed-nets use. A total of 338 episodes of malaria were recorded in this period in the untreated children, a rate of 0.63 episodes per child per month. Efficacy against malaria (fever with any parasitaemia) was high in both groups, 74% (DHAPQ) and 80% (SPAQ), (table 4.6). For malaria defined as fever with parasite density $\geq 3000/\mu\text{L}$, there were 229 episodes in the untreated cohort, a mean of 0.92 episodes per child, compared with 108 episodes in the DHA group and 78 in the SPAQ group over the same period, efficacy adjusted for the same covariates was 79% (70%,85%) and 84% (76%,90%) respectively. To estimate duration of protection, incidence after the last round of SMC in each treatment group was compared with the incidence in controls over the same period, adjusted for the same covariates. In both groups protection persisted at a high level for 3-4 weeks in each group and decreased rapidly thereafter (Fig 4.2).

Table 4.6 Protective efficacy of SMC

Cox regression analysis of malaria incidence (fever with parasite density $>0/\mu\text{L}$) over two months from the second round of SMC showed a protective efficacy of 74% in DHAPQ and 80% in the SPAQ group.

	No. of children	Cumulative Hazard	Crude hazard ratio	Adjusted hazard ratio (95% CI)	Efficacy (95% CI)
Untreated	250	0.95	1	1	
DHAPQ	753	0.18	0.19	0.26 (0.19,0.35)	74% (65%, 81%)
SPAQ	735	0.14	0.15	0.20 (0.14,0.28)	80% (72%, 86%)
Site:					
Kadomba	644	0.18	1	1	
Balla	549	0.55	3.01	1.12 (0.79,1.59)	
Satiri	545	0.11	0.59	0.60 (0.42,0.85)	
ITN use:					
No	1279	0.29	1	1	
Yes	464	0.24	0.84	0.82 (0.67,1.01)	
Age					
<12 months	328	0.22	1	1	
12-23 months	374	0.37	1.68	1.54 (1.18,2.01)	
24-35 months	360	0.44	2.01	1.91 (1.47,2.49)	
36-47 months	328	0.20	0.90	0.91 (0.66,1.25)	
48-59 months	347	0.12	0.55	0.58 (0.39,0.85)	

A cohort of untreated children was recruited as a control group, at the time the main cohort received the second round of SMC. The y-axis shows the mean number of episodes per child since the start of surveillance. Malaria episodes were detected by passive detection, and at cross-sectional survey performed just thirty days after the last round of SMC. Incidence of malaria was much higher in the comparator group who started just few days after the randomised group had received its second dose of SMC. The cumulative hazard was almost three times the hazard in the treated cohort where it remained under 0.40 (figure 4.3).

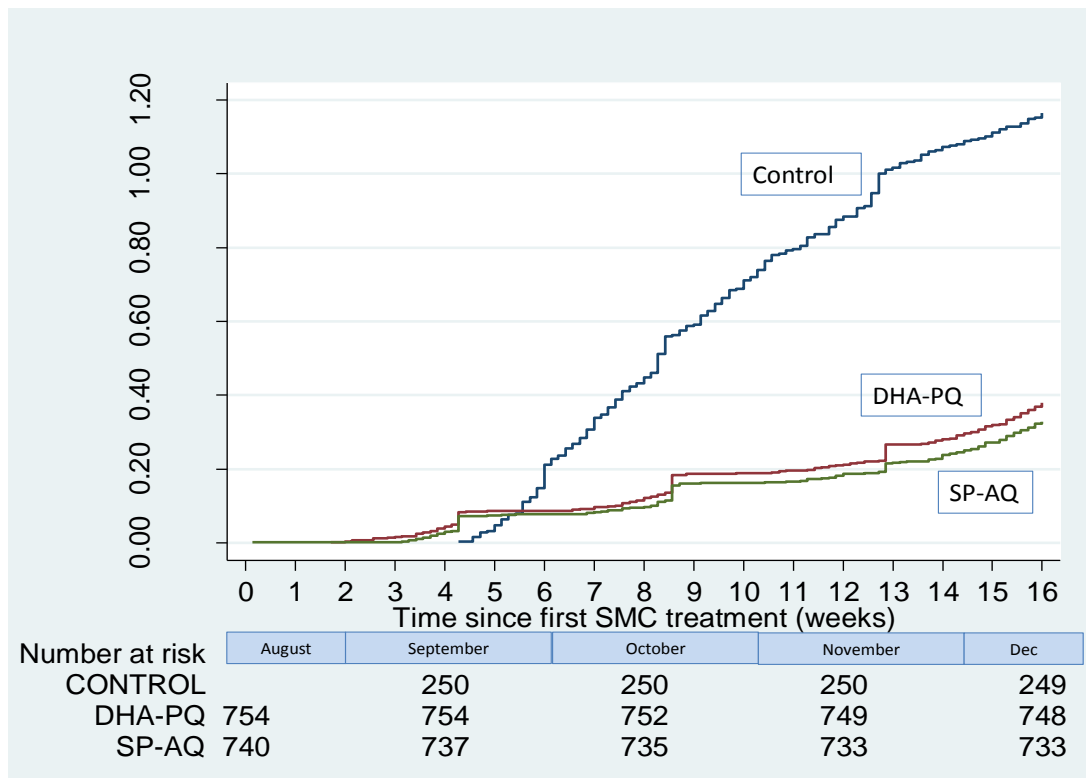


Figure 4.3: Cumulative hazard of malaria (fever or history of fever with any parasitemia) in children who received SMC with DHAPQ or SPAQ on three occasions in August, September and October.

4.5.4 Incidence of severe malaria

Four cases of severe malaria were recorded, two in the DHAPQ arm and one respectively in SPAQ and untreated groups. In the DHAPQ group, two female participants respectively 7 and 5 months on admission presented with positive smear, neurologic signs (convulsions) and severe anemia (hb<5 g/dl). In the comparator group a female aged 9 months was admitted for severe anaemia and neurologic malaria. In the SPAQ group, a male, 19 months was admitted for haemoglobinuria. All children received intravenous quinine and recovered.

4.5.5 Impact of SMC on the prevalence of *Plasmodium falciparum* infection and gametocyte carriage at the end of the transmission season survey

The prevalence of parasitaemia and of gametocyte carriage was similar in both SMC groups, and substantially lower in the SMC groups than in the untreated controls (Table 4.7). In the control group, the prevalence of parasitaemia at the end of the transmission season was lower than when subjects were enrolled (36% compared to 61% at enrolment, Table 4.1), presumably reflecting the effect of antimalarial treatment received for clinical cases in this group.

Table 4.7: Parasitemia and Gametocytemia at the end of the transmission season survey

	Prevalence of parasitaemia			Prevalence of gametocytes		
	No. sampled	No. positive (%)	Risk ratio (95% CI)	No. sampled	No. positive (%)	Risk ratio (95% CI)
Comparator	247	88 (36%)	1	247	18 (7%)	1
DHAPQ	722	88 (12%)	0.34 (0.26,0.44)	721	6 (0.8%)	0.11 (0.05,0.28)
SPAQ	731	89 (12%)	0.34 (0.26,0.44)	727	6 (0.8%)	0.11 (0.05,0.28)

4.5.6 Impact of SMC on nutritional status

At enrolment, severe malnutrition was an exclusion criterion; therefore any class of malnutrition found did not appear to influence the intervention outcome as tested in Cox regression.

The proportion of underweighted and wasted decreased over the course of the trial but not the stunting stunted which in fact increased. In the treated groups, the proportion of wasted increased slightly 12%; other parameters improved. The treated and comparator groups were not comparable in regard to the differences in time of their recruitment (table 4.8).

Table 4.8: Evolution of nutritional parameters over the follow up period

	Underweight (%)		Stunting (%)		Wasting (%)	
	Pre	Post	Pre	Post	Pre	Post
Comparator	38%	23%	14%	24%	42%	16%
DHAPQ	25%	20%	24%	13%	22%	25%
SPAQ	26%	16%	25%	12%	22%	25%

4.5.7 Prevalence of anemia at the end of the transmission season survey

The prevalence of severe anaemia was similar in the two treated groups (both 9%). Prevalence was lower in the untreated group, possibly due to the fact that this group was surveyed one

month later, November in the intervention groups and December in the untreated group (table 4.9).

Table 4.9: Haemoglobin concentration at the end of season survey:

	Heamoglobin (g/dL)		
	Number sampled	<10 g/dL (%)	< 5 g/dL (%)
Control	243	114 (47%)	4 (2%)
DHAPQ	719	253 (35%)	66 (9%)
SPAQ	713	231 (32%)	64 (9%)

4.6 Clinical tolerability of the intervention

4.6.1 Mild adverse events

The incidence of mild adverse events was higher in the first round of SMC than in subsequent rounds, and in each round, the incidence was similar in both treatment groups (figure 8). The most commonly reported mild adverse events reported were cough, diarrhoea, vomiting, and fever (table 4.10).

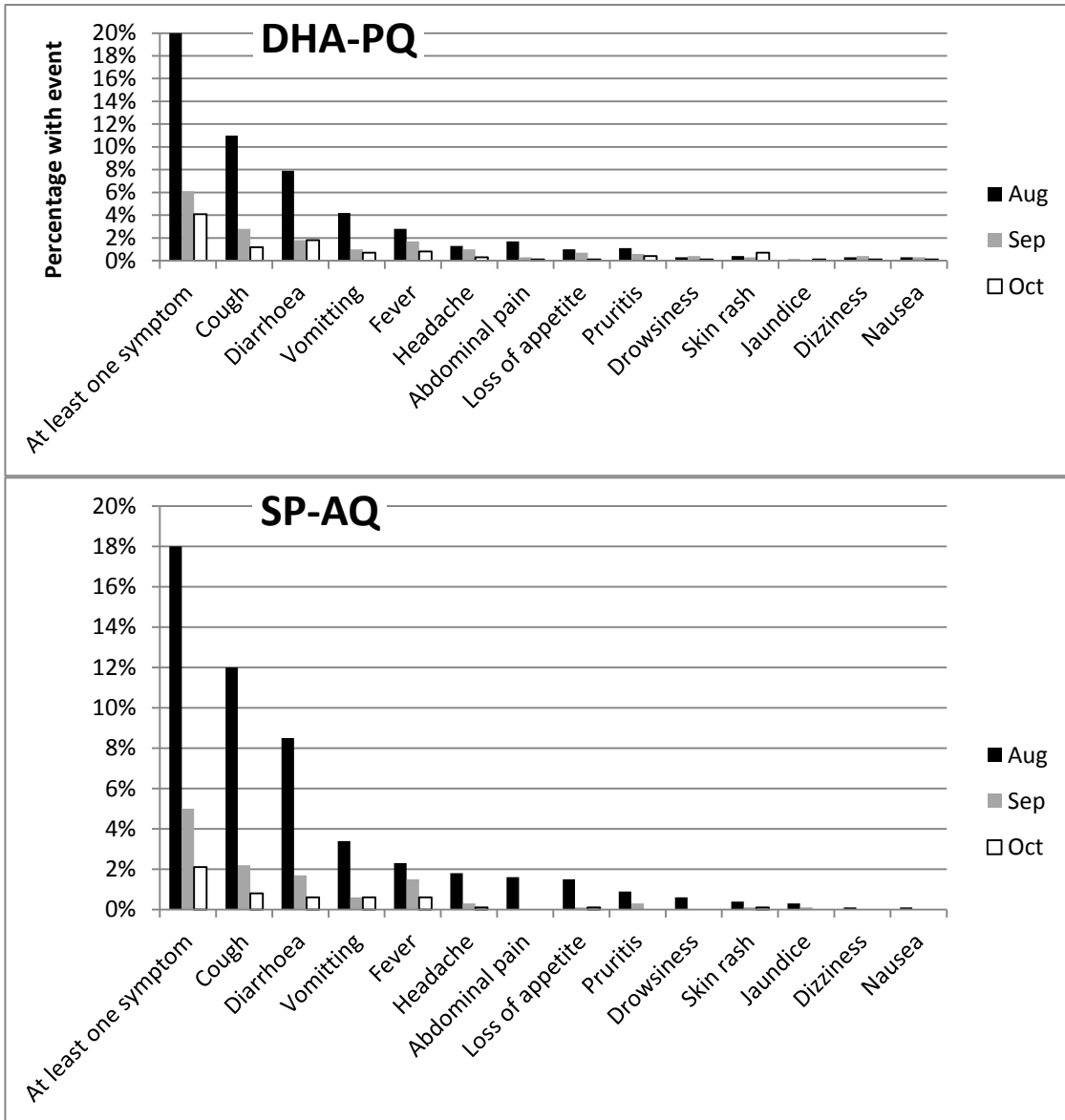


Figure 4.4: Incidence of mild adverse events over the three rounds in the DHAPQ and SPAQ

Table 4.10: Incidence of mild adverse events

Symptom/signs	DHAPQ			SPAQ					
	Aug	Sep	Oct	Aug	Sep	Oct			
	N=707	N=726	N=735	N=685	N=713	N=709	August	September	October
	Proportion (%) with mild adverse events						Risk difference % with (95%CI)		
At least one symptom	20	6.1	4.1	18	5.0	2.1	2.80 (1.20, 12.10)	-1.00 (-3.40,1.40)	-2.00 (-3.70,-0.20)
Cough	11	2.8	1.2	12	2.2	0.80	1.80 (1.80, 8.50)	-0.50 (-2.10,1.10)	-0.40 (-1.40,0.70)
Diarrhoea	7.9	1.8	1.8	8.5	1.7	0.60	1.00 (0.70,3.40)	-0.10 (-1.50,1.20)	-1.20 (-2.30,-0.10)
Vomiting	4.2	1.0	0.70	3.4	0.60	0.60	1.70 (0.80,2.30)	-0.40 (-1.30,0.50)	-0.10 (-0.90,0.70)
Fever	2.8	1.7	0.80	2.3	1.5	0.60	1.00 (0.30,1.80)	-0.10 (-1.40,1.20)	-0.30 (-1.10,0.60)
Headache	1.3	1.0	0.30	1.8	0.30	0.10	0.30 (0.10,1.60)	-0.70 (-1.50,0.10)	-0.10 (-0.60,0.30)
Abdominal pain	1.7	0.30	0.10	1.6	0.00	0.00	0.70 (0.10,1.50)	-0.30 (-0.70,0.10)	-0.10 (-0.40,0.10)
Loss of appetite	1.0	0.70	0.10	1.5	0.10	0.10	0.60 (0.40,0.90)	-0.50 (-1.20,0.10)	0.00 (-0.40,0.40)
Pruritis	1.1	0.60	0.40	0.9	0.30	0.00	0.40 (0.10,0.60)	-0.30 (-0.90,0.40)	-0.40 (-0.90,0.10)
Drowsiness	0.30	0.40	0.10	0.6	0.00	0.00	0.30 (0.70,0.40)	-0.40 (-0.90,0.10)	-0.10 (-0.40,0.10)
Skin reaction	0.40	0.30	0.70	0.4	0.10	0.10	0.00 (0.10,0.30)	-0.10 (-0.60,0.30)	-0.50 (-1.20,0.10)
Jaundice	0.10	0.00	0.10	0.3	0.10	0.00	0.40 (0.10,0.10)	0.10 (-0.10,0.40)	-0.10 (-0.40,0.10)
Dizziness	0.30	0.40	0.10	0.1	0.00	0.00	0.30% (0.10,0.10)	-0.40 (-0.90,0.10)	-0.10 (-0.40,0.10)
Nausea	0.30	0.30	0.10	0.1	0.00	0.00	0.00 (0.00,0.00)	-0.30 (-0.70,0.10)	-0.10 (-0.40,0.10)

4.6.2 Serious adverse events

Four children died in the DHAPQ group: a boy aged 20 months died in September, 24 days after the August round of SMC, with respiratory distress; a girl aged 5 months died in September, 14 days after the September round of SMC, with gastro enteritis; a girl aged 1 year died in November, 34 days after the October round, with gastro-enteritis; and a girl aged 21 months died in October 8 days after the October round, with pneumonia. Two children died in the SPAQ group, a boy aged 2 years, died in September, 26 days after August round, with dehydration, and a boy aged 3 years died in October, 19 days after the September round, with gastro-enteritis. In the untreated cohort, a girl aged 3 years died in November, with malnutrition. None of these deaths was considered related to SMC.

4.7 Biological safety

4.7.1 Pooled analysis

To assess the impact of the SMC intervention on the haematological and biochemical parameters a subset of children in the treated cohort was selected to give 5 ml of venous blood on day 0 before the round dose administration and again on day 7.

A target of 45 children in each arm was calculated for the study (15 per arm and per round). To improve adherence, the sample size was split between the rounds and each participant child was sampled on a single round; the advantage being that children sampled on the last round may have accumulated the impact (positive or negative) of the intervention and this could be reflected in the parameters measured. Venous blood sampling was very challenging: despite repeated explanations and meetings with the mothers and local leaders, parents remained reluctant to attend for the remaining sampling; certain parents after the day 0 sampling collection refused to give the second sample; for some of the children, the amount withdrawn was not enough to be processed. In addition to these challenges in the field, haemolysis, breakage of a few tubes and insufficiency of plasma after centrifugation further limited the number of valid samples for analysis. For the purpose of the analysis, all data were pooled and all day 0 samples over the three rounds were analysed together. The results obtained indicated a decrease in the PVC and haemoglobin values in the children treated with DHAPQ between day 0 and day 7; the adjusted difference on day 7 was highly significant for the haemoglobin (1.03 95% CI [0.51,1.55], $p=0.000$) and for the PVC (2.31 95% CI [0.86,3.77], $p=0.002$) (table .4.11).

4.7.2 Parameters values outside the normal range

In this section we examined each parameter to detect any value outside the adopted normal range for the haematological and biochemical values.

4.7.2.1 White Blood Cell count

High values: One participant treated with DHAPQ had a WBC 18900 cells per mm³ on day 0 and 17200 cells per mm³ on day 7 in September. The participant R0648-7 treated with SPAQ on September round had 16300 cells per mm³ on day 0 and 12100 cells per mm³ on day 7 (fallen within the normal range).

Low values: Seven (7) patients in the DHAPQ group and eight (8) patients had a day 0 WBC count less than the normal value of 5300/mm³. By day 7 only one patient in the SPAQ group still had a low WBC count 3500 per mm³.

4.7.2.2 Red Blood Cell count

High values: Four (4) patients in the DHAPQ and two (2) patients in the SPAQ had a RBC count above the normal range (3.05 to 5.12x10⁶) on day 0. By day 7 two patients (2) in the DHAPQ and one (1) patient in the SPAQ group remain slightly above the normal range (5.41 for the patient in the SPAQ group, and 5.16 & 5.20 for those in the DHAPQ group).

Low values: Three patients (2 in the SPAQ and 1 in the DHAPQ) were below the normal range on day 0, by day 7 all of them fall into the normal range

4.7.2.3 Haemoglobin

On day 0 two (2) patients in the SPAQ group fell below the normal range (7.4 to 12.4 g/dl), their values were 4.6g/dl & 5.5 g/dl; in the DHAPQ group one patient was below the normal 7.3g/dl.

By day 7 only one patient's value in the SPAQ group remained low, even though it increased to 4.8g/dl. In our context, a haemoglobin above 12.4g/dl is not abnormal, therefore only unusual two high values were reported in patients treated with DHAPQ (17.7g/dl & 19.8g/dl) on day 0 but they fall into common range of values on day 11g/dl & 12.8g/dl respectively, so these high values may be due to measurement errors.

4.7.2.4 Creatinine

On day 0, five (5) patients in the DHAPQ group and eight (8) in the SPAQ group had a creatinine values above the normal range (< 60 µmol/liter); only four (4) of them all in the SPAQ group accepted to be sampled again on day 7. Of the four, one patient had normal value and the three other had values slightly above 60 respectively 73.6, 60.8 & 64 µmol/liter.

4.7.2.5 TGOA

Fifteen (15) patients in the DHAPQ and thirteen (13) in the SPAQ group had an absolute TGOA value above 60 UI/L on day 0. On day 7 ten (10) patients in the DHAPQ and six (6) in the SPAQ group refused further sampling. Of those who accepted the sampling, respectively one and three in the DHAPQ and SPAQ were still above the normal range on day 7.

4.7.2.6 TGPA

Seven (7) patients all in the SPAQ group had day 0 value above the normal range (<60 UI/l). On day 7 three (3) refused further sampling; of the four (4) only one had a normal value.

4.7.2.7 Platelet count

Three patients (3) all in the SPAQ group had day 0 platelets above the normal range (133-523 000/mm³) and this trend was maintained upon day 7.

4.7.2.8 The Lymphocytes

Three patients in the SPAQ group had a proportion of lymphocytes outside the normal range (two above 70.7% and one less than 36.7%) but all of them come down within the normal range on day 7.

Table .4.11: Haematological and biochemical variables measured before and after SMC

Variable	DHAPQ			SPAQ			Adjusted difference	
	Day 0	Day 7	N [#]	Day 0	Day 7	N [#]	SPAQ-DHPQ (95%CI)*	P-value
Haemoglobin g/dL	10.9	10.4	37	10.6	11.3	47	1.03 (0.51,1.55)	0.000
PCV %	33.1	32.5	37	32.4	34.4	47	2.31 (0.86,3.77)	0.002
Platelets10 ³ / mm ³	234	319	37	276	304	47	-28 (-84,28)	0.316
White blood cells10 ³ / mm ³	8151	8714	37	7665	9035	48	599 (-767,1963)	0.386
Red blood cells10 ⁶ /mm ³	4.284	4.242	37	4.082	4.370	48	230 (48,412)	0.014
Neutrophils, cells/ mm ³	3083	3474	32	2994	3505	41	53 (-925,1031)	0.914
Eosinophils, cells/ mm ³	233	173	32	194	223	41	54 (-42,151)	0.266
Basophils, cells/ mm ³	3.00	0.00	32	1.24	3.61	41	3.6 (-2.2,9.3)	0.219
Lymphocytes, cells/ mm ³	4752	4830	32	4283	4974	41	393 (-360,1147)	0.302
Monocytes, cells/ mm ³	129	118	32	118	129	41	15 (-38,69)	0.575
Creatinine, µmol/litre	34.2	34.3	17	42.6	50.2	26	13 (-2.1,29)	0.089
Tgoa, IU/litre	54.4	53.1	17	56.2	59.5	25	6.4 (-10,23)	0.449
Tgpa, IU/litre	29.8	35.9	17	45.5	43.4	25	5.4 (-12,23)	0.534
Vgm, Fl	77.6	77.1	37	78.6	79.0	47	1.3 (-0.81,3.3)	0.229
Tcmh pg/GR	25.6	24.8	37	26.2	26.0	47	0.70 (-0.06,1.5)	0.069
Ccmh g/dL	32.8	32.1	37	32.8	33.0	47	0.95 (0.03,1.9)	0.044

[#]No. of children sampled on both days, *difference between group means on day 7, adjusted for the day 0 value

Summary

SMC with SPAQ was highly effective, the protective efficacy was 74% & 80% respectively for DHAPQ and SPAQ for malaria of any parasitemia and 79% & 84% for malaria with 3000 parasites per microliter). SPAQ was more efficacious than DHAPQ, but the difference was within the margin set for non-inferiority. Both regimens gave a very high level of protection lasting 4 weeks and the children were again at risk between two rounds. Both regimens were well tolerated, incidence of mild adverse events decreased in successive months, consistent with tolerance to study drugs. A limitation was that incidence of mild adverse events was not assessed in controls, some of these events may not have been drug-related.

There was a very high burden of malaria in the untreated children.

Chapter 5: Association of piperazine concentration with treatment dosage and incidence of malaria

5.1 Overview of the chapter

This chapter reported relevant results from the nested pharmacokinetics study undertaken in the cohort of children treated with DHAPQ (part of the main clinical trial) and described in the section 3.2.15 of the methods chapter (chapter 3). Drug efficacy is a function of multiple factors; one of them is directly related to the drug itself. Among other factors we can include the dose administered orally (based on weight), the plasma concentration and the duration of action of the active metabolite. These factors play a central role in the growing SMC strategy, particularly plasma concentration and duration of protection are insufficiently explored. The following points are successively presented:

- A brief review of the statistical methods used to analyse the data
- The relationship between the drug administration in mg per kg and the age;
- The relationship between the dose in mg per kg and the plasma concentration on day 7
- The relationship between the age and the plasma concentration on day 7
- The incidence of clinical malaria in relation with the plasma concentration on day 7

In this paragraph, we estimated the duration of protection after one round of SMC.

5.2 Statistics methods

Day 7 piperazine concentration has been predictive of treatment outcome with dihydroartemisinin-piperazine. In this analysis, we selected only the samples collected on day 7 following the dose of the round.

The relationship between the age and the dose of piperazine per kg body weight and the drug concentration measured on day 7 was assessed using plots (groups of age against the dose in mg per kg and the age against the day 7 piperazine concentration).

Then the concentration of piperazine measured on day 7 were plotted against the dose administered in mg per kg will be plotted to assess the relationship between these parameters (concentration on day 7 in Y axis and the doses in mg per kg in X axis).

To assess the incidence of malaria in relation to the day 7 piperazine concentrations (duration of protection of one round of SMC), the follow up time was restricted from October.

October corresponded to the last administration of the SMC but the follow up continued up to the first two weeks of January; more episodes were expected on this period exceeding one month after the last dose of the SMC.

For the relationship between day 7 concentration and incidence of malaria analysis (the duration of protection), the time of entry in the survival analysis is set to be the day 7 date.

Time period to evaluate the SMC duration of protection: For the participants sampled in August, the exit date was the date of the first dose of September; those sampled in September round exited the date of the first dose in October; the exit date of children sampled in October was the first day of the final survey date. Attempt was made to maintain 30 days between two rounds of SMC in this analysis as it is planned in the interval time for the SMC delivery.

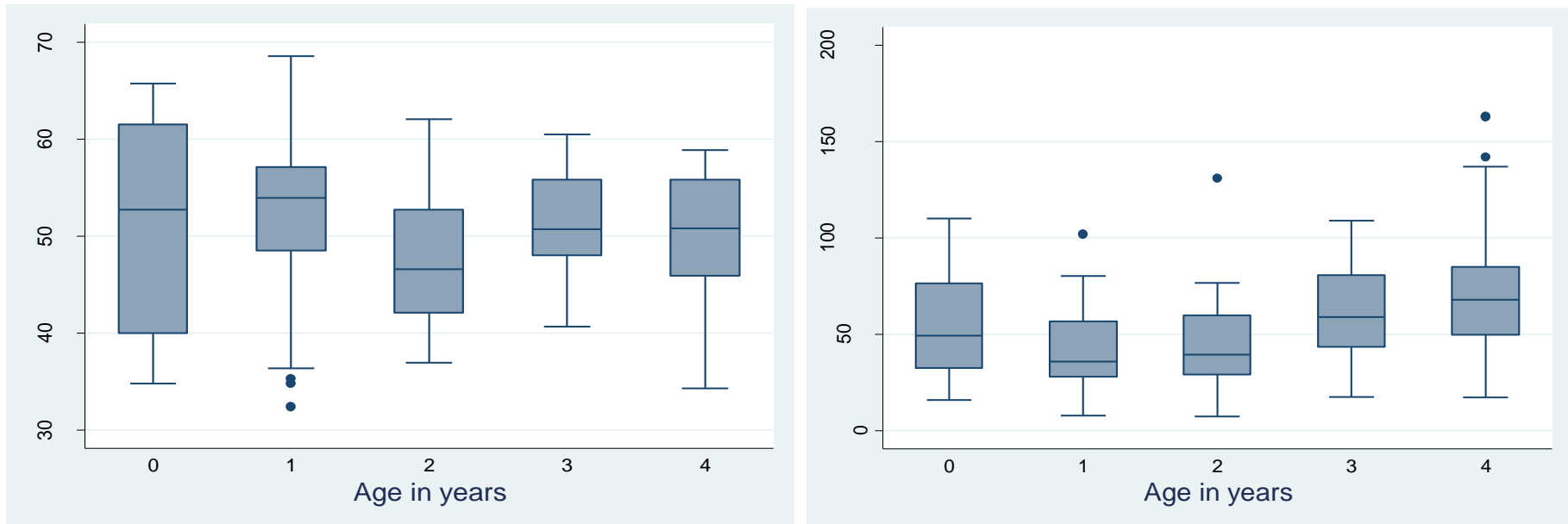
To assess how the number of the clinical cases varies with the level of the day 7 concentrations of piperazine was split into four quartiles to elucidate any variation of incident cases between different levels.

5.3 The relationship between the piperazine administered in milligram per kilogram body weight and the day 7 plasma concentration of PQ in ng/ml given with the DHA with the age.

The mean dose of piperazine was 50.5 ± 8.1 mg per kg body weight.

Children less than 24 months received the highest daily dose of piperazine per kg body weight and the lowest in those aged more than 24 months. (Figure 5.1). In linear regression, each increase in one unit of age (which in fact corresponds to 1 year) increased the PQ day 7 plasma concentration of 0.014 ng/ml, 95% CI [0.010 to 0.017], $p=0.0001$

Children 12-23 months that had high dose of piperazine per milligram per kilogram body weight appeared to exhibit low plasma concentration of piperazine on day 7. In linear regression model, there is a decrease of 0.008 ng/ml in plasma concentration of piperazine on day 7 for an increase in one unit of age.



* 0 [3-11 months], 1 [12-23 months], 2 [24-35 months], 3 [36-47 months], 4 [48-59 months]

Figure 5.1: Bar plot of the relationship between piperaquine administrated in milligram per kilogram of body weight and the day 7 plasma concentration of PQ in ng/ml with the age group

5.4 The relationship between dose in mg per kilogram and plasma concentration of Piperaquine on day 7

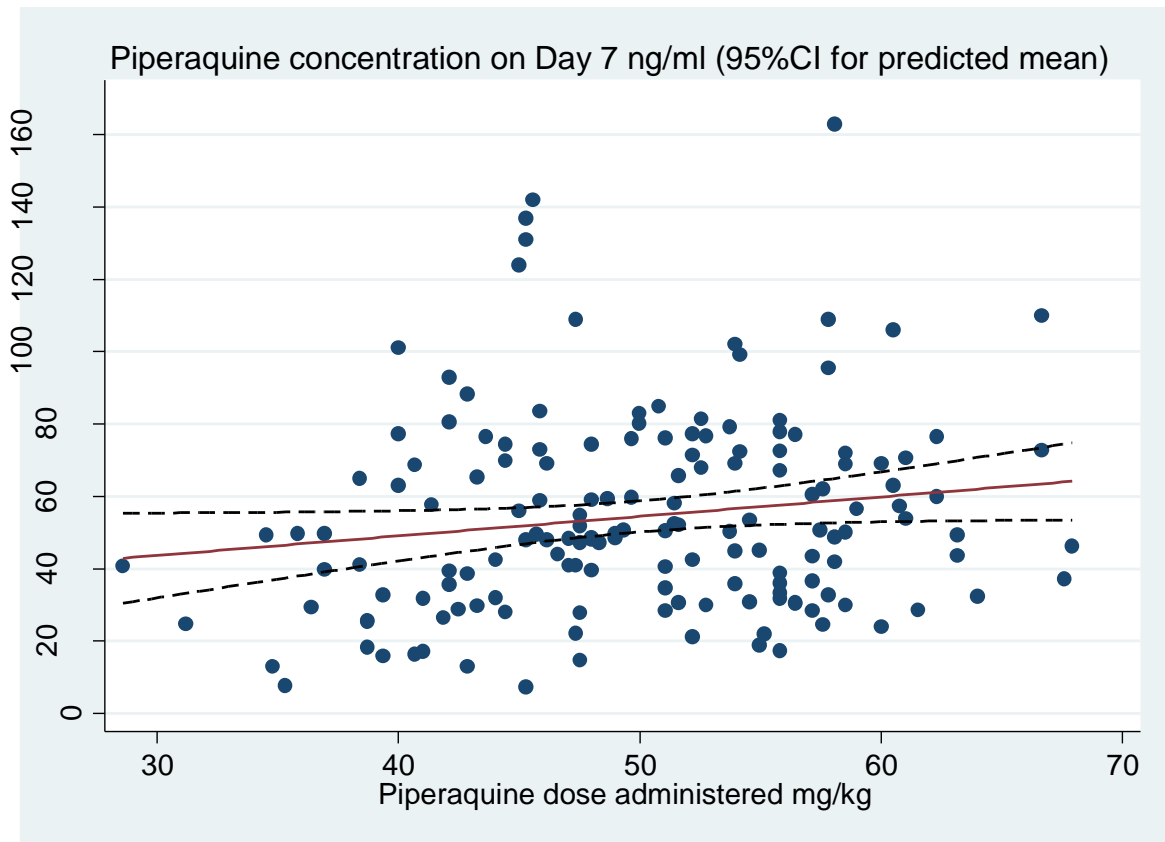


Figure 5.2: Relationship between dose in mg per kg and day 7 concentration of PQ

The concentration of piperazine on day 7 is a function of the dose of piperazine administered in milligram per kilo of body weight (figure 5.2).

5.5 The incidence of clinical malaria in relation with the plasma concentration on day 7

Fifty five children were sampled; sixteen experienced clinical malaria through the whole period of the surveillance. Most of the cases occurred in the first quartile representing the lowest concentration of piperazine as measured on day 7; the third quartile had the second largest number of cases (table 5.1).

Table 5.1: Incidence rate of malaria per quartile PQ concentration

	Mean ng/ml	Concentration range ng/ml	Cases	N	Days at risk	Rate	95% CI
<40	23.4	7.79 to 32.1	6	12	1103	0.005	2.10^{-3} to 12.10^{-3}
40 - 50	44.3	40.5 to 49.5	3	10	911	0.003	10^{-3} to 10^{-2}
50 -70	59.7	49.7 to 70.5	5	18	1648	0.003	10^{-3} to 7.10^{-3}
> 70	96.47	71.3 to 163	2	15	1376	0.001	4.10^{-4} to 6.10^{-4}

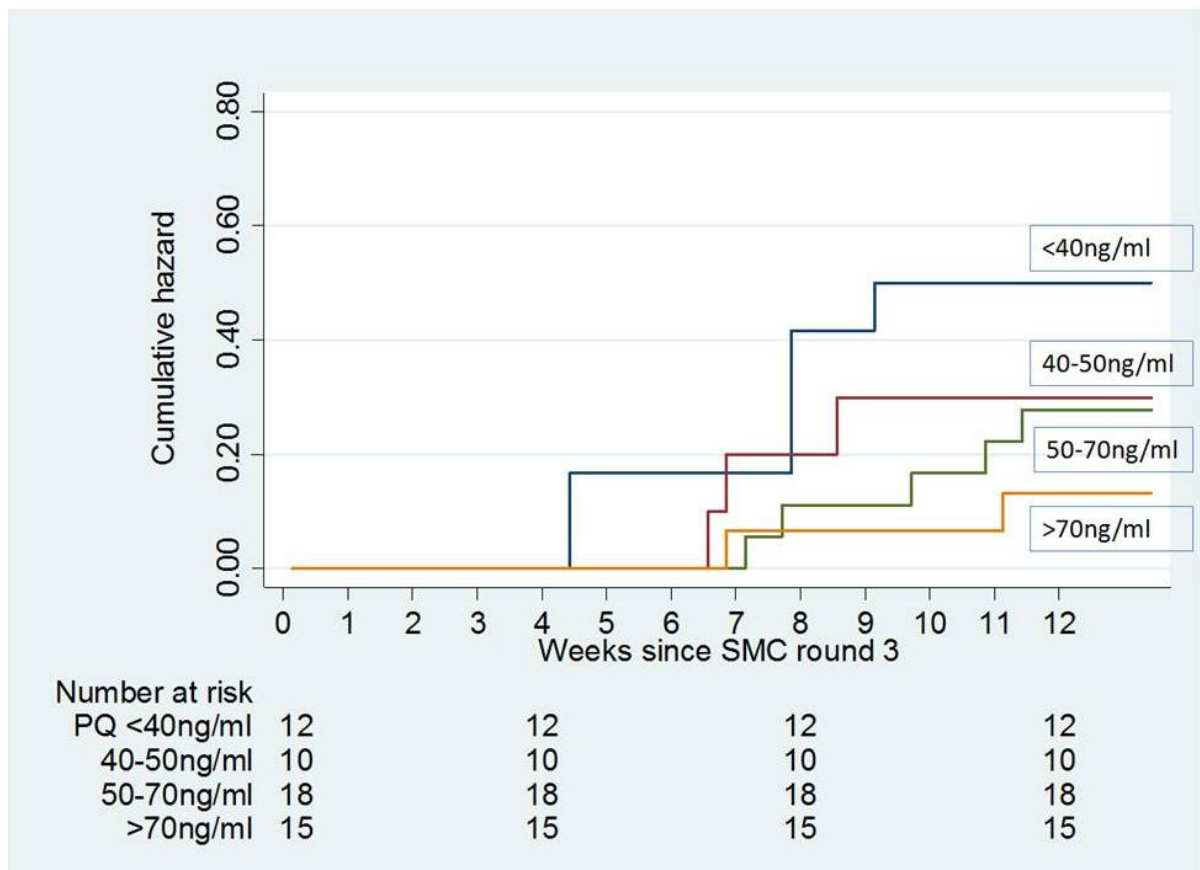
The delay appearance of first case of malaria in this group ranged from 30 days to 77 days following the administration of the drug (Table 15). Among the recurrent 16 cases, 7 children had a dose in mg per kg body weight less than the targeted dose of 18 mg/kg; none had vomited the drug. The lower doses ranged from 13.3 mg/kg a 5 years old boy to 16.9mg/kg in 5 years old boy (table 5.2).

Table 5.2: List of patients with day 7 piperazine concentration measured presenting clinical malaria after the last SMC in October

Patient	Day of malaria occurrence (day)	PQ concentration ng/ml	PQ doses Mg/kg	Vomiting within 30 minutes
R0379-5	30	18.8	18.46	No
R0318-9	54	25.7	13.3	No
R0450-5	54	28.8	14.55	No
R0450-5	30	28.8	14.55	No
R0336-2	63	29.9	20.5	No
R0317-7	54	31.8	18.9	No
R0394-0	47	41.9	18.8	No
R0374-2	45	47.2	16.2	No
R0447-4	59	48.1	15.1	No
R0315-3	49	50.5	16.9	Yes
R0499-6	67	56	15.4	No
R0403-1	53	56.6	19.8	No
R0400-4	79	59	16	No
R0323-9	75	69.1	19.8	No
R0393-5	47	76.6	14	No
R0448-8	77	163	18.9	No

5.6 Kaplan-Meier estimate of the cumulative incidence of malaria in four quartiles of PQ concentration on day 7

The cumulative incidence risk of malaria is significantly higher in children whose day 7 concentration of PQ was below 40 ng/ml, the better protection was achieved with more than 70 ng/ml of plasma concentration. The delay in the appearance of the cases increases as the day 7 concentration is high (figure 5.3).



P value for a test for trend of survivor functions with PQ dose group was $P=0.0873$.

Figure 5.3: Incidence of malaria in relation to piperaquine concentration measured on day 7, in 55 children treated DHA-PQ

5.6 Duration of protection after the last round of SMC in SPAQ and DHAPQ groups

The duration of protection estimated from the day 7 date of sampling was maximal in SPAQ treated patients; up to 4 weeks the protection persisted up to more than 70% and decreased rapidly to 50% by day 35. This proportion was reduced to 40% between day 35 and 42 and weak protection was available after 6 weeks; more than 80% of the children are again at risk. The decrease in the protection with the DHAPQ followed a similar trend but was more marked during the first four week and suddenly fell thereafter. By seven weeks after the SMC dose no protection was observed for all groups (figure 5.4).

A smoothed estimate of the hazard ratio was obtained using regression splines using the method of Lambert and Royston (2009), the efficacy (1-hazard ratio) with 95% confidence band is plotted against time since the final round of SMC.

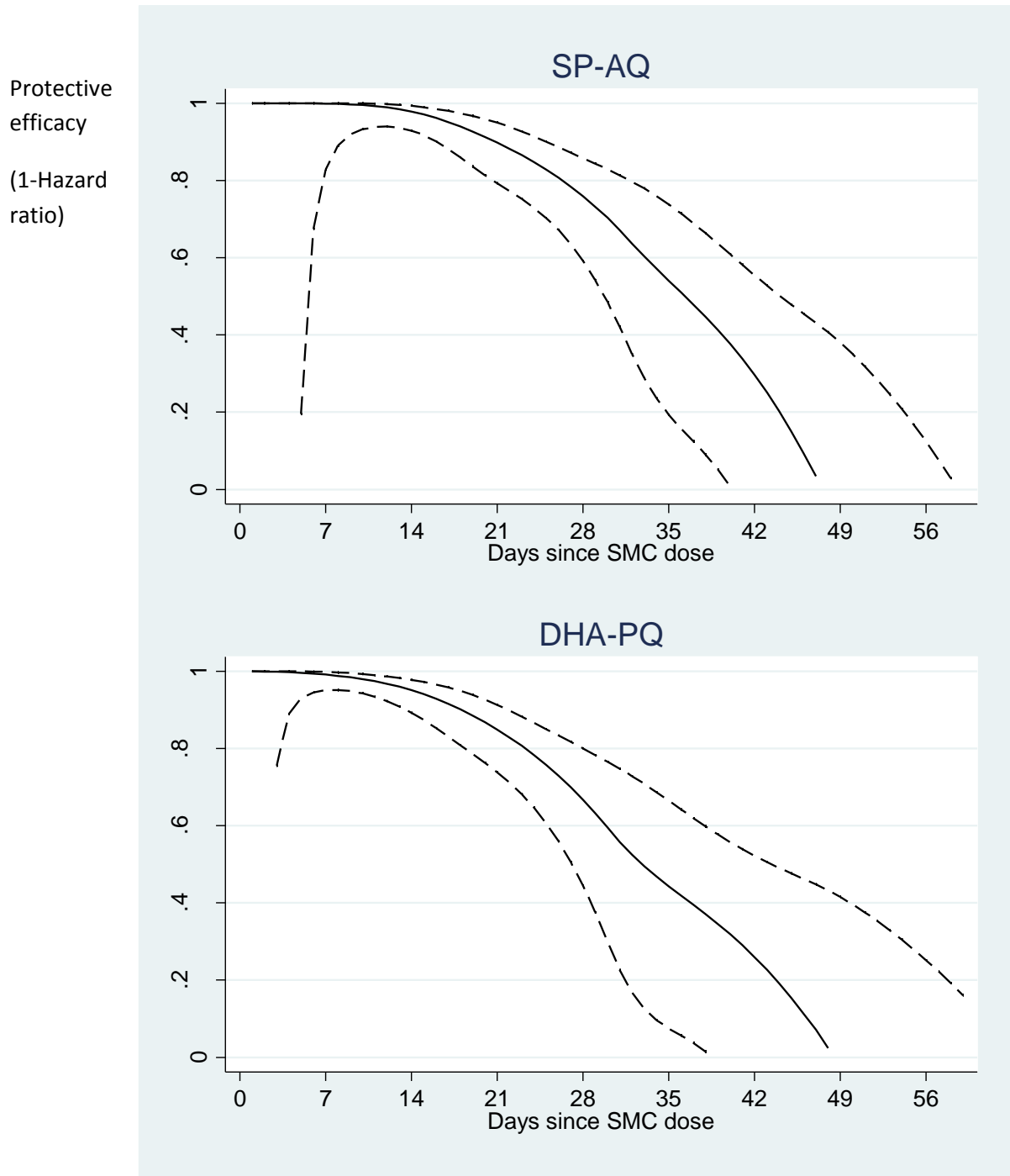


Figure 5.4: Duration of protection (malaria with any parasitemia).

5.7 Summary

Plasma disposition of piperazine on day 7 is an important indicator of the efficacy of dihydroartemisinin-piperazine administered during this SMC study. Children less than 24 months of age received high dose of piperazine administered in milligram per kilogram.

Plasma concentration of PQ on day 7 was dose in milligram per kilogram dependent. The duration of protection for all regimen was maximal over 3 to 4 weeks and then decreased steadily. Very low protection was seen from week 5 and none from week 7 onwards. The drug concentration after 4-5 weeks was not enough to provide a substantial protection but persisted up to 7 weeks. This persistence of low concentrations of antimalarial may lead to the exposure of survival parasites to very low concentration of the drug and offer a scope for selection of drug resistance which will be investigated in more details in the next chapter.

Chapter 6 Drug resistance and SMC with DHAPQ or SPAQ

6.1 Introduction

The present chapter 6 will investigate any possible evidence of selection by either SMC regimen of molecular markers to sulfadoxine-pyrimethamine (*dhfr* N51I, C59R, S108N, *dhps* S436A/F, A437G, A613S), to chloroquine of resistance *pfmdr-1* N86Y, Y184F, D1246Y, *pfprt* haplotypes CVMNK & CVIET at positions 72_76. The analysis was done in the first instance on first episodes samples by passive cases detection prior to the clinical case was treated with artemether-lumefantrine; then subsequent episodes were analysed

The chapter is organised in accordance with the objectives set in the fourth paragraph of the literature review chapter:

- (i) The comparison prevalence of mutations of interest at baseline among the groups.
- (ii) The comparison of the above mutations among the groups in all episodes detected by passive case detection.
- (iii) The comparison of the selection profile of these markers between the first and subsequent weeks following the administration of SMC.
- (iv) The comparison of mutations of interest at the end of the transmission season survey among groups.
- (v) The comparison of the prevalence of different combination of mutations or new identified mutations among the groups (exploratory analysis).
- (vi) The comparison of key markers on pre and post intervention.

6.2 Baseline prevalence of key molecular markers

These data were provided by Mr Somé for inclusion in the analysis. Of 360 samples tested in San Francisco, 260 were found to be PCR positive: 90 in the DHAPQ, 87 in the SPAQ and 83 in the untreated groups (table 6.1).

Table 6.1: Baseline proportion of molecular markers prior to SMC round in 260 PCR positive participants

		DHAPQ	SPAQ	Non SMC	Total
(PCR positive)		90	87	83	260
		Proportion (%) carrying the mutant alleles			
<i>dhfr</i>	51-Ile	55.6	55.2	63.9	58.1
	59-Arg	56.7	50.6	57.1	55
	108-Asn	77.8	89.7	80.7	82.7
<i>dhps</i>	436-Ala	34.8	30.3	40.5	35.1
	437-Gly	59.6	52.3	58.3	56.8
<i>pfmdr-1</i>	Y86	29.2	33.7	24.1	29.1
	F184	53.9	61.6	60.2	58.5
	Y1246	7.9	8.1	7.1	7.7
<i>pfcr</i> CVIET		78.7	67.8	58.3	68.5
<i>pfcr</i> CVIET+Pfm _{dr} 1 Y86		25.5	25.3	16.7	22.6
<i>dhfr</i> (51/59/108)		20	10.3	16.9	15.8
(triple <i>dhfr</i> + <i>dhps</i> G437)		12.2	5.7	12.1	10

All baseline data kindly provided by Mr Somé

At baseline, the prevalence of molecular markers of antifolate and chloroquine resistance were common in the intervention area. The prevalence of the *pfcr* CVIET haplotype was quite high in the study area which could be an indication of CQ pressure in this population. Parasites carrying multiple mutations were however less frequent. The prevalence of the quadruple mutant *pfdhfr/pfdhps* was rare not exceeding 20% irrespective of the treatment group.

6.3 Prevalence of molecular markers in the first episode after SMC administration

DNA for malaria genotyping was extracted for 42.7% (149/349), 35.6% (116/326) and 36.7% (81/221) in the DHAPQ, SPAQ and non SMC groups respectively. Incident episodes were compared between groups using the odds ratio.

Genotype results: The proportion of participants carrying the mutant allele either in *pfmdr-1* gene or *pfcr* haplotype CVIET haplotype thought to be Piperaquine resistant related mutations was similar in all groups. There was no association with their selection and Piperaquine treated clinical resistance. The odds ratio for patients treated with SPAQ carrying the single mutation in *pfdhfr* 51-Ile, 59-Arg, 108-Asn, the *pfdhps* 437-Gly, was higher compared to the untreated group (table 6.2). The triple mutant in *dhfr* or the quadruple in *dhfr* plus *dhps* were commonly reported in the participant treated with Sulfadoxine-Pyrimethamine compared to the untreated group.

Table 6.2: Prevalence of molecular markers of resistance among samples from malaria cases (first episode)

Genotype	DHAPQ	SPAQ	Non SMC	Odds ratio 95% CI SPAQ DHAPQ	Odds ratio 95% CI DHAPQ Non SMC	Odds ratio 95% CI SPAQ Non SMC
	Proportion (%) carrying the mutant allele					
<i>pfert</i> CVIET	62.9	61	61.5	0.9 (0.5 to 1.7), p=0.79	1.1 (0.5 to 2.4), p=0.88	0.98 (0.4 to 2.3), p=0.97
<i>pfmdr-1</i> Y86	33	44.4	30.4	1.6 (0.8 to 3.3), p=0.14	0.7 (0.3 to 1.5), p=0.33	1.8 (0.9 to 3.9), p=0.08
<i>pfmdr-1</i> F184	36.4	32.3	39.2	1.2 (0.6 to 2.5), p=0.6	0.9 (0.5 to 1.7), p=0.7	0.7 (0.3 to 1.6), p=0.3
<i>dhfr</i> 51-Ile	40.6	64	51.3	2.6 (1.5 to 4.6), p<0.001	0.7 (0.4 to 1.2), p=0.1	1.6 (0.9 to 3.2), p=0.08
<i>dhfr</i> 59-Arg	43.6	71	53.75	3.2 (1.8 to 5.7), p<0.001	0.7 (0.4 to 1.2), p=0.15	2.1 (1.1 to 4.1), p=0.01
<i>dhfr</i> 108-Asn	58.3	27	70	0.3 (0.1 to 1.5), p<0.001	0.6 (0.3 to 1.1), p=0.08	0.2 (0.1 to 0.3), p<0.001
<i>dhps</i> G437	63.4	84	75	3 (1.5 to 6.1), p<0.001	0.6 (0.3 to 1.2), p=0.10	1.8 (0.7 to 4.1), p=0.15
<i>dhps</i> S613	6.5	6.9	13.6	1.1 (0.3 to 3.3), p=0.9	0.4 (0.1 to 1.3), p=0.09	0.5 (0.4 to 1.5), p=0.1
<i>pfmdr-1</i> Y86 + <i>pfert</i> CVIET	3.3	6	1.2	1.8 (0.5 to 7.6), p=0.29	2.8 (0.3 to 133), p=0.33	5.3 (0.6 to 234), p=0.09
<i>Dhfr</i> (I51/R59/S108)	30.9	53.2	33.9	2.5 (1.4 to 4.6), p=0.001	1.1 (0.6 to 2.4), p=0.68	2.1 (1.1 to 4.6), p=0.01
<i>dhfr</i> (I51/R59/S108) + G437	19.5	41.5	23.7	2.9 (1.5 to 5.6), p<0.001	0.8 (0.3 to 1.8), p=0.5	2.3 (1.1 to 5.1), p=0.02

6.5 Molecular markers profile of the episodes following SMC rounds

We aimed at investigating the molecular markers profile of the clinical episodes occurring early after the SMC administration (within three weeks) during the follow up period. We first identified the number of episodes which occurred per round; then the episodes per round were split into weeks (first, second third, fourth and fifth). We defined the weeks as the period elapsing between the date the episode occurred and the day the patient received the next dose of SMC or artemether-lumefantrine. Preliminary analysis found few episodes within the first three weeks; therefore we categorised the period into two intervals, before and after three weeks.

6.5.1 Clinical episodes per round of SMC

Fewer cases of clinical malaria occurred during the first three weeks of each round in the intervention groups compared to the Non SMC group. These proportions were 14.1% (9/64) and 13.8% (10/72) in September for SPAQ and DHAPQ groups respectively and 27.8% in the Non SMC group. The risk of malaria occurrence was significantly higher in the Non SMC group compared to the SPAQ (OR=2.3, 95% CI 1.1 to 5.6), $p=0.02$) and to the DHAPQ group (OR=2.4, 95% CI 1.1 to 5.4), $p=0.01$. A similar trend was observed in October where there were more significant cases in the Non SMC group (table 6.3).

Episodes of malaria occurring in the first weeks following SMC administration in each treatment round were identified to investigate their molecular marker profile as they were expected to be resistant strains of parasites.

Table 6.3: Number of clinical cases within 3 weeks after the SMC per round

Rounds	Malaria episodes	DHAPQ	SPAQ	Non SMC
August 09	Number of cases	47	36	-
	Number within 3 weeks	20	10	-
September 09	Number of cases	72	64	329
	Number within 3 weeks	10	9	91
October 09	Number of cases	66	59	114
	Number within 3 weeks	13	8	70
November 09	Number of cases	41	29	71
	Number within 3 weeks	0	0	34
December 09	Number of cases	237	232	41
	Number within 3 weeks	0	0	0

6.5.2 Molecular markers profile of incident cases per round

6.5.2.1 First round in August 09

A total of 20 episodes of malaria occurred within 3 weeks following the SMC administration in August (round 1) in the DHAPQ group. Among these episodes, 10 samples were successfully genotyped and their profile is listed in table 6.4. None of these samples carried triple mutant *pfdhfr* or quadruple mutant *pfdhfr/pfdhps*. In the SPAQ treated participants, only 10 recurrent episodes were recorded of whom 6 were successfully genotyped (table 6.4).

Table 6.4: Molecular markers profile of the cases occurring within 3 weeks of SMC initiation in August 09

ID	<i>pfmdr-1</i>			<i>pfert</i>	<i>dhfr</i>			<i>dhps</i>			<i>Dhfr (irn)</i>
	y86	f184	y1246	cviet	i51	r59	n108	a436	g437	s613	
Dihydroartemisinin-piperaquine											
R1020-2	1	0	0	-	1	0	0	0	0	0	0
R0630-0	0	1	0	1	1	1	1	1	1	0	-
R0311-8	0	0	0	0	-	-	-	-	-	-	-
R1496-5	0	1	0	1	0	0	1	1	1	0	0
R1452-6	0	1	0	-	0	0	1	0	1	0	0
R0943-9	0	1	0	1	-	-	-	1	1	0	-
R1320-4	-	-	0	1	-	-	-	-	-	-	-
R0942-2	0	0	0	-	0	0	1	1	0	0	0
R0193-3	0	1	0	1	-	-	-	0	1	0	-
R0948-0	1	1	1	-	0	0	1	0	1	0	0
R1081-2	0	1	0	1	1	1	0	1	0	0	1
R1019-5	0	0	0	-	0	0	1	1	0	0	0
Total	2	7	1	6	3	2	6	6	6	0	2
Sulfadoxine-Pyrimethamine plus Amodiaquine											
R0401-5	0	1	0	1	-	-	-	1	1	0	-
R0140-6	0	1	0	0	-	-	-	-	-	-	-
R0894-8	0	0	0	-	-	-	-	-	-	-	-
R0156-4	1	-	1	0	-	-	-	-	-	-	-
Total	1	2	1	1	-	-	-	-	-	-	-

6.5.2.2 Second round in September 09

In September, 10 and 9 episodes occurred within the first three weeks in the DHAPQ and SPAQ groups respectively. All samples from the episodes were successfully genotyped except 2 samples in the DHAPQ group. The triple mutant *dhfr* (I51/R59/S108) and the quadruple mutant *dhfr* (I51/R59/N108) and *dhps* G437 were found in two samples in the SPAQ group and only in one sample in the DHAPQ treated participants (table 6.5).

Table 6.5: Molecular markers profile of the cases occurring within 3 weeks of SMC initiation in September 09

ID	<i>pfmdr-1</i>			<i>pfprt</i>	<i>dhfr</i>			<i>dhps</i>			Triple <i>dhfr</i>	<i>dhfr +dhps</i>
	y86	f184	y1246	cviet	i51	r59	n108	a436	g437	s613		
Dihydroartemisinin-piperaquine												
R0615-8	1	0	0	-	0	0	1	1	1	0	0	0
R0786-3	0	1	0	1	1	1	1	1	1	0	-	-
R1019-5	0	1	0	1	1	1	0	0	1	0	-	-
R1120-5	0	0	0	1	1	1	1	0	1	0	-	-
R1147-0	1	1	0	-	0	0	1	1	1	0	0	0
R1189-9	0	1	0	1	1	1	0	0	1	-	1	1
R1244-1	0	1	0	-	0	1	0	1	0	0	0	0
R1247-6	0	0	0	1	0	0	1	1	0	0	0	0
R1461-9	0	1	0	-	0	1	0	0	0	1	0	0
Total	2	6	0	5	4	6	5	5	6	1	1	1
Sulfadoxine-Pyrimethamine plus Amodiaquine												
R0342-9	0	1	0	-	0	0	1	1	1	0	0	0
R1134-7	0	1	0	-	1	1	0	0	1	0	1	1
R1135-0	0	1	0	1	0	0	1	1	0	0	0	0
R1241-2	0	0	0	1	1	0	1	0	1	0	0	0
R1349-8	0	1	0	1	1	1	1	1	1	0	-	-
R1365-9	0	1	0	-	1	0	1	1	1	1	0	0
R1388-6	0	1	0	-	-	-	-	1	1	0	-	-
R1431-2	0	1	0	1	1	1	0	1	1	0	1	1
Total	0	7	0	4	5	3	5	6	7	1	2	2

6.5.2.3 Third round in October 09

The molecular marker profile of the recurrent cases in October in the intervention groups did not reveal significant association between mutations in the recurrent cases (table 6.6).

Table 6.6: Molecular markers profile of the cases occurring within 3 weeks of SMC initiation in October 09

ID	<i>pfmdr-1</i>			<i>pfprt</i>	<i>dhfr</i>			<i>dhps</i>			<i>Dhfr(irn)</i>	<i>Irn+g</i>
	y86	f184	y1246		cviet	i51	r59	n108	a436	g437		
Dihydroartemisinin-piperaquine												
R0022-3	-	-	-	0	1	1	0	1	0	0	1	0
R0164-4	-	-	-	0	1	1	0	0	1	0	0	0
R0265-9	-	-	-	0	0	0	1	0	1	0	0	0
R0489-7	-	-	-	1	-	-	-	-	-	-	-	-
R0795-1	-	-	-	0	0	0	1	0	1	0	0	0
R0837-1	-	-	-	0	0	0	1	0	1	0	0	0
R0948-0	-	-	-	0	0	0	1	1	1	0	0	0
R1020-2	-	-	-	1	0	0	1	1	1	0	0	0
R1147-0	0	0	1	-	1	1	0	1	0	0	0	0
R1189-9	-	-	-	1	1	1	1	1	1	0	-	-
R1238-7	-	-	-	0	1	1	0	1	0	0	0	0
Total	0	0	1	2	5	5	6	6	7	0	1	0
Sulfadoxine-Pyrimethamine plus Amodiaquine												
R0031-7	-	-	-	1	0	0	1	0	1	0	0	0
R0034-4	-	-	-	-	-	-	-	-	-	-	-	-
R0087-2	1	1	0	-	-	-	-	0	1	0	-	-
R0489-7	-	-	-	0	-	-	-	-	-	-	0	0
R0959-1	-	-	-	0	0	0	1	0	1	0	0	0
R1203-0	-	-	-	0	1	1	0	0	1	0	1	1
Total	1	1	0	1	1	1	2	0	4	0	1	1

6.6 Exploratory analysis

The direct sequencing of the blood spot gave the opportunity to explore points of mutations not yet detected in the study area. The mutations in *dhps* K540E and A581G and *dhfr* I164L were not found in any of the samples. Overall, 8.9% (57/644) of the samples carried *dhps* 613S mutation irrespective of the number of malaria episodes. Specifically, its prevalence was 8% (19/237), 8.3% (17/204) and 10.3% (21/203) in the DHAPQ, SPAQ and untreated groups respectively (table 6.7).

The *dhps* 613S mutation was associated with the single mutations in low proportion in the intervention arms but more in the non SMC arm.

Table 6.7: Association with 613S mutation per intervention arm after the first episode

	DHAPQ	SPAQ	Non SMC
613S+51I	3.1% (4/129)	3.2% (3/95)	6.1% (4/66)
613S+59R	3.9% (5/129)	4.2% (4/95)	6.1% (4/66)
613S+108S	2.3% (3/128)	3.2% (3/95)	4.6% (3/66)
436A+613S	1.5% (2/134)	4% (4/100)	6.1% (4/66)
437G+613S	1.5% (2/132)	4% (4/99)	6.2% (4/64)
51/59/108+613S	2.5% (3/120)	2.2% (2/89)	8% (4/50)
51/59/108/437+613S	0.9% (1/120)	1.1% (1/89)	6% (3/50)

6.7 End of the transmission season survey The prevalence of parasitemia at the end of transmission by microscopy was 12% (88/722) in the DHAPQ, 12% (89/731) in the SPAQ and 36% (88/247) in the non SMC group. To perform the molecular analysis of the mutations, 120 samples were randomly selected from each group for PCR. Positive samples from PCR were used for the prevalence assessment. At the end of the transmission season, all positive cases harboured the studied mutations. The proportions of molecular markers remained high in particular for the *pfprt* CVIET haplotype in all treatment groups as well as the *pfmdr-1* (Y86, 184F). The triple mutant dhfr and quadruple mutant dhfr/dhps were particularly high in the anti-folate containing regimen. To evaluate the evolution of key mutation between baseline and the post intervention, we selected key mutations *dhfr* 51I, 59R, *dhps* 437G, *pfprt* CVIET, the triple mutant dhfr and quadruple mutant dhfr/dhps in each of the treatment group. Histograms of the key mutations before and after intervention gave an informative view on the trend of selection in each group (figures 6.1, 6.2 & 6.3).

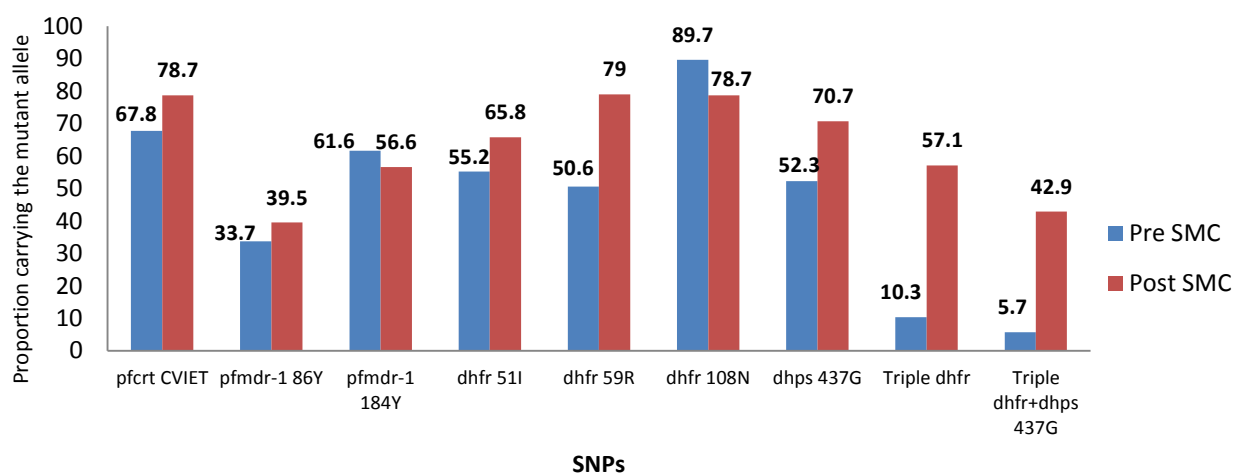
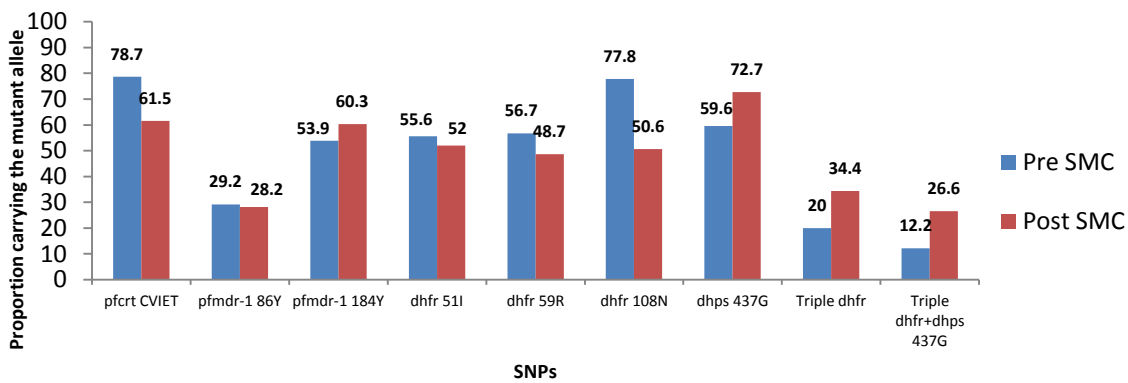


Figure 6.1: Selection of mutations of interest at baseline and at the end of the transmission season survey in the SPAQ treatment group

In the SPAQ group, all selected molecular markers increased in frequency between the pre intervention and the end of the transmission season survey, one month after the administration of the last dose of SMC (figure 6.1). The increase in the *dhfr* 59 R, the triple mutant *dhfr* and quadruple *dhfr/dhps* was statistically significant between the post intervention and pre intervention groups (*dhfr* 59 R, OR=3.6, $p<0.001$, triple mutant *dhfr*, OR=11.5, $p<0.001$ and quadruple mutant *dhfr/dhps* OR=12.3, $p<0.001$).

Figure 6.2: Selection of mutations of interest at baseline and at the end of the transmission season survey in the DHAPQ group



In the DHAPQ group, there was a significant decrease in the prevalence of *pfcr1* CVIET haplotype (OR=0.4, $p=0.01$). For all paired comparison of the selected mutations, there was a slight increase (non-significant) in comparison to baseline proportion (figure 6.2). In the non SMC group, selected mutations slightly increased but none was significant in comparison with the baseline. The mutations in *dhfr* 59 R and 51 I decreased while others increased slightly (figure 6.3).

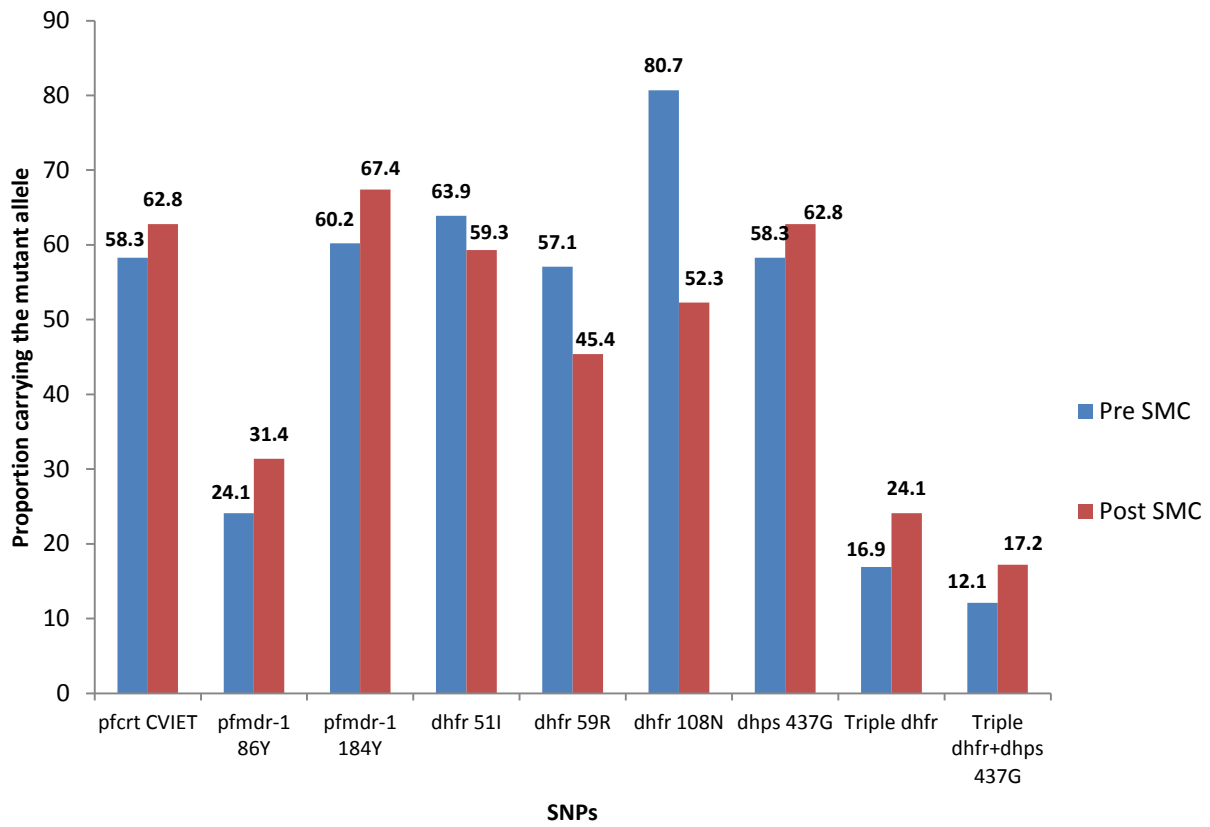


Figure 6.3: Selection of mutations of interest at baseline and at the end of the transmission season survey in the non SMC group

The mutations in the *dhfr* 59 R, 108 S, the *pfert* CVIET, the *pfert* CVIET plus *pfmdr-1* Y86, the triple mutant *dhfr* and quadruple mutant *dhfr/dhps* were significantly associated with the selection in the SPAQ treated children compared to those who received DHAPQ or Non SMC (table 6.8).

Table 6.8: Final survey molecular markers selection profile

Mutations	DHAPQ	SPAQ	Non SMC	OR, 95% CI SPAQ DHAPQ	OR, 95% CI DHAPQ Non SMC	95% CI SPAQ Non SMC
	Proportion carrying the mutant allele					
<i>pfert</i> CVIET	61.5	78.7	62.8	2.3 (1.1 to 5.1), p=0.02	0.9 (0.5 to 1.9), p=0.8	2.2 (1.02 to 4.7), p=0.02
<i>pfmdr-1</i> Y86	28.2	39.5	31.4	0.6 (0.3 to 1.2), p=0.1	0.9 (0.4 to 1.8), p=0.6	1.4 (0.7 to 2.9), p=0.2
<i>pfmdr-1</i> F184	60.3	56.6	67.4	0.9 (0.4 to 1.7), p=0.6	0.7 (0.4 to 1.5), p=0.3	0.6 (0.3 to 1.3), p=0.1
<i>pfmdr-1</i> Y1246	2.6	2.6	8.1	1 (0.1 to 14.5), p=0.9	0.3 (0.02 to 1.6), p=0.1	0.3 (0.03 to 1.6), p=0.1
<i>dhfr</i> 51-Ile	52	65.8	59.3	1.77 (0.9 to 3.6), p=0.08	0.7 (0.4 to 1.5), p=0.3	1.3 (0.7 to 2.7), p=0.4
<i>dhfr</i> 59-Arg	48.7	79	45.4	3.9 (1.8 to 8.6), p<0.001	1.1 (0.6 to 2.2), p=0.6	4.5 (2.1 to 9.7), p<0.001
<i>dhfr</i> 108-Asn	50.6	78.7	52.3	0.3 (0.1 to 0.6), p<0.001	0.9 (0.5 to 1.8), p=0.8	3.3 (1.6 to 7.2), p<0.001
<i>dhps</i> A436	40.3	38.7	32.6	0.9 (0.5 to 1.9), p=0.8	1.4 (0.7 to 2.8), p=0.3	1.3 (0.6 to 2.6), p=0.4
<i>dhps</i> 437G	72.7	70.7	62.8	0.9 (0.4 to 1.9), p=0.7	1.5 (0.7 to 3.1), p=0.2	1.4 (0.7 to 2.9), p=0.2
<i>dhps</i> 613 S	13.7	27.1	12.2	0.4 (0.2 to 1.1), p=0.04	1.1 (0.4 to 3.4), p=0.7	2.7 (1.04 to 7.3), p=0.02
<i>pfmdr-1</i> Y86 + <i>pfert</i> CVIET	34.4	57.1	24.1	2.5 (1.2 to 5.6), p=0.01	1.6 (0.7 to 4), p=0.2	4.2 (1.8 to 9.9), p<0.001
<i>dhfr</i> (51I/59R/108N)	26.6	42.9	17.2	2.1 (0.9 to 4.7), p=0.05	1.7 (0.7 to 4.7), p=0.2	3.5 (1.4 to 9.1), p=0.002

6.8. Overall prevalence of the mutant allele in the study population

Despite an apparent increase in the proportion of parasites carrying the mutant strains at the end of the transmission season, the overall prevalence of these parasites was low in the study population. The prevalence the parasitemia at the end of the transmission season was significantly reduced to 12% in each treatment group. For example, the proportion of children carrying the triple mutant in *dhfr* in the SPAQ treatment group was 57.1%, 79% for the *dhfr* 59R and 70.7% for the *dhps* 437G. At the end of the transmission season survey, the proportion of parasitemic participant was 12% in this group; overall, when we multiplied the proportion of carrying the mutants by the prevalence of parasitemic participant, we found respectively 9% and 8.5% the prevalence of the mutant alleles in the whole studied population.

Caution should be applied when interpreting the relevance of the selection of mutant allele's data to avoid misleading presentation of high proportion of mutant alleles while the overall prevalence of the parasitemic patient is significantly reduced.

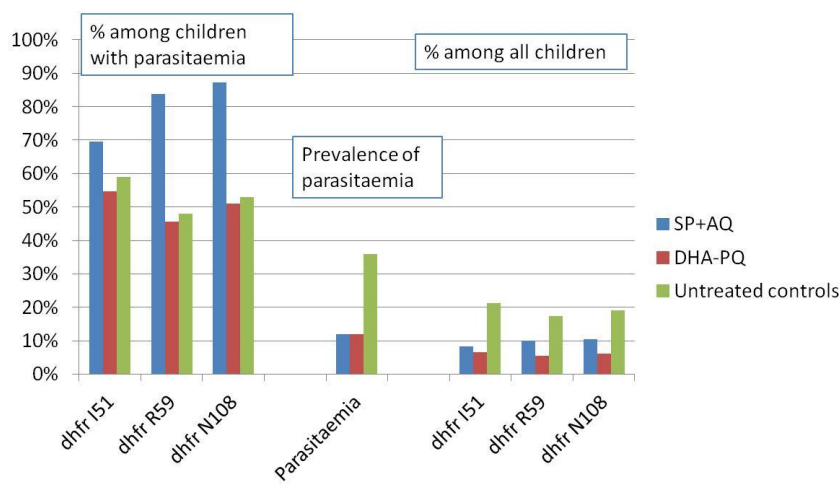


Figure 6.4 Prevalence of molecular markers in children with malaria, then prevalence of parasitemia at the end of the transmission season survey, and in the study population

6.9 Incident cases of clinical malaria in subsequent episodes of malaria

Among the subsequent cases, the trend of the selection differed from that of the first episode. Indeed the mutation in the *dhfr* 51I was significantly selected in the SPAQ group compared to the DHAPQ and Non SMC group while the *dhfr* 108N was mostly selected by the DHAPQ and SPAQ compared to the Non SMC group. The risk of selection of the *dhfr* 51I/59R /108N and the

quadruple mutations (*dhfr* 51I/59R /108N plus *dhps* 437G) was significantly associated with SPAQ in comparison with DHAPQ and the Non SMC groups (table 6.9).

Table 6.9: Prevalence of molecular markers of resistance among samples from malaria cases (subsequent episode)

Genotype	DHAPQ	SPAQ	Non SMC	Odds ratio 95% CI SPAQ DHAPQ	Odds ratio 95% CI DHAPQ Non SMC	Odds ratio 95% CI SPAQ Non SMC
	Proportion carrying the mutant allele					
<i>pfprt</i> CVIET	63.4	50.7	48.8	0.6 (0.3 to 1.2), p=0.1	1.8 (0.9 to 3.6), p=0.06	1.1 (0.5 to 2.1), p=0.8
<i>pfmdr-1</i> Y86	18.3	11.6	21.1	0.6 (0.2 to 1.7), p=0.2	0.8 (0.4 to 1.9), p=0.6	0.5 (0.2 to 1.2), p=0.09
<i>pfmdr-1</i> F184	47.4	50.7	40.1	1.1 (0.5 to 2.5), p=0.7	1.3 (0.7 to 2.6), p=0.3	1.5 (0.8 to 2.9), p=0.1
<i>dhfr</i> 51-Ile	35.8	51	43.5	1.9 (1 to 3.4), p=0.03	0.7 (0.4 to 1.3), p=0.2	1.4 (0.8 to 2.3), p=0.2
<i>dhfr</i> 59-Arg	43.4	53.1	49.4	1.5 (0.8 to 2.7), p=0.1	0.8 (0.5 to 1.4), p=0.3	1.2 (0.7 to 2.0), p=0.5
<i>dhfr</i> 108-Asn	57.8	45.8	71.4	0.6 (0.3 to 1.1), p=0.1	0.5 (0.3 to 0.9), p=0.02	0.3 (0.2 to 0.6), p<0.001
<i>dhps</i> 437G	60.2	71	72.5	1.6 (0.9 to 3.1), p=0.1	0.6 (0.3 to 1.04), p=0.04	0.9 (0.5 to 1.7), p=0.7
<i>dhps</i> 613 S	10.2	8.8	8.3	0.9 (0.3 to 2.7), p=0.9	1.3 (0.6 to 3.4), p=0.6	1.1 (0.4 to 2.9), p=0.8
<i>pfmdr-1</i> Y86 + <i>pfprt</i> CVIET	0.9	0.9	1.3	1 (0.01 to 80.7), p=0.9	0.7 (0.01 to 13.9), p=0.7	0.7 (0.01 to 14.2), p=0.7
<i>dhfr</i> (51I/59R/108N)	27.2	39.3	25.9	1.7 (0.9 to 3.5), p=0.09	1.1 (0.5 to 2.1), p=0.8	1.9 (1 to 3.5), p=0.04
<i>dhfr</i> (51I/59R/108N) + 437G	13.6	29.2	12.9	2.6 (1.1 to 6.4), p=0.01	1.1 (0.4 to 2.6), p=0.8	2.8 (1.3 to 6.1), p=0.003

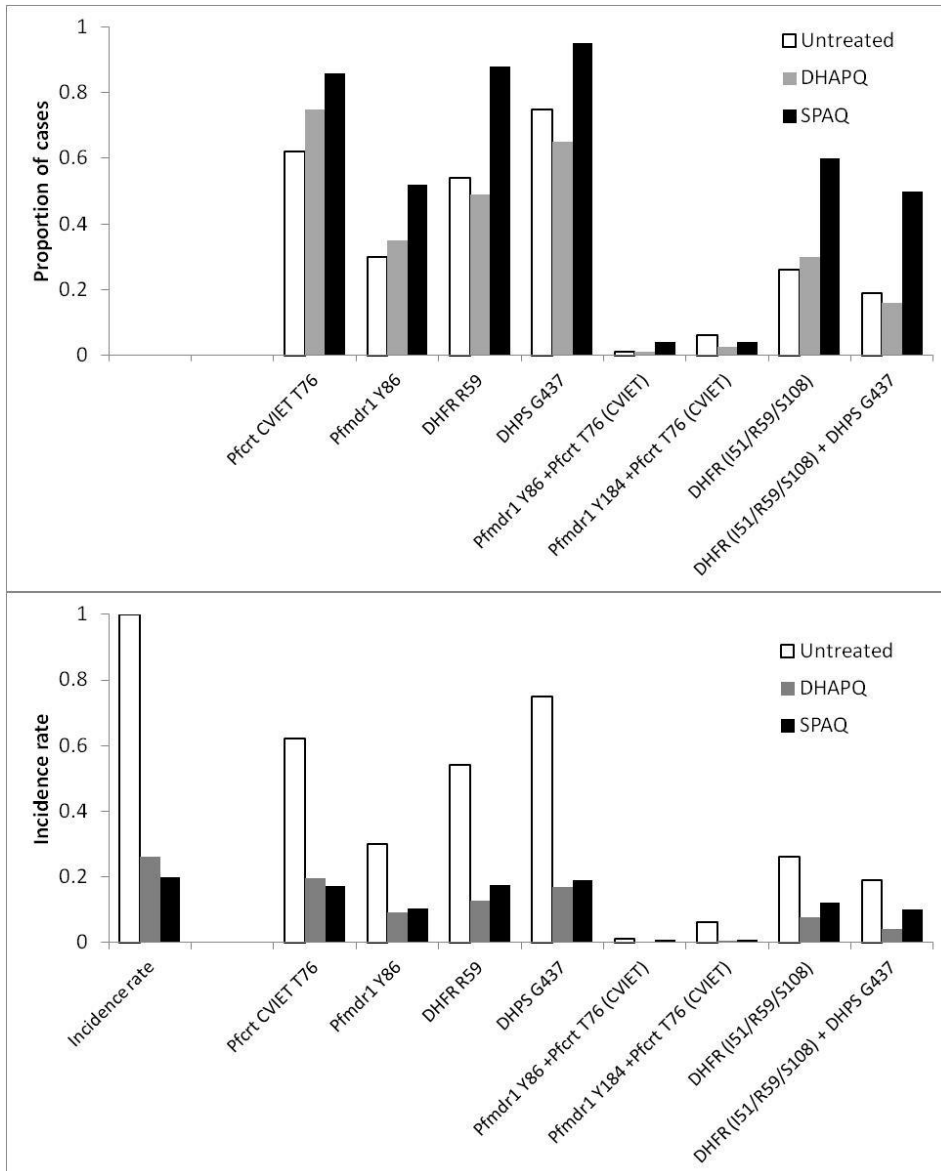


Figure 6.5. Molecular markers carriage in the study groups The upper figure shows the proportion of clinical cases carrying mutations associated with resistance to amodiaquine, sulfadoxine and pyrimethamine, in untreated children and in children receiving SMC with SPAQ or DHAPQ. The lower figure shows, on the left, the relative incidence rate in children who received SMC with SPAQ or DHAPQ relative to untreated controls, and then for each marker, the relative incidence rate in each group. Clinical malaria cases are more likely to carry the triple and quadruple mutations if the child has received SPAQ, but there are far fewer clinical cases if children receive SMC with SPAQ than in untreated children. The net effect is that SMC reduces the incidence of cases carrying these mutations. For example, 24% of cases in the untreated group carried the quadruple mutation, whereas in children receiving SMC with SPAQ, 42% of cases carried the quadruple mutation, but the relative incidence rate was 1 in the untreated group to 0.2 in the SPAQ group, so the overall incidence of the quadruple mutation is $0.42:0.2 \times 0.42$ or $1:0.35$, a reduction of 65%.

Chapter 7 Discussion

7.1 Key findings

Efficacy of SMC was slightly higher with SPAQ, reflecting the advantage of using a combination of two long-acting drugs. Both regimens were well tolerated, and both regimens were highly efficacious. The incidence of adverse events decreased in each successive month which may reflect gradual tolerisation. There was a very high level of protection lasted approximately 4 weeks. In children treated with DHAPQ, protection was associated with the PQ dose received, and this emphasise the importance of maintaining a 4-week interval between SMC doses and avoiding under-dosing. Among children with parasitaemia at the end of the transmission season, the proportion carrying markers of resistance to SP was increased in the SPAQ group, but the overall prevalence of these markers was low due to the low prevalence of parasitaemia in children who received SMC. There is a potential risk of resistance to PQ developing as SMC with DHAPQ exposes parasites to monotherapy with PQ.

There was an enormous burden of malaria in untreated children. 250 children experienced 338 malaria episodes in 2 months, 229 with parasite density $>3000/\mu\text{L}$. SMC with SP+AQ reduced the number of malaria cases by 84%. SMC with SPAQ should be introduced for children in Burkina Faso without delay.

7.2 Limitations of these results

One limitation of this study was the absence of a proper randomised control. By the time the protocol was drafted in the second semester of 2008, a control group was included.

While the protocol was finalized, the preliminary results about the additional benefit of ITNs use and SMC and other results from Senegal and The Gambia (Konate et al., 2011, Cisse et al., 2009, Bojang et al., 2010) have added substantial evidence on the benefit of the SMC and therefore, randomizing a group of children not to receive the intervention would be unethical. However the need of data on the incidence of malaria in the study area was critical. To overcome the ethical constraint and obtain data on the incidence of malaria, we did recruit a sample of 250 children filling similar criteria for study entry as the randomized group. To minimize the differences in the baseline characteristics, the untreated group was recruited in one of the SMC site and followed on the same fashion than the intervention group; this cohort was recruited one month after the start of the intervention trial due to late ethical clearance approval following the revision of the protocol. We did not perform a comparison of baseline

characteristics between the three arms and no safety data was collected for the control group. For the efficacy analysis, we considered only the period from when the untreated group was recruited up to one month after the end of the intervention.

A minor limitation was the absence of quality test (test of dissolution of the tablets). We received the DHAPQ tablets from the Holley Cotec Limited Company, Beijing China along with a Good Manufacturer Practice. The AQ sirup (Flavoquine® Parkin Davis) and SP (Fansidar® Roche) tablets were bought from a central drug provider who insured that the storage, the transports were done in conformity with the manufacturer's recommendations.

7.3 Overall interpretation

7.3.1 Efficacy of SMC with SPAQ and DHAPQ against clinical malaria

In this study, seasonal malaria chemoprevention resulted in a high level of protection against the clinical attacks of malaria. The intervention achieved 79% and 84% reduction of malaria with more than 3000 *Plasmodium falciparum* parasites per micro-litre respectively in SPAQ and DHAPQ group. The protective effect remained substantial even for the case definition of malaria with any parasite density. The protective efficacy reported in this study for DHAPQ was lower than that reported in Senegal, The Gambia, in Uganda and Thailand (Bojang et al., 2010, Cisse et al., 2009, Lwin et al., 2012, Nankabirwa et al., 2010); the protective effect for SPAQ was lower compared to The Gambia and Senegalese studies but higher than of the Ugandan study.

In this study likewise in Senegal and The Gambia, the transmission of malaria is seasonal; the intervention targeted the high transmission period and was given monthly over three months (August September and October) when the cases were at their peak.

In Uganda and Thailand, the transmission was less seasonal and the population targeted differed from the population treated in West African reports (the present report included); the population was school children in Uganda (over 5 years old in general) and adult male in Thailand. In these studies, the intervention was given once and followed up for 28 days in Uganda and given either monthly or bimonthly in Thailand.

The main reason explaining the differences in the protective efficacy in the reports in West Africa compared to the present outcome relied on the baseline incidence of clinical malaria in the different sites. Despite the absence of reliable data collected in routine of from the National Malaria Control Program, results from research studies showed the incidence of clinical malaria remains high in Burkina Faso. Indeed, this incidence was 2.88 person year at risk in Bousse (Konate et al., 2011) and 0.92 per child per month in the present study compared to the situation in The Gambia where field data combined with statistical modelling of trends confirmed significant decline over time in the slide positivity (Ceasay et al., 2010) and Senegal (Trape et al., 2012).

Our results differed with the findings from Uganda and Thailand, the most plausible explanation stained to two aspects: The age range of the intervention participant; In Uganda and Thailand, patients over 5 years old at least and adults were enrolled. While recent studies provided evidence data on the low efficacy of DHAPQ in young children and arguing for the revision of the dosage in these classes of age (Tarning et al., 2012) and the unacceptably high rate of clinical failure of SP and AQ in Uganda combined with the presence of key molecular markers establishing the resistance to SP and AQ finished to explain to low efficacy of SPAQ (Dorsey et al., 2007) and the better protection of DHAPQ.

The protective outcome reported in our project was similar to that observed in 2011 in a previous study to evaluate the additional benefit of ITNs use in children receiving SMC in the centre of Burkina Faso. The protective efficacy was 70% for a malaria definition of fever plus 5000 *P.f* parasites per micro litre of peripheral blood and we would expect the reduction of malaria cases to increase as the malaria definition came to a similar parasite density threshold than this study. Despite high protective efficacy in the overall trials referred in this thesis, to which extent could we still continue with these regimens:

Sulfadoxine-pyrimethamine plus Amodiaquine

SPAQ in opposite to the ACTs combines two “old” drugs which have been in use over the past two or three decades for the treatment for uncomplicated falciparum malaria when chloroquine fails or was not well tolerated. Consequently, there was a long and sustained pressure on these regimens so they have started to lose their efficacy at least when used in monotherapy in areas such as West Africa and had even lose any efficacy in East Africa.

Efficacy trials reported that SPAQ efficacy was extremely low in East Africa but retained some efficacy in areas of West Africa and recent SMC trials in Senegal (Cisse et al., 2009), The Gambia (Bojang et al., 2010), Burkina Faso (Konate et al., 2011) and Mali (Dicko et al., 2011) revealed that the drug could still be used to prevent malaria in children less than 5 years old. Despite these results, mention should be made that the efficacy of individual drug is probably continuously decreasing as part of its use either in combination with an artemisinin derivative (Amodiaquine – Artesunate) for the first line therapy of uncomplicated falciparum malaria or as the dedicated monotherapy used for the intermittent preventive treatment of malaria in infancy and pregnancy (sulfadoxine-pyrimethamine). In the light of these evidences, we could argue that the remaining useful lifespan of the combination appeals for the investigation of alternative possibilities.

Dihydroartemisinin-Piperaquine

This ACT evaluated in this project was highly effective for the SMC in children as demonstrated in previous trials in Senegal (Cisse et al., 2009), The Gambia (Bojang et al., 2010), in Uganda (Dorsey et al., 2007) and in Thailand (Lwin et al., 2012). It combines the very short acting artemisinin derivative eliminated within two hours and the long acting Piperaquine; therefore, DHAPQ does not seem to be an adequate regimen for the SMC though the combination becomes a mono therapy with Piperaquine after two hours with a scope for drug resistance selection. Piperaquine is an effective and suitable regimen for SMC in combination with a partner drug, but to date the only available co-formulated combination and licenced containing the Piperaquine is the DHAPQ. Luckily SMC is given over three rounds and stopped at the end of the transmission season when only few cases are susceptible to occur; DHA is highly effective and would clear most of the parasites including any Piperaquine resistant strain on the next round of the SMC; therefore there is a scope for DHAPQ use for SMC if the intervention is correctly given up to the end of transmission season and all measures taken to insure a high efficacy of the intervention with the DHAPQ.

7.3.2 Efficacy against *Plasmodium falciparum* infection

Thirty days after the last dose of SMC in November 2009, a cross sectional survey was conducted to determine the prevalence of *Plasmodium falciparum* infection among the participant in the intervention and untreated cohorts. SMC with SPAQ and DHAPQ reduced significantly the *Plasmodium falciparum* infection by respectively 73% and 72%. This reduction is similar to that seen for the clinical malaria. This achievement is an additional benefit for the SMC as the intervention is clearing the reservoir of parasites while allowing

continued building of the immunity considering the continued exposure of the children to the infective bites and the discontinuation of the intervention after three to four months of administration; furthermore it reduces the potential of transmission of resistant parasites.

7.3.3 Impact on the gametocyte carriage

The prevalence of gametocytes was low prior the initiation of the intervention and was even low at the end of the transmission season survey. Gametocytes carriage was reduced significantly between the onset and the end of the intervention, at least 93% reduction in each treated group and only 80% in the untreated cohort; this reduction in the untreated cohort was probably due to the close surveillance of the enrolled children which allowed a diagnostic of clinical case on time hence the administration of the effective ACT namely the artemether-lumefantrine (Coartem®). More generally, the substantial reduction in the gametocytes carriage contributed to lower the transmission potential of the disease. The reduction in the gametocytes carriage was found in Senegal at the end of the transmission season survey in both groups of treatment (Cisse et al., 2009).

7.3.4 Impact on anemia and nutritional status

Haemoglobin was not measured upon enrolment and systematic treatment of anaemia was not given; therefore any change in the level through the follow up could not be measured. Anaemia has multifactorial causes and malaria could be a major contributor; it is anticipated that effective control of malaria could improve the haemoglobin level. Previous studies have reported an improvement but DHAPQ use has been associated with a slight drop in haemoglobin level in Senegal and in the present study.

The Z scores height for age, weight for age and weight for height were computed using the reference population defined by the WHO in 2006. Based on their parameters, the nutritional status of the children in the intervention cohort has improved greatly and the proportion of stunted and underweighted decreased at the end of the transmission survey; however the proportion of the wasted increased slightly without any obvious explanation.

7.3.5 Clinical tolerability

While considering a drug regimen for SMC, tolerability should be regarded as a key component of the choice. SPAQ was as tolerated as DHAPQ and this tolerance has improved over the three rounds of SMC administration; vomiting within 30 minutes following the drug administration was rare and similar in the two groups. Furthermore the concern over the use of SPAQ holds on the poor tolerance of amodiaquine due to its bitter taste and this has

resulted in more frequent vomiting but also pruritis. Indeed in this report SPAQ was well tolerated and most of the adverse events were mild; importantly, vomiting which has been the most common side effect in previous SMC studies (Cisse et al., 2009, Bojang et al., 2010) with SPAQ was not more frequent in the present study as compared to the DHAPQ group. The better tolerance was mainly due to the paediatric formulation (sirup improved significantly the tolerability especially in younger children) used but also the precise dose given based on the weight rather than based on age as did the study by Cisse et al. in Senegal (Cairns et al., 2010). In this study, the remaining two doses were given at home under the supervision of the field workers; none of the mothers refused to administer the drug to a child for any reason.

Serious adverse events recorded in this trial did not seem to be drug related as detailed in the result section. Deaths were due to respiratory disorders and severe malnutrition.

However how enough care will be made in the handling of paediatric formulation and correct weighting of the children is a matter of consideration when malaria endemic countries will come to a large scale implementation of the strategy even in settings where the basic logistics (scales) is missing. At least a pharmaco-vigilance component could be set to monitor closely any adverse events which will occur as part of the large implementation of SMC given ad tablets and based on the age to stick with logistic limitations.

7.3.6 Biological safety

Biological safety of repeated doses of drug as it is the case in the SMC has been less investigated. This study aimed to assess the potential effect on the haematological and biochemical parameters over debate on artemisinin derivative and haemolysis (WHO, 1998). Reluctance of the mother for the repeated blood sampling has limited our evaluation. However our data confirmed the findings of previous study in Senegal where a drop in haemoglobin level was seen with DHAPQ (Cisse et al., 2009). Conversely children treated with SPAQ had a better haemoglobin recovery over the three rounds of SMC. Further investigations may be needed to evaluate the biological safety of the SMC. Surprisingly over 4400 patients received full course of DHAPQ or SPAQ over three rounds in Burkina Faso, Senegal and The Gambia totalising 39600 doses of DHAPQ or SPAQ closely monitoring without clinical complaints which had revealed a biochemical or haematological parameter failure; therefore, does it matter to investigate asymptomatic and transient perturbation of biological parameters? If further investigations are needed, the challenge will be to obtain venous blood samples on repeated occasions (optimal interval) in young children; the interval

of 7 days following the drug intake was a reasonable interval but the adherence of the mothers will remain difficult. An alternative would be either to conduct the evaluation in older children (around 48-59 months) and expect a possible inference to the youngest or to enlarge the interval between samples; sample size of such evaluation will account for the vulnerability of the study population and should stick to the possible minimum of children.

7.3.7 Relationship between dose – day 7 concentration and duration of protection

SMC achieved its objective through mainly a prophylactic effect of the drug regimen highlighting the crucial contribution of long half-life drugs. In the present study, the drugs were given 30 days apart but the protection lasted approximately four weeks. Other monthly SMC studies reported higher efficacy as compared to more than 30 days interval (Lwin et al., 2012, Dicko et al., 2008); in Senegal, Cisse et al. gave the intervention at exactly 4 weeks apart and reported a high protective efficacy (Cisse et al., 2009). In the present study, the Kaplan Meier estimates of the cumulative incidence of malaria of any parasitemia showed an increase in the incident cases around the next round (before the intervention was given) confirming the weakness of the protection after four weeks so, 30 days between two doses might be longer and contribute to explain our less effective outcomes.

Day 7 piperazine concentration was predictive of the efficacy of DHAPQ and was a function of the dose given in mg per kg per body weight, though children with higher day 7 concentration were less likely to experience the recurrence of malaria.

In this study, younger children (1-2 years) received high dose of Piperazine in mg per kg per body weight but end up with the lower day 7 concentration of Piperazine hence the intervention was less effective in this sub-population. The lower efficacy of the SMC in this group was already reported in a previous study (unpublished); it has been suggested that CYP450 contributing to the metabolism of Piperazine is not fully mature until the age of 10 years (Johnson et al., 2006)

Our SMC findings were also consistent with population pharmacokinetics studies (either for Sulfadoxine-Pyrimethamine or Piperazine) which reported in young children the low exposure of the parasites to the drugs (Barnes et al., 2006, Tarning et al., 2012, Creek et al., 2013). In this group of patients, arguments have been made about the necessity to revise the current dosage in order to deliver more drugs given in mg per kg so that a higher C_{max} (maximum concentration) can be achieved and therefore a better exposure of parasites. Beside the need to optimize the dosage, sufficient care should be made to insure an accurate dosing

and the adherence to the full course of the treatment over three days; these are key point to maintain, improve or optimize the efficacy of these drugs but also a need to protect the regimen from development of tolerance by the parasite and ultimately the apparition and spread of resistant strains and shorten the lifespan of these useful drugs. Of note the main parameter seen susceptible to affect the day 7 concentration of Piperaquine was the young age due to the immaturity to metabolise the CYP450 which intervene in the transformation of the drug; children presenting with vomiting or diarrhoea were withdrawn from the population pharmacokinetics study. As mentioned in the tolerability section, the intervention presented well tolerated profile which improved over the rounds and after adjustment, mild vomiting and diarrhoea which occurred during the course of the population pharmacokinetics study did not affect the day 7 concentration of the drug.

7.3.8 Drug resistance selection during the SMC intervention

The selection of resistant parasite with chemotherapy has been one of the challenges endemic countries are being faced as the prompt treatment of malaria cases with effective drug is a key component of the control programs. As SMC uses drugs, there is a fear over the possible increase in drug pressure and consequently the loose of their efficacy. The present data on drug resistance showed either on incident or end of the transmission survey cases the association of key mutations in *dhfr* and *dhps* with the SPAQ regimen but the selection was not strong. Nonetheless SPAQ still retained a good efficacy meaning their presence does not sign an established lack of efficacy of the drug though the mutations with strongest association with high failure rate (*dhps* 540E, *dhps* 581G, *dhps* L164) were not found in this study and even the high prevalence of quintuple mutation in *dhfr/dhps* was associated with high protection of infant receiving SP for IPTi in Mozambic (Mayor et al., 2008). This epidemiology of SP resistance is common in West Africa in general where SP remains effective either in efficacy or preventive studies (Cisse et al., 2006, Sokhna et al., 2008, Dicko et al., 2008, Cisse et al., 2009). While key mutations establishing the resistance to SP were not found in the study area, a new one already detected in other parts of Africa (Mberu EK, 2002) but not Burkina Faso was revealed, the DHPS S613. The contribution of this mutation to the drug resistance either as a single mutation or in combination seemed limited as despite the detection of this mutation, SP efficacy remained high during this SMC study.

Up to now, no firm molecular marker was associated with an artemisinin derivative resistance and the very short action combined with the high efficacy of the DHA put this drug less at risk of selection. However the partner drug Piperaquine reduced to a mono-therapy will be

more exposed and offers a scope for drug resistance selection. The *pfprt* CVIET haplotype and *pfmdr-1* gene are potent markers for selecting Piperaquine resistance but this study did not identify any selection by Piperaquine.

Overall, all regimen were highly effective resulting in at least 70% reduction of clinical malaria, over 90% of asymptomatic infection and gametocytes carriage. What would be the impact of resistance selection for regimen which achieved massive reduction in the parasite biomass? The massive reduction in the gametocytes carriage is likely to limit the transmission of resistant parasite; furthermore, a study by Diakite et al. 2010 reported that some resistant parasites were cleared by their host (Diakite et al., 2010).

It therefore can be argued that the beneficial effect of SPAQ in the overall reduction of malaria burden when used for SMC outweighed the slight selection of resistance markers meanwhile any use of DHAPQ will surely exert a selective pressure but we unfortunately don't have the right markers (to Piperaquine) to assess the magnitude of this selection . Nonetheless a close eye should be kept on the evolution of the key known molecular marker along with the identification of gene conferring the resistance to Piperaquine and especially those not yet reported so far in order to detect the level of resistance and to alert the National Malaria Control Programs authorities for immediate actions to be taken. Monitoring of resistance to these drugs where SMC is used will require direct measurement of efficacy, through case control studies.

7.4 SMC, an opportunity for malaria control in Burkina Faso?

SMC with SPAQ in children 3 to 59 months as per the World Health Organisation recommendation suits to the Sahel and Sub-Sahel regions with high seasonal transmission of malaria where at least 70% of the clinical cases occurred within 3-4 months. The case definition of areas susceptible to benefit from the SMC is not unique and depending on which criteria were considered, most of West African countries will be covered by SMC and even parts of East Africa could be included(Cairns et al., 2012).

Burkina Faso as most of the Sahel and Sub-Sahel countries is one of these areas susceptible to benefit from the seasonal prevention of malaria.

The epidemiology of malaria in Burkina Faso is characterised by three transmission areas:

- A short transmission area over three months in the North and extreme North
- A medium transmission area over 4-6 months in the middle

- A longer transmission area over 6 months. In all these settings the transmission is highly seasonal and most of the cases occur within the rainy season. This description of malaria situation in Burkina Faso is rather old; yet there is no reliable parasitological confirmed data country wide to guide the definition of areas which could benefit from the SMC. Data provided by the National Malaria Control Program is incomplete and generally most of the cases reported were unconfirmed. In addition to that, the age group definition is unclear since a majority of children do not have birth certificate and ended up with a best guess to estimate their age with possible underestimation or overestimation; in children in these cases could under or over dosed and consequently more adverse events or inefficacy of the treatment. Owing these limitations, the criteria to set the SMC scope will rely on this old classification of the country into the three epidemiology settings for malaria transmission.

The short and medium transmission areas cover two third of the country and is likely to be suitable for SMC and even the long transmission area might be covered upon reconsideration of some criteria. Populations in the low income countries are characterised by a large majority of young persons and the children less than 5 years old are well represented. They are most at risk of malaria and are susceptible to benefit from SMC.

The situation described above justified that the Ministry of Health through the National Malaria Control Program introduced SMC with SPAQ in their strategic plan from 2011 to 2015 to fight malaria (earlier than WHO recommendation to introduce SMC). Before a large scale implementation, the NMCP initiated a pilot introduction of the strategy in 6 health districts during the 2013 high transmission period (NMCP, 2013). The selected health districts are:

- The district of Seguenega in the North
- The district of Zorgho in the centre
- The district of Sebba at the Sahel region
- The district of Bogande in the East
- The district of Nouna in the North-West
- The district of Kaya in the centre-North;

There has been a tentative to cover the country rather than a random selection. The objective of this pilot was to reduce the incidence of malaria in children 3-59 months by 60% in 2013 compared to the situation of 2012 and to learn how best to proceed for the large scaling up (step wise or full deployment once). The project aimed at covering the population of 3-59 months living within the 6 selected districts

and 90% of compliance by mothers. SMC with SPAQ is expected to be delivered over four days of mass drug administration per month by a pair of community health workers trained for this purpose. The intervention was scheduled to last four months (July to October) but up to the end of August no start has been made though the resources were not yet available.

In the implementation process, a concise plan for training, supervision and evaluation is in place to insure the success of the pilot. The budget of this pilot, approximately 1 million US dollars was submitted for funding to international bodies such as UNICEF, WHO, Plan, etc...

The application went through a first round of selection and was shortlisted; the final decision of funding is expected at the end of 2013 or early in 2014. It is therefore confirmed that SMC pilot implementation in Burkina Faso is postponed for the next 2014 transmission season. Furthermore, worse than postponing the pilot study, there is no firm plan in 2014; all plans being subject to funding availability either from the government or from the donors. In the best of the options of fund secured, does it stand to conduct a pilot implementation or should the NMCP go straight for national scaling up program? This might be the best option to avoid a waste of time in scaling up the strategy.

7.5 Challenges surrounding the large scale deployment of the SMC in the Sahel region:

Whatever suitable part of Africa for SMC strategy, several challenges are foreseen:

- Endemic and low income countries of Africa need to get ready to translate this SMC recommendation of WHO into a policy. Political commitment is highly sought to lead large campaign of information and guidelines redaction to facilitate adequate training of the staff in the field and to support any modest additive running costs. Such commitment includes also sustainable provision of high quality of drug to cover the number of treatments needed in a timely manner.

- The delivery of SMC unlike in the IPTi is not supported by any existing channel but can be achieved at cost effectively in clinical trial settings or in areas with existing community health workers in Ghana and in The Gambia (Bojang et al., 2011, Kweku et al., 2009). The challenges (a) and (b) necessitate financial resources which may be beyond the states capacities; multilateral donors and NGOs are therefore welcomed to sustain the states through the Ministry of Health but how far these donors can support such strategy will be a matter of negotiation and the states should ultimately take the responsibility and raise fund internally.

- The evaluation of SMC impact. We would expect a dramatic decrease of the burden of malaria in children after the implementation of SMC as a public health tool but how to assess the impact when there is no reliable data prior the introduction of the strategy. A part limited data provided by clinical studies, most of the data on malaria are from the routine collection in the health facilities and compiled in the National Malaria Control Programs annual report to WHO. We believe that pre SMC main activity should be a robust collection of routine data in the health facilities (irrespective of the cause of consultation or admission); in this respect the Data Surveillance System (DSS) or similar but less costly systems are urgently needed along with staff (physicians, nurses but also field/community health workers) training and simple data collection forms.

Confirmation of malaria cases through the use of the rapid Diagnostic Tests (RDTs) or microscopy if available should be the rule in the health facilities; again this calls for a sustainable provision of the RDTs to avoid shortage and absence of reliable data.

Having set these variable aspects, the impact evaluation has two components: - Assessment of drug efficacy and selection of resistance through case control studies, - the assessment of the whole program impact on malaria related morbidity/mortality parameters: incidence of clinical cases, prevalence of anaemia of any grade, proportion of admission and death due to malaria, prevalence of infection and of gametocyte carriage.

- Future of SMC. There is a real hope that SMC in conjunction with the available and foreseen tools (ITNs, IRS, effective treatment of the clinical cases, vaccine) can curve the course of malaria in endemic countries and improve the survival of population at risk especially children and pregnant women. We foresee the future of SMC in three angles:

- SMC is maintained till the elimination of malaria in a given area. When malaria related morbidity and mortality parameters fell significantly, the main question becomes it is efficient to provide SMC to all children under five when few of them remain at risk? Probably the approach needs to be rethought.
- SMC is stopped when the incidence of malaria falls below a certain cut-off in a given area

It is anticipated that SMC will impact significantly the incidence of clinical malaria and other morbidity parameters. Therefore any discontinuation of SMC might create a gap and offer a

scope of return back to higher level. If when and where to introduce SMC seems quite well defined, the time of its discontinuation is more difficult to determine.

- SMC is switched to an intermittent screening and treatment when a cut-off in the incidence of malaria is reached. The concept of SMC is the administration of a full course of treatment to a subject irrespective of its parasitological status. As SMC goes and is effective, the number of treated children who are slide negative and unlikely to acquire malaria will increase. From such point of time, it is worth asking if whether SMC should continue in that format. Our conviction is to switch from the SMC to an intermittent screening and treatment of positive cases; this is justified in the sense that negative slide are really likely to be negative as the impact on asymptomatic infection and gametocytes carriers is huge. At this stage, the challenge will be the adequate diagnostic of malaria cases either by microscopy or RDTs, though more sensitive RDTs are needed to pick up even the very low parasitemia. Although children bore the high burden of malaria, there is still a reservoir in adults but it is anticipated that an indirect effect of the effective SMC along with the direct effect of the other measures (ITNs, IRS, and effective treatment of clinical cases) will be a decrease in the reservoir in adults. Overall if direct and indirect effects of SMC curved significantly the burden, we can argue the extension of the intermittent screening and treatment to the whole population. The advantages of targeted treatment at this phase reduction of drug consumption, which in turn reduces the cost of the strategy but it also put less drug pressure susceptible to generate parasite mutations hence the appearance and widespread of drug resistance. Intermittent screening and treatment could ultimately be part of the malaria elimination/eradication agenda.

Despite the challenges and other difficulties, SMC remains a serious tool susceptible to change the course of the ever disastrous infectious disease over the past century.

Malaria parasites exist in the nature in equilibrium with other pathogens. Other diseases will emerge to fill the gap caused by the declining malaria such as bacterial diseases, viral infections which can bring up the mortality in young groups.

7.6 Areas for future research

This thesis has explored an alternative, effective and safe drug regimen for SMC. However several research questions remain and call for further investigation:

- 1) The monitoring and evaluation of the impact of SMC as it is introduced, including pharmaco-vigilance studies and monitoring of drug resistance.
- 2) The evaluation of child-friendly formulations, dispersible formulations and taste-masked formulations.
- 3) In some countries SMC may be given to a wider age range, (it is currently given to children up to 10yrs in Senegal), safety and efficacy of DHAPQ could therefore be evaluated in a wider age range. In areas of seasonal malaria in South East Africa, SMC with DHAPQ could potentially be used; a superiority trial of the safety and efficacy of DHAPQ compared to SPAQ in these areas should be conducted as a priority.

Chapter 8 Conclusion and recommendations

The time has more than ever come to curve the course of malaria as several effective tools are accessible at once to made available to the policy makers, the funders, the patients and finally to the scientists working on malaria. More than ever, the challenge has come to implement in an integrated manner and settings specific epidemiology the strategies to fight malaria.

As part of my PhD program, we undertook to investigate different drug regimens for chemoprevention of malaria from August 2009 to January 2010. We designed a non-inferiority clinical trial described in the chapter 3 to primarily determine if whether efficacy of DHAPQ is non-inferior to that of SPAQ. We also determined the incidence of malaria in the study area and investigated how day 7 Piperaquine concentration is related to the level of protection. Secondary endpoints of the study included biological safety assessment, the prevalence of adverse events, malaria infection, carriage of gametocytes, anemia and the profile of drug resistance selection due to each drug regimen over the follow up period and again at the end of the transmission season. A total of 1499 children 3-59 were randomly assigned to receive either monthly full treatment dose of SPAQ or DHAPQ; an untreated and non-randomized cohort of 250 children in the similar age range was recruited in one of the site where the randomized cohort was selected. The study took place in Satiri, Balla and Kadomba in the Lena Health district at approximately 30 miles from the capital city of Bobo-Dioulasso; the intervention was given over three (3) months in August, September and October. Sub-studies for day 7 Piperaquine concentration measurement and the biological safety assessment were undertaken beside the main trial. An epidemiological survey was carried out in November for the randomized cohort and in December for the untreated cohort. Tolerability assessment was set on day 3 and fourth nightly following each round of the intervention administration.

The main results were reported in the chapters 4, 5 and 6. SMC with SPAQ or DHAPQ led to a reduction of 84% and 79% in the incidence of malaria with 3000 parasites per microliters and above. The incidence of malaria was 229 episodes in the untreated cohort, a mean of 0.92 episodes per child per month.

The other relevant findings of this study included:

- (i) The adverse events were moderate and common in all regimen and decreased over the rounds of SMC administration; no serious adverse event did appear to be drug related

- (ii) The day 7 Piperaquine concentration was dose related and a predictor of the intervention outcome; the young children had a lower protective efficacy (low day 7 Piperaquine concentration).
- (iii) The prevalence of asymptomatic malaria infection and gametocytes carriers were dramatically reduced (over 93% in the treated groups).
- (iv) The use of SPAQ exerts a selective pressure; however, the overall prevalence of molecular markers to this regimen was low due to the massive reduction of the prevalence of parasitemia at the end of the transmission season.
- (v) The selection pressure of DHAPQ use was not assessed due to the absence of firm gene conferring the resistance to Piperaquine but it is anticipated that its use will increase the presence of resistant parasite to the Piperaquine.

A full assessment of the suitability of a drug regimen to consider for the Seasonal Chemoprevention of Malaria relies not only on the efficacy, but rather include the tolerability and ease of use by the mothers, the potential impact on the hematological and biochemistry parameters and ultimately balancing the drug efficacy and its potential to select resistant parasites. On these grounds, SPAQ stands to be suitable drug for the SMC as recommended by the World Health Organization.

The concept of SMC has proven to be effective in reducing the incidence of clinical malaria; its targets the high risk period in the Sahel and Sub-Sahel regions of Africa. Successive and step wise exploration of the efficacy, the number of doses, the interval between doses and the timing have demonstrated that monthly administration of a full course of an effective drug is the optimum combination to obtain the maximum of efficacy. The potential for SPAQ and DHAPQ has been investigated in Senegal, The Gambia, Ghana, Burkina Faso and finally the present study (Cisse et al., 2009, Bojang et al., 2010, Konate et al., 2011). Although the incidence of malaria was low in Senegal and The Gambia to yield solid conclusion, the findings in the present study along with that reported in the centre of the country (Konate et al., 2011) have definitively confirmed the benefit of SMC for malaria prevention among children. Studies on how SMC could be delivered since there is no previous channel for delivery have concluded that SMC can be delivered successively through systems outside the health facilities at a cost effective way; community health workers or villages strikers in The Gambia have delivered SMC even better than the health facility based strategy. For all aspects considered, SMC as a potent in clinical trials setting to reduce clinical malaria.

Time has come to translate the hope into the reality in the communities of the Sahel region; this is a big challenge for public health policy makers. Since SMC recommendation by WHO in March 2012, countries are moving into implementation phase at different speed depending on how they faced and overcome the challenges; Senegal has formally adopted SMC and is in an implementation stage; Togo is also piloting SMC in selected health districts in 2013. In Mali SMC is ongoing in an MSF (Médecin Sans Frontière) site, Koutiala (500 km from Bamako). In Burkina Faso the process hasn't start yet; a technical guide on SMC introduction in 6 selected districts exists but the fund was not available.

SMC is an advocated tool which can along with other measures contribute to a sensible reduction of the burden of malaria. For the perspective of large scaling up and to maintain the efficacy of the strategy as tool, the following recommendations can be drawn:

1. A political will to commit them for a sustainable support of the program. SMC introduction requires substantial financial resources, infrastructures and logistics.
2. SMC with SPAQ was adopted in a context where SP retained some efficacy especially in West Africa but could rapidly decline as SPAQ is used in children and SP alone for the intermittent preventive treatment in pregnancy. We recommend a close monitoring of the drug efficacy to guide any change of regimen
3. New drug regimens are needed. While monitoring the efficacy of the current drugs, new components should be evaluated to allow a switch when the current drug will fail. The evaluation will include the determination of the pharmacokinetic and the biological safety profile. Pediatric formulation are welcome to be evaluated as they contribute to lower the adverse events
4. Molecular markers studies are needed to follow the change in the markers profile; they will inform about the efficacy before the clinical stage.
5. The measurement of SMC impact. This measure is critical but is challenging in view of lack of reliable data on malaria morbidity in health facilities prior SMC implementation.
6. More research is needed to guide the future of SMC as its implementation progresses and the incidence and other morbidity parameters of malaria are decreasing. It that pertinent to establish trend of cut off values of the malaria morbidity parameters from which SMC should be rethought?

7. Careful designed studies to evaluate the impact of SMC on the transmission of malaria. In the present report, the gametocyte carriage has decreases but the proportion of children with gametocyte was low and could not yield any significant conclusion on SMC potential for transmission reduction.

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Appendixes

Appendix 1 Information sheet and informed consent forms (randomized & untreated cohorts)

FICHE D'INFORMATION DU PATIENT

TRAITEMENT PREVENTIF INTERMITTENT SAISONNIER DU PALUDISME CHEZ LES ENFANTS DE 3 A 59 MOIS A BOBO DIOULASSO, BURKINA FASO: UN ESSAI CLINIQUE RANDOMISE ET CONTROLLE

Le paludisme est un problème très préoccupant dans notre pays des recherches ont montré en Afrique que la prise d'un médicament contre le paludisme une fois par mois (IPT) peut diminuer le risque de contracter le paludisme chez les enfants de moins de 5 ans. Ici nous voulons savoir quel est le meilleur médicament pour cela. Si vous acceptez que votre enfant participe à l'étude nous allons vous demander d'amener votre enfant au centre de santé une fois par mois (en Août, Septembre et Octobre) ; le docteur va examiner votre enfant et l'infirmier lui donnera le médicament (flavoquine® plus du fansidar® ou duocotexcin®) au dispensaire, et vous remettra le restant pour lui donner à la maison pour les deux jours suivants. Ces médicaments sont efficaces, bien tolérés et utilisés au Burkina Faso pour le traitement du paludisme simple. Un agent vous visitera deux fois par semaine à la maison pour s'assurer que vous avez donné le médicament à l'enfant mais aussi demandera si l'enfant va mieux et s'il n'a pas de plaintes depuis la prise du médicament. Si votre enfant a la fièvre, l'agent va faire un examen sanguin pour voir s'il a le paludisme pour commencer rapidement le traitement, mais vous devez emmener l'enfant au dispensaire. Environ 1500 enfants rentreront dans cette étude. Pour comprendre comment les médicaments agissent et sont efficaces, nous allons faire des prélèvements sanguins chez votre enfant au début et en fin d'étude. Votre enfant aura des prélèvements sanguins au bout du doigt ou au pli du coude (de très petites quantités de sang) deux fois au jour 2 et 7 er parfois au jour 30. Nous vous dirons si votre enfant est concerné. Vous pouvez amener votre enfant à la formation sanitaire chaque fois que vous sentez qu'il est malade ou si vous avez des craintes sur sa santé. Les prélèvements sanguins ne feront rien à votre enfant, seulement une douleur légère et passagère due à la piqure pour le prélèvement. Nos équipes sont bien formées et ferons de leur mieux pour minimiser la douleur lors du prélèvement, le matériel utilise est à usage unique. Les médicaments que nous donnons à votre enfant sont efficaces et bien tolérés ; des effets indésirables sévères (comme une réaction sévère de la peau ou du foie) peuvent être observés chez certains enfants mais cela est très rare. Vous êtes libre de ne pas participer à cette étude

et nous respecterons votre choix ; vous pouvez aussi a tout moment retirer votre enfant de l'étude sans avoir à vous justifier, et cela n'affectera pas la santé ou la demande de soins de votre enfant. Les connaissances issues de cette étude aideront le Burkina à déterminer le meilleur médicament pour la prévention du paludisme chez les enfants. Les prélèvements de sanguins de votre enfant seront gardés à l'IRSS Bobo et pourront être utilisés plus tard pour des recherches sur le paludisme.

Les données collectées sur votre enfant resteront confidentielles et ne seront accessible qu'à ceux qui travaillent dans le projet, le nom de votre enfant ne sera en aucun cas utilisé lors d'un rapport ou un écrit dans les journaux. .

Si vous avez des questions complémentaires, vous pouvez contacter le Principal Investigateur du projet Dr Issaka Zongo, téléphone 226-20-98-18-80 à l'Institut de Recherche en Sciences de la Santé.

**TRAITEMENT PREVENTIF INTERMITTENT SAISONNIER DU PALUDISME
CHEZ LES ENFANTS DE 3 A 59 MOIS A BOBO DIOULASSO, BURKINA FASO:
UN ESSAI CLINIQUE RANDOMISE ET CONTROLLE**

Consentement éclairé

En signant ce formulaire, vous dites à l'équipe du projet que:

Nous vous avons bien expliqué l'étude

Vous avez bien compris ce que nous demandons à votre enfant

Votre participation à l'étude est entièrement volontaire

Vous pouvez arrêter à tout moment votre participation

Vous avez l'occasion de poser toutes vos questions

FICHE DE CONSENTEMENT

Nom de l'enfant _____

Numéro d'Identification _____

J'ai compris les explications qui m'ont été données et j'accepte que mon enfant participe à l'étude.

Nom du parent ou du gardien de l'enfant

Relation avec l'enfant _____

Signature ou *empreinte du parent ou du gardien de l'enfant

*Si le parent ou le gardien ne peut pas signer, un témoin signera à son nom.

Nom du témoin

Signature du témoin

Signature de l'investigateur administrant le consentement éclairé

Date/Heure

**ETUDE TRANSVERSALE SUR LA PREVALENCE DE LA PARASITEMIE ET DE
L'ANEMIE CHEZ L'ENFANT DE 3-59 MOIS A BOBO-DIOULASSO, BURKINA
FASO**

Le paludisme est un problème très préoccupant dans notre pays, les enfants de moins de 5 ans les femmes enceintes sont les plus susceptibles de contracter la maladie. Des recherches menées dans beaucoup de pays africains ont montré la prise d'un médicament contre le paludisme une fois par mois (IPT) peut diminuer le risque de contracter le paludisme chez les enfants de moins de 5 ans.

Nous voulons savoir combien d'enfants ont les microbes du paludisme dans leur sang mais aussi chez combien d'enfants la quantité du sang a diminué. Si vous acceptez de participer à cette étude, nous allons faire un prélèvement de sang au bout du doigt de votre enfant, nos docteurs ou infirmiers poseront des questions sur l'âge, le statut vaccinal et nutritionnel, l'utilisation ou non de moustiquaires (imprégnées ou pas) puis examineront votre enfant. Si votre enfant souffre du paludisme ou n'a pas beaucoup de sang, nous lui donnerons des médicaments. Vous êtes libres de participer ou non à cette étude et nous respecterons votre volonté ; cela n'empêchera pas votre enfant d'être traité dans le centre de santé ou nous faisons l'étude et vous pouvez aussi arrêter votre participation à l'étude sans avoir à nous donner les raisons.

Les connaissances issues de cette étude aideront le Burkina à déterminer le meilleur médicament pour la prévention du paludisme chez les enfants.. Les prélèvements de sangs de votre enfant seront gardés à l'IRSS Bobo et pourront être utilisés plus tard pour des recherches sur le paludisme.

Les données collectées sur votre enfant resteront confidentielles et ne seront accessibles qu'à ceux qui travaillent dans le projet, le nom de votre enfant ne sera en aucun cas utilisé lors d'un rapport ou un écrit dans les journaux. .

Si vous avez des questions complémentaires, vous pouvez contacter le Principal Investigateur du projet Dr Issaka Zongo, téléphone 226-20-98-18-80 à l'Institut de Recherche en Sciences de la Santé.

Consentement éclairé

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Vous pouvez arrêter à tout moment votre participation

Vous avez l'occasion de poser toutes vos questions

FICHE DE CONSENTEMENT

Nom de l'enfant _____

Numéro d'Identification _____

J'ai compris les explications qui m'ont été données et j'accepte que mon enfant participe à l'étude.

Nom du parent ou du gardien de l'enfant _____

Relation avec l'enfant _____

Signature ou *empreinte du parent ou du gardien de l'enfant

*Si le parent ou le gardien ne peut pas signer, un témoin signera à son nom.

Nom du témoin _____

Signature du témoin _____

Signature de l'investigateur administrant le consentement éclairé

Date/Heure

Appendix 3 Enrolment form

FICHE CLINIQUE (1): 1. Numéro de randomisation: _ _ _ _ _ _ - _ _		
Nom et prénoms de l'enfant:		Nom Prénoms de la mère :
5. Sexe: M ____ F ____	2. Jour 0 Date: _ _ _ _ / _ _ _ _ / _ _ _ _ <i> Jour mois années</i>	2. Date de naissance de l'enfant : _ _ _ _ _ / _ _ _ _ / _ _ _ _ ou Age : _ _ _ _ <i>(Années)</i> <i> Jour mois années</i>

Enumérer tous les médicaments injérés les deux dernières semaines

Médicament (si nom inconnu ,lister par des lettres – "Inconnu medic A") (a)	Dose (b)	Date de la dernière dose (c)
8.	<input type="checkbox"/> Complete <input type="checkbox"/> Incomplete <input type="checkbox"/> Inconnu <input type="checkbox"/> N/A.	
9.	<input type="checkbox"/> Complete <input type="checkbox"/> Incomplete <input type="checkbox"/> Inconnu <input type="checkbox"/> N/A.	

Cotation 0-4: absent = 0; moyen = 1; modéré = 2; sévère = 3, menace vitale = 4, N/A = ne peut être évalué

	AOUT			SEPTEMBRE			OCTOBRE			AUTRES JOURS IMPREVUS			
	JOU R 0	JOU R 1	JOU R 2	JOU R 0	JOUR 1	JOU R 2	JOU R 0	JOU R 1	JOU R 2	JOU R..	JOUR ..	JOU R..	JOU R..
DATE													
12. Histoire febrile (O/N)													
13. Vomissement < 30 mns													
14. Faiblesse													
15. Moustiquaire (Oui/Non)													
16. MII (Oui/Non)													
16. Céphalées*													
17. Anorexie													
18. Nausée*													
19. Vomissement													
20. Dleur abdominale*													
21. Diarrhée													
22. Toux													
23. Prurits													
27. Autres _____													
29. Effets secondaires† (O/N)													
Initials													

****Noter seulement pour patients ≥ 3ans. Pour enfants < 3 ans incapable de répondre, noter N/A. † Effet secondaire: si nouvellement apparu ou aggravant et si cotation ≥ 2. Notifier au CE Muraz et sponsor tout effet secondaires grave.***

ICHE CLINIQUE (2):		Numéro de randomisation: _ _ _ _ _ _ - _ _
Nom et prénoms de l'enfant:	Nom et prénoms de la mère :	

5. Sexe: M ____ F ____	2. Jour 0 Date: _ _ / _ _ / _ _ _ <i>Jour</i> <i>mois années</i>	2. Date de naissance de l'enfant : _ _ / _ _ / _ _ ou Age : _ _ (Années) <i>Jour mois années</i>											
<i>SIGNES PHYSIQUES Cotation 0-4: absent = 0; moyen = 1; modéré = 2; sévère = 3, menace vitale = 4, N/A = ne peut être évalué</i>													
	AOUT	SEPTEMBRE	OCTOBRE	AUTRES JOURS IMPREVUS									
	JOU R 0	JOU R 1	JOU R 2	JOU R 0	JOU R 1	JOU R 2	JOU R 0	JOU R 1	JOU R 2	JOUR ..	JOUR ..	JOUR ..	JOUR ..
DATE													
30. Temperature (°C)													
31. Poids (kg)													
32. Taille (cm)													
33. Deshydratation													
34. Jaunice													
35. Thorax													
36. Abdomen													
37. Peau													
39. Autres _____													
40. Effets secondaires† (O/N)													
ABNORMAL EXAM RECORD													
En cas d'anomalie rencontrés à l'examen physique les décrire													
Initials													

* Tablet test – ≥ 9 mo; Heel-toe – ≥ 2 years; Romberg – ≥ 4 years. * Suivre les guides d'évaluation et de cotation. Noter N/A pour les jeunes enfants et les patients non adhérents. † Enregistrer l'effet second si nouveau ou aggravation avec cote ≥ 2. . Notifier au CE Muraz, et au sponsor immédiatement tout effet secondaire grave.

FICHE CLINIQUE (3):	Numéro de randomisation: _ _ _ _ _ - _
Nom et prénoms de l'enfant:	Nom et prénoms de la mère:

5. Sexe: M ____ F ____	2. Jour 0 Date: _ _ / _ _ / _ _ _ Jour mois années	2. Date de naissance de l'enfant : _ _ / _ _ / _ _ ou Age : _ _ (Années) Jour mois années
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RESULTAT DE LABORATOIRE

	AOUT			SEPTEMBRE			SEPTEMBRE			AUTRES JOURS IMPREVUS			
	JOUR 0	JOUR 1	JOUR 2	JOUR 0	JOUR 1	JOUR 2	JOUR 0	JOUR 1	JOUR 2	JOUR ..	JOUR ..	JOUR ..	JOUR ..
DATE													
40. Densité parasitaire (asexual parasites/ul)													
41. Espèces													
42. Heure d'administration du médicament													
Heure du prélèvement													
43. Densité Gametocytaire													
44. Hémoglobine*† (g/dL)													
Initials													

*(Cotation 0-4: normal = 0; mild abnormality = 1; modere = 2; severe = 3, menace vitale = 4)

† Tx d'hémoglobine \leq 5g/dl mesure après le jour 0 est un effet secondaires grave. Notifier au CE Muraz, et au sponsor immédiatement tout effet secondaires grave.

L'enfant a-t-il reçu du TP ce mois? Oui Non NSP

Si oui, précisez la date mentionnée sur la carte ____/____/____

Si non, pour quelle raison l'administration n'a pas été faite ? _____

Nous voudrions maintenant avoir vos analyses sur le TPI administré à votre enfant :

1 ^{ere} dose			
L'enfant a-t-il reçu la première dose du TPI administré?	<input type="checkbox"/> Oui/Non/Nsp		
Si oui, l'enfant a-t-il: (<i>cochez la réponse</i>)			
Bien avalé le médicament ?	<input type="checkbox"/>		
Avalé le médicament mais l'a aussitôt rendu?	<input type="checkbox"/>		
Refusé de prendre le médicament?	<input type="checkbox"/>		
L'enfant a-t-il vomi après prise du médicament ?	<input type="checkbox"/> Oui/Non/Nsp		
Si oui, la dose a-t-elle été re-administrée ?	<input type="checkbox"/> Oui/Non/Nsp	2 ^{eme} dose J1 (à domicile par l'agent de terrain)	3 ^{eme} dose J2 (à domicile par l'agent de terrain)
Avez-vous administré les 2 nd et 3 ^{eme} doses ?	<input type="checkbox"/> Oui/Non/Nsp	<input type="checkbox"/> Oui/Non/Nsp	<input type="checkbox"/> Oui/Non/Nsp
Si non, pour quelle raison? (Mentionnez la raison dans la case)			
Si oui, l'enfant a-t-il: (<i>cochez la réponse</i>)			
Bien avalé le médicament ?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Avalé le médicament mais l'a aussitôt rendu?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Refusé de prendre le médicament?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
L'enfant a-t-il craché le traitement?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Appendix 5 Active malaria case detection form

Projet TPI Lena2009/Diagnostic et suivi de la morbidité
(Chaque enfant devra être visité dans un délai de 14 jours suivant l'administration du TPI)

1. Identification : Date de la visite : ____/____/____/

Round du TPI : Aout : Septembre Octobre

Date : ____/____/____/

Nom de l'enfant: _____ ID ||| Sexe: M /F

Date Naissance : ____/____/____ ou Age : années mois

Nom de la Mère : _____

2. Examen clinique et para clinique

Température axillaire: . °C L'enfant se sent-il/elle bien aujourd'hui? Oui /Non/NSP

Symptômes lors des dernières 48 heures: (1=oui, 2=non, 3=ne sait pas)

	<i>Oui/Non/Nsp</i>	<i>Oui/Non/Nsp</i>
Histoire febrile	<input type="checkbox"/>	
Toux	<input type="checkbox"/>	
Difficulté respiratoire	<input type="checkbox"/>	
Convulsions	<input type="checkbox"/>	
Vomissements	<input type="checkbox"/>	
Diarrhée	<input type="checkbox"/>	
Eruption	<input type="checkbox"/>	
Autres Symptômes		

Précisez : Si l'enfant est fébrile, (température axillaire non corrigée $\geq 37.5^{\circ}\text{C}$) ou antécédents de fièvre dans les 48 dernières heures, faire si possible un prélèvement.

Un TDR a-t-il été fait ? *Oui/Non* Si oui, indiquez le résultat : Positif/Négatif/Ininterprétable

Si non, pourquoi?

La goutte épaisse a-t-elle été faite? *Oui/Non* (1=oui, 2=non)
Si oui, indiquez le résultat (nb *Pf* par μl): _____ Si non, pourquoi?

Une goutte épaisse sur papier buvard a-t-elle été recueillie ? *Oui/Non* (1=oui, 2=non)

EN CAS DE TDR NEGATIF, EN PRESENCE DE FIEVRE OU D'HISTOIRE FEBRILE, REFERER L'ENFANT A LA FORMATION SANITAIRE IMMEDIATEMENT

Le traitement à administrer en cas de TDR positif est le Coartesiane sirop (La posologie du Coartesiane sera conforme à la fiche posologique contenue dans votre kit.

L'enfant a-t-il/elle été référé(e) au centre de santé ? *Oui/Non* (1=oui, 2=non)

5. Nom et fonction du personnel soignant : _____

Préciser aux parents que l'enfant doit être ramené au poste de santé si son état ne s'améliore pas.

Appendix 6 Guide for signs, symptoms and biological results cotation

Guide pour la cotation des symptômes, signes physiques et résultats de laboratoire

Tableau A. Guide pour la cotation des symptômes du patient.

	Grade 1 MOYEN	Grade 2 MODERE	Grade 3 SEVERE	Grade 4 MENACE VITALE
Histoire fébrile	N/A	Présent (Oui)	N/A	N/A
Faiblesse	Légère baisse de l'activité, asthénie pour les enfants, mais continue à jouer	Diminution modérée de l'activité, asthénie et limitation des activités pour les enfants	Non participation aux activités habituelles, Incapacité de jouer pour les enfants	Prostration
Douleur musculaire/ articulaire	Plaintes moyenne et/ou localisée	Douleur diffuse	Asthénie profonde, limitation des activités	N/A
Cephalées*	Moyenne, ne nécessite pas de traitement	Transitoire et modéré mais nécessite un traitement	douleur sévère, constant; requiert un traitement narcotique	Douleur très sévère nécessite un traitement narcotique régulier
Anorexie	Baisse de l'appétit mais accepte les aliments solides	Baisse plus importante de l'appétit, admet les aliments liquides et évite les solides	Appétit très diminué, refuse le lait évite les aliments liquide et solide (< 2 ans ≤ 12 h; > 2 ans ≤ 24 h)	Appétit très diminué, refuse le lait évite les aliments liquide et solide (< 2 ans > 12 h; > 2 ans > 24 h)
Nausée*	Moyenne, transitoire, sensation d'envie de vomir. Peut toujours prendre les aliments	Modérée et/ou envie constant de vomir, baisse de la prise alimentaire	Sévère, constante envie de vomir avec baisse importante de la prise alimentaire	N/A
Vomissement	1 épisode par jour	2-3 épisodes par jour	Hypotension orthostatique ou réhydratation par voie veineuse	Collapsus cardio vasculaire, requiert une réhydratation par voie intra veineuse
Douleur abdominale *	Moyenne (1-3 sur une échelle de 1 à 10)	Modérée (4-6 sur une échelle de 1 à 10)	Modérée à sévère (≥ 7 sur une échelle de 1 à 10)	Severe – nécessite un traitement
Diarrhée	Transitoire 3-4 selles liquides par jour	5-7 selles liquides par jour	Hypotension orthostatique ou > 7 selles liquides par jour ou nécessite de réhydratation intra veineuse	Collapsus cardio vasculaire, requiert une réhydratation par voie intra veineuse
Toux	Transitoire / intermittent	Persistent / constant	Incontrôlée	Cyanoses, stridor, baisse sévère de la capacité respiratoire
Prurit	Prurit transitoire	Prurit perturbant le sommeil	Prurit constant insomniant	N/A
Acouphènes*	Mild, transient ringing or roaring sound	Moderate, persistent ringing or roaring sound	Severe ringing or roaring sound with associated hearing loss	N/A
Changement du comportement	Difficulté de concentration, légère confusion ou agitation sans perturber les activités quotidiennes ne requérant pas de traitement	Confusion ou agitation modérées, limitation des activités quotidiennes, requiert un léger traitement	Confusion sévère ou agitation; Besoin d'assistance pour les activités quotidiennes, nécessite un traitement	Psychose toxique; Requiert hospitalisation pour prise en charge
“Rhinite” (IRA viral)	Légère congestion nasale, rhinorrhée moyenne	Congestion nasale et rhinorrhée modérées	N/A	N/A
Reaction allergique	N/A	N/A	Urticaire	urticaire severe choc anaphylactique, angioedeme
Convulsion	N/A	N/A	Crises localisées ou généralisées	Crises épileptiformes

* Evaluer seulement pour les enfants de plus de 3 ans. N/A pour les moins de 3 ans ou incapable de répondre

Référence – Base sur l'échelle de toxicité de l'OMS pour déterminer la sévérité des effets secondaires

Table B. Guide pour les signes physiques

<i>Déshydratation</i>	Apprécier l'état de la peau, les muqueuses, les globes oculaires, la fontanelle, le pouls, et les urines
Jaunice	Rechercher une coloration jaune de la sclera, les conjonctives les téguments et la peau
Thorax	<p>Apprécier la respiration: rythme, profondeur, et un éventuel effort respiratoire. Rechercher une cyanose. Rythme respiratoire maximal acceptable selon l'âge: < 2 mois = 60, 2-12 mois = 50, 1-5 ans = 40, > 5 ans = 30.</p> <p>Inspecter le cou pour apprécier la position de la trachée, une éventuelle rétraction supra claviculaire et une contracture du muscle sterno mastoïdien ou des autres muscles respiratoires accessoires.</p> <p>Inspecter a la recherche d'une déformation évidente, palper a la recherche d'une crépitation neigeuse et percuter a la recherche de zones d'hyper sonorité.</p> <p>Auscultar la région antérieure et postérieure du thorax a la recherche de bruits respiratoires normaux et tout autre bruit surajoutés: Râles crépitant, sous crépitant, sibilants, ronchus.</p> <p>En cas d'anomalies évaluer le degré</p>
Abdomen	<p>Inspecter et ausculter l'abdomen. Rechercher les bruits (borborygmes) avant toute palpation. Listen for bowel sounds in the abdomen before palpating it. Palper tous les quadrants de l'abdomen superficiellement et profondément. Evaluer la taille du foie et de la rate.</p> <p>Rechercher des signes d'inflammation ou d'irritation péritonéale: Baisse de la mobilité intestinale, une défense abdominale, une contracture localisée ou généralisée ou une rigidité.</p>
Peau	Inspecter la peau: couleur, rougeur, lésion évidente. En présence de lésions noter leur localisation, distribution (localisée ou diffuse), type (macules, papules, vésicules) et leur couleur.
Tablet test	<p>Pour les enfants ≥ 9 mois, demander à l'enfant de prendre un objet posé sur une surface plane avec le pouce ou l'indexe (bic par exemple). <i>Ce test pour apprécier la coordination des extrémités distales évalue la fonction motrice, le système vestibulaire (coordination des yeux et des mouvements du corps), le cerceau et le système sensoriel. Attention : Pendant ce test soyez attentif car l'enfant a tendance à tout emporter à la bouche</i></p>

Table C. Grading Physical Examination Findings

	Grade 1 MOYEN	Grade 2 MODERE	Grade 3 SEVERE	Grade 4 MENACE VITALE
Temperature* (axillaire)	37.5-37.9°C	38.0-39.5°C	> 39.5°C	Fièvre continue, \geq 40.0°C pendant plus de 5 jours
Déshydratation	Less than 2 of the following: Restless, irritable Sunken eyes Drinks eagerly, thirsty Skin pinch goes back slowly	2 of the following: Restless, irritable Sunken eyes Drinks eagerly, thirsty Skin pinch goes back slowly	Deux des signes suivants: Léthargie ou inconscience Yeux secs (enfoncement des globes oculaires) Incapacité de boire ou boisson très limitée. Pli cutané s'effaçant très lentement	Deux des signes suivants + choc: Léthargie ou inconscience Yeux sec avec enfoncement des globes oculaires Incapacité de boire ou boisson très limitée. Pli cutané s'effaçant très lentement
Jaunice	Légère coloration jaune des conjonctives et de la sclère	Coloration jaune modérée de la sclère, des conjonctives et des téguments	Ictère flamboyant des conjonctives et de la sclère, coloration jaune de la peau	N/A
Thorax	Légère augmentation de la fréquence respiratoire (pour l'âge, température), bruits anormaux transitoires ou localisés	Augmentation modérée de la fréquence, persistance de bruits anormaux diffus	Fréquence respiratoire rapide (< 2 mois > 60, 2-12 mois > 50, 1-5 ans > 40, adultes > 30)* mouvement des ailes du nez	Cyanose
Abdomen	Bruits normaux, réaction abdominal localisée et/ou foie palpable a 2-4 cm au dessous de rebord costal droit et/ou une rate palpable et/ou une hernie ombilicale	Bruits normaux ou légèrement anormaux, réaction abdominale modérée, hépatomégalie modérée (4-6 cm sous le rebord costal droit) et/ou une splénomégalie palpée a mi chemin entre l'ombilic et la symphyse pubienne	Contracture abdominale Signes évidents d'irritation péritonéale avec une hépatomégalie importante (> 6 cm sous le rebord costal droit) et/ou une rate en dessous d'une ligne passant a mi chemin entre l'ombilic et la symphyse pubienne	Absence de mouvements intestinaux. Rigidité abdominale
Peau†	Rash cutané localisé, érythème ou prurit	Reaction maculo papuleuse diffuse, peau desquamant	Présence de vésicules, desquamation cutanée plus importante, ulcération cutanée.	Dermatose exfoliative ou érythème multiforme ou suspicion de syndrome de Stevens-Johnson ou nécrose nécessitant une intervention chirurgicale

	Grade 1 MOYEN	Grade 2 MODERE	Grade 3 SEVERE	Grade 4 MENACE VITALE
Audition	< 4 ans: N/A \geq 4 ans: Baisse de l'audition au niveau d'une oreille	< 4 ans: N/A \geq 4 ans: Baisse de l'audition au niveau au niveau des deux oreilles ou baisse sévère au niveau d'une oreille	< 4ans: Pas d'évidence de baisse de l'audition \geq 4 ans: Baisse très importante de l'audition au niveau des deux oreilles	N/A
Tablet test	Difficulté à prendre l'objet mais parvient quand même à le prendre	Incapable de prendre un objet sans qu'il ne retombe	Incapable de prendre un objet	N/A
Autres signes cliniques	Ne nécessite pas un traitement; surveillance du signe	Besoin de traitement	Besoin de traitement et d'une éventuelle hospitalisation	Requiert une intervention médicale active, voire une hospitalisation

Référence – The Harriet Lane Handbook, 15th edition, 2000

† Référence – Echelle de cotation de la sévérité des effets secondaires de l'OMS

TABLE D. Guide pour la cotation des anomalies de Laboratoire (taux d'hémoglobine)

	Grade 1 MOYEN	Grade 2 MODERE	Grade 3 SEVERE	Grade 4 MENACE VITALE
Hémoglobine (g/dL)	9.0 – 9.9	7.0 – 8.9	5.0 – 6.9	< 5.0

Critères de paludisme grave/Signes de Danger

Paludisme grave

- Coma (*si après convulsions, > 30 min*)
- Convulsions répétées (*> 2 en 24 h*)
- Anémie sévère (*Hb < 5.0 g/dL*)
- Détresse respiratoire (*dyspnée de repos*)
- Ictère (*coloration jaune des yeux*)

Signes de danger

- Convulsions récentes (*1-2 en 24 h*)
- Altération de la conscience (*confusion, delirium, psychose*)
- Léthargie
- Incapacité de boire ou de téter
- Vomissements incoercibles
- Incapacité de s'asseoir / se tenir debout du fait de l'asthénie

Appendix 7 Weight based dose of the study drugs

Amodiaquine (AQ)

Poids par kg	Nombre de cuillères mesures par jour
5.0-5.6	1 cuillère mesure + $\frac{1}{4}$
5.7-7.5	1 cuillère mesure + $\frac{1}{2}$
7.6-8.7	1 cuillère mesure + $\frac{3}{4}$
8.8-10	2 cuillère mesures
10.1-10.7	2 cuillères-mesures + $\frac{1}{4}$
10.8-12.5	2 cuillères-mesures + $\frac{1}{2}$
12.6-13.7	2 cuillères-mesures + $\frac{3}{4}$
13.8-15	3 cuillères-mesures
15.1-15.7	3 cuillères-mesures + $\frac{1}{4}$
15.8-17.5	3 cuillères-mesures + $\frac{1}{2}$
17.6-18.7	3 cuillères-mesures + $\frac{3}{4}$
18.8-20	4 cuillères-mesures
20.1-20.7	4 cuillères-mesures + $\frac{1}{4}$
20.8-22.5	4 cuillères-mesures + $\frac{1}{2}$
22.6-23.7	4 cuillères-mesures + $\frac{3}{4}$
23.8-25	5 cuillères-mesures
25.0-25.7	5 cuillères-mesures + $\frac{1}{4}$
25.8-27.5	5 cuillères-mesures + $\frac{1}{2}$
27.6-28.7	5 cuillères-mesures + $\frac{3}{4}$
28.8-30	6 cuillères-mesures
30-30.6	6 cuillères-mesures + $\frac{1}{4}$
30.7-32.5	6 cuillères-mesures + $\frac{1}{2}$
32.6-33.7	6 cuillères-mesures + $\frac{3}{4}$
33.8-35.0	7 cuillères-mesures
35.1-35.6	7 cuillères-mesures + $\frac{1}{4}$
35.7-37.5	7 cuillères-mesures + $\frac{1}{2}$
37.6-40.0	5 cuillères-mesures

Sulfadoxine-pyrimethamine (SP)

Poids en kg	Nombre de comprimés
5.0-7.4	¼
7.5-12.4	1/2
12.5-17.4	3/4
17.5-22.4	1
22.5-27.4	1 1/4
27.5-32.4	1 1/2
32.5-36.2	1 3/4

Dihydroartemisinin-Piperaquine (DHAPQ)

Poids en kg	Nombre de comprimés par jour
5-7.1	1/4
7.2-11.9	1/2
12.0-16.6	3/4
16.7-21.4	1
21.5-26.1	1 1/4
26.2-30.9	1 1/2
31.00-35.70	1 3/4
35.8-36.2	2

Appendix 8 Pharmacokinetics of sulfadoxine

A clinical study was carried out to assess the pharmacokinetics of Sulfadoxine-pyrimethamine and amodiaquine when used for SMC. Drug measurement was done using HPLC for sulfadoxine (Appendix 8) but no peak of drug was detected for Amodiaquine.

1 Baseline characteristics of the enrolled patient.

A total of 150 children were enrolled and followed up over 28 days; the percentage of compliance at each scheduled visit was very high: 98.7% (148/150) for the second sample visit, 94.7% (142/150) for the third and 90% (135/150) for the last visit. The study was conducted in May-June 2011 in an area of low incidence of malaria and no slide was positive for *Plasmodium falciparum*. Other characteristics are described in the table 27.

Table 0-1: Characteristics of patients treated with SP at entry

	6 months	6-11 months	12-23 months	24-35 months	36-47 months	48-59 months
Population	10	21	40	17	43	19
Male n (%)	6 (60%)	13 (62%)	16 (40%)	8 (47%)	21 (49%)	12 (63%)
Mean temperature (°C)	36.4	36.6	36.5	36.5	36.4	36.7
Mean Weight in kilogram (sd)	7.2	8.6	9.1	10.9	12.0	14.0
Mean Height in cm (sd)	66.2	71	75.9	86.2	91.6	98.1
Dose in mg/kg (Mean)	33.4 (25-41.7)	31.8 (25.41.7)	29.6 (24.8-41.7)	28.9 (24.5-41.7)	29.3 (23.8-34.1)	28.7 (24.2-34.1)

2 Treatment dosage characteristics in different groups of age

Table 28 Treatment characteristics

	Mean dose	Median dose	IQR	Range	Under-dosed (<25mg/kg/day)		Target range (25-70 mg/kg/day)	
Age group					N	%	N	%
<=6 months	33.4	35.7	13.5	16.7	0	0%	10	100%
7-11 months	31.8	31.3	5.6	16.7	0	0%	21	100%
12-23 months	29.6	28.1	3.4	16.9	1	2.5%	39	97.5%

24-35 months	28.9	28.8	6.0	17.2	3	17.6%	14	82.4%
36-47 months	29.3	28.8	4.5	10.3	4	9.3%	39	90.7%
48-59 months	28.7	28.8	5.4	9.9	1	5.3%	18	94.7%
Total	29.9	29.2	4.5	17.9	9	6%	141	94%

The mean dose of SP administrated was 29.9 mg per kilogram body weight; the targeted dose is 25-70 mg per kilogram body weight; only 6% of the children were under-dosed (table 28).

3 The dosage given in mg per kg and the age

Children aged 6 months received the higher dosage of drug in mg per kg and this dosage was slightly less in those aged 24 to 36 months; but overall none was over-dosed (figure 16).

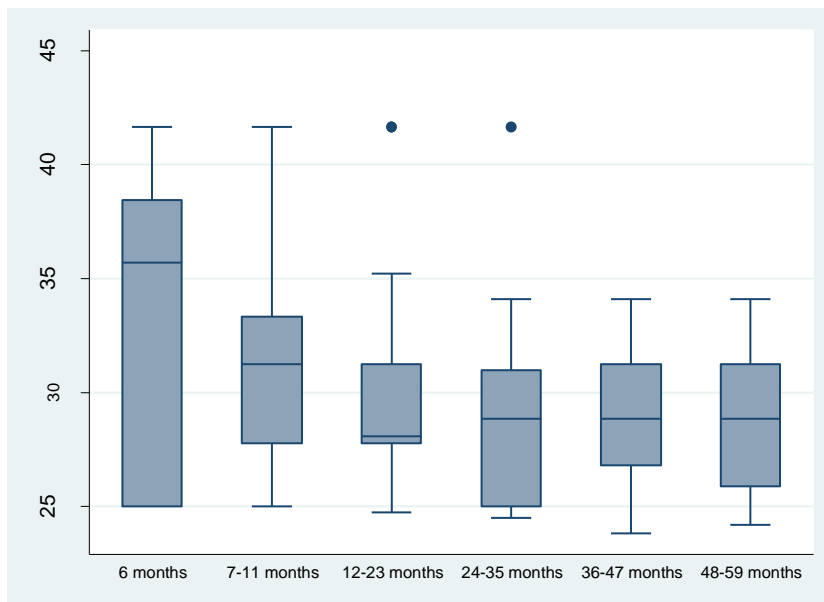


Figure 16 Distribution of dose in mg per kg and the group of age in months

4 Relationship between Sulfadoxine concentration in mg/ml and the age in months

Sulfadoxine concentration on days 1-5 was higher in old age group (24-35 months and 48-59 months) and lower in the participant 7-11 months. The same pattern was seen with the next window of sampling but the concentration was lesser in the 12-24 months children. Over the last window, the drug concentration was almost null in two age groups, those under 6 months and those in the 24-35 months group (figure 17).

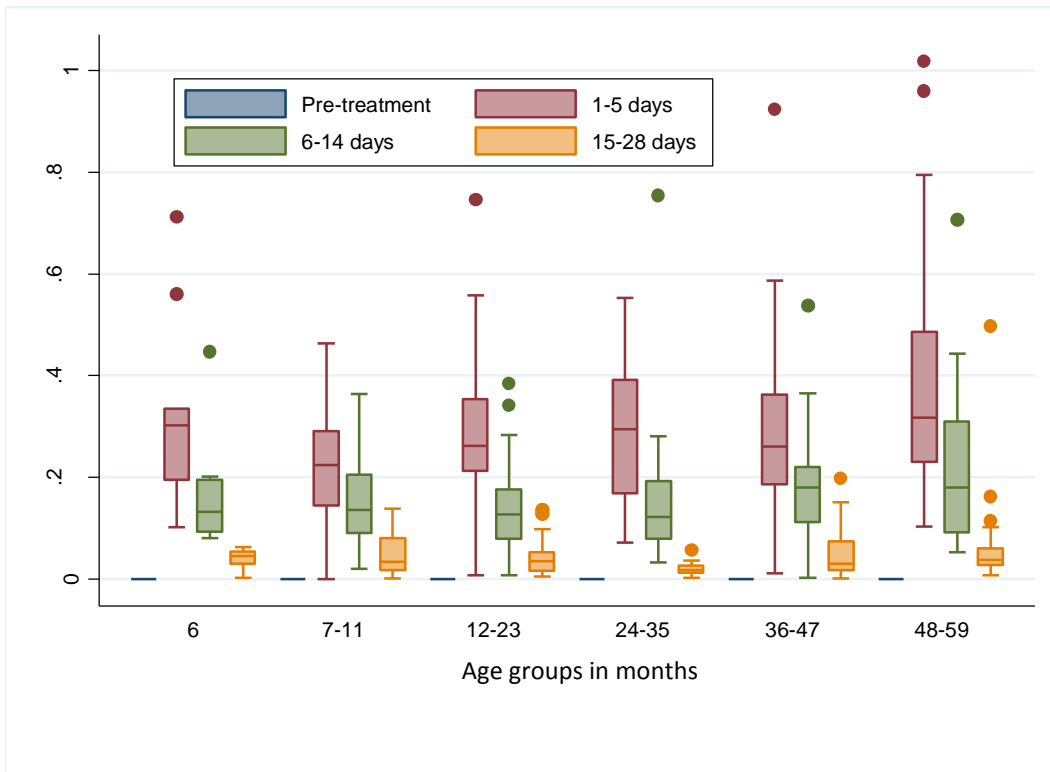


Figure 17 Distribution of sulfadoxine concentration and age groups

5 Sulfadoxine concentration in mg/ml and dose in mg per kg

6 Measure of amodiaquine

Samples were collected for desethylamodiaquine concentration measurement.

Unfortunately no drug was detected during the analysis with the HPLC.

Appendix 9: Alternative drug regimen for SMC

1. Sulfadoxine-Pyrimethamine plus Piperaquine (SP+PQ)

This combination is ideal for SMC in regard of the long half-life of the two partners and the objective of the SMC which is to clear the existing parasites while preventing the acquisition of new ones (prophylactic effect). SPPQ has been trialled in Senegal and in The Gambia (Bojang et al. 2010) and in Senegal (Cisse et al., 2009). The combination was as effective as or even better than SPAQ and better tolerated. The disadvantages of this regimen are that (i) it does not exist as co-formulated and (ii) the partner SP suffers from loss of efficacy in areas of East Africa. Future research are needed to investigate the efficacy of co-formulated regimen ideally given as a single dose (like SP) to maximise the efficacy, the adherence and protect each of the component as longer as possible.

2. Artemether-Lumefantrine

Artemether-Lumefantrine is another ACT not primarily suitable for use in SMC for similar reasons as for the DHAPQ. Artemether-lumefantrine is a highly effective anti-malarial as demonstrated in efficacy trials through endemic countries of Africa and Asia (Katrak et al., 2009, McGready et al., 2008, Nambozi et al., 2011). However the post treatment prophylactic effect is very limited due to the relatively short half-life of Lumefantrine (14 days) allowing recurrence of parasites few weeks following the initiation of a therapy with artemether-lumefantrine (Zongo et al., 2007b) AL might be used for SMC only in areas of low transmission and probably the timing would be reduced to every three weeks owing most frequent adverse events.

3. Mefloquine-artesunate (AS-MQ)

AS-MQ is a combination used for the first line treatment of malaria in the Cambodia border since 2000 at the dose of 12 mg per kg of Artesunate and 20 mg/kg of mefloquine or a maximum total of 600 mg of Artesunate and 1000 mg of mefloquine; by 2002 emergence of resistance restricted its use to the in Eastern Cambodia and in Thailand (Carrara et al., 2009, Rogers et al., 2009). With the introduction of ACTs in the treatment of malaria there was renew of interest in the combination Mefloquine plus Artesunate (MQ-AS) and series of studies in Asia reported the combination is effective for the treatment of uncomplicated falciparum malaria owing more frequent adverse events (Mayxay et al., 2006, Smithuis et al., 2006). An efficacy study conducted in patients with uncomplicated falciparum malaria in Benin showed a high efficacy of MQ-AS with a good tolerance profile (Massougbdji A, 2002). Mefloquine in monotherapy is

now being considered as a possible alternative to the failing monotherapy with sulfadoxine-pyrimethamine for the intermittent preventive treatment of malaria in pregnancy (Denoeud-Ndam L, 2012). Artesunate is safe in the second and third trimester of the pregnancy and its use in combination with mefloquine may be an option to protect mefloquine; of note Artesunate appears to decrease the maximum mefloquine concentration and its half-life (Karbwang et al., 1994). From there we can argue for the investigation of MQ-AS for a possible replacement of SPAQ for the SMC in children less than 5 years old in the Sahel and Sub-Saharan region.

4. Mefloquine plus Sulfadoxine-Pyrimethamine (MQ-SP)

An old study comparing the efficacy of MQ-SP (Fansimef®) and SP (Fansidar®) in Zambian adults with symptomatic malaria reported better efficacy with MQ-SP compared to SP alone and the side effects was common in both groups (Ekue JM, 1987); in a phase I safety and tolerance study of mefloquine adult Brazilian adverse events included dizziness, nausea, vomiting that were mild and did not require specific care (de Souza JM, 1987). These studies are precursors and may serve a baseline for further investigation about the potential of this combination for the SMC in replacement to SPAQ.

The alternative drug regimen to consider in a given area should account for the current lines for the treatment of acute malaria and avoid being the similar regimen as this will increase the drug pressure and may contribute to speed the spread of drug resistance. Furthermore, the potential replacement should be easier of use, preferably as a single dose once, finally very well tolerated and accessible.