PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Out-of-sequence DTP and measles vaccinations and child mortality in Guinea-Bissau – a reanalysis
AUTHORS	Thysen, Sanne; Rodrigues, Amabelia; Aaby, Peter; Fisker, Ane

VERSION 1 - REVIEW

REVIEWER	Standaert Baudouin GSK Vaccines, Wavre, Belgium GSK is a vaccine producer. There could be conflict of interest here.
REVIEW RETURNED	03-Aug-2018

GENERAL COMMENTS	The paper is unclear and confusing about what the authors want to demonstrate. From a previous analysis of data reported in 2000 it was found that DTP vaccinated children had a higher overall mortality than unvaccinated children. The period of assessment was 1.5 to 6 months old and the MRR reported was after one dose of DTP only. The vaccine coverage rate was 17% in time 1st dose. The others did not get it right. The reported value for MRR was 1.84 mentioned in the text but it was not possible to get that number from Table 4 of reference 11. We obtained lower values in the range of 1.25. This new paper here tries to evaluate -if we understand the paragraph well at the end of the introduction- that wrong sequential administration of DTP-vaccines and measles could be associated with an increased mortality. The formulation of that question is not very clear. I guess the authors want to demonstrate that a normal vaccination schedule of DTP and measles should give the best overall MR results over time and that different sequential administration and no administration should give worse overall MR results. The sequential order the authors prone is that non-live vaccines like DTP should be given before live vaccines like measles vaccine in order to get best overall MR results. The problem is that there is one big confounding factor in the analysis of sequential vaccine order: if you change the sequential order of vaccine introduction, it means that you delay the appropriate administration of vaccines in time and that will also impact the overall MR results. This is what Figure 2 reports. At month 9, the MR of in-sequence versus non-in-sequence is much higher for non-in-sequence as expected. After 9 months the MR drops dramatically after the administration of MV with DTP. As such, this combination of

	vaccines given at month 9 is doing a good job (the rate of decrease seems higher than in the in-sequence vaccination group). The dose of DTP given at month 9 could be considered as a booster dose by which the reduction in MR is maintained over time after 18 months as seen in the graph. This is maybe not the case for in-sequence vaccination where not everyone received the DTP booster dose at 18 months and that could explain the slight increase of overall MR over time. This is much a different interpretation of the results as what the authors are proposing. What we miss however as additional info is the MR of the no-MV vaccinated group with and without DTP in Figure 2. I guess that for that group we will also see a drop in MR during the 9 to 17 months window given the pooled results in Figure 1. If that is the case, than the interpretation of the results observed in Figure 2 becomes more difficult and an analysis of cause-specific mortality drop could help, I guess …. The point I want to make is that what the authors would like to document that sequential order of vaccines is critical, can't be concluded from the analysis and the data they present. The reason is that what brings the MR at a high rate for non-in-sequence vaccinate group at 9 months time should be analysed and that has not been done.. They analyse the data after 9 months and not before. And there we see that after 9 months the combination of DTP with MV is doing a good job. This is of course in opposition to what the authors try to claim, but this is what comes out from the graphs and I would be very interested in getting more info about the group that has not been vaccinated with MV at that period… I'm not saying that the hypothesis the authors have is wrong about the need of a good sequential order of vaccines. They could be right. But the analysis they propose is not adequate to answer that question that is very difficult to respond with retrospective data unless we can control for completion and compliance. In addition we need to ask the authors to calibrate the figures to the right scale of age (shift to the left is needed). The Tables should specify in greater detail what is presented in between brackets and the units of evaluation (days for ages in table 1). There are also too many references to their own research (32 of the 39 references are from their own group). That makes the analysis suspicious that they only look at what they think is right whereas maybe a more neutral view is needed. If we analyse and evaluate the results from the perspective that vaccines not given in time give a bad MR-result as compared with 'given in time' (high completion and compliance and high coverage),the analysis presented here fits perfectly well. But that message is not so new...

REVIEWER	Carina King Karolinska Institutet, Sweden
REVIEW RETURNED	17-Sep-2018

GENERAL COMMENTS	This was a well written and interesting paper, addressing a really important topic of vaccine sequence and the impacts of this on childhood mortality. The paper took advantage of an existing data set to conduct a secondary analysis of the order of DPT and measles vaccine receipt in Guinea-Bissau. My main comments are around more explanation and justification of a few of the methodological decisions, but otherwise minor!

	Major Comments: - If the analysis is restricted to children with a written vaccination document, or assumed no vaccination, then why was a landmark approach favoured over using actual dates of vaccination? My assumption is that one of the benefits of a landmark approach would be the inclusion of those children without a documented date of vaccination (i.e. caregiver recall) in a way that minimises recall bias? Considering that almost half the children are not included in the analysis, this needs to be more clearly explained/justified, and the various biases of not including these children explored (especially considering they do differ based on Supplementary Table 1). - I was not clear on why adjusted analyses were not presented - there are several results where +/-10% in effect would make a clinically relevant difference, even if not a statistical difference. From Table 1 MUAC/education/age/ethnicity would qualify as confounders (assuming they are also associated with mortality)? Minor comments: Introduction: - Line 6, pg. 4 - it might be more helpful for readers in the future to put a year, rather than "last decades", this will mean something different in 10 years time! - Line 46-54, pg. 4 - this sentence was hard to follow, consider splitting or rephrasing. Method: - Line 24, p.g. 6 - the sentence "it will be seen in Figure 1" did not make sense to me - Line 45, p.g. 6 - age in what scale, days, weeks or months? - Lines 25-29, pg.g. 7 - this paragraph seemed to be a repeat? - Line 33, p.g. 7 - it wasn't clear to me what the term "follow-up" referred to. Was this the period between household visits? Discussion - it would be useful to put these results in the context of current (as well as planned) vaccine programmes, including HiB roll-out and PC and rotavirus vaccines having been widely introduced. Figures 2 and 3 - the x-axis seems to be off, with the line(s) starting at 12 months rather than 9. Also, would it be possible to include confidence intervals on these graphs? Supplementary table 1 - for the the median age, I presume this is in days? As everywhere else age is presented in months, this would be more helpful.

REVIEWER	Edward Goldstein Harvard TH Chan School of Public Health Boston, MA 02215 USA
REVIEW RETURNED	18-Dec-2018

GENERAL COMMENTS	The paper under review studies the effect of the measles vaccine (MV) and the diphtheria-pertussis-tetanus (DTP) vaccine on mortality in young children in Guinea-Bissau. The results are a

	useful addition to the literature, providing evidence about the effects of scheduled (in-sequence) vaccine administration on childhood mortality. At the same time, the reviewer has some questions related both to the presentation, as well as to the paper's findings (particularly point 3. below). 1. The Abstract mentions that the participants were children aged 9-17 months (recommended age of MV) and 18-35 months (recommended age of booster DTP). Subsequently, the Results section of the Abstract calls the in-sequence vaccinations those that had DTP followed by MV, which might not seem compatible with the earlier sentence. The Methods section does indicate that the initial doses of DTP are scheduled at 6,10 and 14 weeks of age; MV at 9 months, and DTP booster at 18 months. The reviewer would suggest including a brief description of the vaccination schedule in the Abstract to make the results better interpretable. 2. The Abstract says: after 18 months, the mortality rate increased, and the differential effect of out-of-sequence vaccinations disappeared. One only learns after reading the Methods that two analyses were performed in the study: one for the effect of vaccination on mortality at ages 9-17 months, the other is on the effect of vaccination on mortality at ages 18-35 months (as far as the reviewer could understand). It is important to explain in the Abstract that two analyses were performed, and the results reported in the Results section of the Abstract refer to the first analysis, with the DTP booster vaccination mentioned earlier in the Abstract not relevant to the notion of in-sequence/out-of-sequence vaccination mentioned in the Results section of the Abstract. 3. This is relevant to point 2. It says in the Abstract: after 18 months, the mortality rate increased. Examination of Figure 2 suggests that mortality increased after 18 months only in the in-sequence arm of the study, and this should be pointed out in the Abstract. Secondly, and probably more importantly, mortality in the in-sequence arm increased after 18 months of age, and the booster DTP dose is scheduled at age 18 months. This brings into question the relation between the booster DTP at age 18 months and the subsequent mortality, with the booster potentially contributing to higher mortality. The reviewer would suggest performing analyses of the effect of the booster DTP on mortality after 18 months of age, including the effect of the booster in those children who had in-sequence vaccination prior to the booster (receipt of booster vs. no receipt of booster in those children and subsequent mortality) 4. The Results section of the Abstract says: among 5937 observations in children aged 9-17 months included in the main analysis, 1590 were classified as in-sequence, and 1984 were out-of-sequence. This leaves out 2363 children, and the results of the mortality analyses for some of those children are reported in Table 2. The reviewer suggests placing some of the results comparing mortality in children who received in-sequence vaccinations vs. under-vaccinated children in the Abstract. 5. It wasn't very clear to the reviewer how were the socio-economic status of children vaccinated in-sequence vs. children vaccinated out-of-sequence compared. Also, was there geographic clustering for children not vaccinated in-sequence which could further explain the spread of infection/associated mortality? 6. There could be biases in the results related to vaccination during the study period. For example, for enrolled children who

	weren't vaccinated with MV at the time of enrollment, those who died prior to the receipt of the MV dose were included in mortality counts for vaccinated out-of-sequence; those who did receive MV during the study period were deemed vaccinated in-sequence – thus this would create an association between receipt of vaccination and survival. The reviewer was wondering if additional sensitivity analysis could be performed that included all children enrolled by the age of 12 months, comparing subsequent mortality outcomes in those who were vaccinated in-sequence by the age of 12 months vs. those who weren't regardless of the subsequent vaccination history of those children.

VERSION 1 – AUTHOR RESPONSE

Reviewers' Comments to Author:

Reviewer: 1

Reviewer Name: Standaert Baudouin

Institution and Country: GSK Vaccines, Wavre, Belgium Please state any competing interests or state 'None declared': GSK is a vaccine producer. There could be conflict of interest here.

The paper is unclear and confusing about what the authors want to demonstrate.

From a previous analysis of data reported in 2000 it was found that DTP vaccinated children had a higher overall mortality than unvaccinated children. The period of assessment was 1.5 to 6 months old and the MRR reported was after one dose of DTP only. The vaccine coverage rate was 17% in time 1st dose. The others did not get it right. The reported value for MRR was 1.84 mentioned in the text but it was not possible to get that number from Table 4 of reference 11. We obtained lower values in the range of 1.25.

Response: The reported MRR of 1.84 in the previous paper was obtained using a Cox proportional hazards model with age as underlying time scale adjusting for BCG vaccination status and cluster. As older children are more likely to have received DTP vaccine as demonstrated in Table 1 of Reference 11, where the DTP1 coverage between 0 and 2 months was 17% as the reviewer mention, and the DTP1 coverage between 3-5 months of age was 65%. It is therefore important to adjust for age, and the crude rates obtained from Table 4 of reference 11 are thus not representative of the estimated effect of DTP. If the proposed estimate of 1.25 comes from Table 4 of reference 11 that is an invalid estimate.

This new paper here tries to evaluate -if we understand the paragraph well at the end of the introduction- that wrong sequential administration of DTP-vaccines and measles could be associated with an increased mortality. The formulation of that question is not very clear. I guess the authors want to demonstrate that a normal vaccination schedule of DTP and measles should give the best overall MR results over time and that different sequential administration and no administration should give worse overall MR results. The sequential order the authors prone is that non-live vaccines like DTP should be given before live vaccines like measles vaccine in order to get best overall MR results.

Response: We are not sure we follow the reviewer's comment and whether it is a critique. We planned to evaluate the effect of the sequence of DTP and MV vaccines on mortality. We have tried to clarify this in the last paragraph of the introduction by adding DTP and MV to the sentence, which now reads: "We took advantage of this historical dataset to test if the different sequences of DTP and MV vaccinations were associated with mortality" (Page 8).

The problem is that there is one big confounding factor in the analysis of sequential vaccine order: if you change the sequential order of vaccine introduction, it means that you delay the appropriate administration of vaccines in time and that will also impact the overall MR results.

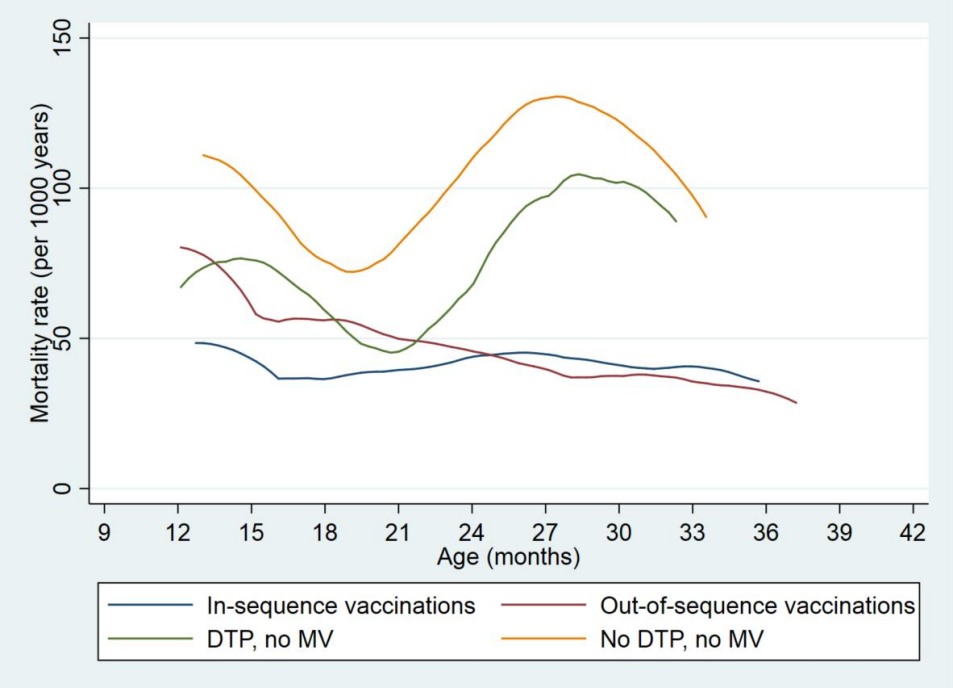
This is what Figure 2 reports. At month 9, the MR of in-sequence versus non-in-sequence is much higher for non-in-sequence as expected. After 9 months the MR drops dramatically after the administration of MV with DTP. As such, this combination of vaccines given at month 9 is doing a good job (the rate of decrease seems higher than in the in-sequence vaccination group). The dose of DTP given at month 9 could be considered as a booster dose by which the reduction in MR is maintained over time after 18 months as seen in the graph. This is maybe not the case for in-sequence vaccination where not everyone received the DTP booster dose at 18 months and that could explain the slight increase of overall MR over time.

Response: This study assesses the potential effect of the sequential order of vaccines in real-life. The reviewer is correct that sequence of vaccines and age of administration are highly correlated. However, in the group of children classified as in-sequence vaccinated (DTP<MV) and out-of-sequence vaccinated (DTP=MV or DTP>MV), all children have received both DTP and MV, and no child enters the analysis before 9 months of age (when MV is scheduled). Furthermore, in the analysis we use age as underlying timescale. Thus, differential mortality patterns are not caused by younger children missing one of the vaccines.

We think the reviewer refers to figure 3, and based on figure 3 in isolation, the reviewer´s interpretation could be plausible for the mortality up to 18 months. However, as no study without survival bias suggests that DTP decreases all-cause mortality[1], and no study suggests that repeated doses of DTP should further decrease mortality, we fail to see how not receiving the booster DTP could explain the increasing mortality after 18 months. As we mention in the paper, we speculate that the increase in mortality after 18 months of age for children vaccinated in-sequence could be due to DTP booster, but we do not have data to be able to assess this. Thus, we make no conclusions on the effect of DTP booster, but merely suggest this as an explanation, which others may have the data to test elsewhere.

This is much a different interpretation of the results as what the authors are proposing. What we miss however as additional info is the MR of the no-MV vaccinated group with and without DTP in Figure 2. I guess that for that group we will also see a drop in MR during the 9 to 17 months window given the pooled results in Figure 1. If that is the case, than the interpretation of the results observed in Figure 2 becomes more difficult and an analysis of cause-specific mortality drop could help, I guess ….

Response: Adding the DTP, no MV and no DTP, no MV groups demonstrates that mortality declines with age as expected, but we also see an increase in mortality after 18 months of age. This could be due to the DTP booster dose, but as previously explained, we do not have sufficient information to make such conclusions.



The point I want to make is that what the authors would like to document that sequential order of vaccines is critical, can't be concluded from the analysis and the data they present. The reason is that what brings the MR at a high rate for non-in-sequence vaccinate group at 9 months time should be analysed and that has not been done.. They analyse the data after 9 months and not before. And there we see that after 9 months the combination of DTP with MV is doing a good job. This is of course in opposition to what the authors try to claim, but this is what comes out from the graphs and I would be very interested in getting more info about the group that has not been vaccinated with MV at that period… I'm not saying that the hypothesis the authors have is wrong about the need of a good sequential order of vaccines. They could be right. But the analysis they propose is not adequate to answer that question that is very difficult to respond with retrospective data unless we can control for completion and compliance.

Response: We agree that with an observational study, we cannot guarantee that no other factor than the sequence of vaccines causes the differential mortality. We have detailed this in the discussion: "To limit confounding, we assessed whether available background factors changed the estimate by more than 10%. As no background factor changed the estimate by more than 10%, we did not present adjusted estimates. However, there may be residual confounding not adjusted for." (Page 13). We cannot conclude on the effect of sequence of MV and DTP before children start receiving MV, therefore, we think our analysis should start after 9 months after which it is possible to analyse the association between sequence of vaccines and subsequent mortality.

We have difficulties understanding what the reviewer means with the sentence: ”They analyse the data after 9 months and not before. And there we see that after 9 months the combination of DTP with MV is doing a good job. This is of course in opposition to what the authors try to claim, but this is what comes out from the graphs and I would be very interested in getting more info about the group that has not been vaccinated with MV at that period”. Between 9 and 17 months (before booster DTP was due), “the combination of DTP and MV” did not do a good job, but was associated with higher mortality than MV after DTP.

In addition we need to ask the authors to calibrate the figures to the right scale of age (shift to the left is needed).

Response: The mortality curve only starts shortly before 12 months of age, as there are very few children under study between 9 and 12 months of age, and the first event is shortly before 12 months of age. Since our study starts at first seen card after 9 months, we still think it is appropriate to retain the scale. This was not clear from the figure and we have therefore added a footnote to clarify. “The smoothed mortality curve starts shortly before 12 months of age, where the first event occurs. Only few children contribute with observation time between 9 and 12 months.”

The Tables should specify in greater detail what is presented in between brackets and the units of evaluation (days for ages in table 1).

Response: We have added further details to Table 1 and changed age in days to age in months.

There are also too many references to their own research (32 of the 39 references are from their own group). That makes the analysis suspicious that they only look at what they think is right whereas maybe a more neutral view is needed.

Response: The reviewer is correct that many references are to work from our own group. Unfortunately, the vast majority of published studies within this field were conducted by our group or in collaboration with members from our group. This is therefore not a signal that we selectively report the literature, but rather that few have sought to test the observations. Please note that in the WHO/SAGE sponsored review of the non-specific effects of vaccines on child survival[7] a very large part of the studies are also from our group.

An independent Dutch study conducted by Tielemans and colleagues[8] set out to replicate the findings from a Danish study, which assessed the NSEs of live MV and non-live DTP-containing vaccine on admission rates[9]. We quote the Danish study, which in addition to beneficial effects of MV found a 7% (0-15%) higher infectious disease admission rate for children vaccinated out-of-sequence. However, Tielemans and colleagues exclude children with out-of-sequence vaccinations, and we have therefore not referred to the study in the present manuscript. In our revised manuscript, we urge others to test our findings.

If we analyse and evaluate the results from the perspective that vaccines not given in time give a bad MR-result as compared with ‘given in time’ (high completion and compliance and high coverage),the analysis presented here fits perfectly well. But that message is not so new...

Response: Our results also suggest that emphasis should be given to the sequence of vaccinations, and thus our results do add an important public health message. This was relevant in the 1990s when the data was collected, but with many new (mainly non-live) vaccines being introduced now, we believe that our paper is still highly relevant.

Reviewer: 2

Reviewer Name: Carina King

Institution and Country: Karolinska Institutet, Sweden Please state any competing interests or state ‘None declared’: None declared

This was a well written and interesting paper, addressing a really important topic of vaccine sequence and the impacts of this on childhood mortality. The paper took advantage of an existing data set to conduct a secondary analysis of the order of DPT and measles vaccine receipt in Guinea-Bissau. My main comments are around more explanation and justification of a few of the methodological decisions, but otherwise minor!

Major Comments:

- If the analysis is restricted to children with a written vaccination document, or assumed no vaccination, then why was a landmark approach favoured over using actual dates of vaccination? My assumption is that one of the benefits of a landmark approach would be the inclusion of those children without a documented date of vaccination (i.e. caregiver recall) in a way that minimises recall bias? Considering that almost half the children are not included in the analysis, this needs to be more clearly explained/justified, and the various biases of not including these children explored (especially considering they do differ based on Supplementary Table 1).

Response: Thank you for the comment. We did not compare survival from date of vaccination as this would have added risk free survival time between the date of vaccination and the date of inspecting the vaccination card. This happens because if the child had died in the interval between vaccination and the home visit, we would be unlikely to get the information since mothers commonly dispose the vaccination card of dead children and the child who had died would therefore be likely to be classified as “unvaccinated” due to the lack of information. This extra risk-free time would be added for both in- and out-of-sequence vaccinated children and could dependent on the distribution dilute or even distort a differential survival.

Furthermore, the landmark approach is a good way of dealing with information, which is differentially obtained for survivors and children who die during follow up. In the landmark approach, the vaccines given during follow-up are ignored: vaccination status remains fixed until a vaccination card is inspected again. This is important since information on subsequent vaccination is more likely to be captured from surviving children than from children who have died. The landmark approach therefore gives a conservative estimate.

Relying on inspected vaccination cards limits the effect of recall bias on date of vaccines received, and thus allows a classification of sequence of vaccines. In the BHP HDSS, we register vaccines based on documented vaccines (as per information from the vaccination card) or where the mother confirms that the child has never been vaccinated. By using vaccination cards to limit recall bias, and the landmark approach to eliminate survival bias, we have obtained conservative results. We have added this clarification to page 10: “If we had used the actual vaccination dates obtained at subsequent home visits to change the vaccine status, we would get better vaccination information for children who survived and had kept their vaccination cards, whereas the families of children who died between visits were likely to have discarded the vaccination card. As a consequence, the survivors would be given risk-free survival time for their new vaccination status, whereas it would not be known if the dead child had been vaccinated, and the child would therefore be misclassified as less vaccinated or unvaccinated. Such “risk-free” survival time will strongly inflate the estimated benefit of the last vaccination. To avoid such survival bias, we have therefore chosen the landmark approach.”

- I was not clear on why adjusted analyses were not presented - there are several results where +/- 10% in effect would make a clinically relevant difference, even if not a statistical difference. From Table 1 MUAC/education/age/ethnicity would qualify as confounders (assuming they are also associated with mortality)?

Response: We agree that differences could indeed be clinically relevant even if the estimates were not statistical significantly different, but our criteria for selecting potential confounders were not statistical significance. Instead, we included the background factors one by one to adjust for estimates that changed the estimated effect (Hazard Ratio) by more than 10%, not using statistically significance as a criterion; however, as no effect estimate changed by more than 10%, adjusted estimates were not presented.

In table 1, we have presented the distribution of background factors, and indeed some of the factors differ significantly between groups. However, all the background factors (regardless of whether differently distributed or not) have been included one-by-one in the Cox model. We have emphasised this on page 10: “All available baseline characteristics (Table 1) were included in the analyses one by one. No variable changed the main estimate by more than 10% and adjusted estimates are therefore not presented”.

The reviewer has an important point that there could be clinically relevant confounding that we have not captured using this method, as we do not capture the combined effects of potential confounders and furthermore there could be unmeasured confounding. This was not clear from our manuscript, we have therefore added: “To limit confounding, we assessed whether available background factors changed the estimate by more than 10%. As no background factor changed the estimate by more than 10%, we did not present adjusted estimates. However, there may be residual confounding not adjusted for.” (Page 14).

Minor comments:

Introduction:

- Line 6, pg. 4 - it might be more helpful for readers in the future to put a year, rather than "last decades", this will mean something different in 10 years time!

Response: We have adapted accordingly and the text now reads “Child mortality has declined significantly between 2000 and 2015.”

- Line 46-54, pg. 4 - this sentence was hard to follow, consider splitting or rephrasing.

Response: We have adapted and the text now reads: “Randomised trials have compared inactivated vaccine after medium- or high-titre MV with standard-titre MV after inactivated vaccine. A meta-analysis of the trials indicates that receiving an inactivated vaccine after a live MV was associated with a mortality rate-ratio (MRR) of 1.38 (95% CI: 1.05-1.83) compared with receiving live MV after an inactivated vaccine, the negative effect being particularly strong for females.”

Method:

- Line 24, p.g. 6 - the sentence "it will be seen in Figure 1" did not make sense to me

Response: We have changed to: “Figure 2 depicts the combined mortality rate of all study children. Mortality declines with age as expected in the beginning, but in the months following 18 months of age the mortality rate increases.”

- Line 45, p.g. 6 - age in what scale, days, weeks or months?

Response: Age as underlying time scale is a technical way of explaining how the analysis is performed, and means that children are always compared with children of the same age. Since our data is based on exact dates this transforms into days.

- Lines 25-29, pg.g. 7 - this paragraph seemed to be a repeat?

Response: We have retained the paragraph to underline the exposure groups as we believe this helps the reader.

- Line 33, p.g. 7 - it wasn't clear to me what the term "follow-up" referred to. Was this the period between household visits?

Response: Follow-up refers to the period in which the child contributed with observation time. In our revised manuscript, we have clarified this and the paragraph now reads: “Since many children were vaccinated during follow up, i.e. after the inspection of their vaccination card, which allowed their exposure group to be classified, we conducted two sensitivity analyses to limit the effect of vaccines administered during follow-up. In the first sensitivity analysis, we censored observation time at 2 months after entry. In the second sensitivity analysis, we included only children who had completed three DTP vaccinations and were therefore not eligible for further doses during follow-up.”

Discussion - it would be useful to put these results in the context of current (as well as planned) vaccine programmes, including HiB roll-out and PC and rotavirus vaccines having been widely introduced.

Response: We very much agree with this point. Unfortunately, there is not much evidence on the effects of these vaccines on all-cause mortality yet. Furthermore, since the vaccines are introduced in combination with other vaccines, assessing the effects of the vaccines in isolation is difficult. The topic of our paper is non-live DTP vaccines given after the live MV – and though we would caution that the effects may be generalizable to other non-live vaccines after MV, we do not yet have much data. Thus, our discussion would rely more on assumptions than evidence. We have therefore focused on the effect of sequence of DTP and MV vaccines and have not included a discussion of other antigens in the manuscript although we also find this very interesting.

Figures 2 and 3 - the x-axis seems to be off, with the line(s) starting at 12 months rather than 9. Also, would it be possible to include confidence intervals on these graphs?

Response: The mortality curve only starts shortly before 12 months of age, as there are very few children under study between 9 and 12 months of age, and first event is shortly before 12 months of age, but since our study starts at first seen card after 9 months, we still think it is appropriate to retain the scale. This was not clear from the figure and we have therefore added a footnote to clarify. “The smoothed mortality curve starts shortly before 12 months of age, where the first event occurs. Only few children contribute with observation time between 9 and 12 months.”

We have added confidence intervals to the graphs.

Supplementary table 1 - for the the median age, I presume this is in days? As everywhere else age is presented in months, this would be more helpful.

Response: We have changed the scale from days to months.

Reviewer: 3

Reviewer Name: Edward Goldstein

Institution and Country: Harvard TH Chan School of Public Health, Boston, MA 02215, USA Please state any competing interests or state ‘None declared’: None declared

The paper under review studies the effect of the measles vaccine (MV) and the diphtheria-pertussis-tetanus (DTP) vaccine on mortality in young children in Guinea-Bissau. The results are a useful addition to the literature, providing evidence about the effects of scheduled (in-sequence) vaccine administration on childhood mortality. At the same time, the reviewer has some questions related both to the presentation, as well as to the paper’s findings (particularly point 3. below).

1. The Abstract mentions that the participants were children aged 9-17 months (recommended age of MV) and 18-35 months (recommended age of booster DTP). Subsequently, the Results section of the Abstract calls the in-sequence vaccinations those that had DTP followed by MV, which might not seem compatible with the earlier sentence. The Methods section does indicate that the initial doses of DTP are scheduled at 6,10 and 14 weeks of age; MV at 9 months, and DTP booster at 18 months. The reviewer would suggest including a brief description of the vaccination schedule in the Abstract to make the results better interpretable.

Response: We have added the recommended vaccination schedule to the setting section in the abstract: “The recommended vaccination schedule was BCG and oral polio vaccine (OPV) at birth, DTP and OPV at 6, 10 and 14 weeks, MV at 9 months, and booster DTP and OPV at 18 months of age”.

2. The Abstract says: after 18 months, the mortality rate increased, and the differential effect of out-of-sequence vaccinations disappeared. One only learns after reading the Methods that two analyses were performed in the study: one for the effect of vaccination on mortality at ages 9-17 months, the other is on the effect of vaccination on mortality at ages 18-35 months (as far as the reviewer could understand). It is important to explain in the Abstract that two analyses were performed, and the results reported in the Results section of the Abstract refer to the first analysis, with the DTP booster vaccination mentioned earlier in the Abstract not relevant to the notion of in-sequence/out-of-sequence vaccination mentioned in the Results section of the Abstract.

Response: We have adapted the abstract: “Children aged 9-17 months (main analysis) and 18-35 months (secondary analysis: age of booster DTP) with vaccination status assessed between April 1991 and April 1996.”

3. This is relevant to point 2. It says in the Abstract: after 18 months, the mortality rate increased. Examination of Figure 2 suggests that mortality increased after 18 months only in

the in-sequence arm of the study, and this should be pointed out in the Abstract. Secondly, and probably more importantly, mortality in the in-sequence arm increased after 18 months of age, and the booster DTP dose is scheduled at age 18 months. This brings into question the relation between the booster DTP at age 18 months and the subsequent mortality, with the booster potentially contributing to higher mortality. The reviewer would suggest performing analyses of the effect of the booster DTP on mortality after 18 months of age, including the effect of the booster in those children who had in-sequence vaccination prior to the booster (receipt of booster vs. no receipt of booster in those children and subsequent mortality)

Response: We have clarified that mortality increases in the in-sequence vaccinated group in the abstract, and the sentence now reads: “Between 18-36 months the mortality rate increased among children vaccinated in-sequence and the differential effect of out-of-sequence vaccinations disappeared.” We agree with the reviewer that the suggested analysis would be useful, and would also have conducted that analysis if we had the data to do so. However, as stated in the manuscript, booster doses of DTP was unfortunately not systematically registered during the time period included in the manuscript, and we are therefore not able to perform this analysis, which would have allowed for more firm conclusions on the effect of booster DTP.

4. The Results section of the Abstract says: among 5937 observations in children aged 9-17 months included in the main analysis, 1590 were classified as in-sequence, and 1984 were out-of-sequence. This leaves out 2363 children, and the results of the mortality analyses for some of those children are reported in Table 2. The reviewer suggests placing some of the results comparing mortality in children who received in-sequence vaccinations vs. under-vaccinated children in the Abstract.

Response: We have rewritten the abstract to make the message clear.

5. It wasn’t very clear to the reviewer how were the socio-economic status of children vaccinated in-sequence vs. children vaccinated out-of-sequence compared. Also, was there geographic clustering for children not vaccinated in-sequence which could further explain the spread of infection/associated mortality?

Response: Socio-economic status of children vaccinated in-sequence and out-of-sequence were not compared as such data was not collected in the period, which the manuscript covers. We compared available background factors (sex, median age at start of follow-up, MUAC at start of follow-up, Region, Ethnicity, median maternal age, education of caretaker, and time since MV/DTP) (Table 1). All analyses were stratified by village cluster, and thus, geographic clustering should not explain the results.

6. There could be biases in the results related to vaccination during the study period. For example, for enrolled children who weren’t vaccinated with MV at the time of enrollment, those who died prior to the receipt of the MV dose were included in mortality counts for vaccinated out-of-sequence; those who did receive MV during the study period were deemed

vaccinated in-sequence – thus this would create an association between receipt of vaccination and survival. The reviewer was wondering if additional sensitivity analysis could be performed that included all children enrolled by the age of 12 months, comparing subsequent mortality outcomes in those who were vaccinated in-sequence by the age of 12 months vs. those who weren't regardless of the subsequent vaccination history of those children.

Response: Apparently our description of the survival analysis methods has not been sufficiently clear. The analyses described by the reviewer would indeed lead to biased estimates. To avoid this type of bias, we chose the landmark approach, which is very close to what the reviewer has suggested. In a landmark analysis, children enter the analyses at the time the vaccination status is assessed, and only change vaccination group once the vaccination status is assessed again to avoid survival bias. If we understand the reviewer correctly, the proposed sensitivity analysis is indeed the same kind of analysis. To make our analytical approach clearer we have written "Survival during the six months after assessing vaccination status was compared by vaccination sequence in Cox proportional hazards models with age as underlying time scale" in the abstract. We have furthermore emphasised this in the methods section (page 9): "Children entered the analysis at the date of inspection of the vaccination card and remained in the analysis in the same vaccination group until the subsequent village visit, 6 months after the visit, death or migration, whichever came first." We have also added a section to the methods to further explain the landmark approach: "If we had used the actual vaccination dates obtained at subsequent home visits to change the vaccine status, we would have better vaccination information for children who survived and had kept their vaccination cards, whereas the families of children who died between visits were likely to have discarded the vaccination card. As a consequence, the survivors would be given risk-free survival time for their new vaccination status, whereas it would not be known if the dead child had been vaccinated, and the child would therefore be misclassified as less vaccinated or unvaccinated. Such "risk-free" survival time will strongly inflate the estimated benefit of the last vaccination. To avoid such survival bias, we have therefore chosen the landmark approach.[10]" (page 10)

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VERSION 2 – REVIEW

REVIEWER	Baudouin Arnould Standaert GSK Vaccines Health economist in a company that produce vaccines
REVIEW RETURNED	17-Apr-2019

GENERAL COMMENTS	I have problems with this article. The authors are convinced about their hypothesis that it is critical that the right sequence of vaccines should be followed for obtaining the best reduction in overall mortality in children. Maybe they are right, I don't want to dispute that. But they want to demonstrate that point with retrospective data analysis while it could be very difficult to proof it the way they are presenting the analysis and the results. I have too much the impression that they force the analysis of the data they have, the way to support their case while it could be that analyzing the data in a different way could lead to maybe different findings. The problem is Figures 1. By the way Figure 1 and 3 were not reported to the right scale of what they should have done as mentioned in the text – there is a shift in 3 months time that is confusing and the X-axis on Figure 3 has not the right numbers (twice 18). Figure 1 demonstrates a gradual decline in mortality during the first 17 months of children while Table 2 may predict no real decrease... This is contradictory and the real increase in mortality as reported in Figure 1 is observed in the second part of the analysis that could be explained by other factors than what they claim. Why this discrepancy between the Figure and the Table results? I don't understand unless there is something that has not been reported yet. Table 2 indicates what causes the highest mortality increase in the 9 to 17 months old period in the group that received DTP and MV. The highest mortality is when DTP is given together with MV. They

	like to add the non-significant mortality increase of those children who received DTP after MV into that group as well. I don't know why they want to pool that together as non-sequential -it is like forcing us to think in non-sequential conditions. The point is that maybe the group that received the two vaccines together (DTP with MV) didn't receive them in time and that delay may be the cause of the higher mortality rate, but we don't know. Those data are not reported. What would have been interesting to simulate but it has not been reported is what could have been Figure 1 if everyone should have received the vaccines as appropriate in sequence. Then we could have seen a difference between what is observed and what could have been ideal and that may have trigger the question of the difference in mortality. Now, if I should have received Figure 1 as such for the first 17 months with no further knowledge on the importance of vaccine sequence on mortality rates, I would have said great, everything works well, there is a nice decrease in mortality over time, I would never have question that there is a problem. Sounds like no, the decline seems like not steep enough -my personal thinking. It seems that the level of decrease has triggered the authors additional questions related to vaccine sequence. That is a little bizarre to me, I would never have thought about that. Unless we can demonstrate that the decline is not steep enough, I would never have thought about the issue of vaccine sequence when looking at Figure 1 first 17 months… I would have question the increase after month 21 if I'm right with the shift in the Figure, but that was not the initial trigger of their analysis. That is for me the main reason that the authors who have an idea -a hypothesis-, wanted to have the data fitting their hypothesis… This is weak from a scientific point of view… If they can better argue that looking at Figure 1 -first part (up to month 17)-, what has triggered their subsequent analysis, then, I guess, they could be on the right track… So difficult to conclude what they claim..

REVIEWER	Carina King Karolinska Institutet, Sweden
REVIEW RETURNED	28-Mar-2019

GENERAL COMMENTS	Thank you for the clarifications and the paper is now very clear!

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Baudouin Arnould Standaert Institution and Country: GSK Vaccines Please state any competing interests or state 'None declared': Health economist in a company that produce vaccines

I have problems with this article. The authors are convinced about their hypothesis that it is critical that the right sequence of vaccines should be followed for obtaining the best reduction in overall mortality in children. Maybe they are right, I don't want to dispute that. But they want to demonstrate that point with retrospective data analysis while it could be very difficult to proof it the way they are presenting the analysis and the results. I have too much the impression that they force the analysis of the data they have, the way to support their case while it could be that analyzing the data in a different way could lead to maybe different findings.

Response: We have thoroughly described the analysis done in the methods section. The methods for the data analyses were chosen prior to data analyses. It is difficult to disprove the reviewer's impression, since it is just an impression and not a critique of how we have handled and analysed the data. We agree that we cannot prove causality using observational data, but we do not claim to have demonstrated causality. We also agree that different data analysis could result in different findings and that is one of the main reasons for choosing the methods prior to data analyses. As long as the reviewer does not provide us with direct critique of our methods, but only states that we have forced the analysis of the data without specific points to back up this criticism, the critique is not very helpful nor appropriate.

The problem is Figures 1. By the way Figure 1 and 3 were not reported to the right scale of what they should have done as mentioned in the text – there is a shift in 3 months time that is confusing and the X-axis on Figure 3 has not the right numbers (twice 18).

Response: We thank the reviewer for pointing out the error in Figure 3. It has now been corrected. The intention with the text was to make the reader aware that the shift was after 18 months of age (the age of DTP booster and therefore where the analysis was split). However reading the section again, we agree that this could be misinterpreted as if the mortality rate increases directly after 18 months of age, we have therefore adapted the text to: "Mortality declines with age as expected in the beginning, but around 21 months of age the mortality rate increases."

Figure 1 demonstrates a gradual decline in mortality during the first 17 months of children while Table 2 may predict no real decrease... This is contradictory and the real increase in mortality as reported in Figure 1 is observed in the second part of the analysis that could be explained by other factors than what they claim. Why this discrepancy between the Figure and the Table results? I don't understand unless there is something that has not been reported yet.

Response: We are not sure that we understand the reviewer correctly here. But figure 1 depicts the mortality of all children plotted against age, and as the reviewer mentions demonstrates a gradual decline. In table 2, we compare mortality according to vaccination status, comparing children with different vaccination status in Cox proportional hazards models with age as underlying time scale, thus comparing children of same age. Table 2 and Figure 1 are therefore not directly comparable. An overall decline in mortality over age as depicted in Figure 1, will not be revealed in a comparison of groups as shown in Table 2. It is therefore unclear how the reviewer can say "Table 2 may predict no

real decrease…This is contradictory" Please clarify if we have misinterpreted what the reviewer intended with this comment.

Table 2 indicates what causes the highest mortality increase in the 9 to 17 months old period in the group that received DTP and MV. The highest mortality is when DTP is given together with MV. They like to add the non-significant mortality increase of those children who received DTP after MV into that group as well. I don't know why they want to pool that together as non-sequential -it is like forcing us to think in non-sequential conditions.

The point is that maybe the group that received the two vaccines together (DTP with MV) didn't receive them in time and that delay may be the cause of the higher mortality rate, but we don't know. Those data are not reported.

Response: The two groups "DTP with MV" and "DTP after MV" are pooled as they are out-of-sequence vaccinations (vaccines given, but not in the intended order), and as this was the objective of the paper we have pooled the estimates. However, they are also presented independently for transparency. The groups are not pooled based on the results, but due to the objective of the paper.

We have conducted this kind of analysis in previous papers1 2 and the WHO review of the potential non-specific effects of childhood vaccines on child mortality under 5 years of age also presented combined estimates for out-of-sequence vaccinations3.

It is correct that based on Table 2, it seems worse to receive DTP with MV than receive DTP after MV. In both groups, vaccines are delayed, as the last DTP is scheduled at 14 weeks and MV is scheduled at 9 months of age, children receiving DTP with MV or DTP after MV have had their vaccines delayed. In this study, we only follow children after vaccination status has been assessed and after 9 months of age, thus, the effects are not due to the fact that the vaccines have not been received.

What would have been interesting to simulate but it has not been reported is what could have been Figure 1 if everyone should have received the vaccines as appropriate in sequence. Then we could have seen a difference between what is observed and what could have been ideal and that may have trigger the question of the difference in mortality.

Response: We are not sure we understand the purpose of this comment. The Cox model estimates are estimates of the differences in mortality between the children, who received vaccines in the prescribed sequence (DTP<MV) and children who received the vaccines in other sequences. This is depicted in Figure 3, where the mortality rate stratified by whether vaccines have been received in-sequence or out-of-sequence is shown. We believe that this is more accurate than simulating the mortality rates (as this would require several additional assumptions).

Now, if I should have received Figure 1 as such for the first 17 months with no further knowledge on the importance of vaccine sequence on mortality rates, I would have said great, everything works well, there is a nice decrease in mortality over time, I would never have question that there is a problem. Sounds like no, the decline seems like not steep enough -my personal thinking. It seems that the level of decrease has triggered the authors additional questions related to vaccine sequence.

That is a little bizarre to me, I would never have thought about that. Unless we can demonstrate that the decline is not steep enough, I would never have thought about the issue of vaccine sequence when looking at Figure 1 first 17 months… I would have question the increase after month 21 if I'm right with the shift in the Figure, but that was not the initial trigger of their analysis.

That is for me the main reason that the authors who have an idea -a hypothesis-, wanted to have the data fitting their hypothesis… This is weak from a scientific point of view… If they can better argue that looking at Figure 1 -first part (up to month 17)-, what has triggered their subsequent analysis, then, I guess, they could be on the right track… So difficult to conclude what they claim..

Response: As mentioned previously, the idea for this study was not based on the present data, but on the results of numerous studies from the past 20 years. The plans for data analysis were made prior to conducting the analysis, and figure 1 (generated during data analysis) depicts the mortality pattern observed, but does not drive the hypothesis or the choice of methods. If we understand the reviewer correctly, he suggests that we should have based our analysis on figure 1. This whole paragraph sounds more like that "I would have liked it differently". It is hard to see this as a factual scientific critique.

Reviewer: 2

Reviewer Name: Carina King

Institution and Country: Karolinska Institutet, Sweden Please state any competing interests or state 'None declared': None declared

Thank you for the clarifications and the paper is now very clear!

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