Diagnosis of malaria in pregnancy: evaluation, new developments and implications

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Chapter 1

General Introduction
CHAPTER 1. GENERAL INTRODUCTION

1.1 Background

Malaria infection during pregnancy is a major public health problem in (sub-)tropical regions. Malaria is caused by infection with protozoa of the genus *Plasmodium* [1,2]. Of the five human malaria species, *Plasmodium falciparum* and *Plasmodium vivax* cause the most severe effects during pregnancy [1,3,4]. Infections with *Plasmodium malariae* and *Plasmodium ovale* during pregnancy are rarely seen in the placenta, and are often reported as a mixed infection with *P. falciparum* [1,5]. *Plasmodium knowlesi*, a malaria infection common in monkeys, can also infect humans, but so far there are no studies reporting infections with this species in pregnant women. Malaria parasites are transmitted by mosquitoes, in the case of humans exclusively by female mosquitoes of the genus *Anopheles* [6,7]. Pregnant women are more attractive to malaria mosquitoes and more likely to be bitten than other adults, most likely due to two physiological factors: increased heat and increased release of volatile substances from the skin surface [8–10]. In areas where malaria is endemic, children carry the heaviest burden of malaria infection, but pregnant women are the main adult risk group.

The severe effects of malaria on pregnant women and their (unborn) infants, described in detail in section 1.2, make early detection and subsequent treatment of great importance. Measures to prevent malaria during pregnancy include the use of insecticide treated bed nets (ITNs) and intermittent preventive treatment (IPT) in high transmission areas. IPT is the administration of several therapeutic doses of antimalarial drugs irrespective of complaints or diagnosis. Despite the application of these measures, malaria can occur and early diagnosis and treatment are essential for case management, both in low and high transmission areas.

1.2 Epidemiology and effects of malaria in pregnancy

In 2007 approximately 55 million women living in areas with stable *P. falciparum* transmission and around 70 million living in areas with low malaria transmission or with *P. vivax* only became pregnant [11]. The incidence and thus the risk of getting malaria in the areas of low transmission is much lower than in stable transmission areas, so only a small proportion of the 70 million women actually acquired malaria. It was estimated that in areas with stable transmission in Africa at least one in four pregnant women have parasitological evidence of malaria at delivery [12]. In areas of low or seasonal transmission in Africa the median prevalence of peripheral and placental parasitaemia were approximately 14% and 7%, respectively [12]. Outside Africa these estimates were 6% and 10% [12]. Independent of the transmission in-
1.2. EPIDEMIOLOGY AND EFFECTS OF MALARIA IN PREGNANCY

tensity, the risk of infection with peripheral parasites is highest in the second trimester and although women of all parities can become infected, prevalence of parasitaemia is more frequent among women with lower parity, which will be described in more detail in a later section [12, 13]. Lower maternal age might be an additional risk factor [12].

**Low or unstable P. falciparum malaria transmission**

The symptoms and complications of malaria in pregnancy differ with the transmission intensity, which is related to the level of immunity that the women have acquired against the infection [13, 14]. In low or unstable transmission areas, malaria in pregnancy usually presents as a symptomatic, severe disease that can result in death of mother and foetus (Figure 1.1). Most people in these areas, including pregnant women, have not acquired a significant level of immunity against malaria and are at risk of developing severe disease. Frequently observed symptoms of malaria are fever, headache, abdominal pain, nausea and vomiting. If left untreated the disease can progress rapidly to severe disease and possibly cerebral malaria. Maternal death may result from severe malaria directly or indirectly due to severe anaemia [12, 13]. In addition, malaria in pregnancy can result in a variety of adverse pregnancy outcomes, including spontaneous abortion, neonatal death, preterm delivery and low birth weight [12, 13].

**High or stable P. falciparum malaria transmission**

In areas of high (stable) malaria transmission, most adults have acquired sufficient immunity to protect themselves to severe disease, and in these cases malaria infection rarely results in symptomatic disease (Figure 1.1). During the first and second pregnancy women are more susceptible to the effects of malaria infection and may develop symptomatic and even severe disease. In later pregnancies, immunity prevents development of severe disease unless the woman is infected with HIV as well, as described below. The main impact in high-transmission areas is malaria-related maternal anaemia and low birth weight and stillbirth [12, 13]. In Africa, 5 - 10% of pregnant women may develop severe anaemia and it was estimated that in 26% of these cases severe anaemia is attributable to malaria [12]. Severe anaemia is directly linked to maternal mortality and is a risk for intrauterine growth retardation leading to low birth weight [12]. Additionally, excessive postpartum blood loss is a possible complication of placental malaria [15, 16].
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Figure 1.1 Symptoms and complications of malaria according to transmission intensity
Adapted from [14].

Effect of *P. vivax* malaria during pregnancy

*P. vivax* is more prevalent than *P. falciparum* in many areas of Asia and South America [11]. Although *P. vivax* infection during pregnancy is considered to be more benign than *P. falciparum* infections, recent evidence associates significant adverse effects with *P. vivax* infections during pregnancy [3,17–21]. The main difference between two malaria species is that *P. vivax* infected erythrocytes are incapable to sequester in the placenta as is the case with *P. falciparum* [2,22]. In several studies from India, Thailand, Papua New Guinea, Colombia and Venezuela, *P. vivax* infection during pregnancy was associated with (severe) anaemia, low birth weight and preterm delivery; albeit to a lesser extent than with *P. falciparum* infections [3,17–21].

Effect of maternal malaria on infant health

Annually an estimated 75 000 - 200 000 infant deaths are linked to malaria in pregnancy in high transmission areas [12,23]. Newborns may have low birth weight (LBW; birth weight < 2500 g) due to intrauterine growth retardation
or premature delivery because of malaria infection of the placenta. Overall, neonates born to mothers with an infected placenta are twice as likely to be of LBW as neonates born to mothers with uninfected placentas and LBW is a significant risk factor for neonatal and infant mortality [24–26]. Another consequence of (malaria-induced) LBW is an increased risk of metabolic diseases such as type 2 diabetes [27]. Already, diabetes is hugely under diagnosed in Africa, leading to an estimated 344 000 deaths in 2011 and health systems are slow to respond to this burden [28]. In addition to LBW, children are more susceptible to malaria infection themselves when the placenta was infected during their gestation [4]. Maternal malaria infection during pregnancy can result in an infected neonate (congenital malaria) as well, but in endemic areas this type of infection is often asymptomatic and clears spontaneously, probably because of passive immunity from maternal antibodies in breast milk [4].

**Effect of HIV infection on malaria in pregnant women**

Although several infections can occur at the same time as malaria in pregnant women, co-infection with HIV is quite prevalent due to a high prevalence of HIV infections in sub-Saharan Africa. It merits specific discussion, because it affects the pregnancy-specific immunity to malaria and the response to intermittent preventive treatment [29]. HIV-infected pregnant women are more frequently infected with malaria and with higher parasite densities and more often have symptomatic malaria than HIV-uninfected pregnant women [29]. Anaemia and maternal death are more common in HIV-malaria co-infected pregnant women and very often an increased occurrence of placental malaria is seen [29]. In addition, the viral load is increased in malaria infected patients and in their placentas. The difference in the presentation of a malaria infection between HIV-infected and HIV-uninfected women is more pronounced after the third pregnancy and data from immunological studies indicate that HIV impairs parity-specific immunity [29, 30]. The co-infection is associated with an increased risk of low birth weight and preterm delivery compared with pregnant women with a single infection of either disease [29]. HIV infection impairs responses to chloroquine-based and sulfadoxine-pyrimethamine (SP)-based intermittent preventive treatment regimens [29]. Only IPT strategies during pregnancy with monthly SP treatment, which will be described in more detail in section 1.4, achieved the same efficacy on placental malaria in HIV-infected and HIV-uninfected women [29]. The clinical benefits, however, of monthly SP treatment versus two doses of SP on adverse effects such as premature delivery, low birth weight, and perinatal mortality remains to be determined for HIV-infected women [29].
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1.3 Pathogenesis of malaria in pregnancy

Both *P. falciparum* and *P. vivax* infections during pregnancy cause adverse effects, although the mechanisms are somewhat different. Susceptibility of pregnant women to malaria probably represents a combination of immunological and hormonal changes associated with pregnancy, combined with the unique ability of *P. falciparum*-infected erythrocytes to sequester in the placenta. The pathogenesis of *P. vivax* infection during pregnancy is not well studied and most knowledge of pathogenesis of and immunity to malaria comes from areas of high transmission.

Parasite life cycle

To understand the pathogenesis of malaria in pregnant women it is necessary to have some knowledge of the parasite life cycle in general, which is summarised in this section and described in detail in 'Essential Malariology' [2].

Malaria infection in the patient starts with sporozoite entry into the bloodstream with the saliva of the mosquito during its blood meal. The sporozoites migrate to the liver with the bloodstream where they invade liver hepatocytes and there the parasite undergoes asexual multiplication resulting in merozoites. *P. vivax* and *P. ovale* parasites can remain in a dormant stage in the liver for many weeks to months, and even up to several years, which are called hypnozoites, and when triggered by a stimulus (possibly febrile illness) the hypnozoites re-enter the cell cycle causing a so-called relapsed infection [31]. The liver cells burst and release the merozoites into the bloodstream where they invade red blood cells (erythrocytes). The merozoites that have infected erythrocytes undergo a second round of asexual multiplication after which the erythrocytes burst and the merozoites can infect other red blood cells, dramatically increasing the amount of infected cells. *P. falciparum* can infect all erythrocyte stages, whereas *P. vivax* and *P. ovale* can only infect reticulocytes and *P. malariae* only mature erythrocytes, thus limiting the amount of infected cells in infections by the latter three parasite species. A small proportion of the young merozoites develop into male and female gametocytes that circulate in the blood and can be taken up by a mosquito in its blood meal. In the mosquito gut they mature into gametes and sexual reproduction can occur. This results in a motile ookinete that penetrates the gut wall of the mosquito and becomes an oocyst, in which another phase of asexual multiplication occurs. The resulting sporozoites migrate to the salivary glands of the mosquito, ready to be injected into a new person to repeat the cycle.
1.3. PATHOGENESIS OF MALARIA IN PREGNANCY

*P. falciparum*

During pregnancy, *P. falciparum* parasites, in more detail the trophozoite and schizont stages, can sequester in the maternal vascular area of the placenta (the intervillous space), which is mainly caused by a specific interaction of a plasmodial variant surface antigen (named VAR2CSA) with chondroitin sulphate A (CSA); a ligand expressed only in the placenta [32, 33]. This leads to several placental changes central to the pathogenesis of placental malaria. The sequestration of *P. falciparum* infected erythrocytes is different than sequestration in other tissues in non-pregnant individuals. First of all, the sequestration occurs through a different receptor (CSA) than in non-pregnant individuals and sequestration occurs throughout the intervillous space, instead of in close apposition to the vascular wall [32, 33]. Secondly, placental infected erythrocytes do not show so-called rosetting, the adhesion of non-infected erythrocytes, and they do not agglutinate in the presence of serum from prior exposed individuals [33].

The surface antigens on infected erythrocytes of non-pregnant and pregnant individuals are the main targets of the antibodies that mediate protective immunity, which explains the susceptibility to malaria infection of previously immune primigravidae in high transmission areas, since women have not been exposed to the pregnancy related antigen VAR2CSA before their first pregnancy [32, 33]. Immunity to the non-pregnancy antigens is associated with a reduction in placental malaria infections as well, which explains why young age is an independent risk factor for malaria during pregnancy when immunity is not fully developed yet [33, 34].

In addition to the sequestration of infected erythrocytes, an increased number of maternal phagocytic cells, i.e. monocytes and macrophages, are found in the intervillous space and haemoglobin (also called malaria pigment; a metabolic waste product of haemoglobin degradation) is found inside phagocytic leucocytes and within fibrin deposits in the intervillous space [35–37]. Chronic infection of the placenta (defined as the presence of parasites and substantial amounts of pigment in fibrin or cells) has been associated with decreased birth weight and anaemia, whereas acute infection (defined as the presence of parasites, with absent or minimal pigment deposition within fibrin) was more closely associated with preterm delivery [38, 39]. The sequestration of infected erythrocytes triggers the secretion of β-chemokines that are chemotactic for monocytes and macrophages, explaining the predominance of these types of leucocytes in the intervillous space [33, 40, 41]. Malaria infection of the placenta shifts the placental cytokine balance from Th2-type response to a Th1-response, helping to eliminate the parasites, but at the same time this Th1-response is a risk for the pregnancy [33, 40, 41]. A strong
Th1-response during pregnancy has been associated with maternal anaemia, abortions, stillbirth and premature deliveries [33, 40, 42].

Anaemia during malaria infection in non-pregnant individuals is caused by a combination of effects, including bone marrow dysfunction and destruction of both infected and uninfected erythrocytes [2, 33]. During pregnancy, anaemia is further caused by disrupted levels of the cytokines TNF-α and IL-10 [33, 40, 41]. Elevated levels of TNF-α can suppress erythropoiesis in the absence of sufficient IL-10 and alternatively, by inflicting oxidative stress that alters erythrocyte membranes, leading to increased erythrocyte destruction [33, 40, 41].

There are several ways in which malaria infection can contribute to foetal growth restriction leading to low birth weight (LBW). During the first trimester of pregnancy, trophoblasts invade the uterus, transforming the maternal spiral arteries and increasing placental blood supply, and malaria infection during this period can cause placental insufficiency, altered angiogenesis and possibly hypertension, causing symmetric growth restriction [33, 43]. Infection late in the first trimester and in the second trimester, when peak incidences of infection occur, is associated with monocyte sequestration in the placenta that alters cortisol metabolism and nutrient transport and cause deregulation of insulin-like growth factors, resulting in asymmetric growth restriction [43]. Additionally, foetal growth is dependent on placental delivery of nutrients such as amino-acids and glucose, and during malaria infection of the placenta, transport of nutrients can be decreased by down regulation of transporter protein activity by cytokines such as IL-1β [33, 41, 43]. Maternal anaemia is an independent risk factor for LBW, but the exact mechanisms are not well understood [43]. Possible contributing factors are deficiencies in haematinic factors such as iron and folic acid, but also impaired oxygen delivery [43]. Similarly, the mechanisms leading to preterm delivery, which in turn leads to high risk of death in early life, are not well understood, but have been associated with malaria parasite density, maternal anaemia and high levels of cytokines (TNF-α and IL-10) [33].

Malaria infection during pregnancy is not always associated with placental pathology and it has been proposed that women who are able to control parasite density (due to immunity or treatment) might not experience the adverse consequences [33].

**P. vivax**

In contrast to *P. falciparum*, *P. vivax* infected erythrocytes do not seem to sequester in the placenta, even though *P. vivax* infections have been associated with severe consequences, but the number of studies is very limited [44, 45].
1.4. PREVENTION AND TREATMENT OF MALARIA IN PREGNANCY

One study in Thailand clearly showed that antenatal *P. vivax* infections were only associated with the presence of haemozoin in the placenta, but inflammatory and other pathological changes were very modest, especially compared to *P. falciparum* infections [44]. The exact mechanisms with which *P. vivax* causes the adverse effects during pregnancy, such as low birth weight, remain to be elucidated, but might be related to maternal anaemia and systemic and local inflammatory responses [43–45].

1.4 Prevention and treatment of malaria in pregnancy

Fortunately, malaria during pregnancy can be prevented and treated. Control measures to be deployed during pregnancy depend on the epidemiological exposure patterns. The World Health Organization (WHO) has developed guidelines for areas with stable malaria transmission, which consist of prompt and effective treatment combined with prevention of infection by intermittent preventive treatment during pregnancy (IPTp) and use of insecticide treated nets (ITNs) [1,46–48]. In lower transmission areas there are no specific guidelines for prevention of malaria in pregnant women and malaria control is primarily based on case management and prevention of mosquito bites [46,48,49].

IPTp is the administration of a full course of antimalarial treatment with a drug that is suitable and safe for pregnant women at specified time points during the pregnancy, regardless if the woman is infected at that time [1]. In this way it should reduce the malaria burden in the target population. In areas of stable malaria transmission, pregnant women should receive at least two doses of sulfadoxine-pyrimethamine (SP) at the first and second scheduled antenatal visit after quickening (first noted movement of the foetus) [1]. The doses should be taken at least a month apart and under direct observation during antenatal visits. Unfortunately, resistance against SP is becoming more and more widespread, making IPTp with SP less effective at preventing malaria infection in pregnant women and a change in policy to artemisinin-based combination therapies (ACTs) is being more and more supported by the scientific community [50,51]. Large studies assessing the safety and efficacy of ACTs for pregnant women are underway.

The use of insecticide-treated nets (ITNs) is being widely advocated to prevent pregnant women from getting infected and its impact has been studied, mainly in Africa. These studies have shown that the ITNs have a beneficial effect on pregnancy outcome in malaria-endemic areas in Africa in the first few pregnancies [52]. Infants born from pregnant women with ITNs showed higher mean birth weights and there was a reduced risk of placental infection, LBW and stillbirths compared to pregnant women without ITNs [52]. Their effect in areas outside Africa has not been extensively studied, but a trial from
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Thailand showed a reduction in anaemia and foetal loss in pregnant women from all gravidities when using ITNs [52,53]. ITNs are treated with pyrethroid insecticides, but the widespread use of these insecticides in malaria vector control through the scale up of ITN distribution programs and indoor residual spraying campaigns, inevitably lead to the development of resistance of the major malaria vectors, which is spreading rapidly throughout Africa [54].

Case management for pregnant women in stable transmission areas, where infection is often asymptomatic, include screening and treatment of anaemia with iron supplements and effective antimalarial treatment [1,48]. The advised treatments for malaria infection in pregnant women are beyond the scope of this thesis.

Alternative strategies than IPTp are being suggested and used, for example at the Shoklo Malaria Research Unit clinics on the Thai-Burmese border where many pregnant women receive weekly antenatal care, including malaria screening [44,55]. Another study has shown that IST (screening for malaria infection using a malaria RDT at scheduled antenatal clinic visits and treatment of positive women with an effective antimalarial drug) was not inferior to IPTp with SP in preventing maternal anaemia and low birth weight in women who used long-lasting bednets in an area of moderately high malaria transmission in Ghana [56]. Screening and subsequent treatment of women during pregnancy might be more effective than a preventive approach in areas with low levels of transmission or highly seasonal transmission and limits the number of women receiving drugs unnecessarily through IPTp in the low transmission season [44,56].

1.5 Diagnosis of malaria in pregnancy

An essential element of effective case management of malaria in pregnancy and screen-and-treat strategies is the diagnosis of the infection. Even with control measures to prevent malaria infection during pregnancy, such as IPTp and ITNs, diagnosis is essential. IPTp greatly reduces prevalence of malaria and severe consequences, but women are not protected throughout the entire pregnancy and can still become infected between doses or after the final dose, especially when other protective measures such as ITNs are not being used, or parasites are resistant to the drugs used for IPTp.

Unfortunately, diagnosis of malaria infection during pregnancy is often complicated by the very low number or even absence of parasites in peripheral blood due to placental sequestration [57,58]. The infection is often asymptomatic, but not without adverse effects, especially in high transmission areas, and the absence of symptoms complicates diagnosis even further [13]. Therefore, accurate diagnostic tools are necessary to confirm infection. While
1.5. DIAGNOSIS OF MALARIA IN PREGNANCY

Microscopic examination of venous (peripheral) blood slides is considered the gold standard for diagnosis in non-pregnancy related malaria, accurate detection of parasite infection in the placenta requires examination of placental tissue [39,59]. A detailed description of the diagnostic tools that are available for the diagnosis of malaria in pregnancy is presented in Chapter two, and are shortly introduced in this section.

Histological examination of the placenta

Histological examination of a stained biopsy from the maternal side of the placenta is considered the gold standard for diagnosis of placental malaria. The biopsy is examined for the presence of malaria parasites and pigment in the placental tissue and a classification system was developed to standardise the diagnosis of placental malaria [39]. Placental histology slides can be classified in active, active-chronic, past and no infection [39,59]:

**active infection** Parasites in maternal erythrocytes in the intervillous space, pigment in erythrocytes and monocytes in the intervillous space, but no pigment in fibrin or cells within fibrin.

**active-chronic infection** Parasites in maternal erythrocytes in the intervillous space, pigment in erythrocytes and circulating monocytes within the intervillous space and pigment in fibrin or cells within fibrin and/or chorionic villous syncytiotrophoblast or stroma.

**past-chronic infection** Parasites not present, pigment confined to fibrin or cells within fibrin.

The categories were based on assumption of the progression of infection during the early stages of infection; pigment is detected within circulating erythrocytes and monocytes and later accumulates in fibrin as well [39]. After clearance of placental parasites and pigment from circulating erythrocytes and monocytes, the pigment persists in fibrin [39].

Placental histology can only be performed after delivery, when the placenta is available for examination, which is too late to prevent adverse effects on the mother and foetus.

Microscopy

An alternative to placental histology is the examination of placental or peripheral blood slides with normal light microscopy. Slides are made from placental blood from the intervillous space of the placenta, which can be collected in different ways, for example by aspiration with a syringe, as impression smear
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of placental tissue or by washing a placental biopsy [60]. For peripheral blood
microscopy, venous or capillary blood is collected and thick and thin smears
are made. Slides from blood of both sources are dried, fixed with methanol
(thin smear), Giemsa stained and examined microscopically for the presence
of malaria parasites. Placental blood microscopy can only be performed af-
after delivery, like placental histology. Accuracy of microscopical examination
of both types of blood has been reported to be relatively low compared to
placental histology [57, 60, 61].

Rapid diagnostic tests

Rapid diagnostic tests (RDTs) have the advantage of being quick and easy-to-
use in remote settings. RDTs are based on the detection of parasite antigens in
the blood by specific monoclonal antibodies. Currently available RDTs come
in various formats (dipstick, cassette or card) and contain bound antibodies
to specific antigens, which are abundant in all asexual and sexual stages of
the parasite. They detect one or more of the following antigens: histidine
rich protein 2 (HRP2) (specific for P. falciparum), Plasmodium lactate dehy-
drogenase (pLDH), common to all species, or Plasmodium aldolase from the
parasite glycolytic pathway found in all species [62, 63]. pLDH-RDTs can de-
tect all species (PAN-specific) or detect a specific species, and aldolase-RDTs
detect all species (PAN-specific). The number of RDTs that are commercially
available has grown rapidly since their introduction in the late 1990s and
there are approximately 60 brands and over 200 different tests available to-
day detecting a single or multiple antigens, with an estimated 50 - 70 million
tests used for malaria diagnosis in 2008 [62, 63]. Depending on the man-
ufacturer, the quality of the RDT in terms of accuracy and stability can be
high [64, 65]. HRP2-based tests detect only P. falciparum, and antigenic vari-
ation of this antigen may cause false negative results [66]. A different problem
often encountered with HRP2-based RDTs is that the antigen can persist in
the blood for several weeks even though parasites have been cleared after
treatment [67–74]. Occasionally, this is observed for pLDH-based tests as
well, although to a much smaller extent, and often related to the presence of
gametocytes [67, 69, 75]. Nevertheless, HRP2-RDTs are most commonly used,
because of their lower cost, better stability and lower detection threshold com-
pared to pLDH-based tests [62, 63, 76, 77]. RDTs, especially those detecting
HRP2, show much potential to accurately diagnose malaria in non-pregnant
and pregnant individuals [57, 62, 63, 78]. The HRP2 antigen is excreted from
the infected red blood cell, which can be beneficial for the diagnosis of placen-
tal malaria, as the antigen can be detected in the circulation even when the
parasite is sequestered in the placenta [79].
Molecular methods

The diagnostic challenges associated with microscopy and RDTs led to the introduction of DNA- or RNA-based detection techniques for the diagnosis of malaria, particularly for research purposes and epidemiological evaluation [57]. There are several different DNA- or RNA-based detection techniques of which the polymerase chain reaction (PCR) is the most widely used [80–82]. There is a wide variety of PCR-based detection assays for *Plasmodium* parasites, most of which are based on the single-stranded ribosomal RNA genes, but extensive comparative studies are not available [80, 81, 83]. There are two basic approaches for species detection, nested PCR or single PCR [80, 81, 83]. In general, nested PCR is more sensitive than single PCR, but multiple reactions are needed in order to establish diagnosis [82]. For the analysis of the PCR amplification, various methods are available as well, with gel electrophoresis as historically most used, but recently real time methods with Sybr-green or Taqman probes have become more into use. Irrespective of the methodology used, the sensitivity of any PCR assay relies on the quantity and quality of the sample (DNA) used in the reaction.

The actual contribution of PCR on the diagnosis of malaria resides mainly in its ability to detect very low numbers of parasites, either in low-grade sub-microscopic infections or as a minor species in mixed infections [82]. Sub-microscopic infections detected by PCR have been associated with the adverse effects of malaria in pregnancy, such as anaemia, low birth weight and premature delivery, although very low level infections have also been shown to produce no major foetal impairment [84, 85].

Although PCR is considered to have the most sensitive detection level of parasites (for both regular peripheral malaria and placental malaria), it requires highly trained staff and specialised equipment, which are not always available in resource-poor settings [57, 58, 86]. Additionally, PCR assays are more labour-intensive and expensive than microscopy and RDTs and there is a risk of contamination, leading to false-positive results [57, 87]. Moreover, the sensitivity of a given PCR assay can vary between laboratories, making good quality control and standardisation essential to retain the added benefit of PCR to detect low-level and mixed infections [82].

1.6 Thesis aims and outline

Both PCR and RDTs are reported to have a higher sensitivity for placental malaria in peripheral and placental blood than microscopy, but are not considered to be as accurate as placental histopathology. However, evidence for this conclusion has not been analysed systematically [57]. Besides accuracy
there are many other reasons influencing the choice to use a certain type of
diagnostic test, such as affordability, number of tests to be performed, equip-
ment and resources, trained staff, etc. that depend on the setting where the
test will be used. Without a sufficient level of accuracy, however, there is no
justification of using a certain test in any circumstance. Therefore, the pub-
lished diagnostic accuracy of RDTs and PCR for the diagnosis of malaria in-
fecion in pregnant women compared to a suitable reference standard should
be reviewed systematically. To merit use as a diagnostic test for malaria in
pregnancy tests should at least have a similar or preferably better sensitivity
and specificity than peripheral blood microscopy.

Additionally, there is a need for the development of new RDTs or other
quick and easy-to-use diagnostic tests [57, 62, 63, 78]. Concerns about test
stability, accuracy, antigen persistence and antigen genetic diversity prompt
the need for improvement of test performance characteristics, and therefore
new antigens should be studied as targets for RDTs [62, 63, 88–90].

The gaps in the knowledge concerning the diagnosis of malaria in preg-
nancy and the need for new developments in diagnostics are the basis of
the research described in this thesis. This thesis contributes to two lines of
malaria diagnostic test research. The specific aims of this thesis are:

- To provide an evidence based conclusion on the currently available di-
agnostic tests that can reliably be used for the diagnosis of malaria in
pregnant women
  - by reviewing published diagnostic accuracy of RDTs, PCR, mi-
  croscopy and histology for the diagnosis of malaria in pregnancy
  - by directly comparing diagnostic tests for malaria in pregnant wo-
  men.

- To select new antigens that can be targeted by diagnostic tests and to
develop new antibodies against them for diagnostic test purposes.

Therefore, in Chapter two currently available diagnostic tests for malaria
in pregnancy are systematically reviewed and a meta-analysis of the diag-
nostic accuracy of these tests is described. Chapter three describes the
development and characterisation of monoclonal antibodies against the Plas-
modium antigens, glutamate rich protein (GLURP), dihydrofolate reductase-
thymidylate synthase (DHFR-TS) and heme detoxification protein (HDP). A
selection of the antibodies is evaluated in ELISA for their ability to detect
Plasmodium falciparum and Plasmodium vivax in Chapter four. In Chap-
ter five the persistence of several Plasmodium antigens after treatment is
evaluated and different diagnostic tests are compared during follow up af-
ter treatment of pregnant women in Burkina Faso. Chapters six and seven
describe the performance of RDTs, PCR and the HDP and DHFR-TS antibodies in ELISA compared to peripheral microscopy during pregnancy (Chapter six) and compared to peripheral- and placental blood microscopy at delivery (Chapter seven). In the final chapter (Chapter eight) the implications of the results described in this thesis are discussed and an outlook on future research themes is provided.

The field studies were conducted in two villages, Nanoro and Nazoanga, in Boulikemde Province, Burkina Faso. This area is considered holoendemic for malaria transmission with *P. falciparum* as the main infecting species. Transmission is perennial, with a peak during the rainy season (June to December) [1]. These studies were performed in conjunction with an ongoing study assessing the safety and efficacy of three ACTs for the treatment of malaria in pregnant women (clinicaltrials.gov: NCT00852423). Ethical approval to conduct this study in conjunction with this trial was obtained from the Ethical Committee of the University Hospital in Antwerp (registration number ITG 10 30 2 732), and from the Institutional Ethics committee of Centre Muraz, Burkina Faso (registration number 019-2010/CE-CM).