

## Peer Review Information

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**Journal:** Nature Microbiology

**Manuscript Title:** Placental *Streptococcus agalactiae* DNA is associated with neonatal unit admission and fetal pro-inflammatory cytokines in term infants

**Corresponding author name(s):** D. Stephen Charnock-Jones, Gordon C. S. Smith

### Editorial Notes:

**Transferred manuscripts** This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments, rebuttal and decision letters for versions considered at Nature Microbiology.

### Reviewer Comments & Decisions:

<b>Decision Letter, initial version:</b>	
Subject:	Decision on Nature Microbiology manuscript NMICROBIOL-23061561-T
Message:	16th August 2023

\*Please ensure you delete the link to your author homepage in this e-mail if you wish to forward it to your co-authors.

Dear Gordon,

Thank you for your patience while your manuscript "Placental *Streptococcus agalactiae* causes fetal cytokine storm and neonatal sepsis" was under peer-review at Nature Microbiology. It has now been seen by 3 referees, whose expertise and comments you will find at the of this email. You will see from their comments below that while they find your work of interest, some important points are raised. We are very interested in the possibility of publishing your study in Nature Microbiology, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication. If we are satisfied with your responses, we will not send the revision back to referees.

In particular, you will see that referees #1 and #3 have some relatively minor comments that will need to be addressed with text edits and some additional statistical analyses. The rest referees' reports are clear and the remaining issues should be straightforward to address.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

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Some reduction could be achieved by focusing any introductory material and moving it to the start of your opening 'bold' paragraph, whose function is to outline the background to your work, describe in a sentence your new observations, and explain your main conclusions. The discussion should also be limited. Methods should be described in a separate section following the discussion, we do not place a word limit on Methods.

Nature Microbiology titles should give a sense of the main new findings of a manuscript, and should not contain punctuation. Please keep in mind that we strongly discourage active verbs in titles, and that they should ideally fit within 90 characters each (including spaces).

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Please include a data availability statement as a separate section after Methods but before references, under the heading "Data Availability". This section should inform readers about the availability of the data used to support the conclusions of your study. This information includes accession codes to public repositories (data banks for protein, DNA or RNA sequences, microarray, proteomics data etc...),

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Figure legends must provide a brief description of the figure and the symbols used, within 350 words, including definitions of any error bars employed in the figures.

When submitting the revised version of your manuscript, please pay close attention to our [Digital Image Integrity Guidelines](https://www.nature.com/nature-research/editorial-policies/image-integrity) and to the following points below:

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- that control panels for gels and western blots are appropriately described as loading on sample processing controls

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Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

Please include a statement before the acknowledgements naming the author to whom correspondence and requests for materials should be addressed.

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- \* ensure it complies with our format requirements for Letters as set out in our guide to authors at [www.nature.com/nmicrobiol/info/gta/](http://www.nature.com/nmicrobiol/info/gta/)

- \* state in a cover note the length of the text, methods and legends; the number of references; number and estimated final size of figures and tables

- \* resubmit electronically if possible using the link below to access your home page: [REDACTED]

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We hope to receive your revised paper within three weeks. If you cannot send it within this time, please let us know.

We look forward to hearing from you soon.

Yours sincerely,

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Reviewer Expertise:

Referee #1: neonatal and maternal health, microbiome and infection [original referee #3 at Nature]

Referee #2: maternal and neonatal health, microbiome and infection [original referee #1 at Nature]

Referee #3: biostatistics, clinical [new referee]

Reviewers Comments:

Reviewer #1 (Remarks to the Author):

The manuscript is improved with clarification on the primary outcomes, discussion of statistical power for achieving this primary outcome, and validation of GBS detection by PCR. However, in many instances the conclusions still seem exaggerated, and do not fit the data and study design.

1. The authors clarify that neonatal unit admission is the primary outcome in the response to reviewers. However, this important detail is not clear. For example, neither the current title (Placental *Streptococcus agalactiae* causes fetal cytokine storm and neonatal sepsis) nor abstract reflect this

critical aspect of the study design. A suggestion for a more appropriate title that describes the data is “Presence of Placental Streptococcus agalactiae DNA is associated with neonatal unit admission in term infants”. Using the word “causes” in the title is inaccurate and misleading, since as the authors point out in the response to reviewers that retrospective analysis can only define “associations”, and not cause and effect relationships.

2. The conclusions stated in the abstract still seem exaggerated and somewhat misleading without clearly stating in the very limited number true GBS bacteremia cases. I would suggest re-focusing the abstract to neonatal unit admissions since this is what this study was designed and powered to evaluate. Mentioning secondary outcomes in the abstract need to highlight the very limited sample size. For example, strong association with proven GBS sepsis (OR=66.7% CI=7.3-963.7) is misleading and should be reworded to state... “among the 3 cases of proven GBS neonatal sepsis, two had placental GBS detection”. Probable sepsis should state, “among the 80 cases of probable neonatal sepsis 10 had placental GBS detection”.

3. Supplementary Table 3. What were the specific parameters in the full blood count, CRP, and chest and abdominal X-ray findings that distinguish between probable and possible sepsis?

4. The different terminologies for infection certainty are not congruent, and make the paper difficult to understand. For example “proven GBS sepsis (abstract)” vs. “confirmed sepsis (line 120)”, vs. “overt GBS (Table 2)”. Since sepsis is a clinical definition, “confirmed GBS bacteremia” should be used instead? More confusing is “probable but culture negative sepsis (abstract)” and “covert GBS (table 2)”. I understand that changing the terminology to overt-covert is limited to only those cases where GBS DNA was detected in the placenta (as opposed to all neonatal unit admissions in the “probable vs. confirmed” comparison). What is especially confusing with this change in definition, is why this change in classification would not also apply to “overt” with limitation by the same criteria (GBS DNA was detected in the placenta).

5. Criteria for discovery and validation. Why were the same criteria not applied with regards to time for admission and duration of stay? Since the focus is on early onset sepsis, would relaxing the case definition for infant admissions “without time limits” still be evaluating early onset sepsis? What is the age cut-off for when “infants” are still admitted to the neonatal unit? (One week? One month? Three months?)

Reviewer #2 (Remarks to the Author):

The authors have addressed the criticisms raised and have improved the manuscript substantially with the addition of the RT-qPCR data on GBS and with better description of controls.

Reviewer #3 (Remarks to the Author):

This is a very nice study assessing the association between placental *Streptococcus agalactiae* and neonatal sepsis. Just one major comment regarding the statistical analyses and a minor comment on Figure 2:

1. Since the outcomes in these studies are fairly common (e.g., >10% occurrence) please consider using modified Poisson regression (rather than logistic) to get prevalence ratios rather than odds ratios (which are difficult to interpret when outcomes are more common).
2. Figures 2a & 2c: please put error bars on these graphs so reader can see variability.

#### Author Rebuttal to Initial comments

Reviewer #1 (Remarks to the Author):

The manuscript is improved with clarification on the primary outcomes, discussion of statistical power for achieving this primary outcome, and validation of GBS detection by PCR. However, in many instances the conclusions still seem exaggerated, and do not fit the data and study design.

1. The authors clarify that neonatal unit admission is the primary outcome in the response to reviewers. However, this important detail is not clear. For example, neither the current title (Placental *Streptococcus agalactiae* causes fetal cytokine storm and neonatal sepsis) nor abstract reflect this critical aspect of the study design. A suggestion for a more appropriate title that describes the data is "Presence of Placental *Streptococcus agalactiae* DNA is associated with neonatal unit admission in term infants". Using the word "causes" in the title is inaccurate and misleading, since as the authors point out in the response to reviewers that retrospective analysis can only define "associations", and not cause and effect relationships.

**R1. We have edited the title to remove the language of causality.**

2. The conclusions stated in the abstract still seem exaggerated and somewhat misleading without clearly stating in the very limited number true GBS bacteremia cases. I would suggest re-focusing the abstract to neonatal unit admissions since this is what this study was designed and powered to evaluate.

Mentioning secondary outcomes in the abstract need to highlight the very limited sample size. For example, strong association with proven GBS sepsis (OR=66.7% CI=7.3-963.7) is misleading and should be reworded to state... “among the 3 cases of proven GBS neonatal sepsis, two had placental GBS detection”. Probable sepsis should state, “among the 80 cases of probable neonatal sepsis 10 had placental GBS detection”.

**R2. We have added the absolute numbers of events to the abstract.**

3. Supplementary Table 3. What were the specific parameters in the full blood count, CRP, and chest and abdominal X-ray findings that distinguish between probable and possible sepsis?

**R3. We have added the laboratory and imaging findings which were used to define probable versus possible sepsis (Supplementary Table 3).**

4. The different terminologies for infection certainty are not congruent, and make the paper difficult to understand. For example “proven GBS sepsis (abstract)” vs. “confirmed sepsis (line 120)”, vs. “overt GBS (Table 2)”. Since sepsis is a clinical definition, “confirmed GBS bacteremia” should be used instead? More confusing is “probable but culture negative sepsis (abstract)” and “covert GBS (table 2)”. I understand that changing the terminology to overt-covert is limited to only those cases where GBS DNA was detected in the placenta (as opposed to all neonatal unit admissions in the “probable vs. confirmed” comparison). What is especially confusing with this change in definition, is why this change in classification would not also apply to “overt” with limitation by the same criteria (GBS DNA was detected in the placenta).

**R4. We have edited the manuscript to use the following definitions consistently:**

**Cases with positive culture of GBS in the neonatal period are now consistently referred to as “proven GBS sepsis”.**

**Cases of neonatal unit admission where there was a clinical diagnosis of sepsis (based on clinical and laboratory/imaging indicators) or histopathological evidence of intra-uterine inflammation (chorioamnionitis or funisitis) but no organism was cultured are now consistently referred to as “probable but culture negative sepsis”.**

**The terms are a bit more cumbersome but we agree with the reviewer that clarity is the priority.**

5. Criteria for discovery and validation. Why were the same criteria not applied with regards to time for admission and duration of stay? Since the focus is on early onset sepsis, would relaxing the case



definition for infant admissions “without time limits” still be evaluating early onset sepsis? What is the age cut-off for when “infants” are still admitted to the neonatal unit? (One week? One month? Three months?)

**R5. The discovery study used a pre-defined outcome of neonatal unit admission which we have employed in the first output from the POPS cohort in the Lancet in 2015. We used this outcome in the discovery study as it was already defined in our study dataset. Having observed the association with the presence of GBS DNA in the placenta we made a decision to re-analyse all of the other neonatal unit records of the infants admitted to determine the evidence supporting sepsis and to perform blinded histopathology on the placenta and membranes. As the hypothesis being tested was focused on sepsis and we were ascertaining cases by detailed examination of the medical record and biological samples, we included all neonatal unit admissions. All of these cases were still early onset as this analysis was confined to the delivery admission. i.e. we did not include infants who were discharged home and readmitted. We have now clarified this in the methods.**

Reviewer #2 (Remarks to the Author):

The authors have addressed the criticisms raised and have improved the manuscript substantially with the addition of the RT-qPCR data on GBS and with better description of controls.

**R6. We are grateful to the reviewer for their positive comments.**

Reviewer #3 (Remarks to the Author):

This is a very nice study assessing the association between placental *Streptococcus agalactiae* and neonatal sepsis. Just one major comment regarding the statistical analyses and a minor comment on Figure 2:

1. Since the outcomes in these studies are fairly common (e.g., >10% occurrence) please consider using modified Poisson regression (rather than logistic) to get prevalence ratios rather than odds ratios (which are difficult to interpret when outcomes are more common).

**R7. We understand the point being made by the reviewer. In analysis of cohort studies with common outcomes, the rare disease assumption is violated and the odds ratio is no longer a reliable approximation of the relative risk (the odds ratio is systematically further from unity than the relative risk and the magnitude of the difference between the two ratios is positively associated with the**

frequency of the outcome). However, this analysis is a nested case control study, i.e. we have selected all cases and a sample of controls from the cohort. The outcomes only appear to be common because we analyse all of the cases and a sample of the controls. Conventionally, associations in case control studies are quantified by odds ratios. Please see Chapter 8 (Case-Control Studies) in *Modern Epidemiology Third Edition 2008* (edited by Kenneth J. Rothman, Sander Greenland, Timothy L. Lash). This describes the use of odds ratios in case control studies and on page 114 it states “the rare disease assumption is not needed in case control studies”.

2. Figures 2a & 2c: please put error bars on these graphs so reader can see variability.

**R8. We have added bars to indicate the 95% confidence intervals.**

**Decision Letter, first revision:**

**Subject:** Your manuscript, NMICROBIOL-23061561A

**Message:** Our ref: NMICROBIOL-23061561A

21st September 2023

Dear Gordon,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Microbiology manuscript, "Placental Streptococcus agalactiae DNA is associated with neonatal morbidity and fetal cytokine storm at term" (NMICROBIOL-23061561A). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within two weeks). Please get in contact with us if you anticipate delays.

When you upload your final materials, please include a point-by-point response to any remaining reviewer comments.

If you have not done so already, please alert us to any related manuscripts from your group that are under consideration or in press at other journals, or are being written up for submission to other journals (see: <https://www.nature.com/nature-research/editorial-policies/plagiarism#policy-on-duplicate-publication> for details).

In recognition of the time and expertise our reviewers provide to Nature Microbiology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Placental Streptococcus agalactiae DNA is associated with neonatal morbidity and fetal cytokine storm at term". For those reviewers who give their assent, we will be publishing their names alongside the published article.

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If you have any further questions, please feel free to contact me.

Best regards,

**Final Decision Letter:**

**Subject:** Decision on Nature Microbiology manuscript NMICROBIOL-23061561B

**Message:** 16th October 2023

Dear Gordon,

I am pleased to accept your Article "Placental Streptococcus agalactiae DNA is associated with neonatal unit admission and fetal pro-inflammatory cytokines in term infants" for publication in Nature Microbiology. Thank you for having chosen to submit your work to us and many congratulations.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Microbiology style. We look particularly carefully at the titles of all papers to ensure that they are relatively brief and understandable.

Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required. Once your paper has been scheduled for online publication, the Nature press office will be in touch to confirm the details.

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Due to the importance of these deadlines, we ask you please us know now whether you will be difficult to contact over the next month. If this is the case, we ask you provide us with the contact information (email, phone and fax) of someone who will be able to check the proofs on your behalf, and who will be available to address any last-minute problems.

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With kind regards,