Author’s response to reviews

Title: The epidemiology of sexually transmitted co-infections in HIV-positive and HIV-negative African-Caribbean women in Toronto

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Author’s response to reviews: see over
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Editor
BMC Infectious Diseases
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236 Gray's Inn Road
London WC1X 8HB, United Kingdom

Re: Manuscript entitled “The epidemiology of sexually transmitted co-infections in HIV-positive and HIV-negative African-Caribbean women in Toronto”

Dear Sir/Madam,

Thank you very much for considering our manuscript. We have revised the manuscript in response to the reviewers’ comments.

Attached, please find:

• Authors’ responses to reviewers’ comments and, as appropriate, description and location of changes made. Please note the reviewer’s comments are in “bold” and our responses are in normal font.
• Revised manuscript with tracked changes
• Revised manuscript in final format

Sincerely,

Robert S. Remis MD, MPH, FRCPC
Response to Reviewers’ comments

Reviewer's report 1

Title: The epidemiology of sexually transmitted co-infections in HIV-positive and HIV-negative African-Caribbean women in Toronto

Version: 1 Date: 26 July 2013

Reviewer: Thomas Peterman

Reviewer's report:

1. This paper describes the results of extensive STI testing of African and Caribbean women recruited from a community health center in Toronto. A strength of the paper is the large number of tests that were done. One weakness, mostly acknowledged by the authors, is that STI incidence varies by age, and a cross-sectional study can tell about cumulative risk for viral infections, but only recent risk for bacterial infections. The median age of HIV-positive women is 40 and HIV-negative women is 31, so both groups are beyond the usual high-risk age for chlamydia or gonorrhea.

The reviewer’s observation with respect to age distribution of the participants is correct. Nevertheless, we had enough participants in the younger age strata to examine prevalence of these pathogens among younger women, at least for HIV-negative women.

I have several suggestions for minor and discretionary revisions:

2. I think the authors should discuss more about whether (or not) women selected from this site could be considered representative of all African and Caribbean women in Toronto.

Participants were recruited from a community health center in downtown Toronto and thus, subjects may not be representative of African-Caribbean population in Toronto. However, they do represent a broad sample of women by age, region of birth, and education. Furthermore, the distribution by these variables is very similar to that of the women from Africa and the Caribbean in Toronto according to the 2006 Canada Census (data not shown). A statement to this effect is now included in the Discussion (please see Page 16, second paragraph).

Others:

3. Page 7. Laboratory methods should include the method used to diagnose BV.
We apologize for this oversight. BV was diagnosed by the Nugent criteria using the results of Gram stain of the vaginal swab. The method is now included in the Laboratory methods section and added a reference (please see Page 7, last paragraph to Page 8, first paragraph and reference 16).

4. Page 8, Study population (or page 10 Viral infections). I would mention that more of the HIV-infected women are African (81%) compared to the HIV-uninfected women (36.4%)

Good suggestion. We have added a statement to this effect in the Study population section in Results (please see Page 9, second paragraph).

5. Page 10, covariates of chlamydial infection: Did all of the 15-19 year-olds report having sex at some time during their lives?

70% of 15-19 year-old women reported having had sex during their lifetime. All women who were positive for chlamydia reported having sex during their lifetime. In contrast, 94% of 20-24 year old participants had had sex. Thus, as the reviewer implicitly suggests, the difference in CT prevalence observed between these two groups may be explained by a lower proportion of the younger group who had been sexually active. In fact, the prevalence of chlamydia was slightly higher in sexually active 15-19 year old women then in sexually active 20-24 year old women (12.5% versus 10.4%). The multivariate analysis presented in Table 4 takes into account sex in the previous six months although we do not specifically compare these two groups.

6. Page 11, covariates of bacterial vaginosis: Why are different age groups used for HIV-positive women (less than 45 vs. 45 and older) and HIV-negative women (… and 40+)? I think the same age groups should be used for both.

We agree. The same age strata (15-24, 25-44 and 45+ years) for both groups are now presented in the revised manuscript (please see Page 11, last paragraph).

7. Page 12, paragraph 1. “…other interventions, including HPV vaccination, might both reduce the high viral STI burden and reduce HIV transmission in these communities.” I would mention current HPV vaccine recommendations in Toronto. Do the authors recommend changing current recommendations for these women?

Ontario public health units offer HPV vaccination free-of-charge to girls in Grade 8 through school-based clinics. The vaccine currently given is Gardasil® which contains only four HPV strains, namely 6, 11, 16 and 18. We found many high-risk HPV genotypes not included in Gardasil among the women studied. Therefore
including several additional genotypes in an expanded vaccine would be desirable and we have now included this recommendation in the Discussion (please see Page 12, second paragraph, last three sentences).

8. Page 12, paragraph 2. Gonorrhea and chlamydia infections will clear (within months to a couple years) without treatment, so I would not attribute the lack of current infection to the availability of antibiotics. Many of these women are beyond the age at highest risk for chlamydia and gonorrhea.

Point well taken. This reason for the prevalence observed has been added to the text (please see Page 13, third paragraph).

9. Page 13, paragraph 1. “…chlamydial infection among HIV-negative women was significantly higher than in older women under 25 years;” I am not sure what is meant by “older women under 25 years”.

Thanks for finding this error. This sentence has been corrected (please see Page 13, fourth paragraph).

10. Page 15, paragraph 2. It is helpful to have the statement that the study was unable to reach the targeted sample size of 300 HIV-positive women. It would also be helpful for the methods section to mention the sample size target and the rationale for choosing it.

A statement about the sample size targeted is now included in the Methods section (please see Page 6, first paragraph).

11. Table 2. What is meant by “Abnormal vaginal flora?”

The definitions of ‘abnormal vaginal flora’ and bacterial vaginosis are now included in the Laboratory methods section. A score of four to six in Nugent criteria on Gram-stain of the vaginal smear was classified as abnormal vaginal flora (please see Page 7, last paragraph to Page 8, first paragraph).

12. Table 3. Many of the HIV-infected women had not had sex in the previous 6 months (52%). That seems unlikely, but possible. I am more surprised by HIV-negative women--42% did not have sex in the previous 6 months? That seems too high.

We are not surprised by the high proportion of HIV-positive women who had not had sex in the previous six months. HIV-infected women are often ambivalent about
becoming sexually active following HIV diagnosis due to their concern about secondary transmission. Furthermore, in Canada, HIV-infected persons are legally required to disclose their HIV status to prospective and some women may be concerned about stigma and rejection.

As the reviewer notes, we agree the 42% of HIV-negative women (mean age 33.9 years) who reported not having had sex in the previous 6 months intuitively seems high. While the accuracy of self-reported sexual behaviour is always a concern, we are reassured that this finding is comparable to that of a previous study in the same community. Specifically, the East African Health Study found that 36% of East African women (with a mean age 32.8 years) in Toronto reported not having sex in the previous year.

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

Quality of written English: Acceptable

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
Reviewer's report 2

Title: The epidemiology of sexually transmitted co-infections in HIV-positive and HIV-negative African-Caribbean women in Toronto

Version: 1 Date: 8 August 2013

Reviewer: David Andresen

Reviewer's report:

This is a nice well-conducted study and the discussion is generally appropriate:

A few issues need to be addressed though:

1. Why was CMV tested? (MER)

   Our interest in CMV stems from previous studies which found that CMV infection and/or reactivation was associated with HIV disease progression, higher T-cell immune activation and increased HIV levels in the genital secretions (Gianella S, et al, *J Infect Dis* 2013 and Griffiths PD *J Clin Virol* 2006). These are references 5 and 7 in the manuscript.

2. What testing modality was used for BV? The abstract implies PCR but this is clearly not correct. No mention in methods section (MER)

   The method used to diagnose BV is now included in the Laboratory methods section (Please see Page 7, last paragraph to Page 8, first paragraph). “PCR” in abstract was an error and has been removed (please see Page 3, second paragraph).

3. Is HPV "infection" the correct terminology? Wouldn't "shedding" or "detection" be a better description for HPV PCR positivity? (DR)

   The reviewer makes an excellent point. In fact, HybridCapture2, not PCR, was used to detect HPV. It is possible that a small subset of individuals might be infected by HPV and yet shed HPV DNA at a level below the threshold of detection. The frequency of such an occurrence is not known, but this possibility is now formally noted in the limitations section of the Discussion (please see Page 15, first paragraph).

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

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: No
Reviewer's report 3

Title: The epidemiology of sexually transmitted co-infections in HIV-positive and HIV-negative African-Caribbean women in Toronto

Version: 1 Date: 23 August 2013

Reviewer: Marc Steben

Reviewer's report:

1. Title: I do not understand the concept of co-infection in HIV negative ... never heard of such nomenclature...

   The reviewer is correct that “co-infection” in the HIV-uninfected participants cannot refer to HIV co-infection. However, since all participants – even those who were HIV uninfected – were diagnosed with at least two or more simultaneous infections (such as CMV and HSV-2), we feel that the term “co-infection” still applies.

2. p3 methods in abstract: typos++ with repeat herpes simplex virus type 1 and herpes simplex virus type 1 (HSV-2) = herpes simplex virus type 2 (HSV-2)

   As this reviewer indicates, this was an error and has been corrected (please see Methods in Abstract, Page 3)

3. p3 results: why look for vaginal HPV?

   Participants self-sampled for all genital diagnostics – i.e. the provision of urine and vaginal swabs - and so formal cervical samples were not obtained. However, vaginal self-sampling has been used as a reliable surrogate. We have included this point in the Discussion section and added a reference to support it (please see Page 15, first paragraph and reference 32).

4. HSV-2 serology: you need to discuss asymptomatic vs symptomatic patients, cut-off for positivity. The PPV in patients with positive serology is not great. Maybe better in higher prevalence population but in general population...

   The reviewer is correct that the PPV of the Focus HSV2 ELISA may be poor in populations with a low prevalence of HSV-2. For example, it was only 38% in a student population with an HSV-2 prevalence of under 4% (Mark P, Sex Trans Dis 2007). However, the PPV was over 93% in a population with a higher HSV-2 prevalence of over 40% (Turner K, Sex Trans Dis 2002). Therefore, we believe that the very high HSV-2 prevalence in our participants (48% in the HIV-uninfected
women and higher in HIV-infected women) means that this is less likely to be a concern in our study. This point is now included in the Discussion including new references (please see Page 15, second paragraph to Page 16, first paragraph, and references 34 and 35).

5. **How do you ascertain positivity for BV? Nugent score on a wet mount? If so, you should put intermediate flora and not only BB.**

   The methods used to diagnose BV is now described in the Laboratory methods section (please see Page 7, last paragraph and Page 8, first paragraph). We have presented results on intermediate flora as “Abnormal vaginal flora” in Table 2 (please see Page 27).

6. **In your table 2, what is abnormal vaginal flora? How do you define this? How do you test for Trichomonas? This should also be in your lab section the paper is well written and gives an insight of the interaction between the microbiome of the vagina and HIV.**

   Abnormal vaginal flora is now defined in the in the Laboratory methods section (please see Page 7, last paragraph and Page 8, first paragraph) as noted in Point 5 above.

   We used PCR to detect Trichomonas. This is now included details on the methods used in the Laboratory methods section and added two references (please see Page 8, first paragraph and references 17 and 18).

**Level of interest: An article of importance in its field**

**Quality of written English: Acceptable**

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

**Declaration of competing interests: I personally know many of these researchers and Rob Remis was my boss more than 10 years ago**