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## CHAPTER 1

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### GENERAL INTRODUCTION



## Background

Africa, a continent of nearly one billion people has the world's largest mortality rate at every age group, and it accounts for half the world's burden of maternal, new-born and child mortality (1,2). Among the most pressing public health challenges is the improvement of maternal and child health in Africa.

According to the World Health Organization (WHO) every year, an estimated 15 million infants are born preterm (before 37 weeks of gestation), more than 60% occurring in Africa and South Asia, and according to the same source more than 20 million infants worldwide (15% of all births) are born with low birthweight, of which 95% occur in developing countries (3).

The Millennium Development Goals 4 and 5 were targeted at reducing by two-thirds, between 1990 and 2015, the under-five mortality rate and improving maternal health by reducing by three-quarters the maternal mortality rate within the same time period (4). While the most severe adverse outcome of pregnancy is the death of the mother or her offspring, even if both the mother and infant survive, pregnancy complications or problems at delivery or during the neonatal period can lead to severe maternal or infant morbidity (5). Preterm birth and low birthweight complications are the leading causes of death among children under 5 years of age, responsible for nearly one million deaths in 2013. Many of those surviving face a lifetime disability, including learning disabilities and visual and hearing problems (6). Also, there is epidemiological evidence that intrauterine growth restriction (IUGR) is associated with a substantially greater incidence of adult hypertension, insulin resistance/type 2 diabetes and cardiovascular diseases deaths (7–9).

Africa is hit with a double burden of disease. Primarily seen as Africa's major health burden were infectious diseases including tuberculosis, malaria, and HIV/AIDS. But now, in addition to these, there is a rising epidemic of chronic, non-communicable diseases such as heart disease, mental illnesses, trauma, cancer, diabetes, and obesity (10,11). In sub-Saharan Africa, the observed high rates of maternal and neonatal morbidity and mortality derive from multiple causes including endemic infectious diseases; malnutrition and micronutrient deficiencies; gynaecologic and obstetric complications; new-born illness associated with inadequate antenatal and perinatal care as well as suboptimal postnatal care due to financial and logistic constraints in these resource-limited regions (2,12,13).

Targeted public health interventions such as Intermittent Preventive Treatment of malaria during pregnancy (IPTp), vitamin and micronutrient supplementation, provision of long-lasting insecticide-treated nets (LLITNs), the prevention of mother-to-child HIV transmission (PMTCT), and improved frequency and quality of antenatal and postnatal health care are the

cornerstone of current strategies to reduce adverse pregnancy and birth associated outcomes in sub-Saharan Africa (14–18).

The studies of pregnancy and neonatal health are important as the human foetus and the infant can respond to unbalanced nutrition and other adverse influences by changing their developmental and growth trajectories (19–21). The processes involved may include induction of attributes that adapt the individuals for the type of environment in which he or she is likely to live later in life. People who are born particularly small have health disadvantages both in infancy and in later life (20). In addition, the causes of foetal-neonatal deaths are a complex mix of medical and social factors that vary by settings. Local new-born health problems need to be examined and prioritized on the basis of data and community perceptions. There is no one-size-fits-all new-born health program. Evidence-based intervention packages and lessons learned from field-based programs need to be adapted to fit the local setting.

Taken together, the identification of additional - yet underappreciated - preventable risk factors for adverse pregnancy outcome is necessary to further strengthen current efforts to reduce maternal and neonatal mortalities and morbidities. In areas like sub-Saharan Africa, it is important to address the issues surrounding adverse pregnancy outcomes and to identify risk factors. Moreover, in the region where parasitic infections challenge the immune system continuously, it would be important to design and conduct studies that aim to fully grasp the influence of a total burden of micro- and macro-organisms on the shaping of the immune system and the implications for birth outcomes including preterm birth and low birthweight. These studies will strive to understand the biological mechanisms which control pregnancy and will identify underlying mechanisms responsible for adverse outcomes. This would set the stage for steps towards generating data and specimens that are analysed by high dimensional systems biology techniques. The ultimate aim of such studies would be to exploit the findings for development of biomarkers, and new prevention and treatment strategies that will address the burden of low birth weight and preterm birth around the globe.

### **Epidemiology of adverse pregnancy outcomes**

In most developed countries pregnancy outcomes are generally favourable for both mother and infant and complications are few, while conversely, adverse pregnancy outcomes are far more frequent in the developing world (5). Maternal or offspring death is the most severe adverse pregnancy outcome. Every day, approximately 800 women die from preventable causes related to pregnancy and childbirth, 99% of all maternal deaths occur in developing countries. In 2013, the maternal mortality ratio in developing countries was 230 per 100,000 live births versus 16 per 100,000 live births in developed

countries (22). Maternal mortality is higher in women living in rural areas and among poorer communities, and young adolescents face a higher risk of complications and death as a result of pregnancy than older women. In 2015, 4.5 million (75% of all under-five deaths) occurred within the first year of life, with the risk five times higher in Africa compared to Europe, 55 deaths per 1000 live births and 10 per 1000 live births, respectively (23). Though the importance of mortality and severe morbidity is recognized as measures of adverse pregnancy outcome, proxy outcomes for mortality and severe morbidity are often studied. The most commonly studied of these proxies has been low birthweight, including features associated with it, namely preterm birth, intrauterine growth restriction (IUGR) and congenital anomalies (5) [Figure 1].

Birthweight, is the first weight of the foetus or new-born obtained after birth, for live births, it should ideally be measured within the first hour of life before significant postnatal weight loss occurs (3). Birthweight is often used as a health indicator, not only of the mother's health and nutritional status, but also of the new-born's chances for survival, growth, long term health and psychosocial development. Low birthweight is defined by WHO as birth weight below 2500g (24). Low birthweight is generally determined by a short duration of gestation or prematurity, defined as gestational age less than 37 weeks, or intrauterine growth retardation (IUGR) also referred to as small for gestational age (SGA) [Figure 1]. Whatever the type, the consequences of low birthweight are more or less the same, though its clinical importance may also depend on the primary cause as an infant who has IUGR as a result of intrauterine rubella infection will have a much poorer prognosis than another of similar weight at birth who is small because his or her mother is short (25).

Low birthweight can be measured with excellent validity and precision. This is one of the reasons it continues to be studied by epidemiologists and health practitioners. Conversely, measuring preterm birth or IUGR requires a valid estimate of gestational age, which is often difficult in developing countries.

The etiologic determinants of preterm birth and IUGR differ, so lumping them together as low birthweight can hinder progress in developing preventive interventions (5). For example, preterm birth may be caused by genital tract infection, multiple birth, pregnancy induced hypertension and other complications of pregnancy, while IUGR may be due to low energy intake, low gestational weight gain, short stature, etc. However, in countries where the prevalence of low birthweight is very high, most low birthweight infants are growth restricted rather than preterm (26,27). The global prevalence of low birthweight is 15.5%, which amounts to about 20 million low birthweight infants born each year, 96.5% of them in the developing countries (3).

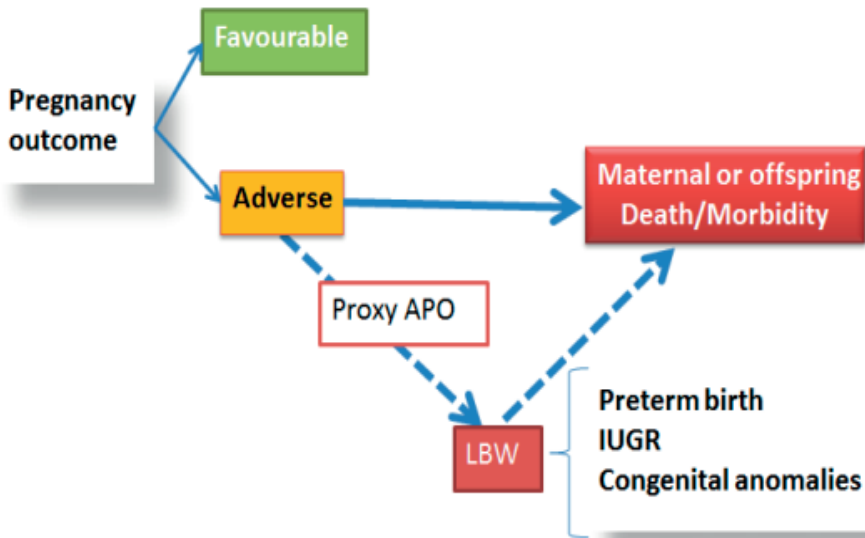


Figure 1: **Adverse Pregnancy Outcome (APO).**

Though the importance of mortality and severe morbidity is recognized as measures of adverse pregnancy outcome, proxy outcomes for mortality and severe morbidity are often studied. The most commonly studied of these proxies have been low birth weight (LBW), including its features, preterm birth, intrauterine growth (IUGR) and congenital anomalies (Kramer 2003). Birth weight is often used as a health indicator of both the mother's health and nutritional status, and also of the newborn's chances for survival, growth, long term health and psychosocial development

### **Determinants and risk factors of low birthweight**

It is in general recognized that low birthweight can be caused by many factors. In an effort to identify potential determinants, Kramer conducted a critical assessment and meta-analysis of medical literature in English and French published from 1970 to 1984 (25). Restricting the assessment to singleton pregnancies and excluding extremely rare factors as well as complications of pregnancy, 43 potential determinants were identified. According to the author, in developing countries, the major determinants of IUGR were Black or Indian racial origin, poor gestational nutrition, low pre-pregnancy weight, short maternal stature, and malaria, while in developed countries, the most important single factor, by far, was cigarette smoking, followed by poor gestational nutrition and low pre-pregnancy weight (25).

## Parasitic infections during pregnancy

“Humans are hosts to nearly 300 species of parasitic worms and over 70 species of protozoa, some derived from our primate ancestors and some acquired from animals we have domesticated or come in contact with during our relatively short history on Earth” (28). Among the major tropical infectious diseases in sub-Saharan Africa are malaria, schistosomiasis, and filariasis with each affecting more than 200 million people in the world including pregnant women (Table 1) (29).

Table 1: **Our knowledge on major Parasitic Infections during pregnancy**

Parasitic Infections during pregnancy
<p><b><i>Malaria</i></b></p> <ul style="list-style-type: none"> <li>• Substantial risk to the pregnant woman, her foetus, and the new-born child</li> <li>• Established interventions: LLITNs, IPTp, case management</li> </ul>
<p><b><i>Schistosomiasis</i></b></p> <ul style="list-style-type: none"> <li>• Little known about the schistosome-specific morbidities during pregnancy</li> <li>• Animal models: schistosomiasis infections lead to deleterious pregnancy outcomes</li> <li>• Case reports: association with adverse pregnancy outcome</li> <li>• No established intervention during pregnancy</li> </ul>
<p><b><i>Loiasis</i></b></p> <ul style="list-style-type: none"> <li>• Paucity of studies regarding loiasis during pregnancy</li> <li>• No information on its impact on maternal and foetal health outcome</li> <li>• Anecdotal evidence indicates possible invasion of the placenta</li> <li>• No established intervention during pregnancy</li> </ul>
<p><b><i>Co-infections</i></b></p> <ul style="list-style-type: none"> <li>• Helminth may increase susceptibility to clinical malaria</li> <li>• How combination of these infections affect pregnancy outcome has hardly been studied</li> </ul>

Note: Table 1 shows the recognised public health importance of the three major parasitic (co)infections; LLITNs: Long Lasting Insecticide Treated Nets; IPTp: Intermittent Preventive Treatment of malaria during pregnancy.

It is thought that “so long as Woman has walked the earth, malaria may have stalked her” (30). Malaria infection during pregnancy is a significant public health problem with substantial risks for the pregnant woman, her foetus, and



the new-born child. Owing to as yet not fully established reasons, pregnant women are more susceptible to the effects of malaria infection with increased associated morbidity and mortality both in the mother and her new-born (31). In the African region, nearly 30 million pregnancies occur every year in areas with stable transmission of *Plasmodium falciparum* (32). Both maternal anaemia and placental parasitaemia can lead to low birthweight. In high-transmission settings, the adverse effects of *P. falciparum* infection in pregnancy are most pronounced for women in their first pregnancy. Malaria is the only tropical disease in Africa with an established prevention strategy in place during pregnancy. WHO recommends the following package of interventions for the prevention and treatment of malaria during pregnancy: use of long lasting insecticidal nets (LLINs); intermittent preventive treatment of malaria in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP); and, prompt diagnosis and effective treatment of malaria infections.

Although it is estimated that about 40 million women of childbearing age are currently infected with schistosomes, little is known about the schistosome-specific morbidities that are experienced by pregnant and lactating mothers and their offspring (33). Animal models provide evidence that schistosomiasis infection leads to deleterious pregnancy outcomes. In an experimental group of mice infected with *S. mansoni* compared to an uninfected control group, there was significantly higher proportion of abortion, maternal and offspring deaths as well as a lower weight of the offspring (33,34). These findings indicate that there is likely to be an interaction between schistosome infection and pregnancy such that the former is potentially more pathogenic affecting the outcome of pregnancy. Although there are many case reports that associate infection of the female genital tract with schistosomiasis in pregnant women, studies that address schistosomiasis and subsequent birth outcomes are scarce and only limited causal inference can be made from case reports. Of the many case reports of pregnant women with schistosomiasis who experienced an adverse birth outcome, inflammation of the placenta and of the cervix has been reported in some (35–37). A review by Nawal Nour thoroughly describes the health effect of schistosomiasis on women (38). In endemic areas, women in their reproductive years are thought to suffer from severe morbidity and even mortality because of female genital schistosomiasis, predominantly caused by *S. haematobium*. As the eggs penetrate the urinary system, they can find their way to the female genital region and form granulomas in the uterus, fallopian tube, and ovaries (39). Studies have demonstrated increased risk of anaemia associated with *S. mansoni* infection in pregnant women (40), and higher number of preterm deliveries and low birth weight infants (41). Schistosomiasis has been detected in the placenta and new-borns have been diagnosed with the disease, thus confirming congenital infection. One study reported on a case of urogenital and placental

schistosomiasis in a 28 year-old German woman returning from Malawi. Urogenital schistosomiasis was diagnosed based on typical vesical lesions revealed by cystoscopy and confirmed ova by histopathology of the bladder and the placental stroma (42). Another case reported an African woman with genital pathology who upon examination was found to have ectopic pregnancy and *S. haematobium* ova were identified in the patient's fallopian tube (43). Additional case reports have indicated recurrent ectopic pregnancy associated (44), and spontaneous abortion to be associated with *S. haematobium* infection (37).

There is also paucity of studies regarding loiasis during pregnancy. Despite the high prevalence of *Loa loa* in Central African communities, there is to date no information on its impact on maternal and foetal health outcomes during pregnancy. Since loiasis is a chronic infection, it may be speculated that a considerable proportion of pregnant women are harbouring this infection during gestation. Anecdotal evidence indicates the potential of *L. Loa* to invade the placenta (45,46). In addition, in the context of infection, there is growing evidence that *in utero* exposure to helminths that affect the mother during pregnancy, such as filariasis, may even have an impact on the natural history of these specific parasites during childhood (47). It has to be noted that for filariasis and schistosomiasis during pregnancy there is no established screening, treatment or prevention programs, so they are rarely diagnosed and treated during pregnancy (48).

Of public health importance in schistosomiasis and filariasis endemic areas is the co-infection with malaria during pregnancy as studies have reported that helminths may increase susceptibility to clinical malaria (49), although there are also reports of conflicting results as reviewed by Mwangi and colleagues (50). Briefly, early studies from Comoros Island provided evidence that *Ascaris Lumbricoides* infection in general is protective against clinical malaria (51,52). This was followed by a study conducted in 1998 by Jambou *et al.* in Madagascar reporting that the incidence of malaria attacks was three-fold reduced among children receiving the anthelmintic drug Levamisol (quoted from Spiegel *et al.* 2003)(49), while later on in a series of observational studies it was shown that children infected with soil-transmitted helminths had an increased risk of clinical malaria compared to those uninfected (49). Similarly, a study of severe malaria in rural Senegal showed that children infected with *A. lumbricoides* had an increased risk of severe malaria (53). From two further studies in Senegal comparing the relationship between schistosome and *falciparum* infections, one reported that the incidence of clinical malaria was higher in children infected with *S. mansoni* than those uninfected (54), while the other found a negative association between the two infections and reported a lack of association between soil-transmitted helminths and *P. falciparum* infection (55). Regarding clinical malaria, Lyke and co-workers

reported for children aged 4 to 8 years that *S. haematobium* delayed time to first infection (74 vs. 59 days), fewer numbers of malaria episodes (1.55 vs. 1.81 infections) and lower geometric mean parasitic densities (6.359 vs. 9.874 asexual forms/mm) at first infection (56). How combination of these infections affect pregnancy outcome has hardly been studied. A review from Adegnika and Kremsner in 2012 summarized that hookworm, *A. Lumbricoides* and schistosomiasis species were associated differently with malaria, which might explain the conflicting reports on interaction between helminths and malaria (57).

### **Immunology of parasitic infections and pregnancy**

A simplification of cellular immune responses provides a workable framework to understand health and disease. A number of T cell subsets have been identified, which play different roles to ensure a healthy outcome. The type-1 immune responses spearheaded by Th1 cells, are characterized by the production of pro-inflammatory cytokines such as interferon (IFN)-gamma and tumour necrosis factor (TNF), whereas type-2 responses which encompass the Th2 cells, lead to increased interleukin (IL)-4, IL-5, and IL-13 along with eosinophilia and high levels of immunoglobulin (Ig) E. Discovered, later, there are also cells that represent the Th17 response which represents increased IL-17 and IL-22 and associates with expansion of neutrophils. To keep effector Th1, Th2 and Th17 cells under control, the regulatory cells exert suppressory activities. The regulatory cells of T cell (Treg) and B cell (Breg) lineages have been described and are important to halt a strong inflammatory response to prevent tissue damage (all reviewed in Wammes et al. 2014)(58).

Studies of the immunology of pregnancy have shown evidence of an increase of Tregs during pregnancy, and that the expansion of the Treg population is of importance for the allogenic foetus to evade immune attack from the mother (59). Tregs play a dominant role in the maintenance of immunological self-tolerance by preventing immune and auto-immune responses against self-antigens. Several functional studies have shown that unexplained infertility, miscarriage and pre-eclampsia are often associated with deficit in Treg cell number and function while normal pregnancy selectively stimulates the accumulation of maternal FOXP3<sup>+</sup> Treg cells with foetal specificity (60). During pregnancy, the balance of Th1 and Th2 cytokines is more toward Th2 cytokines, followed by a progressive shift toward Th1 later in gestation, that when abnormal, may initiate and intensify the cascade of inflammatory cytokine production involved in adverse pregnancy outcomes. Inflammation is a process by which tissues respond to various insults. It is characterized by up-regulation of chemokines, cytokines, and pattern recognition receptors that sense microbes and tissue breakdown products. Moreover, maternal and

placental hormones may affect the inflammatory pathway (61). In parallel, it is also known that parasitic infections can skew immune responses. Whereas malaria is associated with pro-inflammatory cytokines (62), the hallmark of helminth infections is a strong skewing of immune responses towards Th2 (63), and regulatory mode (64). Indeed, helminths as masters of regulation stimulate the induction of regulatory T cells and B cells and inhibit antigen presenting cells (63). The Th2 and suppressor cells are able to also alter responses to bystander antigens. Such modulatory effects are thought to have both negative and positive impact on health. On the one hand, this could potentially have detrimental consequences for co-infections or vaccination programs (65), and maybe to some extent on growth and cognition, while on the other hand it could exert beneficial effects on diseases that stem from strong inflammatory reactions. The immunological alternations exerted by malaria parasites and helminth infections, could interfere with the immunological processes that change during pregnancy and therefore affect pregnancy outcome.

In areas like sub-Saharan Africa, it is important to address the issues surrounding adverse pregnancy outcomes and to identify risk factors. Moreover, in the region where parasitic infections insult the immune system continuously, it would be important to design and conduct studies that aim to fully grasp the influence of a total burden of micro- and macro-organisms on the shaping of the immune system and the implications for birth outcomes including preterm birth and low birth weight. These studies will strive to understand the biological mechanisms which control pregnancy and will identify underlying mechanisms responsible for adverse outcomes. This would set the stage for steps towards generating data and specimens that are analysed by high dimensional systems biology techniques. The ultimate aim of such studies would be to exploit their findings for development of biomarkers, and new prevention and treatment strategies that will address the burden of adverse pregnancy outcomes including low birth weight and preterm birth around the globe.

## Scope and Aims of the thesis

From the fact that adverse pregnancy outcomes are major problems in developing countries, it is important to get reliable data on the extent of the problem in country specific manner and then to work on identifying the major risk factors. Moreover, parasitic infections are still highly prevalent in sub-Saharan Africa and millions of women of childbearing age are exposed to these infections. Studies of parasitic infections during pregnancy will help to grasp the influence of a total burden of parasites on shaping the birth outcomes and also will allow us to understand the biological mechanisms which control pregnancy and identify the underlying immunological processes that might explain the birth outcomes.

This thesis explores the burden of adverse pregnancy outcomes including low birthweight and preterm birth in sub-Saharan Africa and investigates their risk factors. It examines the burden during pregnancy of the major parasitic infections endemic in Gabon including malaria, loiasis and urogenital schistosomiasis and their implications for birth outcomes in addition to already identified risk factors. The thesis explores existing and potential alternative preventive strategies against parasitic infections to be integrated in the continuum of care for maternal, newborn and child care. In addition, it starts dissecting some immune mechanisms to understand how parasites are shaping the immune system via induction of regulatory T cells in the infected hosts and in the cord blood of offspring from parasite infected mothers to pave the way towards analysis of the immune system during pregnancy and its effect on birth outcomes.

## The outline of the thesis

In **Chapter 2**, we have conducted a multicentre study in four sub-Saharan African countries from Western (Benin), Central (Gabon), Eastern (Tanzania), and Southern (Mozambique) African areas. We determined the global and specific burden of low birthweight infants, preterm deliveries, and maternal anaemia as well as their respective risk factors when restricted to singleton live births in the context of standardized and freely provided antenatal care including distribution of long lasting insecticide treated nets and administration of effective intermittent preventive treatment against malaria during pregnancy. The aim of the study is to use identical methods to assess the burden of adverse pregnancy outcomes in Gabon and compare it with what is seen in other African partner countries.

**Chapter 3** is on the epidemiology of loiasis in pregnant women in Gabon. The prevalence of *L. loa* microfilaria is determined in peripheral and placental blood among pregnant women in a highly endemic region of Gabon and related to pregnancy and birth outcomes.

In **Chapter 4**, pregnant women are screened for urogenital schistosomiasis and related to pregnancy and birth outcomes.

In **Chapter 5**, the effectiveness of alternative preventive treatment of malaria is studied comparing mefloquine and sulphadoxine-pyrimethamine. The outcomes measured are low birth weight, *Plasmodium falciparum* parasitaemia, maternal anaemia and other morbidities during pregnancy.

**Chapter 6** investigates the efficacy of mefloquine against urogenital schistosomiasis while administered for prevention of malaria during pregnancy, exploring the concept of combined strategies to combat these co-infections together.

**Chapter 7** explores the function of regulatory T cells by studying CD4<sup>+</sup>CD25<sup>hi</sup>FOXP3<sup>+</sup> T cells in cord blood of neonates born to mothers with loiasis compared to their uninfected counterparts.

In **Chapter 8**, the mechanisms of immune modulation used by parasites is assessed by determining the proportions and phenotype of regulatory T cells (CD4<sup>+</sup>CD25<sup>hi</sup>FOXP3<sup>+</sup>) during urogenital schistosomiasis. The suppressive capacity of Tregs is measured by an established field-applicable assay.

At the end, in **Chapter 9**, the main findings of the studies listed above are summarized and discussed along with the limitations of the studies and future directions.

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