Author's response to reviews

Title: Cervical human papillomavirus infection among young women engaged in sex work in Phnom Penh, Cambodia: prevalence, genotypes, risk factors and association with HIV infection

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Title: Cervical human papillomavirus infection among young women engaged in sex work in Phnom Penh, Cambodia: prevalence, genotypes, risk factors and association with HIV infection (formerly, Human papillomavirus infection among young women engaged in sex work in Phnom Penh, Cambodia: prevalence, genotypes and risk factors)

Dear Dr. Sepehr Tabrizi,

We are very pleased that the BMC Infectious Diseases journal has sent such favorable reviews and valuable suggestions in regard to our manuscript referenced above. In response to the reviewers’ very helpful comments, we have revised the manuscript accordingly. Below, we duplicate the Reviewers’ comments and include our point by point response, in the order in which the reviewers commented.

The comments and requested edits and clarifications have substantively improved the manuscript and we hope that it will be positively reviewed and accepted for publication.

Regards,
Marie-Claude Couture

REVIEWER #1:

MAJOR COMPULSORY REVISIONS

Methods
1. Specify if data was from a single visit
We have specified that the data were from a single visit in the Methods section (page 5; paragraph 2, line 1).

2. What happened to participants who were found to be positive for HIV? Did they receive counseling, were they offered treatment, were they referred for treatment?
Women infected with HIV received counselling and were referred for free medical evaluation and treatment. This has been added to the Methods section (page 6, paragraph 2, last line).
3. Explain why the ‘oral review of written informed consent’ was done, perhaps with low literacy rates? Were participants also given a chance to read the document?

An oral review of the written consent was conducted due to low literacy rates among participants. However, women were also given an opportunity to read the informed consent form, and were offered copies if desired.

4. HPV quadrivalent vaccine is approved in the US for women 9-26 years of age. Were women 26-29 given vaccine in this study, and are the regulations different in Cambodia? Cervarix in the US is given to women 9-25 years.

We also vaccinated older women (26-29) with the HPV quadrivalent vaccine to avoid discrimination. The FDA's recommended age guidelines are largely based on cost-effectiveness analysis, and not on safety. To our knowledge, there is no express limitation on their receipt of the vaccine, and older women who have not already been exposed to all four strains may gain some benefit from the quadrivalent vaccine.

5. All women negative for HIV were given vaccine, but you also recruited HIV positive participants correct?

We recruited women regardless of HIV status into the study. However, only those who were antibody-negative for HIV (and not pregnant) were offered the HPV quadrivalent vaccine.

6. Under data collection, specify that questionnaires were administered in a private area if done so. Was this a paper questionnaire?

The paper questionnaire was administrated by an interviewer in a private room. We have included additional information on questionnaire administration as recommended by the reviewer (page 6, paragraph 2, first line).

7. Was the technique used the ‘standard spatula/cytobrush technique’?

We have specified in the section “HPV and HIV testing” that we used standard cytobrush technique to collect cervical samples.

8. Please give more details on the HPV testing. What subtypes were detectable.

MY-09/MY-11 L1 consensus primer PCR was performed as described previously (Palefsky, JNCI 1999; Strickler, JNCI, 2003).

This is a well-validated system that has been used for over 10 years to test women participating in the Women’s Interagency HIV Study and many other cohorts around the U.S. and internationally. Beta-globin primers were used as an internal positive control for the presence of human DNA. Samples were dot-blotted and probed for HPV DNA using a chemiluminescent procedure with a consensus probe mixture. Samples that are determined to be consensus-probe positive were probed for the presence of 29 individual HPV types, as well as a mixture of 10 less common HPV types. Samples were classified as HPV-positive or HPV-negative based on the results with the consensus probe. If a sample was consensus probe-positive but negative for all 39 HPV types, it was considered to have an unknown HPV type. Samples that were negative for beta-globin DNA were excluded from analysis.

9. If this is the first time HPV testing has been done at NIPH, how was quality control assessed

Cervical samples were transported to University of California at San Francisco for HPV testing. We have clarified this in the “HPV and HIV testing” section (page 6, paragraph 3, first line).
10. Under measures, how was HPV infection assessed (line blot, linear array, etc). The presence of HPV was determined using a well-validated dot blot system as described in our response to question 8 above.

11. Were HPV results given back to participants? The HPV results were not given to participants, and this was stated in the informed consent form. However, women were referred to a local collaborating physician at Maternal and Child Hospital for free PAP cytology testing, with referrals for treatments as necessary.

12. Under analyses or measures, explain how you got from the data collection method to Stata Data were double entered into a database software (Access, Microsoft Corp., Redmond, WA, USA) and transferred into STATA 11.0 (STATA, College Station, TX, USA) for statistical analyses. We have added an explanation in the “Statistical analysis” section (page 8, paragraph 2, first line).

13. Explain in methods section what is included in HR and LR HPV HPV infection was classified as high risk (types 16, 18, 26/69, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 or 82) or low risk (types 6, 11, 32/42, 54, 61, 62, 67, 70, 71, 72, 81, 83 or 84) based on the strength of association of specific HPV types with invasive cervical cancer. This classification was based on the analysis published by Munoz et al (Munoz, N Engl J Med 2003). We have added information in the methods section as suggested by the reviewer (page 7, paragraph 1, lines 1-3).

Discussion
1. Under limitations, if no quality control was done or no gold standard examined, the HPV results may need to be examined before additional testing is done. The system we employed has been in use for over a decade, is highly validated, and the results of many studies using this system have been published. Each dot blot contains negative water controls and positive controls for each individual HPV type tested. Recently, our laboratory participated in the World Health Organization HPV LabNet HPV testing proficiency program and the system performed very well.

MINOR ESSENTIAL REVISIONS

Abstract
1. Specify the procedure used for HPV testing and how many types were detected, this will help inform the results section of the abstract for prevalence. L1 consensus primer-based PCR with probing for 29 individual HPV types and a mixture of 10 other HPV types was performed. We have added additional information to the abstract and methods section in response to this comment, to explain that we conducted PCR and genotyping using type-specific probes for 29 individual HPV types, as well as a mixture of 10 less common HPV types.

2. In conclusions, rephrase first sentence to say this is the first Cambodian study on HPV. As suggested, we have rephrased the first sentence to state: “This is the first Cambodian study on HPV prevalence and genotypes”.

Background:
1. Check sentence ‘…few treatment options exist mon[2]
We have corrected this sentence. (page 3, paragraph 5, line 5).

2. Change the word ‘effective’ to ‘efficacious’ and ‘reducing HPV infection’ to ‘reducing chronic HPV infection’
We have made the changes accordingly (page 4, paragraph 1, line 2).

3. Grammar check sentence ‘factors detected so far include…’
We have checked the sentence and modified it (page 4, paragraph 2, lines 2-3).

4. Final paragraph, grammar check first sentence and specify risk factors for incident HPV.
We have checked the sentence and modified it.

5. Change final sentence to ….‘we undertook a study to EXAMINE HPV genotypes..’
We appreciate the reviewer suggestion and have modified this sentence (page 4, paragraph 1, line 1).

Methods
1. Fix grammatical errors to include ‘s’ for plural items
This has been done.

2. Rephrase first sentence to ‘Cross sectional data were obtained….’
We have rephrased this sentence according to the reviewers’ comment (page 5, paragraph 2, line 1).

3. Mention the geographic data of those recruited, all from one city, etc.
We specified in the “Study Setting” section that this was part of a prospective study among women engaged in sex work in Phnom Penh, the largest city in Cambodia.

4. Was HPV prevalence done before vaccination?
HPV prevalence and genotype results were not available at the time of vaccination.

5. Under ethical review, change ‘Humans’ to ‘Human’
The sentence has been corrected (page 7, paragraph 3, line 2).

6. Change ‘and’ to ‘or’ in the section of classification ‘either 1)…….OR 2)’.
We have changed this, as suggested by the reviewer (page 7, paragraph 4, line 4).

7. Define STI before using acronym.
The acronym STI is now defined previously in the “Background” section (page 3, paragraph 3, line 1).

Results
1. 15.8% had HIV of the 41.1% with HPV?
The HIV prevalence was 15.8% among all women. We agree with the reviewer that it was confusing and have revised this sentence (page 9, paragraph 2, lines 1-2).

2. Recommend reporting HPV prevalence in HIV negatives and positives separately in addition to overall
We appreciate the reviewer’s comment and have added a sentence reporting HPV prevalence among HIV negative versus HIV positive participants in the “Results” section (page 9, paragraph 2, lines 1-2).
3. Move socio-demographics above HPV DNA results
We agree with the reviewer that it is common in the literature to present the population characteristics first. However, HPV prevalence and genotypes were our main interest, and the outcome of our statistical analysis in this paper. Further, we wanted the text to follow the order of the tables for overall clarity and consistency.

Discussion
1. Useful to describe the assays used (types detected) in your study and perhaps a few of the ones mentioned
We have added information in the “Methods” section on the assay and type of HPV detected as recommended by the reviewer.

2. Specify cross protection from reference shown in Cervarix not Gardasil.
We added a sentence about the cross-protection related to Cervarix according to the reviewer’s suggestion (page 12, paragraph 1, lines 12-13).

3. Mention that cervical HPV may not necessarily represent total HPV infection, which could also include oral, vaginal/vulvar, and anal HPV, plus serology
We appreciate the reviewer’s suggestion and have added information to address this concern in the study limitations section. (page 14, paragraph 2, lines 3-4).

4. Discuss limitations if any of measuring consistent condom use via condom use with last paying partner.
We agree with the reviewer that measuring condom use with the last partner may not be representative of general condom use behaviours, and have added a sentence in the limitations section addressing this (page 14, paragraph 2, lines 5-6).

5. A limitation might be that you don’t have any cervical screening data to pair with these HPV results, to see if the most prevalent types are associated with cancer precursors.
Women were referred to a local collaborating physician at Maternal and Child Hospital for free PAP cytology testing, and treatment was provided as indicated. However, results from the Pap tests were not available for this study.

6. You may reference recent work from Brown et al on cervical HPV prevalence found in FSW’s in Peru of 65.8% (Int J STD AIDS. 2011 Nov;22(11):655-8). The study has a similar sample size.
We appreciate the reviewer’s suggestion and have added the reference in the manuscript.

Conclusions
1. Fix sentence ‘routine screening testing’
This sentence has been fixed. (page 14, paragraph 3, line 2).

Tables
1. In table 1, missing information on condom use with last non-paying partner
We apologize for this missing data and have now added the missing information to the table.

2. In table 2B, drop header ‘HIV and STI infections’ since STIs not reported
We have dropped the header as suggested.

Figure
1. Only include genotypes with a prevalence
We have modified the figure and included only genotypes with a corresponding prevalence, as recommended by the reviewer.

DISCRETIONARY REVISIONS

Title
1-Much of your analysis focuses on the relationship between HPV and HIV. Consider somehow adding HIV to the title.
We appreciate the reviewer’s suggestion and have included “HIV infection” in the title.

Abstract
1-Restate the sentence ‘All participants were also screened for HIV status’ to include type of HIV test.
We added that the participants were tested for HIV using blood samples. The specific HIV tests and algorithm are described in the “Methods” section.

2-Not sure that looking at HPV in FSW’s can inform urgent need for vaccine programs, if general population subtypes are unknown, but surely the high cancer rate in Cambodia from other studies speaks to this need
It is unknown whether our assessment of HPV in this sample of high risk women can inform the need for urgent vaccine programs, especially given the lack of HPV data in the general population including the prevalence of infecting subtypes. However, the high rates of cancer seen in other studies in Cambodia, and presumably, high mortality, speaks to this significant need.

Background
1-You might want to specify between genital HPV types and other types that cause the outcome of interest in this paper
We have further specified this in the manuscript as recommended by the reviewer.

2-Change ‘this’ to ‘the’ country
We have made this change (page 3, paragraph 4, line 3).

Methods
1-Move inclusion criteria sentence after convenience sample sentence
We have moved the sentence as suggested by the reviewer.

Results
1-It might be interesting to know what the previously diagnosed STIs were.
We agree with the reviewer that it would have been interesting to know what the previously diagnosed STI were. Unfortunately, most of the participants did not know the specific STI.

Discussion
1-Regarding access perhaps mention cost of vaccine in Cambodia, and also that knowledge of HPV vaccines may be limited among FSW’s in developing countries (Vaccine. 2010 Nov 16;28(49):7743-7). [Of women who heard about HPV, only 10% heard of a vaccine].
To our knowledge, no published studies have been conducted to date in Cambodia on knowledge of HPV vaccine. However, in our study among FSW, only 23.7% had heard of HPV infection at their baseline visit. At this time, the HPV vaccine is available in private clinics in Cambodia at a cost that is likely prohibitive for this population. The situation is changing rapidly however, and increasingly,
non-governmental organizations are partnering with governmental clinics to increase availability and access.

2. Check definition of OR and how this translates to odds when talking about a specific result
We agree with the reviewer that OR should not be interpreted as a RR. We have corrected the sentence accordingly.

3. In paragraph ‘this is the first study’ and after, be careful with distinguishing increased risk and association.
The reviewer is right and we should be careful in distinguishing ‘risk’ and ‘association’. We have modified the sentence to address this issue (page 13, paragraph 2).

4. Maybe mention the estimated number of FSW’s in Cambodia since this comes up in generalizability and also in conclusions
We have added information on the number of women engaged in sex work in Cambodia as recommended by the reviewer (page 15, paragraph 1, line 9).

Tables
1. In table 1, the more drinking the more HPV?
The reviewer is correct, but this association is related to the type of sex work. Women working in entertainment establishments usually drink more alcohol. These women are also less likely to be infected with STI.

2. Consider replacing ‘last paying partner’ with ‘last client’ in table 1.
We appreciate the suggestion but we believe that “last paying partner” is more appropriate term for our study.

REVIEWER #2:

MAJOR COMPULSORY REVISIONS: NONE.

MINOR ESSENTIAL REVISIONS
1) As described in the section “methods” the HPV detection and typing was achieved by general primer PCR followed by specific probe hybridization and references n 21 (Palefsky et al 1999) was cited for technical details. Nonetheless neither sequences nor references are given for type specific probes, leaving the point of typing unresolved. A list of sequences used, or at least a clarifying reference for each of them is to be included for the adequate evaluation of results and conclusions as well as for the convenient dissemination of the expertise described.
We used the MY09/MY11 primer and probe method, which is a well-validated system that has been in use for over 10 years to test women participating in the Women's Interagency HIV Study and many other cohorts around the U.S. and internationally. Results of many studies using this system have been published, and the technical details are described in Burk, J Infect Dis, 1996; Qu, J Clin Microbiol, 1997.

2) HPV prevalences in Cambodian prostitutes are discussed in comparison with those obtained from prostitutes cohorts in Philippines; Viet-Nam; Thailand; Senegal and Bulgaria and the reported variation attributed to differences in HPV assay, FWS population and sampling. No mention was done on patients' age and climate. Considering the very low mean age of enrolled population and that a young age is believed to have a relevant impact on HPV infection
the mean ages of patients in cited papers should be reported whenever available and discussed. As far as climate, in addition to those from unrelated countries such as Senegal and Bulgaria, literature data from non South-East Asia “mediterranean countries” should be cited and commented (as for instance those from Tunisian prostitutes published on J. Med. Virol. 78:950–953, 2006).

We agree with the reviewer that younger women are more at risk of being infected with HPV and this should be addressed when comparing the HPV prevalence in other studies. Our participants mean age was similar to other studies conducted among FSW, which range from 24-31 years old. We have added more information and other references to address the reviewer's comments in the discussion section (page 11, paragraph 2, line 7).

3) Discussion appears to be too long and speculative. Authors should focus on the paucity of data from their region and on their specific features avoiding lengthy discussion about pathological implication of HIV/HPV interplay as well as multiple infections, as no experimental contribution shedding light on their mechanisms is provided.

We appreciate the reviewer general comment on the discussion. We agree with the reviewer that it is important that the discussion focus on the limited information on HPV prevalence and genotypes in Cambodia and the specific characteristics related to this population. However, we believe that discussing the possible interactions between HIV and HPV, as well as the interplay between HPV infection and drug use allow discussion on the potential mechanisms involved, and give insights for future research which we believe are of interest to the reader.

4) In the present version the absolute number of enrolled patients (i.e.: 220) is reported just in the abstract and in table 1. Thus the results are somewhat hard to be read and difficult to figure out. For the sake of clarity the number of enrolled patients should be openly stated in the section “results” too. Accordingly the actual number patients in each subgroup should given in addition to its percentage. e.g.: HPV positive 41.1% (90 patients). HPV 51 and 70 were the most common (5.0%, 22 patients) ... as authors did for the number of patients suffering from multiple infections (see the results at lines 6-8).

As suggested by the reviewer, we added information on the number of participants (n=220) in the “Results” section as well as on the number of women infected for each HPV subtype.

5) Figure 1 is difficult to be read in its present form. Arranging type specific column bars in decreasing prevalence order (namely 51;16; 52;53, …) would probably give a clear idea of HPV types prevalence at first glance, making the paper core message much easier to grasp.

We appreciate the reviewer’s suggestion. After reviewing the literature, we believe that it is easier for the reader to interpret a graph where we separate the column bars by type of HPV (high; low; unknown).

6) A few spelling and grammar errors must be corrected with the help of an English mother-tongue scientist.

This has been conducted, and corrections have been made accordingly.

Results, line 14: the word “sector” has been typed twice.

We have removed this duplication.

Discussion line 65 “women working as freelance FSW or in brothels were…” it is not clear if brothel are still in use despite current laws or if authors wanted to refer to women which had been working in brothels up to recent years.

We described in the “Methods” section under “measure” that the promulgation of anti-sex-trafficking and sexual exploitation laws enacted in 2008 has resulted in the closure of brothels and the displacement of many FSW to entertainment establishments or outdoor settings. At the time this
study was conducted (2009-2010) most of the brothels were closed and only five women reported working in brothels.

DISCRETIONARY REVISIONS

1. Wrong expression such as “numbers of sex partners”, “age at first sex”, “new sex partners” should be replaced by the correct ones namely: “numbers of sexual partners, age at first sexual intercourse, new sexual partners”. Although largely used in spoken language the above colloquial expressions may largely reduce the efficacy of scientific communications. We appreciate the reviewer’s suggestion and have changed the expressions accordingly.