The effect of *falciparum* malaria prevalence on the effectiveness of intermittent preventive treatment with Sulfadoxine-Pyrimethamine during pregnancy in reducing low birth weight in southern Mozambique

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DECLARATION

I, Yasmin Cassam, hereby declare that this report is my own work and that it has not been submitted for any degree or examination in others Universities. All the sources that have been used or quoted have been indicated and acknowledged by means of complete references.

Date:

Signature:

DEDICATION

During the course of this research, I had the luck and pleasure of being surrounded by wonderful and very special people, one of them already out of this world but always present in my heart, and to them I dedicate this thesis:

My mother, Fatima Amade

And

My late father, Cassam Mussa (Jamal)

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ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
AIC	Akaike´s Information Criteria
ANC	Antenatal care
BIC	Bayesian Information Criteria
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethene
DHFR	Dihydrofolate Reductase
DHPS	Dihydropteroate Synthetase
DHS	Demographic Health Survey
DPS	Direção Provincial de Saúde (Provincial Directorate of Health)
Hgb	Haemoglobin
HIV	Human Immunodeficiency virus
INS	Instituto Nacional de Estatística (National Statistics Institute)
IPT	Intermittent Preventive Treatment
IPTp	Intermittent Preventive Treatment during Pregnancy

IPTi	Intermittent Preventive Treatment infant
IRS	Indoor Residual insecticide Spraying
ITNs	Insecticide Treated bed Nets
IUGR	Intra Uterine Growth Retardation
LBW	Low Birth Weight
LMP	Last Menstrual Period
LSDI	Lubombo Spatial Development Initiative
MDO	Millenium Development Objectives
MoH	Ministry of Health
NHS	National Health System
P.	Plasmodium
PDAs	Personal Digital Assistants
PES	Plano Económico e Social (Economic and Social Plan)
PF	Plasmodium Falciparum
PTV	Prevenção de Transmissão Vertical (Prevention of Vertical Transmission)
SLBW	Low Birth Weight of Singleton

SP	Sulfadoxine-Pyrimethamine
SVLBW	Very Low Birth Weight of Singleton
TLBW	Low Birth Weight of Twin
TVLBW	Very Low Birth Weight of Twin
VLBW	Very Low Birth Weight

ABSTRACT

Malaria infection is a major cause of morbidity and mortality in tropical countries, and particularly in Mozambique. Recently substantial resources have been used to reduce the burden of malaria in Mozambique. These include the distribution of insecticide treated bed-nets, indoor residual insecticide spraying, access to artemisinin-based combination treatment (ACT), and intermittent preventive treatment of pregnant women with sulfadoxine-pyrimetamine (SP-IPTp). The most important benefit of SP-IPTp in malaria endemic areas has been the increase in birth weight, thus increasing the probability of child survival. The SP-IPTp policy was based on evidence of its effectiveness in areas of high intensity malaria transmission. The effect of SP-IPTp has been less evident in the presence of high coverage with insecticide treated bed-nets. It is not know whether reducing the risk of malaria through effective vector control using indoor residual insecticide spraying and large-scale deployment of ACTs has a similar effect in reducing the impact of SP-IPTp on birth weight. At the same time, increasing resistance of SP could be compromising the effect of SP-IPTp on birth weight, as could co-infection with HIV.

The aim of this study was to determine if the effect of SP-IPTp on reduction in risk of low birth weight is modified by Plasmodium *falciparum* malaria prevalence. This retrospective antenatal record review, analyzed 20867 antenatal records from 2005 to 2007 from public health facilities in Maputo and Gaza provinces, southern Mozambique. One or two doses of SP-IPTp does not have any effect on reducing the risk of low birth weight, while women who had at least three doses of SP-IPTp had a 15% lower risk of their babies being born with low birth weigh compared with fewer doses, (OR=0.85; 95% CI 0.73 – 1.00; p=0.053). The risk of babies being born with low birth weight was reduced by 28% when both malaria prevalence and *dhfr / dhps* mutation prevalence are low, (OR=0.72; 95% CI 0.51 – 1.00), but this effect was no longer significant with higher malaria prevalence and or mutation prevalence.

SP-IPTp has an effect on reducing low birth weight with three or more doses, and in areas where malaria prevalence and mutation prevalence are low.

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INTRODUCTION

1. BACKGROUND

Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions throughout the world resulting in complications such as maternal anaemia, maternal clinical malaria, low birth weight (LBW), abortion, premature delivery and stillbirth.[1, 2, 3] This can result in maternal and infant mortality and increased health care expenditure as well as loss of productivity.[4]

Recently substantial resources have been used to reduce the burden of malaria in Mozambique. These include distribution of insecticide treated bednets (ITNs), indoor residual insecticide spraying (IRS), access to artemisinin-based combination treatment (ACT), and most recently, intermittent preventive treatment of pregnant women with sulfadoxine-pyrimethamine (SP-IPTp). IPTp describes the administration of a full therapeutic course of an anti-malarial to subjects at risk at specified times regardless of whether or not they are infected with malaria parasites.[5] IPTp using SP in areas of high intensity malaria transmission has been associated with a decline in placental infection and in the proportion of low birth weight babies.[6]

Many countries in Africa are currently succeeding in reducing their malaria burden.[7] As the risk of malaria decreases, the effectiveness of IPTp in reducing the incidence of low birth weight would be expected to decrease. However, the level of malaria transmission below which the risks of IPTp outweigh its benefits remains to be defined.

A retrospective antenatal record review was conducted to determine whether the impact of SP-IPTp is influenced by the prevalence of falciparum malaria in two provinces in Southern Mozambique where there has been a recorded reduction in malaria burden following the phased implementation of community-based IRS and ACTs by the Lubombo Spatial Development Initiative (LSDI).[8]

2. LITERATURE REVIEW

This chapter includes the following topics:

2.1. The burden of Malaria (globally, in sub-Saharan Africa and in Mozambique);

2.2. Malaria and its prevention in pregnant women;

2.3. HIV and Malaria during pregnancy;

2.4. Low Birth Weight and its associated risk factors;

2.5. Malaria prevention by insecticide treated bed nets and indoor residual insecticide spraying;

2.6. Summary of socio-economic and demographic data:

- 2.6.1.1. Mozambique;
- 2.6.1.2. Maputo province;
- 2.6.1.3. Gaza province;

2.7. Description of National Health System in Mozambique

2.1 The burden of Malaria

Malaria is the fifth most common cause of death from infection diseases worldwide (after respiratory infection, HIV/AIDS, diarrhoeal diseases and Tuberculosis) and the second in Africa, after HIV/AIDS.[9] It is estimated that as many as 3.3 billion people live in malaria risk areas in 109 countries or territories.[9]

Each year, up to a million deaths occur due to malaria and approximately five billion episodes of clinical illness possibly meriting antimalarial therapy occur throughout the world, with Africa having more than 90% of this burden.[10, 11]

Malaria and poverty are intimately connected. 58% of the malaria cases occur in the poorest populations, which generally receive the worst care and are most likely to have catastrophic economic consequences from their illness.[10]

Malaria is one of the principal health problems in Mozambique and, as in many African countries, is one of the principal causes of absenteeism from school and

work, perpetuating the vicious cycles of disease and poverty.[12] The majority of Mozambique's populations live in areas at high risk of malaria infection.

In Mozambique malaria transmission occurs throughout the year with peaks during and after the rain season, between December and April. The intensity of transmission varies depending on annual rainfall and temperature and also on specific environmental conditions in the different regions.[12]

The factors contributing to the high risk of malaria in Mozambique include favorable climate and environmental conditions, especially the temperature and rainfall patterns that increase the abundance of mosquitoes and their breeding sites; and socio-economic conducive conditions, including poverty and lack of access to prevention strategies.[12]

Unlike the LSDI area, malaria is increasing in the rest of Mozambique. During 2006, 6,227,719 cases of malaria were reported in Mozambique with 4,985 malaria related deaths.[13] According to UNICEF, malaria is the primary cause of ill health in Mozambique, accounting for 40% of out-patient consultations, 60% of paediatric inpatients and a third of hospital deaths.[13] About 90% of children under 5 years of age are infected with malaria parasites in Mozambique.[13] Malaria is also a major problem affecting pregnant women in rural areas. Approximately 20% of women are parasitaemic, with primigravidae having the highest prevalence (31%).[13] Anemia, often associated with malaria, is a major problem and 68% of children surveyed had a packed cell volume of less than 33%.[14]

The Strategic Plan of the National Malaria Control Program (NMCP), prepared in collaboration with several implementation partners in Mozambique, delineates malaria prevention and control activities as well as coverage targets for number of interventions with a view to expanding efforts to combat malaria at the household level.[15]

2.2 Malaria and its prevention in pregnant women

Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions throughout the world. In most endemic areas of Africa, pregnant women are the main adult risk group for malaria.[12] In Mozambique, as in many African countries, malaria is a major cause of death among pregnant women.[16]

Delivery of cost-effective interventions to reduce the burden of malaria in pregnant women will require:

- Increased awareness of the problem among communities affected by malaria;
- Integration of malaria control tools and interventions within maternal and newborn health programmers including provision of IPTp and insecticide treated nets;
- Strengthened antenatal care services;
- Involvement of community health workers in promoting the following interventions at the community level:
    - The use of antenatal clinics and insecticide treated nets (ITNs),
    - Acceptance of Indoor Residual Insecticide Spraying (IRS) and
    - Early treatment seeking for malaria-related symptoms.[17]

In areas of stable malaria transmission, pregnant women with *Plasmodium falciparum* are often asymptomatic, but the parasite sequesters in the placenta and these infection are strongly associated with maternal anaemia and low birth weight, accounting for an estimated 26% of severe maternal anaemia in Sub-Saharan Africa and causing an estimated 100,000 – 200,000 infant deaths each year as a result of low birth weight.[18,19,20]

In areas of unstable malaria transmission, malaria in pregnancy is associated with severe syndromes, such as cerebral malaria and pulmonary oedema, and the risk on malaria is 1.5 times higher in the third trimester of pregnancy.[21]

The current WHO recommended strategies for controlling malaria in pregnancy include both curative (effective case management) and preventive (mosquito vector control using indoor residual insecticide spraying and or insecticide treated nets, and preventive chemotherapy) measures.[22] Preventive chemotherapy entails the administration of an effective antimalarial medicine at specified times to a population at risk regardless of whether or not they are infected with malaria.[22] One of the early uses of preventive chemotheraphy was the prophylactic use of chloroquine by pregnant women living in malaria endemic areas – a strategy which has been abandoned in many situations because of the widespread resistance of Plasmodium falciparum to chloroquine and the low compliance with the weekly regimen of administration.[22]

In 2000, the WHO Technical Advisory Group, recommended that intermittent treatment with an effective antimalarial drug should be made available as a routine part of antenatal care to women in their first and second pregnancies in highly endemic areas and should be given at least twice after quickening (the first noted movement of the foetus), no less than 4 weeks apart, alongside the use of insecticide-treated bed nets (ITNs) and effective case management.[5, 15, 18, 19, 23, 24] IPT with SP delivered at routine antenatal care visits is policy in several African countries.

Intermittent Preventive Treatment during Pregnancy (IPTp), describes the administration of a full therapeutic course of antimalarials to risk subjects at specified times regardless of whether or not they are infected with malaria parasites. IPT differs from chemoprophylaxis, which aims to sustain blood levels above the mean inhibitory concentration for a prolonged period.[5] By contrast, IPT aims at producing therapeutic concentrations to clear parasitaemia and providing post-treatment prophylactic concentrations for shorter periods of time separated by periods when drug concentrations are below the concentration necessary to inhibit parasite growth.[5]

Sulfadoxine - Pyrimethamine (SP) is a fixed dose antimalarial combination tablet containing a long-acting sulphonamide combined with pyrimethamine.[6] SP has the distinct operational advantage of the complete treatment course being administered

as a single dose, facilitating directly observed therapy and patient adherence. SP is no longer recommended for prophylaxis, given its cumulative toxicity and decreasing antimalarial efficacy due to the rapid spread of SP resistance.[6]

There has been widespread programmatic use of SP during pregnancy, which is thought to be safe, although overdose and idiosyncratic reactions could lead to detrimental effects on the mother and/or the foetus.[25] Sulphonamides are generally considered safe in the second and third trimesters of pregnancy.[25] There is very limited evidence that sulpha drugs may be associated with kernicterus when given to premature neonates, this problem has not yet been noted in studies of SP-IPTp.[25] Studies examining the risk to the foetus from in utero exposure to SP combination have generally not found any increased risk in spontaneous abortions or congenital defects.[25] However, with the spread resistance to SP, IPTp with SP exacerbate placenta malaria and also IPTp was associated with decreased cord haemoglobin. [26, 27] Concomitant use of SP with high doses of folic acid (5 mg/day) in the first trimester of pregnancy is associated with neural tube effects.[19]

In Malawi, where SP-IPTp has been part of national health policy since 1993, pregnant women receiving SP had a decline in placental infection (from 32% to 23%) and in the proportion of low birth weight babies (from 23% to 10%). Also in Malawi, 75% of all pregnant women took advantage of IPTp when offered.[28] SP-IPTp reduced early neonatal mortality. [29] Another study shows that IPTp, protects against maternal anemia as well as low birth weight, especially in primigravidae and secundigravidae.[30] Women with more than three pregnancies and who took SP-IPTp had a lower prevalence of babies with low birth weight. [31]

Sulphadoxine-pyrimethamine acts in two enzymes in the parasite´s folate synthesis pathway.[32, 33] Resistance to SP results from accumulation of mutations in the dihydrofolate reductase (DHFR) gene and dihydropteroate synthetase (DHPS) genes of Plasmodium falciparum.[34] According to an study carried out in Maputo province, the dhfr triple mutations had reached fixation (91.7%) in 2004, the prevalence of parasites carrying the quintuple mutation (triple dhfr mutations plus double dhps mutations) had increased markedly from 11.0% in 2004 to 75.0% in 2008 (OR=42.2; 95% CI: 21.3-83.7; $p<0.0001$).[35] The same study showed a

positive relationship between quintuple mutation prevalence and months since artesunate plus SP deployment (OR=1.11; 95% CI: 1.10-1.12; p<0.0001), years since deployment of SP IPTp policy (OR=4.40; 95% CI: 3.43-5.65;p<0.0001), and fever (OR=2.26; 95% CI: 1.32-2.87; p=0.003).[35] Factors that contributed to the alarmingly rapid spread of SP-resistant parasites in Maputo province after the systematic large-scale deployment of artesunate plus SP, included the pre-existing fixation of the *dhfr* triple mutation before the commencement of artesunate plus SP deployment in 2004, and the use of SP monotherapy for IPT of pregnant women since 2006.[35]

With increasing drug resistance, the minimum inhibitory concentration at which parasite growth is inhibited increases and the time taken for drug concentrations to fall below these levels after a treatment dose shortens.[36] This results in a progressive shortening of the duration of the suppressive prophylactic post-treatment effect.[36] Parasites with triple DHFR mutations have an approximate 1000-fold reduction in *in vitro* susceptibility to pyrimethamine, which translates into a reduction in the duration of post-treatment prophylaxis, compromising the efficacy of the 2-doses SP-IPTp regimen, which can have a 3-month interval between doses.[36] Despite this, in areas in which 1 of 4 treatments with sulfadoxine-pyrimethamine fail in children by day 14, the 2-dose IPT with sulfadoxine-pyrimethamine regimen continues to provide substantial benefit to HIV-negative semi-immune pregnant women.[36] SP-IPTp reduce the risk of babies born with low birth weight, however many studies shown that the reduction is significantly with an increasing of the number of doses [37, 38, 39, 40]

2.3 HIV and Malaria in pregnancy

The prevalence of HIV/AIDS infection adds to the burden of low birth weight globally, and particularly in sub-Saharan Africa where the prevalence of HIV is high. Pregnant women infected with HIV are at increased risk of delivering low birth weight infants, of preterm delivery, and intrauterine growth retardation.[41]

More frequent dosing of SP-IPTp is required in HIV positive women residing in endemic areas.[32] If HIV prevalence exceeds 10% and HIV testing is not available,

all women should receive three doses of SP-IPTp.[42] HIV positive women who had one dose of SP-IPTp are in more risk of being babies with low birth weight when compared with others in the same groups who had more than one dose. [43] HIV infection diminishes a pregnant woman´s ability to control Plasmodium falciparum infection.[44] The prevalence and intensity of malaria infection during pregnancy is higher in women who are HIV-infected, who are more likely to have symptomatic infections and to have an increased risk of malaria-associated adverse birth outcomes.[44] Compared to women with either malaria or HIV infection, women who are co-infected have a higher risk of preterm birth and intrauterine growth retardation and therefore are more likely to have low birth weight infants. Reports suggest that there is no consistent effect of maternal HIV on congenital malaria or on the relationship between maternal and infant malaria.[45]

In the most severely affected countries (such as the Central Africa Republic, Malawi, Mozambique, Zambia and Zimbabwe) more than 90% of the population is exposed to malaria, and HIV prevalence among adults between 15-49 years of age is above 10%.[28] Anaemia can result from infection with malaria or HIV (or both) and anaemia during pregnancy contributes to higher levels of maternal morbidity and mortality. [45]

IPTp with SP is recommended to HIV-positive women receiving cotrimoxazole or antiretroviral drugs.[36, 46] In areas with high HIV seroprevalence, for SP-IPTp to have an effect on reducing low birth weight it must be give in more than two doses.[47] Widespread cotrimoxazole use could also accelerate the development of resistance in malaria parasites to SP.[45] Cotrimoxazole contains the antifolates sulfamethoxazolemand trimethoprim, so could select for mutations in the enzymes that are inhibited by the sulphonamide and pyrimethamine components of SP.[45] This potential impact emphasizes the importance of drug resistance monitoring, in particular in communities where prophylaxis with cotrimoxazol is common.[45] The risk of serious adverse events, such as Steven Johnson Syndrome, increases if sulfadoxine-pyrimethamine and cotrimoxazole are used concomitantly, further justifying avoiding SP-IPT in pregnant women taking cotrimoxazole.

2.4 Low Birth Weight and its associated risk factors

Low birth weight is defined by the WHO as birth weight below 2500g irrespective of gestational age.[48, 49] Low birth weight is caused by either a short gestational period or retarded intrauterine growth, or a combination of both.[48, 50] Most extremely low birth weight infants are also the youngest of premature newborns, usually born at 27 weeks gestational age or younger.

Birth weight and gestational age each have an important effect on fetal and neonatal mortality.[50] Birth weight and neonatal care visits are also correlated, the prevalence of low birth weight reduce with a increase of the numbers of antenatal care visits.[51, 52]

Low birth weight infants have an increased risk of developing cerebral palsy, although prematurity appears to carry a greater risk. Also they have a risk of developing hyaline membrane disease, apnea, and other conditions related to physiological immaturity.[50] Low birth weight may also result in a wide spectrum of diseases in later life such as hypertension, ischemic heart disease, stroke, metabolic syndrome, diabetes, malignancies, osteoarthritis, and dementia.[48]

Intra uterine growth retardation and low birth weight infants represent a significant health problem worldwide.[48] low birth weight is most prevalent in developing countries, where the burden of malnutrition and infectious diseases is heavy.[41, 53] The rate of Intra uterine growth retardation and low birth weight births is highest in South-Central Asia (with about 72%), followed by Middle and western Africa, Oceania, and Latin America.[41, 53] Level of low birth weight babies in sub-Saharan Africa range from 10% to 20%.[53]

Birth weight is influenced by many factors, i.e. the causes can be "multi-factorial",[50] including gestational length, fetal sex, maternal nutrition and weight, maternal height, maternal age, gravidity, tobacco smoking, substance abuse, socioeconomic status, and a range of viral, bacterial, and parasitic infections.[41, 49]

Most studies showed the relationship between teenage pregnancy women and adverse pregnancy outcome as low birth weight, this group of women has the highest risk of delivering an low birth weight infant.[32, 54, 55]

As noted above, malaria is independently associated with a lower birth weight, which is progressively lower with increasing density of Plasmodium falciparum malaria.[41] In regions of Africa, where malaria is endemic, it is estimated that about 100,000 infants die of low birth weight due to malaria during pregnancy.[42, 56]

Prevention and control of malaria in pregnant women will contribute immensely to improving the outcomes of pregnancy and the health of mothers and newborns.[17] Reducing the risk of low birth weight is considered the most important public health gain achieved by SP-IPTp, given the strong correlation of low birth weight with neonatal and infant mortality.

The impact of SP-IPTp on birth weight is likely to be influenced by a number of factors, including malaria transmission intensity, number and timing of SP-IPTp doses administered, DHFR / DHPS mutation prevalence and maternal HIV-status.

Maternal nutritional deficiencies of protein, folic acid, vitamin A, B, C, zinc, calcium, magnesium, copper or selenium may all lead to intra uterine growth retardation. Eating disorders are among the many reasons for malnutrition that are associated with intra uterine growth retardation;[49] an Australian retrospective case-control study found that 30% of women with babies born with low birth weight suffered from eating disorders.[57]

Low maternal level of insulin can lead to decreased fetal growth, since there will be a decrease in the absorption and utilization of nutrients by the fetus.[49]

Maternal anaemia increases the risk of low birth weight.[58] Anaemia is highly associated with under-nutrition, and an insufficient maternal caloric intake will produce intra uterine growth retardation. Thus anaemia, may merely be a sign of poor maternal nutrition.[50]

In an study conducted in Varanasi district in India, pregnant women who received supplementation of 60 mg ferrous sulphate combined with 500 mcg folic acid, had a lower birth weight incidence of 20.4% compared to control group of 37.9%.[59]

Maternal age has a correlation with birth weight. Women aged below than 20 year olds are more likely to have babies with low birth weight.[60]

Maternal weight and height and midupper arm circumference, in a prospective cohort study from 1995 to 1997 in Tanzania, showed a significant association with lower relative odds of low birth weight in univariate analysis, but after adjusted for other covariates, only weigh remained a significant (AOR: 0.96; 95% CI 0.93, 0.99).[59]

Infants born to women who were severely under-weight before pregnancy (BMI <18.5 kg/m²) were at increased risk of intra uterine growth retardation. Compared with a normal maternal BMI, severely under-weight was associated with mean reduction of 219 g in infant birth weight, and 80% increased risk of intra uterine growth retardation (OR 1.8; 95% CI 1.0 – 3.3).[49]

Low socioeconomic status and their correlation with low birth weight are well recognized. Social class, maternal education, and income have been used as individual and household-based socioeconomic status measures in the study of low birth weight.[61] A positive linear relation between the proportion of babies born weighing ≥3500 g and increasing socioeconomic status.[62]

Twins or multiple births are 11 times more likely to have a low birth weight than singleton births (OR=10.08; p<0.001).[63] In twin pregnancies, 15 - 30% are complicated by intra uterine growth retardation and premature delivery.[49]

Male sex in univariate analyses was strongly associated with the lower relative odds of low birth weight but the strength of this association was reduced after adjusted for the other risk factors.[41]

Tobacco smoking produces deleterious effects in both the mother and fetus. Cigarette smoke causes vascular constriction, reduces the utero-placental blood flow and may cause decidual necrosis, increased placenta apoptosis, placental infarcts and consequently growth restriction and even placental abruption and stillbirth.[49] Babies born to mothers who smoke weigh less than babies whose mothers do not smoke.[63]

In a cohort of healthy pregnant Norwegian women studied over an 11-year period found a strong correlation between smoking habits and low birth weight, with 47% of newborn infants of heavy smokers failing in the lowest birth weight quartile.[64]

Maternal drug abuse may induce intra uterine growth retardation either by a direct effect on fetal growth or through inadequate diet, lack of prenatal care, and other socioeconomic factors.[49] Cocaine is a central nervous system stimulant. The vasoconstrictor effects of cocaine can lead to maternal and fetal hypertension, which can cause hemorrhages or placenta infarcts at any time of pregnancy.[65]

Infants born to cocaine abusers are reported to have significantly lower births than infants of controls.[65, 66] However, a prospective multicenter cohort study (1984 to 1989) found that neither cocaine (OR 0.7; 95% CI 0.4 to 1.3), nor marijuana (OR 1.1; 95% CI 0.9 to 1.5) were associated with LBW, while cigarette smoking was positively associated with low birth weight (OR 1.5; 95% CI 1.2 to 1.8).[67] These authors concluded that 15% of all cases of low birth weight in the study could have been prevented if the women had not smoked cigarettes during pregnancy.[67]

Alcohol is a teratogen that causes a broad variety of developmental anomalies, including fetal growth retardation, craniofacial anomalies, poor growth and neurobehavioral function.[68, 69] These multiple defects are known as the fetal alcohol syndrome (FAS).[68, 69]

Alcohol passes freely through the placenta and reaches concentrations in the fetus that are as high as those in the mother. The fetus has limited ability to metabolize alcohol. Alcohol and acetaldehyde can damage developing fetal cells can also impair placenta/fetal blood flow, leading to hypoxia.[69]

Other maternal infections have been associated with low birth weight. Viral infections other than HIV, including Cytomegalovirus (CMV), Herpes simplex virus, varicella and adenovirus may also cause intra uterine growth retardation.[49]

Periodontal diseases represent a previously unrecognized and clinically significant risk factor for preterm labor or premature rupture of membranes.[70]

A systematic review of 25 studies reveled that oral prophylaxis and periodontal treatment may lead to almost a 60% reduction in preterm low birth weight (pooled RR 0.43; 95% CI 0.24 – 0.78).[49]

In a study from Denmark, low birth weight was associated to the severity of maternal chronic regional enterocolitis (OR 2.4; 95% CI 1.6 – 3.7).[49]

Among intestinal parasites species *E. histolytica* and *S. stercolis* were associated with 4.68 (95% CI 1.46, 14.94) and 5.97 (95% CI 1.23, 28.98) times the adjusted relative odds of low birth weight, respectively. The occurrence of any helminthic infection was also associated with higher risk of low birth weight (AOR: 1.85; 95% CI 1.02, 3.35).[41]

Maternal lung and heart disease are associated with intra uterine growth retardation. Observations from Norwegian Birth Registry in 1972 suggested increased risks of preterm birth, low birth weight and neonatal death due to maternal asthma.[49] **Vascular diseases** such as preeclampsia, diabetes mellitus, renal disease or collagen vascular are also causes of intra uterine growth retardation.[49] Severe essential hypertension (diastolic blood pressure >110 mm Hg) before 20 weeks gestation increased the risk of early intra uterine growth retardation and premature delivery.[49] A large population-based study revealed that preeclampsia mean weight by 4.4% overall; in preterm births, mean differences in birth weight ranged from -11% to -23% against near-equal birth weight in term births.[65]

Depression and residing at an elevated altitude are others factors reported to be associated with low birth weight.[71, 72]

2.5 Malaria prevention by insecticides treated bed nets and indoor residual insecticide spraying

Success demonstrated with insecticides treated bed nets(ITN) use during pregnancy to reduce both maternal and infant mortality due to malaria infection provides a powerful prevention approach for Africa.[73] Insecticide treated nets are mosquito nets that repel, disable and / or kill mosquitoes coming into contact with insecticide on the netting material.[74] Insecticides treated bed nets reduce malaria cases by 50% and infant mortality by 18% (range 14 – 29%) in sub-Saharan Africa.[74] The general implication of this is that 5.5 lives could be saved per year for every 1000 children under 5 years of age protected by insecticides treated bed net.[74]

Pregnant women who slept under insecticides treated bed nets have about half (range 39 - 62%) as many clinical episodes of malaria (caused by Plasmodium falciparum and/or Plasmodium vivax infections), a reduce prevalence of high-density peripheral parasitaemia, and a 23% reduction in placental parasitaemia (RR 0.77, CI 0.66 – 0.90).[74] Most importantly, the use of insecticides treated bed nets by pregnant women in Africa increased mean birth weight by 55 g (95% CI 21 – 88), reduced the risk of low birth weight by 23% (RR 0.77, CI 0.61 – 0.98), and reduced miscarriages/stillbirths by 33% (RR 0.67, CI 0.47 – 0.97) in the first few pregnancies.[74, 75] Insecticides treated bed nets use also increases maternal haemoglobin concentration and decreases the risk of abortion and stillbirths in women in their first to fourth pregnancies.[75]

Despite these benefits, coverage rates with bed nets is often too low to confer benefits to the community; in Mozambique, according to National malaria indicator survey conducted in 2007, the coverage with mosquito nets remained low, only 15.8% of all households owned at least one insecticides treated bed net, and about 18.5% of those households with pregnant women and or child under 5 years owned at least one insecticides treated bed net.[12]

Indoor residual insecticide spraying (IRS) is one of the primary vector control interventions for reducing and interrupting malaria transmission.[76] Indoor residual insecticide spraying is the application of long-acting chemical insecticides on the

walls and roofs of houses and domestic animal shelters in a given area, in order to kill the adult vector mosquitoes so as to reduce malaria transmission.[77] Some insecticides (N,N diethyl-m-toluamide (DEET),[34,78] Permethrim,[78]) also repel mosquitoes and by so doing reduce the number of mosquitoes entering into the sprayed room, and thus reduce human-vector contact.[76] Scientific evidence of indoor residual insecticide spraying efficacy in reducing or even interrupting malaria transmission in different epidemiological settings has been available since the 1940s and has confirmed the effectiveness of indoor residual insecticide spraying in reducing levels of infection and incidence of clinical malaria.[76] Evidence confirms that malaria control using indoor residual insecticide spraying has made epidemics less frequent and reduced malaria transmission from hyper to meso-endemicity in tropical Africa and from meso to hypo-endemicity at the southern fringe of transmission.[79] Indoor residual insecticide spraying is a method for community protection, and given its mode of action, the highest possible level of coverage is required to achieve the maximum impact on malaria transmission.[76]

Dichlorodiphenyltrichloroethane (DDT) and its breakdown product dichlorodiphenyldichloroethylene (DDE) pass into breast milk and across the placenta. Although in uterus exposure may be lower than exposure through lactation because of the low solubility of DDT/DDE in plasma, the fetus may be more vulnerable than the infant to the impact of neurotoxins.[80]

In Mozambique, indoor residual insecticide spraying was first introduced in 1945 in the southern part of country. After indoor residual insecticide spraying was introduced, malaria admissions dropped from 16% to 8% in 1947 and to a low of 3% and 1% in 1953 and 1954, respectively.[79] The prevalence of Plasmodium falciparum malaria reduced from 86% to < 5% with annual indoor residual insecticide spraying with DDT during the malaria eradication campaign in 1960s (figure 1).[81] The abandonment of the global malaria eradication programm followed by the escalation of civil war in the late 1970s led to a complete breakdown of malaria control measures.[8] Following the cessation of the war in the 1990s, indoor residual insecticide spraying was re-introduced, but only in the suburban areas of most provincial capitals.[79]

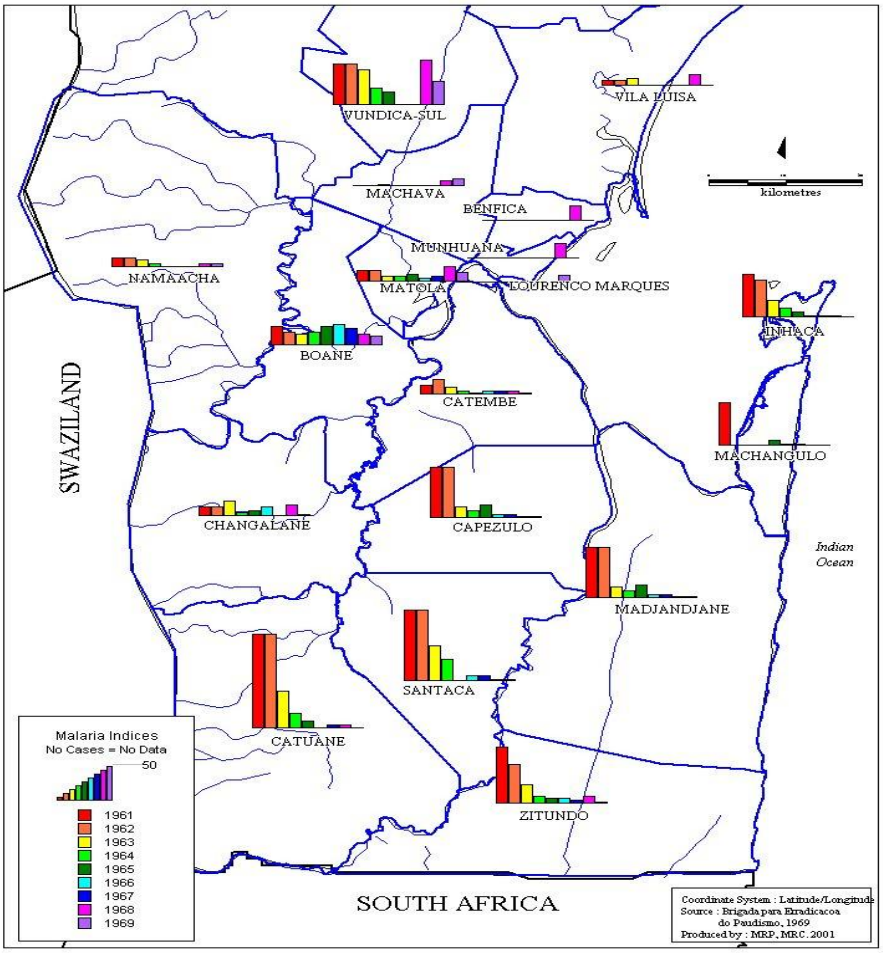

Figure 1: The prevalence of Plasmodium falciparum malaria reduced during the malaria eradication campaign in 1960s.

In 2000, community-based indoor residual insecticide spraying was re-introduced in the rural parts of Maputo province as part of the Lubombo Spatial Development Initiative (LSDI), a trilateral agreement between Mozambique, Swaziland and South Africa aimed at protecting communities in the Lubombo region against malaria in order to create a suitable environment for economic development and promotion of eco-tourism.[79] In Maputo province, bi-annual indoor residual insecticide spraying was introduced incrementally since 2000 across five zones, which comprise an area of approximately 21,000 km², with a population of approximately 800,000, in seven districts.[8] The LSDI regional malaria control initiative has markedly reduced Plasmodium falciparum malaria among children in southern Mozambique, with a reduction in parasite prevalence in all zones with OR of 0.48 (95% CI, 0.42 – 0.56) per intervention per year.[8] Following this success, it was decided to extend the LSDI area to incorporate Gaza province. In Gaza

Province, as was done in Maputo Province, indoor residual insecticide spraying was phased in over a five year period, starting with Zone 4 in 2006.

While SP-IPTp is currently recommended in areas of moderate to high malaria transmission, its role in areas of lower intensity transmission is unclear. Early evidence has shown that the impact of SP-IPTp becomes less apparent in pregnant women in the presence of hight coverage with insecticide treated bed nets.[22] Another study in Western Kenya showed that the effect of IPT with SP during pregnancy was significantly reduced in women who slept under insecticide treated bed nets, both in terms of the effect on placental malaria as well as on the mean birth weight.[36] This might suggest that the benefits of IPTp de crease as the malaria risk decreases. However, there is insufficient data currently available to inform a cutoff bellow which IPTp is no longer effective / cost effective.

2.6 Summary of socio-economic and demographic data

2.6.1 Mozambique

Mozambique is located on the eastern coast of Southern Africa, between the parallels 10º 27´ and 26º 52´south latitude and 30º 12´and 40º 51´longitude East (Figure 2: Map of Mozambique). The country has a coastline of over 2700 Km; the land surface is 799 390 Km², embraces rainforest and mountains, flat and arid terrains, marshlands, valleys, lakes, rivers crossing the country from the mountains in the west into the Indian Ocean in the east, and coastal swamps.

According to the preliminarily 2007 general population census, total population of Mozambique is about 20.5 million people. This represents an average annual growth of 32.4% compared with the 1997 census. However the full data from the census in 2007 are not yet available.

In 1997, the total population was about 16.1 million people with about 44.5% under 15 years old, and the overall fertility rate was 5.6.[82] About 13.4% of all births in the country were to adolescent mothers and 28.9% of adolescent women (15 to 19 years old) had at least one child.[82]

According to the demographic health survey (DHS) conducted in 2003, only 18% of pregnant women attended their first antenatal clinic when they were at less than four months of gestation and the majority first attended when four to seven months pregnant.[63] Most of the pregnant women (53.1%) attended antenatal clinics four or more times, 27.9% attended two to three times, 3% attended only once and 14.9% never attended an antenatal clinic.[83]

According to the report on the millennium development goals (MDG 2010), the average annual mortality rate under five year olds, increased to 2.3 per cent during the period of 2000 - 2008, compared with 1.4 per cent in the 1990s. Mozambique has seen an absolute reduction of more than 100 per 1000 live births since 1990.[84]

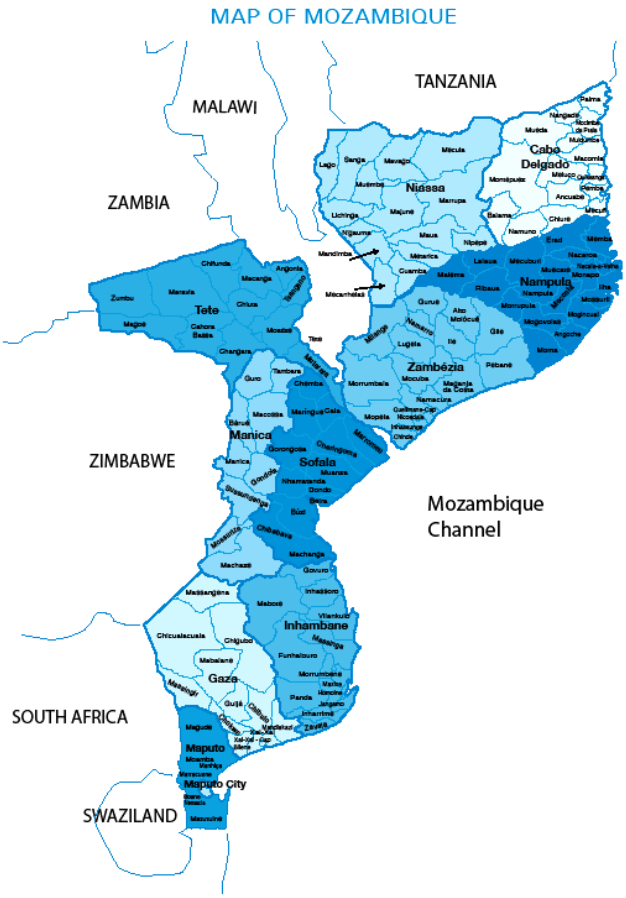

Figure 2: Map of Mozambique

2.6.2 Maputo province

The size, structure and population growth are the three basic variables used to describe the demographic characteristics of a region. In 1997, Maputo province had 806.2 thousand people, with 62.7% living in urban areas. Matola city is the district with the largest proportion of the province´s population, 52.7%.[82]

Tabela 1: Population distribution by sex and by district, Maputo province, 1997

Districts of Maputo Province	Male		Female		Total	
	N $X(10^3)$	%	N $X(10^3)$	%	N $X(10^3)$	%
Matola City	204.0	53.7	220.4	51.7	424.9	52.7
Boane	26.6	7.0	29.8	7.0	56.4	7.0
Magude	18.2	4.8	24.7	5.8	42.7	5.3
Manhiça*	57.7	15.2	72.5	17.0	130.6	16.2
Marracuena	19.7	5.2	21.7	5.1	41.9	5.2
Matutuíne	17.5	4.6	17.9	4.2	35.5	4.4
Moamba	20.5	5.4	23.0	5.4	43.5	5.4
Namaacha	15.6	4.1	15.8	3.7	31.4	3.9
Total	379.8	100	426.4	100	806.2	100

* Not an LSDI intervention or survey site, so not included in this study.

The province's population was predominantly young, with 42.1% under the age of 15 years. The annual average population growth rate was 0.8%, well below that of the country.[82]

According to a demographic and health survey conducted in 2003, most (77.2%) of the pregnant women in Maputo Province attended antenatal care four or more times, 19.9% attended two or three times, 0.7% attended only once and 0.1% never attended antenatal care.[70] The time that the women start their first antenatal care varies, but only 16.8% started at less than four month gestation.[83]

Early motherhood was common in both urban and in rural areas, with 24.2% of adolescent women (15 to 19 years old) having had at least one child.[83]

According to the same survey (2003), 12.5% of births in Maputo province occurred at home, 84.8% occurred in the private hospital and only 0.6% occurred in a public health facility.[83] Regarding to the type of care received during delivery in this province, about 10.7% of births were assisted by a relative of the mother, 75.3% were assisted by a nurse or midwife, 9.9% by a doctor and 3.7% had no assistance.[83]

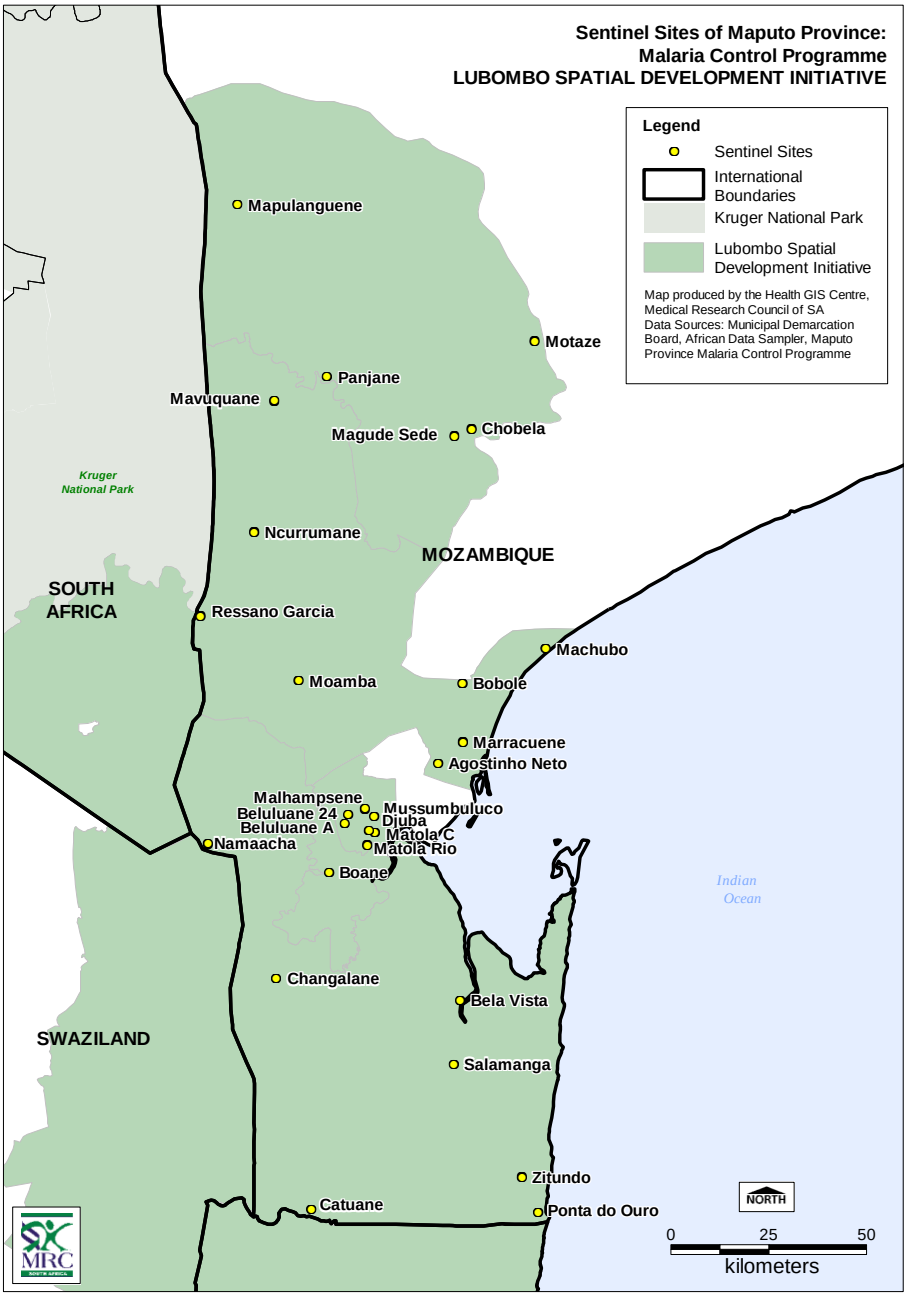

Figure 3: Geographical location of study sites, Maputo province

2.6.3 Gaza province

In 1997, Gaza province had a population of 1.1 million people, with 24.7% living in urban areas. Most of the population was concentrated in the following six districts: Chokwe, Xai-Xai, Chibuto, Manjacaze, Bilene Macia and Xai-Xai city (Table 2).[82] The districts in the northern half of Gaza Province are very sparsely populated.

Table 2: Population distribution by sex and by district, Gaza province, 1997

Districts of Gaza Province	Male		Female		Total	
	N $X(10^3)$	%	N $X(10^3)$	%	N $X(10^3)$	%
Xai-Xai City	45.7	10.0	53.9	8.9	99.9	9.4
Bilene Macia	56.7	12.4	76.3	12.6	132.8	12.5
Chibuto	69.0	15.1	95.7	15.8	164.7	15.5
Chokwe	74.5	16.3	98.7	16.3	173.2	16.3
Guijá	24.7	5.4	32.7	5.4	47.4	5.4
Manjacaze	68.5	15.0	92.6	15.3	161.5	15.2
Massingir	10.1	2.2	12.1	2.0	22.3	2.1
Xai-Xai	71.3	15.6	94.5	15.6	165.7	15.6
Mabalane*	11.0	2.4	14.5	2.4	25.5	2.4
Chicualacuala*	14.6	3.2	18.8	3.1	32.9	3.1
Chigubo*	5.5	1.2	7.9	1.3	13.8	1.3
Massangena*	5.5	1.2	7.9	1.3	13.8	1.3
Total	456.9	100.0	605.5	100.0	1,062.4	100.0

* Not an LSDI intervention or survey site, so not included in this study.

The province's population was predominantly young, with 43.9% under the age of 15 years. The average annual population growth rate was 0.5%, well below the national average.[82]

According to the demographic and health survey conducted in 2003, most (65.7%) of the pregnant women attended more than four antenatal care visits, 28.3% attended two or three times, 1.6% attended only once and 2.5% never attended antenatal care.[83] The time that the women started their first antenatal care visit varied, but 17.8% started before four months gestation.[83]

Early motherhood was common in both urban and in rural areas, with 31.5% of adolescent women (15 to 19 years old) having had at least one child.[83]

According to the same survey (2003), 35.2% of births in Gaza province occurred at home, 62.6% occurred in the private hospital and only 0.6% occurred in a public health facility.[83] Regarding to the type of care provided at delivery in this province, about 31.2% of births were assisted by a relative of the mother, 58.5% were assisted by a nurse or midwife, 2.1% by a doctor and 2.8% had no assistance.[83]

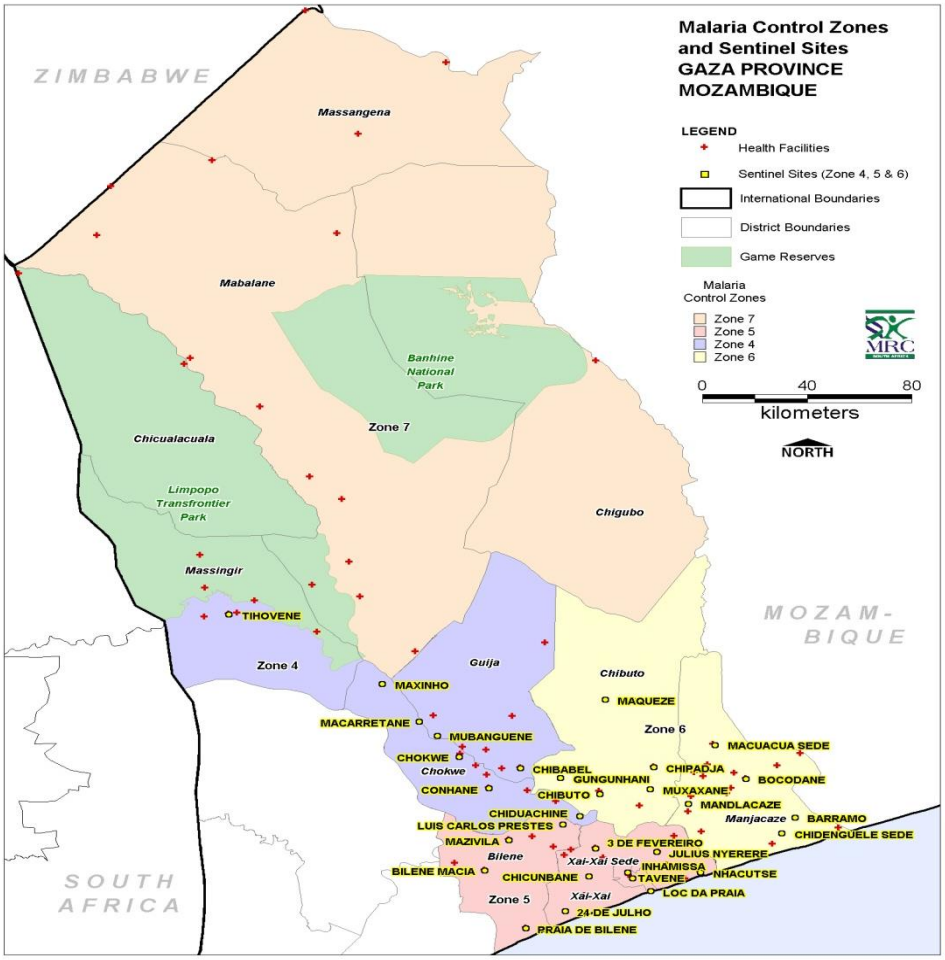

Figure 4: Geographical location of study sites, Gaza province

2.7 Description of the National Health System

The National Health system (NHS) of Mozambique is organized into three different levels, namely the National Level, the Provincial Level and the District Level. The national level is responsible for policy design, planning and negotiation with bilateral and multilateral agencies. Almost 50% of the budget supporting the NHS is covered by the donors, but the budget is insufficient for even just providing primary health care to the entire population. The provincial level is responsible for

the translation of the centrally planned policies into activities in the provinces, and for close supervision of the districts. At the district level the activities are implemented as this is where the HFs is located.

Because of cultural reasons and lack of confidence in the public health system, in Mozambique the majority of births takes place in a private health facility or at home.

a) Cultural reasons:
    a. Women depend on their mothers in law for decision making, and in most of the communities they have more confidence in delivering at home
    b. Myths and taboos in relation to some cultural practices soon after birth regarding the mother and the newborn.
b) Lack of confidence in the public health system:
    a. There is a perception that the public health personnel are mistreating patients
    b. Lack of medicines in the public health facilities
    c. Poor access due to an inadequate health facility network Lack of health personnel with capacity in rural areas, there is only one midwife and no one doctor.

2.8 Rationale for the study

Malaria is a major cause of morbidity and mortality in tropical countries, and particularly in Mozambique. Recently substantial resources have been used to reduce the burden of malaria in Mozambique. These include the distribution of insecticide treated bed nets, indoor residual insecticide spraying, access to artemisinin-based combination treatment (ACT), and intermittent preventive treatment of pregnant women with sulfadoxine-pyrimethamine (SP-IPTp). The most important benefit of SP-IPTp in malaria endemic areas has been the increase in birth weight, thus increasing the probability of child survival.[76] The SP-IPTp policy was based on evidence of its effectiveness in areas of high intensity malaria transmission.[76] The effect of SP-IPTp has been less evident in the presence of high coverage with insecticide treated bed-nets.[44] It is not known whether reducing the

risk of malaria through effective vector control using Indoor Residual Insecticide Spraying and large-scale deployment of artemisinin-based combination treatment has a similar effect in reducing the impact of SP-IPTp on birth weight. At the same time, increasing resistance to SP could be compromising the effect of SP-IPTp on birth weight, as could co-infection with HIV. This study aims to explore the effect of these factors on the impact of SP-IPTp on birth weight in southern Mozambique.

The study was designed to assess if the impact of sulphadoxine-pyrimethamine intermittent preventive treatment is influenced by the prevalence of falciparum malaria in reducing low birth weight. Maputo and Gaza provinces have a lower prevalence of malaria as compared to the other provinces in Mozambique. This is due to the high coverage of vector control interventions (nets and indoor residual spraying) in these provinces. This study was to see if there was any added value in providing IPT to pregnant women in the context of high coverage of vector control interventions and if SP had any impact on birth weight. Birth weight was chosen as an outcome because malaria is one of the contributing factors to low birth weight. Previously SP was used as first line malaria treatment but the policy later changed due to the resistance observed in many countries in Sub-Saharan Africa and SP was recommended for malaria prophylaxis in pregnant women.

The results of the study will be useful in demonstrating that sulphadoxine-pyrimethamine intermittent preventive treatment led to improved birth weight. This calls for a need to revise the policy and once the study demonstrates that SP influences for low birth weight will be need for changes in policies on the use of SP as IPTp to improve the rates of low birth weight and provincials through the National Malaria Control program would benefit from improvements on the prevention malaria in pregnant women as well on the newborn outcomes.

STUDY OBJECTIVES AND HYPOTHESIS

1. AIM

The aim of this study was to determine if the effect of intermittent preventive treatment in pregnant women (IPTp) with Sulfadoxine-Pyrimethamine (SP) on the risk of low birth weight is modified by Plasmodium falciparum malaria prevalence. To achieve this aim the following outcomes were evaluated:

2. PRIMARY OBJECTIVE

- To determine the influence of P. falciparum prevalence on the effect of SP-IPTp on the risk of low birth weight, adjusting for potential confounders.

3. SECONDARY OBJECTIVES

- To determine the influence of number (and timing) of SP-IPTp doses on the risk of low birth weight, adjusting for potential confounders.

- To determine the influence of the HIV-status on the effect of SP-IPTp on the risk of low birth weight, adjusting for potential confounders.

- To determine the influence of Plasmodium falciparum dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS) mutations on the effect of SP-IPTp on the risk of low birth weight, adjusting for potential confounders.

4. HYPOTHESIS

4.1 *Null Hypothesis*

The effect of intermittent preventive treatment with sulfadoxine-pyrimethamine (SP-IPTp) is not influenced by the prevalence of *falciparum* malaria.

4.2 *Alternative Hypothesis*

The effect of intermittent preventive treatment with sulfadoxine-pyrimethamine SP-IPTp) is altered by a decrease in the prevalence of *falciparum* malaria.

MATERIAL AND METHODS

Although used earlier in some areas, the policy of SP for IPTp was implemented nationally in Mozambique in mid 2006 for all women attending antenatal care, which is provided primarily through public sector health centers and health posts. Three tables of SP (Fansidar®, Roche, Gauteng, South Africa, 500mg/25mg each tablet) are given at the first antenatal visits after 20 weeks gestation and then one dose per month is given until a maximum of three doses.

1. STUDY DESIGN

This study was a retrospective antenatal record review, comparing birth weights among women who did and did not receive SP-IPT in public sector health facilities in selected sentinel sites in Maputo and Gaza provinces in southern Mozambique. These two provinces were selected as those where the LSDI program had conducted a phased implementation of intensive malaria control interventions (since 2001 in Maputo Province and since 2007 in Gaza Province).

2. INCLUSION AND EXCLUSION CRITERIA

All available antenatal records from January 2005 to December 2007 were collected from the health facilities within each selected sentinel site. Heath facilities with fewer than 140 deliveries during the study period, and any antenatal records without birth weight, were excluded from the study.

3. DEFINITIONS

New born weight:

- Normal Birth Weight (NBW) – New born weighing more or equal to 2500 grams, for newborn twins, normal birth weight was classified as both twins weighing more than or equal to 2500 grams.

- Low Birth Weight (LBW):
  - Singleton - New born weighing less than 2500 grams but greater or equal to 2000 grams.
  - Twins:
    - LBW one - at least one twin weighing less than 2500 grams but greater or equal to 2000 grams.
    - LBW both - both twins weighing less than 2500 grams but greater or equal to 2000 grams.
- Very Low Birth Weight (VLBW):
  - Singleton - New born weighing less than 2000 grams.
  - Twins:
    - VLBW one - at least one of the twins weighing less than 2000 grams.
    - VLBW both - both twins weighing less than 2000 grams.

Preterm - Any delivery that occurred before 37 weeks gestation.

Congenital Anomaly - any record that a congenital anomaly was observed on the newborn; the type of congenital anomaly is not usually specified.

Abortion – Any stillborn delivery before 28 weeks gestation.

Maternal age:

- Adolescent – any woman aged less than 20 years.
- Adult – any woman aged 20 or more years.

Gestational age:

- Primigravidae - any woma who is pregnant for the first time.
- Secondigravidae - any woman who is pregnant for the second time.
- Multiparum – any pregnant woman with more than two previous pregnancies.

High blood pressure – any blood pressure where the systolic blood pressure was greater than or equal to 140 mmHg and / or diastolic blood pressure was greater than or equal to 90 mmHg.

HIV Positive - Any women whose antenatal record indicated participation in the mother-to-child- transmission (MTCT) programme.

Anaemic women – any women with anaemia by pallor and or haemoglobin less than or equal to 10 g/dl.

4. DATA COLLECTION AND MANAGEMENT

For data collection, six data collectors were trained over three days, and the data were collected using Personal Digital Assistants (PDAs). The data collectors were divided into two teams, each with three people and one supervisor; one team collected data in Maputo province while the other collected data in Gaza province.

All pregnant women have an antenatal care card in which all the information regarding the current pregnancy and the newborn is recorded. This card is kept by

the pregnant woman during pregnancy. After delivery, the forms are stored in the health facility. These cards are not duplicated.

The process of data recording has shortcomings. Data may not be recorded or stored adequately.

The team of data collectors arranged prenatal records in boxes by month then by year and then separated the antenatal records from other documents. All boxes were clearly labeled with markers with referencing on what type of document existed in the box done.

The collection of data was based on antenatal records in 53 antenatal care facilities, selected by their location within the 38 LSDI sentinel sites (Figures 3 and 4).

All LSDI sentinel sites are public health facilities in the National Health System and are no different compared to other public health facilities that are not LSDI sentinel sites. We do not have the data on the population covered by each health facility or each sentinel site, but tables 1 and 2 shows the population distribution in each district of both provinces.

5. SAMPLE SIZE CALCULATION

Sample size was calculated based on the following assumptions:

- That 12.5% of infants have birth weights lower than 2.5 Kg in the absence of IPTp and Indoor residual insecticide spraying.[82]

- In areas with a high prevalence of Plasmodium *falciparum* the proportion of low birth weight will be reduced to 9.5% in mothers that have been given 2 or more doses of SP-IPTp and that in areas of low Plasmodium *falciparum* prevalence, SP-IPTp has not had a significant difference on the proportion of low birth weight.

At least 1700 deliveries would be needed in each strata of malaria prevalence, (850 per group, IPTp vs no IPTp). In healthcare facilities with fewer deliveries during the study period, all eligible deliveries were included. However, sites with fewer than 140 deliveries during this period were excluded from this study. Given uncertainty of these estimates and multi-factorial determinants of both birth weight and IPTp impact, it was decided to include all eligible records at all LSDI sentinel site (53-56 antenatal care facilities) in this study.

6. DATA MANAGEMENT AND ANALYSIS

Following pilot testing data were collected from the antenatal records directly onto personal digital assistants (PDAs). Data were downloaded daily into a MS Access (2003) relational database on two laptops, one in Maputo province and the other in Gaza province. At the end of data collection, these two provincial data sets were merged into a single database for tabulation and analysis. Data were analyzed using STATA (Intercooled version 10; Stata Corp., College Station TX).

Data on asexual parasite prevalence and the prevalence of dhfr / dhps mutations were collected annually from the 43 LSDI sentinel sites, these values were constant for all women attending antenatal care within each sentinel site that year. Similarly indoor residual insecticide spraying coverage and insecticide treated bed nets coverage were constant by sentinel site and year.

Indoor residual insecticide spraying coverage data were collected at the LSDI offices in Maputo and in Gaza provinces and these data are matched against sentinel site sprayed, while insecticide treated bed nets coverage data were collected from the Provincial Health Directorate in Gaza province and these are matched against bed nets distributed to pregnant women at ante natal care visit.

As these variables refer to health facilities or sentinel sites and not to the individual pregnant women, analyses were clustered by sentinel site using linearized variance estimation (command: svyset sentinelsite, vce (linearized) singleunit (missing) in STATA 10).

Continuous variables were mostly non-parametrically distributed therefore they were summarized using median and interquartile range (IQR). Trend tests were used to compare the three years of the study. Categorical variables were compared using the Chi-squared test (Pearsons or Fischer´s exact, as appropriate). Univariate analyses used LR-test to determine predictors and multivariate analyses used logistic regression (svy linearized: logistic in STATA10), to explore the determinants of categorical variables.

Model building proceeded by the step-wise adding of variables with significant univariate associations with birth weight, adding each of the covariates separately. The best model was selected based on the lowest Akaike´s information criteria (AIC). Because most of the variables included missing data, model selection also considered the lowest Bayesian information criteria (BIC) when the numbers of observations were reduced.

To assess whether the effect of SP-IPTp was modified by malaria prevalence or mutation prevalence or both, interaction terms were generated and added to the final multivariate model. $P<0.05$ was deemed statistically significant.

7. ETHICAL CONSIDERATIONS

Ethical clearance was obtained from the Research Ethics Committee of Pretoria University, the University of Cape Town and from the Mozambican National Ethics Committee. Furthermore, consent from the Provincial Directorates of health and the clinics were obtained to access the records. As this study only accessed the patients' routine antenatal records retrospectively, no written or verbal consent was required from the patients. All information obtained during the conduct of study regarding the subject's state of health was regarded as confidential, and the patients' names were not recorded on the PDAs nor in the study database.

RESULT

In this chapter the following results will be presented:

1. Findings per study sentinel site
2. Description the study population
3. Prevalence of low birth weight and its associated risk factors
4. The effects of SP-IPT on:
    a. Effect on birth weight
    b. Effect on malaria in pregnancy
    c. Effect on birth outcomes
    d. Multivariate analysis
        i. The influence of number of SP-IPTp doses on the risk of low birth weight
        ii. The influence of HIV status on the effect of SP-IPTp on the risk of low birth weigh
        iii. The influence of Plasmodium falciparum prevalence and quintuple DHFR / DHPS mutation prevalence n the effect of SP-IPTp on risk of low birth weigh

1. Findings per study sentinel site

The data collected are for women who gave birth at 50 HFs in 48 sentinel sites in 15 districts of Maputo and Gaza provinces, from January 2005 to December 2007. In Gaza province, two pre-selected HFs were unable to collect data: Licilo HF (in the sentinel site of Chikotane), where no babies were delivered as women in that area attend ANC at other HFs, and in the Provincial hospital (in the sentinel site of Tavena). Despite delivering in this hospital, no records for consultations are kept here; instead the mothers seek antenatal or postpartum care at other HFs where their antenatal records are stored.

Of the 50 HF´s where the records were collected, 18.0% (9 / 50) had less than 140 antenatal records over the three-year study period, and according to the protocol, these were excluded from the analysis. In one sentinel site (Chiduachine) there

were two health facilities, thus the analyzed data were from 39 HFs in 38 sentinel sites (14 and 24, Maputo and Gaza provinces). In both provinces the number of antenatal records that were found did not match the number of deliveries for each facility; some HFs had burnt the antenatal records because they thought that they were no longer needed, while in other HFs the antenatal records were damaged by moisture or insects, or were lost during renovations. In the remainder the mothers did not return the antenatal records to the HF.

The prevalence of malaria, *dhfr / dhps* quintuple mutations and of indoor residual insecticide spraying / insecticide treated bed nets coverage study area (Maputo and Gaza provinces) between 2005 and 2007 are summarized in Table 3.

Malaria prevalence and *dhfr* / *dhps* quintuple mutation prevalence data was only available for the 14 sentinel sites in Maputo Province in 2005, as Gaza province only started to measure malaria prevalence in 2006, prior to implementation of indoor residual insecticide spraying in 2007. In Maputo province, indoor residual insecticide spraying coverage decreased from a median of 83% in 2005 to 75% and 74% in 2006 and 2007, respectively (p=0.048). Indoor residual insecticide spraying coverage in Gaza increased from zero until 2006 to a median of 83% in 2007 (p<0.0001).

Maputo province did not have data available regarding the distribution of insecticide treated bed nets during the period under study. As the distribution of insecticide treated bed nets has been very limited in this area, we assumed that the coverage at each sentinel site was zero. Insecticide treated bed nets coverage in Gaza province increased from a median of 20.6% in 2005 to 76.1% in 2006 (p=0.013) but this was not sustained and decreased to 44% in 2007 (p=0.615), Table 3.

Despite the decrease in indoor residual insecticide spraying coverage in Maputo province, we found a statistically significant decrease in malaria prevalence between 2005/2006 (10.5%) and 2007 (3.9%); (p=0.046). In Gaza Province, there was a decrease in median malaria prevalence from 32% to 20% between 2006 and

2007, when indoor residual insecticide spraying was introduced, although this decrease did not achieve statistical significance; Table 3.

Table 3: Sentinel site Plasmodium falciparum malaria and dhfr / dhfs quintuple mutation prevalence, indoor residual insecticide spraying and insecticide treated bed nets coverage, by province and year (2005 - 2007)

		Malaria prevalence			Quintuple mutation prevalence			IRS cover			ITN cover		
		2005	**2006**	**2007**	**2005**	**2006**	**2007**	**2005**	**2006**	**2007**	**2005**	**2006**	**2007**
Maputo	**N**	14	14	14	14	14	14	14	14	14	14	14	14
	Median	8.0	10.5	3.9	13.1	56.0	50.0	82.9	74.9	73.9	NA	NA	NA
	IQR	5.1 - 21.7	4.9 - 19.7	1.7 - 6.5	8.3 - 21.4	46.0 - 66.7	0 - 87.5	78.5 - 84.9	71.7 - 80.7	72.2 - 79.3	NA	NA	NA
	Range	1.6 – 65.0	0.8 - 70.8	0 - 61.6	0 - 33.3	26.5 - 100	0 - 100	58.7 - 95.5	54.1 - 96.6	58.9 - 94.4	NA	NA	NA
	p-value*		0.046			0.004			0.048			-	
		2005	**2006**	**2007**	**2005**	**2006**	**2007**	**2005**	**2006**	**2007**	**2005**	**2006**	**2007**
Gaza	**N**	0	24	24	0	24	24	21	24	24	21	24	24
	Median	NA	32.0	20.0	NA	50.0	63.5	NA	NA	83.1	20.8	76.1	43.7
	IQR	NA	14.0 - 65.5	12.0 - 54.5	NA	39.1 - 67.2	40.1 - 85.0	NA	NA	35.9 - 87.6	0 - 55.6	16.1 - 112.3	22.9 - 87.8
	Range	NA	8.0 – 92.0	2.0 – 87.0	NA	0 - 86.2	0 - 100	NA	NA	0 – 95.0	0 - 98.1	0 - 288.6	0 - 255.5
	p-value*		0.362			0.299			< 0.0001			0.019	

* nptrend test
NA: Data not available

The relationship between malaria prevalence and quintuple mutation prevalence at each study year in all study sites is shown in Figure 5.

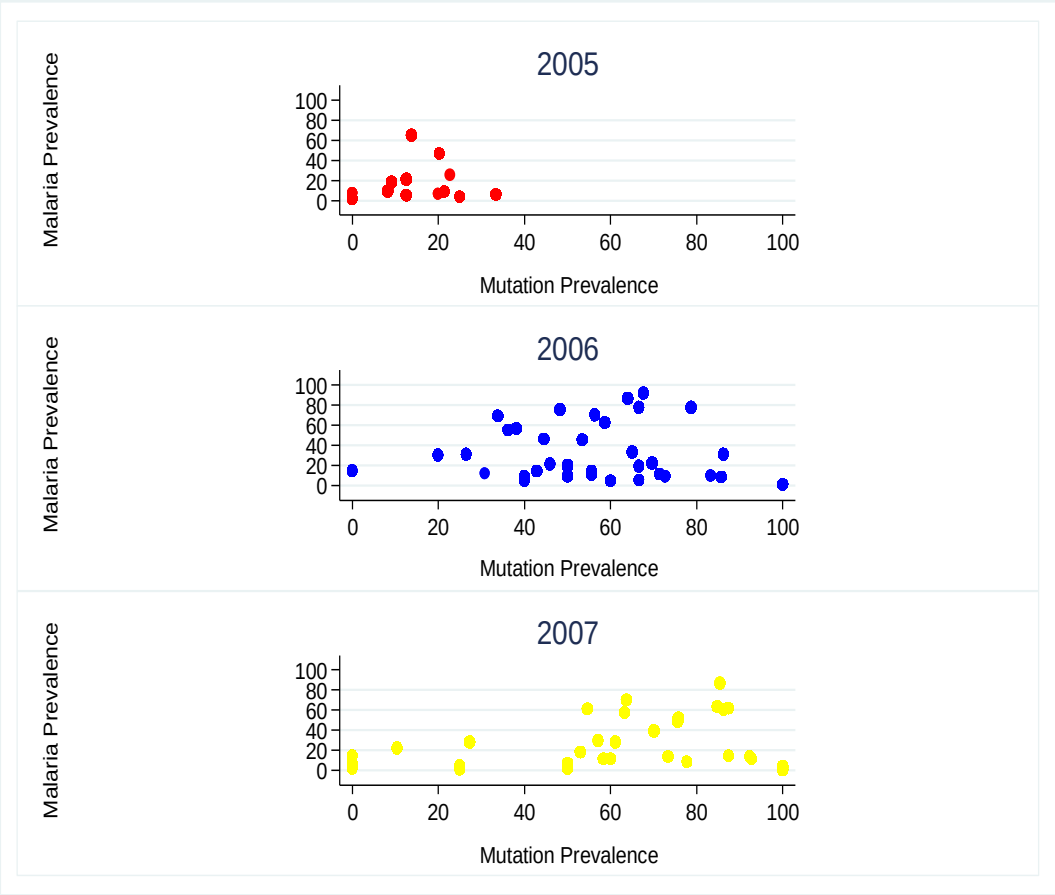

Figure 5: Relationship between malaria prevalence and quintuple mutation prevalence at each study year in all study sites

With respect to the insecticide used for indoor residual insecticide spraying, three different types were used in both provinces: dichlorodiphenyl trichloroethane (DDT), bendiocarb (FICAM) and deltamethrin. Gaza province only introduced indoor residual insecticide spraying in 2007. The various combinations of insecticide used in the different study areas over the study period are summarized in Table 4 (A and B).

Table 4A: Insecticide types used for Indoor Residual spraying (IRS) across the different sentinel sites, by study year

		Both provinces			Maputo			Gaza		
		2005	**2006**	**2007**	**2005**	**2006**	**2007**	**2005**	**2006**	**2007**
Sites sprayed with Ficam only	**N**	14 / 35	3 / 38	6 / 38	14 / 14	3 / 14	5 / 14	0 / 21	0 / 24	1 / 24
	% (95% CI)	40.0 (25.1 – 57.0)	7.9 (2.5 – 22.1)	15.8 (7.2 – 31.3)	100 (-)	21.4 (6.7 – 50.8)	35.7 (15.1 – 63.5)	-	-	4.2 (0.5 – 25.3)
	p-value *		0.003			<0.0001			0.392	
Sites sprayed with DDT only	**N**	0 / 35	2 / 38	0 / 38	0 / 14	2 / 14	0 / 14	0 / 21	0 /24	0 / 24
	% (95% CI)	-	5.3 (1.3 - 19.1)	-	-	14.3 (3.4 – 44.3)	-	-	-	-
	p-value *		0.144			0.130			-	
Sites sprayed with Ficam and DDT	**N**	0 / 35	9 / 38	19 / 38	0 / 14	9 / 14	7 / 14	0 / 21	0 / 24	12 / 24
	% (95% CI)	-	23.7 (12.7 – 39.9)	50.0 (34.4 – 65.6)	-	64.3 (36.5 – 84.9)	50.0 (25.1 - 74.9)	-	-	50.0 (30.6 – 69.4)
	p-value *		<0.0001			0.002			<0.0001	
Sites sprayed with Ficam and Deltametrina	**N**	0 / 35	0 / 38	1 / 38	0 / 14	0 / 14	1 / 14	0 / 21	9 / 24	0 / 24
	% (95% CI)	-	-	2.6 (0.4 – 16.9)	-	-	7.1 (0.9 – 39.1)	-	-	-
	p-value *		0.383			0.368			-	
Sites sprayed with Ficam, DDT and Deltametrina	**N**	0 / 35	0 / 38	5 / 38	0 / 14	0 / 14	1 / 14	0 / 21	0 / 24	4 / 24
	% (95% CI)	-	-	13.2 (5.5 – 28.3)	-	-	7.1 (0.9 – 39.1)	-	-	16.7 (6.2 – 37.6)
	p-value *		0.007			0.368			0.020	
Sites sprayed with DDT and Deltametrina	**N**	0 / 35	0 / 38	1 / 38	0 / 14	0 / 14	0 / 14	0 / 21	0 / 24	1 / 24
	% (95% CI)	-	-	2.6 (0.4 – 16.9)	-	-	-	-	-	4.2 (0.6 – 25.3)
	p-value *		0.383			-			0.392	

*χ^2 test

Table 4B: Insecticide types used for Indoor Residual Spraying (IRS) across the different sentinel sites, by year

		Both provinces			Maputo			Gaza		
		2005	**2006**	**2007**	**2005**	**2006**	**2007**	**2005**	**2006**	**2007**
Sites sprayed with any DDT (alone or combined)	**N**	0 / 35	11 /38	25 / 38	0 / 14	11 / 14	8 / 17	0 / 21	0 / 24	17 / 24
	% (95% CI)	-	29.0 (16.7 – 45.4)	65.8 (49.3 – 79.2)	-	78.6	57.1	-	-	70.8
	p-value *		<0.0001			<0.0001			<0.0001	
Sites sprayed with only non-DDT insecticides	**N**	14 / 35	3 / 38	7 / 38	14 / 14	3 / 14	6 / 14	0 / 21	0 / 24	1 / 24
	% (95% CI)	40.0 (25.1 – 57.0)	7.9 (2.5 – 22.1)	18.4 (8.9 – 34.2)	100	21.4 (6.7 – 50.8)	42.9 (19.9 – 69.3)	-	-	4.2 (0.6 – 25.3)
	p-value *		<0.0001			0.0002			0.3916	
Sites sprayed with Ficam and DDT	**N**	21 / 35	24 / 38	6 / 38	0 / 14	0 / 14	0 / 14	21 / 21	24 / 24	6 / 24
	% (95% CI)	60.0 (43.0 – 74.9)	63.2 (46.7 – 77.0)	15.8 (7.2 – 31.3)	-	-	-	100	100	25.0 (11.4 – 46.2)
	p-value *		<0.0001			-			<0.0001	

* χ^2 test

2. Description of the study population

Overall, 21,304 (12,474 and 8,830, in SP-IPTp and no SP-IPTp groups, respectively) antenatal records were collected, of which 2.1% (437) were withdrawn, corresponding to the HFs that had less than 140 antenatal records over the study period. The remaining 20867 records were included for analysis.

Among these antenatal records, 32.0% (6,685) of the women had three (or more) SP-IPTp doses, 16.4% (3,431) had two doses and 9.8% (2,047) had only one dose, with 8704 not treated with SP-IPTp. The mean (SD) gestational age at the first, second and third SP-IPTp doses was 25 (4), 29 (4) and 32 (3) weeks respectively, with ranges of 11 – 41, 15 – 41 and 19 – 44 weeks.

The baseline characteristics of the mothers, by SP-IPTp treatment group are summarized in Table 5. There were no differences in baseline characteristics of the mothers between treatment groups, except in terms of supplementation of ferrous salt with folic acid, which occurred more commonly in those given IPTp, $p<0.0001$.

Table 5: maternal characteristics, by treatment group and the effect of SP-IPTp, clustering by sentinel site and year

	N	No SP-IPTp	1 + dose SP-IPTp	Unadjusted OR	95% CI	p-value
Women lived in Maputo Province (vs. Gaza Province)	20867	3451/8704 (39.7)	4026 / 12163 (33.1)	0.75	0.29 – 1.93	0.551
Women tested positive for HIV (amount tested)	7160	850 / 1891 (45.0)	1753 / 5269 (33.3)	0.61	0.21 – 1.77	0.361
Adolescent women	20799	2282 / 8666 (26.3)	2983 / 12133 (24.6)	0.912	0.82 – 1.01	0.089
Primiparas women	20834	2453 / 8684 (28.3)	3272 / 12150 (26.9)	0.94	0.81 – 1.08	0.366
Number of antenatal visita Mean (IQR)	20833	4 (3 -5)	4 (3 – 5)	1.02	0.95 – 1.09	0.568
Anaemic women (Hg <10g/dL / pallor)	8285	521 / 3384 (15.4)	750 / 4901 (15.3)	0.99	0.66 – 1.50	0.973
Iron / folate supplementation	20867	8106 / 8704 (93.1)	11933 / 12163 (98.1)	3.83	2.0 – 7.33	<0.0001
Hypertension (among tested)	17682	90 / 6960 (1-3)	287 / 10722 (2.7)	2.10	0.93 – 4.75	0.075
Resident in rural vs. urban and periurban area	20867	4086 / 8704 (46.9)	5284 / 12163 (43.4)	0.87	0.36 – 2.09	0.750
Women delivered out of maternity	20798	796 / 8675 (9.2)	996 / 12123 (8.2)	0.89	0.46 – 1.73	0.720

3. Prevalence of low birth weight and its associated risk factors

The mean (SD) birth weight for live singleton newborns was 3051.4 (476.6) grams, ranging between 500 to 5000 grams. The birth weight was categorized in low birth weight (LBW < 2500 grams and >= 2000 grams), very low birth weight (VLBW less than 2000 grams) and normal birth weight (NBW >=2500 grams).

Table 6 shows the association of maternal characteristics with categorical birth weight outcomes, low birth weight (LBW, Table 6A) and very low birth weight (VLBW, Table 6B) for singletons; data on twins was deemed insufficient for an informative analysis of factors associated with birth weight.

There was no evidence of a difference in birth weight between women who delivered in **Maputo or in Gaza Province**. Compared with women who delivered in periurban and urban areas, women who delivered in a **rural area** had 33% lower odds of having a singleton newborn with low birth weight (OR=0.67; 95% CI 0.53 – 0.84; p=0.001) 44% lower odds of having a singleton newborn with very low birth weight (OR=0.54; 95% CI 0.39 – 0.74; p<0.0001), table 6.

Table 6A: Association of maternal characteristics with low birth weight

	Nº observation		LBW N / N (%)	OR	95% CI	p-value
Province	20733	Maputo	691 / 1816 (38.1)	1.10	0.86 – 1.42	0.440
		Gaza	1125 / 1816 (62.0)			
Delivery	20664	Community	228 / 1816 (12.6)	1.61	1.33 – 1.94	<0.0001
		Health facility	1582 / 1810 (87.4)			
Parity	20700	Primiparous	829 / 1814 (45.7)	2.41	2.13 – 2.74	<0.0001
		Secundiparous /Multiparus	985 / 1814 (54.3)			
HIV status (among tested)	7112	HIV Positive	294 / 710 (41.4)	1.27	1.08 – 1.49	0.003
		HIV Negative	416 / 710 (58.6)			
Hypertension (among tested)	17583	Hypertensive	46 / 1526 (3.0)	1.52	1.14 – 2.04	0.005
		Normotensive	1480 / 1526 (97.0)			
Anaemia	8236	Anaemia	145 / 803 (18.1)	1.25	1.02 – 1.52	0.030
		Not anaemic	658 / 803 (81.9)			
Iron folate supplement	20733	Iron-folate	1754 / 1816 (96.6)	1.17	0.85 – 1.62	0.328
		No supplement	62 / 1816 (3.4)			
Area of residence	20733	Rural	653 / 1816 (36.0)	0.66	0.53 – 0.84	0.001
		Peri-urban / Urban	1163 / 1816 (64.0)			
Malaria in pregnancy	207333	Malaria in pregnancy	90 / 1816 (5.0)	1.30	1.00 – 1.68	0.053
		No malaria	1726 / 1816 (95.0)			
New born sex	20390	Male	788 / 1775 (44.4)	0.76	0.70 – 0.84	<0.0001
		Female	987 / 1775 (49.4)			

Table 6B: Association of maternal characteristics with very low birth weight

	Nº observation		VLBW N / N (%)	OR	95% CI	p-value
Province	20733	Maputo	114 / 306 (37.3)	1.06	0.73 – 1.54	0.766
		Gaza	192 / 306 (62.8)			
Delivery	20664	Community	40 / 306 (13.1)	1.61	1.19 – 2.17	0.002
		Health facility	266 / 306 (86.9)			
Parity	20700	Primiparous	139 / 305 (45.6)	2.23	1.69 – 2.93	<0.0001
		Secundiparous /Multiparus	166 / 305 (54.4)			
HIV status (among tested)	7112	HIV Positive	65 / 135 (48.2)	1.65	1.14 – 2.38	0.008
		HIV Negative	70 / 135 (51.9)			
Hypertension (among tested)	17583	Hypertensive	8 / 251 (3.2)	1.56	0.76 – 3.19	0.224
		Normotensive	243 / 251 (96.8)			
Anaemia	8236	Anaemia	33 / 148 (22.3)	1.60	1.13 – 2.28	0.009
		Not anaemic	115 / 148 (77.7)			
Iron folate supplement	20733	Iron-folate	292 / 306 (95.4)	0.85	0.40 – 1.82	0.676
		No supplement	14 / 306 (4.6)			
Area of residence	20733	Rural	94 / 306 (30.7)	0.54	0.39 – 0.74	<0.0001
		Peri-urban / Urban	212 / 306 (69.3)			
Malaria in pregnancy	207333	Malaria in pregnancy	20 / 306 (6.5)	1.71	1.10 – 2.66	0.018
		No malaria	286 / 306 (93.5)			
New born sex	20390	Male	131 / 301 (43.5)	0.75	0.60 – 0.94	0.012
		Female	170 / 301 (56.5)			

Women that **delivered in the community** had 1.61 times the odds of having a singleton newborn with low birth weight, (OR=1.61; 95% CI 1.33 – 1.94; p<0.0001) or low very birth weight, (OR=1.61; 95% CI 1.19 – 2.17; p=0.002). **Adolescent women** had double the odds of having a singleton newborn with low birth weight (OR=2.11; 95% CI 1.89 – 2.34; p<0.0001) or very low birth weight (OR=2.02; 95% CI 1.62 – 2.52; p<0.0001), compared with adult women. When compared with secundiparous and multiparous women, **primiparous women** had 2.41 times the odds of having a singleton newborn with low birth weight (OR=2.41; 95% CI 2.13 – 2.74; p<0.0001) and 2.23 times the odds of having very low birth weight, (OR=2.23; 95% CI 1.69 – 2.93; p<0.0001), table 6.

Among the 7112 (34.3%) women who were tested for HIV, the odds of **HIV positive** women having a singleton low birth weight newborn was 1.27 times higher when compared with those whose HIV test result was negative, (OR=1.27; 95% CI 1.09 – 1.49; p=0.003) and 1.65 times higher for having a newborn with very low birth weight, (OR=1.65; 95% CI 1.14 – 2.38; p=0.008).

Among women that had their blood pressure measured (n = 17,583), those with **high blood pressure**, had 1.52 times the odds of having a singleton newborn with low birth weight (OR=1.52; 95% CI 1.14 to 2.05; p=0.005). **Anaemic women** (defined as any women with anaemia by pallor or haemoglobin less than or equal to 10 g/dL) had 1.25 and 1.6 times the odds of having a singleton born with low birth weight (OR=1.25; 95% CI 1.02 to 1.52; p=0.030) and very low birth weight (OR=1.60; 95% CI 1.13 to 2.28; p=0.009), respectively compared with those without anaemia. Supplementation of **iron and folate** had no statistically significant effect on birth weight; Table 6.

The number of antenatal care visits that the mothers attended during pregnancy was associated with the risk of low birth weight. The number of antenatal visits varied between zero and fourteen, with the mean (SD) of 4.2 (1.8). The odds of delivering a baby with low birth weight was reduced by 16.0% (OR=0.84; 95% CI 0.81 – 0.87), and the odds of delivering a very low birth weight baby reduced by 37% (OR=0.63; 95% CI 0.58 – 0.69), for each antenatal care visit that mothers attended (p<0.0001 for both).

Male sex newborns had 24% higher odds of having low birth weight (OR=0.76; 95% CI 0.70 – 0.84; p<0.0001) and 25% higher odds of having very low birth weight (OR=0.75; 95% CI 0.60 – 0.94; p=0.12) compared with female sex newborn; Table 6.

Women with **malaria during pregnancy** (n= 831) had 1.71 times the odds of having a singleton newborn with very low birth weight (OR=1.71; 95% CI 1.10 – 2.66; p=0.018) compared with women that did not have any malaria episodes during pregnancy. The effect of malaria in pregnancy on the risk of low birth weight did not reach statistical significance (OR=1.30; 95% CI 1.0 – 1.68; p=0.053).

4. The effects of SP-IPTp

Overall, of the 20,733 singleton births, 58.3% (12083) mothers received at least one dose of SP – IPTp, with 32.0% (6,685) of women having three (or more) SP – IPTp dose, 16.4% (3,431) having two doses and 9.8% (2,047) having had only one dose. 8,650 (41.7%) of the mothers received no SP-IPTp.

4.1 The effects on birth weight

Table 7 shows the number and proportion of singleton low birth weight and very low birth weight newborns, by treatment group, after adjusting for clustering by sentinel site and year.

Receiving at least one dose of SP-IPTp did not reduce the risk of low birth weight or very low birth weight. The risk of low birth weight did not decrease with each additional SP-IPTp dose (OR=0.95; 95% CI 0.88 – 1.02; p=0.176), the risk of very low birth weight was reduced by 16.0% for each additional dose (OR=0.84; 95% CI 0.74 – 0.95; p=0.005). Without adjusting for covariates, three doses of SP-IPTp was found to decrease in 24% the odds of having a low birth weight (OR=0.76; 95% CI 0.63 – 0.92; p=0.004) and in 54% the odds of having very low birth weight (OR=0.46; 95% CI 0.33 – 0.64; p<0.0001) baby, Table 7.

Table 7: The effect of at least three doses of SP-IPTp on birth weight category for singleton and twins, clustering by sentinel site and year

	n	3 + SP-IPTp n/N (%)	No SP-IPTp n/N (%)	Unadjusted OR	95% CI	pv
Singleton:						
LBW	20733	488 / 6645 (7.3)	756 / 8650 (8.7)	0.76	0.63 – 0.92	0.004
VLBW	20733	55 / 6645 (0.8)	136 / 8650 (1.6)	0.46	0.33 – 0.64	<0.0001
Twins:						
LBW one	133	31 / 40 (77.5)	38 / 54 (70.4)	1.56	0.60 – 4.05	0.352
VLBW one	132	13 / 40 (32.5)	15 / 54 (27.8)	1.29	0.56 – 2.99	0.545
LBW both	132	19 / 40 (47.5)	27 / 54 (50.0)	1.08	0.53 – 2.21	0.836
VLBW both	132	8 / 40 (20.0)	11 / 54 (20.4)	1.03	0.42 – 2.50	0.951

4.2 Effect on malaria in pregnancy

The risk of maternal malaria was halved among women who received at least one dose of SP-IPTp (OR=0.53; 95% CI 0.29 – 0.99; p=0.047). This effect was not increased by the use of additional doses of SP-IPTp (OR=0.72; 95% CI 0.41 – 1.26; p=0.239).

4.3 Effect on birth outcomes

Adverse birth outcomes were generally more prevalent among those given at least one dose of SP-IPTp. While the risk of abortion and preterm births were similar between treatment groups, the odds of a **term stillborn** being delivered was significantly higher in the SP-IPTp group (OR=4.66; 95% CI 1.70 – 12.78; p=0.003), Table 8. There were 298 **preterm** births (< 37 weeks of gestation), the proportion of still births preterm were 5.6% and 2.2% for SP–IPTp and no SP-IPTp groups respectively, although this difference was not statistically significant, (OR=2.64; 95% CI 0.55 – 12.78; p=0.223), Table 8.

Table 8: The frequency of adverse birth outcomes in those receiving at least one dose of SP-IPTp, clustering by sentinel site and year

	N	≥ 1 doses SP-IPTp n/N (%)	No SP-IPTp n/N (%)	Unadjusted OR	95% CI	p-value
Abortion	20636	3 / 12034 (0.02)	2 / 8602 (0.02)	1.07	0.19 – 6.20	0.937
Preterm still birth (among preterm)	298	9 / 161 (5.6)	3 / 137 (2.2)	2.64	0.55 – 12.78	0.223
Term still birth	20619	26 / 12022 (0.2)	4 / 8597 (0.05)	4.66	1.70 – 12.78	0.003
Preterm live birth	20636	11996 / 12034 (99.7)	8593 / 8602 (99.9)	0.33	0.15 – 0.73	0.006
Congenital anomalies (among live birth)	20589	4 / 11996 (0.03)	0 / 8593	-	-	-

Congenital anomalies were recorded for only four singleton newborns and all were in the SP – IPTp group; unfortunately the types of congenital anomaly were not recorded on antenatal cards.

4.4 Multivariable analysis

The multivariate analyses explored the determinants of singletons being born with a weight less than 2500 grams (not separated from very low birth weight), and data were analyzed only in singletons, since the data for twins were insufficient.

When the best model was found, survey analysis was done clustering by sentinel site and year. Covariates were generally dropped if they did not have a significant effect on the risk of low birth weight or effect of SP-IPTp on low birth weight. IRS had no impact on birth weight, but was included together with supplementation of ferrous salt / folic acid, because these changed the results on the effect of the third dose of SP-IPTp.

4.4.1 The influence of number of SP-IPTp doses on the risk of LBW

In areas where HIV infection is prevalent, previous studies have established that at least three-doses of SP-IPTp are needed to reduce the risk of low birth weight, so we decided to use this dose (third dose) to compare with less than three doses of SP-IPTp. This generated a better fitting model than comparing two or more doses of SP-IPTp with fewer.

The best model is presented on Table 9. After adjusting for covariates, women taking at least three doses of SP-IPTp had 15% lower risk of babies being born with low birth weight compared with women receiving less than three doses of SP-IPTp during pregnancy (OR=0.85; 95% CI 0.73 – 1.00; p=0.053).

4.4.2 The influence of HIV status on the effect of SP-IPTp on the risk of low birth weight

Among HIV tested women (n=7112; 34.3%), those who tested HIV positive had 1.41 times the odds of having a baby born with low birth weight compare with those tested negative (OR=1.41; 95% CI 1.31 – 1.76; p=0.002). Women with high blood pressure, among those who had their blood pressure measured (n=17583; 84.8%), had 1.74 times the odds of their baby born with low birth weight (OR=1.71; 95% CI 1.25 – 2.41) and women with at least one episode of malaria during pregnancy had 1.80 times the odds of having a newborn weighing low birth weight (OR=1.80; 95% CI 1.38 – 2.35; p<0.0001), Table 9.

Table 9: Best multivariable model of factors associated with singleton low birth weight

	Adjusted Odds Ratio	95% CI	P-value
At least 3 doses of SP-IPTp	0.85	0.73 – 1.00	0.053
Resident in peri-urban area	1.35	1.00 – 1.82	0.051
Resident in urban area	1.22	0.83 – 1.79	0.303
HIV positive	1.41	1.13 – 1.76	0.002
Gestational hypertension	1.74	1.25 – 2.41	0.001
Clinical malaria in pregnancy	1.80	1.38 – 2.35	<0.0001
Antenatal visit	0.81	0.77 – 0.86	<0.0001
Secundiparous women	0.45	0.36 – 0.55	<0.0001
Multiparous women	0.43	0.37 – 0.49	<0.0001
Adult women	0.82	0.70 – 0.95	0.008
Delivere in community	1.51	1.28 – 1.77	<0.0001
Male newborne	0.79	0.71 – 0.88	<0.0001
P. falciparum malaria prevalence	0.99	0.99 – 1.00	0.068
Prevalence of quintuple *dhfr*/*dhps* mutation	1.00	1.00 – 1.01	0.088
IRS with any DDT	0.89	0.63 – 1.25	0.499
Non DDT IRS	0.79	0.53 – 1.19	0.250

For each additional antenatal care visit, the risk of babies born with low birth weight was reduced by 19% (OR=0.81; 95% CI 0.77 – 0.86; p<0.0001). The odds of low birth weight reduced by 55% (OR=0.45; 95% CI 0.36 – 0.55; p<0.0001) and 57% (OR=0.43;

95% CI 0.37 – 0.49; p<0.0001) in secundiparous and multiparous women, compared with primiparous women. Compared with adolescent mothers, adult women had an 18% lower risk of their babies being born with low birth weight (OR=0.82; 95% CI 0.70 – 0.95; p=0.008). Compared with women who had delivered in a maternity facility, women who had delivered in the community had 1.51 times the odds of their babies being born with low birth weight (OR=1.51; 95% CI 1.28 – 1.77; p<0.0001). The risk of babies born with low birth weight was 21% lower in male compared with female newborns (OR=0.79; 95% CI 0.71 – 0.88; p<0.0001), Table 9.

4.4.3 The influence of P.falciparum prevalence and quintuple dhfr / dhps mutation prevalence on the effect of SP-IPTp on the risk of low birth weight

After adjusting for all other significant covariates, the influence of malaria prevalence and quintuple *dhfr / dhps* mutation prevalence on the effect of SP-IPTp on the risk of low birth weight was complex, because of interactions between these terms. Without adjusting for these interactions, there was unexpectedly a slight trend towards the risk of low birth weight decreasing, with an increase in malaria prevalence, and as quintuple mutation prevalence increased, there was a trend towards an increased risk of LBW (Table 9). To explore the three way interactions between malaria prevalence, mutation prevalence and the effect of SP-IPTp on low birth weight, malaria and mutation prevalence were divided into quartiles. As the effects of the lower and upper quartiles were similar, sites were divided into those below (low prevalence) and above (high prevalence) the median prevalence (26.4% for malaria prevalence, and 48.2% for quintuple mutation prevalence). Once these interaction terms were included, SP-IPTp reduced the risk of babies being born with low birth weight only when both malaria prevalence and quintuple *dhfr / dhps* mutation prevalence are low (OR=0.72; 95% CI 0.51 – 1.00). The effect of SP-IPTp was no longer significant with higher malaria prevalence and / or mutation prevalences; as can been seen from the Table 10, however the confidence intervals of various categories do overlap. A more detailed analysis of these complex inter-relationships is beyond the scope of this Dissertation.

Table 10: The influence of community malaria and dhfr / dhps mutation prevalence on the effect of SP-IPTp on the risk of low birth weight, after adjusting for other covariates

	Low malaria prevalence (<26.4%)	High malaria prevalence (>26.4%)
Low quintuple *dhfr / dhps* mutation prevalence (<48.2%)	0.72 (0.51 - 1.00)	0.98 (0.51 - 1.89)
High quintuple DHFR and DHPS mutation prevalence (>48.2%)	0.78 (0.39 - 1.56)	1.07 (0.39 - 2.94)

DISCUSSION

The study took the form of a retrospective antenatal record review, comparing birth weight among babies of women who did and did not receive SP-IPTp in public sector health facilities in southern Mozambique during their pregnancy. The aim of this study was to determine the influence of *Plasmodium falciparum* malaria prevalence on the effect of SP-IPTp on the risk of low birth weight, adjusting for potential confounders. This study also explored the influence of the number (and timing) of SP-IPTp doses on the risk of low birth weight, the influence of HIV-status on the effect of SP-IPTp on the risk of LBW, and the influence of of Plasmodium *falciparum* dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS) mutations on the effect of SP-IPTp on the risk of low birth weight, adjusting for potential confounders.

1. The effect of SP-IPTp on low birth weight

We found that receiving at least one dose of SP-IPTp does not reduce the risk of low birth weight, but increasing the number of SP-IPTp doses for singleton newborns significantly reduces the risk of low birth weight by 16.0% for each additional dose.

This finding agrees with the results of a study conducted in western Burkina Faso between 2004 and 2006, which showed that by increasing the numbers of SP-IPTp doses, the weight among singleton live-births increased from 2563 g (95% CI 2400 - 2706) with 0 doses to 2723 g (95% CI 2656 - 2789) with one dose and 2899 g (95% CI 2871 - 2928) with two or more doses ($p<0.001$).[37] At least two other studies support our findings – namely that the risk of low birth weight was significantly reduced as the number of SP-IPTp doses increased.[38, 40]

As the timing of administration of SP-IPTp in our study was largely dependent on the timing of the initial antenatal visit, which in turn correlated strongly with the number of antenatal visits and SP-IPTp doses, the effect of timing of SP-IPTp administration on birth weight could not be further explored in this study.

In our study, we found an increased risk of low birth weight/ very low birth weight among HIV-positive women when compared with HIV-negative women. The risk of

low birth weight (very low birth weight) was 1.27 (1.65) times higher in HIV-positive women, and this risk increased to 1.41 times for low birth weight after adjusting for other significant co-variants. Our study did not find any additional benefits for HIV-positive women who took at least three doses of SP-IPTp in relation to the risk of low birth weight (OR=0.90; 95% CI 0.78 – 1.04; p=0.138).

In a study of HIV-seropositive pregnant women in Zambia, those women who received a single-dose of SP-IPTp were significantly more likely to have infants born with low birth weight and lower mean birth weight when compared with those who had more than one dose of SP-IPTp.[43]

2. Possible detrimental effects of SP-IPTp

In relation to pregnancy outcome, we found that women who took at least one dose of SP-IPTp had a 4.7 times higher risk of stillborn babies (95% CI 1.70 – 12.78; p=0.003). This result was not the same as that found in Manhiça district in southern Mozambique, between August 2003 to April 2005, where researchers found that SP-IPTp reduced early neonatal mortality by 61.3% (95% CI 7.4 – 83.8; p=0.024).[29] This discrepancy between studies may be explained by lower prevalence of SP-resistant mutations at the time that the Manhiça study was conducted. It is also probable that in the Manhiça study all the recruited women received a treated bed net, and those who received SP were given at least two doses of SP-IPTp.[29] Also, a more systematic follow up of all birth outcomes took place than was possible in our retrospective antenatal record review.

In our study we had very few reported stillbirths in the antenatal records, possibly because women who have a stillbirth in the community do not return to the health facility for consultation. Similarly, we found an increased risk of abortion in women receiving at least one dose of SP-IPTp. Again our study design may not detect all abortions. A large percentage of women attend antenatal care for the first time after the first trimester of pregnancy when most cases of abortion occur. However, this information bias should not necessarily affect treatment groups differently, so this may not explain the association of adverse birth outcomes associated with SP-IPTp in our study. When SP-IPTp is ineffective, because fewer doses are given or because SP

resistance is prevalent, then the risks may actually outweigh the benefits. SP acts by interrupting the folate synthesis pathway and has been assigned to pregnancy category C by the FDA. Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans. However, the potential benefits may warrant the use of the drug in pregnant women despite the potential risks.

3. The effect of malaria prevalence and DHPS and DHPS mutation prevalence on LBW

In our study, the prevalence of quintuple *dhfr* and *dhps* mutations increased across the study period, despite the prevalence of malaria decreasing. This increased prevalence of resistant strains may have further contributed to the need for: a) at least three doses of SP-IPTp; and b) for women to be resident in communities where there is a low malaria and DHFR/DHPS mutation prevalence for SP-IPTp to be effective. SP reduces placental malaria and thus decreases the risk of low birth weight via competitive action of two enzymes in the parasite´s folate synthesis pathway (sulphadoxine inhibiting DHPS and pyrimethamine inhibiting DHFR). [33]

In this study, we found that the reduction in risk of low birth weight was 28.0% in women who had taken at least three doses of SP-IPTp during pregnancy and who also lived in areas where malaria, DHFR and DHPS prevalence was low compared to the reduction in risk for women who lived in areas where malaria prevalence and/or DHFR and DHPS was high. The lack of effect of fewer than three doses of IPTp was expected, given the high HIV prevalence in pregnant women in Mozambique. Previously published data from areas of high antenatal HIV seroprevalence indicated that one or two doses of SP-IPTp during pregnancy do not have a significant effect in reducing the risk of low birth weight. [47]

4. Effect of other factors on low birth weight

Our study confirms the results found in other studies regarding the risk reduction of 16.0% for low birth weight and 37.1% for low birth weight for each additional antenatal care visit that the mothers attended. Regarding twins, we found that the risk of low birth weight was similarly reduced with each additional antenatal care visit – by 25.0% (at

least one twin with low birth weight), by 29.0% (at least one twin with very low birth weight), and by 46.0% (both twins with very low birth weight).

Most studies show that birth weight has a positive correlation with the frequency of antenatal care visits; women who had no antenatal care visits were found to have a significantly higher incidence of low birth weight.[51, 52]

In our study we found that the risk of low birth weight (very low birth weight) was 1.25 (1.6) times higher in anaemic women (defined as any woman with anaemia by pallor and/or haemoglobin less than or equal to 10 g/dl). Maternal anaemia is a major risk factor for low birth weight.[58] In pregnancy the main cause of anaemia is inadequate nutrition or a lack of certain nutrients that are essential for preventing iron deficiency.

In relation to malaria, in our study the risk of low birth weight (very low birth weight) was 1.30 (1.71) times higher in women who had malaria during pregnancy, and this effect on low birth weight increased when adjusted for other co-variables (OR 1.80). Malaria in pregnancy is a major cause of maternal- and fetal morbidity and mortality.

Adolescent pregnancy is more prevalent among the poorest socio-economic classes and among women with low educational backgrounds. The risk of adolescent pregnancy is both biological and psycho-social. In our multivariate analysis this risk was reduced by 18.0% in adults compared with adolescent women. Many studies have shown that adolescent women (less than 20 years old) have a higher risk of their babies being born with low birth weight compared with adult women. [51, 60]

In our study primiparous women had a 2.41 and 2.33 times higher risk of their babies being low birth weightand very low birth weight respectively. The risk was reduced by 55% and 57% in secondiparous and multiparous women after adjusting for other covariates. For twins, the risk was high (10.83 times) in both twins weighing very low birth weight when the women are primiparus. Primigravidae are at higher risk of having babies with low birth weight, probably because this group of women are more prone to malaria during pregnancy and pre-eclampsia or eclampsia, both of which are predisposing factors for low birth weight.[51]

In Manhiça, southern Mozambique, women who had had four or more pregnancies and who received SP-IPTp had a reduction in the prevalence of low birth weight of their newborns. [29]

Based on univariate analysis, our study showed that high blood pressure results in an increased risk of 1.52 times for delivering singleton very low birth weight newborns compared to normal blood pressure. In our multivariate analysis, the risk of singleton low birth weight was 1.74 times higher if their mothers had high blood pressure during pregnancy. In Nigeria between 2007 and 2008, pregnant women with hypertensive disease accounted for 22.5% of low birth weight babies, followed by malaria in pregnancy 16.0%, anaemia in pregnancy 13.4%, and HIV/AIDS 1.9%.[51]

The risk of women having singleton babies with low birth weight and very low birth weight was 1.61 higher if delivered in the community, and this relative risk was attenuated slightly in the multivariate analysis to 1.51. Many births in the community are risk prone, because the deliveries are not performed according to the recommendations of the WHO. In Mozambique most pregnant women attend antenatal care and receive the necessary information for a safe delivery. However, for cultural reasons there are still many cases of birth in the community, where deliveries are generally performed by the pregnant woman`s family members. There are some trained women at the community level in Mozambique who have received a basic training and delivery kit to do safe deliveries in the community. However, these women are not able to solve certain complications that may arise during labour.

CONCLUSION

The present work represents the culmination of the Master degree course (MsC) in Clinical Epidemiology.

Collecting and storing data in Mozambique is challenging, however the motivation for this study was the fact that malaria remains as a major cause of hospitalization and outpatient consultations, causing high mortality rates principally in children and pregnant women. Malaria in pregnancy is associated with poor perinatal and maternal outcomes (such as maternal anaemia which in turn also contributes to poor perinatal outcomes). In 2007 Mozambique adopted the policy of using SP as an intermittent preventive treatment for malaria during pregnancy as recommended by WHO, despite some therapeutic efficacy studies showing high resistance of SP.

Because of the high and increasing prevalence of SP resistant strains of Plasmodium falciparum, there is a need for Mozambique National Malaria Control Programme (NMCP) and possibly the WHO to review the policy of SP-IPTp and think of other alternatives to reduce the burden of malaria during pregnancy.

The risk of having a newborn with low birth weight in women who have had SP-IPTp is reduced only if women received more than two doses of SP-IPTp, and lived in areas where malaria prevalence and dhfr and dhps mutation prevalences are low. In sites where malaria prevalence and / or dhfr and dhps mutation prevalences are high, no effects of SP-IPTp on the risk of low birth weight are observed, even with three doses. As mentioned before, in these cases the use of bed nets can contribute to improve pregnancy outcomes.

The cause of low birth weight is complex and multifactorial, and these factors may well be inter-related.

HIV-positive women are at greater risk of having newborn with low birth weight, but this effect does not change in women who received SP, even after increasing the number of SP doses. Pregnant women with anaemia, malaria during pregnancy, high

blood pressure, primiparus, and / or adolescent women (less than 20 years old) are more likely to have infants with low birth weight. Deliveries in the community without any assistance from a certified midwife increased the risk of having an LBW infant, as does living in peri-urban areas.

Attending antenatal care reduces the risk of low birth weight, and this benefit increases with the number of antenatal care visits.

What is of concern in our findings is that women who received at least one dose of SP-IPTp are at greater risk of stillbirth and abortions. Given the limitations of our retrospective record review study design in terms of the small sample size of specifically stillbirths and abortions, we feel that further studies within this area are needs to conclusively evaluate the risk-benefit profile of this treatment in pregnancy.

Given these potential harms, and benefits being seen only in one quarter of the pregnant women receiving at least three doses of SP-IPTp (i.e. those residing in communities with malaria and mutation prevalence below the median) and the operational challenges of ensuring that at least three-doses of SP are administered (32.0%), our suggestion is that the Mozambican National Malaria Control Program reconsiders its policy on the use of SP as IPTp. Alternatives for consideration could include other malaria control interventions, such as indoor residual insecticide spraying, insecticide treated bed nets and artemisinin-based combination therapy, which could reduce the malaria burden across the population while simultaneously improving pregnancy outcomes and maternal anaemia. The risk-benefit profile of other long-acting antimalarials could also be evaluated for their use as IPTp.

Our suggestion is for an urgent change in the use of SP as IPTp and the use of bed nets to improve pregnancy outcomes and maternal anaemia.

The results of this study will be presented to local, provincial and central health authorities. Change implementation could happen at district or provincial depending on the policy changes needed.

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