Author's response to reviews

Title: Association of HIV infection with distribution and viral load of HPV types in Kenya: a survey with 820 female sex workers

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Author's response to reviews:

From: Prof. Matthew Chersich, MBBCh, M.Sc., Ph.D.
International Centre for Reproductive Health (ICRH)

To: The Editor
BMC Infectious Diseases

Subject: Resubmission of manuscript on HPV type specific distribution and viral load, and its association with HIV among female sex workers in Mombasa, Kenya.

21st November 2009

Dear Editor,

We thank you for your extensive review and constructive comments on our manuscript entitled “Association of HIV infection with distribution and viral load of HPV types in Kenya: a survey with 820 female sex workers”. As per your suggestion, we have addressed each of the reviewers’ comments point by point and have amended our manuscript accordingly. Please find below a detailed response.

Reviewer one (Claire Vajdic)
Major compulsory revisions

Comment: Abstract. I recommend adding the years of the study, the median age of the participants, and adding the qualification ‘cross-sectional’ to the methods.
Response: The change has been implemented and the first sentence of the method section now reads: “Between July 2005 and January 2006, a cross-sectional community-based survey in Mombasa, Kenya, enrolled female sex workers (FSW) using snowball sampling.” The first sentence of the results section provides the median age of the FSW study population and reads: “Median age of the 820 enrolled FSW was 28 years (IQR=24–36 years).” We tightened the remainder of the abstract text to ensure we remained within the 350 word limit for abstracts.

Comment: Methods. Do the authors have any data from the field with which to judge the representativeness of their study population, even if only with respect to the age distribution?

Response: We have done two other recent unpublished studies among female sex workers (FSW) in Mombasa, which give broadly similar population characteristics as those reported here. The median age of FSW in 400 women enrolled in 2006 in a microbicides feasibility cohort in Mombasa was 25 (IQR 21-29) and the identical figures in a behavioural survey among 297 FSW in July 2007. The median age in those studies was a few years lower than the study reported in this paper. Sampling this population group is difficult and the sample obtained snowball sampling will doubtless differ somewhat from a truly random representative sample. We have included this limitation in the discussion section.

Comment: Results. Please check the data given in the first sentence of the section headed ‘Prevalence of HPV infection’. Do you mean that in 786 women the samples were of sufficient quality for cytology and in 789 women they were of sufficient quality for HPV testing?

Response: Indeed, the sentence was intended to mean those figures, but it was ambiguous. The sentence was rephrased and now specifies this more clearly: “Of the 820 participants, study samples of adequate quality were obtained from 786 women for Pap smear assessment and from 789 women for HPV typing.”

Minor essential revisions
Comment: Background. HPV is causally associated with anogenital and oropharyngeal cancers (see Bouvard V et al Lancet Oncology 2009)

Response: This textual change has been made and the suggested reference added.

Comment: Results. For the final paragraph of the ‘Prevalence of HPV infection’ sub-section, I would recommend making the qualification ‘Having one or more children...’.

Response: This revision has been implemented and the sentence (in final paragraph of the new subsection: “Predictors of high-risk HPV infection”) now reads: “Having one or more children was also associated with high-risk HPV; these women had a 2.0 fold higher odds of high-risk HPV (95%CI AOR=1.3-3.3; P=0.003) than women with no children.”
Comment: In the subsection ‘Effect of HIV status on prevalence of specific high-risk HPV types’, does stepwise mean that you tested for a linear trend and it was significant? If that is the case, perhaps it should be described as a linear decrease?

Response: Indeed, we found a linear decrease in the odds of infection with an increase in years, using the chi-square test for trend. The odds of infection decreased by 0.7 with each increase in decade of age, 95%CI=0.52-0.96; P=0.03. This has been re-worded in the manuscript and the name of the test provided. It now reads: “Similarly, a linear decrease in the prevalence of HPV 16 and/or HPV 18 was noted in HIV-negative women, from a prevalence of 40.5% (32/169) in women below 25 years to 7.6% (6/57) in women above 40 years (odds of infection decreased by 0.71 with each increase in decade of age, 95%CI=0.52-0.96; P=0.03, chi-square test for trend).” This is in the new subsection of the results called “Predictors of high-risk HPV infection”.

Comment: Was HPV subtyping performed on the squamous cell carcinoma?

Response: We have added the results of HPV typing for the woman with squamous cell carcinoma as follows: “High-risk HPV type 52 (907 copies/cell) and low-risk type 67 were detected in the woman who had invasive carcinoma on cytology.” (see subsection Cervical cytology).

Comment: Discussion. Please clarify whether the cited studies #27 and #28 included FSW.

Response: The study in reference 27 (Ng’andwe et al 2007) was among female hospital patients but the reference number 28 (Figueroa and co-authors 2001), was among female sex workers. This detail has been added to the discussion text for reference 27. Note that since we included an additional reference, the numbering of references has changed. The meta-analysis of HPV types in HIV-infected women by Clifford (previous reference 26) does not report whether the women in the studies were mostly sex workers. But this seems unlikely given that most of the women included in the meta-analysis were in studies in North America and Europe.

Comment: Page 10. I would suggest moving the sentence about the study of family planning clinic attendees in Kenya one sentence up, so that the commentary about this study is continuous.

Response: The sentence has been moved up as suggested.

Comment: In that same paragraph, it might be helpful to emphasise the study population. For example, replace ‘in this context’ with ‘in the context of FSW’.

Response: It is now emphasized that the context concerns the context of female sex work. The sentence now reads: “However, that most cervical lesions were negative for HPV 16 and/or 18, suggests that other high-risk HPV types might play a more important role in this context of female sex work.”

Comment: The authors might like to speculate why they found a significant
association with one or more children. Could it be that childbirth makes the cervix more susceptible to HPV/HIV infection, or that it is an indirect measure of sexual activity prior to FSW, or are women with children more likely to engage in risky sexual behavior with clients so they can feed their children? The increase in the OR after adjustment for condom use with clients and the apparent independent effect suggests the latter may not be important. Has any other study noted this before, in FSW or women in general?

Response: We have added some text on this issue and background evidence on this issue, please see first paragraph on page 10 in the discussion.

Reviewer two

Discretionary Revisions

Comment: Abstract. The results section of the abstract needs some minor revisions. The first sentence should read “One third of women were infected..., and these women were more likely...”

Response: The sentence has been changed and now reads: “One third of women were HIV infected (283/803; 35.2%) and these women were more likely to have abnormal cervical cytology than HIV-negative women (27%, 73/269, versus 8%, 42/503; P<0.001).”

Comment: Background. Suggest a stronger rationale for the study in the final sentence of the background. Authors begin with “Additional information about the HPV types...” but do not specifically state the type of information that would be necessary to assist in planning for vaccine implementation and strategy. We are left to believe that the data the authors have collected will address this aim.

Response: We agree that the rationale for the study was not clearly stated and have revised this text. Essentially, the study rationale is to describe the prevalence of high-risk HPV types and their association with cervical disease, with specific relevance to the HPV vaccine types.

Comment: General. Multivariable logistic regression (not multivariate)

Response: This suggestion has been implemented.

Comment: Data management and analysis. More description on backwards fitting model

Response: The paragraph describing the statistical analysis has been amended to include a brief description of the backward fitting process. The sentences now read: “A multivariable logistic regression model was constructed, in which the presence of any high-risk HPV infection was the dependent variable. Fitting of the regression model was done using stepwise backward elimination, with variables retained if their removal markedly altered model parameters”. We include a reference to this method, in which backward elimination and alternative methods are discussed in detail, concluding that: “None of these methods
provides infallible tactics in the difficult problem of selecting predictor variables (page 358).

Comment: Results. Add sentence on missing data (number surveyed, number with complete HIV data, number with complete HPV data, number with cytology data, number with complete data on all factors, etc...)

Response: Missing data is now clearly described for important variables in the first sentence of the respective paragraph. For example, the first sentence of the paragraph on “Prevalence of HPV infection” describes the following about the availability of results: “Of the 820 participants, study samples of sufficient quality were obtained from 786 women for Pap smear assessment and from 789 women for HPV typing.” Also, the first sentence of the paragraph on “HIV infection” describes: “A third of participants were HIV infected (283/803; 35.2%); HIV status was unknown in 17 women.”. Readers can also note information on missing data for each variable from the tables.

Comment: Results. Spell out standard deviation the first time it is used
Response: This suggestion has been incorporated into the manuscript.

Comment: Results (General). In the “Prevalence of HIV section”, when reporting a p value comparing two proportions, both proportions should be presented in the parentheses, not just one. For example, “Prevalence of HIV was lower in Muslim than other women (25.4% vs XX%, p < 0.001)”
Response: This change has been made as suggested.

Comment: Results. “...786 had a Pap smear”. More information on Pap smears. Not clear what this variable means. A Pap smear in the previous year, in the lifetime, etc...
Response: The Pap smear was obtained as one of the study investigations. This is described in the Methods section of the manuscript and has been further clarified in the above mentioned sentence which now reads: “Of the 820 participants, study samples of sufficient quality were obtained from 786 women for Pap smear assessment and from 789 women for HPV typing.”

Comment: Results. Paragraph that begins “Among the whole study population...” should be a separate section. Authors are no longer reporting data on Prevalence of HPV Infection, but rather Predictors of HPV Infection. For this same paragraph, please also describe the trend in HIV positive women (not only HIV negative women).
Response: The suggested paragraph is now a separate section entitled: “Predictors of high-risk HPV infection”. Also, predictors of high-risk HPV infection among HIV-infected women is described as suggested. A few
sentences looking at the association between age and high-risk HPV were moved up into this section and describes:” Similarly, a linear decrease in the prevalence of HPV 16 and/or HPV 18 was noted in HIV-negative women, from a prevalence of 40.5% (32/169) in women below 25 years to 7.6% (6/57) in women above 40 years (odds of infection decreased by 0.71 with each increase in decade of age, 95%CI=0.52-0.96; P=0.03, chi-square test for trend). Among women with HIV infection, the prevalence of HPV 16 and/or HPV 18 did not vary across age groups.”

Comment: Results. The few sentences just before the HPV Viral Load section (beginning with “Among women with HIV infection”) are repetitive. This portion could be moved up in the results section into the recommended section, Predictors of HPV Infection, and combined with the detail that is already presented earlier. Note: the authors are discussing effect modification of age on HPV infection within strata of HIV status. This is different than the effect of HIV status on HPV infection.

Response: The proposed sentences are now moved up in the new section on “predictors of high-risk HPV infection”. This improved the flow and the consistency of the sections and discusses the association with age and high-risk-HPV separately for HIV-infected and uninfected women, as suggested.

Minor Essential Revisions

Comment: Abstract. The sentence that begins with “Prevalence of all 15...” reports a ‘median age adjusted risk ratio’. There is no such mention of this risk ratio in the manuscript. Furthermore, the parenthesis for this sentence contains an ‘IQR RR’.

Do the authors mean to suggest this is the interquartile range for the risk ratio? I suspect this is a typo. Please correct. If not a typo, than please clarify.

Response: We have removed this text as it was ambiguous. The relative risk of each of the 15 high-risk HPV subtype was higher in HIV-infected women, we now give the range of these relative risks as this is easier to understand.

Comment: Discussion. Please discuss the limitations of snowball sampling, and how missing data may have affected your results. This might also help explain why the authors results differ from what has been previously published.

Response: In the limitations section of the discussion, we have included information about the limitations of snowball sampling and how the sample may differ from a truly random sample of the target population (should that have been possible to obtain). Also, as suggested we mention the missing data and possible consequences thereof, though we argue that this is likely to be mostly non-differential missing and thus not bias the findings.

Major Compulsory Revisions

Comment: None
Response: We thank the reviewer for the constructive and kind comments.

Many thanks for considering this manuscript for your journal.

Yours sincerely,

Matthew Chersich, on behalf of authors