Author's response to reviews

Title: Seroprevalence of Human Papillomavirus Types 6, 11, 16 and 18 in Chinese Women

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Version: 3 Date: 17 May 2012

Author's response to reviews: see over
Dear Sir or Madam,

My colleagues and I are pleased to re-submit our manuscript entitled “Seroprevalence of Human Papillomavirus Types 6, 11, 16 and 18 in Chinese Women” for consideration by *BMC Infectious Disease*. This paper presents original research from China and has not been previously published nor is it being considered by another journal. All of the authors have been involved in the preparation of the manuscript and agree with the conclusions.

Please kindly find a point by point response to the editorial comments attached. We take the opportunity to thank you for helpful comments, which have certainly improved the quality of our manuscript.

We thank you for your consideration of our manuscript and look forward to your further review. Jennifer and I will serve as the corresponding authors.

Sincerely yours,

You-Lin Qiao

Jennifer S. Smith
Please kindly find a point by point response to the reviewers’ comments attached. We take the opportunity to thank the reviewers for their helpful comments, which have certainly improved the quality of our manuscript.

To address both reviewers’ comments about language improvement, Dr. Smith, a native-English speaker with scientific expertise, has reviewed this manuscript and corrected any language problems. Besides, this manuscript has been revised for several times by two co-authors, Dr. Smith and Dr. Belinson, including language checking. Both Dr. Smith and Dr. Belinson are fluent English speaking people. They have agreed this manuscript is well written in English. Besides, Dr. Esser, another co-author and native English speaker, also corrected language problems and approved this manuscript.

Reviewer: Xiang-Sheng Chen

MAJOR COMMENT
1. The study reports the seroprevalence of antibodies to HPV types 6, 11, 16 and 18 in Chinese women. The authors indicate that there are few studies on estimation of HPV seroprevalence and conclude that the findings from this study can be used for designing and implementing cervical cancer control and prevention programs via future prophylactic vaccination programs. However, rationale of this conclusion is based on the relationship between the seroconversion of the high-risk HPV types and the cervical infections, especially persistent infections, with the high-risk HPV types. Are there any evidences (or studies) to validate the relationship?

Thank you for making this very good point. A global review of HPV antibody and DNA prevalence by Tiggelaar et al. (J Adolesc Health. 2012 Feb;50(2):110-31) showed that populations with low HPV seroprevalence generally had low DNA prevalence. Another recent worldwide HPV prevalence review by IARC (Cancer Epidemiol Biomarkers Prev 2010;19(9):2379-88) concluded that the correlation between HPV DNA and HPV serology was not very good at an individual woman level, but high at a population level. We added “Carter et al. [48] studied HPV 16 and 18 antibody response following incident infections over years and found out that women in whom HPV DNA is detected at several visits may be significantly more likely to seroconvert than are women with only one HPV DNA-positive visit.” in the second last paragraph of Discussion section to reinforce this point.

METHODS
2. Different terms, seroprevalence, prevalence, seropositivity, were used. Are there any differences? If not, the most appropriate term should be sued throughout the manuscript.

Thank you for this comment. We have now changed prevalence to seroprevalence throughout the manuscript unless the former needs to refer to DNA prevalence.

3. What was the definition of a virgin in this study? Was it based on self-reported information collected through asking the participants?

If a subject declares to have no prior history of sexual intercourse, she is defined as a virgin in this study. It is defined in the 3rd paragraph of Methods section.
4. It is not quite clear for me in sampling strategies? Based on the “target population”, what did the authors do in terms of sampling? In each of age-group strata, a maximum of 125 women were recruited. In this case, non-probability sampling method was used. What percentage of women in each stratum was recruited actually?

This study indeed used non-probability sampling method. Because census data for each stratum at study time is not available now, the percentage of women actually recruited in each stratum is not estimable. In the last paragraph of Discussion section, we stated that “women were recruited through posters or advertisement, but not by random sampling, indicating possible selection bias in our study. This bias is, to some extent, offset by the relatively large sample size, wide age range of participating women, as well as the inclusion of multiple geographical sites.”.

5. Was the information about husband’s behaviors of extramarital sex collected through interviewing the women participants? If so, how much information bias did the authors estimate? Based on responses of the participants excluding those responded “unknown”, 30% of the husbands had extramarital sex, which is much higher than the previous studies conducted in China. Information bias is a concern.

The information about husband’s behaviors of extramarital sex was collected through interviewing the women participants. However, the information bias is hard to estimate or quantify. In response to the reviewer, we have now added “Self-reported sexual behavior and their husbands’ extramarital sexual behavior by female subjects may also have introduced some bias” in the last paragraph of Discussion section to acknowledge this potential bias.

6. Some part of the text should be re-organized. E.g.,
   a) In 3rd paragraph of “Study population”, some descriptions to limit the participants for data analysis (… women who had complete HPV DNA data and cytology results; one woman had missing serological results …) should be reorganized as a part of statistical analysis.
   b) In 1st paragraph of “Sample collections”, laboratory assays related to determining HC2 positivity and cytology examination should be under a separate subtitle. Particularly, a statement of study sites is inappropriate to be included in this section.

   a) We now moved the following sentence to the statistical section: “Among 4,372 sexually active subjects, 4,212 women had finished questionnaire and complete HPV DNA data and cytology results. One woman had missing serological result and was therefore not included in the analysis. Thus 4,211 women (age 17-54, median 37) were included for final statistical analyses restricted to sexually active women. Of 649 virgins, 520 subjects (age 14-36, median 18) provided blood samples and had serological results available for analyses.”
   b) In response, we created a separate subtitle called “HPV DNA measurement and cytology/histology evaluation” and moved these statements under this subtitle.

RESULTS
7. HPV seroprevalence of high-risk types was much lower in virgins (2.5%) than sexually active women (15.8%). Is the difference attributed to sexual behaviors or age difference in these 2 subgroups because a difference among age-groups was found as well in this study?

HPV infection has been found strongly associated with sexual behaviors in many previous studies. The global review of HPV antibody and DNA prevalence by Tiggelaar et al. (J Adolesc Health. 2012 Feb;50(2):110-31) presented HPV 16 or 18 seroprevalence was in the range of 1-12% or 0.5-5% at age of 18 (median age of virgin in our study), respectively, when pooling data from female populations in Asia areas. These percentages are higher than ours is because those studies were conducted in populations mostly comprising of sexually active women. HPV 16 peaked in women aged 25-40 years and HPV 18 seroprevalence had a slightly late peak. Therefore, the difference is also attributed to age difference, which is strongly correlated with sexual behaviors difference as well. We adjusted odds ratio by age, HPV DNA positivity and geographic sites in this study.

8. In results section, seroprevalence and its 95% confidence intervals were used and also comparisons of two seroprevalences based on univariate analyses were conducted but these statistical methods were not mentioned in the “Statistical analysis” section. P value used for determining statistical significance was not mentioned and p value of each comparison of seroprevalences in results section was not provided to show the significance level.

We used odds ratio and its 95% confidence interval to evaluate the association between two variables. Chi-square test was conducted to evaluate the significance. We added “Chi-square test was conducted at significance level of 0.01.” in Statistical analysis section of Material and Methods. We added P value when statistical significance was reported.

9. The study subjects include 4211 sexually active women and 520 virgins, but only were the sexually active women included in the analysis of socio-demographic and behavioral characteristics. Why?

HPV infection has been found strongly associated with sexual behaviors in many studies. Only sexually active women were potentially exposed to HPV and HPV prevalence among virgins was low as previously cited (HPV 16 or 18 seroprevalence was in the range of 1-8% or 0.5-2% at age of 18 (median age of virgin in our study) in Asia areas, respectively). Therefore, we focused on investigating risk factors of HPV seroprevalence in sexually active women only.

DISCUSSION
10. The statement “Although reports of husbands’ extramarital relationship were provided by wives via questionnaire and might be inaccurate, associations between HPV seropositivity and husbands’ sexual activity were strong” seems very subjective.

We changed to “Though reports of husbands’ extramarital relationship were provided by wives via questionnaire and might be inaccurate, associations between HPV
seropositivity and husbands’ sexual activity were still observed when husbands were reported to have extramarital sexual relationships”.

11. Regarding the strengths of the study, relatively large sample size may one of them but it is not necessarily indicating that it can provide a sound basis for future planning of a vaccination program. Furthermore, the authors indicate that their study provides a fair estimate that HPV seroprevalence is intermediate in China because sample size of the study is large. However, a fair estimate is not only subject to sample size of the study but its representativeness.

Thank you for pointing this out. We changed to “The number of virgins we recruited was also relatively large, providing source data for future planning of a vaccination program.”. We also stated in the last paragraph of Discussion section that “Among study limitations, data obtained from the five geographic sites might not be representative of the entire nation of China, particularly given the variation of HPV seropositivity across the sites or age groups.”. We also removed “However, given our large sample size, our study provides a fair estimate that HPV seroprevalence is intermediate in China.”.

12. The study found that the Uyghur minority and Shanghai had higher seroprevalences than their counterparts (Han and Beijing). What are the implications of these findings for interventions?

It suggests regional difference or ethnic group related difference of HPV6/11/16/18 seroprevalence. HPV seroprevalence provided a picture of cumulative exposure to HPV over a certain period, rather than cross-sectional transient exposure that could be measured by HPV DNA detection. Vaccination program implementation might need to consider the difference of HPV exposure in difference regions or ethnic groups, especially when the starting age of HPV exposure might be different in various areas or among different ethnic groups.
Reviewer: Fengyi Jin

Results:
1. The results section in its current form is difficult to follow for its lack of coherence. I would strongly recommend the use of subheadings to make better organisation, such as seroprevalence, risk factors, etc. Under risk factors, this can be further divided by age, geographic, ethnic, and sexual exposure, etc.

Thank you for this helpful comment. We have now added subheadings “Overall seroprevalence”, “Seroprevalence and its risk factors in sexually active women”, and “Seroprevalence and cytology/histology” in results section.

Methods:
2. One of the strengths of the paper is its population-based sampling. Nevertheless, from the methods, the sampling is rather convenient. It would ease the concern for the bias introduced by this recruitment method by comparing a few key demographic statistics in the discussion section between the samples and the consensus data to ensure a fair representation of whole population.

This study indeed used non-probability sampling method. Because census data for each stratum at study time is not available now, comparison of a few key demographic statistics between the samples and the consensus data is not achievable. As this was a screening study, random sampling was not easy to implement. In order to enhance the representativeness of the study and minimize the bias of this sampling method, we tried to conduct this multi-center study with large sample size and balance the sample size between urban and rural population. In the last paragraph of Discussion section, we stated that “women were recruited through posters or advertisement, but not by random sampling, indicating possible selection bias in our study. This bias is, to some extent, offset by the relatively large sample size, wide age range of participating women, as well as the inclusion of multiple geographical sites.”.

3. In the results section, the authors compared the prevalence of HPV seropositivity after controlling for geographics and HPV DNA status. One would think that HPV seropositivity is highly correlated with HPV DNA status. The rational for this adjustment should be explained in the methods section under ‘statistical analysis’.

HPV DNA status is a transient marker and reflects current HPV infection. HPV seropositivity is a persistent marker and reveals cumulative exposure of HPV infection. Though HPV DNA status and HPV seropositivity is associated to some extent, they are not necessarily highly correlated. We calculated Kappa estimate to assess the correlation between HPV DNA status and HPV seropositivity in our study. The HPV serology results had correlation with HC2 results but not strongly correlated (Kappa=0.190). The similar results was also found between HPV serology and Linear Assay results (Kappa=0.168). Nevertheless, a recent worldwide HPV prevalence review by IARC (Cancer Epidemiol Biomarkers Prev 2010;19(9):2379-88) concluded that the correlation between HPV DNA and HPV serology was not very good at an individual woman level, but high at a population level. We added “The adjustment over HPV DNA status
considers potential association between HPV serology and HPV DNA results.” in “statistical analysis” section of Material and Methods.

Other minor suggestions are as follows:

Abstract:
4. It reads seroprevalence increased with age in sexually active women. Is this statistically significant? If so, the p value for trend should be given.

We performed Chi-square test and P value was 0.02. We added “Chi-square test was conducted at significance level of 0.01.” in Statistical analysis section of Material and Methods. This age trend was therefore not significant at significance level of 0.01.

Material and Methods:

5. Under sample collections, it reads that all the cervical cytological specimens were physician-collected. However, it also mentioned that in rural areas there were “self or physician obtained samples”. These seem contradictory.

Thank you for pointing this out. We changed to “Consenting sexually active women also provided self- or physician-collected exfoliated cervical cells for HPV DNA detection by HC2” in sample collection part. This is consistent with descriptions in paragraph followed, where it stated that “Women in rural areas who were HPV positive within the self or physician obtained samples by HC2 ...”.

6. There is a need for the definition of LGSIL. What is included, ASCUS and LSIL? If it is greater that LGSIL, would it be clearer and more consistent just to use high-grade SILs instead?

Per Bethesda classification, LGSIL or HGSIL in this manuscript is changed to LSIL or HSIL. LSIL doesn’t include ASCUS. Subjects with LSIL or greater were considered, i.e. both subjects with LSIL and those with HSIL were considered.

7. The referral standard for urban area is unclear to me. Was it any cytological abnormalities and/or HPV positivity to any of the four types tested?

The referral standard in urban area considers (1) subjects with both HPV positivity and ASCUS or (2) LSIL or greater. We re-worded as “In urban sites, the referral standard was (1) atypical squamous cells of undetermined significance (ASCUS)/HPV positive or (2) LSIL or greater”.

Results:
8. HPV6 is “notably more common than” HPV11. Is the difference statistically significant? If so, the p value should be given. The same applies to the comparison between sexually active women and virgins.

We performed Chi-square test and P value is less than 0.001. This is statistically significant. Thank you for pointing this out. We added P value throughout this manuscript when statistical significance was reported.
9. It looks like the risk factors analyses were restricted to sexually active women only. It would be clearer to mention this in the methods section.

   We added “Thus 4,211 women (age 17-54, median 37) were included for final statistical analyses (i.e. risk factor analyses) restricted to sexually active women.” in Methods section.

10. The last paragraph in the results section: the authors should define high-grade cervical lesion in the methods section. Is it just CIN2/3 considered as being high-grade lesions in this analysis?

   We added “High-grade cervical lesions were defined as CIN2 or greater in histology or high-grade squamous intraepithelial lesions (HSIL) in cytology.” in Methods section.

11. The same paragraph, the authors introduced a new term HGSIL. What is regarded as HGSIL? This should be defined in the methods section.

   We added definition of “high-grade squamous intraepithelial lesions (HSIL)” in Methods section and used HSIL in this paragraph.

Discussion:
12. Second paragraph: the association between HPV6 seroprevalence and HPV DNA detection (the second last sentence). Could this be due to the higher clearance of HPV6 than other types as well?

   Higher clearance of HPV6 may be one important reason. There may be other reasons such as lower induction or persistence rate of antibody of other HPV types upon their infection. We added “Some possibilities include higher clearance of HPV 6 than other types or lower induction or persistence rate of antibody of other HPV types upon their infection.”.

13. Third paragraph: the comparison of peak age of HPV16 between China and the US should be referenced.

   We added reference 28, which was also cited in the second paragraph in Discussion section.

14. Fifth paragraph: the authors speculated the role of nonsexual contact in HPV transmission, or should it be non-intercourse sexual contact, such as oral sex?

   We changed to “Given that HPV can be acquired at other non-genital sites, these findings may reflect possible non-intercourse sexual transmission.”.