

## PEER REVIEW HISTORY

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### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	The phase-out of smallpox vaccination and the female/male HIV-1 prevalence ratio: an ecological study from Guinea-Bissau
<b>AUTHORS</b>	Rieckmann, Andreas; Villumsen, Marie; Hønge, Bo; Sørup, Signe; Rodrigues, Amabelia; da Silva, Zacarias; Whittle, Hilton; Benn, Christine; Aaby, Peter

### VERSION 1 – REVIEW

<b>REVIEWER</b>	Luiz Antonio Bastos Camacho Fundação Oswaldo Cruz, Rio de Janeiro, Brazil
<b>REVIEW RETURNED</b>	15-Jun-2019

<b>GENERAL COMMENTS</b>	<p>This is an interesting study with results supporting a possible role of vaccine-induced immunity to smallpox in cross-protection against HIV-1 infection. I recommend that the issues below be addressed before the manuscript is accepted for publication.</p> <p>The prevailing mode of HIV transmission in Guinea-Bissau and eventual changes across birth cohorts is relevant to the interpretation of the findings and should be explained in the paper. Page 6, lines 31-37 seem to imply that forms of HIV transmission other than the heterosexual are not relevant in Guinea Bissau. Differential survival could affect prevalence data of males and females. For instance, young males could be more prone to premature deaths from external causes. Is it worth considering in Guinea Bissau?</p> <p>Page 6, lines 57-60: specificity is also an issue although it could not be estimated in the article by Aaby et al. (Vaccine 2006; 24:5718–5725). In page 10, lines 11-13 addressed differential misclassification in male and female. The impact of false-positive and false-negative rates of scar ascertainment across surveys should be elaborated.</p> <p>Page 8, lines 1-16, described a component of the study that is based on individual data. It seems that the characterization of the study design (page 5, line 54; page 10, line 27) should be revised. In page 10, line 23, differential participation entails a potential for selection bias rather than confounding.</p>
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<b>REVIEWER</b>	Dr Olanrewaju Oladimeji University of Namibia, Namibia
<b>REVIEW RETURNED</b>	18-Jul-2019

<b>GENERAL COMMENTS</b>	<p>Good information with high potential contribution to knowledge domain. But there are few things the author may wish to re-do to improve the quality of the manuscript</p> <ol style="list-style-type: none"> <li>1) The title could be revised</li> <li>2) Explore additional plausible variable</li> </ol>
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	<p>3) Clearly explain access to data and justify why permission to the dataset was not obtained. Was the data in the open source? Is the author familiar with the data protection policy?</p> <p>4) Method section could be succinctly re-written and coherently re-phrased.</p> <p>5) Conclusion may be expanded a little more than just a statement. What are the policy implication and benefit to the society?</p>
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## VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Luiz Antonio Bastos Camacho

Institution and Country: Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Please state any competing interests or state 'None declared': None declared

This is an interesting study with results supporting a possible role of vaccine-induced immunity to smallpox in cross-protection against HIV-1 infection. I recommend that the issues below be addressed before the manuscript is accepted for publication.

The prevailing mode of HIV transmission in Guinea-Bissau and eventual changes across birth cohorts is relevant to the interpretation of the findings and should be explained in the paper. Page 6, lines 31-37 seem to imply that forms of HIV transmission other than the heterosexual are not relevant in Guinea Bissau.

Response 3: This is a good point and correctly interpreted. We now explain this in the Methods under Estimates of female/male HIV-1 prevalence ratios: "In Guinea-Bissau, injection drug use is virtually nonexistent,(10) and blood transfusions have been screened for HIV since 1987 (4); thus, HIV-1 is almost exclusively sexually transmitted."

Differential survival could affect prevalence data of males and females. For instance, young males could be more prone to premature deaths from external causes. Is it worth considering in Guinea Bissau?

Response 4: Thank you for the consideration. Young males would likely be prone to premature death from external causes. Our understanding is that smallpox vaccination status would not be associated with the likelihood of death from external causes, and thus the analysis of changes in smallpox vaccination coverage and F/M HIV-1 prevalence over time should still be valid.

Page 6, lines 57-60: specificity is also an issue although it could not be estimated in the article by Aaby et al. (Vaccine 2006; 24:5718–5725).

Response 5: Correct. We highlight this in the strengths and limitations now: "As no central smallpox vaccination register exists in Guinea-Bissau, we used smallpox vaccination scars as a proxy for the smallpox vaccination coverage. We have previously shown that smallpox scars have a sensitivity of >90% in correctly identifying smallpox vaccinated individuals (no specificity measure available).(7)"

In page 10, lines 11-13 addressed differential misclassification in male and female. The impact of false-positive and false-negative rates of scar ascertainment across surveys should be elaborated.

Response 6: Good point. We have now considered this and added the following: "Potential variation in false-positive and false-negative rates of scar across surveys would likewise not be expected to be sex-differential. "

Page 8, lines 1-16, described a component of the study that is based on individual data. It seems that the characterization of the study design (page 5, line 54; page 10, line 27) should be revised.

Response 7: Yes, this could be clearer in the first Methods section. We now write "We used individually-based data from a smallpox vaccination scar survey in Bissau in 2005 to model smallpox vaccination coverages at the time of the HIV-1 surveys."

In page 10, line 23, differential participation entails a potential for selection bias rather than confounding.

Response 8: Thank you for catching this. We have corrected it to "Hence, differential participation in different study years is unlikely to have caused selection bias."

Reviewer: 2

Reviewer Name: Dr Olanrewaju Oladimeji

Institution and Country: University of Namibia, Namibia

Please state any competing interests or state 'None declared': None

Good information with high potential contribution to knowledge domain. But there are few things the author may wish to re-do to improve the quality of the manuscript

1) The title could be revised

Response 9: Thank you. We have changed the title to "The termination of smallpox vaccination may have increased the female/male HIV-1 prevalence ratio: an ecological study from Guinea-Bissau testing a hypothesis".

2) Explore additional plausible variable

Response 10: This is a very good point for further studies. Currently, we were limited by the published data. We have now added a request for studies addressing multiple potential causes simultaneously in the conclusion. It says: "Our hypothesis that termination of smallpox vaccination may have increased the female/male HIV-1 prevalence ratio was compatible with our results. More research is needed to test this hypothesis, and we hope other research groups will test the hypothesis and other potential explanations for the change in female-male HIV prevalence ratios over time it in individual-based data."

3) Clearly explain access to data and justify why permission to the dataset was not obtained. Was the data in the open source? Is the author familiar with the data protection policy?

Response 11: The data sources on HIV prevalence and smallpox scares would not be linkable on the individual level because it came from different populations. Reproduction of the results of F/M HIV-1 prevalence ratios can be based on summary results of each of the previously published papers.(4-6) We explain access to data in the Patient and public involvement section as: "As this study was based on previously published data,(4-6) neither patients or the public were involved in conducting this research."

4) Method section could be succinctly re-written and coherently re-phrased.

Response 12: We have revised the method section and updated accordingly.

5) Conclusion may be expanded a little more than just a statement. What are the policy implication and benefit to the society?

Response 13: We have revised the conclusion as Reviewer #2 suggests. It now reads: "Our hypothesis that termination of smallpox vaccination may have increased the female/male HIV-1 prevalence ratio was compatible with our results. More research is needed to test this hypothesis, and we hope other research groups will test the hypothesis and other potential explanations for the

change in female-male HIV prevalence ratios over time it in individual-based data. While it may not be possible to reintroduce smallpox vaccine, if more support for the hypothesis that smallpox vaccine protected females against HIV can be obtained, from epidemiological and immunological studies, it could provide important information for HIV-1 vaccine research.”

## VERSION 2 – REVIEW

<b>REVIEWER</b>	Luiz Antonio Bastos Camacho Fiocruz, Rio de Janeiro, Brazil
<b>REVIEW RETURNED</b>	15-Aug-2019
<b>GENERAL COMMENTS</b>	Considering the study design, it does not seem appropriate to characterize cessation of smallpox vaccination as intervention. As a non native English speaker I shall abstain from making specific comments on the standard of written English. Yet, I think the text needs revision before it is published.