PLOSMODIUM FALCIPARUM MALARIA IN PREGNANCY AND FETAL, NEWBORN, AND MATERNAL OUTCOMES AMONG A COHORT OF PREGNANT WOMEN IN COASTAL KENYA, 2006 - 2009

Elizabeth Mary McClure

A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology, Gillings School of Global Public Health.

Chapel Hill
2013

Approved by:

Steven R. Meshnick, MD, PhD

Anna Maria Siega-Riz, PhD

Michael G. Hudgens, PhD

Carla Hand-Cerami, MD, PhD

Arlene E. Dent, MD, PhD

Abstract

ELIZABETH MARY MCCLURE: *Plasmodium falciparum* malaria in pregnancy and fetal, newborn, and maternal outcomes among a cohort of pregnant women in coastal Kenya, 2006 – 2009
(Under the direction of Steven R Meshnick, MD, PhD)

Plasmodium falciparum malaria in pregnancy causes adverse pregnancy outcomes, most notably reduced birth weight and maternal anemia. Preventive treatment that is safe during pregnancy has been shown to effectively reduce rates of malaria in pregnancy, yet in malaria-endemic regions rates of adverse pregnancy outcomes remain high.

We sought to explore the association of malaria in pregnancy and other risk factors with poor outcomes, among a cohort of pregnant women who received the recommended preventative treatment for malaria at antenatal care. The prevalence of malaria at the first antenatal care visit was 11%, and malaria infection was associated with lower measures of fetal growth, as measured by ultrasound. Among live, term births, the mean birth weight was not significantly different for malaria-positive vs. malaria-negative women. However, among women with under-nutrition, as measured by low body-mass-index, malaria exposure was associated with significantly decreased birth weight (mean difference -370 grams, 95% CI -728, -12 g). The rates of maternal anemia (hemoglobin <11.0 g/dL) and moderate/severe anemia (hemoglobin < 9.0 g/dL) at antenatal care were 70% and 27%, respectively. Moderate/severe maternal anemia at the first antenatal care visit was associated with malaria as diagnosed by microscopy (aRR 2.06, 95% CI 1.24, 3.44) as was high-intensity hookworm infection in multivariate regression (aRR 2.37, 95% CI 1.44, 3.91).

Our findings suggest the importance of good preventative treatment for malaria in pregnancy to minimize the impact of exposure to malaria on fetal and newborn growth. However, under-nutrition has an important role and research and programs to improve maternal nutritional health may be important to important to further improving birth outcomes in low-resource settings. Furthermore, given the high prevalence of anemia observed in our study, also associated with under-nutrition, as well as hookworm, and malaria, further research is needed to optimize interventions around pregnancy to improve maternal and newborn health in malaria-endemic regions.

For James and William

Acknowledgments

This would not have been possible without the support of many people. First, I thank my research advisor and dissertation committee chair, Steve Meshnick, for his thoughtful guidance, patience, and mentorship throughout this research project. I thank the other members of my dissertation committee for their time and assistance: Anna Maria Siega-Riz, my academic advisor, for her encouragement and support throughout my doctoral program; Michael Hudgens, for patiently helping me appreciate and understand the signal and the noise; Carla Hand-Cerami, for her encouragement and support, and finally to Arlene Dent, for entrusting her study data with me, her very helpful advice, and her availability throughout this process.

I am indebted to many good friends for their support over the years. Especially, I thank Robert Goldenberg, my mentor, who has been helpful to me in so many ways, for encouraging me to begin when I thought it was too late, and who usually gave me good advice (even if I often didn't follow it). I also thank Alan Jobe for continuous encouragement (and nagging), and especially friends from the Global Network for their ongoing friendship and support of my education and professional development.

I am very thankful to the faculty, friends, and colleagues in the UNC School of Public Health, Department of Epidemiology, who have supported my education and professional development, in a stimulating and supportive environment. Especially, the UNC Reproductive and Perinatal Epidemiology Training grant (funded by NICHD) and fellow students have been supportive throughout and members of Meshnick's lab, especially Jennifer Griffin, for their assistance in understanding the data and appropriate analyses.

I am grateful for the love and support of my family, who always remind me of what is important, my sons, William and James – I will remember the many nights doing our homework and learning together - and my husband, Robert McClure, for his patience and perseverance. I thank my younger sister, Sonja - who led the way - and her family. I am especially thankful for my parents, Natalie and Gary Boorman, for their unconditional love, words of wisdom, and the many ways they have supported my growth over the years. I am indebted to my grandparents, for the sacrifices they made for their families, with fond memories of my grandfather, George, a great teacher in my life.

Finally, I thank the research team at Kenya Medical Research Institute and Case Western Reserve University, who conducted the parent study, with special thanks to Dr. Christopher King. I especially thank the women, doctors, and nurses of Msambweni District, Kenya who participated in this study, and without whom this study would not have been possible. I sincerely hope that our efforts, together with those of many others, will contribute to improving the health of women and children everywhere, so that fewer may die of preventable diseases.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANC	Antenatal care
aRR	adjusted risk ratio
BMI	body mass index
BW	birth weight
CI	confidence interval
CS	Cesarean section
G	grams
GA	Gestational age
Hb	Hemoglobin
HIV	Human immune-deficiency virus
IPTp	Intermittent preventive treatment in pregnancy
ITN	insecticide-treated bednet
IUGR	Intrauterine growth restriction
LBW	Low birth weight
MIP	Malaria in pregnancy
PCR	Polymerase chain reaction
PI	Pulsatility index
PM	Placenta malaria
PR	Prevalence ratio
RR	Risk ratio
SP	Sulphadoxine-pyrimethamine
US	Ultrasound
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION AND SIGNIFICANCE

Plasmodium falciparum malaria and pregnancy outcomes

Malaria in pregnancy affects more than 25 million pregnant women who give birth in malaria-endemic areas each year, primarily in Sub-Saharan Africa, as well as many others who deliver in areas of low or unstable malaria transmission (Figure 1-1) [1]. Although those living in endemic areas generally develop immunity to malaria, pregnancy is a period of increased vulnerability. Beginning in the late 1960s, published studies described lower birth weights among women who were infected with malaria during pregnancy.

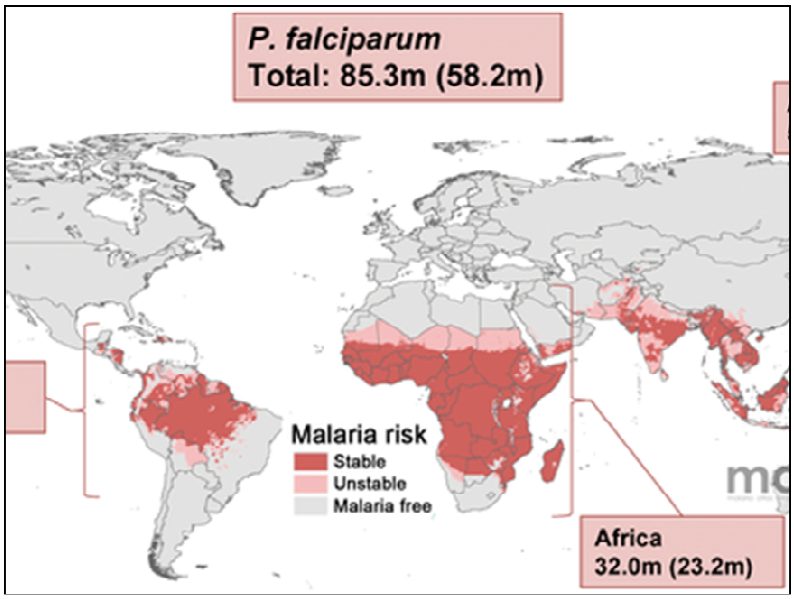

Figure 1-1. Malaria risk in pregnancy (Malaria Atlas Project) [1]

Since then, adverse outcomes which have been associated with malaria for both mother and newborn include maternal anemia, stillbirth, preterm birth, and low birth weight (LBW). Of these outcomes, maternal anemia and LBW are the most commonly documented serious effects associated with malaria in pregnancy [1]. Eisele et al. [2] estimated that malaria

infection was responsible for up to 14% of all LBW infants worldwide and 11% of LBW-related infant mortality in Sub-Saharan Africa—probably the single largest cause of adverse newborn outcomes in this region.

Prevention strategies

Because of the adverse outcomes associated with malaria in pregnancy, several strategies to reduce malaria have been studied [2–4]. A 2006 Cochrane review of 16 trials evaluating antimalarial treatment concluded that the administration of antimalarials to all pregnant women significantly reduced prenatal parasitemia compared with no treatment (relative risk [RR] 0.53; 95% confidence interval [CI], 0.33–0.86) [4]. Numerous antimalarial treatments have been tested; for example, the Cochrane review included trials of proguanil, chloroquine, and sulfadoxine-pyrimethamine (SP). However, for low-resource settings, where the highest burden of malaria remains, treatment ideally is low-cost and effective with limited dosing, and has a good safety profile. In particular, the relatively low-cost SP had promising results in clinical trials [5]. In 2004, WHO recommended intermittent preventive treatment for pregnant women with 2 doses of SP (IPTp-SP) administered at least 1 month apart beginning in the second trimester (3 doses for women with HIV) as standard care in malaria-endemic areas [5]. With the increased availability of SP and the 2004 WHO endorsement, many countries in Sub-Saharan Africa implemented IPTp-SP programs [3].

However, there are potential limitations to IPTp-SP. First, adherence is often suboptimal. Second, geographic areas of SP resistance, which are becoming increasingly common, were found to be associated with reduced effectiveness [6–8]. Third, there may be a differential impact based on other risk factors; most notably, primigravidae, who are at higher risk in general, also have a higher risk of adverse birth outcomes associated with

malaria [1,2]. Fourth, the interactions of malaria with other common infections such as HIV are important—especially in Sub-Saharan Africa, where HIV remains prevalent and optimal treatment has not been determined [9]. Fifth, there are different ways to measure malaria during pregnancy, including peripheral or placental blood smears, polymerase chain reaction (PCR), and placental histopathology; these measures are often not concordant [1].

Since the WHO guidelines were issued, a number of studies have evaluated the impact of intervention programs (IPTp-SP and/or insecticide-treated nets [ITNs]) on birth weight and other pregnancy outcomes. Given these efforts, the aim of this review was to review recent studies evaluating the impact of IPTp-SP, with emphasis on birth weight and anemia, to determine the effectiveness of SP to reduce adverse outcomes associated with malaria in pregnancy and factors influencing these outcomes.

Methods

A review of malaria in pregnancy and pregnancy outcomes in Sub-Saharan Africa was undertaken, with emphasis on recent studies of peripheral or placental malaria, antimalarial treatments (IPTp-SP and/or ITNs), and outcomes of birth weight and maternal anemia. English literature in PubMed, WHO publications, and the Cochrane database published since 2002 and relevant source publications were reviewed. Search terms included “malaria in pregnancy,” “sulfadoxine-pyrimethamine,” “insecticide-treated bed nets,” “anemia,” and “birth weight.” Studies conducted in Sub-Saharan Africa that included rates of LBW, maternal anemia, and maternal malaria infection were included; studies that did not quantify birth outcomes associated with the intervention or that did not quantify the treatment were excluded.

Studies of malaria and pregnancy outcomes: results

Of the 197 papers screened, 84 studies were reviewed in-depth and 33 were included in the present review. Of these, 18 were observational studies, including cohort studies, and 15 were randomized clinical trials (Figure 1). The randomized clinical trials generally compared standard SP dosing (2 doses, as recommended by the 2004 WHO guidelines) with a number of different strategies: placebo; other antimalarials (chloroquine, mefloquine); and alternate SP-dosing strategies. Five trials also evaluated the impact of ITNs. Across both trials and observational studies, the primary outcomes evaluated were rates of placental malaria, LBW (defined as <2500 g), and maternal anemia, with several studies also examining preterm birth and perinatal mortality.

Table 1 summarizes the randomized clinical trials of SP published since 2004 [8–22]. The rates of ITN use and peripheral malaria at enrollment are included for trials that reported these data. Next, the outcomes associated with 2 doses of SP (IPTp-SP) versus an alternate SP regime are given. At enrollment, the rate of untreated bed net use was approximately 50% while rates of ITN use were significantly lower (5%–25%). The rates of malaria at baseline ranged from 7% to 58%. Next, the outcomes associated with the treatment are reported. The placental malaria rates among those treated with IPTp-SP ranged from 2% to 29%. Higher dosing strategies (≥3 doses) showed lower rates of placental malaria infection, ranging from 2% to 8%. The alternate drug treatment groups (chloroquine, mefloquine) generally had similar rates of placental infection to those receiving IPTp-SP, while the placebo groups had significantly higher rates of infection. A similar relationship existed for LBW rates, which ranged from 2% to 13%, with reductions in LBW prevalence in the IPTp-SP group equivalent to the reductions associated with alternate drug treatments. Women who received more than 2 SP doses had decreased risk of LBW

outcome. For example, Maiga et al. [19] found that LBW rates in Mali were significantly lower with 3 versus 2 doses of SP (adjusted RR 0.5; 95% CI, 0.32–0.79). A study from Côte d'Ivoire also found that a third dose decreased risk for LBW (adjusted odds ratio [OR] 0.12, 95% CI, 0.05–0.31) [8]. Among HIV-positive women, monthly SP dosing was superior to 2 doses in the trial reported by Filler et al. [17] and there was a non-significant trend for better outcome with monthly doses compared with 2 doses in the trial reported by Hamer et al. [9]. Finally, maternal anemia rates ranged from 2% to approximately 30%; these differences in part reflected different hemoglobin levels defining moderate–severe anemia, but in general within each study the SP-treated group had lower anemia rates than the alternate treatment or placebo group.

Several common findings were reported in the randomized trials. Timing of infection was related to risk of adverse outcomes in several studies. For example, a Burkina Faso cohort study evaluated outcomes by gestational age at infection and found a trend for decreased birth weight among women infected at less than 4 months of gestation and/or more than 6 months (mean birth weight decreased by 68 g [*P*=0.08] and 105 g [*P*=0.02], respectively) [13]. A cohort study of women in Malawi [18] found that LBW risk was higher among women with second-trimester infection (prevalence ratio [PR] 1.7; 95% CI, 1.1–2.7) than among those with third-trimester infection (PR 1.5; 95% CI, 0.9–2.7). The risk for first-trimester infection was not evaluated [18]. Primigravidae treated with IPTp-SP were consistently more likely to have decreased risk for LBW compared with multigravidae. For example, in a Burkina Faso study, IPTp-SP use was associated with reduced risk of LBW among primigravidae (adjusted OR 0.11; 95% CI, 0.07–0.17) but not secundigravidae [12]. Finally, ITNs had an interactive effect with SP treatment in decreasing the risk of adverse outcomes in some, but not all, trials that examined their impact together with SP treatment [14,16].

Next, the non-randomized studies of IPTp-SP treatment were reviewed [7,23–40] (Table 2). These studies included cohort, cross-sectional, and pre–post studies that evaluated the impact of IPTp-SP scale-up on rates of malaria in pregnancy, birth weight, and maternal anemia. In general, they found IPTp-SP use to be associated with reduced malaria rates in pregnancy. However, the impact of IPTp-SP on LBW and maternal anemia rates varied. Feng et al. [30] conducted a 10-year observational study in Malawi; they noted increasing trends of any SP use, use of more than 2 SP doses, and ITN use over time and corresponding decreases in rates of maternal malaria, LBW, and maternal anemia over the same period. By contrast, several studies noted the impact only among subpopulations—in particular, primigravid women and women who also used ITNs.

Several explanatory variables for the variation in impact of IPTp-SP were examined. A Tanzanian study, which found no differences in birth weight between infants born to women who received IPTp-SP and those born to women who did not receive SP treatment, found evidence of SP-resistant malaria parasites in the geographic area in which the study was conducted [39]. Additionally, one of the most commonly reported modifiers was gravidity; primigravidae were generally more likely to experience significant benefit from SP treatment than were multigravidae [26,30]. Adherence to and delivery of treatment are important. Receipt of 2 or more doses of SP was found to be beneficial in several studies. For example, Aziken et al. [36] observed significantly decreased risks of maternal peripheral malaria, placental malaria, and LBW with 2 doses versus 1 dose versus no treatment. Furthermore, consistent with trials, timing of dose was associated with outcome in several studies, with early identification and treatment associated with decreased risk of LBW. However, women were often identified only late in pregnancy, and coverage of IPTp-SP—although varied—was generally lower among the observational studies than the clinical

trials. Most observational studies did not conduct specific analyses of HIV-positive women; however, a cross-sectional study found that malaria and anemia were both reduced among HIV-positive women who were taking co-trimoxazole, regardless of SP treatment [31]. Finally, some studies observed that seasonality was a factor in effectiveness of treatment, with women who were pregnant during high-transmission season generally benefiting more from IPTp-SP treatment than those who were pregnant during low-transmission season.

Several studies examined the risk of LBW stratified by gravidity [8,11,19,35] for IPTp-SP compared with no treatment. Among primigravidae, SP treatment was consistently protective against LBW (RR ranged from 0.1 to 0.5). Among multigravidae, SP treatment showed less association with risk of LBW (RR ranged from 0.70 to 0.98). In several of these trials, there was no significant difference in risk of placental malaria by gravidity; however, SP treatment reduced the risk of placental malaria overall in several trials (RR 0.04 [95% CI, 0.003–0.6] in the study by Gies et al. [11]; RR 0.48 [95% CI, 0.27–0.85] in the study by Maiga et al. [19]).

Discussion

The present review found that, in general, the use of IPTp-SP was beneficial, which is an important finding given the concerns about its impact. One of the limitations of the analysis was the comparison of outcomes across different types of study that had used various methods for determining exposure and outcome assessment. Malaria was often an exposure of interest as well as an outcome in relation to the impact of IPTp-SP. Additionally malaria was diagnosed in different studies either by blood smear or by PCR. The latter test is generally more sensitive but less commonly available in clinical settings. Some studies tested for malaria infection in peripheral blood during pregnancy or at delivery, as well as in

the placenta. Furthermore, maternal anemia was defined by different hemoglobin cutoff values and ITN use was generally determined by self-report, which may be less reliable. However, common elements could be determined across both randomized and non-randomized studies, thus enabling evaluation of the relationship of SP treatment to malaria in pregnancy and several pregnancy outcomes.

Several trends were noted across both the randomized trials and the non-randomized studies with regard to malaria infection in pregnancy. Most studies found that peripheral malaria and placental malaria were both associated with adverse pregnancy outcomes among primigravidae, including higher risk for LBW. By contrast, multigravidae generally had a lower risk of LBW and other adverse pregnancy outcomes, regardless of malaria and SP treatment status [26,35,36,39]. Another observation was that the timing of infection was associated with birth outcome. Studies from Burkina Faso that evaluated outcomes according to gestational age at infection found a trend for decreased birth weight among women infected early or late in pregnancy [11,12]. The authors of a study conducted in Benin also reported that malaria infection detected early in pregnancy was associated with a lower mean birth weight compared with infection detected in mid-pregnancy [10].

Additionally, pregnancy outcomes associated with IPTp-SP use varied with regard to gravidity, number of SP doses received, and timing of treatment. For example, when examining the impact of IPTp-SP, several studies that did not find overall differences in birth weight associated with IPTp-SP did report a significant impact in subpopulations. A study from Nigeria found that placental malaria rates differed between the SP-treated group and the placebo group among primigravidae but not among women in their second or later pregnancies [33]. A randomized controlled trial that restricted enrollment to primigravidae found a substantial reduction in LBW among women who received SP treatment [21]. Thus,

first pregnancy seemed to be a relatively consistent risk factor for adverse outcomes, and SP treatment appeared to reduce LBW among these women. With regard to the timing of treatment, earlier treatment—where examined—seemed to be beneficial; however studies with additional late treatment also found benefit. For example, the trial in Mali [19] and a Côte d'Ivoire study [25] both found that a third dose of SP—late in the third trimester, when significant fetal weight gain occurs—reduced risk for LBW. Additionally, trends indicated that an increased number of SP doses was associated with decreased risk of LBW and maternal anemia.

The use of ITNs in addition to IPTp-SP decreased the risk of malaria infection and LBW in several studies. In a study in Malawi, for example, SP treatment alone was not associated with reduced malaria infection rates, whereas concurrent ITN use provided significant protection [30]. However, in a trial in the Gambia, while those who used bed nets had similar birth weights regardless of treatment, the mean birth weight among women who did not routinely use a bed net was greater for those who received SP than those who did not [14]. The authors speculated that, especially in geographic areas with increasing resistance to SP, the use of ITNs may be more protective for malaria infection.

There was an overall difference in findings between clinical trials and non-randomized studies, including those evaluating the impact of the IPTp-SP policy. As expected, randomized clinical trials, which had higher adherence to treatment, generally reported significantly better outcomes associated with SP treatment than did observational studies attempting to document the effectiveness of IPTp-SP in a population. Although non-randomized studies still generally found benefit of treatment, the impact was lower than observed in the trials. For example, Le Port et al. [24] found that IPTp-SP significantly

improved outcomes in a clinical trial setting in Benin; however, when the Benin Government scaled-up the IPTp-SP program, although pregnancy outcomes were still improved, the results were far less impressive than in the trials. Increasing SP resistance has been reported in several areas of Africa and has been hypothesized to be a potential factor in lower-than-anticipated reductions in LBW and other adverse pregnancy outcomes when SP alone is used; however, few of the trials specifically examined this issue. As observed in several studies, alternate strategies, including addition of ITNs, may increase effectiveness.

Steketee and Campbell conducted an ecologic evaluation of neonatal and childhood mortality prevalence rates associated with malaria control strategies, observing that, while there are numerous potential factors associated with recent health improvements in African countries, high coverage of malaria control intervention, especially with ITNs, has been an important contributor to reduced childhood mortality [41]. In light of the results from studies comparing the effectiveness of SP related to doses, the WHO revised its guidelines in 2012 to recommend monthly SP treatment beginning in the second trimester for pregnant women in Africa [42]. Given the reduction in malaria prevalence in many geographic areas, one of the considerations is at what prevalence this level of presumptive treatment should be provided. Thus, the specific components of malaria prevention strategies are important to overall public health impact.

Gaps in research

Although concerns have been raised about the effectiveness of SP in improving pregnancy outcome (e.g., because of potential resistance and lack of adherence), studies conducted since 2002 collectively indicate that interventions such as IPTp-SP and/or ITNs during pregnancy are associated with measureable improvements in maternal and infant health, especially among primigravidae. Increasing the number of SP doses administered

improves effectiveness. However, to reduce mortality substantially, optimal strategies to implement IPTp-SP effectively—taking into account important common comorbidities such as HIV—must be developed. With concerns about increasing resistance to SP, additional strategies, including alternate treatments and use of ITNs, may be important for improving outcomes during pregnancy. It is clear that increasing standardization of both measurement of malaria and evaluation of implementation strategies can lead to improvements in the data needed to address important questions regarding strategies to reduce malaria in pregnancy and associated adverse outcomes.

Maternal anemia and malaria and other parasitic infections

In many of the geographic areas where malaria is endemic, high rates of anemia in pregnancy are common [43]. Estimates suggest that 55.8% (95% CI, 51.9, 59.6%) of women in sub-Saharan Africa have anemia in pregnancy (Figure 1-2) [43] with some countries within Africa having rates of maternal anemia as high as 75%. Pregnant women and children are particularly among those at risk for anemia in low-resource countries. Maternal anemia, itself a morbidity, also increases risk for mortality from obstetric conditions such as postpartum hemorrhage, a leading causes of maternal mortality [44]. Additionally, maternal anemia has been associated with fetal, neonatal, and early childhood anemia, potentially increasing risk for neurodevelopmental delays in studies from both developed and developing countries [45-48].

Measurement of anemia

Anemia is measured by hemoglobin (Hb) level. In pregnancy, Hb levels decrease over the course of the pregnancy mostly due to increased volume expansion during pregnancy, thus diluting the red cells and decreasing the proportion of hemoglobin [49-54]. The WHO thus has classified anemia as Hb <11.0 g/dL during pregnancy (compared with Hb < 12.0 g/dL for the non-pregnant, adult population) [43,49]. While Hb levels are an efficient way to approximate anemia levels at the population level, it does not distinguish iron-deficiency anemia from anemia which is not associated with iron deficiency [50].

In Sub-Saharan African countries, high rates of low cord Hb levels, known as fetal anemia, have been documented, with less research on newborn Hb levels [45-47]. However, international cut-offs for neonatal and fetal Hb levels parallel to those for maternal anemia have not yet been established, in part due to the challenges of standardized collection of fetal and neonatal blood samples [49]. In high-resource countries, 13.5 – 19.5 g/dL is considered to be the normal range of cord Hb levels [49]. Several studies from Sub-Saharan Africa have used 12.5 g/dL as the cut-off to indicate fetal hemoglobin (based on cord blood) [45,47]. With increasing recognition of the importance of fetal and newborn iron levels in relation to growth and development, further research may be warranted on fetal

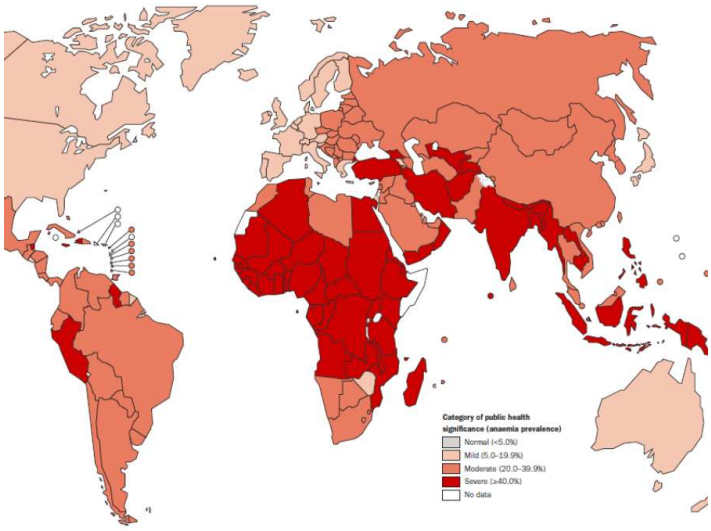

Figure 1-2. Prevalence of anemia in pregnancy [43]

and newborn anemia and to establish reference ranges, incorporating data from Africa, which may differ from high-resource countries [46].

Etiology of anemia in pregnancy

Gestational anemia progresses during pregnancy as the increasing needs of the mother and fetus are met, the plasma expands and the concentration of hemoglobin decreases. However, malaria-endemic regions, many women are anemic at or prior to pregnancy and as a result of a number of risk factors, are at increased risk to develop moderate or severe anemia in pregnancy [56-64]. Anemia results from a number of conditions – often inter-related – prevalent in low-resource countries, infections (including malaria, hookworm, and other parasitic infections), inadequate nutrition, and genetic conditions (e.g., sickle cell anemia) [54-65]. The chronic conditions/diseases associated with anemia are summarized in the panel below [54].

Chronic conditions/diseases associated with anemia

Infection	Malaria, HIV, tuberculosis, osteomyelitis, bacterial endocarditis, pulmonary abscess
Parasitic infection	Hookworm, ascaris, schistosomiasis
Chronic noninfectious diseases	Diabetes, rheumatoid arthritis, Systemic Lupus Erythematosus, Chrohn's disease, ulcerative colitis, chronic liver disease, cirrhosis, hemoglobinopathies
Malignancy	Carcinoma, sarcoma, lymphoma, myeloma

(from Gandopadhyay et al, 2011 [54])

However, nutritional deficiency and infections, especially *P. falciparum* malaria and other parasitic infections, appear to account for the majority of anemia found in Sub-Saharan Africa [55].

Infectious etiology of anemia

While studies addressing the association between *P. falciparum* malaria and maternal anemia are described above and summarized in Table 1-2, malaria infection in pregnancy increases risk for maternal anemia. For example, Huyn et al founded an adjusted OR 1.7 ($p = 0.001$) for anemia among those women with malaria in pregnancy [23]. As another example, in a study in Malawi, maternal anemia was related to the number of malaria episodes, with higher anemia risk among women with 3 or more infections [60]. Malaria may also relate to fetal anemia. For example, a recent study in Malawi, found that the prevalence OR for fetal anemia was 1.41 (95% CI 1.05, 1.90) for malaria-positive vs. negative women [45]. Malaria causes anemia through hemolysis of red cells combined with suppression of erythropoiesis, but is not thought to cause iron-deficient anemia [66], although this is an area of ongoing research.

Hookworm

In many geographic areas where malaria is endemic, other parasitic infections, most notably hookworm, are also prevalent [58,67-71]. An estimated 37 million pregnant women are infected with hookworm each year [71]. In one example, a cross-sectional study of pregnant women conducted from 2000-2005 in Kenya where 42.7% were infected with *P. falciparum*, rates of infections were 30.6% for *Schistosoma haematobium* and 31.5% for hookworm [67].

Hookworm may cause maternal anemia through intestinal blood loss [69-71]. Hookworms are generally measured through stool samples, by intensity of infection as measured by number of eggs per gram of stool. The WHO has classified hookworm as

light, moderate or high infection, with moderate/high infections most closely associated with anemia in the population [70]. However, research has also suggested that there may be the potential for a lower threshold of infection to increase infection risk, especially in association with under-nutrition or among vulnerable populations [70].

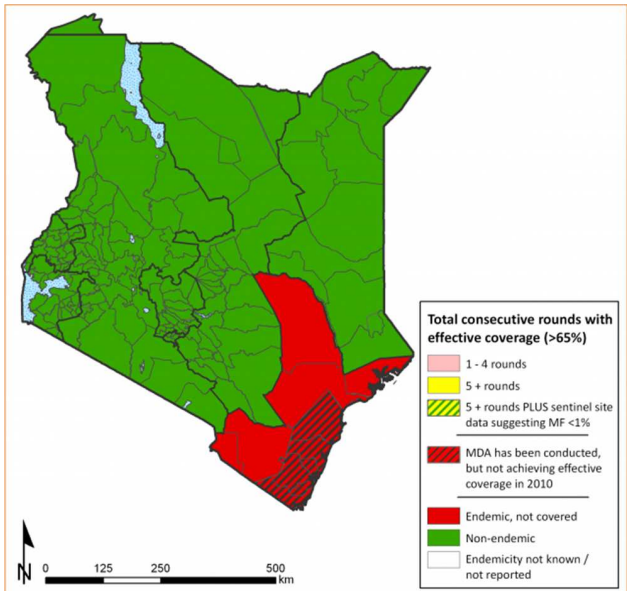

Figure 1-3. Status of elimination of Lymphatic filarisis in Kenya, 2010 (WHO)

In 1994, the WHO first recommended that hookworm treatment be included in health care for women of reproductive age in geographic areas where hookworm was endemic; however the treatment (albendazole) was not recommended during pregnancy or lactation [69]. In 2002, the WHO reconsidered their recommendation, given that there was no evidence of toxicity [70,71]. To date, three randomized controlled trials have assessed treatment for hookworm during pregnancy (Peru; Sierra Leone; Uganda), with results suggesting that the recommended anti-helminthic treatment effectively reduced prevalence of hookworm [73-77]. However, regarding impact on maternal anemia, the trial in Uganda which assessed anemia by treatment arm, found no effect of treatment on anemia status [77]. In Kenya, as elsewhere, coverage of the WHO recommended program to eliminate hookworm has been inconsistent, as shown in Figure 1-3.

Schistosomiasis and other parasitic infections

In addition to malaria and hookworm, the primary infections associated with maternal anemia, a number of other infections have been associated with anemia in studies [46,57,58,62]. For example, urogenital schistosomiasis (*S. haematobium*), a parasite present in urine which is measured as eggs/mL, has also been associated with anemia in some studies [78]. Treatment for schistosomiasis (praziquantel) is safe during pregnancy and recommended by the WHO and other organizations.

Besides hookworm, other helminthes, including *T. trichuria* and others, have been associated with anemia [58,79]. As with hookworm, high-intensity infections have been most closely correlated with moderate/severe anemia, most commonly in studies conducted with school-age children.

Finally, HIV has an important role in maternal anemia in Sub-Saharan Africa [e.g., 46,62,64], but is beyond the scope of this dissertation.

Role of nutrition in parasitic infection

Nutritional deficiency, another important cause of maternal anemia, is common in malaria-endemic regions [49-57]. Malnutrition causes anemia in a number of ways. First, iron deficiency anemia, characterized by poor iron absorption or inadequate iron intake, is prevalent in low-resource countries [66]. However, iron deficiency is commonly seen in combination with deficiency of other micronutrients such as folic acid [54,55], which together contribute to anemia.

In addition a direct contribution to increased risk of anemia, under-nutrition may also be associated with increased vulnerability to infections, and other morbidities [52,59]. An example of the potential factors influencing nutrition and infection is illustrated in Figure 1-4 [59]. In addition to the relationship with infections, nutrition and especially inadequate intake of iron-rich foods, is a source maternal anemia, especially in low-resource countries [49,59,66]. As a result, international guidelines recommend iron/folic acid intake during pregnancy, and especially among women who are iron-deficient [49,66]; however, research is ongoing to determine optimal strategies to reduce nutrition-related anemia.

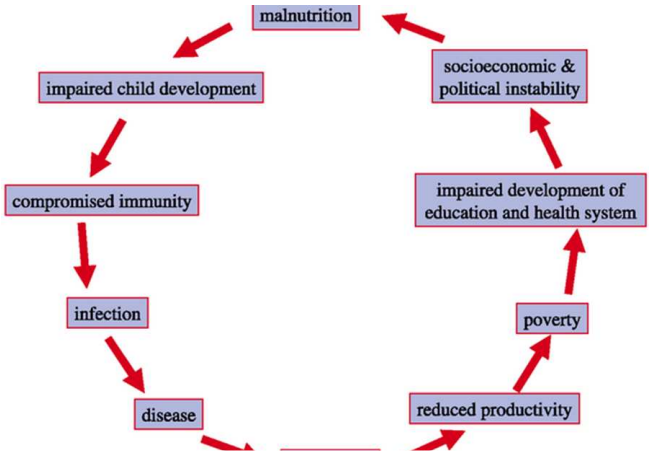

Figure 1-4. Interaction of nutrition and infection [59]

Other factors associated with anemia

As noted, other maternal conditions, including chronic disease, as well as socio-demographic factors may increase risk of maternal anemia. Reduced time between pregnancies also may be a risk factor for anemia, as time is required for a woman to restore depleted iron stores following pregnancy [49]. Thus, multigravida status compared to primigravida status has been associated with risk of anemia in studies [e.g., 57]. Finally, chronic non-infectious disease, environmental and genetic factors (e.g., sickle cell disease) clearly contribute to anemia [54], but are beyond the scope of this review.

Research gaps and conclusions: anemia and infections

Maternal anemia remains an important and common condition associated with maternal morbidity and increased risk of maternal mortality. Maternal anemia is also associated with fetal and neonatal anemia, conditions which increase the risk of long-term neurodevelopmental impairment. However, the cut-offs for defining fetal/neonatal anemia is an area of ongoing research.

P. falciparum malaria has long been recognized as a cause of anemia in pregnancy and effective malaria preventive treatment programs have been implemented in recent years. However, in many geographic areas where malaria is endemic, other parasitic infections, most notably hookworm, are also prevalent. While high-intensity infections are associated with anemia in the general population, their contribution to maternal and fetal anemia has been less well studied. Finally, under-nutrition is also recognized to have an important role in anemia, and may contribute both directly to increased risk of anemia and as well as indirectly as a risk factor for infections. The etiology of anemia, especially in low-resource countries where many risk factors are prevalent, is complex and despite strategies to reduce factors related to anemia, including malaria and hookworm treatment, as well as provision of iron/folic acid during pregnancy, rates of maternal anemia remain high in Sub-Saharan Africa.

Table 1-1 Randomized clinical trials of SP treatment in pregnancy in Sub-Saharan Africa

Country	Study period	Sample size	Other treatment	ITN use, %	Peripheral malaria, baseline %	Placental malaria			LBW			Maternal anemia		
						SP, %	No IPT, %	Other, %	SP, %	No IPT, %	Other, %	SP, %	No IPT, %	Other, %
Benin [10]	2005–2008	1601	MQ	53[a]	7.2	4.4	—	1.7	9.8	—	8.0	20	—	16
Burkina-Faso [11]	2004–2006	1441	Weekly CQ	35.5	20.0	19.2	—	18.8	12.8	36.5	24.8	14	—	35
Burkina-Faso [12]	2005	423	IPT-CQ	5.4	50.8	10.6	—	15.9	11.4	—	15.6	—	—	—
Burkina Faso [13]	2006–2008	1034	Three-dose SP	—	—	23.8	—	46.1	—	—	—		—	
Côte d'Ivoire [8]	2009	400	SP (no schedule)	—	9	4	—	7	12	—	15	9.8	—	14.6
Gambia [14]	2002–2004	2688 multi	Placebo	50.2 [a]	14.9	3	9	—	5.4	7.1	—	10.6	8.9	—
Ghana [15]	2004–2007	3643	AQ; SP-AQ	24.8	58.0	28.7	—	20.8 (AQ); 27.6 (SP-AQ)	23.8	—	19.3 (AQ); 22.6 (SP-AQ)	4.8	—	4 (AQ); 16 (SP-AQ)
Ghana [16]	2007–2008	3333	AQ-AS; ITN-SP	52.1 [a]	23	12.3	—	12.4 (ITN-SP); 11.3 (AQ-AS)	10.7	—	10.7 (ITN-SP); 12.7 (AQ-AS)	1.4	—	1.7 (ITN); 1.7 (AQ-AS)
Malawi [17]	2002–2005	432 HIV-negative; 266	Monthly SP	16.2	32.9	6.3	—	2.3	13.4	—	10.6	3.4	—	1.0

		HIV-positive												
Malawi [18]	2003–2006	1320	Monthly SP; AZI-SP	60.4[a]	8.8	2.5	—	2.1 (monthly SP); 1.8 (AZI-SP)	12.9	—	9.1 (monthly SP); 7.9 (AZI-SP)	—	—	—
Mali [19]	2006–2008	814	Three-dose SP	16.7	26.7	16.7	—	8.0	13.3	—	2.1	3.3	—	—
Mozambique [20]	2003–2005	1030	Placebo	—	9	4	4	—	10	10	—	—	—	—
Mozambique [21]	2001–2002	600 <21 years of age, primiparous	Placebo	—	33	2.4	13.3		9.5	13.3	—	—	—	—
Uganda [22]	2004–2007	5775	ITN-SP; ITN alone	—	—	4.3	—	3.1 (ITN-SP); 2.6 (ITN)	6.5	6.3	6.8 (ITN-SP); 6.3 (ITN)	14.2	—	13.6 (ITN-SP); 16.3 (ITN)
Zambia [9]	2003–2004	224 HIV-positive	Monthly SP	25	17	26	—	29	15	—	12	49	—	43

Abbreviations: AQ, amodiaquine; AS, artesunate; AZI, azithromycin; CQ, chloroquine; IPT, intermittent preventive treatment; ITN, insecticide-treated net; LBW, low birth weight; MQ, mefloquine; PMR, perinatal mortality rate; SP, sulfadoxine/pyrimethamine.

[a] Bed net (not insecticide treated).

Table 1-2 Non-randomized studies of SP treatment in pregnancy in Sub-Saharan Africa

Country	Study period	Sample size	ITN use, %	≥1 SP dose, %	Peripheral malaria, %	Placental malaria, %	Birth weight results	Maternal anemia
Benin [23,24]	2008–2010	1037	100 [a]	IPTp-SP	24	11.5	10.9% LBW, not associated with infection; 16% early positive vs 9% negative	Infection associated with increased risk of anemia (aOR 1.6 and 1.7, respectively)
Côte d'Ivoire [25]	2008	2044	47.9	83.8	—	4.8	10.6% LBW, associated with primigravidae and placental malaria	—
Democratic Republic of Congo [7]	2007	1393	—	—	—	—	6.8% LBW (1 dose) vs 11.4% LBW (2 doses); not significantly different after adjustment	—
Gabon [26]	2005–2006	203	21	24	34.4	53.6	13% LBW overall; mean birth weight decreased if PM present (–315 g)	53%
Gabon [27]	2003–2004; 2005–2006	1403	51.6; 50.2	0 vs. 83.2	—	—	11.7% LBW (2004) vs 10.3% LBW (2006). 12.6% LBW (no IPTp) vs 8.7% LBW (IPTp)	4.6% (2004) vs. 5.3% (2006)
Ghana [28]	2000; 2006	1065	—	76.5	—	—	26.0 LBW (2000) vs 16.2 LBW (2006) (*P*=0.03) in primigravidae; multigravidae NS	35% for primigravidae; 33.5% (2000) vs. 17.8% (2006) in multi
Ghana [29]	2009	363	6.3	55.6	28.4	38.6	—	Severe anemia 3.5% (IPTp) vs. 12.4% (no IPTp)
Malawi [30]	1997–2006	8131	14.4 65.6	77 (1997); 95 (2004)	24(1997); 5 (2006)	25 (1997); 7 (2006)	18% LBW (1997) vs 15% LBW (2006)	—
Malawi [31]	2005–2009	1142	59.6	49.7	10	—	—	61.4% (no treatment) vs. 52.4% (IPTp-SP only),

								34.7% (IPTp-SP and co-trimoxazole)
Mozambique [32]	2003–2004	7911	13.5	92.5	—	—	12.5% LBW (no SP) vs 7.3% LBW (≥2 doses)	3.4% (no SP) vs. 0.8% (≥2 doses)
Nigeria [33]	2003–2004	983	1.1	—	13	13	LBW RR 1.30 (95% CI, 0.64–2.61) in presence of PM	—
Nigeria [34]	2005–2006	500	—	—	—	64.4	LBW not associated with PM (*P*=0.08)	—
Nigeria [35]	2007–2008	800	20.1	SP	—	—	Increased risk of LBW with non-use of IPTp-sp (aOR: 2.27 [95% CI, 0.98—5.28])	—
Nigeria [36]	2009	741	—	Case–control	8.9 (2 doses); 16.3 (1 dose); 19.1 (none)	5.2 (2 doses); 19.3 (1 dose); 22.6 (none)	11% (IPTp-SP) vs. 23% (none)	1.6% (IPTp-SP) vs. 10.8% (none)
Senegal [37]	2004	692	—	—	—	10	2684 g (malaria positive) vs. 3085 g (malaria negative)	—
Senegal [38]	2000; 2007	965	0.7–0.8	—	—	—	9.5% pre- vs. 7.7% post-IPTp-SP	
Tanzania [39]	2002; 2005	880	15.5	—	—	—	9%–12.8% vs. 15.7% (aOR for IPTp vs. none 0.53 (0.23–1.19)	4.6% vs. 7.4%
Uganda [40]	2003–2005	2785	—	—	49.5; 17.6	—	—	—

Abbreviations: aOR, adjusted odds ratio; IPTp, intermittent preventive treatment in pregnancy; LBW, low birth weight; PM, placental malaria; RR, risk ratio; SP, sulfadoxine/pyrimethamine.

[a] All women received an ITN and IPTp-SP, per country guidelines.

CHAPTER TWO

BACKGROUND AND SPECIFIC AIMS

Background

More 100 years ago adverse pregnancy outcomes were first reported in association with malaria and in the 1960's, the specific association of malaria and low birth weight was reported in Sub-Saharan Africa [1,78]. Since then, numerous studies have confirmed an association between malaria in pregnancy and low birth weight as well as other adverse neonatal and maternal outcomes, but most notably maternal anemia [2,59]. Treatments to reduce malaria were established to be safe during pregnancy and, since 2004, international guidelines have recommended routine preventive treatment during pregnancy [42]. However, in the context of preventative treatment, few studies have examined the role of malaria in pregnancy on pregnancy outcomes. This study sought to describe the association of malaria and birth weight and maternal anemia, among a cohort who receive the recommended preventive treatment for malaria in pregnancy.

Specific Aims

Given the potential pathways by which malaria infection during pregnancy may affect birth outcomes, the primary objective of this study is to evaluate the associated risk of malaria infection in pregnancy on maternal, fetal and birth outcomes in a malaria-endemic area, in the context of preventive treatment for malaria in pregnancy.

Specific Aim I

To describe the association between malaria infection during pregnancy and fetal growth, uteroplacental measures, and birth weight among a cohort in a malaria-endemic region with preventive treatment in pregnancy. The relationships between malaria exposure and these outcomes will be examined in association with other parasitic infections, maternal nutritional status (hemoglobin level, low body mass index [BMI]), and socio-demographic factors (use of bednets, gravidity, and socio-demographic status).

1. Evaluate the association between malaria exposure and fetal growth, evaluating the timing of exposure and fetal growth patterns.

   Hypothesis: Malaria infection early in pregnancy (<22 weeks gestation) is associated with lower estimated fetal weight compared to late infection or no infection.
2. Evaluate the associations between maternal nutritional status and malaria infection on birth outcome.

   Hypothesis: Malaria infection and poor maternal nutritional status, as measured by low BMI, are both associated with reduced birth weight.
3. Evaluate the association between malaria prevention (bed net use) and treatment, malaria infection and birth outcomes

   Hypothesis: Those women who have not received preventive treatment prior to first study visit will have poorer birth outcomes compared to those who received preventive treatment prior to study entry.

Specific Aim II

To estimate the association of malaria infection and hookworm infection during pregnancy on maternal anemia, specifically moderate/severe anemia, during pregnancy.

1. Evaluate the association between malaria exposure, low BMI and moderate/severe maternal anemia during pregnancy, as measured at antenatal care.
   Hypothesis: Malaria infection during pregnancy and low BMI are both associated with moderate/severe maternal anemia.
2. Given the control of malaria infection, explore the association between hookworm and other helminth infections and maternal anemia during pregnancy and at delivery
   Hypothesis: Women with hookworm in pregnancy have increased risk of maternal anemia.

CHAPTER THREE

METHODS

Study overview

The dataset utilized for this dissertation was from the Case Western Reserve University (CWRU) and Kenya Medical Research Institute (KEMRI) *Fetal Immunity to Falciparum Malaria* study (Christopher R. King, MD, Principal Investigator; Arlene Dent, MD, PhD, Co-Investigator). The *Fetal Immunity* study, funded by the US National Institute of Allergy and Infectious Disease (National Institute of Allergies and Infectious Disease; NCT00314899), will examine how prenatal malaria exposure affects development of humoral and cellular immune responses to malaria blood stage antigens from birth to 3 years of age and to assess how prenatal exposure to malaria affects malaria infection during infancy, as well as growth and development during infancy.

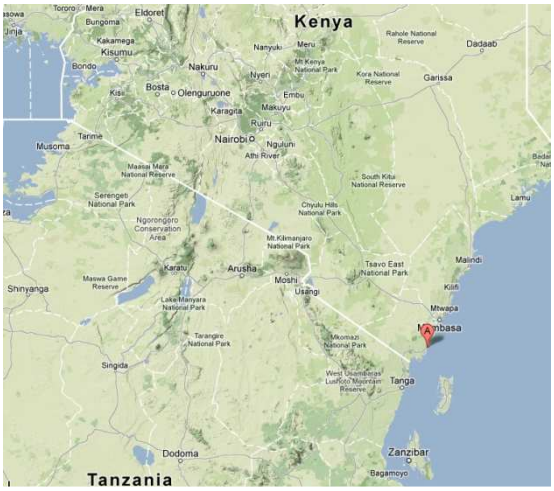

Figure 3-1. Study Site: Msambweni District, Kenya

Study population

For the study, a cohort of pregnant women was recruited in the Mswambweni Hospital in Msambweni, Coast District, located on the coast of Kenya (Figure 3-1, Study Site), a geographic area where the prevalence rate of malaria was approximately 10% at the time of

the study. From 2006 to 2009, the study team recruited pregnant women who presented for antenatal care (ANC) for at the study hospital and obtained follow-up for the women who delivered at the hospital (detailed description of the parent study available [79]). At the time of the study, widespread distribution of insecticide-treated bednets (ITN's) was ongoing, as part of Kenyan national policy [80].

Study recruitment and enrollment

Women who presented for antenatal care (ANC) at the Mswambweni Hospital between 16 and 28 weeks gestation were recruited for this study (however, women at any gestational age, prior to delivery were enrolled). Per Kenya's national policy, women received intermittent presumptive treatment (IPTp) for malaria, beginning in the second trimester as part of routine antenatal care, as well as iron/folate supplementation.

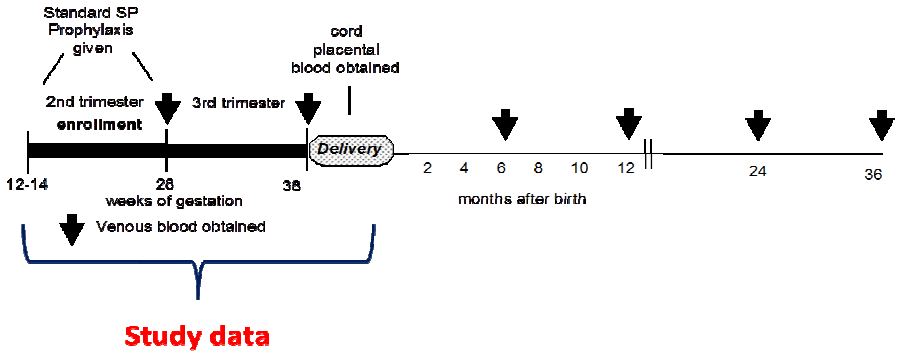

Figure 3-2. Study Timeline and Data Collection, Mswambweni Hospital, Kenya

Women with evidence of helminth infection were treated, but treatment for schistosomiasis was deferred until post-delivery. The birth outcomes collected were for consenting women with term, live births who delivered at the study hospital (Figure 3-2). (For the parent *Fetal Immunity* study, infants were followed to 36-months post-delivery, which is beyond the scope of this thesis.)

Inclusion and exclusion criteria

Inclusion criteria

Pregnant women were allowed to enroll irrespective of their gestational age, although they could not enroll at delivery because of the inability to undergo adequate consent. However, potential participants were strongly encouraged to come to the clinic for prenatal care early in the second trimester (ideally <16 weeks gestation) both to ensure adequate prenatal care for the mother and unborn infant. Additional inclusion criteria for the main study were as follows:

- Greater than or equal to 15 years of age
- Willingness to provide informed consent
- Confirmed pregnancy
- Apparent good health
- Residence of study region
- Consent for lab and stool samples

Exclusion criteria

Exclusion criteria for the main study included the following criteria:

- Preterm delivery less than 34 weeks gestation
- Failure to deliver in the hospital
- Evidence of placenta previa
- Maternal chorioamnionitis
- Receipt of immunosuppressive drugs during pregnancy
- Hemoglobin less than 6.07 g/dL

Additional inclusion/exclusion criteria

The analysis set for the birth outcomes was restricted to term, singleton births. HIV infection was low among this cohort and thus not otherwise evaluated.

Study recruitment

The number recruited and inclusion/exclusion are summarized in Figure 3-3.

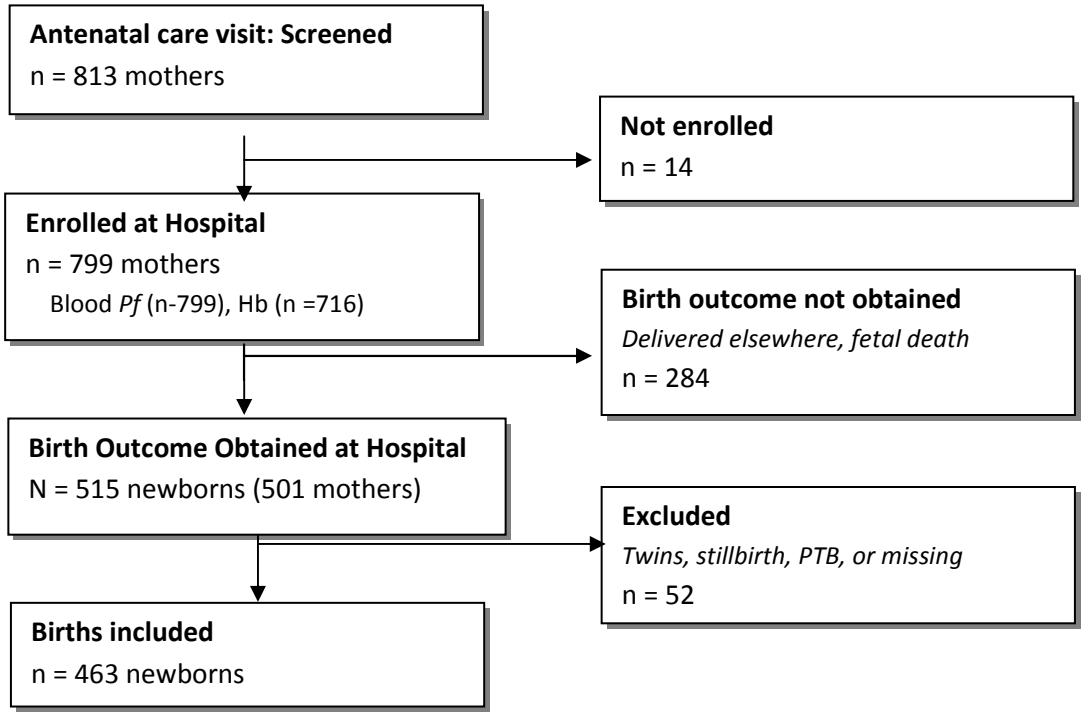

Figure 3-3. Study recruitment of pregnant mothers at first antenatal care visit, Msambweni District Hospital, coastal Kenya, 2006-2009

The demographic and exposure variables were compared for the women enrolled with birth outcomes to those without birth outcome data available. Overall, the groups did not have significant differences in these measures (Table 3-1).

Data collection

Antenatal care (ANC) visit: At the first antenatal care visit, demographic data (including maternal age, marital status, educational level, tribe, household expenditure), medical history (Cesarean section, maternal conditions), medications used in the past 3 months (iron, folic acid, blood, herbal medicine, malaria treatment and helminth treatment), date of last menstrual period, and pregnancy history (parity, and previous outcome) were obtained.

A maternal physical exam (height, weight, blood pressure) was performed. Finally, prenatal labs were obtained:

- Malaria by blood smear
- Hemoglobin by Coulter counter (Beckman Coulter)
- Dipstick (haematuria, proteinuria, and glucose)
- Hookworm by stool (eggs per gram)
- Schistosomiasis (eggs per mL)

Ultrasound: at least one fetal ultrasound (US) exam which measured fetal head circumference (HC), biparietal diameter, abdominal circumference, and femur length to generate fetal weight was performed at enrollment. Ultrasound examinations were performed using a SonoSite 180 Plus ultrasound machine (SonoSite FujiFilm, Bothell, WA).

The ultrasound variables include:

- Placenta assessment (location, previa, grade, thickness)
- Fetal assessment (biparietal diameter, head circumference [HC], abdominal circumference, fetal length, estimated gestational age, scalp thickness, organ calcification)
- Doppler assessment (maximum velocity, pulsity index (PI), resistance index (RI), notching, and abnormalities) for umbilical and right/left artery

Delivery: At birth, the following were obtained:

- Neonatal (birth weight, length and head circumference, Apgar scores)
- Maternal (hemoglobin and labs including blood, urine, and stool, described above)
- Placenta assessment (weight, length, height, proportion classified 'abnormal')
- Malaria (cord, placenta or maternal by blood smear and PCR)

Definitions of study variables

Exposure

Malaria: The assessment of malaria during pregnancy has important implications. While there are several documented types of malaria infection (*P. falciparum, P. vivax*, *P. ovale,* and *P. malariae)*, *P. falciparum* is most closely associated with adverse pregnancy outcomes and the most prevalent in Sub-Saharan Africa. Studies have shown that in most areas of Africa more than 80% of women testing positive for malaria were infected with *P. falciparum* [81,82].

Women were tested for peripheral parasitemia during pregnancy as well as at delivery for placental malaria infection. These both may be assessed using microscopy or PCR techniques. PCR is generally considered to be the gold standard, demonstrating greater sensitivity to detect malaria parasitemia, while misclassification is more common with blood smear techniques. However, blood smear may provide a measure of clinically meaningful malaria infection as it detects only higher parasitemia levels, and to date is more feasible in field settings [83-85]. Accurate testing is important as pregnant women with malaria are often asymptomatic, especially those residing in malaria-endemic regions; however, recent studies have also suggested that there may be sub-groups of women, with particular co-morbidities, who are symptomatic during pregnancy [83-85].

For this study, malaria exposure was assessed at antenatal care visits (peripheral) and at delivery (placental, cord and maternal). Malaria was assessed at a KEMRI lab on-site in Kenya by microscopy using the standard Gibson stain (thick and thin slices). Parasitemia density was counted and also categorized as asexual and gametocytes. Additionally, samples were analyzed using PCR-ligase detection reaction/fluorescent microsphere assay at the CWRU laboratory for *P. falciparum, P. malaria and P. vivax,* as previously described [86]. For analyses of the primary outcome, peripheral malaria *P. falciparum* infection at the antenatal care visit and at delivery will be defined using the CWRU PCR results. Polymerase chain reaction (PCR) has been shown to be the gold standard and is able to detect malaria parasitemia that are not detected by blood microscopy [83,84]. For quality control, a sub-set of the 25 samples were reviewed by an independent lab (University of North Carolina at Chapel Hill) (Table 3-2). With UNC as gold standard, the CWRU-PCR had 78% sensitivity and 88% specificity.

Furthermore, we also compared the microscopy results to the PCR-results for the samples with both results available. The sensitivity and specificity of the microscopy, using the CWRU PCR-test as a gold standard was 22% and 99% respectively, as shown in a sub-sample with both types of diagnostic tests available (Table 3-3).

Hookworm and other soil-transmitted helminthes (STH): Maternal stool samples were collected at ANC and delivery. Stool samples were then evaluated for presence and number of eggs per gram by specific organism hookworm (*Ancylostoma duodenale*) and other STH (*Ascaris lumbricoides*, *Trichuris trichuria, Strongyloides stercoralis)*.

Urogenital schistosomiasis: Maternal urine samples were collected and measured as eggs/mL to assess presence of schistomiasis and intensity of infection. Urine samples were evaluated for presence of urogenital schistosomiasis (*Schistosomiasis haematobium).*

Neonatal outcomes and covariates

Gestational age: Gestational age was determined at enrollment using ultrasound measurement and date of last menstrual period. The study team at CWRU validated these estimates.

Fetal anthropometrics**:** Fetal measurements included head circumference, abdominal circumference, biparietal diameter (BPD) and femur length to generate fetal weight using Hadlock's formula [87]. At delivery, within 24 hours of birth, birth weight, head circumference and length were obtained by trained study staff, with 2 measurements taken and the mean used.

Birth weight: Weight was measured at birth by trained staff using a tray scale within 24 hours of delivery. Weight was measured twice.

Rohrer's Ponderal Index (PI): The PI is calculated as birth weight / birth length cm^3 and is a measure to approximate symmetrical vs. asymmetrical growth patterns. The following cut-points are used to define the newborn growth pattern [88]:

Symmetrical growth restriction: PI = 2.32 – 2.85

Asymmetrical growth restriction: P<2.32

Fetal anemia: Hemoglobin levels were measured at delivery from the cord. Fetal/neonatal anemia was defined as cord hemoglobin < 12.5 g/dL, corresponding to 2 standard deviations below the mean level in developed countries [89].

Maternal outcomes and covariates

Anemia: Hemoglobin levels were measured at antenatal care visits and at delivery by Coulter counter (Beckman Coulter Inc.). Maternal anemia is defined as a hemoglobin level < 11 g/dL [43,90].

Anemia was also categorized by severity according to guidelines:

None to mild: Hb >=9 g/dL

Moderate to severe anemia: Hb< 9 g/dL

Maternal body mass index (BMI): A measure of maternal weight divided by $height^2$, the calculated BMI, adjusted for gestational age, was defined as an indicator of maternal nutritional status. Maternal weight was taken at the prenatal care visit by trained staff and height measured at the initial visit. While low BMI *pre-pregnancy* BMI is defined as <18.5

kg/m^2 (per Institute of Medicine [91]) and pre-pregnancy obesity for women is defined as BMI > 30 kg/m^2, in this study BMI was taken at the first ANC visit (approximately 20-32 weeks gestation), thus we defined low-BMI as the lowest percentile (less than or equal to the 10th percentile for the gestational age at which it was taken). The median BMI ranged from 23.9 kg/m^2 to 25.3 kg/m^2 with the range of 10th and 90th percentiles summarized in Table 3-4. Further description of the analyses to define this variable is included in Appendix 1; however, overall, these results suggest that this was a relatively homogenous group regarding BMI status, with most being relatively thin and no women considered to be obese.

Additionally, we conducted exploratory analyses to determine whether women with the low BMI were at increased relative risk of infection, compared to women without low-BMI (Table 3-5). Based on unadjusted analyses, low-BMI (10th percentile) did not appear to be an important risk factor for the main infections of interest for the study analyses (malaria, hookworm, and schistosomiasis).

Primigravid: Defined as a woman in her first pregnancy, a potential risk factor for adverse birth outcomes and generally associated with increased risk of malaria infection.

Preventive treatment in pregnancy (IPTp): Preventive treatment of women with SP beginning in the second trimester was standard of care and all women in the study received the recommended 2 doses. IPTp prior to the first ANC visit was self-reported and whether it was provided at the visit was also collected.

Table 3-6 summarizes the key exposure and outcome variables as they were defined for this study. The primary exposure and outcome variables were obtained at two time

points, at the first ANC visit and, for a sub-set of women with term births who delivered at the study hospital (n= 501), at delivery for the mother, and the neonate.

Data analyses

Statistical analyses were performed in SAS 9.3 (SAS Institute, Cary, NC). Descriptive statistics (means, standard deviations, and frequencies) were computed for study variables. Based on evaluation of the variables, co-variates were categorized or collapsed into dichotomous variables for analyses. The potential confounders and effect measure modifiers were identified from literature review [See Chapter 2]. Finally, multivariate linear and log-risk models were developed to assess the association of malaria on the study outcomes, birth weight and anemia.

Data analyses for Aim I. Maternal anemia and birth weight

To evaluate the relationship of malaria at ANC with birth weight, descriptive analyses were performed to determine the association between potential confounders and malaria and birth weight (identified through literature review and directed acyclic graph, Figure 3-4). Linear and log binomial regression models were fitted to estimate risk ratios (RR) and 95% confidence intervals (CI) for estimated fetal and newborn anthropometrics, before and after adjusting for potential confounders. Based on previous studies, the potential modifying effects of maternal BMI and primagravida status were evaluated [11]. A p-value of <0.15 for the interaction term was selected to indicate statistical significance. Additional potential confounders evaluated were socio-demographic status (household expenditures,

educational level, maternal age), and bednet use. A backward elimination strategy was used to fit the multivariate models for birth weight. Ultrasound measurements were stratified by 3-week gestational age groups (18-35 weeks gestation) and compared malaria infected to not infected women.

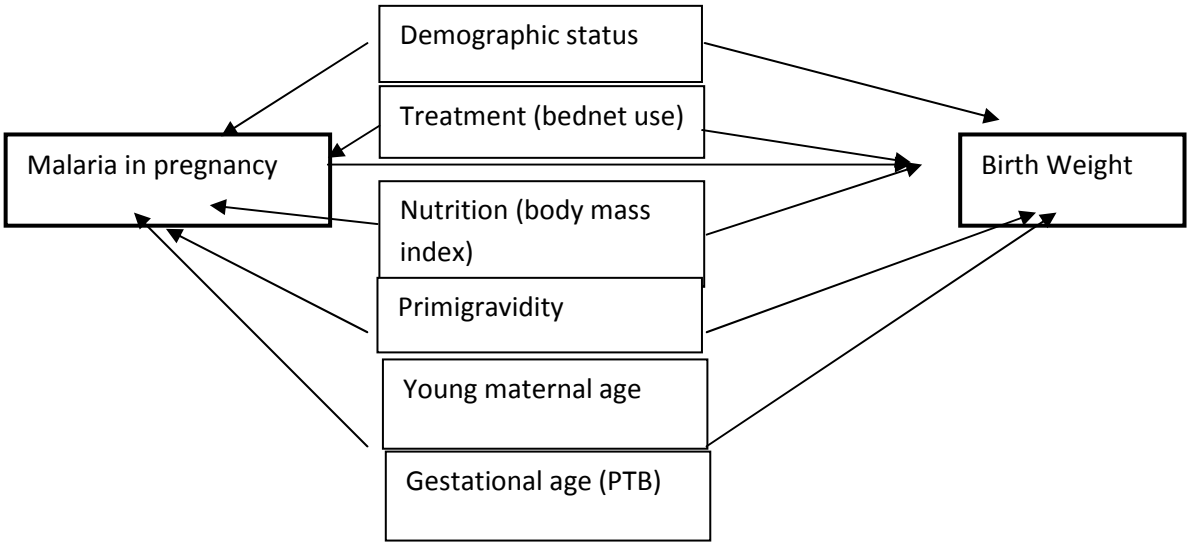

Figure 3-4 Directed Acyclic graph: Malaria and birth weight

Data analyses for Aim II. Maternal malaria, hookworm and anemia

This analysis explored the associations of malaria and hookworm at the first antenatal care visit with moderate/severe maternal anemia, considering other parasitic infections associated with maternal anemia, maternal under-nutrition (as measured by low BMI), and other potential confounders (identified through literature review and directed acyclic graph, 3-5).

Primigravidity, maternal age, maternal education, bednet use, and marital status were evaluated as potential confounders, based on previous research [See Chapter 2] [92]. Confounders with a change estimate >10% were kept in the final log-risk model to evaluate the contribution of hookworm, malaria, and other infections to maternal anemia. Furthermore, maternal anemia in pregnancy was adjusted for gestational age at measurement. The estimated risk ratios for maternal anemia for each infection were estimated, with and without the potential confounders. Finally, a log-risk model was developed to estimate the association of the common infections (*P. falciparum* malaria, hookworm, schistosomiasis, *T. trichuria*) and moderate/severe maternal anemia.

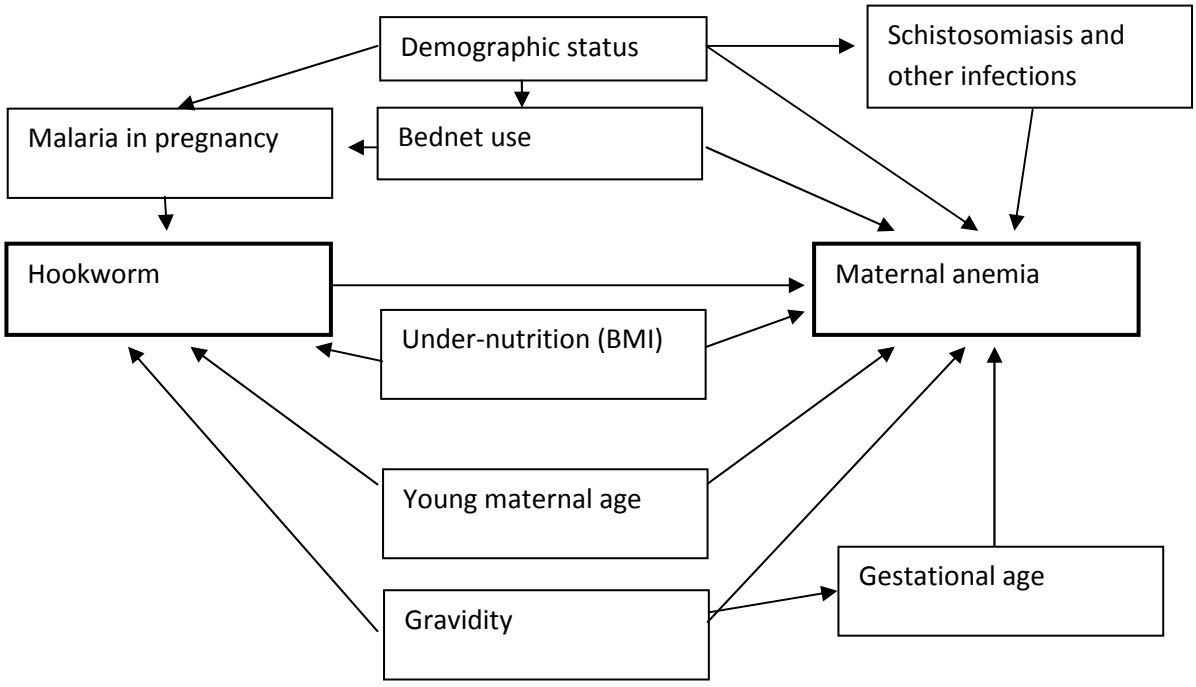

Figure 3-5. Directed acyclic graph: Infection in pregnancy and anemia

3.4 Sensitivity analyses for missing birth outcome data

For the study, as described, women who had term births (n = 501/799 or 63% of those enrolled at ANC) were subsequently enrolled for the parent study, whose goal was to assess effect of fetal immunity. Thus, for the present study, the association of malaria and reduced birth weight associated with preterm birth was unable to be assessed, only birth weight associated with intra-uterine growth restriction among term births. Since there were a significant proportion (n=298 or 37%) of women enrolled at antenatal care but whose infants were not enrolled at delivery for various reasons (including delivering outside the catchment hospital, preterm birth, stillbirth, voluntary withdrawal or other unknown reasons) (shown in Figure 3-3), we examined the demographic variables at antenatal care for those who delivered vs. who did not deliver at the study hospital for demographic variables with key study variables displayed (Table 3-1). Of those who were PCR- malaria-positive at ANC, 38% (n=23) had missing delivery data, while for those who were PCR-malaria negative at ANC, 38% (n=275) also had missing data at delivery. While there were slightly reduced rates of anemia at ANC ($p<0.05$), other demographic variables did not differ significantly between those women with outcomes obtained at delivery at the study hospital compared to those without data at delivery available. One important limitation is that the specific reason for lack of delivery outcomes (e.g., preterm birth, fetal death, lost to follow-up) was unavailable.

Additionally, a sensitivity analyses was conducted to examine the possible range of differences in mean birth weight for those with PCR-malaria vs. no PCR-malaria at antenatal

care, but with missing birth weight. As a first step, the median, 10th, 25th and 75th percentiles birth weights were calculated for those with birth weight measurements available, stratified by PCR-malaria status as follows:

- PCR-malaria positive:10th percentile 2300 g; 25th 2600 g; 75th 3250
- PCR-malaria negative: 10th percentile 2400 g; 25th percentile 2750 g; 75th 3750

Second, the sensitivity analyses were conducted to assess the impact of missing data for the 38% without birth weight (38% of both PCR-positive and PCR-negative groups were missing) under different assumptions for the value of missing birth weight to evaluate the potential ranges of birth weight differences. As shown in Table 3-8, at the most extreme, assuming all missing PCR-positive birth weights were at the 10th%ile and PCR-negative birth weights were at the 75%ile, there would have been mean difference of -907 g (95% CI -983, -831 g). Similarly, the opposite extreme, finds a similar magnitude of difference with malaria-positive women having approximately an 856 g mean higher birth weight. If both groups with missing birth weight data had had the actual median birth weights for PCR-positive and negative, PCR-malaria exposure would have had a non-significant impact on birth weight, mean difference 32 g (95% CI -34, 98 g), as was the actual scenario (described in subsequent chapters).

Table 3-1 Demographics of women who delivered at the study hospital vs. birth outcome not recorded

	Delivered at study hospital N (%)	Delivered elsewhere N (%)	Chi^2 p-value
Maternal Age			0.08
< 20 yrs	171 (38.3)	141 (37.1)	
≥20 yrs	276 (61.7)	239 (62.9)	
Gravidity			0.1
Primigravida	118 (26.5)	81 (21.5)	
Multigravida	296 (78.5)	239 (62.9)	
Used bednet past 3 mos			0.4
No	115 (26.0)	103 (27.1)	
Yes	327 (27.0)	277 (72.9)	
Moderate/severe Anemia			0.01
No	286 (74.9)	237 (71.0)	
Yes	96 (25.1)	97 (29.0)	
PCR-malaria			0.1
No	45 (10.1)	42 (11.3)	
Yes	398 (89.9)	328 (88.7)	
Hookworm			0.4
No	257 (76.5)	242 (75.4)	
Yes	79 (23.5)	79 (24.6)	

Table 3-2 Comparison of CWRU-PCR to UNC-PCR for *P. falciparum* malaria parasitemia detection

	UNC- Positive	UNC-Negative	Total
CWRU – Positive	7	1	8
CWRU – Negative	2	15	18
Total	9	16	25

Table 3-3. Sensitivity and specificity of microscopy (MS) compared to CWRU PCR for *P. falciparum* malaria parasitemia detection

	Pf-MS Positive	*Pf*-MS Negative	Total
Pf- PCR Positive	13	46	59
Pf- PCR Negative	5	368	373
Total	18	414	432

Table 3-4 Maternal Body-Mass-Index (BMI) by Gestational Age (GA) at Measurement

GA, weeks	N (%)	BMI, Median	BMI, 10^{th} percentile	BMI, 90^{th} percentile
18-20	64 (9)	24.3	19.5	30.1
21-23	92 (13)	23.9	20.4	28.2
24-26	135 (19)	24.9	20.7	29.9
27-29	182 (26)	24.5	20.7	29.7
30-32	140 (20)	25.3	20.8	30.2
33-35	89 (13)	24.4	21.3	31.4

Table 3-5. Relative risk of low BMI by infection (RR and 95% CI)

	Low BMI, RR (95% CI)	P-value
PCR-Malaria	0.8 (0.4, 1.7)	0.4
Microscopy-malaria	1.5 (0.5, 4.3)	0.5
Hookworm	0.8 (0.5, 1.4)	0.5
Hookworm >100 eggs/g	0.7 (0.9, 5.7)	0.7
Schistosomiasis	1.2 (0.8, 1.9)	0.4

Table 3-6. Summary of key exposure and outcome study variables

Variable	Description	Defined for study
Antenatal Care		
P. falciparum malaria	PCR-positive	Dichotomous
Hookworm	Positive/negative -	Dichotomous
	Continuous (eggs/g)	Burden - dichotomous (≥ 100 eggs/mL)
Other parasitic infection *(T. trichuria, S. stercoralis, A. lumbricoides)*	Positive/negative -	Dichotomous
	Continuous (eggs/g)	Burden - dichotomous (≥ 100 eggs/g)
Schistosomiasis (*S.haematobium)*	Positive/negative -	Dichotomous
	Continuous (egg/mL)	Burden - dichotomous (≥ 50 eggs/mL)
Table 3-5 continued		
Body-mass-index	Kg/m^2 as continuous variable	Low BMI defined as lowest 10%ile for GA
Ultrasound measurements	Biparietal diameter, femur length, head circumference, estimated fetal weight	Continuous
Maternal anemia	Hemoglobin (Hg) - g/dL	Anemia – Hb < 11.0 g/dL Moderate/severe< 9.0 g/dL
Delivery		
P. falciparum malaria	PCR-positive	Dichotomous
Hookworm	Positive/negative -	Dichotomous
	Burden of infection (eggs/mL)	Moderate burden - dichotomous (> 100 eggs/mL)
Schistosomiasis	Positive/negative - Burden of infection (egg/mL)	Dichotomous
Birth weight	Continuous (grams)	Continuous
Anthropometrics	Length, head circumference	Continuous
Maternal anemia	Hemoglobin (Hg) - g/dL	Anemia: Hb < 11.0 g/dL Moderate/severe: Hb < 9.0 g/dL
Fetal anemia	Cord hemoglobin (Hg) - g/dL	Anemia: Hb < 12.5 g/dL

Table 3-7. Difference in birth weight (Mean, g, and 95% CI) with imputed missing birth weights using available birth weights, by PCR negative vs. PCR positive malaria*

	Assumptions about missing birthweight (BWT) for PCR-malaria negative Mean difference, 95% CI			
Assumptions about BWT for PCR-malaria positive	10th percentile	25th percentile malaria-negative BWT	Median malaria-negative BWT	75th percentile malaria-negative BWT
10th percentile	31 (-34, 97)	-428 (-486, -371)	-668 (-734, -602)	-907 (-983, -831)
25th percentile	357 (307, 407)	-128 (-186, -71)	-368 (-433, -302)	-607 (-683, -531)
Median	606 (611, 718)	271 (214, 329)	32 (-34, 98)	-207 (-283, -131)
75th percentile	856 (806, 906)	665 (611, 718)	281 (216, 318)	43 (-34, 118)

*values imputed for those with missing birthweight (n= 303) based on malaria status at ANC

CHAPTER FOUR

P. FALCIPARUM MALARIA AND BIRTHWEIGHT

Introduction

Malaria in pregnancy causes low birth weight, a major contributor to neonatal mortality and morbidity worldwide [1,2,93,94]. In Sub-Saharan Africa, where malaria is endemic, malaria in pregnancy may contribute to 25% of low birth weight [95]. Risk of decreased birth weight is generally highest among primigravida women, who may experience more severe clinical illness associated with malaria infection [1,2]. Additionally, while maternal under-nutrition as determined by low body-mass index (BMI) has been associated with low birth weight [96,97], a recent study suggested an interaction between maternal under-nutrition and malaria [60]. The study, conducted in the Democratic Republic of Congo (DRC), found that among those women with malaria during pregnancy, intra-uterine growth restriction (IUGR) and birth weight were significantly reduced among women with under-nutrition [60].

In addition to the relation between maternal characteristics and malaria on birth weight, recent studies point to a role of the timing of malaria infection [13,23,60,98]. Although few studies have evaluated early infection and birth weight, some have suggested that first or second trimester infection increases risk of low birth weight compared to later

infection [98]. However, other studies have found malaria infection late in pregnancy, when fetal weight gain is most rapid, correlated with decreased birth weight [13,23].

Studies of malaria in pregnancy utilizing ultrasound, a method to establish gestational age and fetal growth, have been limited [60,99-101]. In addition to evaluation of fetal growth, ultrasound assesses uterine and umbilical blood flow, both of which may be impaired by malaria in pregnancy [100]. With limited availability of routine ultrasound in Sub-Saharan Africa, few reports have described fetal growth and impairment associated with malaria by ultrasound. One study of 3,779 pregnant women from the Thai-Burmese border found that early malaria infection (<24 weeks) was associated with decreased biparietal diameter (BPD).[100] Another recent study in Tanzania, found that early malaria infection was associated with reduced third trimester fetal growth [101]. Additionally, in DRC, ultrasound evaluations established IUGR among women with malaria [60] and significantly increased uterine and umbilical artery resistance among malaria-positive compared to malaria-negative pregnant women [99]. Umbilical artery resistance has been associated with impaired fetal growth and intra-uterine growth restriction [102].

Given the adverse birth outcomes associated with malaria and the effective treatment now available, in 2004 the World Health Organization (WHO) recommended intermittent preventive treatment with two doses of SP (IPTp-SP) beginning the second trimester for pregnant women residing in malaria-endemic areas [5]; more recent WHO guidelines recommend monthly SP treatment [42]. With the increased SP availability, many countries in Sub-Saharan Africa have implemented programs based on the IPTp-SP strategy and evaluation efforts have been undertaken [26, 27,103]. However, a better understanding of factors related to the impact of malaria on birth outcomes is needed to

optimize public health programs. We therefore sought to explore the impact of malaria in pregnancy on fetal growth and newborn outcomes among a cohort of women enrolled in an IPTp-SP treatment program in a malaria-endemic region.

Materials and methods

Study population and recruitment

From 2006 to 2009, the study team recruited pregnant women who attended antenatal care (ANC) at Msambweni District Hospital, Msambweni, Coast Province, Kenya, a rural area where malaria is endemic [67]. Per Kenya Ministry of Health national policy, women received IPTp beginning in the second trimester as well as iron, folic acid, and bed nets as part of routine care. At the first ANC visit, consented HIV-negative women were tested for peripheral malaria, demographic information was obtained, and a physical examination and an ultrasound examination were performed. Pregnant women with known medical disorders contributing to fetal growth restriction, placental dysfunction, twin pregnancy, and prematurity were excluded. Participating women who delivered a term, live infant at the Msambweni District Hospital had maternal venous, placental and cord blood tested for hemoglobin level and malaria parasites. Neonatal anthropometric measurements were obtained within 24 hours of delivery including birth weight, head circumference and length. All women provided written, informed consent. The study, part of a larger study on fetal immunity to malaria, was approved by the Institutional Review Boards at Kenya Medical Research Institute, Case Western Reserve University, and the University of North Carolina Chapel Hill.

Measurements

All anthropometric measurements were obtained by trained study staff. Maternal BMI was calculated based on maternal weight and height obtained at the first antenatal care visit (kg/m^3). Because a pre-pregnancy BMI was unavailable, to explore the potential effects of under-nutrition, BMI less than the 10 percentile for the gestational age at ANC was calculated and defined as low BMI. Ponderal index (PI), the ratio of infant length to weight, was also calculated with PI <2.32 defined as asymmetrical growth.

Ultrasound examinations were performed using a SonoSite 180 Plus ultrasound machine (SonoSite FujiFilm, Bothell, WA). Fetal biometry was performed according to standard techniques for determination of fetal gestational age and weight. Right and left uterine arteries were interrogated using standard techniques. Notching of uterine arteries was noted in pregnancies greater than 25 weeks gestation. Umbilical artery interrogation was performed on a free flowing segment of umbilical cord.

Presence of malaria parasitaemia was determined by polymerase chain reaction (PCR)-ligase detection reaction/fluorescent microsphere assay as previously described [86]. For purposes of this study, analyses of malaria were restricted to *P. falciparum,* the species most commonly associated with adverse birth outcomes [2].

Data analyses

All analyses were performed in SAS version 9.3 (SAS Institute, Cary, NC, USA). Descriptive analyses were performed with chi-square and t-tests to evaluate differences in estimates. Linear and log binomial regression models were fitted to estimate risk ratios (RR)

and 95% confidence intervals (CI) for estimated fetal and newborn anthropometrics, before and after adjusting for potential confounders. Based on previous studies, the potential modifying effects of maternal BMI and primagravida status were evaluated. A p-value of <0.15 for the interaction term was selected to indicate statistical significance. Additional potential confounders evaluated were socio-demographic status (household expenditures, educational level, maternal age), and bednet use. A backward elimination strategy was used to fit the multivariate models for birth weight. Ultrasound measurements were stratified by 3-week gestational age groups (18-35 weeks gestation) and compared malaria infected to not infected women.

Results

Of the 813 women screened at antenatal care (ANC), 799 provided initial demographic information and were tested for malaria; 676 had an ultrasound examination at the first ANC visit between 18 and 35 weeks and were included in analyses of fetal measurements. Of these, 463 delivered a term, singleton, live birth in the study hospital.

The median gestational age at the first visit was 27 weeks (IQR 24, 31 weeks). Demographic characteristics and relative risk analyses are presented in Table 4-1 for women with and without *P. falciparum* infection at their first ANC visit. Overall, 87 (11%) of women tested positive for *P. falciparum* malaria by PCR. In unadjusted analyses, women who were <20 years of age and primigravida had a higher risk of *P. falciparum* (RR 2.10 95% CI 1.38, 3.18 and RR 1.68 95% CI 1.11, 2.54, respectively), while the socio-economic status (as measured by household expenditures), marital status, and educational status were not significantly associated with malaria. Finally, women who were not using bednets

prior to ANC were more likely to be malaria positive (RR 1.71, 95% CI 1.14, 2.55). Women who did not have malaria treatment prior to ANC had a relative risk of 1.68 (95% CI 0.76, 3.74) of being malaria positive at the first ANC visit. Women with moderate or severe anemia (hemoglobin <9 g/dl) had a higher relative risk of exposure to malaria. The median BMI was 24.2 (IQR 22.2, 26.9) and similar for the malaria-positive women (23.9, IQR 22.1, 26.0). Finally, we examined clinical symptoms of febrile illness and only 4 of the women who were malaria-positive had illness (data not shown).

Analyses adjusted for parity and stratified by gestational age to examine the association of concurrent *P. falciparum* status and fetal growth estimated by ultrasound are reported in Table 4-2. The mean adjusted estimated fetal weight measurements were generally lower for fetuses exposed to *P. falciparum* positive compared to negative mothers; however, except at 30-32 weeks' gestation, these differences did not reach statistical significance. At 30-32 weeks, the fetal weight, head and abdominal circumference were significantly lower ($p<0.05$) for *P. falciparum* positive mothers, while femur length measurements were similar between the groups across all gestational ages (Table 4-5, supplemental tables).

Additionally, we examined measures of uteroplacental blood flow for malaria-positive vs. negative women, adjusted for primigravid status. Umbilical artery resistance index (RI) was higher among the *P. falciparum* positive, compared to negative fetuses, with statistically difference measurements ($p<0.05$) detected at ultrasound available at or prior to 26 weeks gestation (Table 4-2). No statistically significant differences in umbilical artery pulsatility index (PI) or systolic/diastolic (S/D) ratio were found nor were statistically significant

differences in uterine artery notching, RI, PI, or S/D ratio between the groups found (data not shown).

Next, we examined birth anthropometrics and hemoglobin levels for live, singletons. Compared to women whose births were excluded (due to delivery outside the study hospital, voluntary discontinuance of study participation, lost to follow up, premature delivery, or non-collection of samples and measurements), women whose births were included were comparable on incidence of malaria, education level, gravidity, age, and household expenditures to those whose births were excluded from the study, and maternal severe or moderate anemia. However, enrolled women who delivered a live birth in the study hospital were significantly less likely to have a low BMI percentile ($p<0.05$), compared to women whose birth data were not available.

Of women with a live, term birth at the study hospital (n=463), 8% were delivered by cesarean section. Overall, 54.7% of the neonates were male and the median gestational age was 39.0 weeks (IQR, 37.9, 39.9), neither of which differed by malaria status. In a multivariate regression analyses, maternal factors which remained significantly associated (at $p<0.05$) with reduced birth weight were primigravidity, young maternal age (<20 years), and low BMI. Because of the collinearity between primigravidity and young age, only parity remained in the initial model. Anthropometric measurements for newborns of women with *P. falciparum* detected at the first ANC visit or at delivery were similar to those without *P. falciparum* in both adjusted and unadjusted analyses (Table 4-3). While the incidence of malaria at delivery was slightly lower (10%) than at first ANC visit (11%), women who were malaria-positive at first ANC visit were at higher risk of having any malaria at delivery (placenta, cord or peripheral samples) (aRR 2.1, 95% CI 1.0, 4.4, $p = 0.05$).

Finally, we examined the role of maternal BMI ($\leq 10^{th}$ percentile, adjusted for gestational age) and malaria. Low BMI had a significant interaction (p=0.06) with *P. falciparum* status and thus an interaction term was included in the linear regression model to evaluate the association of malaria with birth weight. In the model for term births, adjusted for gravidity, malaria was not significantly associated with lower birth weights among women with normal BMI, but was associated with a decreased birth weight among women with low BMI (-370 g, 95% CI –728, -12, p=0.04) (Table 4-4).

Discussion

Approximately 11% of women were positive for malaria at ANC and, similar to previous studies, risk of malaria was associated with primigravida status and younger maternal age [1]. We also found a significantly higher risk of malaria among women who did not use bednets. In the region, distribution of free insecticide-treated bednets in addition to the IPTp-SP program were active during the study period and likely contributed to relatively low burden of malaria infection, compared to earlier studies. In contrast, in a study from the same region of Kenya conducted from 2000 through 2005 - prior to widespread adoption of the WHO IPTp-SP guidelines - 42% of women were malaria-positive at time of delivery [67].

This is one of the few studies to evaluate concurrent malaria with ultrasound measurements of fetal growth and uteroplacental blood flow. While modest, the estimated fetal anthropometrics suggested potential reduced growth associated with malaria, especially late in the second trimester when fetal growth is most rapid. There was also a

trend for higher umbilical artery RI among women with malaria, consistent with a recent study, which found that early malaria parasitaemia was associated with increased umbilical artery RI among primigravida, but not multigravida women [99]. Increased umbilical artery RI has been associated with intrauterine growth restriction in previous studies [10].

In this population treated with IPTp-SP, malaria detected by PCR at ANC did not have a significant impact on birth weight or other anthropometric measurements in this cohort of live term births. However among those women with the lowest BMI, malaria was significantly associated with reduced birth weight. While factors leading to low BMI are complex, in this study, low BMI appeared to be the maternal factor most highly associated with reduced birth weight. A previous study conducted in the DRC found a similar trend, with significant impact of malaria primarily among those with indicators of under-nutrition [60].

The widespread distribution of bednets, even prior to enrollment which was associated with significant decreased risk of malaria at ANC as well as the provision of IPTp-SP likely contributed to decreased risk for repeat or severe malaria infection, consistent with previous studies [41]. Both of these confirm the important role of treatment programs in reducing the prevalence of malaria in pregnancy.

A limitation of this study was that the women who were seen at ANC but subsequently delivered outside the study hospital or who did not deliver a live, term birth were excluded. Thus, we were unable to determine the contribution of malaria to preterm birth or other potentially adverse pregnancy outcomes. While the women excluded had higher rates of low BMI, they did have comparable malaria rates to those with birth

outcomes. Another consideration is that we evaluated malaria detected by PCR, rather than women with febrile illness or repeated infections, and thus these findings are limited to the impact of potential modest malaria exposure on birth outcomes. However, even among women who received among the best care and had optimal outcomes, malaria and under-nutrition was found to be associated with reduced birth weight.

Conclusions

Risk of malaria at delivery was associated with presence of malaria at first ANC visit, which was reduced among those using bednets. We found that malaria detected by PCR at ANC was associated with only a modest reduction in fetal growth that with IPTp and by term birth, no difference in birth weights was found. This suggests that with good preventive care, the impact of exposure to malaria on fetal and newborn growth may be minimized. However, maternal BMI was highly associated with birth weight, and among women with lowest BMI, the impact of malaria was more pronounced. Additional research and programs to improve maternal nutritional health may be important to important to further improving birth outcomes in low-resource settings. Taken together, these results suggest that mild malaria infection detected at first ANC, among women given IPTp-SP and who delivered at a study hospital, was not associated with significant adverse outcomes among term births.

Table 4-1. Maternal characteristics and relative risk (95% CI) for *P. falciparum* malaria at first antenatal care (ANC) visit, Kenya cohort, 2006–2009

	Total N* (%)	*P falciparum* n (%)	RR (95% CI) †
Maternal Age			
20-45	658 (82.3)	60 (9.1)	Referent
< 20	141 (17.7)	27 (19.2)	2.10 (1.38, 3.18)
Formal education			
No education	163 (20.6)	17 (10.4)	Referent
Primary education	526 (66.4)	58 (11.0)	1.05 (0.63, 1,76)
Secondary/higher level	103 (13.0)	11 (10.7)	1.11 (0.40, 3.11)
Low household income			
No	299 (36.8)	27 (9.0)	Referent
Yes	514 (63.2)	60 (11.7)	1.31 (0.91, 1.88)
Marital status			
Married/partner	698 (87.9)	72 (10.3)	Referent
Widow/divorced/single	96 (12.1)	14 (14.6)	1.41 (0.83, 2.40)
Gravidity			
Multigravida	601 (75.9)	56 (9.3)	Referent
Primigravida	191 (24.1)	30 (15.7)	1.68 (1.11, 2.54)
Bednet use last 3 mos			
Yes	583 (73.6)	54 (9.3)	Referent
No	209 (26.4)	33 (15.8)	1.71 (1.14, 2.55)
Malaria treatment last 3 mos			
Yes	88 (11.1)	6 (6.9)	Referent
No	706 (88.9)	81 (11.5)	1.68 (0.76, 3.74)
Maternal anemia			
Hemoglobin $\geq$ 9	506 (73.3)	38 (7.5)	Referent
Hemoglobin < 9	184 (26.7)	21 (11.4)	1.38 (0.95, 1.99)
Body mass index, median (IQR)	24.2 (22.2, 26.9)	23.9 (22.1, 26.0)	--

*Differences in numbers due to missing data
†Unadjusted risk ratio (RR) and 95% confidence interval (CI)

Table 4-2. Estimated fetal weight and umbilical resistance index stratified by gestational age (GA) and concurrent *P. falciparum* at first antenatal care visit, Kenya cohort, 2006–2009.

		Estimated Fetal Weight*			Umbilical Resistance Index (RI)		
GA at measure	Concurrent malaria† (N)	Mean‡, grams	Mean Difference (95% CI) ‡	p-value	Mean‡ RI	Mean Difference (95% CI) ‡	p-value
18-20 wks	Positive (N=6)	277	-37 (-86, 15)	0.15	0.82	0.042 (0.0098, 074)	0.01
	Negative (N=45)	314			0.78		
21-23 wks	Positive (N=8)	522	16 (-45, 78)	0.61	0.78	0.025 (-0.044, 0.093)	0.5
	Negative (N=85)	506			0.75		
24-26 wks	Positive (N=16)	729	-23 (-84, 38)	0.46	0.75	0.031 (-0.0016, 0.064)	0.06
	Negative (N=118)	815			0.72		
27-29 wks	Positive (N=25)	1190	-33 (-94, 27)	0.28	0.70	0.011 (-0.017, 0.042)	0.3
	Negative (N=160)	1223			0.69		
30-32 wks	Positive (N=12)	1683	-126 (-237, -14)	0.03	0.67	0.015 (-0.022,0.053)	0.4
	Negative (N=121)	1809			0.66		
33-35 wks	Positive (N=7)	2344	-16 (-172, 141)	0.84	0.68	0.029 (-0.022,0.081)	0.4
	Negative (N=73)	2359			0.66		

*Hadlock's formula for estimation of fetal weight, grams (27) †*P falciparum* malaria at antenatal care

‡Estimated mean and mean differences, adjusted for primagravida status

Table 4-3. Birth outcomes and malaria at delivery by *P. falciparum* malaria at antenatal care, Kenya cohort, 2006–2009

	Malaria status* (N**)	Adjusted Mean†	Mean Difference (95% CI) †	p-value
Gestational age (wks)				
	Positive (N=43)	38.8	-0.06 (-1.2, 0.9)	0.8
	Negative (N=388)	38.9		
Birth weight (g)				
	Positive (N=43)	3072	-5 (-131, 121)	0.9
	Negative (N=422)	3077		
Infant length (cm)				
	Positive (N=43)	49.0	0.2 (-0.9, 1.2)	0.7
	Negative (N=417)	48.8		
Head circumference (cm)				
	Positive (N=43)	34.5	0.05 (-0.7, 0.8)	0.9
	Negative (N=417)	34.4		

* *P. falciparum* malaria status at antenatal care visit
† Mean and mean difference from linear regression model, adjusted for primagravida status

Table 4-3. Birth outcomes and malaria at delivery, continued

		N** (%)	aRR (95% CI) ‡	p-value
Ponderal index<2.32 (Kg/cm^3)				
	Positive (N=43)	7 (16)	0.96 (0.47, 1.95)	0.9
	Negative (N=422)	69 (16)	1.0	
Cord hemoglobin <12.5 (g/dl)				
	Positive (N=42)	8 (20)	1.15 (0.57, 2.21)	0.7
	Negative (N=365)	62 (18)	1.0	
Malaria at delivery§ (%)				
	Positive (N=42)	7 (18)	2.1 (1.0, 4.4)	0.05
	Negative (N=365)	29 (8)	1.0	

* *P. falciparum* malaria status at antenatal care visit **Numbers vary due to missing data
† Mean and mean difference from linear regression model, adjusted for primagravida status
‡ Risk ratio and 95% confidence interval from log binomial risk model, adjusted for primagravida status
§ *P. falciparum* malaria at delivery defined as cord, placenta and maternal peripheral determined by PCR

Table 4-4. Associations between birth anthropometrics and maternal malaria, stratified by maternal BMI, Kenya cohort, 2006–2009

	Malaria status* (N)	Adjusted Mean †	Mean Difference (95% CI) †	p-value
Birth weight, g				
BMI ≤ 10%ile	Positive (N = 8)	2658	-370 (-728, -12)	0.04
	Negative (N=26)	3028		
BMI > 10%ile	Positive (N=47)	3161	75 (-91, 241)	0.4
	Negative (N=265)	3087		
Birth length, cm				
BMI ≤ 10%ile	Positive (N = 8)	48.1	-1.3 (-3.6, 2.4)	0.4
	Negative (N=26)	49.0		
BMI > 10%ile	Positive (N=47)	49.1	0.7 (-0.9, 1.4)	0.4
	Negative (N=265)	48.8		
Head circumference, cm				
BMI ≤ 10%ile	Positive (N = 8)	33.7	-0.8 (-3.1, 1.5)	0.4
	Negative (N=26)	34.5		
BMI > 10%ile	Positive (N=47)	34.7	0.2 (-0.9, 1.1)	0.8
	Negative (N=265)	34.5		

**P falciparum* at first antenatal care visit
†Adjusted for primigravida status

Table 4-5. Supplementary ultrasound measurements, Kenya study, 2006-2009.

		Head circumference (cm)				Abdominal circumference (AC)			
		Adjusted±							
GA at measure	Concurrent malaria† (N)	Adjusted Mean±	Mean Difference	95% CI of difference	p-value	Adjusted± Mean	Mean Difference	95% CI of difference	p-value
18-20 wks	Present (N=6)	15.97	-0.65	-2.62, 1.31	0.5	13.77	-0.96	-1.9, 0.0	0.08
	Absent (N=45)	16.62				14.73			
21-23 wks	Present (N=8)	20.21	0.06	-0.79, 0.092	0.9	17.69	0.19	-0.6, 1.2	0.7
	Absent (N=85)	20.15				17.51			
24-26 wks	Present (N=16)	23.24	-0.4	-1.00, 0.200		20.60	-0.28	-0.9, 0.3	0.4
	Absent (N=118)	23.64				20.88			
27-29 wks	Present (N=25)	26.35	-0.11	-0.5, 0.3	0.6	23.75	-0.30	-1.0, 0.5	0.5
	Absent (N=160)	26.46				24.04			
30-32 wks	Present (N=12)	28.58	-0.69	-1.21, -0.16	0.01	26.97	-0.69	-1.4, 0.06	0.06
	Absent (N=121)	29.27				27.66			
33-35 wks	Present (N=7)	31.04	-0.09	-0.65, 0.48	0.8	29.89	-0.15	-1.4, 1.1	0.8
	Absent (N=73)	31.13				30.04			

†*P falciparum* malaria at antenatal care

‡Estimated mean and mean differences, adjusted for primagravida status

		Biparietal diameter (cm)				Femur length (cm)			
GA at measure	Concurrent malaria† (N)	Mean±	Mean Difference	95% CI of difference	p-value	Mean±	Mean Difference	95% CI of difference	P-value
18-20 wks	Positive (N=6)	4.24	-0.29		0.1	2.95	-0.50	-2.06, 1.06	0.5
	Negative (N=45)	4.53				3.44			
21-23 wks	Positive (N=8)	5.42	0.048	-0.21, 0.30	0.7	3.96	0.09	-0.11, 0.29	0.4
	Negative (N=85)	5.37				3.88			
24-26 wks	Positive (N=16)	6.22	-0.05	-0.20, 0.11	0.5	4.76	-0.27	-1.45, 0.91	0.6
	Negative (N=118)	6.27				5.03			
27-29 wks	Postive (N=25)	7.06	0.003		0.96	5.29	-0.14	-0.77, 0.48	0.6
	Negative (N=160)	7.05				5.44			
30-32 wks	Positive (N=12)	7.63	-0.23	-0.40, -0.05	0.01	5.86	-0.11	-0.31, 0.10	0.3
	Negative (N=121)	7.86				5.97			
33-35 wks	Postive (N=7)	8.32	-0.15	-0.32, 0.03	0.09	6.57	0.03	-0.22, 0.27	0.8
	Negative (N=73)	8.47				6.54			

†*P falciparum* malaria at antenatal care

‡Estimated mean and mean differences, adjusted for primagravida status

CHAPTER FIVE

MATERNAL AND NEONATAL ANEMIA

Introduction

Anemia affects nearly 25% of all pregnancies worldwide and more than 40% of those in Sub-Saharan Africa [104]. Defined as hemoglobin <11 g/dL, anemia in pregnancy contributes to maternal morbidities and increased risk for mortality associated with conditions such as post-partum hemorrhage [105-108]. Maternal anemia has also been associated with fetal anemia, which contributes to infant anemia as well as long-term childhood morbidities, including impaired neurodevelopmental outcomes [45, 109-112].

Although anemia is multi-factorial, poor nutrition and infection are common causes. In Sub-Saharan Africa, soil-transmitted helminthes (STH) including hookworm, urogenital schistosomiasis, and other parasitic infections such as malaria contribute to the high anemia rates in women and young children [113-120]. Infection prevalence of up to 50% has been documented in some regions in Sub-Saharan Africa [121]. Estimates suggest that more than 25% of pregnant women are infected with hookworm, which causes intestinal bleeding and blood loss, and has been most commonly associated with anemia [104,122-124]. In a study of parasitic infection in pregnancy conducted in coastal Kenya from 2000 to 2005, about 32% of women were infected with hookworm, 31% with urogenital schistosomiasis (*S.*

haematobium), and almost 43% with malaria (*P. falciparum)*, while more than 46% of women were co-infected [67].

Parasitic infections, including hookworm, may be evaluated by intensity of infection, as measured by the concentration of eggs in the stool. While most morbidity has been seen with high intensity infections, in populations with low iron stores, even low-intensity hookworm infection has been associated with morbidities [125,126]. In addition to hookworm, *P. falciparum* malaria has been shown to increase risk for moderate and severe maternal anemia [113-119]. While urogenital schistosomiasis causes adverse health outcomes including anemia, its association with maternal anemia has been less clearly established [127,128]. Finally, poor nutrition, which contributes to inadequate intake of iron, folate, and other micronutrients, is common in the geographic areas where these parasitic infections are prevalent, and may have an important role in the relationship of infections and anemia [96,129-132].

Many studies have focused on the effects of a single infectious agent on pregnancy outcome and maternal anemia, although a few studies have attempted to understand the relative effects of multiple agents with conflicting results [67,131]. Fetal anemia has been documented in association with maternal anemia, with rates of 10% to 23% reported in recent studies in Malawi [45,108,109], but its association with infection is less well understood.

With preventative treatment during pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) as recommended by the World Health Organization (WHO) in 2004, rates of malaria in pregnancy have decreased [41,42,133]. Thus, other causes of maternal anemia and poor

birth outcomes have become increasingly important. Recent trials show that presumptive hookworm treatment reduces the infection rates in pregnancy, although the impact on pregnancy outcomes such as maternal anemia has varied [134-137]. Where hookworm infection is endemic, the WHO recommends provision of antihelminthic treatment (e.g., albendazole or other treatments safe during pregnancy) in the second trimester [137]. Furthermore, safe, effective treatment is available to treat urogenital schistosomiasis during pregnancy and endorsed by the WHO [139]. However, for various reasons, the WHO recommendations for hookworm and urogenital schistosomiasis treatment during pregnancy have not been widely implemented [140]. In this study, we sought to ascertain the contributions of parasitic infection among a cohort of pregnant women in coastal Kenya to maternal and fetal anemia.

Methods

From 2006 – 2009, pregnant women were recruited for the study at their first antenatal care (ANC) visit at Mswambweni District Hospital, Mswambweni, Coast Province, Kenya. At the first ANC visit, blood, stool and urine samples were collected, in addition to maternal anthropometrics, and basic demographic information. All women diagnosed with helminthes infections were treated with albendazole. Women were not treated for urogenital schistosomiasis during pregnancy, but treatment (e.g., praziquantel) was delayed until after delivery, per standard care in Kenya during the study period. All women enrolled in the study received IPTp-SP, iron, and multi-vitamins per Kenyan national guidelines

All women provided written informed consent prior to study enrollment. Institutional review board approval was received by Case Western Reserve University, Kenya Medical Research Institute, and the University of North Carolina at Chapel Hill.

Measures

Malaria was determined both by microscopy using the standard Gibson stain (thick and thin slices) and by PCR/Ligase Detection Reaction Fluorescent Microsphere Assay as previously described [86] at antenatal care and at delivery for maternal peripheral, cord and placental samples. PCR is considered to have high sensitivity to detect malaria parasitemia; however, microscopy, which is more commonly used in clinical settings, is generally considered reliable to detect malaria in higher concentrations [83]. For this study, we used microscopy as a proxy for higher intensity of malaria infection and PCR-positive malaria as any malaria infection.

Maternal stool and urine sample were collected at the first ANC visit and at delivery. Stool samples were tested for hookworm infection (*Ancylostoma duodenale*) and other STH (*Ascaris lumbricoides*, *Trichuris trichuria, Strongyloides stercoralis)*. STH infections were determined by the presence of intestinal eggs in the stool sample. Burden was also determined by count of eggs/gram. Urine samples were evaluated for presence of urogenital schistosomiasis (*Schistosomiasis haematobium*) and results expressed as number of eggs/mL. Schistosomiasis was also categorized as light (0-<50 eggs/mL) or moderate ($\geq$50 eggs/mL), according to WHO criteria [138].

Hemoglobin (Hb) levels were measured at the first ANC visit and at delivery by Coulter counter (Beckman Coulter Inc.). Women were classified as anemic (Hb < 11 g/dL) and then categorized as being moderately to severely anemic (Hb <9 g/dL) and being mildly to non-anemic (Hb $\geq$9 g/dL) according to the WHO classification of anemia [104]. Cord blood hemoglobin levels were also determined and cord (fetal) hemoglobin defined by hemoglobin <12.5 g/dL, as previously defined [45]. Maternal height and weight were taken at the first ANC visit (generally in the second trimester) and body mass index (BMI) calculated as $kg/m^{2.}$ Since pre-pregnancy BMI was unavailable, to assess BMI, low BMI was defined as the lowest 10^{th} percentile for the gestational age at measurement, which ranged from 19.8 to 20.7 kg/m^2.

Analyses were performed in SAS version 9.3 (SAS Institute, Cary, NC, USA). Descriptive analyses were performed. Parity, gestational age, maternal age, maternal education, and socio-economic status (as measured by monthly household expenditures) were evaluated as potential confounders, based on previous research [119,121-125]. The risk ratios for moderate/severe anemia associated with each of infections evaluated are presented with and without the potential confounders, using a log-binomial regression model. A backward elimination strategy was employed to estimate the adjusted RR of moderate/severe maternal anemia associated with infections and maternal BMI, accounting for the potential confounders.

Results

Of the 813 women screened at ANC, 706 (88%) consented women had blood and urine samples available for anemia, malaria, and schistosomiasis evaluation, respectively.

Of these participants, 544 (71%) provided stool samples at antenatal care for measurement of STHs. The mean gestational age at enrollment was 24.5 weeks (SD 3.8 weeks).

At the first ANC visit, 516 (71%) were anemic (Hb < 11 g/dL) and 190 (27%) had moderate to severe anemia (Hb <9 g/dL). For subsequent analyses, moderate/severe anemia was evaluated as the primary outcome of interest. About 19% of the women were <20 years of age, nearly 88% were married, 12% had no formal education, and about 25% were primagravidas (Table 1). In unadjusted analyses, these factors were not associated with increased risk of anemia. Insecticide-treated bednet use, malaria treatment, and iron/folic acid received 3 months prior to the ANC visit were also not associated with moderate/severe anemia risk. Few women (<2%) received anti-helminth treatment prior to first ANC (data not shown).

The association of demographic characteristics and the prevalence at first ANC of hookworm infection, PCR-positive malaria (*P. falciparum*), and urogenital schistosomiasis (*S. haematobium*) are summarized in Table 2. Risks of *P. falciparum* PCR-positive (RR 2.29, 95% CI 1.38, 3.79), hookworm (RR 1.42, 95% CI 1.02, 1.98), and urogenital schistosomiasis infection (RR 2.25, 95% CI 1.66, 3.07) were higher among those <20 years compared to women $\geq$ 20 years. Risks for infection did not differ significantly by maternal education levels. Primigravidity was associated with increased risk of *P. falciparum* PCR-malaria (RR 1.52, 95% CI 1.11, 2.56) and urogenital schistosomiasis (RR 1.51, 95% CI 1.07, 1.78), but not hookworm infection. About one-fourth (25.7%) of the women reported no use of insecticide-treated bednets (ITNs) prior to enrollment, which was associated with increased risk of malaria infection (RR 2.00, 95% CI 1.23, 3.27), but not other infections.

Hookworm (24%), *P. falciparum* PCR-malaria (8%), urogenital schistosomiasis (17%), and *T. trichuria* (10%) were the most common infections at the first ANC visit. Of women positive for one of these infections, about 10% were co-infected (data not shown). Hookworm intensity ranged from 1 to 1035 eggs/g; thus all were considered 'light' according to the WHO criteria (light defined as <1999 eggs/g). To further evaluate whether relative intensity of infection was associated with outcomes, we also classified the highest intensity of infection (≥100 eggs/g) among the cohort as 'moderate' infection.

We next examined the risk for moderate/severe maternal anemia at ANC associated with these infections, in unadjusted and adjusted analyses (Table 3). In analyses adjusted for gestational age, primagravida status and low BMI, moderate/severe anemia was associated with moderate hookworm infection (aRR 2.53, 95% CI 1.62, 3.92), *P. falciparum* PCR-positive and microscopy positive (aRR 1.45, 95% CI 1.01, 2.08 and aRR1.98, 95% CI 1.17, 3.35, respectively). *S. haematobium* and *T. trichuria*, although common, were not significantly associated with moderate/severe anemia and few women had moderate burden of infection. *S. stercoralis* and *A. lumbricodes* were relatively rare (about 1% or less of the cohort), with no cases of moderate burden; these infections were not significantly associated with moderate/severe anemia at ANC, in adjusted or unadjusted analyses.

For those women who delivered live, term births at the study hospital, we evaluated the association between infection at the first ANC visit with maternal and fetal anemia at delivery, as well as the association of infections detected at delivery for those who had stool (n=210), or urine and blood samples (n=394) available at delivery. Compared to women whose births were excluded (due to delivery outside the study hospital, voluntary discontinuance of study participation, lost to follow up, premature delivery, or non-collection

of samples and measurements), women whose births were included were comparable on socio-demographics (education level, gravidity, age, and household expenditures), maternal characteristics (BMI, anemia) and infection at ANC (malaria, hookworm) to those whose births were excluded from the study (data not shown).

At delivery, 34.2% of the women had moderate/severe anemia and 18.4% of the neonates had fetal anemia (cord Hb <12.5 g/dL). Moderate hookworm burden at the first ANC visit was associated with moderate/severe maternal anemia at delivery (aRR 2.30, 95% CI 1.42, 3.71), but other infections at first ANC visit were not significantly associated with risk of maternal moderate/severe maternal anemia at delivery (Table 4). Fetal anemia was not significantly associated with any of the infections, in adjusted or unadjusted analyses. Of women tested for presence of hookworm, *P. falciparum* malaria (PCR and microscopy) and schistosomiasis at delivery, none of these infections were significantly associated with maternal or fetal anemia at delivery.

We also examined the association of the anemia at ANC with anemia at delivery. Women with moderate/severe anemia at first ANC visit had increased risk of maternal anemia at delivery (unadjusted RR 3.84, 95% CI 2.94, 4.98). Fetal anemia was also associated with moderate/severe maternal anemia at first ANC visit and moderate/severe maternal anemia at delivery (RR 1.58, 95% CI 1.02, 2.45, p=0.05; RR 2.75, 95% CI 1.78, 4.24, p <0.001, respectively) (data not shown).

Finally, in a multivariate regression model to assess the infections identified as risk factors associated with moderate/severe maternal anemia at ANC, moderate hookworm (aRR 2.37, 95% CI 1.44, 3.91, p=0.0007) and *P. falciparum* microscopy-malaria infection

(and aRR 2.06, 95% CI 1.24, 3.44, p =0.005, respectively) remained significantly associated with moderate/severe maternal anemia at ANC, when adjusting for primigravida status and low maternal BMI (Table 5).

Discussion

Few studies have examined the burden of helminthic infection and under-nutrition in pregnancy on maternal and fetal anemia in malaria-endemic regions. This is now especially important in areas with a declining incidence of malaria. In this study of pregnant women at ANC, the prevalence of *P. falciparum* PCR-malaria had fallen to 9% from previous rates of 40% at delivery reported in the same region in Kenya, prior to widespread IPTp-SP [67]. Despite this decline in malaria, 71% of the women studied were anemic, and more than 25% had moderate/severe anemia in pregnancy. Infection with hookworm (24%), and schistosomiasis (17%), which had less significant reductions since the previous study period [67], were also common, although most hookworm infections were light. While PCR-diagnoses is more sensitive to determine malaria infection, we evaluated both microscopy-diagnosed malaria to assess the potential associations with higher-burden parasitemia. Both *P. falciparum* malaria as diagnosed by microscopy and moderate hookworm infections at ANC were associated with moderate/severe anemia at the ANC visit, while urogenital schistosomiasis and trichurisis and light infections were not. This is consistent with previous studies finding an association with anemia among populations with higher intensity parasitic infection [113].

Socio-demographic factors assessed included age, gravidity, education, socio-economic and marital status, and low BMI were not significantly associated with

moderate/severe maternal anemia in this cohort. However, the study was conducted among a relatively homogenous community, and thus these disparities may not have been large enough to be detectable. One limitation was that pre-pregnancy BMI and additional measures of under-nutrition were not available for this cohort and thus a more sophisticated assessment of the relationship of nutritional intake and anemia was not possible. While our findings are also consistent with research suggesting that in the context of low socio-demographic status, even light infections such as hookworm and malaria may be associated with anemia [141]; however, further research is needed to address these relationships.

Maternal anemia significantly affects women and children, especially in low-resource areas where hookworm, malaria, and other parasitic infections, in addition to poor nutritional intake, are common. In this study, maternal anemia was associated with increased risk of fetal anemia. While fetal anemia has been less well studied, emerging research suggests that it may also be common in areas with high-burden of infection [110-112,143,144]. Since fetal and childhood anemia associated with maternal anemia potentially may lead to long-term impaired neurologic function, a better understanding the etiology and effects of fetal anemia is important.

Effective, safe treatments are available to prevent and treat hookworm and malaria, both of which were associated with maternal anemia in this study. While numerous studies have evaluated preventative treatment for malaria in pregnancy, fewer have assessed anti-helminth treatment in the context of malaria treatment. Of those that have assessed hookworm, the results suggested that benefit may be most pronounced among women with higher burden of hookworm infection [124,125,142,143]. Additionally, few studies have evaluated the roles of multiple infections and under-nutrition in pregnancy and interventions.

In a study assessing the role of malaria, hookworm, and nutrition in Uganda, malaria was significantly associated with maternal anemia while hookworm and nutrition were not. The authors speculated that this was in part due to the relatively good nutritional indicators and coverage of helminth treatment in the region, while malaria prevention strategies were limited [142].

In contrast to the Uganda study, in our study, while all women in this study received antenatal care including IPTp-SP for malaria, treatment for hookworm as indicated, and iron/folic acid, most were not enrolled until after 20 weeks gestation. Thus, even with relatively good antenatal care, treatment was not initiated until the second trimester at which time anemia was prevalent in this cohort. Furthermore, unlike the region where this study was conducted and despite the international recommendations, uptake of treatment for hookworm, malaria, and schistosomiasis in ANC is still low in many parts of Africa [138]. In part, this may relate to perceptions that treatment has not been associated with improved pregnancy outcomes or may be harmful [136]. Given the high prevalence of anemia seen in our study and elsewhere and the relationship between maternal and fetal anemia, further research is needed to optimize interventions around pregnancy to reduce anemia and ultimately improve maternal and newborn health.

Table 5-1. Maternal socio-demographic factors by maternal anemia status at first antenatal care visit, cohort of pregnant women in coastal Kenya, 2006-2009

Characteristic*	Moderate/severe anemia (Hg <9) N (%)	No/mild anemia (Hg≥9) N (%)	RR (95% CI)†
Number enrolled	190 (27.0)	516 (73.0)	--
Maternal age			
20 – 44	154 (81.1)	420 (81.4)	Referent
< 20	36 (18.9)	96 (18.6)	1.02 (0.75, 1.39)
Marital status			
Married/partner	163 (86)	453 (88)	Referent
Widow/divorced/single	26 (14)	58 (12)	1.05 (0.95, 1.16)
Formal education			
No education	43 (23)	105 (21)	Referent
Primary education	124 (65)	344 (67)	0.99 (0.68, 1.45)
Secondary/higher education	23 (12)	63 (12)	0.98 (0.46, 2.10)
Gravidity			
Multigravida	145 (77)	385 (75)	Referent
Primigravida	42 (23)	128 (25)	0.97 (0.90, 1.04)
Body Mass Index (BMI), m/kg^2‡			
Low BMI	154 (84)	444 (88)	Referent
Normal BMI	30 (16)	62 (12)	1.30 (0.94, 1.81)
Bednet last 3 mos			
Used bednet	134 (71)	386 (75)	Referent
Not used bednet	53 (28)	127 (25)	1.14 (0.87, 1.50)
Malaria treated last 3 mos			
Treatment provided	18 (9)	53 (10)	Referent
No treatment	171 (91)	460 (90)	1.07 (0.70, 1.63)
Folic acid/iron-last 3 mos			
Folic acid/iron	11 (6)	28 (5)	Referent
No folic acid/iron	178 (94)	487 (95)	0.99 (0.96, 1.03)

*Numbers less than total enrolled reflect missing data

†Unadjusted Risk ratio (RR) and 95% confidence intervals (CI)

‡Adjusted for gestational age

Table 5-2. Maternal characteristics and association with malaria, hookworm and urogenital schistosomiasis infection in pregnancy at first ANC visit, among a cohort of pregnant women, coastal Kenya, 2006 – 2009

	Total	*P. falciparum*†		Hookworm*		*S. haematobium*	
	N (%)	n (%)	RR (95% CI)	n (%)	RR (95% CI)	n (%)	RR (95% CI)
Number	706	59		129		119	
Maternal Age							
20-44	574 (81.3)	39 (7.0)	Referent	96 (22.1)	Referent	80 (14.1)	Referent
< 20	132 (18.7)	20 (16.0)	2.29 (1.38, 3.79)	33 (31.4)	1.42 (1.02, 1.98)	39 (30.5)	2.25 (1.66, 3.07)
Formal education							
Secondary/higher level	86 (12.3)	6 (7.0)	Referent	9 (15.3)	Referent	12 (14.6)	Referent
Primary education	468 (66.7)	41 (9.1)	1.30 (0.57, 2.97)	86 (23.8)	1.56 (0.83, 2.93)	80 (17.4)	1.18 (0.68, 2.08)
No education	148 (21.1)	12 (8.5)	1.69 (0.32, 8.80)	34 (29.1)	2.43 (0.69, 8.58)	27 (18.2)	1.41 (0.46, 4.32)
Marital status							
Married/partner	616 (88.0)	48 (8.1)	Referent	115 (24.4)	Referent	100 (16.5)	Referent
Widow/divorced/single	84 (12.0)	10 (12.1)	1.41 (0.83, 2.40)	12 (18.7)	0.86 (0.54, 1.36)	18 (21.7)	1.18 (0.77, 1.83)
Gravidity							
Multigravida	529 (75.7)	39 (7.6)	Referent	95 (23.6)	Referent	81 (15.9)	Referent
Primigravida	170 (24.3)	19 (11.6)	1.52 (1.11, 2.56)	33 (24.6)	1.04 (0.76, 1.47)	38 (19.4)	1.51 (1.07, 1.78)
Maternal BMI							
Normal BMI	598 (86.7)	51 (8.5)	Referent	94 (24.8)	Referent	79 (15.9)	Referent
Low BMI	92 (13.3)	12 (8.7)	0.83 (0.35, 2.01)	16 (21.0)	1.08 (0.66, 1.78)	19 (19.4)	1.04 (0.60, 1.78)
Bednet use last 3 mos							
Yes	520 (74.3)	24 (13.9)	Referent	94 (23.6)	Referent	84 (16.2)	Referent
No	180 (25.7)	35 (6.9)	2.00 (1.23, 3.27)	33 (23.9)	1.01 (0.72, 1.43)	34 (19.9)	1.23 (0.86, 1.76)

*Analyses restricted to women with stool sample available at antenatal care visit (n= 544)

†PCR-diagnosed malaria

Table 5-3. Prevalence of parasitic infections in pregnancy and association of moderate/severe maternal anemia (Hg<9) at first ANC visit, among a cohort of pregnant women in coastal Kenya, 2006 – 2009

	Infection N* (%)	Moderate/severe anemia Unadjusted RR (95% CI)	p-value	Moderate/severe anemia aRR† (95% CI)	p-value
Hookworm					
Absent	415 (76.3)	Referent		Referent	
Present	129 (23.7)	1.15 (0.86 – 1.55)	0.3	1.17 (0.88, 1.56)	0.3
0-< 100 eggs/g	534 (98.2)	Referent		Referent	
≥100 eggs/g	10 (1.8)	2.45 (1.60, 3.77)	<0.0001	2.53 (1.62, 3.92)	<0.0001
P. falciparum					
PCR-negative	631 (91.4)	Referent		Referent	
PCR-positive	59 (8.6)	1.38 (0.96, 2.00)	0.08	1.45 (1.01, 2.08)	0.05
MS-negative	516 (95.8)	Referent		Referent	
MS-positive	24 (4.6)	2.12 (1.26, 3.57)	0.004	1.98 (1.17, 3.35)	0.01
S. haematobium					
Absent	577 (82.9)	Referent		Referent	
Present	119 (17.1)	1.00 (0.92, 1.09)	1.0	1.00 (0.92, 1.10)	0.9
0-< 50 eggs/mL	670 (96.3)	Referent		Referent	
≥50 eggs/mL	26 (3.7)	1.15 (0.64, 2.08)	0.6	1.23 (0.68, 2.22)	0.5
T. trichura					
Absent	489 (89.9)	Referent		Referent	
Present	55 (10.1)	1.06 (0.70 – 1.62)	0.8	1.10 (0.73, 1.67)	0.5
0-<100 eggs/g	542 (99.6)	--		--	
≥100 eggs/g	2 (0.4)				
S. stercoralis					
Absent	537 (99.1)	Referent		Referent	
Present‡	5 (0.9)	0.68 (0.12 – 3.94)	0.7	0.43 (0.07, 2.70)	0.4
A. lumbricodes					
Absent	535 (98.5)	Referent		Referent	
Present‡	8 (1.5)	0.42 (0.07 – 2.66)	0.4	0.67 (0.12, 3.86)	0.7

*Numbers differ due to missing values; †Adjusted for primagravida status, gestational age at ANC, and low maternal BMI; ‡None of the participants had ≥ 50 eggs/mL; MS = Microscopy-diagnosed malaria

Table 5-4. Association of infections and maternal and fetal anemia at delivery, coastal Kenya, 2006 – 2009

		Maternal moderate/severe anemia (Hb < 9 g/dl) at delivery				Fetal anemia (Hb < 12.5 g/dl)			
Infection at first ANC	N† (%)	RR (95% CI)	p-value	aRR (95% CI)*	p-value	RR (95% CI)	p-value	aRR (95% CI)*	p-value
Hookworm									
Negative	218 (88)	Referent		Referent		Referent		Referent	
Positive	61 (22)	0.76 (0.52, 1.12)	0.2	0.81 (0.55, 1.20)	0.3	1.10 (0.64, 1.90)	0.7	1.13 (0.65, 1.95)	0.6
0-<100 eggs/mL	274 (98)	Referent		Referent		Referent		1.0	
≥100 eggs/mL	5 (2)	2.43 (1.52, 3.90)	0.0002	2.30 (1.42, 3.71)	0.0006	1.91 (0.58, 6.08)	0.3	1.84 (0.58, 5.84)	0.3
P. falciparum									
PCR-negative	310 (89)	Referent		Referent		Referent		Referent	
PCR-positive	37 (11)	1.12 (0.45, 2.78)	0.8	1.02 (0.41, 2.54)	1.0	1.58 (0.44, 5.62)	0.5	1.45 (0.43, 4.91)	0.5
MS-negative	326 (95)	Referent		Referent		Referent		Referent	
MS-positive	17 (5)	1.16 (0.47, 2.89)	0.7	1.08 (0.43, 2.75)	0.9	1.22 (0.44, 3.40)	0.7	1.42 (0.50, 3.97)	0.3
S. haematobium‡									
Negative	310 (83)	Referent		Referent		Referent		Referent	
Positive	62 (17)	1.05 (0.92, 1.19)	0.4	1.05 (0.93, 1.20)	0.4	0.75 (0.21, 2.76)	0.7	0.61 (0.17, 2.21)	0.5
Delivery infection									
Hookworm‡									
Negative	177 (84)	Referent		Referent		Referent		Referent	
Positive	33 (16)	1.07 (0.63, 1.82)	0.8	1.08 (0.64, 1.84)	0.8	1.14 (0.52, 2.50)	0.7	1.10 (0.51, 2.41)	0.5
P. falciparum									
PCR-negative	353 (89)	Referent		Referent		Referent		Referent	
PCR-positive	41 (11)	1.43 (0.94, 2.20)	0.09	1.48 (0.97, 2.27)	0.07	1.58 (0.82, 3.01)	0.2	1.60 (0.84, 3.06)	0.2
MS-negative	384 (97)	Referent		Referent		Referent		Referent	
MS-positive	10 (3)	1.35 (0.69, 2.60)	0.4	1.42 (0.74, 2.74)	0.3	1.66 (0.63, 4.36)	0.3	1.69 (0.64, 4.45)	0.3
S. haematobium‡									
Negative	244 (84)	Referent		Referent		Referent		Referent	
Positive	49 (16)	0.98 (0.63, 1.53)	0.9	0.97 (0.62, 1.53)	0.9	0.80 (0.38, 1.66)	0.5	0.74 (0.36, 1.57)	0.4

*Adjusted for primagravida status and low BMI

†Different denominators reflect missing samples; ‡insufficient number to assess moderate burden

MS = Microscopy-diagnosed malaria

Table 5-5. Factors associated with moderate/severe maternal anemia at first ANC among a cohort of pregnant women, coastal Kenya 2006-2009.

	Maternal moderate/severe (Hb < 9 g/dL) anemia at ANC	
	aRR (95% CI) *	p-value
Hookworm (≥100 eggs/g)	2.37 (1.44, 3.91)	0.0007
P. falciparum (MS-positive)	2.06 (1.24, 3.44)	0.005
Low BMI	1.25 (0.86, 1.81)	0.2
Primigravida	0.78 (0.54, 1.11)	0.2

*Regression model adjusted for all variables listed and gestational age

ANC = Antenatal care; MS = microscopy

CHAPTER SIX

CONCLUSIONS

Overview

Reports of adverse birth outcomes associated with *P. falciparum* malaria in pregnancy were first published nearly 100 years ago, with reduced birth weight and anemia most commonly associated with infection [1]. Since the initial reports, treatment that is safe, effective and low-cost has been developed. Since 2004 the World Health Organization (WHO) has recommended intermittent preventive treatment in pregnancy with sulphadoxine/pyrimethamine (IPTp-SP) and insecticide-treated bednets (ITNs) for all pregnant women beginning in the second trimester in Sub-Saharan Africa and other malaria-endemic regions. While the IPTp-SP is effective in reducing malaria in pregnancy, evaluation of its public health impact on pregnancy has just begun and other risk factors for adverse pregnancy outcomes remain prevalent in malaria-endemic regions, including under-nutrition and other infections. Thus, the aim of this study was to assess the effects of malaria in pregnancy and their relation with known risk factors including under-nutrition and other parasitic infections, on pregnancy outcomes among women receiving the recommended preventive treatment for malaria in pregnancy.

Findings

We examined birth weight and moderate/severe maternal anemia among a cohort of women receiving IPTp-SP at first antenatal care (ANC) visit in coastal Kenya, 2006-2009. Among this cohort of nearly 800 wom nrolled in their second trimester, a reduced prevalence of malaria in pregnancy (approximately 10%) were observed compared to the prevalence of nearly 40% found in a study from the same region in 2000-2005, prior to widespread preventive treatment for malaria. Furthermore, among those women using ITNs, malaria at first ANC visit was significantly reduced. Malaria infection was associated with increased umbilical artery resistance and reduced fetal growth and, associated with impaired placenta, in the second trimester, but not later in pregnancy. The end of the second trimester is when rapid fetal weight gain occurs, and thus it may not be surprising that this was the primary period when significant reduced fetal growth measurements associated with malaria were observed. Previous research from the DRC found significant impact of malaria at the same gestational age. Furthermore, the observation of significant increased umbilical resistance early in the second trimester is also consistent with the gestational age where there is increasing uterine artery blood flow, and our findings are similar to differences reported from a recent study. Among women with low body mass index (BMI), a proxy for under-nutrition, malaria detected at ANC was significantly associated with reduced birth weight (-370 g, 95% CI -728, -12 g, p=0.04). This finding is similar to previous research documenting the interaction of malaria and maternal under-nutrition.

The rates of maternal anemia were high at the first ANC visit (70% and 27% for anemia and moderate/severe anemia, respectively). When we examined the rates of various infections in pregnancy, hookworm (24%), *P. falciparum* malaria (9%), urogenital

schistosomiasis (17%), and *T. trichuria* (10%) were the most common infections. Moderate/severe anemia was higher among those who were microscopy malaria-positive (aRR 2.06, 95% CI 1.24, 3.44), and those with the higher hookworm intensity had increased risk of anemia (aRR 2.37, 95% CI 1.44, 3.91). Neither PCR-positive malaria nor low-intensity infection of hookworm was significantly associated with increased risk of moderate/severe anemia. Furthermore, moderate/severe maternal anemia was also associated with increased risk of fetal anemia, which has been associated with increased risk for neurodevelopment impairment in previous research.

Limitations

This study had several limitations. First, birth outcomes were only measured among term, live births, limiting our ability to generalize the findings regarding malaria-infection and birth weight outcomes to all pregnancies. Thus, the study primarily addressed the association of malaria and IUGR-related low birth weight. Additionally, malaria infection was measured twice, at antenatal care and delivery, but not measured throughout pregnancy, thus limiting the ability to estimate potential impact of re-infection during pregnancy. Maternal BMI was a proxy for under-nutrition, but because maternal weight was measured at first ANC visit, we were unable to use the standard pre-pregnancy BMI or other measures to assess the maternal nutritional status. Finally, while both maternal and fetal hemoglobin were measured, hemoglobin estimates anemia but does not define the type of anemia, and thus further research may be warranted to explore the type of anemia observed in this population to better inform treatment.

Strengths

Despite the limitations noted, this study was among the first to evaluate the associations of malaria at ANC and pregnancy outcomes, among pregnant women receiving IPTp-SP. The study included several measures of malaria, assessed by PCR as the gold standard, as well as assessment of other infections, which afforded us the opportunity to conduct a comprehensive assessment of the roles of a number of prevalent infections during pregnancy. Furthermore, the study utilized the gold standard, ultrasound measurements, which have not been widely used in research in malaria-endemic countries to date, to assess fetal growth measurements concurrent with malaria status. Finally, the study included measures of both maternal and cord hemoglobin levels, which allowed assessment of anemia in both the mother and the fetus/neonate.

Conclusions

Despite safe, effective treatments for malaria and other infections, rates of infection-related adverse pregnancy outcomes remain high in Sub-Saharan Africa. Antenatal care, when many women access the available health care system, is an opportunity to provide interventions. While we observed decreased malaria rates with IPTp-SP, approximately 10% of women were still infected with malaria at the time of their first ANC visit, generally in the second trimester. Women infected at ANC had reduced fetal growth, compared to un-infected women early in pregnancy, although these differences decreased later in pregnancy. While minimal differences were observed at birth between those who were PCR-malaria positive vs. negative at antenatal care, those with under-nutrition and PCR malaria-infection were at increased

risk. Our findings confirm the importance of preventative treatment for malaria to reduce the adverse outcomes associated with *P. falciparum* malaria. In 2012, the WHO recommended IPTp-SP be provided on a monthly basis, given the research suggesting 3 or more treatments are more effective. Additionally, our results point to the opportunity to further reduce rates of malaria pre-pregnancy or early in pregnancy through widespread use of ITNs, as suggested in other studies.

Furthermore, in Sub-Saharan Africa, co-infection with other parasitic infections, most notably hookworm, remains common. Thus, even with IPTp-SP, maternal anemia rates remain high in many areas. In this cohort, while microscopy malaria was associated with moderate/severe anemia, higher-intensity hookworm infection was also an important risk factor that was highly associated with risk of moderate/severe anemia in pregnancy.

Finally, while significant progress has been made to reduce malaria in pregnancy, our results suggest the need to not only continue current efforts, but also to better integrate treatment for other infections, such as hookworm, and interventions for under-nutrition into ANC programs that include IPTp-SP. Comprehensive ANC programs incorporating malaria and prevalent conditions that reach women early in pregnancy are needed to continue to improve maternal and newborn outcomes in Sub-Saharan Africa.

Appendix

Since body mass index (BMI) generally increases over the course of pregnancy, additional analyses were under-taken to assess the association between gestational age in pregnancy and BMI. Figure A1-1 illustrates the mean (SD) of BMI by gestational age at antenatal care.

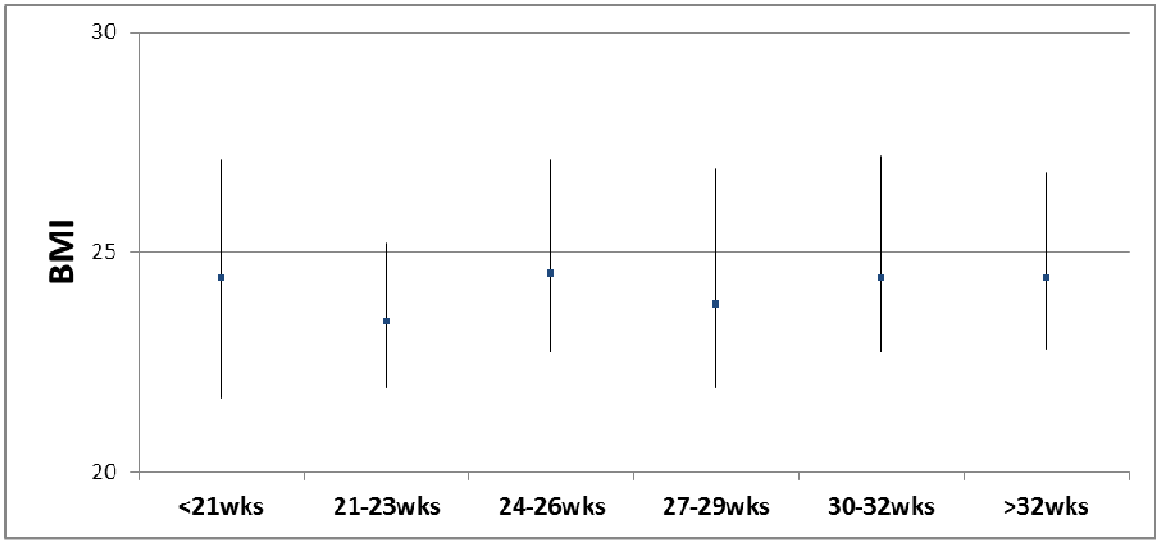

Figure A1-1. Mean BMI (SD) by gestational age in pregnancy at first ANC visit, coastal Kenya, 2006 – 2009.

REFERENCES

[1] Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, et al. (2007) Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis;7(2):93–104.

[2] Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, et al. (2012) Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. Lancet Infect Dis;12(12):942–9.

[3] Newman RD, Parise ME, Slutsker L, Nahlen B, Steketee RW. (2003) Safety, efficacy and determinants of effectiveness of antimalarial drugs during pregnancy: implications for prevention programmes in Plasmodium falciparum-endemic sub-Saharan Africa. Trop Med Int Health;8(6):488–506.

[4] Garner P, Gülmezoglu AM. (2006) Drugs for preventing malaria in pregnant women. Cochrane Database Syst Rev;(4):CD000169.

[5] World Health Organization. (2007) Malaria in pregnancy guidelines for measuring key monitoring and evaluation indictors. http://whqlibdoc.who.int/publications/2007/9789241595636_eng.pdf.

[6] Iriemenam NC, Shah M, Gatei W, van Eijk AM, Ayisi J, Kariuki S, et al. (2012) Temporal trends of sulphadoxine-pyrimethamine (SP) drug-resistance molecular markers in Plasmodium falciparum parasites from pregnant women in western Kenya. Malar J;11:134.

[7] Likwela JL, D'Alessandro U, Lokwa BL, Meuris S, Dramaix MW. (2012) Sulfadoxine-pyrimethamine resistance and intermittent preventive treatment during pregnancy: a retrospective analysis of birth weight data in the Democratic Republic of Congo (DRC). Trop Med Int Health;17(3):322–9.

[8] Offianan AT, Penali LK, Coulibaly M, Tiacoh N, Ako A, Adji E, et al. (2012) Comparative efficacy of uncontrolled and controlled intermittent preventive treatment during pregnancy (IPTp) with combined use of LLTNs in high resistance area to sulfadoxine-pyrimethamine in Côte d'Ivoire. Infect Drug Resist;5:53–63.

[9] Hamer DH, Mwanakasale V, Macleod WB, Chalwe V, Mukwamataba D, Champo D, et al. (2007) Two-dose versus monthly intermittent preventive treatment of malaria with sulfadoxine-pyrimethamine in HIV-seropositive pregnant Zambian women. J Infect Dis;196(11):1585–94.

[10] Briand V, Bottero J, Noël H, Masse V, Cordel H, Guerra J, et al. (2009) Intermittent treatment for the prevention of malaria during pregnancy in Benin: a randomized, open-label equivalence trial comparing sulfadoxine-pyrimethamine with mefloquine. J Infect Dis;200(6):991–1001.

[11] Gies S, Coulibaly SO, Ouattara FT, D'Alessandro U. (2009) Individual efficacy of intermittent preventive treatment with sulfadoxine-pyrimethamine in primi- and

secundigravidae in rural Burkina Faso: impact on parasitaemia, anaemia and birth weight. Trop Med Int Health; 14(2):174–82.

[12] Tiono AB, Ouedraogo A, Bougouma EC, Diarra A, Konaté AT, Nébié I, et al. (2009) Placental malaria and low birth weight in pregnant women living in a rural area of Burkina Faso following the use of three preventive treatment regimens. Malar J;8:224.

[13] Valea I, Tinto H, Drabo MK, Huybregts L, Sorgho H, Ouedraogo JB, et al. (2012) An analysis of timing and frequency of malaria infection during pregnancy in relation to the risk of low birth weight, anaemia and perinatal mortality in Burkina Faso. Malar J;11:71.

[14] Mbaye A, Richardson K, Balajo B, Dunyo S, Shulman C, Milligan P, et al. (2006) A randomized, placebo-controlled trial of intermittent preventive treatment with sulphadoxine-pyrimethamine in Gambian multigravidae. Trop Med Int Health;11(7):992–1002.

[15] Clerk CA, Bruce J, Affipunguh PK, Mensah N, Hodgson A, Greenwood B, et al. (2008) A randomized, controlled trial of intermittent preventive treatment with sulfadoxine-pyrimethamine, amodiaquine, or the combination in pregnant women in Ghana. J Infect Dis; 198(8):1202–11.

[16] Tagbor H, Bruce J, Agbo M, Greenwood B, Chandramohan D. (2010) Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: a randomised controlled non-inferiority trial. PLoS One;5(12):e14425.

[17] Filler SJ, Kazembe P, Thigpen M, Macheso A, Parise ME, Newman RD, et al. (2006) Randomized trial of 2-dose versus monthly sulfadoxine-pyrimethamine intermittent preventive treatment for malaria in HIV-positive and HIV-negative pregnant women in Malawi. J Infect Dis;194(3):286–93.

[18] Luntamo M, Kulmala T, Mbewe B, Cheung YB, Maleta K, Ashorn P. (2010) Effect of repeated treatment of pregnant women with sulfadoxine-pyrimethamine and azithromycin on preterm delivery in Malawi: a randomized controlled trial. Am J Trop Med Hyg;83(6):1212–20.

[19] Diakite OS, Kayentao K, Traoré BT, Djimde A, Traoré B, Diallo M, et al. (2011) Superiority of 3 over 2 doses of intermittent preventive treatment with sulfadoxine-pyrimethamine for the prevention of malaria during pregnancy in mali: a randomized controlled trial. Clin Infect Dis;53(3):215–23.

[20] Bardají A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, et al. (2011) Impact of malaria at the end of pregnancy on infant mortality and morbidity. J Infect Dis;203(5):691–9.

[21] Challis K, Osman NB, Cotiro M, Nordahl G, Dgedge M, Bergström S. (2004) Impact of a double dose of sulphadoxine-pyrimethamine to reduce prevalence of pregnancy malaria in southern Mozambique. Trop Med Int Health;9(10):1066–73.

[22] Ndyomugyenyi R, Clarke SE, Hutchison CL, Hansen KS, Magnussen P. (2011) Efficacy of malaria prevention during pregnancy in an area of low and unstable transmission: an individually-randomised placebo-controlled trial using intermittent preventive treatment and insecticide-treated nets in the Kabale Highlands, southwestern Uganda. Trans R Soc Trop Med Hyg;105(11):607–16.

[23] Huynh BT, Fievet N, Gbaguidi G, Dechavanne S, Borgella S, Guézo-Mévo B, et al. (2011) Influence of the timing of malaria infection during pregnancy on birth weight and on maternal anemia in Benin. Am J Trop Med Hyg;85(2):214–20.

[24] Le Port A, Cottrell G, Dechavanne C, Briand V, Bouraima A, Guerra J, et al. (2011) Prevention of malaria during pregnancy: assessing the effect of the distribution of IPTp through the national policy in Benin. Am J Trop Med Hyg;84(2):270–5.

[25] Vanga-Bosson HA, Coffie PA, Kanhon S, Sloan C, Kouakou F, Eholie SP, et al. (2011) Coverage of intermittent prevention treatment with sulphadoxine-pyrimethamine among pregnant women and congenital malaria in Côte d'Ivoire. Malar J;10:105.

[26] Bouyou-Akotet MK, Nzenze-Afene S, Ngoungou EB, Kendjo E, Owono-Medang M, Lekana-Douki JB, et al. (2010) Burden of malaria during pregnancy at the time of IPTp/SP implementation in Gabon. Am J Trop Med Hyg;82(2):202–9.

[27] Ramharter M, Schuster K, Bouyou-Akotet MK, Adegnika AA, Schmits K, Mombo-Ngoma G, et al. (2007) Malaria in pregnancy before and after the implementation of a national IPTp program in Gabon. Am J Trop Med Hyg;77(3):418–22.

[28] Hommerich L, von Oertzen C, Bedu-Addo G, Holmberg V, Acquah PA, Eggelte TA, et al. (2007) Decline of placental malaria in southern Ghana after the implementation of intermittent preventive treatment in pregnancy. Malar J;6:144.

[29] Wilson NO, Ceesay FK, Obed SA, Adjei AA, Gyasi RK, Rodney P, et al. (2011) Intermittent preventive treatment with sulfadoxine-pyrimethamine against malaria and anemia in pregnant women. Am J Trop Med Hyg;85(1):12–21.

[30] Feng G, Simpson JA, Chaluluka E, Molyneux ME, Rogerson SJ. (2010) Decreasing burden of malaria in pregnancy in Malawian women and its relationship to use of intermittent preventive therapy or bed nets. PLoS One; 5(8):e12012.

[31] Kapito-Tembo A, Meshnick SR, van Hensbroek MB, Phiri K, Fitzgerald M, Mwapasa V. (2011) Marked reduction in prevalence of malaria parasitemia and anemia in HIV-infected pregnant women taking cotrimoxazole with or without sulfadoxine-pyrimethamine intermittent preventive therapy during pregnancy in Malawi. J Infect Dis;203(4):464–72.

[32] Brentlinger PE, Dgedge M, Correia MA, Rojas AJ, Saúte F, Gimbel-Sherr KH, et al. (2007) Intermittent preventive treatment of malaria during pregnancy in central Mozambique. Bull World Health Organ;85(11):873–9.

[33] Falade CO, Tongo OO, Ogunkunle OO, Orimadegun AE. (2010) Effects of malaria in pregnancy on newborn anthropometry. J Infect Dev Ctries;4(7):448–53.

[34] Aribodor DN, Nwaorgu OC, Eneanya CI, Okoli I, Pukkila-Worley R, Etaga HO. (2009) Association of low birth weight and placental malarial infection in Nigeria. J Infect Dev Ctries;3(8):620–3.

[35] Tongo OO, Orimadegun AE, Akinyinka OO. (2011) Utilisation of malaria preventive measures during pregnancy and birth outcomes in Ibadan, Nigeria. BMC Pregnancy Childbirth;11:60.

[36] Aziken ME, Akubuo KK, Gharoro EP. (2011) Efficacy of intermittent preventive treatment with sulfadoxine-pyrimethamine on placental parasitemia in pregnant women in midwestern Nigeria. Int J Gynecol Obstet;112(1):30–3.

[37] Sarr D, Marrama L, Gaye A, Dangou JM, Niang M, Mercereau-Puijalon O, et al. (2006) High prevalence of placental malaria and low birth weight in Sahelian periurban area. Am J Trop Med Hyg;75(1):171–7.

[38] Olliaro PL, Delenne H, Cisse M, Badiane M, Olliaro A, Vaillant M, et al.(2008) Implementation of intermittent preventive treatment in pregnancy with sulphadoxine/pyrimethamine (IPTp-SP) at a district health centre in rural Senegal. Malar J;7:234.

[39] Harrington WE, Mutabingwa TK, Kabyemela E, Fried M, Duffy PE. (2011) Intermittent treatment to prevent pregnancy malaria does not confer benefit in an area of widespread drug resistance. Clin Infect Dis;53(3):224–30.

[40] Mbonye AK, Bygbjerg IC, Magnussen P. (2008) Intermittent preventive treatment of malaria in pregnancy: a new delivery system and its effect on maternal health and pregnancy outcomes in Uganda. Bull World Health Organ;86(2):93–100.

[41] Steketee RW, Campbell CC. (2010) Impact of national malaria control scale-up programmes in Africa: magnitude and attribution of effects. Malaria Journal, 9:299

[42] World Health Organization. Updated WHO Policy Recommendation (2012). Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine/Pyrimethamine (IPTp-SP). http://www.who.int/malaria/iptp_sp_updated_policy_recommendation_en_102012.pdf

[43] de Benoist B, McLean E, Egli I, Cogswell M. (2008) Worldwide prevalence of anaemia 1993–2005 WHO global database on anaemia . Geneva: World Health Organization.

[44] Khan KS, Wojdyla D, Say L, Gülmezoglu AM, Van Look PF. (2006) WHO analysis of causes of maternal death: a systematic review. Lancet 2006;367(9516):1066-74.

[45] Rogawski ET, Chaluluka E, Molyneux ME, Feng G, Rogerson SJ, Meshnick SR. (2012) The effects of malaria and intermittent preventive treatment during pregnancy on fetal anemia in Malawi. Clin Infect Dis;55(8):1096-102.

[46] Mwinga K, Vermund SH, Chen YQ, Mwatha A, Read JS, Urassa W, Carpenetti N, Valentine M, Goldenberg RL. (2009) Selected hematologic and biochemical measurements in African HIV-infected and uninfected pregnant women and their infants: the HIV Prevention Trials Network 024 protocol. BMC Pediatr ;9:49. doi: 10.1186/1471-2431-9-49.

[47] Brabin BJ, Kalanda BF, Verhoeff FH, Chimsuku LH, Broadhead RL. (2004) Risk factors for fetal anaemia in a malarious area of Malawi. Ann Trop Paediatr; 24:311–21.

[48] Felt BT, Peirano P, Algarín C, Chamorro R, Sir T, Kaciroti N, Lozoff B. (2012) Long-term neuroendocrine effects of iron-deficiency anemia in infancy. Pediatr Res.;71(6):707-12.

[49] Cao C, O'Brien KO. (2013) Pregnancy and iron homeostasis: an update. Nutrition Reviews 71(1):35–51.

[50] Mei Z, Cogswell ME, Parvanta I, Lynch S, Beard JL, Stoltzfus RJ, Grummer-Strawn LM. (2005) Hemoglobin and ferritin are currently the most efficient indicators of population response to iron interventions: an analysis of nine randomized controlled trials. J Nutr;135(8):1974-80.

[51] Viteri FE. The consequences of iron deficiency and anemia in pregnancy. (1994) In: Allen L, King J, Lonnerdahl B, eds. Nutrient regulation during pregnancy, lactation and growth. New York: Plenum Press, 127–139.

[52] Goonewardene M, Shehata M, Hamad A. (2012) Anaemia in pregnancy. Best Pract Res Clin Obstet Gynaecol;26(1):3-24.

[53] Scholl TO. (2005) Iron status during pregnancy: setting the stage for mother and infant. Am J Clin Nutr;81(5)1218 S-1222 S.

[54] Gangopadhyay R, Karoshi M, Keith L. (2011) Anemia and pregnancy: a link to maternal chronic diseases. Int J Gync 115: S11-S15.

[55] Alusala DN, Estambale BB, Magnussen P, Friis H, Luoba AI, Mwaniki D. Predictors of serum ferritin and haemoglobin during pregnancy, in a malaria-endemic area of western Kenya.Ann Trop Med Parasitol. 2008 Jun;102(4):297-308.

[56] Adam I, Babiker S Mohmmed AA, Salih MM, Prins MH, Zaki M. (2008) Low body mass Index, anaemia and poor perinatal outcome in a rural hospital in Eastern Sudan. Journal of Tropical Pediatrics; 54 (3): 202-204.

[57] Ouédraogo S, Koura GK, Accrombessi MM, Bodeau-Livinec F, Massougbodji A, Cot M. (2012) Maternal anemia at first antenatal visit: prevalence and risk factors in a malaria-endemic area in Benin. Am J Trop Med Hyg;87(3):418-24

[58] Boel M, Carrara VI, Rijken M, Proux S, Nacher M, Pimanpanarak M, Paw MK, Moo O, Gay H, Bailey W, Singhasivanon P, White NJ, Nosten F, McGready R. (2010) Complex Interactions between soil-transmitted helminths and malaria in pregnant women on the Thai-Burmese border. PLoS Negl Trop Dis ;4(11):e887.

[59] Schaible UE, Kaufmann SHE. (2007) Malnutrition and Infection: Complex mechanisms and global impacts. Plos Med. 115.

[60] Landis SH, Lokomba V, Ananth CV, Atibu J, Ryder RW, Hartmann KE, Thorp JM, Tshefu A, Meshnick SR. (2009) Impact of maternal malaria and under-nutrition on intrauterine growth restriction: a prospective ultrasound study in Democratic Republic of Congo. Epidemiol Infect;137(2):294-304.

[61] OurMason JB, Saldanha LS, Ramakrishnan U, Lowe A, Noznesky EA, Girard AW, McFarland DA, Martorell R. (2012) Opportunities for improving maternal nutrition and birth outcomes: synthesis of country experiences. Food Nutr Bull;33(2 Suppl):S104-37.

[62] Mehta S, Manji KP, Young AM, Brown ER, Chasela C, Taha TE, Read JS, Goldenberg RL, Fawzi WW. (2008) Nutritional indicators of adverse pregnancy outcomes and mother-to-child transmission of HIV among HIV-infected women. Am J Clin Nutr 87(6):1639-49.

[63] Ayoya MA, Spiekermann-Brouwer GM, Traoré AK, Stoltzfus RJ, Garza C. (2006) Determinants of anemia among pregnant women in Mali. Food Nutr Bull;27(1):3-11.

[64] Muhangia L, Woodburn P, Omarab M, Omoding N, Kizito D, Mpairwe H, Nabulime J, Ameke C, Morison LA, Elliott AM. (2007) Associations between mild-to-moderate anaemia in pregnancy and helminth, malaria and HIV infection in Entebbe, Uganda. Trans R Soc Trop Med Hyg; 101(9):899-907.

[65] Baig-Ansari N, Badruddin SH, Karmaliani R, Harris H, Jehan I, Pasha O, Moss N, McClure EM, Goldenberg RL. (2008) Anemia prevalence and risk factors in pregnant women in an urban area of Pakistan. Food Nutr Bull ;29(2):132-9.

[66] Stoltzfus RJ, Dreyfuss ML; International Nutritional Anemia Consultative Group (1998) Guidelines for the Use of Iron Supplements to Prevent and Treat Iron Deficiency Anemia. Geneva: World Health Organization.

[67] Fairley JK, Bisanzio D, King CH, Kitron U, Mungai P, Muchiri E, King CL, Malhotra I. (2013) Birthweight in offspring of mothers with High Prevalence of Helminth and Malaria infection in Coastal Kenya. Am J Trop Med Hyg;88(1):48-5.

[68] Brooker S, Hotez PJ, Bundy DAP. (2008). Hookworm-related anaemia among pregnant women: a systematic review. Plos Neg Trop Dis 2 (9): e291.

[69] WHO. (1994) Report of the WHO informal consultation on hookworm infection and anaemia in girls and women. WHO/CTD/SIP/96.1. World Health Organization, Geneva.

[70] Montresor DWT, Crompton A, Hall DAP, Bundy, Savioli L. (1998) Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. WHO/CTD/SIP/98.1

[71] WHO. (2002) Prevention and Control of Schistosomiasis and Soil-Transmitted Helminthiasis: Report of a WHO Expert Committee. Geneva: World Health Organization; 2002.

[72] WHO. (2002) Report of the WHO informal consultation on the use of praziquantel during pregnancy/lactation and albendazole/mebendazole in children under 24 months. Geneva: World Health Organization. WHO/CDS/CPE/PVC/2002.4.

[73] Haider BA, Humayun, Q, Bhutta ZA. (2009). Effect of administration of antihelminthics for soil transmitted helminths during pregnancy. Cochrane Database of Systematic Reviews (2), CD005547.

[74] Larocque R, Casapia M, Gotuzzo E, MacLean JD, Soto JC, Rahme E, Gyorkos, TW. (2006) A double-blind randomized controlled trial of antenatal mebendazole to reduce low birthweight in a hookworm-endemic area of Peru. Trop Med Int Health 11: 1485–1495.

[75] Elliott AM, Ndibazza J, Mpairwe H, Muhangi L, Webb EL, Kizito D, et al. (2011) Treatment with anthelminthics during pregnancy: what gains and what risks for the mother and child? Parasitology 138: 1499-1507.

[76] Torlesse, H. and Hodges, M. (2001) Albendazole therapy and reduced decline in haemoglobin concentration during pregnancy (Sierra Leone). Trans Royal Society Trop Med Hygiene 95: 195–201.

[77] Ndibazza J, Muhangi L, Akishule D, Kiggundu M, Ameke C, Oweka J, Kizindo R, Duong T, Kleinschmidt I, Muwanga M, Elliott AM. (2010) Effects of Deworming during Pregnancy on Maternal and Perinatal Outcomes in Entebbe, Uganda: A Randomized Controlled Trial. Clin Infect Dis; 50(4): 531–540.

[76] Nour NM. (2010) Schistosomiasis: health effects on women. Rev Obstet Gynecol; 3(1):28-32.

[77] Gyorkos TW, Gilbert NL, Larocque R, Casapía M. (2011) Trichuris and hookworm infections associated with anaemia during pregnancy. Trop Med Int Health;16(4):531-7.

[78] Edmonds FH. (1899) Malaria and pregnancy; Br Med J. 1(2000): 1023.

[79] Study registration available at Clinical Trials.gov: http://clinicaltrials.gov/ct2/show/NCT00314899?term=fetal+immunity%2C+king&rank=1

[80] Mutuku FM, King CH, Mungai P, Mbogo C, Mwangangi J, Muchiri EM, Walker ED, Kitron U. (2011) Impact of insecticide-treated bed nets on malaria transmission indices on the south coast of Kenya. Malar J 10:356.

[81] Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO. (2010) Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. PLoS Med;7(1):e1000221.

[82] Peters PJ, Thigpen MC, Parise M, Newman RD. (2007) Safety and toxicity of sulfadoxine-pyrimethamine: implications for prevention of malaria in pregnancy using intermittent preventive treatment. Drug safety; 30: 481-6.

[83] Kattenberg JH, Ochodo EA, Boer KR, Schallig HD, Mens PF, Leeflang MM. (2011) Systematic review and meta-analysis: rapid diagnostic tests versus placental histology, microscopy and PCR for malaria in pregnant women. Malar J 10:321.

[84] Campos IM, Uribe ML, Cuesta C, Franco-Gallego A, Carmona-Fonseca J, Maestre A. (2011) Diagnosis of gestational, congenital, and placental malaria in Colombia: comparison of the efficacy of microscopy, nested polymerase chain reaction, and histopathology. Am J Trop Med Hyg;84(6):929-35.

[85] Rantala AM, Taylor SM, Trottman PA, Luntamo M, Mbewe B, Maleta K, Kulmala T, Ashorn P, Meshnick SR. (2010) Comparison of real-time PCR and microscopy for malaria parasite detection in Malawian pregnant women. Malar J 9:269.

[86] McNamara DT, et al. (2006) Diagnosing infection levels of four human malaria parasite species by a polymerase chain reaction/ligase detection reaction fluorescent microsphere-bassed assay. Am J Trop Med Hyg 74: 413-421.

[87] Hadlock FP, Harrist RB, Martinez-Poyer J. (1991) In utero analysis of fetal growth: a sonographic weight standard. Radiology 181:129–133.

[88] Rohrer, F. (1921) "Der Index der Körperfülle als Maß des Ernährungszustandes”

[89] Brabin B. (1992) Fetal anaemia in malarious areas: its causes and significance. Ann Trop Paediatr 12:303–10.

[90] McLean E, Egli I, Cogswell M (ed). (2012) Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia. Geneva: World Health Organization.

[91] Institute of Medicine. (2009)Weight gain and pregnancy. Washington DC.

[92] Ngnie-Teta I, Kuate-Defo B, Receveur O. (2009) Multilevel modelling of sociodemographic predictors of various levels of anaemia among women in Mali. Public Health Nutr; 12(9):1462-9.

[93] Steketee RW, Nahlen BL, Parise ME, Menendez C. (2001) The burden of malaria in pregnancy in malaria-endemic areas. American Journal of Tropical Medicine and Hygiene;64(1-2 Suppl):28-35.

[94] Marchant T, Willey B, Katz J, Clarke S, Kariuki S, ter Kuile F, Lusingu J, Ndyomugyenyi R, Schmiegelow C, Watson-Jones D, Armstrong Schellenberg J. (2012) Neonatal mortality risk associated with preterm birth in East Africa, adjusted by weight for gestational age: individual participant level meta-analysis. PLoS Med 9(8):e1001292.

[95] Steketee RW, Campbell CC. (2010) Impact of national malaria control scale-up programmes in Africa: magnitude and attribution of effects. Malaria Journal; 9:299.

[96] Neggers Y, Goldenberg RL. (2003) Some thoughts on body mass index, micronutrient intakes and pregnancy outcome. Journal of Nutrition; 133: 1737S-1740S.

[97] Caulfield LE, Richard SA, Black RE. (2004) Undernutrition as an underlying cause of malaria morbidity and mortality in children less than five years old. Am J Trop Med Hyg. 2004 Aug;71(2 Suppl):55-63

[98] Kalilani L, Mofolo I, Chaponda M, Rogerson SJ, Meshnick SR. (2010) The effect of timing and frequency of Plasmodium falciparum infection during pregnancy on the risk of low birth weight and maternal anemia. Trans Royal Society of Tropical Medicine and Hygiene;104:416-422.

[99] Griffin JB, Lokomba V, Landis SH, Thorp JM Jr, Herring AH, Tshefu AK, Rogerson SJ, Meshnick SR. (2012) Plasmodium falciparum parasitaemia in the first half of pregnancy, uterine and umbilical artery blood flow, and foetal growth: a longitudinal Doppler ultrasound study. Malaria Journal 11:319.

[100] Rijken MJ, Papageorghiou AT, Thiptharakun S, Kiricharoen S, Dwell SL, Wiladphaingern J, Pimanpanarak M, Kennedy SH, Nosten F, McGready R. (2012) Ultrasound evidence of early fetal growth restriction after maternal malaria infection. PLoS One 7:e31411.

[101] Schmiegelow C, et al. (2013) Malaria and fetal growth alterations in the 3(rd) trimester of pregnancy: A longitudinal ultrasound study. PLoS One;8:e53794.

[102] Trudinger BJ, Giles WB, Cook CM, Bombardieri J, Collins LE. (1985) Fetal umbilical artery flow velocity waveforms and placental resistance: clinical significance. British Journal of Obstetrics and Gynaecology 92:23–30.

[103] Kayatentao K, Garner P, van Eijk AM, Naidoo I, Roper C, Mulokozi A, MacArthur JR, Luntamo M, Ashorn P, Doumbo OK, ter Kuile FO. (2013) Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: Systematic review and meta-analysis. JAMA;309:594-604.

[104] McLean E, Egli I, Cogswell M (ed). (2012) Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia. Geneva: World Health Organization.

[105] Ouédraogo S, Koura GK, Accrombessi MM, Bodeau-Livinec F, Massougbodji A, Cot M. (2012) Maternal anemia at first antenatal visit: prevalence and risk factors in a malaria-endemic area in Benin. Am J Trop Med Hyg 87:418-424.

[106] Nwizi EN, Iliyasu Z, Ibrahim SA, Galadanci HS. (2011) Socio-demographic and maternal factors in anaemia in pregnancy at booking in Kano, Northern Nigeria. African Journal of Reproductive Health 15: 33-41.

[107] Hartman TK, Rogerson SJ, Fischer PR. (2010) The impact of maternal malaria on newborns. Ann Trop Paediatr 30:271-282

[108] Koura GK, Ouedraogo S, Le Port A, Watier L, Cottrell G, et al. (2012) Anaemia during pregnancy: impact on birth outcome and infant haemoglobin level during the first 18 months of life. Trop Med Int Health 17:283-291.

[109] Adediran A, Gbadegesin A, Adeyemo TA, Akinbami A, Osunkalu VO, et al. (2011) Haemoglobin and ferritin concentrations in cord blood in a tertiary health centre in Nigeria. Nig Q J Hosp Med 21:284-9.

[110] Chang S, Zeng L, Brouwer ID, Kok FJ, Yan H. (in press) Effect of iron deficiency anemia in pregnancy on child mental development in rural China. Pediatrics.

[111] Tamura T, Goldenberg RL, Hou J, Johnston KE, Cliver SP, et al. (2002) Cord serum ferritin concentrations and mental and psychomotor development of children at five years of age. J Pediatr 140:165-70.

[112] Walker SP, Wachs TD, Gardner JM, Lozoff B, Wasserman GA, Pollitt E, Carter JA; International Child Development Steering Group. (2007) Child development: risk factors for adverse outcomes in developing countries. Lancet 369(9556):145-57.

[113] Brooker S, Hotez PJ, Bundy DA. (2008) Hookworm-related anaemia among pregnant women: a systematic review. PLoS Negl Trop Dis 2:e291.

[114] McClure EM, Goldenberg RL, Dent AE, Meshnick SR. (2013) A systematic review of the impact of prevention of malaria in pregnancy on low birth weight and maternal anemia. Int J Gynec Obst. doi:pii: S0020-7292(13)00051-9. 10.1016/j.ijgo.2012.12.014

[115] Uneke CJ. (2007) Impact of placental *Plasmodium falciparum* malaria on pregnancy and perinatal outcome in sub–Saharan Africa: II: effects of placental malaria on perinatal outcome; malaria and HIV. Yale J Biol Med 80:95–103.

[116] Ouédraogo S, Bodeau-Livinec F, Briand V, Huynh BT, Koura GK, et al. (2012) Malaria and gravidity interact to modify maternal haemoglobin concentrations during pregnancy. Malar J 11:348.

[117] Valea I, Tinto H, Drabo MK, Huybregts L, Sorgho H, et al; FSP/MISAME study Group. (2012) An analysis of timing and frequency of malaria infection during pregnancy in relation to the risk of low birth weight, anaemia and perinatal mortality in Burkina Faso. Malar J 11:71.

[118] Degarege A, Legesse M, Medhin G, Animut A, Erko B. (2012) Malaria and related outcomes in patients with intestinal helminths: a cross-sectional study. BMC Infect Dis 12:291.

[119] Ouma P, van Eijk AM, Hamel MJ, Parise M, Ayisi JG, Otieno K, et al. (2007) Malaria and anaemia among pregnant women at first antenatal clinic visit in Kisumu, western Kenya. Trop Med Int Health 12(12):1515-23.

[120] Bustinduy AL, Parraga IM, Thomas CL, Mungai PL, Mutuku F, et al. (in press) Impact of polyparasitic infections on anemia and undernutrition among Kenyan children living in a *schistosoma haematobium*-endemic area. Am J Trop Med Hyg 20.

[121] Sousa-Figueiredo JC, Gamboa D, Pedro JM, Fançony C, Langa AJ, Magalhães RJ, et al. (2012) Epidemiology of malaria, schistosomiasis, geohelminths, anemia and malnutrition in the context of a demographic surveillance system in northern Angola. PLoS One 7: e33189.

[122] Adegnika AA, Ramharter M, Agnandji ST, Ngoa UA, Issifou S, et al. (2010) Epidemiology of parasitic co-infections during pregnancy in Lambarene, Gabon. Trop Med Int Health 15: 1204-9.

[123] Awasthi S, Bundy DAP, Savioli L. Helminthic infections. (2003) BMJ 327: 431-3.

[124] Roberts T, Gravett CA, Velu PP, Theodoratou E, Wagner TA, Zhang JS, et al. (2011) Epidemiology and aetiology of maternal parasitic infections in low- and middle-income countries. J Glob Health 1:189-200.

[125] Hillier SD, Booth M, Muhangi L, Nkurunziza P, Khihembo M, et al. (2008) Plasmodium falciparum and helminth coinfection in a semi urban population of pregnant women in Uganda. J Infect Dis 198:920-927.

[126] Yatich NJ, Jolly PE, Funkhouser E, Agbenyega T, Rayner JC, et al. (2010) The effect of malaria and intestinal helminth co-infection on birth outcomes in Kumasi, Ghana. Infect Dis Obstet Gynecol 350763.

[127] Montresor A, Crompton DWT, Hall A, Bundy, DAP Savioli L. (1998) Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. Geneva: WHO.

[128] Friedman JF, Mital P, Kanzaria HK, Olds GR, Kurtis JD. (2007) Schistosomiasis and pregnancy. Trends Parasitol ;23(4):159-64.

[129] Ogbodo SO, Nwagha UI, Okaka AN, Ogenyi SC, Okoko RO, Nwagha TU. Malaria parasitaemia among pregnant women in a rural community of eastern Nigeria; need for combined measures. Niger J Physiol Sci. 2009 Dec;24(2):95-100.

[130] Bechir M, Schelling E, Hamit MA, Tanner M, Zinsstag J. (2010) Parasitic infections, anemia and malnutrition among rural settled and mobile pastoralist mothers and their children in Chad. Ecohealth;9(2):122-3127.

[131] Woodburn PW, Muhangi L, Hillier S, Ndibazza J, Namujju PB, Kizza M, Ameke C, Omoding NE, Booth M, Elliott AM. (2009) Risk Factors for helminth, malaria, and HIV infection in pregnancy in Entebbe, Uganda. Plos Neg Trop Dis; 3(6): e473.

[132] van Eijk AM, Lindblade KA, Odhiambo F, Peterson E, Rosen DH, Karanja D, et al. (2009) Geohelminth infections among pregnant women in rural western Kenya; a cross-sectional study. PLoS Negl Trop Dis;3(1):e370.

[133] Ouédraogo S, Koura GK, Bodeau-Livinec F, Accrombessi MM, Massougbodji A, Cot M. (in press) Maternal anemia in pregnancy: Assessing the effect of routine preventive measures in a malaria-endemic area. Am J Trop Med Hyg.

[134] Haider BA, Humayun Q, Bhutta ZA. (2009) Effect of administration of antihelminthics for soil transmitted helminths during pregnancy. Cochrane Database Syst Rev 2:CD005547.

[135] Ndibazzza J, Muhangi L, Akishule D, Kiggundu M, Ameke C, et al. (2010) Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. CID; 50: 531-539.

[136] Elliott AM, Ndibazza J, Mpairwe H, Muhangi L, Webb EL, Kizito D, et al. (2011) Treatment with anthelminthics during pregnancy: what gains and what risks for the mother and child? Parasitology 138: 1499-1507.

[137] WHO (1994) Report of the WHO informal consultation on hookworm infection and anaemia in girls and women. WHO/CTD/SIP/96.1. World Health Organization, Geneva.

[138] WHO (2002) Prevention and Control of Schistosomiasis and Soil-Transmitted Helminthiasis: Report of a WHO Expert Committee. Geneva: World Health Organization; 2002.

[139] WHO (2002) Report of the WHO informal consultation on the use of praziquantel during pregnancy/lactation and albendazole/mebendazole in children under 24 months. Geneva: World Health Organization 2002. WHO/CDS/CPE/PVC/2002.4.

[140] Basra A, Mombo-Ngoma G, Melser MC, Diop DA, Würbel H, et al. (in press) Efficacy of Mefloquine intermittent preventive treatment in pregnancy against *Schistosoma haematobium* infection in Gabon: A nested randomized controlled assessor-blinded clinical Trial. Clin Infect Dis. 40.

[141] Gyorkos TW, Gilbert NL, Larocque R, Casapía M, Montresor A. (2012) Re-visiting *Trichuris trichiura* intensity thresholds based on anemia during pregnancy. PLoS Negl Trop Dis ;6(9):e1783.

[142] Muhangi L, Woodburn P, Omara M, Omoding N, Kizito D, Mpairwe H, Nabulime J, Ameke C, Morison LA, Elliott AM. (2007) Associations between mild-to-moderate anaemia in pregnancy and helminth, malaria and HIV infection in Entebbe, Uganda. Royal Soc Trop Med Hyg 101: 899-907.

[143] Makhoul Z, Taren D, Duncan B, Pandey P, Thomson C, Winzerling J, et al. (2012) Risk factors associated with anemia, iron deficiency and iron deficiency anemia in rural Nepali pregnant women. Southwest Asian J Trop Med Public Health;43(3):735-46.