Author's response to reviews

Title: Kaposi's sarcoma associated herpesvirus infection among female sex workers and general women in Shanghai, China: a cross-sectional study

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Author's response to reviews: see over
Dear Editor,
We sincerely appreciated your and reviewers’ comments regarding our manuscript entitled 'Kaposi’s sarcoma associated herpesvirus infection among female sex workers and general women in Shanghai, China: a cross-sectional study' (MS: 4023354259994612). These encouraging and thoughtful comments enabled us to greatly improve the quality of our manuscript.

We have fully addressed all of the comments in the revised manuscript. We also asked a native English speaker to proofread and edit the manuscript carefully to correct grammatical and typographical errors. Enclosed please also find our point by point responses to the referees.

We hope that this improved manuscript will be acceptable to you and BMC Infectious Diseases. Thank you very much for your kind considerations of our revised manuscript.

Sincerely yours,

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Point-to-point responses to comments

Editorial requirement

Comment 1. Please include a copy of your questionnaire as additional file.

Response: The questionnaire used in this study was included in as an additional file

Reviewer 1 (Dr. Denise Whitby)

Comment 1. The discussion of transmission routes in the introduction is overly simplistic, especially considering that the subject matter of the manuscript concerns KSHV transmission routes.

Response: Thank you for your suggestion. Heterosexual transmission of KSHV is still not consistent, data from different regions have not shown consistency and need to be further investigated. We have strengthened the transmission routes of KSHV in the introduction. Meanwhile, female sex worker is of importance in the prevention of HIV and STIs in China and all over the world. Thus far, to explore KSHV seropositivity among FSW is crucial for the future prevention of KSHV. Per reviewer’s suggestion, we have revised the introduction section and mentioned the importance of study population. Please see Page 4 Para 1 to Page 5 Para 2.

Comment 2. I applaud the authors for the inclusion of power calculations to estimate the number of subjects needed to study the research question adequately and for the detail provided on the recruitment procedures. It is still unclear however, how and where the “general women” were recruited.

Response: We thank the reviewer for the encouraging words. A detailed description of the subject selection procedure for both FSW and general women was provided in the revised manuscript. (Page 6 Para 2 to Page 7 Para 1).

Comment 3. Although the study population is relatively robust and recruitment procedures are well described, the authors should be more cautious in generalizing their observations to the entire Chinese population

Response: Thank you for your suggestion. The present study has been conducted in
a relatively large sample of participant but we agree that caution should be given in
generalizing their observations to the entire Chinese population. Therefore, we state
this limitation in the discussion section of the revised manuscript to provide
transparency and help the readers understand the generalizability of the present
study (Page 17, Para 2).

Comment 4. The conclusions regarding transmission in MSM are grossly overstated
and cannot be substantiated based on reported data.
Response: We appreciate the reviewer’s concern. To address it, we have rephrased
the description in the discussion and try not to overstate on the homosexual
transmission of KSHV (Please see Page 16, Para 2 to Page 17 Para 1).

Reviewer 2 (Dr. Jesse Clark)
Comment 1. Introduction: Overall, the authors do not make a convincing case for the
importance of their analysis. While the epidemiology of KSHV infection among
MSM has been an important topic of research, due to the high prevalence of KS
among HIV-infected MSM, it is not as clear what the importance of understanding its
epidemiology among women would be. As stated above, the authors present an
interesting, well-conducted analysis that, if nothing else, would be important as an
academic exercise. But it would be helpful to have more information to highlight the
importance of conducting this analysis in the first place (e.g., public health impact,
importance for understanding patterns of STI transmission among women in China,
etc.)
Response: We thank the reviewer for his suggestion and apologize for not making
the rationale for the present study more clear. In the revised manuscript we have
strengthened it in the Introduction section (Page 4 Para 1 to Page 5 Para 2).

Comment 2. Methods: i) How was “commercial sex” defined (Page 5, last
paragraph)?
ii) The sampling methods are unclear: If the minimum number of subjects estimated
for 95% power was 430, then why were 600 subjects recruited? If 600 subjects were recruited, then why was the total sample size 1200 subjects? How were the FSW participants and the general population participants matched?

iii) What cut-off value was used to determine seropositivity on the HerpeSelect ELISA? iv) How was the multivariate analysis designed and what analytic techniques were used?

Response: i) In the present study, commercial sex was defined as the exchange of sex for money. This has now been clarified in the text (Page 5, Para 2).

ii) We apologize for not being clear. Based on sample size estimation, approximately 430 subjects were required for each group. Based on our previous experience it was anticipated that some participants may refuse to attend. Thus, to address this, the sample size was increased by 30% to 559 subjects. Finally, this number was rounded up to 600 subjects in each group. So the total sample size is 1200, including 600 female sex workers and 600 general women, respectively. We have now revised the text accordingly (Page 6, Para 2).

A frequency matching method was employed in the study design, which involves matching an entire stratum of reference subjects with an entire stratum of index subjects based on the matching factors. In the present study, the criteria for the matching factor is broad, for instance matching females whose ages are between 18-65 year old. Clearly a subject (such as, FSW) in such a scenario can be matched with control subject (such as, general women) from that stratum and hence making this frequency match easy conducing and high efficient. (Please see the reference below).

Reference


iii) The cutoff value applied in the study was as suggested by the manufacturer. An index value of $>1.10$ was considered positive; $\geq 0.90$ and $\leq 1.10$ was equivocal and;
<0.90 was considered negative. The specific information of this product is also available on the Manufacturer’s website (http://www.focusdx.com/pdfs/pi/OUS/EL0920G.pdf).

iv) Previous researches have shown that univariate analysis alone is not sufficient, multivariate techniques are mandatory (please see reference 1-2). Given that potential confoundings may also have influence on KSHV seropositivity in this study, we further carried out multiple logistic regression by forcing potential influencing factors into the final model. To make it clear, we rephrased the description in the statistical analysis section (Please see Page 10 Para 1.).

Reference:


Comment 3. Results: i) The comment on Page 15 at the end of Paragraph 1 (“The consistent observations among MSM studies in China…”) is dubious. Although KSHV prevalence is higher among MSM and associated with men who engage in receptive anal intercourse, I don’t think it is correct to state that KSHV infection is associated with “homosexual activities, especially receptive anal sex acts,” but rather that KSHV/HHV-8 is more common within MSM sexual networks. None of the studies cited provide any evidence to suggest that specific sexual acts are more likely to result in HHV-8 transmission.

Response: i) We thank the reviewer for his comment. In the revised manuscript, we have rephrased the expression in the discussion and tried not to overstate the homosexual transmission of KSHV (Please see Page 16, Para 2 to Page 17 Para 1).

Comment 4. Conclusions: Again, the authors don’t make a convincing case of the importance of their findings. The impact of HHV-8 infection on health outcomes
among non-MSM populations is rather small, and so it is not clear that additional public health efforts to control the infection are needed. Instead, it may be helpful to outline how this analysis contributes to the understanding of patterns of transmission of different viral infections and differentiating STIs from other viral infections.

**Response:** Although KSHV/HHV8 infection seems to cast relatively smaller impact on other groups when compared with MSM, previous researches have shown that when person infected with both HIV and KSHV concurrently, the outcome could be serious, including manifestation of KS etc.. Moreover, female sex workers had a higher risk of HIV infection in China and other countries. Thus far, to explore KSHV infection status and its transmission routes is of public health importance in this population. The present study could better the understanding of KSHV epidemiology in China and shed a light on the mystery of this virus. Per reviewer’s suggestion we have revised in the INTRODUCTION section, and have further discussed this issue in the DISCUSSION section. Please see Page 4 Para 1. to Page 5 Para 2 and Page 14 Para 2 to Page15 Para 1.

Meanwhile, we have also talked about the pattern of STIs and KSHV further in the discussion section. Please see Page 15 Para 2 to Page 16 Para1.

**Comment 5.** Tables: In general, the Tables present a large amount of data with little apparent consideration of what information was important to include in the analysis of in the presentation of the results. Table 3: What criteria were used to design the multivariate regression model? It appears that the authors simply included all of the variables available to see what came up significant.

**Response:** We apologize for not being clear. The variables included in the multivariate analyzed were based on our prior knowledge and previous literature search. Previous reports have shown that it is not always appropriate to select only those variables that are "statistically significant (usually P<0.05)" in the univariate analyses into the final multiple regression model for adjustment. Odds ratios and "p-values" for each variable in the univariate analyses might be biased estimates due to potential confounders. (Please see the reference)
Thus, all the potentially influencing factors were forced into the final model for adjustment. Such a research strategy is probably appropriate when we have no logical or theoretical basis for considering one variable over another, either in terms of a hypothetical causal structure of the data or in terms of its relevance to the research goals. In the present study, our aim was to explore the potential risk factors for KSHV infection among study population, thus it is reasonable to employ this independent variable forced entry method. To make this clear, we have revised the statistical analysis section and mentioned the forced entry of potential variables. Please see the Page 10 Para 1.

Reference:


Comment 6. General: The manuscript should be carefully edited by an English speaker to correct spelling and grammatical errors.
Response: We have requested a native English speaker to proofread and edit the manuscript carefully to correct grammatical and typographical errors.

Comment 7. Minor Essential Revisions.
i) Table 2: “Years of prostitution” Row 3 reads “>56”
Response: Thank you for your careful review. We apologize for the typographical error and now it has been changed into “>5”(Page 29, row 3).

ii) Table 3: Row 5: “Monthly Income (Yuan RMB, P<0.001)” Why is a p-value
included in the title of the row?; Row 7 “Number of sex partns in the past 12 months” should be “partners”

**Response:** We apologize for the error. We have removed a P-value from the table. Meanwhile, the ‘Number of sex partns in the past 12 months’ has been changed into ‘Number of sex partners in the past 12 months’.

**Reviewer 3 (Dr. Anne Buve)**

**Comment 1.** Page 6, paragraph on sampling. I am not sure what is meant by “Participants from these sites were recruited until expected samples were finally met”. Does this mean that the research team visited households till the required sample size was reached? That procedure seems to contradict random sampling of households.

**Response:** We apologize for not being clear. In the present study, a multiple stage sampling method was applied to recruit study participants. Initially, four townships were randomly selected from the total of 12 townships in Minhang district, Shanghai, China. Second, the working venues for both female sex works and general women were sampled, respectively. Finally, all the individuals working in these selected sites, were invited to attend the present study consecutively. Only those who gave their consent were actually interviewed till the required sample size was eventually reached. The first and second step were random, and third were not. The similar procedure has been employed by other researchers (Please see the reference).

We have added more details in the method section of the revised manuscript (Page 6 Para 2 and Page 7 Para 1).

**Reference**

Comment 2. Page 8, last paragraph on KSHV serology. Can you please provide some data on the sensitivity and the specificity of this serological test?

Response: Thank you. We have evaluated the performance of serological test of KSHV. The sensitivity and specificity of the IFA test applied in our study was 93.9% and 96.3%, respectively. We have cited a previous report in the method section as well (Please see the reference below). Please also see Page 9 Para 2.

Reference:


Comment 3. Page 9, statistical analysis. How were the variables for the multivariate analysis selected? On the basis of the p value in the univariate analysis?

Response: Thank you for your question. Initially, univariate analyses were conducted and followed by a multivariate analysis with ‘forced entry’ of all variables examined in the univariate analyses into the multivariate regression model. These variables were considered to be potential confounding variables based on our ‘prior knowledge’ about the causal relationship between risk factors or independent variables and the dependent variable as well as the significance of examined variables in univariate analyses.

To make this point clear, we have rephrased the statistical analysis please see Page 10 Para 1.

Comment 4. Page 11, last paragraph, last sentence. This suggests that the databases of the female sex workers and the women in the general population were merged and that a multivariate analysis was done on it. If that is the case that should be explained in the methods section.

Response: Thank you for your suggestion. As per the reviewer’s request, we explain this merge in the statistical analysis of methods section (Page 10 Para 1).
Comment 5. Table 2 and Table 3. Please also show the univariate analyses.

Response: Thank you for your suggestion. Given the limited space, we show the multivariate analysis results in the table. Per reviewer’s request, univariate analyses are shown as an additional file.

Comment 6. DISCRETIONARY REVISIONS
Discussion section. Could the authors comment on the difference between HSV-2 and KSHV serology regarding trend with age. Many studies, including this one, have documented an increase in HSV-2 prevalence with age. This is explained by the fact that HSV-2 infection is a lifelong infection and that acquisition goes on throughout life. There is no such trend for KSHV. What do the authors make of this? Is KSHV serology also lifelong? If that is the case could one say that a KSHV prevalence that does not increase with age is suggestive of vertical transmission of KSHV?

Response: Thank you for your interesting comments.

All herpesviruses establish lifelong infections. Previous data have shown that HSV2 infection increase with age and several studies have shown it to be true for KSHV infection too (Please see references below), especially in endemic areas where primary infection occurs during childhood. Vertical transmission of KSHV among children is indeed possible as shown by Mantina et al (International Journal of Cancer). In the present study, likely due to a relatively narrow age range of the sampled population we did not observe and increase in KSHV seropositivity with age.

Reference:
