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Burden, pathology, and costs of malaria in pregnancy: new developments for an old problem

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Abstract: Over the last ten years, our understanding of the burden, economic costs and consequences of malaria in pregnancy has deepened, while the prevalence of Plasmodium falciparum malaria has declined significantly in some areas. Studies outside of Africa have increased our knowledge of the burden, consequences and costs of Plasmodium vivax in pregnancy. Rapid diagnostic tests have performed poorly in detecting malaria in pregnant women, while PCR has revealed a high prevalence of low density infection, whose clinical importance remains unresolved. Erythrocytes infected with P. falciparum that express the surface protein VAR2CSA accumulate in the placenta, and VAR2CSA is an important target of protective immunity. A VAR2CSA vaccine has begun clinical trials, but sequence variation requires careful exploration. Health system and household costs still limit access to prevention and treatment services. Within the context of malaria elimination, pregnant women may be valuable sentinels for monitoring malaria transmission. This review summarises recent progress, and highlights unresolved issues relating to the burden of malaria in pregnancy.

Burden, pathology and costs of malaria in pregnancy: new developments for an old problem

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Abstract

Over the last ten years, our understanding of the burden, economic costs and consequences of malaria in pregnancy has deepened, while the prevalence of *Plasmodium falciparum* malaria has declined significantly in some areas. Studies outside of Africa have increased our knowledge of the burden, consequences and costs of *Plasmodium vivax* in pregnancy. Rapid diagnostic tests have performed poorly in detecting malaria in pregnant women, while PCR has revealed a high prevalence of low density infection, whose clinical importance remains unresolved. Erythrocytes infected with *P. falciparum* that express the surface protein VAR2CSA accumulate in the placenta, and VAR2CSA is an important target of protective immunity. A VAR2CSA vaccine has begun clinical trials, but sequence variation requires careful exploration. Health system and household costs still limit access to prevention and treatment services. Within the context of malaria elimination, pregnant women may be valuable sentinels for monitoring malaria transmission. This review summarises recent progress, and highlights unresolved issues relating to the burden of malaria in pregnancy.

Introduction

This review summarises progress in understanding of the epidemiology, burden, economic costs, pathogenesis, and diagnosis of malaria in pregnancy over the 10 years since Lancet Infectious Disease published a special issue on this topic. Readers are referred to the earlier reviews for comprehensive analyses of older literature.[1,2] Looking forwards, we discuss how present efforts in malaria elimination may alter the burden and consequences of malaria in pregnancy, and how pregnant women may be used to monitor changing epidemiology of malaria across populations. Finally, we summarise important remaining research questions in this area. Our search strategy is outlined in Panel 1,and major advances over the last 10 years are summarised in Panel 2.

Malaria in pregnancy: progress on epidemiology and burden of *P. falciparum*

In high malaria transmission areas such as sub-Saharan Africa, the risk of *Plasmodium falciparum* infection increases when women get pregnant, with potential adverse consequences for mother and child (Figure 1). Malaria prevalence is highest in the first and second trimester of pregnancy (AM van Eijk, personal communication),[1] and the risk may not immediately return to pre-pregnancy levels after delivery.[3] In these high transmission areas, defined as parasite prevalence in 2-9 year old children >10%, malaria in pregnancy is more common in younger compared to older pregnant women, in women in first or second pregnancy compared to later pregnancies, and in HIV-infected compared to uninfected women.[1,4] Similar to the non-pregnant population, prevalence is affected by location (higher among rural populations), season, and use of malaria prevention.

Currently, malaria prevention and control in pregnant women in Africa rests on three pillars: insecticide treated nets (ITNs), intermittent preventive treatment (IPTp) with sulphadoxine-

pyrimethamine, and effective case management of malarial illness and anaemia.[5] However, coverage of these interventions is suboptimal: in 2014, only 35% slept under an ITN,[6] and in 2015, only 31.5% of eligible women received three or more doses of IPTp. [7] The range of adverse outcomes among women and their newborns include well-described increased risks of maternal anaemia and low birthweight, and prematurity[1] as documented in trials and observational studies.[8] In a large study using African national survey data, the use of malaria prevention in pregnancy was associated with substantial reductions in neonatal mortality and low birthweight, agreeing with findings from an IPTp trial in Mozambique.[9,10] In order to estimate the positive impact of prevention in Africa in 2015, Walker et al. developed a specific model of malaria in pregnancy which takes the unique feature of parity-specific patterns into account; using this model, it was estimated that, without pregnancy-specific interventions, 9.5 million pregnant women would have been exposed to infection, leading to 750 thousand low birthweight deliveries.[11] However, with IPTp coverage (2 doses) estimated at 21.6% in 2015, potentially 128 thousand low birthweight deliveries could have been averted. [11]

Understanding of the impact of malaria in pregnancy on fetal growth has been facilitated by increased use of obstetric ultrasound in cohort studies, providing accurate estimation of gestational age, sometimes combined with Doppler studies. Using such approaches in longitudinal studies, malaria in the first trimester is now recognised as an important risk factor for miscarriage, fetal growth restriction, low birthweight and maternal anaemia in Africa[12,13] and Asia.[14,15] In studies from Benin, the Democratic Republic of Congo (DRC), Malawi, Thailand and Brazil, malaria infections in early pregnancy, in late pregnancy, and throughout gestation have each been associated with reduced fetal growth,[14,16-20] which worsens with repeated infections.[16-18] This fetal growth restriction can occur either immediately or with a delay after malaria infection,[16,20,21] and it may be parity dependent: in

Tanzania malaria infection was only associated with decreased fetal growth among primi- and secundigravidae.[21] In the DRC, malaria in the first half of pregnancy was associated with decreased umbilical artery resistance in the late third trimester and fetal growth restriction among primigravidae, suggesting that parasitaemia in early pregnancy interferes with the development of the fetal circulation.[22]

Malaria in pregnancy, the fetus, and the growing child

In addition to the adverse impact of maternal malaria on newborn birthweight, it can also affect other measures of infant health. Maternal malaria can affect transplacental transfer of antibodies to the fetus, and thus the infant's resulting level of antibodies to pathogens such as tetanus and measles.[2] Maternal malaria has been associated with an increased risk of congenital malaria,[23,24] infant malaria,[25-29] infant anaemia[1,30] and other febrile illnesses.[28,31] It may reduce the response to vaccination in infants,[32] and a significant increase in systolic blood pressure was documented among infants exposed to maternal malaria [33] In Uganda and The Gambia, infants born to mothers with malaria in pregnancy gained less height and weight in the first year of life.[28,34]

In epidemiological studies it is difficult to independently evaluate the effects of maternal malaria exposure on infant susceptibility to malaria, because mothers and their infants typically share similar exposure by living in the same household and frequently sleeping under the same net, but some studies have controlled for exposure. [29] Exposure to malaria in utero early in pregnancy may tolerize the infant's immune system, whereas later in pregnancy it may prime the infant's immune responses.[35] These exposures appear to affect immune responses through early life, and could alter risks of severe and uncomplicated disease [27,29] by affecting regulation of infection in the young child.

Malaria in pregnancy: *P. vivax*

Studies in Guatemala, Colombia, Brazil, Papua New Guinea, and Indonesia, as well as a review on malaria in pregnancy in Asia, have provided information on the burden of infection outside of Africa, including the importance of *P. vivax* malaria and mixed infections [36-38]. By microscopy, prevalence of any malaria infection varied greatly from very low (<1% in regions in Brazil, India, and Guatemala), to 10-20% in some regions in Papua New Guinea and Indonesia.[36,38,39] *P. vivax* frequently causes anaemia, and has been associated with reductions in infant birthweight[37,38,40] and preterm delivery,[38] and with an effect on fetal growth, including lower fetal head diameters in Thailand and lower birthweight in the Brazilian Amazon.[14,20] In addition, severe disease and maternal and fetal deaths due to *P. vivax* have been reported.[38,41] Submicroscopic and asymptomatic malaria infections occur even in areas of low transmission, but their importance still needs further investigation (Figure S1).[41]

Detection of malaria in pregnancy

The major options for detection of malaria infection in pregnant women remain light microscopy, rapid immunochromatographic diagnostic tests (RDTs), or polymerase chain reaction (PCR); in placental specimens, histopathology can also identify parasites and resulting inflammatory responses. Microscopy requires expertise and equipment and is only moderately sensitive, and alternative methods have strengths and weaknesses. Histology, which can reveal both current, active infections and past infections, is costly and its use is restricted to research settings. Loop-mediated isothermal amplification (LAMP) has been used to diagnose placental infections, [42] and has similar sensitivity to qPCR in pregnant women. [43]

The use of PCR has improved species identification and has permitted studies of low-density parasitaemia. PCR is highly sensitive, but is time-consuming and requires specialized laboratories. Parallel parasite detection by PCR and either an RDT or light microscopy identifies many women with PCR-positive but RDT/microscopy-negative infections. These "subpatent" infections generally have parasite densities that fall between the limits of detection for traditional methods and for molecular assays. Among 20 studies with information, prevalence of submicroscopic infection ranged from 2-55%. When comparing the ratio of microscopy to PCR prevalence, the mean ratio was 0.46 (95% 0.35-0.57) among pregnant women (Figure S1, 9 studies) whereas this was 0.51 (95% CI 0.45-0.57) in 65 studies among mainly non-pregnant populations.[44].

Although subpatent infections detected by PCR but not by RDT or microscopy are common in many holoendemic areas, they are inconsistently associated with clinical outcomes. Infections detected by PCR and not microscopy at antenatal enrollment were strongly associated with an increased risk of low birthweight or prematurity in Benin,[45] whereas infections detected by PCR but not by RDT were not associated with poor infant outcomes in Malawi,[46] [47] Ghana,[48] or India.[49] Submicroscopic infections detected antenatally or at delivery may be associated with a mild increase in the prevalence of maternal anaemia at delivery (Figure S2). Therefore, it remains unclear whether, and to what extent, either *P. falciparum* or *P. vivax* infections detected solely by PCR adversely affect maternal and infant outcomes; a large individual participant data analysis to examine this further is under preparation. In addition, more sensitive diagnostics are under development that have the potential to increase detection of malaria parasites. Whether deployment of these new assays can improve pregnancy outcomes and translate into a public health impact remains unknown.

RDTs are most sensitive for parasite densities above 200/μl for *P. falciparum*, and are less sensitive for other species. Many pregnant women have peripheral blood parasite densities

below this threshold, but may also have placental infections. This has led some groups to propose host biomarkers for occult placental infection.[50] The low sensitivity of current RDTs may have contributed to the low efficacy of intermittent screening and treatment compared to IPTp. [6,51] A further consideration is the emergence of parasite lines that lack histidine rich protein 2,[52] the principal protein used in *P. falciparum* RDTs. Nevertheless, as parasite prevalence is generally highest on first presentation at antenatal clinic, these relatively insensitive tests could be a useful screening tool when pregnant women are first seen

In antenatal specimens, compared to parasite detection by a nested PCR method, the sensitivity of microscopy in a research study varied between 40-70%, while that of commercial RDTs varied from 42-75%.[53] At delivery, peripheral or placental blood testing by microscopy or by RDT may not detect histopathologically-evident placental malaria.[53-56] Owing to this, two studies have applied latent class analysis to such samples in order to quantify the operating characteristics of detection methods independent of any reference standard. This analysis suggested that either PCR of placental specimens or RDT of peripheral specimens may have sufficient sensitivity for routine diagnosis of placental malaria.[57,58]

Malaria and maternal nutrition

Malaria and poor maternal nutrition frequently co-exist, and there are over 0.9 million babies born with low birthweight due to malaria and an estimated 0·8 million infant deaths related to maternal undernutrition each year.[59,60] In the DRC cohort discussed above, and in another study in Kenya, poor maternal nutritional status appeared to exacerbate the deleterious effect of malaria on fetal growth.[17,22,61] Risk factors often overlap, and the rainy season is a period of both high malaria risk and food insecurity. With the frequent overlap between malaria and nutrition, strategies to reduce both these risk factors are needed. [62] To examine this further, a

large study combining data from 14,635 pregnancies from 13 studies conducted in Africa and the Western Pacific from 1996-2015 showed an increased risk of low birthweight when malaria and malnutrition were present, but not a synergistic effect; one in three pregnant women had malaria and/or poor nutition [63].

Concerns that iron supplementation might increase the risk of malaria in pregnant women appear to be unfounded. A recent systematic review of this interaction identified mostly observational studies, in which antenatal iron supplementation was associated with a temporal increase in *P. vivax* infections without a clear effect on *P. falciparum* risk.[64] However, the results of two randomized clinical trials of iron supplementation in pregnancy have been reassuring: in both studies, 60 mg of daily elemental iron did not increase malaria risk and was associated with increased birthweight in Kenya,[65] and with improved haemoglobin in Tanzania.[66]

Pathogenesis of malaria in pregnancy

The increased susceptibility of pregnant women to malaria is due, at least in part, to the sequestration of malaria-infected erythrocytes (IEs) in the maternal blood spaces of the placenta. Placental sequestration is common in *P. falciparum*, and placental changes have been seen in some, but not all, studies of *P. vivax*.[67-69] Neither a direct link between placental changes and adverse birth outcomes nor placental sequestration of IEs have been clearly demonstrated in *P. vivax*. Placental changes in other species are not reported.

In *P. falciparum*, sequestration is mediated by the parasite-derived protein VAR2CSA, expressed on the IE surface, binding to placental chondroitin sulphate A (CSA) on syndecan-1.[70] This was elegantly demonstrated with a placental perfusion model [71] in which perfusion of malaria-infected cells into uninfected placentas resulted in binding of VAR2CSA-expressing parasite lines, but not of other lines. Such a binding phenotype has been observed

even among parasites collected in first trimester, suggesting that circulating IEs express VAR2CSA and preferentially bind CSA from early in gestation.[72,73] VAR2CSA is composed of multiple DBL (Duffy binding-like) domains and interdomain (ID) regions, among which DBL 2, 3, 5, and 6 have been associated with binding to CSA (reviewed in[74]). The length and sulphation of the CSA chain are critical,[75] which may be relevant to development of strategies to block sequestration.

Placental sequestration can lead to placental inflammatory responses, most notably accumulation of monocytes in the placenta. This is especially likely in first-time mothers, who often experience high density infections. Placental inflammation has been associated with fetal growth restriction and maternal anaemia in African women,[2] and with low birthweight in Papua New Guinea.[39] Modelling studies suggest that, in the absence of IPTp, placental inflammation may begin in early pregnancy and persist for months, and that 63% of placental infections may have been initiated by the end of the first trimester.[59] However, longitudinal genotyping of *P. falciparum* isolates during gestation in Cameroonian pregnant women showed that 77% of placental parasites were acquired ≥30 weeks in pregnancy,[76] suggesting that data is still needed to confirm the model.

Severe malaria is an infrequent but definite contributor to maternal mortality in endemic areas of Africa.[77] In maternal autopsy studies, massive sequestration of IE has been observed in organs including both the brain and the placenta.[78] The simultaneous sequestration of IE in cerebral capillaries and the placenta in these fatal cases suggests that infections during pregnancy may consist of multiple parasite populations with diverse binding phenotypes.[79-81]

P. falciparum infection in early pregnancy may interfere with trophoblast invasion into the uterus. Using an in vitro model, plasma from malaria-infected women inhibited trophoblast migration.[82] Effects on the development of the maternal placental circulation may contribute to reduced fetal growth rates reported from early to mid gestation, discussed above.[16,17]

Placental malaria can also affect development of the fetal circulation, possibly through activation of the complement cascade, leading to disordered placental angiogenesis.[83] In mice, complement activation led to underdevelopment of the villous vascular tree.[83] In humans, malaria leads to small placental size, which may be due to similar impairments of placental angiogenesis affecting villous growth and development.[84]

Malaria-associated placental inflammation has also been associated with disorders of amino acid and glucose transport[85,86] and dysregulation of the insulin-like growth hormone axis,[87] probably driven by increased cytokine release into the intervillous space.[2] Inflammatory cytokines can alter the expression and distribution of nutrient transporter molecules and growth hormones, and it is likely that disordered placental physiology – rather than mechanical obstructions from IEs – drives many of these effects.

Protective antibodies against malaria in pregnancy

Protection against malaria in pregnancy, low birthweight and anaemia has been associated with development of antibodies to the surface of CSA-binding IEs,[2 88] and the dominant target of such antibodies is VAR2CSA. By ELISA, IgG against DBL1-DBL2 is associated with protection against low birthweight,[89] while depletion studies suggest antibodies that most efficiently block IE adhesion are directed against DBL2 and its flanking inter-domain (ID) regions and DBL4.[90] Antibodies that block adhesion to CSA are associated with reduced risk of placental infection and preterm delivery,[89] while antibodies that opsonise IE for clearance by phagocytic cells may protect from maternal anaemia (reviewed in [91]). The acquisition of antibodies to components of VAR2CSA may be impaired by the receipt of IPTp, which is broadly recommended in malaria-endemic Africa.[92]

Sequence diversity within VAR2CSA has, until now, undermined efforts to define immunogenic and pathogenic motifs. Two recent studies have identified reproducible

dimorphism in sequences encoding the ID1-DBL2 region from diverse parasite populations, which may differentially contribute to increase the risk of low birthweight.[93,94] Two VAR2CSA-based vaccines are currently in early-stage clinical trials (NCT02647489 and NCT02658253, clinicaltrials.gov), but it remains unclear how VAR2CSA-based vaccines may need to be adapted to address VAR2CSA sequence diversity and to offer broad protection against placental malaria.[93]

The economic burden of malaria in pregnancy

The economic burden of malaria in pregnancy can be considered to include: (i) provider costs: the cost to the health system of providing prevention and treatment services; (ii) user costs: the cost to households of accessing these services; and (iii) broader economic impacts arising from several channels such as the reduced labour productivity of women who have experienced malaria in pregnancy and the short- and long-term consequences of low birthweight and other adverse newborn outcomes.

Limited resources constrain the management of malaria in pregnancy and hinder the achievement of optimal intervention coverage. From the perspective of healthcare providers, adequate management of malaria in pregnancy absorbs significant proportions of the total available budget. For example, in 2010, provider costs associated with the management of confirmed cases of malaria in pregnancy reported by a tertiary hospital in a low-endemic area of the Brazilian Amazonas constituted 1% of the monetary value of the total activities undertaken that year. [95] This is a remarkable proportion given that pregnant women are a sub-set of the total population at risk and the study area is characterized by low-transmission.

A number of studies have estimated unit costs associated with the provision of interventions in pregnancy (Table 1). Only a few studies evaluated costs of treatment, [95-97] whereas most studies reported either actual or projected costs of preventive interventions. [97-106] These costs differed according to delivery strategy and, in the case of IPTp, the drug used.

In many countries, public healthcare providers have required financial contributions from pregnant women (also known as cost-sharing). Such contributions may exacerbate barriers to access. Even when healthcare is free, pregnant women still incur high transportation and indirect costs, which by themselves can prevent women from seeking malaria prevention and treatment. Qualitative studies have largely identified direct patient cost to be a major barrier to management of malaria in pregnancy. [107-119] In quantitative studies, removing user fees significantly improved coverage of interventions,[120] and improved IPTp delivery.[121] There are concerns that women may not use nets that are provided free-of-charge, wasting resources [122] but on the other hand cost-sharing schemes for ITNs, some of which employ loans, discourage demand and reduce access for the poorest potential users. [123,124]

A number of studies have estimated the costs incurred by pregnant women for both prevention and treatment, classifying these as direct costs (fees, drugs, ITNs transportation) or as indirect, opportunity costs [95,96,101,104,105,125] (Table 2). The highest costs, which were for treatment, were observed in a study from Brazil, arising from both its higher gross domestic product (reflected in high indirect costs) and the presence of *P. vivax* infection which, during pregnancy, requires additional interventions compared to *P. falciparum* to achieve radical cure.[95] In order to offset these costs, and thereby improve coverage with ITNs in pregnant women, a number of strategies have been implemented. Vouchers have been distributed to incentivise ITN purchase and use. [105,126] Social marketing increased supply of nets as well coverage.[127] From the provider perspective, social marketing was found to be more costly than procuring and selling nets in the private retail market, but also as costly as distributing free of charge to the community.[104] Another strategy to increase IPTp and ITN coverage consists of delivering the interventions through community approaches.[101,128,129] Such community interventions allow coverage to be increased by shifting delivery costs from women (transport and time to seek IPTp) to the provider. For example, when women were

promised a box of personal medical supplies at the delivery facility, this increased adherence to IPTp and rates of institutional delivery by offsetting delivery costs. A complementary community-directed intervention in which volunteers delivered ITNs and IPTp as well as basic counseling services to pregnant women was effective and the additional costs generated were smaller compared to larger health campaigns.[129]

Although the evidence base on the economic burden of malaria in pregnancy has expanded in recent years, there are still three main gaps to fill: (i) very little is known about the economic burden outside sub-Saharan Africa, where different *Plasmodium* species co-exist; (ii) the impact of malaria in pregnancy on indicators of economic development has never been investigated: for example, it is unknown how malaria in pregnancy impacts women's employment and wages; and (iii) costs and consequences associated with malaria in pregnancy are underexplored: only a few studies have estimated, for example, cost associated with low birthweight in malaria endemic countries.[130,131] Additional knowledge on (i), (ii) and (iii) may, respectively: guide decisions on optimal allocation of resources in areas where malaria control tools and strategies depend on *Plasmodium* species and where conditions to achieve elimination exist; inform economic growth, development and women's empowerment strategies; further promote investments towards improvement of prevention and treatment of malaria in pregnancy.

Malaria in pregnancy and the elimination agenda

Variations in malaria transmission, whether geographic or temporal, result in variable exposure to infection, affecting the course of disease.[132,133] Where malaria transmission has declined substantially, increases in parasite density[134,135] and in malaria-related adverse impacts have been observed[134,135]. Taken together, these data suggest that measures that reduce malaria transmission, and thus reduce opportunities to acquire immunity, may lead to

a change in both the burden and clinical spectrum of disease. For this reason, it becomes important to estimate changing trends[134-136] and potential rises in the frequency of severe malaria syndromes,[135] especially in areas with a high prevalence of HIV, which can impair the maintenance of effective immune responses. For malaria control programs, these rapid changes in immunity and susceptibility necessitate ongoing monitoring of the burden of malaria disease in at-risk populations, including pregnant women. This is especially critical in areas embarking on malaria elimination, as understanding the determinants and clinical consequences of malaria declines and resurgences, as well as the time-scales over which antimalarial immunity is gained and lost, has become a priority to prevent reinfection and resurgence of malaria infections.[137]

Antenatal malaria infections may undermine elimination efforts that rely on mass administration of ACTs. Trials based on mass drug administration with an antimalarial such as dihydroartemisinin-piperaquine (DHA-PPQ) are in progress in several African countries (ClinicalTrials.gov Identifier: NCT02329301). ACTs are yet to be approved by WHO for treatment of malaria in the first trimester of pregnancy, and there may be concerns over the use of DHA-PQ, in mass drug administration campaigns that could result in women in the first trimester receiving the drug who are not infected or at risk. Women in the first trimester need to be excluded from mass drug administration programs and instead managed according to a malaria RDT result. Given the low sensitivity of malaria RDTs,[55] that IPTp with SP is contraindicated in first trimester, and that pregnant women frequently do not use ITNs[59] and often carry gametocytes in their blood,[138] there is a risk that undetected and untreated infections in this group can constitute a reservoir of malaria with the potential to sustain transmission during malaria elimination efforts. Recent modeling analysis suggests that pregnant women might constitute up to 23.9% of all infectious individuals following MDA to 90% of the non-pregnant population[139]. Further studies are needed to understand the impact

of omitting pregnant women in MDA campaigns. Use of antimalarials that are known to be safe during pregnancy,such as chloroquine, may be an alternative in areas where the parasite population has regained susceptibility to the drug.[140] Development of new anti-malarials for radical cure of *P. vivax* which are safe in pregnancy are also needed. In absence of these antimalarial alternatives, monitoring pregnant women in areas where malaria elimination activities are conducted can inform the risk of unsuccessful interruption of transmission due to undetected foci of malaria among pregnant women.

Pregnant women might be used as a sentinel population to monitor malaria transmission with sufficient sensitivity to guide malaria elimination activities. Compared to other target populations (e.g. children), pregnant women are relatively easy to access because of their high rate of attendance to antenatal clinics in malaria endemic countries.[141] Parasite prevalence and seroreactivity to parasite antigens at delivery correlate with measures of overall *P. falciparum* transmission,[135] and parasite prevalence in pregnant women correlates with that in children in Africa.[142] In Southern Mozambique, malaria hospital admissions followed similar trends to malaria prevalence among pregnant women,[135] suggesting that detection of malaria parasites with highly sensitive diagnostic tests such as PCR in pregnant women at their first antenatal visit may be useful to assess malaria trends in the underlying community. In addition to the value of surveillance in pregnant women as a surrogate for parasite transmission in general populations,[103] the distribution of molecular markers of parasite drug resistance in parasites collected from untreated African pregnant women closely resembled that observed in children or non-pregnant adults.[143]

Immunologically, sera collected from pregnant women may be used as a proxy for population-level exposure to *P. falciparum*. As transmission fell in Southern Mozambique, antimalarial IgG responses against VAR2CSA declined.[135] Antibodies against VAR2CSA develop after exposure to placental parasites in a parity-dependent manner, increasing over

successive pregnancies. They are affected by variables that influence the risk of exposure to *P. falciparum* such as season, household location,[144] use of IPTp[92,145] or ITNs.[146] Measures of antibody during pregnancy integrate malaria exposure over time (a pregnancy) in which cumulative exposure to VAR2CSA can increase from zero to very high levels. However, the longevity of antibody responses againt VAR2CSA as well as the impact of *var2csa* diversity needs to be evaluated to assess the utility of such a serological approach. The use of pregnant women as a sentinel group for malaria transmission, and the use of antibodies against VAR2CSA as markers of cumulative exposure to *P. falciparum* during pregnancy[147], although still needing further validation, may constitute a feasible alternative to generate sensitive metrics of malaria transmission intensity for malaria elimination.[137]

Conclusion

The global burden of malaria in pregnancy remains substantial. In sub-Saharan Africa, access to recommended ITNs and IPTp with SP and adherence to recommended policies remain poor, and there is an urgent need to identify programmatic bottlenecks and gaps for optimal coverage of preventive tools. Globally, there are challenges in the prompt diagnosis and treatment of malaria infections with both *P. vivax* and *P. falciparum*. Some of these challenges include the low sensitivity of diagnostic tests and restrictions on anti-malarial agents to be used for treatment, prevention and elimination in pregnant women, such as anti-relapse therapy for *P. vivax*. The identification of VAR2CSA as a major target of protective immunity against *P. falciparum* malaria in pregnancy has led to efforts to develop a pregnancy-specific malaria vaccine, but understanding of the importance of different genetic variants of this protein is still very limited. Studies are uncovering the relationship between the longevity of antimalarial immune responses, and the impact of waning immunity on malaria-related adverse outcomes in settings of declining malaria transmission. Such studies

can help to tailor existing diagnostic and preventive tools to different transmission settings, and to guide appropriate clinical practice in situations of changing malaria transmission. Not only this, a deeper understanding of the biology of malaria infection in pregnancy will contribute to the development of new approaches for the successful implementation of malaria elimination strategies in order to achieve a malaria free world. A proposed future research agenda is outlined in Panel 3.

Author Contributions

SJR coordinated the manuscript scope and design. All other authors contributed equally to the manuscript. All authors drafted individual sections of the manuscript, and revised the final version critically for content. All authors read and approved the final version of the manuscript.

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Conflict of interest:

We declare that we have no conflicts of interest.

Panel 1. Search strategy and selection criteria

We examined literature on malaria in pregnancy published over the last 10 years as cumulated in the malaria in pregnancy library (MiPL; http://library.mip-consortium.org/). The keywords “Ultrasound” and “Doppler” were used to screen on publications on fetal growth and malaria. Using the terms stillbirths, perinatal death, and neonatal death, and infant we screened on articles on impact on the infant. For subpatent malaria in pregnancy, we screened on the key words polymerase chain reaction or submicroscopic or subpatent up to May 2016, and articles which presented data in a format which could be extracted and combined were included for Figure S1 and Figure S2. For diagnosis, we searched the MiPL and Pubmed using keywords “malaria”, “pregnancy,” and “diagnosis.” For pathophysiology, we searched the MiPL and Pubmed using keywords “placenta” and “plasmodium” or “malaria” For elimination, we searched publications using keywords “elimination”, “trends” with “malaria ” and “pregnancy”. For the economic burden we searched in PubMed using (malaria[Title/Abstract] AND (pregnan*[Title/Abstract] OR gestat*[Title/Abstract]) AND cost*[Title/Abstract]). Original search on 03/08/2016. Two additional articles were taken from EconLit data base with no specific search criteria but with the aim of facilitating the interpretation of articles extracted from PubMed.

Panel 2. Progress in the last 10 years for burden, pathogenesis and economics of malaria in pregnancy (previous table 1)

Burden of malaria in pregnancy

- Declining prevalence of *P. falciparum* in parts of Africa
- Low prevalence of *P. vivax* in Asia and Latin America
- Modelling to demonstrate the high burden in Africa of low birthweight attributable to malaria
- Established importance of malaria in the first trimester
- Evidence generated indicating impact of malaria in pregnancy on infant health

Diagnosis

- Subpatent antenatal infections are common but their impacts on pregnancy outcomes remain unclear.
- Current rapid diagnostic tests lack sensitivity for diagnosis of placental malaria.

Malaria and nutrition

- In clinical trials, iron supplementation does not increase the risk of malaria in pregnancy
- Both malaria and poor nutrition associated with low birth weight

Malaria pathogenesis

- A vaccine based on VAR2CSA has entered phase I clinical trials.
- Placental malaria can interfere with placental development and function

Economics

- Malaria in pregnancy absorbs significant proportions of the total available healthcare budget
- Direct and indirect costs incurred by pregnant women for malaria prevention and treatment are high.
- Strategies to lower costs incurred (e.g., vouchers, social marketing and delivery through community approaches) are effective, but they need to be scaled up

Changing transmission

- Decreasing exposure to malaria parasites is associated with exacerbated impacts of malaria in infected women

Panel 3: Knowledge gaps and research priorities (Previous table 4)

- Are there long term consequences of malaria in pregnancy for offspring health?
- How do co-morbidities such as helminthiasis and HIV, and treatments such as ivermectin or ARVs, modify the effects of malaria in pregnancy?
- What are the timescales over which antimalarial immunity is gained and lost in situations of transitioning malaria epidemiology?
- What proportion of miscarriages and stillbirths are attributable to malaria in pregnancy in different epidemiological settings?
- Do subpatent antenatal malaria infections contribute to adverse pregnancy outcomes?
- Will deployment of more sensitive, next generation rapid diagnostic tests improve the detection of malaria in pregnancy including placental infections?
- Could combinations of malaria and nutrition interventions result in improved birth weights and decreased maternal anaemia?
- How does malaria in pregnancy affect the development of immunity in the fetus and infant?
- Can vaccines based on VAR2CSA or other antigens prevent malaria in pregnancy and its adverse consequences for offspring?
- Are antenatal women suitable as a sentinel population to monitor malaria transmission, control or elimination in the general population?
- Are parasite prevalence and antibody serology useful monitoring tools?
- How can pregnant women be effectively and safely integrated into malaria elimination efforts?
- How can antenatal malaria prevention be effectively extended to protect women in the first trimester?
- What is the economic burden of malaria in pregnancy in areas with co-existing Plasmodium species?
- How does malaria in pregnancy affect indicators of economic development?
- What are the costs and consequences associated with low birth weight due to malaria?

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Figure 1: Effect of malaria in pregnancy on maternal, newborn, infant and child health

Figure S1 Prevalence of submicroscopic malaria in pregnancy in 20 studies with information available in 15 countries, 1995-2013

* This study only included HIV-infected pregnant women

Note that studies are ordered by prevalence of malaria by microscopy.

Figure S2: The association between microscopic or submicroscopic malaria and anaemia in 6 studies with information available in 5 African countries, 1995-2013

*This study only included HIV-infected pregnant women

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Table 1: Provider costs of malaria in pregnancy prevention and treatment, US$

Country	**Uganda**	**Tanzania**	**Sub-Saharan Africa**	**DRC**	**Mozambique**	**Burkina Faso**	**Uganda**	**Tanzania**	**DRC**	**Kenya and Mali**	**Brazil**	**Tanzania**
Reference	Mbonye et al, 2008	Mulligan et al, 2008	Chico et al, 2008	Becker-Dreps et al, 2009	Sicuri et al, 2010	De Allegri et al, 2010	Hansen et al, 2012	Renggli et al, 2013	Matangila et al, 2014	Fernandes et al, 2015	Botto-Menezes et al, 2016	Willilo et al, 2016
Year in which US$ are expressed	2005	2006	2008	2005	2007	2006/7	2004/5	2010	2013	2011	2011	2013
Outpatient (US$)					4.87		0.7				103.51 (*P. vivax*) 83.59 (*P. falciparum*)	
Admission per night (US$)					40.73						118.51	
ITN - delivery approach		voucher scheme		anc		social marketing or free distribution	anc	campaign				
ITN - cost (US$)		financial cost=7.66/ITN delivered; economic cost =7.57/ITN delivered		8 (donated)		financial cost, (social marketing) =8.08/ITN distributed; (free distribution)=7.21/ITN. Average economic cost 4.81 for both	(impregnation and distribution) 1.71	5.30 (financial)				

						strategies						
IPTp or chemoprophylaxis-delivery approach	anc or community		azithromycin-chloroquine administered 2/3 times				anc			anc		
IPTp or chemoprophylaxis (US$)	at the anc/woman = 1.36; community/ woman=1.57		14 - 21/pregnancy				0.79 (2 dose)		anc	0.63/dose		anc
Other (US$)							(ITN+IPTpSP)= 2.48		One thick smear = 2.62; One RDT = 1.16			RTD=1.28/ woman; 10.70/positive RDT

Notes: anc=antenatal clinic; ITN=insecticide-treated net; IPTp=intermittent preventive treatment of malaria in pregnancy; RDT=rapid diagnostic test; In Chico et al, 2008 drug costs only retrieved from the International Drug Price; In Botto-Menezes, 2016 outpatient cost for Pvivax and Pfalciparum are different as for Pvivax outpatient care; In Fernandes et al, IPTp costs were assumed equal to 25% of total ANC costs.

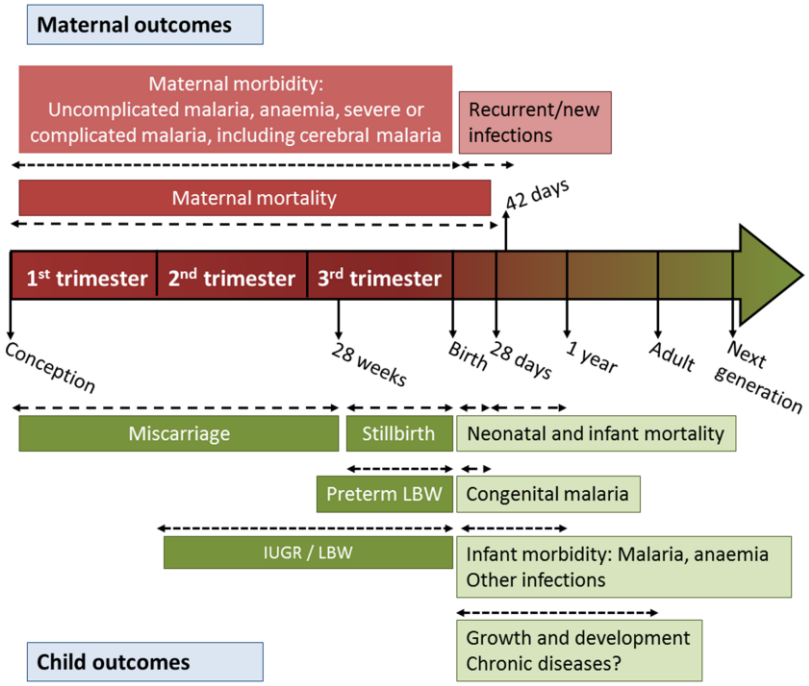
Table 2: User's cost of malaria in pregnancy prevention and treatment

Country	**Uganda**	**Tanzania**	**Mozambique**	**Burkina Faso**	**Brazil**	**Tanzania**
Reference	Mbonye et al, 2008	Mulligan et al, 2008	Sicuri et al, 2010	De Allegri et al, 2010	Botto-Menezes et al, 2016	Sicuri et al, 2015
Year in which US$ are expressed	2005	2006	2007	2006/7	2011	2012
Outpatient (US$)			average direct=0.61; average indirect=1.49		total=45.91; indirect=approx. 50%	Total=6.20; indirect=4.46
Inpatient (US$)			average direct=5.10; average indirect=5.01		total=216.29; indirect=approx. 50%	Total=35.35; indirect=8.45
ITN delivery approach		voucher		social marketing		
ITN (US$)		0.83		0.75		
IPTp or chemoprophylaxis-delivery	anc; community					
IPTp or chemoprophylaxis-cost (US$)	transportation= 0.98; time=1					

Notes: anc=antenatal clinic; all costs are expressed as median apart from Sicuri et al 2010 (mean values); in all studies total costs = direct + indirect, thus when total and indirect are reported, indirect are included in total.

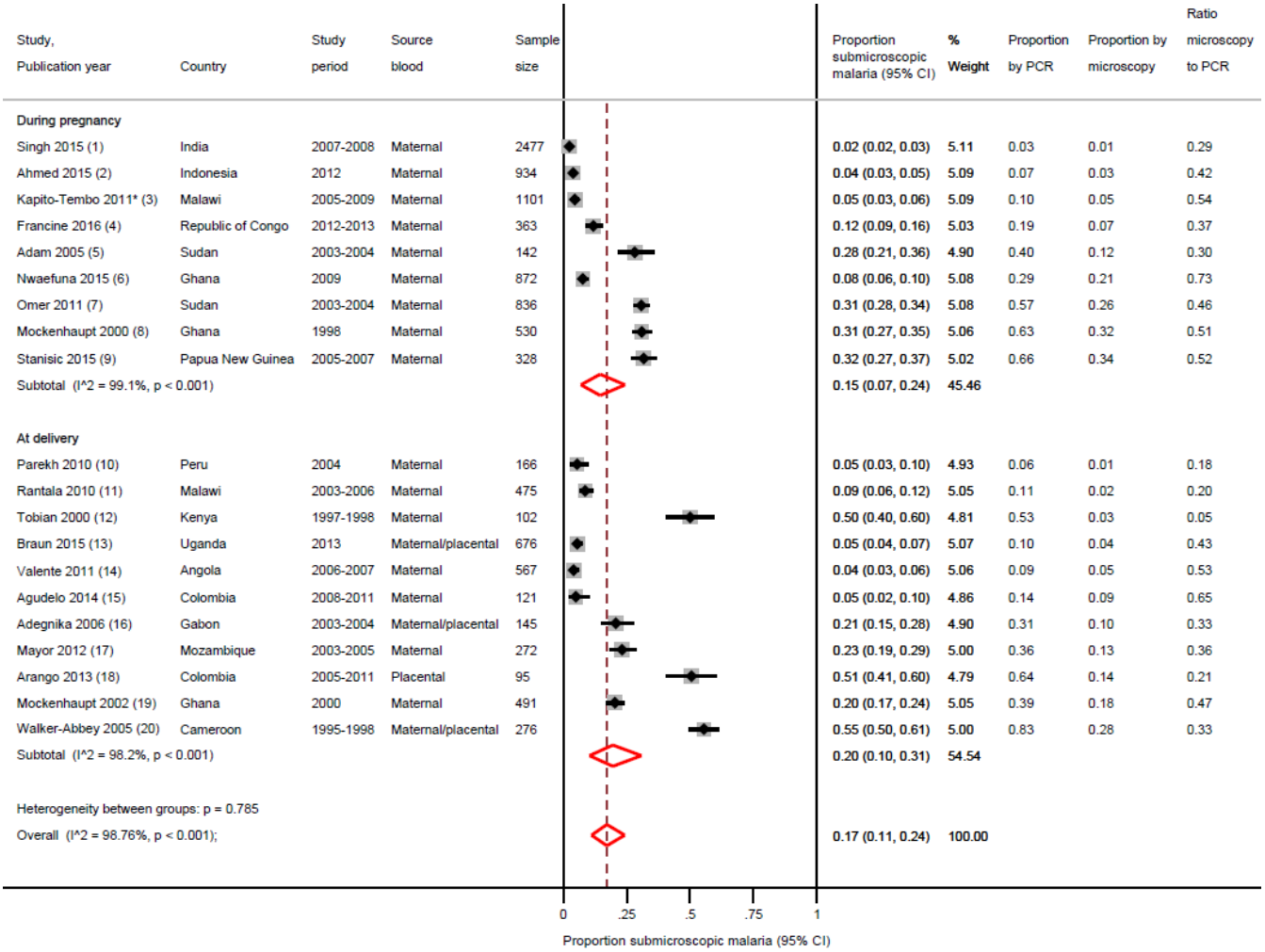
Figure 1

***Figure 1*: Effect of malaria in pregnancy on maternal, newborn, infant and child health** (adapted from Desai et al 2007, LID)



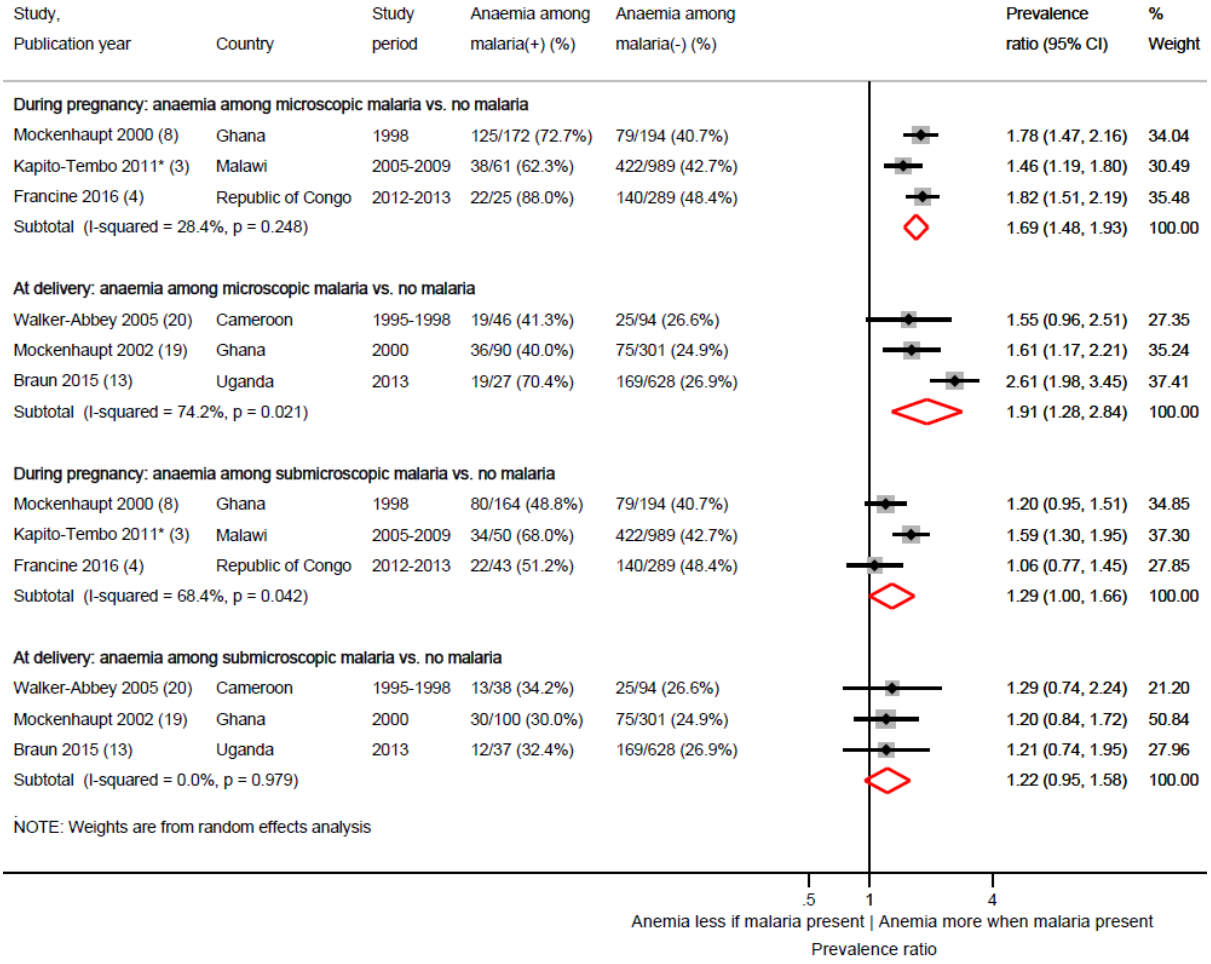
Supplement to: **Burden, pathology and costs of malaria in pregnancy: new developments for an old problem**

Figure S1: Prevalence of submicroscopic malaria in pregnancy in 20 studies with information available in 15 countries, 1995-2013



Note that studies are ordered by prevalence of malaria by microscopy.[1-20]
* This study only included HIV-infected pregnant women

Figure S2: The association between microscopic or submicroscopic malaria and anaemia in 6 studies with information available in 5 African countries, 1995-2013



*This study only included HIV-infected pregnant women

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Notes from the editor (general editorial points follow the reviewers comments):

* Please add a "Search strategy and selection criteria" panel (for detailed guidance see https://protect-au.mimecast.com/s/GN1VBvtnw31ai3?domain=thelancet.com). This will reassure readers that your handling of the topic is comprehensive. Note, this panel will not add to your wordcount and will not be counted as one of your non-text items

Response: Please see the added Panel 1 for the Search strategy and selection criteria

* Please highlight best practise and/or knowledge gaps outlined in your paper in a key points panel; this should just be a simple brief list of bulleted points. As above, this panel will not add to your wordcount and will not be counted as one of your non-text items

Response: Knowledge gaps are now presented as Panel 3 instead of Table 4 and progress is now presented in Panel 2 (previous Table 1).

* It is essential that any figures are submitted in an editable format or at sufficient resolution to allow clear reproduction (for detailed guidance see https://protect-au.mimecast.com/s/YZ52Bmiad3NXH5?domain=thelancet.com). What are the sources of figures 2 and 3?

Response: Figure 1 is a Tif file with 300 resolution; it can also be provided as a Power point allowing manipulation. Figures S1 (Formerly Figure 2) and Figure S2 (formerly Figure 3) were made specifically for this review, and the references are in the figure. They are provided as a PDF so they can be manipulated.

* Given tables 1 and 4 comprise only a single column it is our style to present them as panels. Please relabel them accordingly and ensure they are cited as such in the text. Also, please ensure you renumber the remaining tables

Response: see above re knowledge gaps and best practice panels

* For an article of this type we can only accommodate 150 references, please remove at least 10

Response: We have cut the total number of references in the main text as requested.

* It is essential that your revised manuscript includes a conflict of interest statement at the end of the text. Furthermore, please ensure you submit completed authorship and ICMJE forms with your revised manuscript (the forms can be found at https://protect-au.mimecast.com/s/K41rBvSDQn14tK?domain=thelancet.com)

Response: This statement has been added.

Reviewer #1:

It is nearly 10 years since the LID published a series of articles that provided an overview of what was known about malaria in pregnancy at that time. This new review covers a number of rather diverse topics, ranging for pathogenesis to the costs of delivering ITNs without covering key issues such as treatment and prevention which I imagine will be presented in a separate, parallel review. Overall, the paper is comprehensive, well written and easy to follow and will be a useful contribution to the literature,

I have two main comments and a few minor ones.

Main comments.

a. Search strategy. The authors have conducted a comprehensive review of the recent literature on their selected areas for review. It would be helpful to have more information about their search strategy.

Response: This has been added to Panel 1 now.

b. Future challenges. Areas for further research are mentioned in Table 4 but I think that it would be helpful to expand on these a bit more. What research priorities would the authors recommend to a funding authority that they should support during the next few years? Three clear priorities are indicated for research on the economics of malaria in pregnancy but not for other areas. A final section highlighting the main priorities for future research would be useful.

Response: The previous table 4 has been changed to panel 3, and has been revised to reflect the suggestion. Unfortunately we are at our word limit.

Minor comments

a. Coverage with interventions (p.3). There is more up-to-date information on IPTp coverage in the WHO 2016 report which could be cited.

Response: We added and changed this information as follows (page 3): “However, coverage of these interventions is suboptimal: in 2014, only 35% slept under an ITN,[6] and in 2015, only 31.5% of eligible women received three or more doses of IPTp.(ref WHO report)”

b. Parasite detection by RDT or microscopy (p.5). The authors correctly point out that RDTs and microscopy are not very sensitive in diagnosing placental malaria compared with PCR. However, it could be mentioned that because parasite density is usually highest on first presentation at an antenatal clinic, these relatively insensitive tests can be a useful screening tool when pregnant women are first seen.

Response: This was added to the section on malaria diagnosis: “Nevertheless, as parasite prevalence is generally highest on first presentation at antenatal clinic, these relatively insensitive tests could be a useful screening tool when pregnant women are first seen”.

c. LAMP assay. Did the authors find any references to the use of LAMP in the diagnosis of malaria in pregnancy?

Response: We have modified the text to reference two recent papers: “LAMP has been used to diagnose placental infections (Prahl), and has similar sensitivity to qPCR in pregnant women (Tegegne)”

d. Placental histology (p.7) Placental histology is given little attention although this is a frequent end-point in clinical trials. Is this useful? Do the efforts required in obtaining good histology and interpretation warrant the challenges that this poses in many situations? Has anything more been learnt about the relationship between different histological changes and the outcome of pregnancy? What is the justification for the comment 'histology may return false positives due to inflammation'? Surely this would not be the case if parasites were seen.

Response: We are not aware of recent studies that usefully extend the earlier observation of Menendez et al (J Infect Dis 2001) associating different placental histology findings with clinical outcomes. We have added the following statement: “Histology, which can reveal both current, active infections and past infections, is costly and its use is restricted to research settings.” We agree the statement about histology and false positives was incorrect and have removed it.

e. Folate deficiency in pregnant women. The section on nutrition (p.8) mentions iron supplementation and the concerns over whether this increases susceptibility to malaria. I don't think that this debate is entirely over and there is on-going research in this field. Folate, given religiously as a pregnancy supplement, is not mentioned. Has there been any more recent information on how many pregnant women are folate deficient and whether it is still appropriate to recommend folate supplementation routinely? The issue of folate and IPTP with SP is not mentioned and perhaps this topic has been left for another review focussed on treatment and prevention.

Response: Folate interactions with IPTp with SP is more relevant to the review on prevention, we have left this for that review to cover, given our space limitations.

f. Asymptomatic malaria (p.13). It is stated that in low transmission area, malaria infection in pregnancy is usually symptomatic. How good is the evidence to support this statement? It used to be stated that this was a general case for all individuals living in low transmission areas but there is now good evidence that this is not the case and that asymptomatic, low density infections are common in low transmission areas. Are pregnant women an exception?

Response: The only studies we are aware of that compare malaria in situations of different malaria transmission intensities are “Mayor, A., et al., *Changing Trends in P. falciparum Burden, Immunity, and Disease in Pregnancy.* N Engl J Med, 2015. **373**(17): p. 1607-17” and “Feng, G., J.A. Simpson, E. Chaluluka, M.E. Molyneux, and S.J. Rogerson, *Decreasing burden of malaria in pregnancy in Malawian women and its relationship to use of intermittent preventive therapy or bed nets.* PLoS One, 2011. **5**(8): p. e12012”, that we comment on later on in this paragraph. Given the uncertainty regarding the relationships between symptoms, malaria complications and transmission we have edited the text, deleting from “If transmission is high and stable” to “maternal mortality”, and have rephrased the following sentence “Where malaria transmission has declined substantially, increases in parasite density (Feng, Mayor) and in the malaria-related adverse impact have been observed (Mayor).

g. Malaria in pregnancy and elimination (p.13). Mass campaigns need to achieve high coverage in elimination programmes. Has any modelling been done on how important omitting pregnant women overall or those in the first trimester or those in the reproductive age group who had a negative pregnancy test but who might be pregnant might be?

Response: We added the following text: “Recent modeling analysis suggests that pregnant women might constitute up to 23.9% of all infectious individuals following MDA to 90% of the non-pregnant population (Goncalves, B.P., et al., Pregnant Women: An Overlooked Asset to Plasmodium falciparum Malaria Elimination Campaigns? Trends Parasitol, 2017). Further studies are needed to understand the impact of omitting pregnant women in MDA campaigns.”

h. ACTs in pregnancy (p.14). ACTs are now approved by WHO for treatment of malaria in the first trimester of pregnancy but there may still be concerns over their use, especially with DHA-PQ, in mass drug administration campaigns that could result in women in the first trimester receiving the drug who are not infected or at risk. This distinction could be mentioned.

Response: Although WHO’s MPAC have recommended approval of ACTs for treatment in first trimester this is not yet officially treatment policy. We have changed the sentence to read “ACTs are yet to be approved by WHO for treatment of malaria in the first trimester of pregnancy, and there may be concerns over the use of DHA-PQ in mass drug administration campaigns that could result in women in the first trimester receiving the drug who are not infected or at risk. Women in the first trimester need to be excluded from mass drug administration programs and instead managed according to a malaria RDT result”.

i. Pregnant women as a sentinel population for measuring changes in incidence (p.15) As the authors point out, results obtained by routine screening for malaria at ANC clinics could provide useful information in changes in malaria incidence over time obtained at relatively low cost. However, as pointed out in the review, cases will be missed unless PCR is used. An insensitive test may be satisfactory to measure overall trends in a medium or high transmission area but not in areas approaching elimination unless PCR is used and this could be mentioned.

Response: We agree. We have changed the paragraph "suggesting parasite prevalence among pregnant women may also be a useful proxy for malaria morbidity in the underlying community" to "suggesting that detection of malaria parasites with highly sensitive diagnostic tests such as PCR in pregnant women at their first antenatal visit may be useful to assess malaria trends in the underlying community"

j. Tables 2 and 3. These could be presented as supplementary materials if space is an issue.

Response: We have not made this change.

k. Figures 2 and 3. There are more data on asymptomatic infections in pregnancy post 2013.

Response: Please note that Figure S1 and S2 are about submicroscopic malaria, which is not necessarily the same as asymptomatic malaria. There is indeed more data available about subpatent malaria: the search was conducted in May 2016, as has been included in Panel 1, and more publications have emerged since then. We are currently in the process of a large study using individual participant data to evaluate prevalence and importance of subpatent malaria in pregnancy. This information has been added on page 7 as follows (third paragraph of this section): "Therefore, it remains unclear whether, and to what extent, either *P. falciparum* or *P. vivax* infections detected solely by PCR adversely affect maternal and infant outcomes; a large individual participant data analysis to examine this further is under preparation."

Reviewer #2:

This review on malaria in pregnant women, with emphasis of data obtained in the last 10 years, is a good up-date that will be of value to other scientists interested in the topic. Figures 2 and 3 provide a nice comparative analysis of the prevalence and potential influence of submicroscopic malaria infections on pregnant women. Perhaps the biggest disappointment is the section on economic burden (see below), which may simply be due to lack of available information. The concept of using pregnant women as "sentinels" in evaluating malaria elimination is interesting, but may not be practical.

There are no major concerns. The comments below are meant to help improve the manuscript. The reviewer is D.W. Taylor.

Minor Major Comments

1. MiP: page 4 end of paragraph 1. The report of information from ref 10 is for prevalence without intervention. However, today interventions are being used. Thus, the information seems incomplete. That is, we estimate what it would be without interventions, so what IS the prevalence with intervention?

Response: We changed and added this information as follows: "In order to estimate the positive impact of prevention in Africa in 2015, Walker et al. developed a specific model of malaria in pregnancy which takes the unique feature of parity-specific patterns into account; using this model, it was estimated that, without pregnancy-specific interventions, 9.5 million pregnant women would have been exposed to infection,

leading to 750 thousand low birthweight deliveries.[10] However, with IPTp coverage estimated at 21.6% for 2015, potentially128 thousand low birth weights could have been averted.[10]"

2. MiP: Please define "low transmission." That is, how low does malaria prevalence have to be for an area to become a "low transmission" area? (page 4 end of paragraph 1; Page 6 first paragraph)

Comment: According to WHO, high transmission is most commonly defined as parasite prevalence in 2-9 year old children above 10% (WHO: Disease surveillance for malaria elimination: an operational manual, 2012). We have added a sentence at first mention to include this definition, in the section "Malaria in Pregnancy: Progress on epidemiology and burden".

3. MiP, the fetus and the growing child. "... resulting level of antibodies to malaria parasites and other pathogens such as tetanus and measles.[2]" Not sure what was written in the previous Lancet review [2], but there is no evidence that antibody levels to malaria are reduced in neonates born to PM+ mothers, rather they frequently have higher level antimalarial antibody levels. However, the statement that babies born to PM+ mothers may have lower levels of antibodies to other pathogens is correct. Suggest deleting "antibodies to malaria parasites"

Comment: Thank you for this suggestion, text has been amended on P5 to delete the mention of decreased malaria antibody.

4. Page 6, last two lines - top page 7. Submicroscopic infections at antenatal ....India [40]." This sentence is unclear. Can you re-phrase and use the words "PCR+ but RDT-" or indicate level of parasitaemia? In some cases, RTD are more sensitive that microscopy. The term "submicroscopic" means "levels below detection by microscopy." So, this part of the manuscript (MS) is confusing.

Response: We changed the sentences to indicate which studies used PCR+ and microscopy negative infections, and which used PCR+ and RDT negative studies, rather than using the term "submicroscopic". "Although subpatent parasitaemias (infections detected by PCR but not by RDT or microscopy) are common in many holoendemic areas, they are inconsistently associated with clinical outcomes. Infections detected by PCR but not by microscopy at antenatal enrollment were strongly associated with an increased risk of low birthweight or prematurity in Benin,[58] whereas antenatal infections detected by PCR but not by RDT were not associated with poor infant outcomes in either Malawi,[59] Ghana,[60] or India.[40] By contrast, submicroscopic infections detected either antenatally or at delivery may be associated with a mild increase in the prevalence of maternal anaemia at delivery (Figure 3)."

5. Figure 2 and 3: More information is needed in the figure legend to understand the figure. For example: Are the dots and lines □ means +/1 DS? Do they represent prevalence value? That is n/N (%). Why doesn't malaria + no malaria = 100%? What does anemia less - anemia more when sub-microscope mean? What does % weight mean? Same types of questions apply to Fig.

Response: The figures (now figure S1 and figure S2) have been revised using the suggestions of the reviewer. Note that the graph mentions that the weights are from a random effects analysis; they show how each study is incorporated in the overall pooled estimate.

6. Page 7 - mid page: "The low sensitivity of current RDTs may help explain ...." Could it be that parasitaemia <200 IE/ul do not cause pathology, i.e., it is not a problem with the sensitivity of RDTs?

Response: The point we are primarily making is that, with IPTp, ALL women are exposed to drug, with IST a proportion will always be missed, and this proportion is higher with RDTs than other diagnostics. Although in some settings a low parasite density may indeed not be associated with adverse effects of malaria in pregnancy, in a trial in Malawi the malaria infections not detected by intermittent screening may have led to worse delivery outcomes than intermittent preventive treatment. We changed the sentence wording to read “The low sensitivity of current RDTs may have contributed to the low efficacy of intermittent screening and treatment compared to IPTp”.

7. Malaria and nutrition: page 8.

"The scope of interactions between malaria and nutrition is enormous,…" One wishes you had expanded on this statement.

The above statement and the following seem contradictory. "...when malaria and malnutrition were present, but not a synergistic effect..." If there is an enormous interaction, why isn't there a synergistic effect?

Response: The study mentioned is now in press in PLoS Medicine, and has been added to the references. It may be a function of the studies included (in which ANC and especially malaria prevention was good) that there was no synergistic effect, we were also surprised by this finding. We changed the initial wording to read “With the frequent overlap between malaria and nutrition…”

8. Pathogenesis: Page 9. "Modeling studies ..... 63% of placental infections may have been initiated by the end of the first trimester." This is a modeling study. Note: Leke et al. addressed the question by following parasite genotypes (based on polymorphisms of msp1 and msp2) over the course of pregnancy in women living in a hyper-endemic area (Leke et al. 2010, AJTMH). In Fig. 5a, they concluded that only 13-19% of the women had maintained the same parasite genotype from the first trimester and that in ~60% of the women had new infections near term. So, these data do not agree with the prediction. Thus, the authors might want to suggest that data are needed to confirm the model of Walker et al.

Response: we added this sentence: “However, longitudinal genotyping of *P. falciparum* isolates during gestation in Cameroonian pregnant women showed that 77% of placental parasites were acquired ≥30 weeks in pregnancy (Leke et al., Longitudinal Studies of *Plasmodium falciparum* Malaria in Pregnant Women Living in a Rural Cameroonian Village with High Perennial Transmission. *Am. J. Trop. Med. Hyg.,* 83(5), 2010, pp. 996–1004), suggesting that data is still needed to confirm the model”.

9. Page 10. The statement "..., as demonstrated in vitro with plasma from malaria-infected women." is unclear. How does plasma demonstrate interference with trophoblast invasion?

Response: We have reworded the sentence. The point is that using an in vitro system with plasma from pregnant women, we show that Pf-infected plasma can inhibit trophoblast migration relative to uninfected plasma.

10. Protective antibodies: Please check references 98-102. I think something is wrong. Ref 100 is not a review paper.

Response: Thank you, we have checked these references and they are now correct

11. Protective antibodies: page 11 ... "Antibodies against merozoite antigens have been associated with improved birth outcomes." FYI at least 4 studies have evaluated the role of MSP1 is pregnancy, so this section seems incomplete. Suggest deleting the sentence.

Response: The sentence has been deleted.

12. Page 11 - end of section on protective antibodies. This reviewer would have liked to read a summary about on-going clinical trials. Most readers would really appreciate having access to this information. That is, can the basic information be included: 1) what antigen is in the vaccine, 2) who is being vaccinated, and 3) what is the end point being measured? Inclusion of this information would greatly increase the usefulness of the review.

Response: There is no published data, and we have included links to the trial registration sites for the interested reader.

13. The economic burden: One question that needs to be addressed is: Is there an economic benefit to IPTp or other interventions? One assumes there is, but if only ~25% of women are receiving 2 doses of SP, is the cost of developing infrastructure really cost effective? Is there a way to address this question in the review?

Response: We thank the reviewer for this important remark. There are, indeed, economic (in addition to health) benefits associated with both increasing the frequency and scaling-up IPTp (see for example: *Fernandes et al, Cost-effectiveness of two versus three or more doses of intermittent preventive treatment for malaria during pregnancy in sub-Saharan Africa: a modelling study of meta-analysis and cost data. Lancet Glob Health. 2015 Mar;3(3):e143-53* and *Mbonye AK et al, Intermittent reventive treatment of malaria in pregnancy: the incremental cost-effectiveness of a new delivery system in Uganda. Trans R Soc Trop Med Hyg. 2008 Jul;102(7):685-93. doi: 0.1016/j.trstmh.2008.04.016*).

However, in this review we focused only on the economic burden, therefore on costs. Economic benefits were outside the scope of this review. This choice was done in order to be in line with the overall review, which focused on the burden and on the pathology of malaria in pregnancy: the contribution of economist has been to add costs to those two categories.

Major Minor comments

1. Malaria in pregnancy: progress on epidemiology and burden (add) in Africa

The topic of pregnancy in Non-African areas is covered under a different header.

Response: Several issues in this part are also applicable outside of Africa, and some references are also from outside of Africa. We changed the heading to: "**Malaria in pregnancy: progress on epidemiology and burden of** *P. falciparum"* and "**Malaria in pregnancy: other species"**, which is mainly outside Africa.

2. Throughout the Review, the authors refer to previous reviews (usually reference 1, 2) as the "source" for the information, rather than crediting the author(s) who originally reported the information. For example, "Malaria is highest in the first and second trimester of pregnancy (.... personal communication and 1 (a review by Desai et al.). Clearly, B. Brabin reported these results in the 1970's. This reviewer doesn't feel comfortable with quoting pc or review articles when others researchers could be give credit for their contributions. (Just a comment)

Response: We acknowledge the reviewer's point. We are only permitted a limited number of references and tried to cite work published since the last comprehensive reviews where possible, particularly as the review is essentially an update to previous papers.

3. Page 4. "... birthweight, confirming findings from an IPTp trial in Mozambique. [8,9]." Did the results really confirm or just agree with? The authors might consider reversing references 9,8 so that the references line-up with the information provided.

Response: We followed these suggestions and changed the sentence as follows: "In a large study using African national survey data, the use of malaria prevention in

pregnancy was associated with substantial reductions in neonatal mortality and low birthweight, agreeing with findings from an IPTp trial in Mozambique.(Eisele, Menendez)"

4. Detection of malaria: Bottom of page 7. A lot of facts/ideas are presented. Can you provide a conclusion? That is, what is your conclusion related to Detection of malaria in pregnant women? What's the take-home message?

Response: We have added the following summary: "The impact of submicroscopic infections on pregnancy outcomes still remains to be elucidated. More sensitive diagnostics have the potential to increase detection of malaria parasites. Whether deployment of these new assays can improve pregnancy outcomes and translate into a public health impact remains unknown."

5. The abbreviation for MiP (appears on page 13) does not seem to appear in the MS???

Response: Thank you, we have spelled this out in full, as we have done elsewhere.

6. Pathogenesis of Malaria: Does Pv really sequester? Clearly, the term sequester means "an increase in number," but when the term is used in Pf it implies adhesion. So the statement will be confusing to some readers.

Response: We think the original text makes our point clearly: "Neither a direct link between placental changes and adverse birth outcomes nor placental sequestration of IEs have been clearly demonstrated in *P. vivax*".

7. Protective antibodies: page 11 ... "Antibodies against merozoite antigens have been associated with improved birth outcomes." FYI: There are four papers that addressed the possible role of antibodies to MSP1 in immunity to placental malaria. Suggest deleting the sentence.

Response: Thank you, we have deleted the sentence.

8. Economic burden: Perhaps the biggest disappointment is in the section on "economic burden," (see below), but part of the problem may simply be the lack of solid data. Only one example is provided in the text and 1% of monetary value of total activities doesn't sound like very much money.

Response: There are actually data available on costs associated with malaria in pregnancy and these are presented in tables 1 and 2. The choice of the authors has been not to report "numbers" in the text but simply to summarize and comment on values reported in tables plus on other studies which are mainly qualitative rather than quantitative. This was mainly due to lack of space.

However, the 1% mentioned in text has been better specified in this new version of the manuscript: "For example, in 2010, provider costs associated with the management of confirmed cases of malaria in pregnancy reported by a tertiary hospital in a low-endemic area of the Brazilian Amazonas constituted 1% of the monetary value of the total activities undertaken that year. *This is a remarkable proportion given that pregnant women are a sub-set of the total population at risk and the study area is characterized by low-transmission".*

9. Economic burden: Last sentence "Despite being more ..... free distribution.[114]" isn't clear. Can you please revise?

Response: We thank the reviewer for this remark. The original sentence was "Despite being more costly than traditional private sector retail sale strategies, social marketing increased supply of nets and coverage, and, in another study, its cost did not differ substantially from that of free distribution". It now reads "*Social marketing increased*

supply of nets as well coverage. From the provider perspective, social marketing has been found more costly than procuring and selling nets in the private retail market, but also as costly as distributing free of charge to the community".

10. Elimination agenda: Page 15. The text suggests that pregnant women in areas with malaria-elimination programs might be foci of infection. However, wouldn't countries with malaria-elimination programs continue to emphasis the use of IPTp, whereby pregnant women would be protected from infection? Likewise, Page 16 - top: suggesting that monitoring change in antibody levels to VAR2CSA seems equally unlikely.

Response: Given contraindications or limited efficacy and safety data in first trimester pregnant women, efforts to reduce malaria transmission levels using community-based chemotherapy approaches often exclude pregnant women. Although IPTp is a form of demographically-targeted MDA, it is likely to be less effective at reducing transmission compared to community-wide MDA because IPTp is asynchronous (it does not clear all infections over a short time-window), because SP does not clear gametocytes, and because the parasitological efficacy of SP is low in areas of SP resistance whilst it may still be clinically effective. The high parasite levels observed during pregnancy suggest that these 'non-targeted' individuals might harbour infections with higher densities of transmissible gametocytes compared to infections in non-pregnant adults, and this could result in high infectivity. Their attractiveness to mosquitoes also means that pregnant women are frequently sampled by malaria vectors, and their gametocytes have more opportunities to be ingested by mosquitoes. Together, these observations suggest that pregnant women might become an important source of mosquito infections after MDA rounds with high coverage. Detection of IgGs against VAR2CSA could have a programmatic use in the future if a serological rapid test could be developed (in line with other serological tests, such as SD BIOLINE Dengue IgG/IgM test for the qualitative and differential detection of IgG and IgM antibodies to dengue virus serotype DEN-1, 2, 3 and 4).

11. MiP Agenda: page 14 end of page: "... and that pregnant women at first trimester are not eligible, ..." Why are they not eligible? Is it because SP is tetragenic in rats? If so, then saying contraindicated might be better than eligible. OR do you mean that most pregnant women don't attend ANC until the second trimester, so they are not eligible for treatment?

Response: Both these reasons apply, wording now reads: "As SP is contraindicated at first trimester and pregnant women typically do not attend ANC until the second trimester".

Trivial comments

1. Introduction: ".... we summarise outstanding research questions..." Do you mean excellent research questions or questions that need to be addressed?

Response: "outstanding" changed to "important remaining"

2. Detection of malaria: page 6 "... requires specialist laboratories." Change to specialized

Response: Change made

3. MiP: page 4 mid-last paragraph. "... which worsens with repeated infection. [15-17]" Do you mean in the same pregnancy or subsequent pregnancies?

Response: We mean in the same pregnancy, and think the intention is clear from the sentence context.

4. Page 12: Economic burden: Information is in Table 2 (not 1).

Response: Thanks, corrected

5. Page 13: "".....impacts women's labour supply ..." Since this is a study of pregnant women whose pregnancies end in labour, you might consider changing the wording to employment or work supply

Response: changed from “labour supply” to “employment”

6. Is the abbreviation for ANC provided in the text?

Response: This has been changed to antenatal care

Reviewer #3:

General Comments

This manuscript serves as a review of issues of malaria in pregnancy with an emphasis on the understanding gained during the last 10 years on the burden, economic costs and consequences during a time when overall malaria has declined substantially in some areas. The authors have a clear understanding of the breadth and depth of the work that they are undertaking; and many have been involved in previous reviews of the subject. While the topic is certainly of interest to both the malaria community and the reproductive health and newborn care community, it would appear that the manuscript could be more focused in order to address the specific updates and the priorities in our understanding and the implications for next critical steps.

As an example, in the abstract they highlight a set of features:

1) Malaria due to Pf has declined substantially in some areas (fewer infections, cases and deaths);

2) Studies outside of Africa have increased our knowledge of Pv and its burden in pregnancy;

3) Malaria diagnostics are a challenge in pregnant women (or something like this, but it is not clear from the manuscript that diagnostic testing is generally better/worse/same in detecting relevant malaria infections in pregnant and non-pregnant women);

Response: diagnostic tests such as microscopy and RDTs clearly work less well in pregnant women (who generally have low parasitaemia) than in the setting of diagnosis of clinical infection, where they work well. Current literature does not to our knowledge allow a comparison of use in pregnancy with use of RDT at the population screening level.

4) Immunology investigations have focused on VAR2CSA and there is a suggestion that infected erythrocytes with this surface protein may present a target for immunologic intervention strategies

5) Pregnant women may represent an opportune population as sentinels for malaria transmission

Additionally, the body of the manuscript notes some other issues:

1) Costing analyses have been done - but it is not clear what this tells us;

2) Prevention coverage of interventions (LLINs, IPTp) is improving but remains lower than one would like (I believe that this set of issues may be addressed in another manuscript);

Response: Correct, this is discussed in another manuscript

3) First trimester of pregnancy presents a problem: a) it may be the most at risk interval because infection prior to pregnancy can be carried into the 1st trimester in addition to acquisition of new infections in 1st trimester - yet, because of the usual concerns about the critical interval of 1st trimester for fetal development, any new interventions have limited applicability in this time interval until further testing. And even then, no one likes giving drugs or biologics to pregnant women and their fetus in the 1st trimester - so, addressing this in the near or long term remains a huge challenge.

Response: as the reviewer indicates this is a challenging problem, but there are limited published data to address it. Our focus is on published literature

And, there have been long-standing concerns that the needed attention and improvements to address the various aspects of malaria in pregnancy require engagement of several important groups/systems:

1) The reproductive health programs (public and private) who provide the vast majority of information and services to women for the care of the mother and fetus - intervention coverage is largely in their hands;

2) Pregnant women (and women about to become pregnant) have responsibility for entering their pregnancy malaria-free and staying malaria-free; thus they must be willing/able to engage with the general and reproductive health systems and be aware of their options and responsibilities;

3) The malaria program which has responsibility for communicating evidence-based information on prevention, care and treatment to continue to limit the burden of infection during pregnancy.

Response: Another review for the same issue deals with these three important questions of access and coverage.

Given all of this, I would like to make an overall suggestion to the authors regarding the organization and presentation of the material as I do believe that an update is highly relevant at this point, but it needs more focus and useful recommendations/considerations than I see in this manuscript currently.

Consider the following:

1) Introduce the overall issues of malaria in pregnancy. You can rely heavily on the previously published summaries that have been very good in describing the risks, burden, efficacy of prevention, etc. While you can note the progress (incomplete as it is) in prevention coverage, you may leave the greater details to the other manuscript on prevention.

2) Introduce the key list of factors that have been elucidated in the past decade and set up the text to then work through each one of these as a specific section (I have given a listing below, but you may choose a different order and include others or exclude some noted):

a. Pf malaria is declining substantially in some places - but not others

i. Risk of clinical illness and the need to detect infection early and treat appropriately may become increasingly important

ii. Understanding when to persist and enhance prevention versus when to stop prevention may be a critical knowledge gap - if only 1/1000 pregnant women will get infected during her pregnancy, then maybe protecting the other 999 carries more risk than benefit; what about 5/1000; 10/1000; 50/1000 or 100+/1000?

b. Pv has been examined and increasingly explored; in general, Pv infection rates are lower and the management of Pv that includes radical cure is not really viable with current drugs, etc. (can you be clear that we should do a specific set of intervention/prevention for Pv or that this requires further evaluation and review for WHO guidance?)

c. 1st Trimester remains the special problem - it is the highest risk time, yet it is the most challenging time to prevention and intervene. What is the responsibility for pre-pregnant women and for prevention during early pregnancy when they may not yet know that they are pregnant, and in the first trimester when they do know but haven't presented to the antenatal clinic? Do we really want to explore drugs or vaccines for prevention in this interval, or should our prevention be heavy on vector control and dx/tx saved for rapid evaluation of clinical illness and treatment of confirmed cases? What more do we need not know about 1st trimester malaria and preventive or treatment or intervention options to actually have a plan?

d. Immunology in malaria. The article focuses on VAR2CSA and supposes that this is a critical component of immunologic mechanisms - but is it the only immunologic exploration that is relevant now or in the future? The high risk in young pregnant women and in first/second-pregnant women, and the apparent higher risk in 1st and early 2nd trimester where later in the pregnancy (after a duration of early exposure) there appears to be strong evidence of cellular responses and much parasite killing, are there additional explorations that we should be making in the immunologic response within a single pregnancy or a memory system (systemic or uterine resident responses) that carries across pregnancies? Or, we only have some ongoing investigation in the role of immunologic interventions (vaccines?) to alter infected red cell binding proteins.

e. Diagnostics in pregnancy. (***Be careful here because saying that our diagnostics don't work contradicts the notion diagnosis in pregnant women could serve for an important sentinel group to track infection rates/patterns***). While there have been more studies of diagnostics, are the findings that PCR is more sensitive just a reiteration of what we find in all non-pregnant populations? As with malaria diagnostics in general, the critical breakthrough has been the availability of a point-of-care immediate test result that allows one to make a treatment decision - this is the RDT. With the recent evidence from diagnostics, is this good for clinical care and for surveillance (the easy-to-access sentinel population of pregnant women). What else might come soon - a more sensitive RDT assay? What remains to be learned?

Response: We appreciate the point about being consistent. As included in the response to another reviewer we have reworded this section to read. “detection of malaria parasites with highly sensitive diagnostic tests such as PCR in pregnant women at their first antenatal visit may be useful to assess malaria trends in the underlying community.

f. Roles and responsibilities of reproductive health programs and their staff, malaria programs and their staff, and for reproductive-age and pregnant women

Response: See elsewhere. The “health systems” aspects of MiP highlighted by the reviewer are covered in another review.

g. Costs (***here I am cautious because I was not convinced by the section on costs that the manuscript got to a point of addressing what we know and can now use versus what needs to be done to further inform costs, and for what purpose?) I do believe that understanding costs is important, but the text could address how the program uses those costs - for example, for planning, budgeting, engagement with Ministries of Finance and donors, and looking for efficiencies.

Response: please see our reply below (when page 13 is mentioned)

h. Others?

Finally, there is the balance for each of these areas between 1) new knowledge and what that tells us to do, versus 2) advances that require critical additional work for clarification so that progress can be made in the future.

I do believe that the majority of the information requested here is already within the manuscript. Thus, this is more about framing the overall and specific issues so that they are understandable as an "update over the past decade" for the reader. Of note, there are currently more than 160 references and it is not clear that all of these will be required to tell the story. For example, some of the references to individual studies on a specific issue may be more than is required for the text.

Overall response: Both the reviewer's introductory comments and the list provided here are very helpful in drafting the new Panel on key unresolved questions and future directions. We would point out that our remit is to address what we have learnt over the last 10 years, rather than to highlight what we still don't know, which is the subject of Panel 3. Clearly the reviewer has put a lot of thought into their preferred structure for the review. Overall it seems the proposed structure is more appropriate to an article which has its main focus on future challenges in malaria in pregnancy, an article that would undoubtedly be of significant interest, but which is not the one we have been asked to write.

Specific Comments:

Abstract (page 2).

1. The difference in sensitivity between a RDT versus PCR test is not unique to malaria in pregnant women, so this may need to be rephrased and consider the role of a point-of-care test with near immediate results.

Response: Although the difference in sensitivity between RDT versus PCR is not unique for pregnant women and the effect of submicroscopic malaria on anaemia may not be unique as well, the implications for the fetus are unique to pregnant women and the effect is not yet clear. We added information on subpatent malaria among non-pregnant populations to the manuscript (page 7).

2. It is not clear that two sentences are required for the VAR2CSA; and it is not actually clear that this is an important target for protective immunity - that work still remains to be done.

Response: We disagree with the reviewer. Multiple studies over two decades have confirmed CSA binding as intrinsic to placental malaria pathogenesis, and then shown that VAR2CSA is the key ligand for CSA. Further studies show immunity to parasites expressing VAR2CSA or to VAR2CSA correlate with protection, in at least some settings and study groups, including papers from several of the current authors.

Page 3, para 2, line beginning with "Currently": This appears to be a new concept about prevention and control and should be considered as a separate paragraph.

Response: We have broken this section into a separate paragraph as suggested.

Page 3, para 2 last sentence: could use the World Malaria Report 2016 data with 31% of pregnant women in 36 African countries received three or more doses of IPT.

Response: We have added information from the World Malaria Report here as was also suggested by Reviewer 1.

Page 4/page 5. The authors use several different terms for the adverse events associated with malaria in pregnancy: "infant low birthweight", "fetal growth restriction", "newborn birthweight" ("birthweight" doesn't need the qualifier of "infant" or "newborn") ; but not until page 6 is there any mention of "premature or preterm delivery" which has

been shown to be associated with malaria in pregnancy and carries one of the highest risks for reduced newborn/infant survival.

Response: We added prematurity to page 5 as follows: “The range of adverse outcomes among women and their newborns include well-described increased risks of maternal anaemia, low birthweight, and prematurity as documented in trials and observational studies.” We removed “infant” as superfluous.

Page 5, line 12: consider changing "frequently" to "typically".

Response: Change made.

Page 5, line 14: "tolerize" is not in my dictionary.

Response: The term is in Wiktionary and Steadman’s Medical Dictionary, as we use it. It is also found in http://www.yourdictionary.com/tolerize.

Page 6 & 7, section on "Detection of malaria in pregnancy". Per the general comments, perhaps reconsider how the role of an RDT is cast (the RDT is the only quality assured point-of-care test with near immediate results). The sensitivity is certainly imperfect compared to PCR (true for pregnant women and for non-pregnant populations), but we don't quite know what the relevance is for a PCR+ but RDT or microscopy negative test. The last sentence with the "median ratio of 2.6" may be a bit confusing (note that a ratio may not be the best way to look at this because if the microscopy result is 75% positive, and PCR shows 100% positive, then there is a limit on the ratio of 1.33 - but if it is 0.1% positive by microscopy among 1000 tests, 2 additional positives by PCR get you a ratio of 3.). Is there any evidence that this is different during pregnancy? If not, be careful with how this is cast. If the diagnostic is faulty, then the argument for using pregnant women as a

possible sentinel population for tracking confirmed malaria infections is a problem? Finally, as the case is made that PCR and microscopy require time, training, and laboratory support, the more important question is about the quality assurance of the RDTs and the consistency of the results such that they can be used for the possible surveillance role going forward.

Response: Thank you. We have summarized studies comparing PCR to microscopy or RDT in the section on Detection of Malaria in Pregnancy, with changes made here to clarify the comparisons being made. We agree that ratio may be confusing or misleading, but we also want to give the readers an idea how one could estimate PCR results from microscopy results; we changed the ratio to compare microscopy vs. PCR to make it comparable with results among mainly non-pregnant populations as reported by Okell et al 2009, and added the 95% CI as follows: “Among 20 studies with information, prevalence of submicroscopic infection ranged from 2-55%;[38,40-57] comparing microscopy and PCR results during pregnancy the mean ratio was 0.46 (95% 0.35-0.57) (Figure 2) whereas this was 0.51 (95% CI 0.45-0.57) in 65 studies among mainly non-pregnant populations.(ref okell 2009 JID”.

Page 8. Consider the points being made and the message of what is new vis-à-vis maternal nutrition. Perhaps cast these at the beginning or end that 1) good maternal nutrition is important for fetal/newborn outcomes and 2) iron supplementation is good and safe despite previous concerns.

Response: We hold the first point to be self-evident. In relation to the second point, re iron, we do say “Concerns that iron supplementation might increase the risk of malaria in pregnant women appear to be unfounded” which we feel is an appropriate endorsement of the policy based on current data.

Page 9. The pathogenesis section is nearly entirely focused on CSA binding and VAR2CSA (and this comes back on pages 10 and ll with antibody discussion)- is there anything else new or of interest. All the other points seem to be single sentence with

one or two references for placental circulation, fetal circulation, complement activation, amino acid and glucose transport, etc. - but they don't seem to sum to anything critical for intervention or change, is that right?

Response: for the issues we have highlighted and the reviewer summarises, we are pointing out new insights into pathophysiology, which may eventually lead to evidence to support policy change.

The last sentence of the 2nd full paragraph is the quiet statement that 1st trimester is a critical period. This likely requires more attention and to be placed elsewhere on its own.

Response: As we point out earlier in our response, there is limited information about malaria in 1st trimester. We have highlighted this in the Panel 3

Page 13. Last paragraph on the Cost section. This clearly lists 3 critical issues that are under-explored. This format is helpful; but it also demands why one would actually need to have this information - so it might be important to include how this will make a difference. Will it make the programs, the ministries of finance or the donors sit up and take notice?

Response: We agree with the reviewer that there is a need to better specify the relevance and the potential use of the additional information.

Original paragraph:

"Although the evidence base on the economic burden of malaria in pregnancy has expanded in recent years, there are still three main gaps to fill: (i) very little is known about the economic burden outside sub-Saharan Africa, where different Plasmodium species co-exist; (ii) the impact of malaria in pregnancy on indicators of economic development has never been investigated: for example, it is unknown how malaria in pregnancy impacts women's labour supply and wages; and (iii) costs and consequences associated with MiP are underexplored: only a few studies have estimated, for example, cost associated with low birthweight in malaria endemic countries.148,149"

This now reads "*Although the evidence base on the economic burden of malaria in pregnancy has expanded in recent years, there are still three main gaps to fill: (i) very little is known about the economic burden outside sub-Saharan Africa, where different Plasmodium species co-exist; (ii) the impact of malaria in pregnancy on indicators of economic development has never been investigated: for example, it is unknown how malaria in pregnancy impacts women's labour supply and wages; and (iii) costs and consequences associated with MiP are underexplored: only a few studies have estimated, for example, cost associated with low birthweight in malaria endemic countries. Additional knowledge on (i), (ii) and (iii) may, respectively: guide decisions on optimal allocation of resources in areas where malaria control tools and strategies depend on Plasmodium species and where conditions to achieve elimination exist; inform economic growth, development and women's empowerment strategies; further promote investments towards improvement of prevention and treatment of malaria in pregnancy.".*

Page 13 last paragraph, 3rd line: "most usually" might be changed to "often"; last line: "considered to be usually" might be changed to "more often".

Response: In response to comments from another of the reviewers, we have deleted the sentences that included the words pointed out in this comment.

Page 14 and 15, consider checking some of the references. Was the frequency of severe malaria syndromes studied by Feng G et al (ref 152 on page 14); is this ref 152 the correct reference for several statements in the first full paragraph of page 15 and in the second paragraph where these are about Southern Mozambique?; is the article

about ITN distribution (ref 113) the correct reference for surveillance in pregnant women compared to the general population?

Response: Thank you, we have checked the referencing in this section. Note that reference 152 mainly relates to the first part of the sentence (increases in parasite density), and we moved the reference there.

Page 15, paragraph 2. I am concerned that the testing for antimalarial IgG responses to VAR2CSA may be a stretch for program relevance.

Response:

Detection of IgGs against VAR2CSA could have a programmatic use in the future if a serological rapid test could be developed (in line with other serological tests, such as SD BIOLINE Dengue IgG/IgM test for the qualitative and differential detection of IgG and IgM antibodies to dengue virus serotype DEN-1, 2, 3 and 4).

Page 16, last paragraph of the discussion. This feels unrelated to all that has come before and seems like an add on about P vivax. I am not sure it is relevant here.

Response: We have reorganized the last paragraph and included the comment on vivax in the second paragraph of this section: "Use of antimalarials such as chloroquine that are known to be safe during pregnancy, may be an alternative in areas where parasite population has regained susceptibility to the drug (Galatas B, Nhamussua L, Candrinho B, Mabote L, Cisteró P, Gupta H, Rabinovich R, Menéndez C, Macete E, Saute F, Mayor A, Alonso P, Bassat Q, Aide P. In-Vivo Efficacy of Chloroquine to Clear Asymptomatic Infections in Mozambican Adults: A Randomized, Placebo-controlled Trial with Implications for Elimination Strategies. Sci Rep. 2017 May 2;7(1):1356). Development of new anti-malarials for radical cure of *P. vivax* which are safe in pregnancy are also needed. In absence of these antimalarial alternatives, monitoring pregnant women in areas where malaria elimination activities are conducted can inform the risk of unsuccessful interruption of transmission due to undetected foci of malaria among pregnant women".

Page 16-17. The conclusions seem a bit general. If you return to my suggestions in the general comments, you might be able to focus this and the abstract into a more concise paragraph.

Response: we have used this paragraph to give an over view of our findings and conclusions.

Editorial points to be addressed (these are general points, so not all will apply to your paper):

- Please see the end of this email for a list of signed statements from authors and people named in your paper that we will need before we can consider your paper further. Please scan and upload signed author statements and ICMJE conflict of interest forms for all authors with your revised submission.

- You will need to provide permission from the relevant publisher to include any material previously published that you wish to include in your paper.

Noted

- Please provide: one preferred degree qualification per author and indicate any full professors; affiliation details (department, institute, city, state, country) for each author; full institutional correspondence address for corresponding author.

Comment: already done

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Burden, pathology and costs of malaria in pregnancy: new developments for an old problem

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Abstract

Over the last ten years, our understanding of the burden, economic costs and consequences of malaria in pregnancy has deepened, while the prevalence of *Plasmodium falciparum* malaria has declined significantly in some areas. Studies outside of Africa have increased our knowledge of the burden, consequences and costs of *Plasmodium vivax* in pregnancy. Rapid diagnostic tests have performed poorly in detecting malaria in pregnant women, while PCR has revealed a high prevalence of low density infection, whose clinical importance remains unresolved. Erythrocytes infected with *P. falciparum* that express the surface protein VAR2CSA accumulate in the placenta, and VAR2CSA is an important target of protective immunity. A VAR2CSA vaccine has begun clinical trials, but sequence variation requires careful exploration. Health system and household costs still limit access to prevention and treatment services. Within the context of malaria elimination, pregnant women may be valuable sentinels for monitoring malaria transmission. This review summarises recent progress, and highlights unresolved issues relating to the burden of malaria in pregnancy.

Introduction

This review summarises progress in understanding of the epidemiology, burden, economic costs, pathogenesis, and diagnosis of malaria in pregnancy over the 10 years since Lancet Infectious Disease published a special issue on this topic. Readers are referred to the earlier reviews for comprehensive analyses of older literature.[1,2] Looking forwards, we discuss how present efforts in malaria elimination may alter the burden and consequences of malaria in pregnancy, and how pregnant women may be used to monitor changing epidemiology of malaria across populations. Finally, we summarise important remaining research questions in this area. Our search strategy is outlined in Panel 1,and major advances over the last 10 years are summarised in Panel 2.

Malaria in pregnancy: progress on epidemiology and burden of *P. falciparum*

In high malaria transmission areas such as sub-Saharan Africa, the risk of *Plasmodium falciparum* infection increases when women get pregnant, with potential adverse consequences for mother and child (Figure 1). Malaria prevalence is highest in the first and second trimester of pregnancy (AM van Eijk, personal communication),[1] and the risk may not immediately return to pre-pregnancy levels after delivery.[3] In these high transmission areas, defined as parasite prevalence in 2-9 year old children >10%, malaria in pregnancy is more common in younger compared to older pregnant women, in women in first or second pregnancy compared to later pregnancies, and in HIV-infected compared to uninfected women.[1,4] Similar to the non-pregnant population, prevalence is affected by location (higher among rural populations), season, and use of malaria prevention.

Currently, malaria prevention and control in pregnant women in Africa rests on three pillars: insecticide treated nets (ITNs), intermittent preventive treatment (IPTp) with sulphadoxine-

pyrimethamine, and effective case management of malarial illness and anaemia.[5] However, coverage of these interventions is suboptimal: in 2014, only 35% slept under an ITN,[6] and in 2015, only 31.5% of eligible women received three or more doses of IPTp. [7] The range of adverse outcomes among women and their newborns include well-described increased risks of maternal anaemia and low birthweight, and prematurity[1] as documented in trials and observational studies.[8] In a large study using African national survey data, the use of malaria prevention in pregnancy was associated with substantial reductions in neonatal mortality and low birthweight, agreeing with findings from an IPTp trial in Mozambique.[9,10] In order to estimate the positive impact of prevention in Africa in 2015, Walker et al. developed a specific model of malaria in pregnancy which takes the unique feature of parity-specific patterns into account; using this model, it was estimated that, without pregnancy-specific interventions, 9.5 million pregnant women would have been exposed to infection, leading to 750 thousand low birthweight deliveries.[11] However, with IPTp coverage (2 doses) estimated at 21.6% in 2015, potentially 128 thousand low birthweight deliveries could have been averted. [11]

Understanding of the impact of malaria in pregnancy on fetal growth has been facilitated by increased use of obstetric ultrasound in cohort studies, providing accurate estimation of gestational age, sometimes combined with Doppler studies. Using such approaches in longitudinal studies, malaria in the first trimester is now recognised as an important risk factor for miscarriage, fetal growth restriction, low birthweight and maternal anaemia in Africa[12,13] and Asia.[14,15] In studies from Benin, the Democratic Republic of Congo (DRC), Malawi, Thailand and Brazil, malaria infections in early pregnancy, in late pregnancy, and throughout gestation have each been associated with reduced fetal growth,[14,16-20] which worsens with repeated infections.[16-18] This fetal growth restriction can occur either immediately or with a delay after malaria infection,[16,20,21] and it may be parity dependent: in

Tanzania malaria infection was only associated with decreased fetal growth among primi- and secundigravidae.[21] In the DRC, malaria in the first half of pregnancy was associated with decreased umbilical artery resistance in the late third trimester and fetal growth restriction among primigravidae, suggesting that parasitaemia in early pregnancy interferes with the development of the fetal circulation.[22]

Malaria in pregnancy, the fetus, and the growing child

In addition to the adverse impact of maternal malaria on newborn birthweight, it can also affect other measures of infant health. Maternal malaria can affect transplacental transfer of antibodies to the fetus, and thus the infant's resulting level of antibodies to pathogens such as tetanus and measles.[2] Maternal malaria has been associated with an increased risk of congenital malaria,[23,24] infant malaria,[25-29] infant anaemia[1,30] and other febrile illnesses.[28,31] It may reduce the response to vaccination in infants,[32] and a significant increase in systolic blood pressure was documented among infants exposed to maternal malaria [33] In Uganda and The Gambia, infants born to mothers with malaria in pregnancy gained less height and weight in the first year of life.[28,34]

In epidemiological studies it is difficult to independently evaluate the effects of maternal malaria exposure on infant susceptibility to malaria, because mothers and their infants typically share similar exposure by living in the same household and frequently sleeping under the same net, but some studies have controlled for exposure. [29] Exposure to malaria in utero early in pregnancy may tolerize the infant's immune system, whereas later in pregnancy it may prime the infant's immune responses.[35] These exposures appear to affect immune responses through early life, and could alter risks of severe and uncomplicated disease [27,29] by affecting regulation of infection in the young child.

Malaria in pregnancy: *P. vivax*

Studies in Guatemala, Colombia, Brazil, Papua New Guinea, and Indonesia, as well as a review on malaria in pregnancy in Asia, have provided information on the burden of infection outside of Africa, including the importance of *P. vivax* malaria and mixed infections [36-38]. By microscopy, prevalence of any malaria infection varied greatly from very low (<1% in regions in Brazil, India, and Guatemala), to 10-20% in some regions in Papua New Guinea and Indonesia.[36,38,39] *P. vivax* frequently causes anaemia, and has been associated with reductions in infant birthweight[37,38,40] and preterm delivery,[38] and with an effect on fetal growth, including lower fetal head diameters in Thailand and lower birthweight in the Brazilian Amazon.[14,20] In addition, severe disease and maternal and fetal deaths due to *P. vivax* have been reported.[38,41] Submicroscopic and asymptomatic malaria infections occur even in areas of low transmission, but their importance still needs further investigation (Figure S1).[41]

Detection of malaria in pregnancy

The major options for detection of malaria infection in pregnant women remain light microscopy, rapid immunochromatographic diagnostic tests (RDTs), or polymerase chain reaction (PCR); in placental specimens, histopathology can also identify parasites and resulting inflammatory responses. Microscopy requires expertise and equipment and is only moderately sensitive, and alternative methods have strengths and weaknesses. Histology, which can reveal both current, active infections and past infections, is costly and its use is restricted to research settings. Loop-mediated isothermal amplification (LAMP) has been used to diagnose placental infections, [42] and has similar sensitivity to qPCR in pregnant women. [43]

The use of PCR has improved species identification and has permitted studies of low-density parasitaemia. PCR is highly sensitive, but is time-consuming and requires specialized laboratories. Parallel parasite detection by PCR and either an RDT or light microscopy identifies many women with PCR-positive but RDT/microscopy-negative infections. These "subpatent" infections generally have parasite densities that fall between the limits of detection for traditional methods and for molecular assays. Among 20 studies with information, prevalence of submicroscopic infection ranged from 2-55%. When comparing the ratio of microscopy to PCR prevalence, the mean ratio was 0.46 (95% 0.35-0.57) among pregnant women (Figure S1, 9 studies) whereas this was 0.51 (95% CI 0.45-0.57) in 65 studies among mainly non-pregnant populations.[44].

Although subpatent infections detected by PCR but not by RDT or microscopy are common in many holoendemic areas, they are inconsistently associated with clinical outcomes. Infections detected by PCR and not microscopy at antenatal enrollment were strongly associated with an increased risk of low birthweight or prematurity in Benin,[45] whereas infections detected by PCR but not by RDT were not associated with poor infant outcomes in Malawi,[46] [47] Ghana,[48] or India.[49] Submicroscopic infections detected antenatally or at delivery may be associated with a mild increase in the prevalence of maternal anaemia at delivery (Figure S2). Therefore, it remains unclear whether, and to what extent, either *P. falciparum* or *P. vivax* infections detected solely by PCR adversely affect maternal and infant outcomes; a large individual participant data analysis to examine this further is under preparation. In addition, more sensitive diagnostics are under development that have the potential to increase detection of malaria parasites. Whether deployment of these new assays can improve pregnancy outcomes and translate into a public health impact remains unknown.

RDTs are most sensitive for parasite densities above 200/μl for *P. falciparum*, and are less sensitive for other species. Many pregnant women have peripheral blood parasite densities

below this threshold, but may also have placental infections. This has led some groups to propose host biomarkers for occult placental infection.[50] The low sensitivity of current RDTs may have contributed to the low efficacy of intermittent screening and treatment compared to IPTp. [6,51] A further consideration is the emergence of parasite lines that lack histidine rich protein 2,[52] the principal protein used in *P. falciparum* RDTs. Nevertheless, as parasite prevalence is generally highest on first presentation at antenatal clinic, these relatively insensitive tests could be a useful screening tool when pregnant women are first seen

In antenatal specimens, compared to parasite detection by a nested PCR method, the sensitivity of microscopy in a research study varied between 40-70%, while that of commercial RDTs varied from 42-75%.[53] At delivery, peripheral or placental blood testing by microscopy or by RDT may not detect histopathologically-evident placental malaria.[53-56] Owing to this, two studies have applied latent class analysis to such samples in order to quantify the operating characteristics of detection methods independent of any reference standard. This analysis suggested that either PCR of placental specimens or RDT of peripheral specimens may have sufficient sensitivity for routine diagnosis of placental malaria.[57,58]

Malaria and maternal nutrition

Malaria and poor maternal nutrition frequently co-exist, and there are over 0.9 million babies born with low birthweight due to malaria and an estimated 0·8 million infant deaths related to maternal undernutrition each year.[59,60] In the DRC cohort discussed above, and in another study in Kenya, poor maternal nutritional status appeared to exacerbate the deleterious effect of malaria on fetal growth.[17,22,61] Risk factors often overlap, and the rainy season is a period of both high malaria risk and food insecurity. With the frequent overlap between malaria and nutrition, strategies to reduce both these risk factors are needed. [62] To examine this further, a

large study combining data from 14,635 pregnancies from 13 studies conducted in Africa and the Western Pacific from 1996-2015 showed an increased risk of low birthweight when malaria and malnutrition were present, but not a synergistic effect; one in three pregnant women had malaria and/or poor nutition [63].

Concerns that iron supplementation might increase the risk of malaria in pregnant women appear to be unfounded. A recent systematic review of this interaction identified mostly observational studies, in which antenatal iron supplementation was associated with a temporal increase in *P. vivax* infections without a clear effect on *P. falciparum* risk.[64] However, the results of two randomized clinical trials of iron supplementation in pregnancy have been reassuring: in both studies, 60 mg of daily elemental iron did not increase malaria risk and was associated with increased birthweight in Kenya,[65] and with improved haemoglobin in Tanzania.[66]

Pathogenesis of malaria in pregnancy

The increased susceptibility of pregnant women to malaria is due, at least in part, to the sequestration of malaria-infected erythrocytes (IEs) in the maternal blood spaces of the placenta. Placental sequestration is common in *P. falciparum*, and placental changes have been seen in some, but not all, studies of *P. vivax*.[67-69] Neither a direct link between placental changes and adverse birth outcomes nor placental sequestration of IEs have been clearly demonstrated in *P. vivax*. Placental changes in other species are not reported.

In *P. falciparum*, sequestration is mediated by the parasite-derived protein VAR2CSA, expressed on the IE surface, binding to placental chondroitin sulphate A (CSA) on syndecan-1.[70] This was elegantly demonstrated with a placental perfusion model [71] in which perfusion of malaria-infected cells into uninfected placentas resulted in binding of VAR2CSA-expressing parasite lines, but not of other lines. Such a binding phenotype has been observed

even among parasites collected in first trimester, suggesting that circulating IEs express VAR2CSA and preferentially bind CSA from early in gestation.[72,73] VAR2CSA is composed of multiple DBL (Duffy binding-like) domains and interdomain (ID) regions, among which DBL 2, 3, 5, and 6 have been associated with binding to CSA (reviewed in[74]). The length and sulphation of the CSA chain are critical,[75] which may be relevant to development of strategies to block sequestration.

Placental sequestration can lead to placental inflammatory responses, most notably accumulation of monocytes in the placenta. This is especially likely in first-time mothers, who often experience high density infections. Placental inflammation has been associated with fetal growth restriction and maternal anaemia in African women,[2] and with low birthweight in Papua New Guinea.[39] Modelling studies suggest that, in the absence of IPTp, placental inflammation may begin in early pregnancy and persist for months, and that 63% of placental infections may have been initiated by the end of the first trimester.[59] However, longitudinal genotyping of *P. falciparum* isolates during gestation in Cameroonian pregnant women showed that 77% of placental parasites were acquired ≥30 weeks in pregnancy,[76] suggesting that data is still needed to confirm the model.

Severe malaria is an infrequent but definite contributor to maternal mortality in endemic areas of Africa.[77] In maternal autopsy studies, massive sequestration of IE has been observed in organs including both the brain and the placenta.[78] The simultaneous sequestration of IE in cerebral capillaries and the placenta in these fatal cases suggests that infections during pregnancy may consist of multiple parasite populations with diverse binding phenotypes.[79-81]

P. falciparum infection in early pregnancy may interfere with trophoblast invasion into the uterus. Using an in vitro model, plasma from malaria-infected women inhibited trophoblast migration.[82] Effects on the development of the maternal placental circulation may contribute to reduced fetal growth rates reported from early to mid gestation, discussed above.[16,17]

Placental malaria can also affect development of the fetal circulation, possibly through activation of the complement cascade, leading to disordered placental angiogenesis.[83] In mice, complement activation led to underdevelopment of the villous vascular tree.[83] In humans, malaria leads to small placental size, which may be due to similar impairments of placental angiogenesis affecting villous growth and development.[84]

Malaria-associated placental inflammation has also been associated with disorders of amino acid and glucose transport[85,86] and dysregulation of the insulin-like growth hormone axis,[87] probably driven by increased cytokine release into the intervillous space.[2] Inflammatory cytokines can alter the expression and distribution of nutrient transporter molecules and growth hormones, and it is likely that disordered placental physiology – rather than mechanical obstructions from IEs – drives many of these effects.

Protective antibodies against malaria in pregnancy

Protection against malaria in pregnancy, low birthweight and anaemia has been associated with development of antibodies to the surface of CSA-binding IEs,[2 88] and the dominant target of such antibodies is VAR2CSA. By ELISA, IgG against DBL1-DBL2 is associated with protection against low birthweight,[89] while depletion studies suggest antibodies that most efficiently block IE adhesion are directed against DBL2 and its flanking inter-domain (ID) regions and DBL4.[90] Antibodies that block adhesion to CSA are associated with reduced risk of placental infection and preterm delivery,[89] while antibodies that opsonise IE for clearance by phagocytic cells may protect from maternal anaemia (reviewed in [91]). The acquisition of antibodies to components of VAR2CSA may be impaired by the receipt of IPTp, which is broadly recommended in malaria-endemic Africa.[92]

Sequence diversity within VAR2CSA has, until now, undermined efforts to define immunogenic and pathogenic motifs. Two recent studies have identified reproducible

dimorphism in sequences encoding the ID1-DBL2 region from diverse parasite populations, which may differentially contribute to increase the risk of low birthweight.[93,94] Two VAR2CSA-based vaccines are currently in early-stage clinical trials (NCT02647489 and NCT02658253, clinicaltrials.gov), but it remains unclear how VAR2CSA-based vaccines may need to be adapted to address VAR2CSA sequence diversity and to offer broad protection against placental malaria.[93]

The economic burden of malaria in pregnancy

The economic burden of malaria in pregnancy can be considered to include: (i) provider costs: the cost to the health system of providing prevention and treatment services; (ii) user costs: the cost to households of accessing these services; and (iii) broader economic impacts arising from several channels such as the reduced labour productivity of women who have experienced malaria in pregnancy and the short- and long-term consequences of low birthweight and other adverse newborn outcomes.

Limited resources constrain the management of malaria in pregnancy and hinder the achievement of optimal intervention coverage. From the perspective of healthcare providers, adequate management of malaria in pregnancy absorbs significant proportions of the total available budget. For example, in 2010, provider costs associated with the management of confirmed cases of malaria in pregnancy reported by a tertiary hospital in a low-endemic area of the Brazilian Amazonas constituted 1% of the monetary value of the total activities undertaken that year. [95] This is a remarkable proportion given that pregnant women are a sub-set of the total population at risk and the study area is characterized by low-transmission.

A number of studies have estimated unit costs associated with the provision of interventions in pregnancy (Table 1). Only a few studies evaluated costs of treatment, [95-97] whereas most studies reported either actual or projected costs of preventive interventions. [97-106] These costs differed according to delivery strategy and, in the case of IPTp, the drug used.

In many countries, public healthcare providers have required financial contributions from pregnant women (also known as cost-sharing). Such contributions may exacerbate barriers to access. Even when healthcare is free, pregnant women still incur high transportation and indirect costs, which by themselves can prevent women from seeking malaria prevention and treatment. Qualitative studies have largely identified direct patient cost to be a major barrier to management of malaria in pregnancy. [107-119] In quantitative studies, removing user fees significantly improved coverage of interventions,[120] and improved IPTp delivery.[121] There are concerns that women may not use nets that are provided free-of-charge, wasting resources [122] but on the other hand cost-sharing schemes for ITNs, some of which employ loans, discourage demand and reduce access for the poorest potential users. [123,124]

A number of studies have estimated the costs incurred by pregnant women for both prevention and treatment, classifying these as direct costs (fees, drugs, ITNs transportation) or as indirect, opportunity costs [95,96,101,104,105,125] (Table 2). The highest costs, which were for treatment, were observed in a study from Brazil, arising from both its higher gross domestic product (reflected in high indirect costs) and the presence of *P. vivax* infection which, during pregnancy, requires additional interventions compared to *P. falciparum* to achieve radical cure.[95] In order to offset these costs, and thereby improve coverage with ITNs in pregnant women, a number of strategies have been implemented. Vouchers have been distributed to incentivise ITN purchase and use. [105,126] Social marketing increased supply of nets as well coverage.[127] From the provider perspective, social marketing was found to be more costly than procuring and selling nets in the private retail market, but also as costly as distributing free of charge to the community.[104] Another strategy to increase IPTp and ITN coverage consists of delivering the interventions through community approaches.[101,128,129] Such community interventions allow coverage to be increased by shifting delivery costs from women (transport and time to seek IPTp) to the provider. For example, when women were

promised a box of personal medical supplies at the delivery facility, this increased adherence to IPTp and rates of institutional delivery by offsetting delivery costs. A complementary community-directed intervention in which volunteers delivered ITNs and IPTp as well as basic counseling services to pregnant women was effective and the additional costs generated were smaller compared to larger health campaigns.[129]

Although the evidence base on the economic burden of malaria in pregnancy has expanded in recent years, there are still three main gaps to fill: (i) very little is known about the economic burden outside sub-Saharan Africa, where different *Plasmodium* species co-exist; (ii) the impact of malaria in pregnancy on indicators of economic development has never been investigated: for example, it is unknown how malaria in pregnancy impacts women's employment and wages; and (iii) costs and consequences associated with malaria in pregnancy are underexplored: only a few studies have estimated, for example, cost associated with low birthweight in malaria endemic countries.[130,131] Additional knowledge on (i), (ii) and (iii) may, respectively: guide decisions on optimal allocation of resources in areas where malaria control tools and strategies depend on *Plasmodium* species and where conditions to achieve elimination exist; inform economic growth, development and women's empowerment strategies; further promote investments towards improvement of prevention and treatment of malaria in pregnancy.

Malaria in pregnancy and the elimination agenda

Variations in malaria transmission, whether geographic or temporal, result in variable exposure to infection, affecting the course of disease.[132,133] Where malaria transmission has declined substantially, increases in parasite density[134,135] and in malaria-related adverse impacts have been observed[134,135]. Taken together, these data suggest that measures that reduce malaria transmission, and thus reduce opportunities to acquire immunity, may lead to

a change in both the burden and clinical spectrum of disease. For this reason, it becomes important to estimate changing trends[134-136] and potential rises in the frequency of severe malaria syndromes,[135] especially in areas with a high prevalence of HIV, which can impair the maintenance of effective immune responses. For malaria control programs, these rapid changes in immunity and susceptibility necessitate ongoing monitoring of the burden of malaria disease in at-risk populations, including pregnant women. This is especially critical in areas embarking on malaria elimination, as understanding the determinants and clinical consequences of malaria declines and resurgences, as well as the time-scales over which antimalarial immunity is gained and lost, has become a priority to prevent reinfection and resurgence of malaria infections.[137]

Antenatal malaria infections may undermine elimination efforts that rely on mass administration of ACTs. Trials based on mass drug administration with an antimalarial such as dihydroartemisinin-piperaquine (DHA-PPQ) are in progress in several African countries (ClinicalTrials.gov Identifier: NCT02329301). ACTs are yet to be approved by WHO for treatment of malaria in the first trimester of pregnancy, and there may be concerns over the use of DHA-PQ, in mass drug administration campaigns that could result in women in the first trimester receiving the drug who are not infected or at risk. Women in the first trimester need to be excluded from mass drug administration programs and instead managed according to a malaria RDT result. Given the low sensitivity of malaria RDTs,[55] that IPTp with SP is contraindicated in first trimester, and that pregnant women frequently do not use ITNs[59] and often carry gametocytes in their blood,[138] there is a risk that undetected and untreated infections in this group can constitute a reservoir of malaria with the potential to sustain transmission during malaria elimination efforts. Recent modeling analysis suggests that pregnant women might constitute up to 23.9% of all infectious individuals following MDA to 90% of the non-pregnant population[139]. Further studies are needed to understand the impact

of omitting pregnant women in MDA campaigns. Use of antimalarials that are known to be safe during pregnancy,such as chloroquine, may be an alternative in areas where the parasite population has regained susceptibility to the drug.[140] Development of new anti-malarials for radical cure of *P. vivax* which are safe in pregnancy are also needed. In absence of these antimalarial alternatives, monitoring pregnant women in areas where malaria elimination activities are conducted can inform the risk of unsuccessful interruption of transmission due to undetected foci of malaria among pregnant women.

Pregnant women might be used as a sentinel population to monitor malaria transmission with sufficient sensitivity to guide malaria elimination activities. Compared to other target populations (e.g. children), pregnant women are relatively easy to access because of their high rate of attendance to antenatal clinics in malaria endemic countries.[141] Parasite prevalence and seroreactivity to parasite antigens at delivery correlate with measures of overall *P. falciparum* transmission,[135] and parasite prevalence in pregnant women correlates with that in children in Africa.[142] In Southern Mozambique, malaria hospital admissions followed similar trends to malaria prevalence among pregnant women,[135] suggesting that detection of malaria parasites with highly sensitive diagnostic tests such as PCR in pregnant women at their first antenatal visit may be useful to assess malaria trends in the underlying community. In addition to the value of surveillance in pregnant women as a surrogate for parasite transmission in general populations,[103] the distribution of molecular markers of parasite drug resistance in parasites collected from untreated African pregnant women closely resembled that observed in children or non-pregnant adults.[143]

Immunologically, sera collected from pregnant women may be used as a proxy for population-level exposure to *P. falciparum*. As transmission fell in Southern Mozambique, antimalarial IgG responses against VAR2CSA declined.[135] Antibodies against VAR2CSA develop after exposure to placental parasites in a parity-dependent manner, increasing over

successive pregnancies. They are affected by variables that influence the risk of exposure to *P. falciparum* such as season, household location,[144] use of IPTp[92,145] or ITNs.[146] Measures of antibody during pregnancy integrate malaria exposure over time (a pregnancy) in which cumulative exposure to VAR2CSA can increase from zero to very high levels. However, the longevity of antibody responses againt VAR2CSA as well as the impact of *var2csa* diversity needs to be evaluated to assess the utility of such a serological approach. The use of pregnant women as a sentinel group for malaria transmission, and the use of antibodies against VAR2CSA as markers of cumulative exposure to *P. falciparum* during pregnancy[147], although still needing further validation, may constitute a feasible alternative to generate sensitive metrics of malaria transmission intensity for malaria elimination.[137]

Conclusion

The global burden of malaria in pregnancy remains substantial. In sub-Saharan Africa, access to recommended ITNs and IPTp with SP and adherence to recommended policies remain poor, and there is an urgent need to identify programmatic bottlenecks and gaps for optimal coverage of preventive tools. Globally, there are challenges in the prompt diagnosis and treatment of malaria infections with both *P. vivax* and *P. falciparum*. Some of these challenges include the low sensitivity of diagnostic tests and restrictions on anti-malarial agents to be used for treatment, prevention and elimination in pregnant women, such as anti-relapse therapy for *P. vivax*. The identification of VAR2CSA as a major target of protective immunity against *P. falciparum* malaria in pregnancy has led to efforts to develop a pregnancy-specific malaria vaccine, but understanding of the importance of different genetic variants of this protein is still very limited. Studies are uncovering the relationship between the longevity of antimalarial immune responses, and the impact of waning immunity on malaria-related adverse outcomes in settings of declining malaria transmission. Such studies

can help to tailor existing diagnostic and preventive tools to different transmission settings, and to guide appropriate clinical practice in situations of changing malaria transmission. Not only this, a deeper understanding of the biology of malaria infection in pregnancy will contribute to the development of new approaches for the successful implementation of malaria elimination strategies in order to achieve a malaria free world. A proposed future research agenda is outlined in Panel 3.

Author Contributions

SJR coordinated the manuscript scope and design. All other authors contributed equally to the manuscript. All authors drafted individual sections of the manuscript, and revised the final version critically for content. All authors read and approved the final version of the manuscript.

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Conflict of interest:

We declare that we have no conflicts of interest.

Table 1: Provider costs of malaria in pregnancy prevention and treatment, US$

Country	**Uganda**	**Tanzania**	**Sub-Saharan Africa**	**DRC**	**Mozambique**	**Burkina Faso**	**Uganda**	**Tanzania**	**DRC**
Reference	Mbonye et al, 2008	Mulligan et al, 2008	Chico et al, 2008	Becker-Dreps et al, 2009	Sicuri et al, 2010	De Allegri et al, 2010	Hansen et al, 2012	Renggli et al, 2013	Matangila et al, 2014
Year in which US$ are expressed	2005	2006	2008	2005	2007	2006/7	2004/5	2010	2013
Outpatient (US$)					4.87		0.7		
Admission per night (US$)					40.73				
ITN - delivery approach		voucher scheme		anc		social marketing or free distribution	anc	campaign	
ITN - cost (US$)		financial cost=7.66/ITN delivered; economic cost =7.57/ITN delivered		8 (donated)		financial cost, (social marketing) =8.08/ITN distributed; (free distribution)=7.21/ITN. Average economic cost 4.81 for both strategies	(impregnation and distribution) 1.71	5.30 (financial)	
IPTp or chemoprophylaxis-delivery approach	anc or community		azithromycin-chloroquine administered 2/3 times				anc		
IPTp or chemoprophylaxis (US$)	at the anc/woman = 1.36; community/ woman=1.57		14 - 21/pregnancy				0.79 (2 dose)		anc
Other (US$)							(ITN+IPTpSP)= 2.48		One thick smear = 2.62; One RDT = 1.16

Notes: anc=antenatal clinic; ITN=insecticide-treated net; IPTp=intermittent preventive treatment of malaria in pregnancy; RDT=rapid diagnostic test; In Chico et al, 2008 drug costs only retrieved from the International Drug Price; In Botto-Menezes, 2016 outpatient cost for Pvivax and Pfalciparum are different as for Pvivax outpatient care; In Fernandes et al, IPTp costs were assumed equal to 25% of total ANC costs.

Table 2: User's cost of malaria in pregnancy prevention and treatment

Country	**Uganda**	**Tanzania**	**Mozambique**	**Burkina Faso**	**Brazil**	**Tanzania**
Reference	Mbonye et al, 2008	Mulligan et al, 2008	Sicuri et al, 2010	De Allegri et al, 2010	Botto-Menezes et al, 2016	Sicuri et al, 2015
Year in which US$ are expressed	2005	2006	2007	2006/7	2011	2012
Outpatient (US$)			average direct=0.61; average indirect=1.49		total=45.91; indirect=approx. 50%	Total=6.20; indirect=4.46
Inpatient (US$)			average direct=5.10; average indirect=5.01		total=216.29; indirect=approx. 50%	Total=35.35; indirect=8.45
ITN delivery approach		voucher		social marketing		
ITN (US$)		0.83		0.75		
IPTp or chemoprophylaxis-delivery	anc; community					
IPTp or chemoprophylaxis-cost (US$)	transportation= 0.98; time=1					

Notes: anc=antenatal clinic; all costs are expressed as median apart from Sicuri et al 2010 (mean values); in all studies total costs = direct + indirect, thus when total and indirect are reported, indirect are included in total.

Panel 1. Search strategy and selection criteria

We examined literature on malaria in pregnancy published over the last 10 years as cumulated in the malaria in pregnancy library (MiPL; http://library.mip-consortium.org/). The keywords “Ultrasound” and “Doppler” were used to screen on publications on fetal growth and malaria. Using the terms stillbirths, perinatal death, and neonatal death, and infant we screened on articles on impact on the infant. For subpatent malaria in pregnancy, we screened on the key words polymerase chain reaction or submicroscopic or subpatent up to May 2016, and articles which presented data in a format which could be extracted and combined were included for Figure S1 and Figure S2. For diagnosis, we searched the MiPL and Pubmed using keywords “malaria”, “pregnancy,” and “diagnosis.” For pathophysiology, we searched the MiPL and Pubmed using keywords “placenta” and “plasmodium” or “malaria” For elimination, we searched publications using keywords “elimination”, “trends” with “malaria ” and “pregnancy”. For the economic burden we searched in PubMed using (malaria[Title/Abstract] AND (pregnan*[Title/Abstract] OR gestat*[Title/Abstract]) AND cost*[Title/Abstract]). Original search on 03/08/2016. Two additional articles were taken from EconLit data base with no specific search criteria but with the aim of facilitating the interpretation of articles extracted from PubMed.

Panel 2. Progress in the last 10 years for burden, pathogenesis and economics of malaria in pregnancy (previous table 1)

Burden of malaria in pregnancy

- Declining prevalence of *P. falciparum* in parts of Africa
- Low prevalence of *P. vivax* in Asia and Latin America
- Modelling to demonstrate the high burden in Africa of low birthweight attributable to malaria
- Established importance of malaria in the first trimester
- Evidence generated indicating impact of malaria in pregnancy on infant health

Diagnosis

- Subpatent antenatal infections are common but their impacts on pregnancy outcomes remain unclear.
- Current rapid diagnostic tests lack sensitivity for diagnosis of placental malaria.

Malaria and nutrition

- In clinical trials, iron supplementation does not increase the risk of malaria in pregnancy
- Both malaria and poor nutrition associated with low birth weight

Malaria pathogenesis

- A vaccine based on VAR2CSA has entered phase I clinical trials.
- Placental malaria can interfere with placental development and function

Economics

- Malaria in pregnancy absorbs significant proportions of the total available healthcare budget
- Direct and indirect costs incurred by pregnant women for malaria prevention and treatment are high.
- Strategies to lower costs incurred (e.g., vouchers, social marketing and delivery through community approaches) are effective, but they need to be scaled up

Changing transmission

- Decreasing exposure to malaria parasites is associated with exacerbated impacts of malaria in infected women

Panel 3: Knowledge gaps and research priorities (Previous table 4)

- Are there long term consequences of malaria in pregnancy for offspring health?
- How do co-morbidities such as helminthiasis and HIV, and treatments such as ivermectin or ARVs, modify the effects of malaria in pregnancy?
- What are the timescales over which antimalarial immunity is gained and lost in situations of transitioning malaria epidemiology?
- What proportion of miscarriages and stillbirths are attributable to malaria in pregnancy in different epidemiological settings?
- Do subpatent antenatal malaria infections contribute to adverse pregnancy outcomes?
- Will deployment of more sensitive, next generation rapid diagnostic tests improve the detection of malaria in pregnancy including placental infections?
- Could combinations of malaria and nutrition interventions result in improved birth weights and decreased maternal anaemia?
- How does malaria in pregnancy affect the development of immunity in the fetus and infant?
- Can vaccines based on VAR2CSA or other antigens prevent malaria in pregnancy and its adverse consequences for offspring?
- Are antenatal women suitable as a sentinel population to monitor malaria transmission, control or elimination in the general population?
- Are parasite prevalence and antibody serology useful monitoring tools?
- How can pregnant women be effectively and safely integrated into malaria elimination efforts?
- How can antenatal malaria prevention be effectively extended to protect women in the first trimester?
- What is the economic burden of malaria in pregnancy in areas with co-existing Plasmodium species?
- How does malaria in pregnancy affect indicators of economic development?
- What are the costs and consequences associated with low birth weight due to malaria?

  -

Figure 1: Effect of malaria in pregnancy on maternal, newborn, infant and child health

Figure S1 Prevalence of submicroscopic malaria in pregnancy in 20 studies with information available in 15 countries, 1995-2013

* This study only included HIV-infected pregnant women

Note that studies are ordered by prevalence of malaria by microscopy.

Figure S2: The association between microscopic or submicroscopic malaria and anaemia in 6 studies with information available in 5 African countries, 1995-2013

*This study only included HIV-infected pregnant women

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