Author's response to reviews

Title: Seroprevalence and risk factors of herpes simplex virus type-2 infection among pregnant women in Northeast India.

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Version: 2 Date: 18 May 2011

Author's response to reviews: see over
Reviewer’s report and authors response:

Title: Seroprevalence and risk factor of herpes simplex virus type-2 infection among pregnant woman in Northeast India.

Version: 1 Date: 7 January 2010

Reviewer: Christine Johnston

Reviewer’s report:

This is a well conducted study of HSV-2 seroprevalence in antenatal clinics in Northeast India. It provides needed data about HSV-2 seroprevalence and elucidates risk factor for HSV-2 seropositivity in this region. The methods for determining seroprevalence and the statistical methods require some clarification; otherwise a solid paper which answers an important question.

Major Compulsory Revision:

1. **Methods**: specimen collection: I have some concerns about using the Focus ELISA in this low prevalence population, particularly because it can have a low positive predictive value (PPV) when index values are <3.0 (Golden STD, 2005). Higher index values may have better specificity and PPV (but potentially at the cost of sensitivity). Therefore it is important to know what was used as a cut-off in this study. What cut-off was used to determine a positive test on the Focus ELISA? How were equivocal samples handles? If a higher cutoff was used how would your results change? Please add how you determine a positive value and consider a paragraph in the discussion about the use of the Focus ELISA.

Reply: The cut-off value used in this study to determine a positive test on Focus ELISA was >1.10. Index value between 1.1 and 0.9 were considered equivocal and index values below 0.9 were considered negative as per the kit instruction manual. The PPV (74.0%) and NPV (99.6%) in expectant mothers at 10% prevalence are mentioned in the instruction manual with the kit. Higher cut-off was not used.

Five equivocal samples were re-tested with the same kit and found to be equivocal in the second repeat test. So, we collected second samples from the subjects after 1-2 months and found three samples to be positive while two samples were still negative in the retest.

The relative specificity of the assay as compared to Western Blot assay with a low prevalent population (as per instruction manual with the kit) was shown to be 98.7% and relative sensitivity 100%, however higher cutoff value would falsely reduce the prevalence in this setting.

As advised, a paragraph regarding determination of positive ELISA result has been added in the methodology section.
2. Methods: Statistical analyses: I have several question about the univariate and multivariate analysis

a. Please be specific about how odds ratios were calculated (ie logistic regression). Please calculate p-values for groups, especially when using continuous variable (age) rather than for each category.

Reply: Odds ratios were calculated using Binary logistic regression analysis where first category (having lowest seroprevalence) was used as a reference in each group. In the analysis age was used as categorical variable i.e. code 1 for 18-21yrs, 2 for 22-25yrs. 3 for 26-29yrs and 4 for more than 29yrs. However, as per suggestion from the Associate editor, in general as long as outcome (HSV seropositivity) is rare (<10%) the use of odds ratio is appropriate.

b. The univariate analysis includes many fewer variables then collected in the questionnaire. How did you decide to include only these variables? Where all variable tested? For instance, it seems that FSW or exchange of sex for money would be important risk factors to consider, but these are not included.

Reply: We have included only the most relevant variables in the report to avoid unwanted large tables. Also, some of the variables with poor response or insignificant were not included to avoid misinterpretation in statistical analysis, for instance out of 1640, only 8 subjects disclosed to have sex for money which has low power for proper interpretation. Moreover, some of the questions are for knowledge attitude and behavior study which is not relevant for this paper.

c. Multivariate analysis: I am unclear whether the multivariate model includes only age or if it includes all categories. Please clarify in methods, If only age was included in the multi-variate model, why? Were there attempts to build a more complex multivariate model using all significant co-variates? Were there attempts to look for interactions (for instance, state and religion). I don’t think the multivariate model, as it is now, alters any of the results from the univariate analysis, and is not that informative.

Reply: We had used binary logistic regression where only age was adjusted. But now as suggested we have replaced the age adjusted table with generalized binary logistic regression (multivariate analysis) data. We used the Wald Backward elimination method with probability for stepwise entry at p=0.05 and removal at p=0.1. This is now mentioned in the method […] However, some of the variables like religion became insignificant due to confounding interactions with the State.

3. Results: Give numbers in addition to percentages (ie literacy rate: xx (84.5%) etc. A Table 1 with n (%) of general demographic and OB history of your population may be considered.
Reply: As per suggestion a new table with demographic variables has been added [TABLE 1].

4. Results: Seroprevalence: could give 95% confidence intervals around the overall seroprev and the seroprev per state

Reply: As per suggestion 95% confidence intervals has been added in results.

5. Results: Seroprevalence: The difference in Seroprevalence among the states is very interesting: in this study “state” may be the most significant risk factor for HSV-2 seropositivity. Are there differences in these states (religion, male circumcision rates, education) that could explain the differences (see comment 6 in minor essential revisions) - could include in results or discussion?

Reply: As per suggestion, now we have included in the results and discussion [....] There are socio-cultural differences amongst the states which might have resulted in significant state wise difference in prevalence. One important difference is in prevalence with religion (3.8% in Muslim compared to 12.6% among Christians), which may be due to practice of male circumcision at birth amongst the Muslims. Male circumcision lowers the prevalence of HSV-2 or HPV has been reported in some studies. This has been added in the discussion section with reference.

6. Results: Seroprevalence: Age: Multiple epidemiologic studies have shown increasing HSV-2 Seroprevalence with increasing age. The results in this study that HSV-2 “peaks” in the 22-25 year age group seems artificial, and the lack of significance in the > 29 yr group seems to be due to lack of power. How were the age strata chosen? Would it make more sense to keep age as a continuous variable? If you keep it as is, use an overall p-value for the category.

Reply: In our study, the overall prevalence has increased with age (6.5% to 9.4%), but observed a peak in prevalence (10.5%) in the age group 22-25 years. This may reflect a recent introduction of HSV-2 in the community which may have resulted in higher prevalence among sexually active younger subjects rather than the older subjects which didn’t had the exposure to the virus when they were sexually more active in the past. The age strata were distributed at 4years interval taking into consideration to have almost equal distribution of subjects in each group. We prefer to keep age in the categorical variable as it gives age specific prevalence. The overall p value from chi square test for age as a continuous variable is $p = \ldots$ which is mentioned in the text.

7. Income group: Few in high incomes, would it make sense to combine with middle income for more power?

Reply: As per suggestion, we have now combined the high income group and middle income group into one group and have performed re-analysis of the data. The old data is now replaced with the new data in the tables (Table 2) and text as required.
8. Results: Seroprevalence: give numbers for comparisons/95% CI or p-values for 1st compared to 2nd/3rd trimester and GUD compared to no GUD

Reply: As suggested by the reviewer we have now included 95% CI and numerators and denominators for comparison of the mention variables.

Minor Essential Revisions:
Abstract:
Background: 1st sentence Do not capitalize Simplex Virus Type-
Reply: Corrected as suggested.

Methods: Last Sentence: verb: were instead of was… risk factors associated with HSV-2 seropositivity –
Reply: Corrected as suggested.

Results; 1st paragraph: second sentence: Missing verb: was Include comparison group for “Middle income group” (compared to low income group) –
Reply: Corrected as suggested.

Last sentence: genital is misspelled –
Reply: Corrected as suggested. Also, we have reconstructed the last sentence of the Result.

Discussion/reference: Ref 25 could be updated-
Reply: Updated as suggested.

Discretionary Revisions:
1. Abstract: conclusions: Consider removing statement that “routine antenatal screening for HSV-2 may be helpful for safe pregnancy outcome”. This is a controversial statement and there is no data in the current study regarding this issue –
Reply: Removed statement as suggested.

2. Background: The HSV-2-HIV link is mentioned in several parts of the first paragraph. To tighten the paper, I suggest discussing Seroprevalence of HSV-2 in the first paragraph (and perhaps including more information about HSV-2 Seroprevalence studies in India), and the HSV-2/HIV connection in the second paragraph.
Reply: As suggested by the reviewer, we have re-composed the first paragraph to include seroprevalence of HSV-2 with reference studies from India. The second paragraph has also been re-composed to stress the link between HSV-2 and HIV.

3. HIV prevalence rates in Northeast India, particularly in antenatal clinics, would be helpful to include, if available, to give the reader a sense of the burden of HIV in this population.
Reply: As suggested by the reviewer, we have included HIV prevalence in antenatal clinics in northeast India from data from the National AIDS Control Society (NACO) India.

4. Results: Risk factors: The authors state throughout the paper that women with “multiple” sex partners have higher risk of HSV-2. Rather than multiple state “more than one in past year”. It would be most helpful if you had lifetime sex partners- is this available?

Reply: Since, ‘multiple sex partner’ or ‘more than one sex partner’ reflects the same meaning, we feel the word ‘multiple’ can be retained. We don’t have data on lifetime numbers of sex partners. Only the number of sexual partners in previous one year is available. However, we feel that recent sexual behavior will reflect modestly the overall promiscuity.

5. Discussion, 2nd paragraph: Results of this study are different from US, in that in India HSV-2 is associated with higher SES rather than lower. Care to speculate about these differences?

Reply: The reasons may be multi-factorial. We may not be able to compare directly with US population as there is complete difference in socio-economic status of the two countries.

6. Discussion, paragraph 4: As above (comment 3), the difference in Seroprevalence rates by state is intriguing and it may be interesting for the authors to tease out these differences in the multivariate model and/or speculate on reasons for these difference.

Reply: As suggested, we have now used a generalized multivariate regression analysis. However, due to confounding interactions the variables like religion became insignificant. The difference in seroprevalence of the states that we have found is probably due to difference in religion. As mention earlier, there is difference in prevalence with religion (3.8% in Muslim compared to 12.6% among Christians), which may be due to practice of male circumcision at birth amongst the Muslims. Male circumcision lowers the prevalence of HSV-2 or HPV has been reported in some studies. This speculation has now been added in the text.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no completing interests.
Reviewer’s report

Title: Seroprevalence and risk factors of herpes simplex virus type-2 infection among pregnant women in Northeast India.

Version: 1 Date: 13 January 2010

Reviewer: Joshua Schiffer

Reviewer’s report:

This paper is a cross-section Seroprevalence survey in NE India. The authors correctly point out that HSV-2 is a good marker of sexual risk, and may be an independent risk factor for HIV-1 acquisition and transmission. The paper is important because NE India is the current site of an important HIV epidemic. Behavioral surveys do a poor job of estimating sexual risk. Therefore, an HSV-2 serosurvey can be a valuable tool to measure HIV-1 epidemic potential. Indeed, this survey identifies that there is some degree of risk in this population.

However, there are several major issues:

Major compulsory revisions:
First, there is no limitations section describing the many limitations of deriving odds ratios from a cross-sectional survey in which cases are not matched with controls. It should be emphasized in the discussion that the study’s findings are hypothesis generating only. This paper would be considerably stronger if the authors performed a case-control analysis to better define risk factors for HSV-2. At minimum a reasonable justification for not doing so should be included.

Reply: As suggested by the reviewer we have now added a sentence on the limitations of the study in the methods [...]. Odds ratios were calculated using Binary logistic regression analysis where first category (lowest seroprevalence) was used as a reference in each group. As recommended by the assistant editor, generalized binary logistic regression can be applied where outcome is rare i.e. <10% so odds ratios seems to be appropriate for our data (8.7%). Since, there is wide socio-cultural diversity among the five states studied; matched controls which can suffice for all the study groups will be difficult and can be erroneous or biased. We have now added this as a justification for not analyzing our data as case-control study.

Second, a major point of the paper that should be emphasized is the lack of a typical age-cohort effect where the oldest members of the population have highest Seroprevalence. This is somewhat of a rare and interesting finding for a chronic infectious disease and imply that HSV-2 is a relatively recent addition to the population. It also means that the current Seroprevalence of HSV-2 may be a great measure for HIV-1 risk in this community, and that HSV-2 incidence (which is more difficult to measure and probably a better measure of risk), may actually be high.
Reply: As suggested by the reviewer, we have noted this important point and have added a sentence in the discussion probing this angle as suggested by the reviewer. This may reflect a relatively recent introduction of HSV-2 in the community which may have resulted in higher prevalence among sexually active younger subjects rather than the older subjects which didn’t had the exposure to the virus when they were sexually active in the past.

Third, the authors need to speculate on the following: Why is reported genital ulcer disease so low? Potential reasons include cultural stigma of reporting genital ulcers, genetic factors that limit severity of disease, or an inadequate questionnaire/interview. A clinical point of the paper may be that patients and clinicians under recognize herpes lesions: this has been widely observed in other populations.

Reply: We feel that there is under reporting of GUD due to socio-cultural stigma that is related to sexually transmitted disease. In our study, among the 19 GUD cases from 1640 subjects, 15 GUDs has been confirmed during clinical examination by the concerned doctors. Since clinical examination in this cross-sectional study is at most reflecting a somewhat point prevalence of GUD rather than over a period of one year as outlined in the questionnaire. The finding of genital ulcers coinciding with HSV-2 IgG antibodies (the measured criteria in our study) may not always be linked at one point in time. Since IgG antibodies may be detected for life-long whereas genital ulcers are transient. A sentence has been added in the discussion on the limitations of this finding.

Fourth, is there an obvious difference to explain the differences in HSV-2 prevalence across Indian states? This interesting finding survives multi-variate analysis. Are there data on sexual networks in different provinces that may explain this? Please speculate.

Reply: All the five studied states have a diverse cultural and socio-demographic population. So, differences in HSV-2 prevalence may be expected. We have included one new Table with socio-demographic variables to reflect the diversity in the population. The difference in seroprevalence of the states that we have found may be due to religion. There is a difference in HSV-2 prevalence with religion (3.8% in Muslim compared to 12.6% among Christians), which may be due to practice of male circumcision at birth amongst the Muslims. Male circumcision lowers the prevalence of HSV-2 or HPV has been reported in some studies. This speculation has now been added in the text.

Fifth, what is a joint family, and why is this group at lower risk than members of a nuclear family?
Reply: A joint family (or extended family), parents, their children and children's families often live under a single roof. This type of family often includes multiple generations in the family whereas; a nuclear family is a family group consisting of father, mother and their children, who share living quarters. We speculate that in joint family cultural bondage and strict behavioral practices are usually followed which may indirectly limit the exposure to high risk sexual behavior as
compared to nuclear family. A sentence has added in the discussion section speculating this finding.

Finally, there are several grammatical errors that are listed below, though some may have been missed.

Minor revisions:

In the background:

• The statement that HSV-2 facilitates spread of HIV-1 among low-risk population needs at least one, and preferably multiple citations.
  Reply: Included citations as suggested.

* Despite a very nice list of cofactors in Table 1, the reader does not emerge with a general feel for the population demographics in the region. This would help frame the paper. For example, is there a mobile or static labor force? What population of the HIV-1 epidemic is thought to be related to IDU behaviors? Is female sex work common? Are there broad cultural differences between provinces? Are there well-defined sexual networks? Is it common for younger women to engage in sexual debut with older men? A one-paragraph overview of these points would strengthen the paper. Several papers by Laith Abu- Raddad have taken a similar approach in the Middle East: these descriptive are vital for the reader who is unfamiliar with the region.
  Reply: A new table on socio-demographic variables has been added as per suggestion. We found that 11.9% (192/1640) of the subjects were migrant population. This has now been included in the new TABLE 1 and also mentioned in the paragraph on description of the study area. In northeast India, the epidemic of HIV-1 is driven mainly by the IDU population in the State of Manipur, Mizoram, and Nagaland. The state of Manipur has the highest HIV prevalence in the country (1.67% in 2006). In a recent study from this Institute, the HIV-1 prevalence was found to vary between 23%-32% among the IDUs of Manipur. FSWs are common in scattered locations in few states of northeast. One recent study from this Institute shows a HIV-1 prevalence of 13.6% in FSWs of Nagaland, a state in northeast India. The population of northeast India is heterogeneous population and there is a huge socio-cultural difference among the studied states, so differences of HSV-2 prevalence were not unusual. There is no sexual behavioral data on the issue of younger women engaging in sexual debut with older man from the study area. A paragraph on description of the study region has now been included as advised by the reviewer.

In the methods:
• It should be explained why women under 18 were excluded, as adolescents may be a key population to study. If seroprevalence were high among this group, this would be a critical finding.
  Reply: We had excluded women under 18 years because of difficulty of taking consent for enrollment in the study from minors (i.e. <18 years). Also, since this
study was performed in married antenatal women and the legal age of marriage in India is 18 years and above, so adolescents were not included in the study.

• Under data collection, “etc”. Should be removed. All demographic data that were queried should be listed. If the authors selected possible risk factors a priori, then this lends more credibility to the analysis. This should Laos be stated in the methods.

Reply: As suggested by the reviewer ‘etc’ were removed. Demographic data queried were listed. Though we had selected many risk factors associated with sexually transmitted infections, we were not able to analyze that data due to low response on some of the sensitive queries.

• The study should be classified specifically as a cross-sectional study. Odds ratios derived from cross-sectional studies are more tenuous than those from case-control studies.

Reply: As suggested we have now specifically mention the study as a cross-sectional study in the method. Though, case-control studies are ideal for calculation of Odds ratios, but Odds ratios derived from cross-sectional study using generalized binary logistic regression can be used in case of data whose outcome is rare (i.e <10%) as mentioned earlier in a previous query.

In the result:
• The seroprevalence should show numerator and denominator for each of the 5 states.

Reply: Numerator and denominator added for all values.
• The seroprevalence should be listed for all 3 trimesters and denominators.

Reply: As suggested seroprevalence with denominators is now listed for all the trimesters.

• The seroprevalence should be listed for those with and without GUD with numerators and denominators.

Reply: As suggested seroprevalence with numerator and denominators is now listed.

• Comparisons of prevalences between groups should include p-values.

Reply: p-values included as suggested.

• The very low number of study participants who reported history of genital ulcers (19/1640) is fascinating given the moderate HSV-2 seroprevalence. Is data available from gynecological exam on the data of this visit? It would be interesting to see what % of pts had GUD on the day of the exam. Typically, recognition of GUD by patients increases with focused clinician education: however, this is an intensive process that is quit rarely done in most clinics.

Reply: In our study, among the 19 GUD cases from 1640 subjects, 15 GUDs has been confirmed during clinical examination by the concerned doctors. This data reflects typically point prevalence of GUD (0.9%, 15/1640) rather than over a period of one year. A sentence in the discussion has been added in this context.
In the discussion:
• The authors appear to misinterpret the meaning of peak seroprevalence at age 22-25 this in fact contrasts with most other studies of HSV-2. Typically, during a stable epidemic as in Africa, Europe and the US, seroprevalence increases linearly with age. This is not surprising as people accrue infection over time. However, this study shows a peak (albeit a blunted peak) within a relatively young age cohort (22-25). The implications are that HSV-2 is reasonably new in the young population, and the epidemic is an expansion phase in NE India. The authors should speculate on why this is the case. The probable explanation is changing sexual habits and network structure among the generation.

Reply: In our study, the overall prevalence has increased with age (6.5% to 9.4%), but observed a peak in prevalence (10.5%) in the 22-25 years age group. As observed by the reviewer, this may reflect a recent introduction of HSV-2 in the community which may have resulted in higher prevalence among sexually active younger subjects rather than the older subjects which didn’t had the exposure to the virus when they were sexually more active in the past.

• In regards to the above, a key unreported measure of local HSV-2 epidemiology is incidence rather than prevalence. It can be a devilish task to identify the true incidence as this is a non-linear measure requiring multiple serial data point. Yet, the lack of an age-cohort effect may imply high incidence among young women, and early epidemic stage despite seemingly low HSV-2 prevalence. A follow up seroprevalence survey, as well as a partnership with mathematical modelers, would be useful to confirm this expansion. This concept should be included in the discussion.

Reply: The reviewer’s suggestion has been taken into account and we have now added the speculation that HSV-2 epidemic may be at an early stage in this population considering the high prevalence among young women.

• The high seroprevalence cited in ref 30 (33.2%) needs to be explained in reference to the lower prevalence reported throughout India. Is this due to inclusion of other countries, including SE Asia?

Reply: The South Asian countries included in the review article by Looker KJ were Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, Sri Lanka and the studies were from general population. The 33.2% is a consolidated prevalence among females in the 15-49 yrs age group of South Asian countries. There might be wide divergence in prevalence among the South Asian countries.

The paper also had several grammatical errors, some of which are listed below. It will need closer inspection prior to publication.

Abstract:

Last sentence in background should read “located in NE region of India. Due to lack of….from this part of the country, there is a need…”
Reply: Corrected as suggested.
Last sentence in methods should read: “…analyses were performed…”
Reply: corrected as suggested.

2nd sentence in results should read: “Higher seroprevalence was observed…”
Reply: corrected as suggested.

Background:

1st sentence should be “…infection is almost always sexually transmitted and is…."
Reply: corrected as suggested.

Methods:

In “Specimen collection” section, it should read “Plasma samples were subjected to…”
Reply: Corrected as suggested.

Result:

Should read “The majority of subjects were house wives…, belonged to the nuclear family, and from…."
Reply: Corrected as suggested.

Discussion

Should be… “…seroprevalence found in this study has also been observed in earlier studies.”
Reply: corrected as suggested.

Should be: “inner-city communities”
Reply: corrected as suggested.

Should be: “was reported to be 33.2%”
Reply: corrected as suggested.

Should be “higher coitarchal age was associated with a lower chance…”
Reply: corrected as suggested.

Should be “higher seroprevalence was found among Christians versus Muslims, condom non-users versus etc…”
Reply: corrected as suggested.

Should be “Modifiable risk factors included low coitarchal age, low condom usage, multiple sex partners, and alcohol use…”
Reply: corrected as suggested.
**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests

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**Reviewer’s report**

**Title:** Seroprevalence and risk factors of herpes simplex virus type-2 infection among pregnant women in Northeast India.

**Version:** 1  **Date:** 4 January 2011

**Reviewer:** Stuart Berman
Reviewer’s report: This paper is for the most part, a clearly written, description of HSV-2 seroprevalence among population of pregnant women. Unfortunately the authors leave out important of details of their evaluation, seem unaware of recent major publications regarding the interaction of HSV and HIV, and don’t provide a reasonable rationale for the study. Unfortunately, in its current form, there is little reason to recommend it for publication.

The following are the issues I find with paper and which must be addressed:
1) Under methods, the authors indicate that this was performed as a research study – with informed consent in all “selected tertiary hospitals” of five northeastern states. But we have absolutely no idea what sort of population are seen in these facilities, if they are at all representative of pregnant women in general, and (perhaps most importantly!), how many eligible women there were in the 2 years of the study – what % were offered enrollment and what % accepted. And if the % wasn’t very high, how did the participants differ from the overall population of pregnant women. Without such information, the reader has no idea who the data pertain to!

Reply: The tertiary hospitals cater all sections of the population and represent the general population of pregnant women in the respective states. There is a scheme for economic benefit to the patients for hospitalized deliveries in tertiary hospitals so majority of the delivery takes place in those tertiary hospitals. We had enrolled around 300 subjects from each tertiary hospital and it represents averagely between 2-10% of the antenatal clinic attendees across the tertiary hospitals. We have now included a paragraph in the methodology focusing the demography of the population of the study areas and also have added a new table on socio-demographic variable to make a feel of the population to the reader.

2) How, more precisely, was the multivariate model done. How were the variables coded? I ask this because there are a lot of variables listed in table 1, with numerous coding options. And several of the categories have very few observations (ie “high income” n=45; “single” status n=74; “business” as occupation n= 45. Trying to include all these categories in the model can be quite destabilizing. Why weren’t adjusted values for “age” provided? Frankly, the analysis is quit unsatisfying: given the considerable variation State, it would have been more useful and informative to analysis the data by state, providing stratified seroprevalence data by age, by religion, for the states.

Reply: We had initially done age-adjusted binary logistic regression analysis. However, as per suggestion of other reviewer, we have now performed a generalized binary logistic regression analysis. Now, as per reviewer’s suggestion adjusted (multivariate logistic analysis) values for all the variables including ‘age’ has been included in the modified table. The first category in each variables (lowest seroprevalence) were the reference category required to calculate the Odds ratio. As per suggestion of one of the reviewer we have converged the data on income into two categories i.e. Low income and Middle & high income group. However, we feel that it would be difficult to converge the
categories in ‘Occupation’ and ‘Type of family’, so we have kept them as such. The old data is now replaced with the new data in the tables and text as required. We also added a sentence on the methodology stating the coding of the variables […]. We agree with the reviewer that stratified seroprevalence data by age, religion for the state would be more informative. In this context, we have included a paragraph in the result section.

3) The authors include primarily older references – seemingly unaware of the important, landmark studies recently published (ie by Celum etc) that demonstrated that antiviral Rx of HSV-2 didn’t reduce HIV acquisition among those who were HIV (-)/HSV-2(+) nor of the study demonstrated that acyclovir didn’t reduce HIV transmission when provided to the dually-infected HIV(+)partner in HIV discordant couples.

   b. Celum C: Lancet 2008; 371:2109-2119;

Reply: As per suggestion, we have now included citations of the above mentioned studies. Though clinical trials in Africa on use of acyclovir did not reduce the risk of linked transmission of HIV-1 to couples, however it showed a reduction of plasma HIV-1 RNA level as well as 73% reduction in occurrence of genital ulcers due to HSV-2 [Celum et al, NEJM 2010]. The data from such initial controlled trials with HSV-2 suppressive therapy with acyclovir 400 mg twice daily showed no evidence of decreasing the incidence of HIV in Tanzania also. (Watson-Jones D et al. NEJM 2008; 358; 156-1571). However, we would like to point out that this recent study by Watson-Jones et al, had enrolled female workers who were HIV-seronegative and HSV-2–seropositive in a randomized, double-blind, placebo-controlled trial of suppressive treatment with acyclovir. The primary outcome was the incidence of infection with HIV. We think that the design of the controlled trial of enrolling HSV-2 seropositives for suppressive therapy may not yield the true outcome from such intervention as seropositives mostly may not have active disease process nor do they have GUD or virus shedding regularly. Instead, the study would have been more relevant if they would have enrolled subjects based on genital viral shedding also apart from seropositives. So, we cannot discard the role of HSV-2 suppressive therapy, until more conclusive data are available on this.

4) They fail to include the more recent and comprehensive studies assessing the HSV-2 contribution to HIV transmission (meta analyses by Freeman EE AIDS 2006;20:73-83) who went considerably beyond Wald (ref 4).

Reply: As suggested by the reviewer, we have now included the above mentioned citation.

5) The discussion about just what might be possible or appropriate with regard to HSV prevention is essentially nonexistent, with the authors not only failing to cite the above research, but also not citing modeling studies that could provide some direction (White RG STI 2008; 84(suppl2)L:ii12-ii18) or the discussion by Douglas STD 2009;
39: 729-731) (Note: the format for the references is inconsistent --- consider refs 28-31).

Reply: Reference has been rechecked and corrected. We now have included a sentence in the discussion speculating the possibility of prevention of HSV-2.

6) There is no mention of findings regarding circumcision or the CAPRISA tenofovir trial (microbicide gel) and their impact on HSV-2 acquisition. Moreover, the data that are presented aren’t particularly useful, given the authors intent to inform prevention and “advocating HSV-2 awareness” (Again, the authors concluding statement regarding “suppression by antiviral drugs in high HIVHSV-2 areas…” is not reassuring).

Reply: We have now mention the study by Tobian et al (N Engl J Med 2009;360:1298-309) on the role of circumcision on HSV-2 acquisition.

Few other issues that should be addressed:
1) Odd findings: prevalence highest among 22-25 – greater than among those >29. (Not typical – again speaks to questions about just who constitutes the tested population). Likewise the OR > 10 for those with early coitarche.

Reply: In our study, the overall prevalence has increased with age (6.5% to 9.4%), but in our case there was a peak in prevalence (10.5%) in the 22-25 years age group. As observed by the reviewer, this may reflect a recent introduction of HSV-2 in the community which may have resulted in higher prevalence among sexually active younger subjects rather than the older subjects which didn’t had the exposure to the virus when they were sexually more active in the past. Early coitarchal age may be one of the high-risk factors for HSV-2 acquisition. It would be difficult to explain from our study, but we speculate that early coitarchal age reflects a much more sexually active life compared to those that have initiated sexual activity at an older age. A sentence in the discussion has been added to highlight this speculation.

2) What exactly were the subjects asked with regard to condom use (it is just not adequate anymore to have this be a “yes/no” question – too complicated an issue).

Reply: The subjects were asked about regular condom use in every sexual intercourse, occasional use or never. We have converge the data of occasional use and regular use into one category and compared it to those who has never used condoms.

3) The data presented in table 2 – on the handful of individuals with symptoms isn’t very useful – and who are included in the category “cervicitis” – this table and the text suggests “symptoms” not findings. And cervicitis isn’t a symptom. The authors should be clearer here.

Reply: Cervicitis as mentioned here reflects the finding of inflammation of the cervix as recorded by the Gynecologist during clinical examination. In the Table 3 we have now changed the heading as Sign and symptoms of the subjects.
4) P4/para2: “Since up to 70% of genital herpes infections are recognized (14)” – and the reference is an online article from Medscape. When well-established population-based studies (NHANES etc) have shown that more typically 90% of those with HSV-2 infections haven’t been diagnosed. (I appreciate that the authors are looking for data about pregnant women --- but the purposes the authors give for the study are more grandiose, and suggest an effort to inform broader approaches, not limited to a focus among pregnant women).

Reply: As suggested we have now added a sentence in the discussion regarding the finding of the NHANES study.

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests.