PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Investigating Bordetella pertussis colonisation and immunity: protocol for an inpatient controlled human infection model.
AUTHORS	de Graaf, Hans; Gbesemete, Diane; Gorringe, Andrew; Diavatopoulos, Dimitri; Kester, Kent; Faust, Saul; Read, Rob

VERSION 1 – REVIEW

REVIEWER	Thomas C Darton Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, United Kingdom; Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam.
REVIEW RETURNED	24-Jul-2017

GENERAL COMMENTS	Abstract Minor: Pg 3 Line 23. Rate implies a time denominator. Pg 3 Line 23. It would be useful to include the 95%CI around the colonisation target of 70%. Pg 3 Line 24. Should transmission more accurately be, for example, transmission risk factors, as transmission to other human hosts is not being directly investigated? Introduction Minor: Pg 4 Line 29. Typo. “,” missing before point 2). Pg 4 Line 58. “and the next generation of pertussis vaccines.” or “next-generation” ? Methods Major: Pg 5 Line 18: Is this last sentence correct? It is slightly complicated to dissect the stragegy being used from the figures, but I think that Phase A will be completed once a total of 7 or 8 of 10 participants challenged with the same target SI are successfully colonised? If 10 of 10 are colonised then, according to Figure 3, the target dose will be dropped by 0.5 log (presumably log10?). Would it be useful to clarify (preferably in a list or table) the definitions being used for this study – including “challenge” (i.e. what constitutes a successful challenge), “colonisation”, “infection/disease”? Pg 6 Line 53. How is “mental health” defined? E.g. does this include anxiety/depression? This is not specified in the Supplementary Table. Pg 7. Line 48. Please confirm that female participants are required to use effective contraception for the duration of the study (text on Pg26), which is ~56 weeks.

	If correct suggest including this in the eligibility text, with some justification as this will exclude the majority of female volunteers in this age group. Pg 8 Line 20. Will the participants be allowed to mix socially with each other after challenge? Are there any restrictions to their activities whilst out in the community – e.g. any specific instructions about avoiding crowded areas/public transport etc? Pg 9 Line 18. Doesn't this assume that 100% will seroconvert? Is there any risk that some may not, given that antibiotics are being given so promptly? Minor: Pg 5 Line 7. Typo. Remove capitalisation of "Development"? Pg 6 Line 6. So is seroconversion also being used in the 70% colonisation calculation? This seems different from Page 8 Line 54 definition below? Pg 6 Line 13. Typo. ".." Pg 6 Line 19. Which previous studies? Are there references? Pg 7. Line 7. Will all of these recruitment approaches be used? You may only need 10 participants? How will you determine which will be selected? Pg 8 Line 48. As per the abstract, suggest altering to "assess transmission risk", as samples aren't being collected from contacts (which would be interesting!). Pg 9 Line 28. Presumably eradication will need to be assessed by NP swab once the 3/7 course of antibiotics has been completed – will the participants be allowed home first, as (according to National SOPs) Bp culture requires 5-7 days? Pg 10 Line 48. What is the confidence around 70% colonisation of a group of 10 participants? Discussion Major: It would be useful and of interest to know more about the challenge strain being used – in particular the generalisability to other current circulating strains, the genetic relatedness, and any clinical characteristics of the disease/symptoms associated with it. Minor: Pg 11 Line 12. Positive NP cultures? Pg 11 Line 36. What is the basis for this assumption? Because you are including previously vaccinated individuals or the assumption that there is more immunity in adults than children? Pg 11 Line 55. Suggest add "…Phase A is moderate rather than low because…" Pg 12 Line 15. Again, as you are not directly studying transmission, should this be transmission risk? Pg 12 Line 27. Although the risk with a 100% colonisation rate is that it may overwhelm any putative vaccine-generated protection, which has been documented in multiple enteric challenge studies. References Major: Please review, as some of the formatting/abbreviations are non-intelligible (probably an endnote issue). Specifically references: 1 England PH 4 Collaborators GMaCoD 14 Sciences AoM 18 England PH (and typos in the title) 19 England PH

	21 Format of PLoS One 22 Format of Linnemann CC, Jr.,? Figures Is there any way of combining Figures 2 and 3 as they are part of the same decision-making algorithm and might make the process easier to understand.

REVIEWER	Daniela M Ferreira Liverpool School of Tropical Medicine, United Kingdom
REVIEW RETURNED	01-Aug-2017

GENERAL COMMENTS	This protocol is very comprehensive and clearly written. I praise the authors for the great background information including previous bacterial challenge models and rationale for this pertussis study design. This is perhaps the best study protocol I have ever read. I have some minor points for consideration of the authors. Addition of these clarifications to the protocol would benefit the reader audience. 1. Anti-PT levels will be used for screening of volunteers in study part A as a proxy of recently pertussis exposure. Although I agree with the rationale for doing this, I wonder if there is any evidence that particular levels of anti-PT IgG correlate with protection against pertussis acquisition (carriage)? 2. What is the rationale for excluding volunteers who have never been vaccinated against pertussis from this study? 3. It would be great if the authors could clarify in the protocol why they have a sham inoculated arm for part B of the study. I assume that this is to account for natural acquisition of pertussis colonization but it would be great to have the rationale for this arm clear in the protocol. 4. Why will the sham inoculated volunteers also be treated with antibiotics at the end of the study? 5. The inoculum dilution session of the protocol states that 2 aliquots of 500ul will prepared. Please clarify how much of this volume will be used for inoculation in each nostril. Will this be done by instillation and will any special device be used? 6. An important point I would like to raise is the repetitive nasal washes in the Phase A of the study (3 times a week). Although the volume of saline used for inoculation is in the SOP and not stated in the protocol, I assume it will be between 10-30ml. Repetitive nasal washing in a short interval is very likely to cause local inflammation in the nose which could perhaps interfere with carriage. We have unpublished data showing this in the early phases of the Experimental Human Pneumococcal Carriage studies. Nasosorption and nasal swab samples cause less irritation in the upper airways and will probably provide the longitudinal immunology and microbiology endpoints the investigators are seeking without effecting the carriage rates.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Thomas C Darton
Institution and Country: Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, United Kingdom; Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam.
Please state any competing interests: None declared.

Please leave your comments for the authors below

Many thanks for inviting me to review this interesting study protocol. Please find some comments below which I hope are helpful.

Abstract
Minor:
Pg 3 Line 23. Rate implies a time denominator.

Changed it to:
“This dose will be escalated or de-escalated until colonisation is achieved in approximately 70% (95% confidence interval: 47-93%) of the exposed volunteers without causing disease.”

Pg 3 Line 23. It would be useful to include the 95%CI around the colonisation target of 70%.

Response: Included: “95% confidence interval 47%-93%”

Pg 3 Line 24. Should transmission more accurately be, for example, transmission risk factors, as transmission to other human hosts is not being directly investigated?

Response:Changed “transmission” to “shedding”

Introduction
Minor:
Pg 4 Line 29. Typo. “,” missing before point 2).

Response: Added

Pg 4 Line 58. “and the next generation of pertussis vaccines.” or “next-generation” ?

Response: Added`

Methods
Major:
Pg 5 Line 18: Is this last sentence correct? It is slightly complicated to dissect the stragegy being used from the figures, but I think that Phase A will be completed once a total of 7 or 8 of 10 participants challenged with the same target SI are successfully colonised? If 10 of 10 are colonised then, according to Figure 3, f a successful challenge), “colonisation”, “infection/disease”?

Response: Thank you for this comment. I clarified it in the paragraph and adjusted the figures.
“Once a dose of inoculum achieves a colonisation rate of 70% then that dose will be used to inoculate further participants until a total of 10 participants have been colonised. This will require inoculation of approximately 14 participants with that dose, which will then be defined as the standard inoculum dose. “

Comment: Pg 6 Line 53. How is “mental health” defined? E.g. does this include anxiety/depression? This is not specified in the Supplementary Table.

Response: I have clarified in the preceding paragraph that we will use the general health questionnaire to assess this: “Once consent has been given their eligibility will be assessed. This will include a general health questionnaire, which is a screening tool to identify common psychiatric conditions.”

Comment: Pg 7. Line 48. Please confirm that female participants are required to use effective contraception for the duration of the study (text on Pg26), which is ~56 weeks. If correct suggest including this in the eligibility text, with some justification as this will exclude the majority of female volunteers in this age group.

Response: I have added: “Female volunteers are required to use an effective form of contraception for the duration of their participation in this study.”
I do not completely agree this excludes the majority of the female volunteers aged 18-45 years, so I did not include this.

Comment: Pg 8 Line 20. Will the participants be allowed to mix socially with each other after challenge? Are there any restrictions to their activities whilst out in the community – e.g. any specific instructions about avoiding crowded areas/public transport etc?

Response: I have added:
“Participants will be required to wear a surgical mask covering their nose and mouth when outside their personal room, e.g. in the recreational room, unless outside in open air. Participants will be allowed to leave the CRF for a maximum of two hours twice a day, during the daytime. When outside the CRF, participants will be asked to adhere to infection prevention rules based on Public Health England guidelines. These include avoiding contact with people at risk of pertussis, avoiding direct face to face contact and wearing a surgical face mask when inside.”

Comment: Pg 9 Line 18. Doesn’t this assume that 100% will seroconvert? Is there any risk that some may not, given that antibiotics are being given so promptly?

Resoponse: We do assume that 100% of the participants who are colonised do seroconvert based on the N. lactamica studies and the Pneumococcal challenge studies. We agree there is a risk that some participants who do colonise will not seroconvert. This would be valuable information for the design of phase B.

Minor:
Comment: Pg 5 Line 7. Typo. Remove capitalisation of “Development”?

Response: Thank you.

Comment: Pg 6 Line 6. So is seroconversion also being used in the 70% colonisation calculation?

Response: This seems different from Page 8 Line 54 definition below? Removed “and colonisation”

Comment: Pg 6 Line 13. Typo. “..”

Response: Thank you.

Comment: Pg 6 Line 19. Which previous studies? Are there references?

Response: Added: from previous malaria human challenge studies performed in Southampton.

Comment: Pg 7. Line 7. Will all of these recruitment approaches be used? You may only need 10 participants? How will you determine which will be selected?

Response: Added: “A recruitment and volunteer management plan has been formulated to prioritise and co-ordinate these strategies.”
We anticipate recruitment will be difficult because of the admission time of 17 days, and the risk of getting whooping cough. The recruitment period will be 3 years and includes phase B which will require 45 volunteers.

Comment: Pg 8 Line 48. As per the abstract, suggest altering to “assess transmission risk”, as samples aren’t being collected from contacts (which would be interesting!).

Response: I have changed the wording to “shedding” for the environmental samples. We will take samples from nursing personnel to assess for transmission, so I have added: “Nasopharyngeal samples will be requested from CRF staff members with participant contact to monitor for transmission of viable B. pertussis.”

Comment: Pg 9 Line 28. Presumably eradication will need to be assessed by NP swab once the 3/7 course of antibiotics has been completed – will the participants be allowed home first, as (according to National SOPs) Bp culture requires 5-7 days?

Response: Changed to: “A nasopharyngeal swab will be taken prior to discharge which will be 48 hours after the start of eradication therapy. If this sample is positive for B. pertussis within five days, the course of azithromycin will be repeated.”

Comment: Pg 10 Line 48. What is the confidence around 70% colonisation of a group of 10 participants?

Response: Answered above

Discussion
Major:
It would be useful and of interest to know more about the challenge strain being used – in particular the generalisability to other current circulating strains, the genetic relatedness, and any clinical characteristics of the disease/symptoms associated with it.

Response: No studies are available that compare the clinical characteristics of the disease between different strains as far as we know.
I included another reference that genotyped other strains, but haven’t changed the text. The methods state:
Challenge strain
The Bp isolate to be used in this human colonisation model is B1917, which is representative of current isolates in Europe (15). The strain, isolated in 2000 from a Dutch patient with Bp disease, is characterised as ptxP3-ptxA1-prn2-fim3-2, fim2-1 MLVA27, PFGE BpSR11 and expresses pertactin

(PRN), pertussis toxin (PT) and filamentous hemagglutinin (FHA). This strain has been extensively characterised in the mouse model as well as by proteomics and transcriptomics and has a closed genome available (15). It is fully sensitive to azithromycin in vitro.

Minor:
Comment: Pg 11 Line 12. Positive NP cultures?

Response: Changed to: “positive cough plate cultures”

Comment: Pg 11 Line 36. What is the basis for this assumption?
Because you are including previously vaccinated individuals or the assumption that there is more immunity in adults than children?

Response: “The inoculum required to colonise previously vaccinated adults exposed to natural infection with Bp is unknown, but is assumed to be higher than that used in the paediatric challenge study

Added: “because previously exposed and vaccinated adults are included.”

Comment: Pg 11 Line 55. Suggest add “…Phase A is moderate rather than low because…”

Response: Added

Commnet: Pg 12 Line 15. Again, as you are not directly studying transmission, should this be transmission risk?

Response: Changed to “shedding”.

Comment: Pg 12 Line 27. Although the risk with a 100% colonisation rate is that it may overwhelm any putative vaccine-generated protection, which has been documented in multiple enteric challenge studies.

Response: Agree. I left the sentence as it is stating “near 100%”

References : edited accordingly
Major:
Please review, as some of the formatting/abbreviations are non-intelligible (probably an endnote issue). Specifically references:
1 England PH
4 Collaborators GMaCoD
14 Sciences AoM
18 England PH (and typos in the title)
19 England PH
21 Format of PLoS One
22 Format of Linnemann CC, Jr.,?

Figures
Is there any way of combining Figures 2 and 3 as they are part of the same decision-making algorithm and might make the process easier to understand.

Response: Edited accordingly

Reviewer: 2
Reviewer Name: Daniela M Ferreira
Institution and Country: Liverpool School of Tropical Medicine, United Kingdom
Please state any competing interests: None declared

Please leave your comments for the authors below

This protocol is very comprehensive and clearly written. I praise the authors for the great background information including previous bacterial challenge models and rationale for this pertussis study design. This is perhaps the best study protocol I have ever read.

I have some minor points for consideration of the authors. Addition of these clarifications to the protocol would benefit the reader audience.

1. Anti-PT levels will be used for screening of volunteers in study part A as a proxy of recently pertussis exposure. Although I agree with the rationale for doing this, I wonder if there is any evidence that particular levels of anti-PT IgG correlate with protection against pertussis acquisition (carriage)?

Response: I added in the discussion: “Results of two previous studies demonstrated statistically significant correlations between protection against pertussis disease and the presence of anti-PT IgG in pre-exposure sera (Storsaeter,Locht). Although there is no evidence high anti-PT IgG levels correlate with protection against colonisation, we will exclude volunteers with a high anti-PT IgG in phase A. In phase B this will not be an exclusion criterion and the correlation between anti-PT IgG levels and protection against colonisation will be assessed.”

2. What is the rationale for excluding volunteers who have never been vaccinated against pertussis from this study?

Response:This is to reduce the risk of complicated pertussis. I clarified adding: “who are previously vaccinated and probably naturally exposed to Bp” in the following sentence:
“This approach is considered to be acceptable because the risk of severe disease in healthy adults, who are previously vaccinated and probably naturally exposed to Bp, is extremely low”

3. It would be great if the authors could clarify in the protocol why they have a sham inoculated arm for part B of the study. I assume that this is to account for natural acquisition of pertussis colonization but it would be great to have the rationale for this arm clear in the protocol.

Response: Because this paper focuses on phase A we have not included this. We will publish the protocol for phase B and the rationale behind it as soon as possible.

4. Why will the sham inoculated volunteers also be treated with antibiotics at the end of the study?

Response: This is not stated in the paper, but the reason it is in the protocol is that azithromycin is known to have an immunomodulatory effect on innate and adaptive immune responses. It appears to exert a biphasic action which may serve to promote initial host defence and later reduce bystander tissue injury and promote inflammation resolution. To be able to compare the immune response of the intervention and the control group we need to treat them as equally as possible.

5. The inoculum dilution session of the protocol states that 2 aliquots of 500ul will prepared. Please clarify how much of this volume will be used for inoculation in each nostril. Will this be done by instillation and will any special device be used?

Response: The participant will be positioned supine with neck extended, mouth open, and breathing normally through their mouth. 0.5 mL of the inoculum will be gently expelled into each nostril.
The SOP states that a dedicated pipette will be used to expel the inoculum gently into the nostril.

6. An important point I would like to raise is the repetitive nasal washes in the Phase A of the study (3 times a week). Although the volume of saline used for inoculation is in the SOP and not stated in the protocol, I assume it will be between 10-30ml. Repetitive nasal washing in a short interval is very likely to cause local inflammation in the nose which could perhaps interfere with carriage. We have unpublished data showing this in the early phases of the Experimental Human Pneumococcal Carriage studies. Nasosorption and nasal swab samples cause less irritation in the upper airways and will probably provide the longitudinal immunology and microbiology endpoints the investigators are seeking without effecting the carriage rates.

Response: The volume is indeed 20 mL. We share your concerns about the nasal washes, but also realise the sensitivity of the nasal wash might be higher than the nasopharyngeal swab. In order to assess the correlation between the nasosorption sample, the nasopharyngeal swab and the nasal wash as detection of B. pertussis colonisation we have included the nasosorption sample at every time-point a nasal wash or nasopharyngeal swab is taken. This will inform us about the possibility of replacing the nasal wash with a less invasive alternative in phase B.

VERSION 2 – REVIEW

REVIEWER	Thomas C Darton Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, United Kingdom; Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam.
REVIEW RETURNED	29-Aug-2017

GENERAL COMMENTS	No comments.

REVIEWER	Daniela M Ferreira Reader, Liverpool School of Tropical Medicine United Kingdom
REVIEW RETURNED	04-Sep-2017

GENERAL COMMENTS	Thank you for the clarifications.