PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Neonatal BCG vaccination and child survival in TB-exposed and TB-unexposed children: a prospective cohort study
AUTHORS	Thysen, Sanne; Benn, Christine Stabell; Gomes, Victor; Rudolf, Frauke; Wejse, Christian; Roth, Adam; Kallestrup, Per; Aaby, Peter; Fisker, Ane

VERSION 1 – REVIEW

REVIEWER	Abram Wagner University of Michigan, USA
REVIEW RETURNED	26-Nov-2019

GENERAL COMMENTS	Overall, this is an interesting article that provides some nuanced information about the non-specific effectiveness of BCG vaccines. The paper is well written as is and I just have minor comments below. Introduction In the third paragraph it could be helpful for some readers to have a sentence or phrase explaining what NSEs are. In intro line 21 you mention “BCG-Denmark” but you had not referenced it before. I’d either explain the Denmark component or just simplify it to BCG, which I think is fine for an introduction. The paragraph about PPD in the intro is certainly important, but I’d recommend moving the material to the methods and/or limitations. Methods The third paragraph has an explanation of household vs house. I find this culturally interesting but I think using house as a basis for exposure makes sense and the three sentence explanation is perhaps not needed. So if you are looking for words to cut, I feel like you could do that here. You mention that you tested for confounders and added them in to final model if they changed main estimate by >5%. Is there a reason why you didn’t just include all potential confounders to begin with? I also would recommend here just listing (even if parenthetically, or by reference to a table or something) what all these confounders were. I will say that Table 3 is odd in that for two of the models that adjusted HR is the same as the crude because by your criteria there were not significant of enough changes (and if you were committed

	to this analytic strategy, I would maybe change the presentation to just leave those cells blank with a note that you didn't identify any confounders). In the Ethical considerations section I would mention any ethical review committees involved. Results Table 1 presents important info but also might be a bit confusing. The calculation of the %s changes for the Overall number (first row, where the % represents the row %) vs any characteristic (where the % represents the column). As is could be okay, but an explanation of what the percentages mean might be helpful, but I would actually just recommend calculating the row% instead of col% for every characteristic, that way you actually don't need both the "neonatal BCG" and "no neonatal BCG" columns. For example, for male, the columns would be: Male | 1574 | 1355 (86.1%) | (placeholder for P-value) | 18421 | 15485 (84.1%) | (placeholder for P-value) I think this would make it easier to look at vaccination rates across different groups (as is you are looking at how the population differs between those with and without vaccination – which is okay, but in that case maybe the easiest thing to do is to remove the %s from the first row). I'd recommend a similar change for Table 2, but it might just be how my brain works vs others.

REVIEWER	Prof Hazel M Dockrell London School of Hygiene & Tropical Medicine United Kingdom No direct links to this study or its authors. Co-investigator in another study of non-specific effects of neonatal BCG vaccination in Uganda.
REVIEW RETURNED	10-Dec-2019

GENERAL COMMENTS	There is increasing interest in whether BCG vaccination of neonates induces non-specific as well as mycobacteria-specific immunity, and may contribute to protection against other infections and mortality in young children. This study uses data from Guinea-Bissau to compare mortality rates in infants who did or did not get BCG vaccination soon after birth, or later, with a particular focus on those children who were known to be exposed to TB infection or not. Children who had received neonatal BCG vaccination had lower mortality. There was no effect of TB exposure on the protective efficacy of BCG vaccination, further strengthening the view that BCG can induce non-specific protection. It is useful that BCG vaccination status was categorized by reviewing vaccination cards rather than BCG scars, which may underestimate the frequency of vaccination. As noted in the strengths and limitations section, although all the TB-exposed children can be considered to be TB exposed, some of the children classified as TB-unexposed may have been exposed to TB (and children may also be exposed to an infection TB case from outside the household). Specific points to address:

	1. It can be difficult to diagnose TB in young children. This might be noted as a potential issue. 2. The text on page 6 in lines 13-15 and 20-22 is confusing, is the sense that the first sentence refers to TB-exposed adults? If so this should be made clearer. 3. Also on page 6, a child is considered to be unexposed until the point at which a TB case is diagnosed, at which point they are reclassified as TB-exposed. The text is slightly confused as to when such a TB exposed child is considered TB exposed: on page 5 lines 20-21 it says "Children were classified as TB-exposed from the first registered TB exposure and remained classified as TB-exposed throughout the follow-up period". On page 5, lines 55-, it says "Observation time was split at first registered TB exposure and thus, TB exposed children could contribute with observation time in the TB-unexposed group until TB exposure". On page 6 lines 13-14, exposure was considered to take place in the 3 months prior to TB diagnosis of the case. Please clarify. On what basis was the 3 month exposure period prior to diagnosis selected? If there were delays in diagnosis and the start of treatment, children might be being exposed in the pre-exposure period. 4. The study period ran from 2003 to 2017, but two periods during which studies of chemoprophylaxis were carried out, were excluded. A statement should be included about whether chemoprophylaxis is never given routinely in this setting or if some children might have received it during other periods? On page 7, the text in lines 42-43 states that "Excluding children who had potentially been eligible for studies of preventive TB treatment….resulted in an aHR of 0.95 (95% CI: 0.31-2.97)". Thus excluding these children meant that the significant protection given (primarily to boys) by neonatal BCG was lost. This would imply that the effects of BCG were specific rather than non-specific? Yet the protective effects of neonatal BCG were similar in both TB exposed and non-exposed infants. This might be discussed. 5. It is curious that the effects of BCG were restricted to boys in the TB-exposed group, but not affected by sex in the TB-unexposed group. Similarly in the TB-unexposed group there was no evidence that giving BCG early gave greater protection. Are these effects merely an effect of the smaller group size of the BCG-unvaccinated unexposed group? 6. It is more usual to use Mycobacterium tuberculosis (in italics) not Mycobacterium Tuberculosis.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Abram Wagner
Institution and Country: University of Michigan, USA Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below Overall, this is an interesting article that provides some nuanced information about the non-specific effectiveness of BCG vaccines. The paper is well written as is and I just have minor comments below.

Introduction

In the third paragraph it could be helpful for some readers to have a sentence or phrase explaining what NSEs are.
Response: Thank you for the recommendation, we have added this to the paragraph that now reads: "Vaccines are designed to protect against the specific target infections. Increasing evidence supports that vaccines have additional effects affecting the susceptibility to untargeted ifections. These have been coined non-specific effects (NSEs), and increasing evidence suggest that BCG affects mortality by more than can be explained by the protection against TB, that is, BCG may have beneficial NSE."

In intro line 21 you mention "BCG-Denmark" but you had not referenced it before. I'd either explain the Denmark component or just simplify it to BCG, which I think is fine for an introduction.
Response: We have modified to BCG.

The paragraph about PPD in the intro is certainly important, but I'd recommend moving the material to the methods and/or limitations.
Response: We have moved the paragraph to the limitations section.

Methods

The third paragraph has an explanation of household vs house. I find this culturally interesting but I think using house as a basis for exposure makes sense and the three sentence explanation is perhaps not needed. So if you are looking for words to cut, I feel like you could do that here.
Response: Thank you for the suggestion. We have deleted the sentences.

You mention that you tested for confounders and added them in to final model if they changed main estimate by >5%. Is there a reason why you didn't just include all potential confounders to begin with? I also would recommend here just listing (even if parenthetically, or by reference to a table or something) what all these confounders were.
Response: We chose this strategy over controlling for all potential confounders, as the latter would lead to more children excluded from the analysis, as missing value in one of the baseline characteristics would exclude the child from the comparisons even though the baseline characteristic might not be important for the main comparison. Both methods have their limitations, but we preferred the method selected. The baseline characteristics are mentioned in table 1. We have added this to clarify, and the paragraph now reads: "To control for potential confounding, we assessed whether baseline characteristics (Table 1) changed the estimate by including the factors in the analysis one by one. We adjusted for baseline characteristics that changed the estimate by more than 5%."

I will say that Table 3 is odd in that for two of the models that adjusted HR is the same as the crude because by your criteria there were not significant of enough changes (and if you were committed to this analytic strategy, I would maybe change the presentation to just leave those cells blank with a note that you didn't identify any confounders).
Response: We agree with your suggestion and have adjusted accordingly.

In the Ethical considerations section I would mention any ethical review committees involved.
Response: We have modified the ethical considerations section. Please see editorial comment #1.

Results

Table 1 presents important info but also might be a bit confusing. The calculation of the %s changes for the Overall number (first row, where the % represents the row %) vs any characteristic (where the % represents the column). As is could be okay, but an explanation of what the percentages mean might be helpful, but I would actually just recommend calculating the row% instead of col% for every characteristic, that way you actually don't need both the "neonatal BCG" and "no neonatal BCG" columns. For example, for male, the columns would be: Male | 1574 | 1355 (86.1%) | (placeholder for P-value) | 18421 | 15485 (84.1%) | (placeholder for P-value) I think this would make it easier to look at vaccination rates across different groups (as is you are looking at how the population differs between those with and without vaccination – which is okay, but in that case maybe the easiest thing to do is to remove the %s from the first row).
Response: In all categories, except from number, the column % is presented. We have chosen to maintain this approach, as what we wish to present is the distribution of the different baseline

characteristics in the two groups, i.e. if row % were presented the TB-exposed group would only be a small proportion of the total, and this would therefore not reflect where distribution of baseline characteristics are equal of different in the two groups. To make the objective clear we have edited the Table header to read "Baseline characteristics of children by neonatal BCG and registered TB-exposure status."

I'd recommend a similar change for Table 2, but it might just be how my brain works vs others.

Reviewer: 2
Reviewer Name: Prof Hazel M Dockrell
Institution and Country: London School of Hygiene & Tropical Medicine, United Kingdom Please state any competing interests or state 'None declared': No direct links to this study or its authors. Co-investigator in another study of non-specific effects of neonatal BCG vaccination in Uganda.

Please leave your comments for the authors below There is increasing interest in whether BCG vaccination of neonates induces non-specific as well as mycobacteria-specific immunity, and may contribute to protection against other infections and mortality in young children. This study uses data from Guinea-Bissau to compare mortality rates in infants who did or did not get BCG vaccination soon after birth, or later, with a particular focus on those children who were known to be exposed to TB infection or not. Children who had received neonatal BCG vaccination had lower mortality. There was no effect of TB exposure on the protective efficacy of BCG vaccination, further strengthening the view that BCG can induce non-specific protection.
It is useful that BCG vaccination status was categorized by reviewing vaccination cards rather than BCG scars, which may underestimate the frequency of vaccination.
As noted in the strengths and limitations section, although all the TB-exposed children can be considered to be TB exposed, some of the children classified as TB-unexposed may have been exposed to TB (and children may also be exposed to an infection TB case from outside the household).

Specific points to address:

1. It can be difficult to diagnose TB in young children. This might be noted as a potential issue.

   Response: We agree, and as suggested by reviewer 1, we have moved the paragraph detailing this information to the limitations section.

2. The text on page 6 in lines 13-15 and 20-22 is confusing, is the sense that the first sentence refers to TB-exposed adults? If so this should be made clearer.

   Response: It is correct that the first sentence refers to adults, we have added this information and the sentence now reads: "Since 1996, all diagnosed TB cases above 15 years of age living in the study area have been registered and followed"

3. Also on page 6, a child is considered to be unexposed until the point at which a TB case is diagnosed, at which point they are reclassified as TB-exposed. The text is slightly confused as to when such a TB exposed child is considered TB exposed: on page 5 lines 20-21 it says "Children were classified as TB-exposed from the first registered TB exposure and remained classified as TB-exposed throughout the follow-up period". On page 5, lines 55-, it says "Observation time was split at first registered TB exposure and thus, TB exposed children could contribute with observation time in the TB-unexposed group until TB exposure". On page 6 lines 13-14, exposure was considered to take place in the 3 months prior to TB diagnosis of the case. Please clarify. On what basis was the 3 month exposure period prior to diagnosis selected? If there were delays in diagnosis and the start of treatment, children might be being exposed in the pre-exposure period.

   Response: Thank you for pointing out that this was not sufficiently clear. We have modified the information, so it becomes more clear that children are followed from 3 months prior to diagnosis of a TB-case, and once TB-exposed remain TB-

exposed throughout. The section on page 5, is modified to: "We defined inhabitants as exposed to TB from 3 months prior to diagnosis of a TB case in the house (with several households) and until 2 weeks after diagnosis, to account for delay in diagnosis, which we, in line with previous studies, assumed was a median of 3 months. Children were classified as TB-exposed from the first registered TB exposure (3 months prior to TB diagnosis of co-inhabitant) and remained classified as TB-exposed throughout the follow-up period. We expect some TB cases to be undiagnosed. Thus, some children classified as TB-unexposed may have been exposed."

And the section on page 6 is modified to: "Children entered the analysis the first time their vaccination status was assessed at a home visit after the neonatal period. Observation time was split at the first time-point, where the child was considered to be TB-exposed. Thus, TB-exposed children could contribute with observation time in the TB-unexposed group until 3 months prior to diagnosis of a co-inhabitant."

4. The study period ran from 2003 to 2017, but two periods during which studies of chemoprophylaxis were carried out, were excluded. A statement should be included about whether chemoprophylaxis is never given routinely in this setting or if some children might have received it during other periods? On page 7, the text in lines 42-43 states that "Excluding children who had potentially been eligible for studies of preventive TB treatment….resulted in an aHR of 0.95 (95% CI: 0.31-2.97)". Thus excluding these children meant that the significant protection given (primarily to boys) by neonatal BCG was lost. This would imply that the effects of BCG were specific rather than non-specific? Yet the protective effects of neonatal BCG were similar in both TB exposed and non-exposed infants. This might be discussed.

   Response: To the best of our knowledge chemoprophylaxis have never been given routinely in Guinea-Bissau, although it is recommended in the national TB Guidelines. Chemoprophylaxis have only been provided in the study area during the two periods as described in the paper. We have added: "Preventive treatment to TB-exposed children is not routinely provided in Guinea-Bissau."
   Numbers in the no neonatal BCG group are small. The unadjusted HR is 0.46 (0.18-1.20), adjusting twin status, maternal age, and year of birth excludes 46 PYRS and 0 deaths from the neonatal BCG group, and 15 PYRS and 2 deaths from the no neonatal BCG group.
   A possible explanation of the reduced adjusted effect could be that the effects of BCG were specific. However, as the effect disappeared when children who may have been given preventive treatment (i.e. lower risk of TB than those TB-exposed and not given treatment) *are excluded, this would rather support that BCG has NSE. And as numbers are small we would not base strong conclusions on these.*

5. It is curious that the effects of BCG were restricted to boys in the TB-exposed group, but not affected by sex in the TB-unexposed group. Similarly in the TB-unexposed group there was no evidence that giving BCG early gave greater protection. Are these effects merely an effect of the smaller group size of the BCG-unvaccinated unexposed group?

   Response: Based on the current evidence on non-specific effects of BCG, we would expect these effects to be due to the smaller group size of the TB-exposed no neonatal BCG group. However, not much research has been done assessing the non-specific effects of BCG among TB-exposed children.

6. It is more usual to use Mycobacterium tuberculosis (in italics) not Mycobacterium Tuberculosis.

   Response: Thank you, we have corrected accordingly.

VERSION 2 – REVIEW

REVIEWER	Abram Wagner University of Michigan
REVIEW RETURNED	20-Jan-2020

GENERAL COMMENTS	The authors have appropriately responded to reviewer comments.

REVIEWER	Professor Hazel M Dockrell London School of Hygiene & Tropical Medicine,UK
REVIEW RETURNED	06-Feb-2020

GENERAL COMMENTS	The manuscript has been improved by the addition of the new and revised text. In a couple of places minor edits would be beneficial, this could be done at the proof stage. On age 5 third paragraph, "Children were classified as TB-exposed from the first registered TB exposure (3 months prior to TB diagnosis of co-inhabitant)" could be reworded to Children were classified as TB-exposed from three months prior to registration of the index case". Page 6, Ethical considerations. I suggest rewording the final sentence to:" As no additional data was collected for this study, no additional (or further) ethical approval was needed." Page 9, second last paragraph, reword last sentence to "...a lower PPD response " or to "lower PPD responses". Supplementary Figure 1. The positioning of the time periods for the two studies of preventive TB treatment could be changed to clarify the time periods when individuals were included or excluded.