**Author’s response to reviews**

**Title:** Pregnancy-specific malarial immunity and risk of malaria in pregnancy and adverse birth outcomes: a systematic review

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**Author’s response to reviews:**

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Dr Diana Samuel

Associate Editor

BMC Medicine
Dear Dr Samuel,

We would like to submit our revised manuscript entitled “Pregnancy-specific malarial immunity and risk of malaria in pregnancy and adverse birth outcomes: a systematic review” for consideration for publication in BMC Medicine. We would like to thank the reviewers for their considered comments and suggestions. We have responded to the editorial comments and each of the reviewers’ comments in turn, as outlined below. We are confident that having made the changes suggested by the three reviewers, we have improved the manuscript considerably.

Best regards,

Julia Cutts

Editorial comments:

E1

Please add the heading 'Conclusions' to the main text.

Heading has now been added at line 565.

We have also added an additional summary sentence at lines 566-568:

“Overall, this systematic review found that pregnancy-specific P. falciparum antibody responses likely serve as markers of exposure to malaria in pregnancy, rather than correlates of protection.”

E2 Please define all abbreviations inline the first time they are used, and delete the list of abbreviations.

We have now defined all abbreviations in the text and deleted the list of abbreviations.

E3

Please ensure all figures in additional file 4 are cited. Currently, figures 3, 5, 11-13, 16, 16, 18-22 are not cited.

We have now included citations for all figures. Please note that the number and order of figures has now changed, as several figures have been moved to Additional file 7 in response to reviewer comments. In some cases related forest plots have been combined into parts A and B of a single figures. This was done to clarify the presentation of data following the incorporation of additional studies following reviewer comments.
Only 14 studies contributed estimates in a format enabling inclusion in meta-analysis and 12 were included in narrative form only. May this relatively low number of studies have limited the meta-analysis of associations between antibodies and infant outcomes? Authors argue that "Randomized controlled trials of interventions against P. falciparum malaria and vaccine efficacy trials were excluded because rigorous inclusion and exclusion criteria are applied in these studies, such that the participants are, typically, not representative of the general population". It would be worth to explain what do authors mean by "general population" and how authors consider if the women included in a study are representative of that general population.

Some of the 14 studies included in the systematic review either applied strong selection criteria (even more stringent than those applied in clinical trials) or resulted from clinical trials. For example:

- Babakhanyan et al: When referring to the third set of plasma samples included in the study, "all samples from placental malaria positive women and randomly selected samples from PM− women were selected from women who had ≥3 pregnancies, ≥20 years of age, and had term or premature deliveries. All PM− women had been exposed to malaria since they had Ab to DBL5".

- Fowkes et al and Mc Lean et al: women participated in a placebo randomized controlled trial of chloroquine prophylaxis against P. vivax infection during pregnancy

- Guitard et al: "Women pregnant for less than 6 months were enrolled if they were not infected with malaria parasites at that time, declared not to have had malaria since being pregnant, and were likely to be exposed to infective mosquito bites during their pregnancy".

- Teo et al: "In the latter period, women were randomized to receive 3 courses of intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) and azithromycin (AZ) during pregnancy or to receive a single course of SP and chloroquine (CQ)"
Staalose et al (2004): "Sample selection was done on the basis of HIV-1 status, gravidity (primigravid, 2-3 gravid, or >3 gravid), and placental histology (no malaria infection, acute infection, chronic infection, or past infection). We aimed to investigate 25 samples or more from HIV-1-negative women in all 12 categories of gravidity and histology, but could not always do so".

Staalose et al (2001): Authors included 4 groups of pregnant women from these 2 sites. The first comprised 33 primigravid women; the second group consisted of 45 multigravid women; group 3 comprised 36 primigravid women and the final group comprised 55 primigravid and 58 multigravid women.

Also, any study conducted at antenatal clinics or maternity wards (as is the case of most studies reported) has inherent biases with respect to the general community, as the use of ANC clinics may be linked to demographic characteristics (e.g. place of residence, educational level, socioeconomic status or parity) that could also be related to malaria risk (women who do not attend antenatal clinics are often more rural, less literate, and older than women who utilize antenatal clinics).

Overall, inclusion of women participating in clinical trials might not introduce a bias greater than the biases inherent to the 14 papers included in the systematic review and would rather increase the breadth of study designs and antigens examined to be able to pool estimates for the association between a specific antibody response and clinical outcome across different populations, as well as to assess the effect of transmission intensity. I think authors could consider this possibility and provide more clear arguments to assess if the study can be used as representative or not of the general population.

We have closely considered comments by reviewer #1 and reviewer #2 in relation to the exclusion of randomized controlled trials and accept that there are valid reasons for including such studies in the review. We have now adjusted the inclusion criteria to include randomized controlled trials. We had originally considered including these studies and had contacted some of the relevant authors some time ago. One of these studies (Aitken et al 2010) contributed estimates in the required format for inclusion in meta-analysis. We have now contacted the corresponding author of an additional RCT (Cox et al 2005) who was able to provide data for inclusion in our review. Five additional RCTs are now included in narrative terms because data were not available in the required format in the published manuscript and the authors were unable to respond to our request to provide the summary data.
1.2 Several articles have already shown the increase of pregnancy-specific antibodies in women with placental infection and would be worth to mention them in the discussion.

We have added the following to the discussion (lines 457-460):

“Cross-sectional estimates for the association between antibody responses and P. falciparum infections during pregnancy suggest that antibody responses may serve as markers of current infections. Indeed, previous studies have reported a concurrent increase in pregnancy-specific antibodies in women with placental infection [54, 62, 72, 73].”

(References are Beeson et al 2004, Stalaloe et al 2004, Mayor et al 2011, Mayor et al 2013)

1.3 Line 281: "IgGs against" or "antibody responses" is missing in the sentence.

We have corrected this omission as follows, now at line 313-315:

“We included estimates from nine studies that investigated the association between antibodies (Abs) to pregnancy-specific pRBC or VAR2CSA domains measured at delivery, and placental infection (Figure 2A)”

1.4 Line 294: Check reference 67, as it is a review and does not provide the OR mentioned in that line of the discussion

The correct reference has now been inserted at line 327: (Duffy and Fried, Infection and Immunity, 2003)

1.5 "The association of antibodies to pregnancy-specific pRBC, measured at delivery, and low birthweight was examined in three studies (Figure 6) [51, 57, 58]”. Why only two of these 3 studies were included in Figure 6?

The three studies included two that measured total IgG to pRBC (first two estimates on plot) and one that measured CSA adhesion inhibitory antibodies (third estimate on plot) to pRBC. We agree that this is unclear, so we have reworded as follows (line 409-412):

The association of antibodies to pregnancy-specific pRBC, measured at delivery, and low birthweight was examined in three studies (Figure 4A and Supplementary Figures 8A and 9A, Additional file 7): two studies measured total IgG antibodies [62, 63] and one study measured CSA-adhesion inhibitory antibodies [56].

We have also amended the subtitle within the figure to read “Functional responses to pRBC” to clarify.
Pooled analyses indicated that compared to non-responders, IgG responders to CSA-binding pRBC, measured at delivery, had a 26% reduction in the odds of low birthweight delivery (pooled OR using random effects (reOR)=0.74, 95% CI 0.51-1.06) (Figure 6) [57, 58]. Do authors consider this as statistically significant? If not, the sentence may need some change, including the sentence in the abstract ("Antibodies to pregnancy-specific pRBC, but not recombinant VAR2CSA antigens, were associated with protection from low birthweight").

We have amended the text at lines 412-416 as follows, noting the wide confidence interval that captures a scenario of a small increase in odds of low birthweight:

"Pooled analyses indicated that compared to non-responders, IgG responders to CSA-binding pRBC, measured at delivery, had a clinically significant 26% reduction in the odds of low birthweight delivery (reOR=0.74, 95% CI 0.51-1.06, I2=0.0%), but the confidence interval was wide and captured a scenario of a small increase in odds of low birthweight (Figure 4A)"

We have avoided using the term ‘statistically significant’ as this is now not recommended:


We have also amended the abstract at lines 50-52 as follows:

"Antibody responses to pregnancy-specific pRBC, but not recombinant VAR2CSA antigens, were associated with trends towards protection from low birthweight.”

1.7 Reference 55 is incomplete

The reference title has been amended (now reference 60)

Reviewer #2: Dianne Taylor

2.1 Put a summary at the end of each Results section. For example, I got to Line 316 and thought "So? What am I supposed to think?” My brain was begging for a summary or some indication about the bottom-line message. The same was true for the section on peripheral parasitemia and the other sections as well. When several studies that found Ab were beneficial are mentioned, but a lot of studies showed the Ab were not, are we supposed to dismiss the first two studies or focus on them?

We have now added summary sentences to the end of each results sections, at lines 360-367; 401-406; 442-444, for placental infection, peripheral infection, and low birthweight, respectively and in Additional file 7 for anaemia, severe malaria and preterm birth, which have now been moved out of the main manuscript.
2.2 Discuss the data for Ab presence/absence measured at the time of delivery. Then, present a section for the influence of Ab measured during the first and second trimesters on PM and peripheral malaria at delivery. To me, these are two different topics and combining them made it difficult to sort out the information. The presence of Ab at delivery is associated with an increased risk of PM, which makes sense because women with PM are more likely to make Ab to VAR2CSA. However, when Ab are present early in pregnancy (e.g., T2), the question becomes, are the Ab effective in clearing PM by the end of pregnancy? So, the two types of studies, seem to address two questions. Thus, I found combining the data to be confusing.

FYI (comment only - Lines 321-324): In the study by Tutterrow et al. all women in the study were diagnosed as having malaria early in the second trimester and then followed to term. Since the study was conducted before implementation of IPTp, the primary question was, did Ab to VAR2CSA help clear placental parasites during the second half of pregnancy? Ndam et al. also measured Ab at enrollment (<24 weeks), but then IPT-p was used, making it somewhat difficult to interpret the results, but they also finding reduced PM in Ab+ women. I'm not sure one would actually see the effect in a longitudinal study (e.g., the other two reported studies) unless transmission was high and sample size was very large.

We have now rearranged the results section so that we discuss cross-sectional estimates (e.g. for Ab presence/absence at delivery and placental malaria) first, followed by any prospective estimates (e.g. Ab presence/absence at T2 and placental malaria). We have also rearranged the forest plots so that cross-sectional estimates are presented in “A” and prospective estimates are presented in “B” for each figure, where relevant. Prior to the inclusion of RCTs (as suggested by reviewers #1 and #3), there were very few prospective estimates, so we had included this in an additional file. We feel that the inclusion of the prospective estimates in the main manuscript, directly below cross-sectional estimates facilitates comparison of estimates for different time points, and better ties in with the amended results text.

2.3 Discussion point: Was something missed by including secundigravidae with multigravidae (MG)? Duffy and Fried found Ab to VAR2CSA were associated with improved birthweight and length of pregnancy in secundigravidae (only). They may represent a "transition" group? Should they have been considered as a separate group? Likewise, Laar's group found that Ab to VAR2CSA were effective in women with history of past infections. So, in the early literature it appeared that Ab to VAR2CSA were effective in ONLY a subgroup of women. I am not suggesting a full re-analysis, but telling the reader you have taken these points into consideration (Discussion?) might strengthen the MS.
A challenge of performing systemic reviews is combining data in a consistent way. Several of the studies included in this review grouped secundigravidae and multigravidae together, precluding subgroup analysis of secundigravid women alone. In other cases, once women were stratified into secundigravidae and multigravidae, there were too few women with placental and/or peripheral malaria to enable a meaningful analysis.

We have now included a comment in the discussion (line 528-534) as follows:

“Due to the limited availability of stratified data, we only presented estimates for women subgrouped into primigravidae and secundigravidae/multigravidae groups. In some earlier studies, antibodies to pregnancy-specific P. falciparum antigens were associated with improved pregnancy outcomes in secundigravidae only [56], or in women with chronic pregnancy-associated malaria infections [62]. Therefore further studies examining antibody responses in specific parity and clinical groups may be warranted.”

2.4 Trivial point - Line 72: How solid are the data that malaria increases miscarriages? These often occur in the first trimester, before malaria parasites sequester there. I know the literature about reduced blood vessel formation, but ……. 

We have added two references to line 73-74 (McGready et al 2012 Lancet Infectious Diseases and Moore et al 2016 Lancet Infectious Diseases) to provide supporting evidence for the deleterious association between malaria infection and miscarriage. These studies examined antenatal records for women attending Shoklo Malaria Research Unit antenatal clinics on the Thai Myanmar border and found that odds of miscarriage increased in women with asymptomatic malaria.

2.5 Line 109 - or elsewhere: Please define "severe malaria". I'm not sure what the term includes.

We have now included in the footnotes of Table 1 the definition of severe malaria according to the one study that examined it as an outcome in this review, Chandrasiri et al 2014:

“jPregnant women who were blood film positive and diagnosed with one or more of the following clinical manifestations were categorized as having SM (n=39): severe anaemia, haemoglobin levels &lt;7 g/dL; cerebral malaria, unrousable coma; hypoglycaemia, blood glucose levels &lt;40 mg/dL; hypotension, systolic blood pressure &lt;90 mmHg; jaundice, physical diagnosis or bilirubin levels &gt;3 mg/dL and hyperparasitaemia, &gt;10,000 parasites/µL. Pregnant women who were parasitaemic without these features were defined as uncomplicated malaria (UM; n=41). An additional 41 pregnant women with negative blood films and no signs of clinical malaria were enrolled as uninfected controls.”

2.6 Line 148 - Do you want to include CS2 as a source of VAR2CSA-expressing cells?

We have amended the text to explicitly include CS2 (lines 149-152):
“We included studies that measured immunoglobulin G (IgG) responses to placental isolates, pregnancy-specific parasite strains including CS2, and other strains that had been selected for binding to chondroitin sulfate/chondroitin sulfate proteoglycans, and recombinant or synthetic defined pregnancy-specific variant surface antigens.”

2.7 Line 197: As an American, I am not sure what a performa is. Is it a data-entry template?
The only way I have heard the word used refers to an invoice for a commercial company.

We have replaced “proforma” with “data extraction form” and have now included the data extraction form as an Additional file (line 202):

“Contact was established through an initial email explaining the nature of the systematic review and the information required, together with a data extraction form for authors to complete (Additional file 3).”

2.8 Line 235 (and Abstract): "…referred in narrative terms only ,…” I'm not sure what this means. Does it mean that the data were extracted from the original publication? Please explain/define.

We have now added an explanation of this term in line 252-255:

“If antibody or outcome data could not be provided in categorical form, the study’s key findings on the association between antibody responses and outcomes of interest were described in table 2 and in the text, that is, the study was included in narrative terms rather than quantitative terms.”

2.9 Line 243: The abbreviation FV2 appears without explanation. FV2 does not appear in the list of abbreviations. I assume anyone who is reading the manuscript will know it refers to full-length VAR2CSA, but ….. .

The abbreviation has now been defined in line 262:

“Separate estimates were obtained for the OR/RR associated with pRBC VSA, VAR2CSA: DBL1, DBL1+2, ID1-ID2, ID1-ID2a, DBL2, DBL3X, DBL3-4, DBL4, DBL5, and full length VAR2CSA (FV2)”

2.10 Line 267: data were (I said some of the comments were trivial).

Corrected as advised, now line 295.

2.11 Line 284-286: LOVE the summary. A similar statement for the other result sections would be useful for clarify.

We have now added additional summary statements (see response to comment 2.1).
2.12 Line 341: Is the conclusion that Ab to VAR2SA do not appear to reduce prevalence or level of peripheral parasitemia … i) at delivery or ii) at the time the blood was drawn?

We have added a summary at line 404 to clarify:

“Thus, antibodies to VAR2CSA either early in pregnancy or at delivery do not appear to reduce the incidence or level of peripheral parasitaemia throughout pregnancy.”

2.13 Line 355: "… T2/T3 had reduced odds of severe malaria (versus uninfected controls) …" Does this make sense? Uninfected controls don't have malaria. Maybe part of the problem is not being sure how severe malaria was defined.

We have now clarified the text (now in Additional file 7) as follows to provide an explanation of the study design:

A single Sudanese case-control study examined antibody responses in three groups of pregnant women: those with severe malaria, those with uncomplicated malaria, and uninfected controls. Women who were positive for total IgG or opsonic phagocytosis Abs to CS2 pRBC at T2/T3 had reduced odds of severe malaria, when uninfected controls were the reference group, compared to women who were negative for these antibodies (Supplementary Figure 2, Additional file 7); but this association was significant for phagocytic antibodies only [13]. In contrast, DBL5 responders had a non-significant increase in odds of severe malaria compared to non-responders, when uninfected controls were the reference group (Supplementary Figure 2, Additional file 7).

The definition of severe malaria according to Chandrasiri et al has now been provided in the footnote to table 1, as it is too long to include in text:

“jPregnant women who were blood film positive and diagnosed with one or more of the following clinical manifestations were categorized as having SM (n=39): severe anaemia, haemoglobin levels &lt;7 g/dL; cerebral malaria, unrousable coma; hypoglycaemia, blood glucose levels &lt;40 mg/dL; hypotension, systolic blood pressure &lt;90 mmHg; jaundice, physical diagnosis or bilirubin levels &gt;3 mg/dL and hyperparasitaemia, &gt;10,000 parasites/µL. Pregnant women who were parasitaemic without these features were defined as uncomplicated malaria (UM; n=41). An additional 41 pregnant women with negative blood films and no signs of clinical malaria were enrolled as uninfected controls. Estimates included in this review used uninfected women as the comparator group.”

2.14 Line 369: "… because only one primigravid women was positive for ……." Is this worth mentioning? Maybe the wording could be changed, because it sounds like the authors reported a conclusion based on only 1 person. Is that what you meant?

We have reworded this as follows from line 418-422 to clarify:
“This association was also observed in sub-group analysis of secundigravidae/multigravidae (Supplementary Figure 9A, Additional file 7) but not among primigravidae (Supplementary Figure 8A, Additional file 7), most likely because an insufficient number of women had acquired anti-adhesion activity in their first pregnancy in this study population.”

We were trying to explain why the association was significant in sub-group analysis of secundigravidae/multigravidae, but not primigravidae. It is likely because women acquire these functional antibodies with subsequent pregnancies, and so there was only one woman who had antiadhesion activity among primigravidae (and who’s baby had normal BW). Therefore, there was insufficient power to demonstrate an association in this subgroup.

2.15 Line 394-395: "… Ab to ID1-ID2a had an increased odds of PTB…” This point sounds scary for the on-going vaccine trials. So, in the Discussion, you might address this concern. It is likely that the ID1-ID2a domain in the intact molecular is weakly immunogenic and presence of Ab to it may indicate a rather high parasitemia took place that induced the Ab. As far as I know, there is no evidence that Ab to ID1-ID2a actually might do harm. Just a suggestion.

We have added a comment to the discussion as follows (lines 493-495):

“Interestingly, Malian primigravidae who were positive for antibodies to ID1-ID2a at enrolment (during T2/T3) were more likely to deliver preterm babies, suggesting that this antigen may be a marker of parasite exposure.”

2.16 Figures 2 and 3: Information on % weight (from random effect analysis) is presented. What does this mean? As a non-bio-statistician, I’m not sure how to interpret the information.

We have now included an explanation of the weighting approach to the methods section (line 273-278):

“The random effects meta-analyses were weighted using the inverse of the sum of the individual study sampling variances and a between-study variance component [49]. The application of weights to individual study estimates in pooled effect estimation ensure (typically smaller) studies exhibiting higher standard error do not bias point estimates and contribute to under estimation of pooled effect confidence intervals.”

2.17 Line 444: FYI only: With respect to the two Turtterow papers, I would argue that the association with reduced placental parasitemia at delivery was also not associated with Ab to a single domain, but rather to either i) a multiple domains and variants, and ii) high avidity Ab to FV2. The Ab may not be directly involved in protection, but merely serve as a surrogate marker of the maturation of the immune response of the pregnant women to either the entire FV2 molecule or to malaria in general.
We have now included an additional comment in the discussion regarding breadth of response and antibody avidity (line 495-500) to address this point:

“It is likely that protection from placental malaria and its associated adverse pregnancy outcomes develops with the acquisition of an increasingly broad antibody response to different VAR2CSA domains and allelic variants, rather than to a single domain [70, 71]. Furthermore, high avidity antibodies and functional antibody responses are probably more important measures than simple quantification of IgG responses to recombinant proteins [71, 79].”

2.18 Final comment: Since several of our publications were mentioned, I checked the Tables for accuracy. Table 1: Lloyd et al. [55] studied 1377 women (not 137); Table 2: Siriwardhana [63] studied 1337 women not 1377; Table 2: Tutterrow et al. [66] “… PM- had higher Abs to FV2 and 7G8 than …” should be changed to “… PM- had higher Abs to FV2 (FcR3) than ……”.

These errors have now been corrected as advised and we have double checked extractions for all other papers. We would however, like to confirm with the reviewer the numbers for Siriwardhana, because tables 1 and 2 in the original paper include data from n=341 PM+ women and n=1036 PM- women (total=1377).

Reviewer #3: Anna Van Eijk

3.1 The review is long because all outcomes are spelled out: perhaps the authors can focus on a few outcomes and put the description of the others in the supplement. Maternal or placental malaria must be used as outcomes, and to confirm detection by microscopy or histology in the case of placental malaria is reported in the quality criteria. I am not sure if I would have included PCR in the quality criteria, because that brings up the issue of submicroscopic malaria, and its unclear impact on malaria in pregnancy. LBW, preterm delivery, and anaemia can have other causes than malaria. Perhaps LBW and anaemia can be used best as additional outcomes given their common reports in malaria studies, and preterm and severe malaria can be moved to the supplement.

We have now moved the results for anaemia, preterm birth and severe malaria to the supplement. We feel that this makes for a more concise and readable manuscript.

Although in the quality criteria we listed PCR as a method to confirm placental infection, in all studies that were ultimately included (and provided estimates), placental malaria was confirmed by either microscopy or histology. We have now noted this at lines 331-332:

“In all studies placental infection was confirmed by slide microscopy of placental blood or placental histology.”
3.2 Abstract

Methods: dates of last update differ throughout text: in abstract 07jun19, in methods section: "databases were searched for studies published in all years up to and including 12 July 2018", in suppl 2 pubmed search: to 07 june 2018.

Number of studies and number of participants need to be reported for each outcome presented where possible, e.g. for these sentences: "Estimates were mostly from cross-sectional data …" and "Antibody responses to pregnancy-specific pRBC and VAR2CSA antigens, measured at delivery, were associated with placental malaria and may therefore represent markers of infection, rather than correlates of protection. Antibodies to pregnancy-specific pRBC, but not recombinant VAR2CSA antigens, were associated with protection from low birthweight".

Conclusion: perhaps also add something on standardization of laboratory methods?

The correct date is 07 June 2019, we have now updated the methods section and additional file 2 accordingly.

We have now reported the number of studies for each outcome mentioned in the abstract, as well as in the main results text. We are reluctant to add the number of participants to the abstract because we are concerned that this would give the impression that an overall pooled estimate was calculated for each outcome. This was not possible because of the different antigens and study designs. As suggested below (comment 3.17), we have now included in the forest plots the number of participants for each estimate.

We have amended the conclusions section of the abstract to refer to standardization of laboratory methods:

Lines 58-67:

“Pregnancy-specific malarial antibody responses may serve as markers of exposure to placental malaria. Differences in the recombinant VAR2CSA antigens tested, the methodology employed to measure antibodies, and the epidemiological settings of included studies may partly explain the heterogeneity observed in antibody and birth outcome associations. Further prospective cohort studies using standardized laboratory methods to examine responses to a broad range of antigens in different epidemiological settings and throughout the gestational period, will be necessary to identify and prioritize pregnancy specific P. falciparum antigens to advance the development of vaccines and serosurveillance tools targeting pregnant women living in malaria endemic areas.”
3.3 Was the review registered in prospero?

This review was not registered in Prospero. The purpose of Prospero is to document post-hoc changes to the systematic review protocol. The only change we made to the inclusion criteria was to exclude randomized controlled trials due to limited quantitative data, a decision that was reversed based on feedback from reviewers #1 and #3. Subsequently, this review adheres to our original protocol.

3.4 Searching databases: how was Google scholar searched, given that it generally gives an unwieldy output of over &gt;1,000,000? It is a missed opportunity that the malaria in pregnancy database was not used (http://library.mip-consortium.org), which could have helped with assessment of completeness.

We have taken the reviewer’s advice and searched the malaria in pregnancy database and added it to the list of databases searched (line 123). This database search did not return any additional records of interest to those already identified through the other databases but was helpful for confirming completeness.

We have now removed Google scholar from the list of searched databases because its inclusion is unwarranted. Discussion with co-authors who performed the original literature reviews confirmed that Google scholar was used to identify additional studies by some of the senior authors of studies identified through other database searches, but was not used in a systematic manner due to the unwieldy number of records returned using key words outlined in methods (2,070,000). Of those records that were screened, no studies were identified that were not included in those records returned using PubMed, Web of Science, Scopus, African Index Medicus, and LILACS. We have described the use of Google scholar at lines 129-132 as follows:

“Google scholar was used to identify additional studies by senior authors of some studies identified through other database searches but was not used in a systematic manner due to the unwieldy number of records returned using key words listed above.”

3.5 "Randomized controlled trials of interventions against P. falciparum malaria and vaccine efficacy trials were excluded because rigorous inclusion and exclusion criteria are applied in these studies, such that the participants are, typically, not representative of the general population.”
Why were trials excluded? Generally, the inclusion criteria are not much different from surveys and you could also opt to only use the data from the control arm. In addition, this was not consistently maintained, given that e.g. Staalsoe 2004 was included which was part of a trial (IPTp, Salanti 2004 used women from the same study), same for Fowkes 2012 for participants from the Thai-Burmese border (CQ prophylaxis). A study like Cox 2005 could have provided useful information; you could have restricted to only using the control arm. Case-control studies can be not representative as well, given their selection criteria, and they often go for women with laboratory test results available. Some of these case-control studies may have been matched for one or more potential confounders. It would be good to indicate in the table (1 & 2) where that was the case for case-control studies, and which characteristics were matched for.

We have closely considered comments by reviewer #1 and reviewer #2 in relation to the exclusion of randomized controlled trials and accept that there are valid reasons for including such studies in the review. We have now adjusted the inclusion criteria to include randomized controlled trials. We had originally considered including these studies and had contacted some of the relevant authors some time ago. One of these studies (Aitken et al 2010) contributed estimates in the required format for inclusion in meta-analysis. We have now contacted the corresponding author of an additional RCT (Cox et al 2005) who was able to provide data for inclusion in our review. Five additional RCTs are now included in narrative terms because data were not available in the required format in the published manuscript and the authors were unable to respond to our request to provide the summary data.

We have now indicated in the footnotes to table 1 & 2 where matching was conducted in case-control studies. Only Beeson et al 2004 and Hommel et al 2010 (same original study) were matched case-control studies. As indicated by the reviewer, sometimes case-control studies simply include those women with laboratory tests available and were not necessarily designed as case control studies a priori. We have now included in the footnotes additional information about the selection of women included in all case-control studies, where available.

3.6 The inclusion criteria are extensive and have two parts: inclusion criteria and quality criteria. I tried to summarize them here:

1) Inclusion criteria: The primary criterion for study inclusion was pregnant women living in areas endemic for P. falciparum infection. All geographical locations were included. Studies that measured immunoglobulin G (IgG) responses to pregnancy-specific parasite strains or placental isolates and/or recombinant or synthetic defined pregnancy-specific variant surface antigens were considered. We also included studies that measured functional antibody responses to pregnancy-specific pRBC, including CSA binding inhibition (anti-adhesion assay), pRBC cell-agglutination, and phagocytosis. Studies in which antibodies were measured in peripheral blood taken during pregnancy and/or the immediate postpartum period were considered.
2) The minimum quality criteria for inclusion of studies were: for placental malaria, confirmation of P. falciparum placental infection by slide microscopy of placental blood, PCR, or placental histology for the examination of P. falciparum parasites; for peripheral parasitaemia, detection by slide microscopy or PCR; for low birth weight, defined as less than 2500 grams and birth weight was measured within 72 hours of birth; and for preterm birth, defined as delivery at less than 37 weeks gestation, where gestational age must have been confirmed using Crown Rump Length (CRL) from Ultrasound and Robinson's chart or date of last menstrual period (LMP). Antibody levels must have been determined in maternal peripheral blood samples preceding or at the same time as outcome measurement. Studies in which antibodies were measured in cord blood, placental blood or infant peripheral blood were excluded. Cut-offs for positive antibody responses by ELISA or other means must have been defined using unexposed (malaria-naïve) controls or men/children from the malaria-endemic area.

I tried to check if the inclusion is complete, but that is hard to do when it is not clear which studies were rejected and why; e.g. why are the following studies not appearing: Mount 2004 Lancet, Fievet 2006 Infection Genetics and Evolution, Keen 2007. I must admit I am not an immunologist, so there are probably very good reasons, but it would be nice if this is clear. Perhaps the authors can list the studies rejected and the reasons why in the supplement.

We have now included a full record of rejected studies in Additional file 5, and reasons for their rejection, including for those studies mentioned by the reviewer.

3.7 Another concern is the lack of information on potential confounders. Although timing of the assessment of the antibodies is presented in most forest plots (trimester or delivery), and plots by gravidity are available, other confounders have not been presented. Young age is one of them, and level of transmission another. The authors could have linked study site and time with information from the malaria atlas project to have an indicator of malaria transmission level in the area (P. falciparum prevalence among children age 2-10 years of age: https://map.ox.ac.uk/explorer/#/). Are there other potential confounders of the antibodies, e.g. is there an effect of medication on antibody development (quantity), on which studies may have reported? HIV-status? Use of antimalarial prevention (ITNs, IPTp)?

We have now included additional columns in tables 1 and 2 to report on potential confounders, namely: endemicity; use of IPTp; mean age. Only a minority of studies reported on HIV status of participants and ITN use, so we have not included these variables. We have updated the methods section as follows (lines 236-243):
“Basic information about each study, including enrolment years, age of women, and IPTp use was extracted from individual publications where available. P. falciparum endemicity was categorized as low, intermediate, or high using information in the published papers. If insufficient information was provided in the publication, we used the Malaria Atlas Project website (https://map.ox.ac.uk) to obtain estimates of the Plasmodium falciparum parasite rate in 2-10 year olds (globally, 2000-2017) for each study site (longitude and latitude). We then categorized the endemicity of the study sites as follows: low [<10%]; intermediate [≥10% to <50%] or high [≥50%].”

3.8 Risk of bias assessment; how was this incorporated in the review?

We have now completed a formal risk of bias assessment using the ROBINS-I tool (Additional file 4) and updated the Risk of bias section of the methods accordingly.

3.9 Standardization

The study only uses responders/no responders for the presence or absence of antibodies. Was there a specific reason that the authors choose not to use continuous data for their study, e.g. the concentration of antibodies, or is this opening another can of worms because of the lack of standardization?

We took the approach of analysing antibody data as “responders” versus “non-responders” because this enabled us to combine data from different studies that had employed different techniques to measure antibody responses. Substantial variation in the methodology employed between laboratories precludes antibody values from different labs to be combined. For example, the metric for ELISAs is absorbance, given as “optical density”, whereas the output for FACS is “mean fluorescence index”. Even for assays performed using the same technique, the output measure can vary considerably between laboratories. Therefore, to enable the synthesis of findings from different studies, antibody data was collapsed into binary categories by imposing cut-offs as defined in the original publications (e.g. mean+2SD of unexposed controls, or mean+3SD of unexposed controls).

3.10 "when the I2 statistic was ≥75% and/or the lower 95% confidence limit was between 50%-100%, the studies were not combined [47].” I could not find a guideline regarding to this method in the quoted reference. It may be good not to combine or pool the results if the number of studies were low, or if the comparability of exposures was doubtful (or from the same study); Forest plots are still an excellent way to visualize data.

We have added an additional reference at line 281 (Higgins et al 2003) which “tentatively assigns adjectives of low, moderate, and high to I2 values of 25%, 50%, and 75%”. 
3.11 Flow chart: Preferably there would be more information on why studies were not included. E.g. what were common reasons among the 81 excluded studies? Animal studies? Not the right tests etc?

For 19 studies data was not available in required format for meta-analysis or data did not meet inclusion or quality criteria. What was the reason to exclude these 6 studies; was this for quality? If 12 studies were described, there is still one study not accounted for?

Numbers flow chart and text of results don't match.

We have now included reasons why studies were not included in Additional file 5. We have corrected the inconsistencies between numbers included in the flow chart and text of results. We have also updated the numbers in the flowchart and text to reflect the screening and inclusion of randomized controlled trials, as suggested by reviewers #1 and #3.

3.12 Numbers flow chart and text of results don't match.

This has now been corrected.

3.13 Table 1 & 2: it would be nice if more information could be presented, e.g. time period or study, number of primigravidae per study, mean or median age, HIV status, (use of antimalarial prevention: not sure if this would be relevant), level of malaria transmission. Table 1: perhaps make a separate column for timing of assessment of response, so it is clearer (T1/T2/T3 or delivery). Now you have to search for it. Definition of anaemia would be good (I assume it is all <11 g/dl)?

We have now added columns for enrolment period of study, percentage primigravidae per study, mean age, endemicity, and use of IPTp. We have not included HIV status because an insufficient number of studies reported on the HIV status of participants. We have also added definitions of anaemia to table footnotes.

3.14 This sentence in the footnote is not clear: "Similarly, where categorical data for peripheral parasitaemia was available we used that, if not we reported parasitaemia as a continuous variable." Was parasite density used in that case? Does that mean that all women included had parasitemia?

We have now simplified this footnote because the reference to parasitaemia as a continuous variable does not apply to the studies included in Table 1:

dHerein we tabulate only those outcomes that met the inclusion criteria of this review and were available in categorical format (positive or negative for outcome)
The point we were trying to make was that if authors presented data in both categorical and continuous format, we reported findings for the variable in categorical format. However, this point applies to Table 2 (studies for which we could not retrieve data in the requisite 2x2 cross-tabulated format). We have now amended the relevant footnote under table 2):

aWe only tabulate those antibody responses and clinical outcomes that met the inclusion criteria of this review. In some studies, other antibody responses and outcomes were measured. Where outcomes were presented as both continuous and categorical variables in original papers (e.g. birthweight and low birthweight), we report on findings for the categorical variable.

3.15  Footnote d and I: "Women were enrolled up to T3; also measured antibodies at T3 (weeks 30-32), but these estimates are not presented in this review” and footnote i: "Time points correspond to first- (6-29 weeks) and third- (25-41 weeks) antenatal visits; antibodies were also measured at second visit (16-36 weeks) but data is not included in this review." Perhaps for studies with follow up over time, these outcomes can be graphed? Here we have real cohort studies, but the results are not fully utilized (as a comment on the conclusion of the abstract).

We do not have access to all the raw antibody data collected at these additional time points to enable outcomes to be graphed as suggested. We are also reticent to include additional estimates for these timepoints because the timepoints are overlapping, (e.g. 25-41 weeks and 16-36 weeks).

3.16  Figure 2 Forestplot

Although I understand the challenges with making them, they are a bit difficult to read with the differences in line spacing and only clear from the last columns which studies were pooled and which were not.

We have amended the spacing within the forest plots to make it clearer which studies are pooled.

3.17  It would be nice if the numbers could be added to the forestplot, so you can check where the numbers are coming from (can be done in stata). Odds ratio can be added as the effect estimate where applicable.

We have added a column to show the number of women included in each estimate. For each forest plot we have now indicated in the top row where estimates are odds ratios and risk ratios, except for Figure 3B, 6B, and 7B, which included both odds ratios and risk ratios because of the combination of study designs. This is indicated in the footnote.
3.18 Results text
"4 were cohort (two of which contributed only cross-sectional data). As far as I can see, all of them were used as cross-sectional data?"

Fried et al 2018, Guitard et al 2008, and Megnekou et al 2005 contributed longitudinal data (e.g. Ab measurement at enrolment cross-tabulated with PM at delivery), which was previously included in an additional file, but is now presented in part Figures 2B, 3B, 4B along with prospective estimates from RCTs.

3.19 Results text
Please add numbers of participants/studies for each outcome, and I2 for each pooled outcome.

We have added the number of studies for peripheral infection (which was missing). We have added the number of participants to the forest plots, but feel that including this information in the text may be too cumbersome, if we were to include it for each estimate, and misleading if we were to include it for the overall outcome (e.g. PM) because only a few estimates could be combined. We have added the I2 for each pooled outcome (lines 321, 377, 415)

3.20 The discussion covers most of my concerns so nothing to add. My main concern is (that I cannot check) completeness of inclusion.

We have now included a full list of excluded studies in Additional file 5, to enable completeness of inclusion to be determined.

Please also refer to "Response to reviewer comments" file.