Author's response to reviews

Title: A large, population-based study of age-related associations between vaginal pH and human papillomavirus infection

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Diana Marshall PhD, Editor-in-Chief

BMC Infectious Diseases

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Dear Dr. Marshall:

Thank you for reviewing our submission entitled: “A large, population-based study of age-related associations between vaginal pH and human papillomavirus infection”. We have incorporated most of the major and minor revisions as noted below.

With best regards,

Megan Clarke

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Reviewer #1

Major Revisions

1. Considering there are women who would have had multiple visits during the course of the study, are the authors able to comment on whether or not vaginal pH or any other measurement changed over time for these women?

_We observed many changes in pH status over time in individual women, but decided not to do a longitudinal analysis because of the highly variable and sometimes long follow-up intervals._

2. An ethics declaration should be included in the methods of the document.

_Currently, we have a sentence in the methods section stating that “the study protocol was reviewed and reapproved annually by the National Cancer Institute and a Costa Rican Institutional Review Board”_.

3. Results pg 10 line 212 – the authors describe the relationship between vaginal pH 5.0 and LSIL as positive for all age groups but most significant in women aged <25, 25-34, or 65+. The authors should be cautious using the term “most significant” as the relationship is not significant in other age groups even though a trend might be present.

_We have removed the word “most” and only used “significant” to describe those results that achieved statistical significance._

4. Results pg 10 line 220 – the authors report a positive association between vaginal pH and _C. trachomatis_ DNA. Are the authors reporting a positive association between elevated vaginal pH and increasing amounts of _C trachomatis_ DNA or the presence of _C trachomatis_ DNA? This should be clarified. It is also unclear whether the authors are reporting that women <25 are more likely to have a _C. trachomatis_ infection detected or have multiple infections over a follow up period.

_We added the term “presence of” to clarify that we are reporting the association between increased vaginal pH and infection with _C. trachomatis_. We also clarified these results by saying that women under
age 25 with a vaginal pH of >5.0 “were more likely to have a C. trachomatis infection” instead of “having a higher percentage of C. trachomatis infections”.

5. Discussion pg 11 line 245 – the discussion regarding the link between high vaginal pH and BV is important; however the authors should be cautious in liking their findings of high vaginal pH with other research on the associations of BV with HPV and/or C. trachomatis considering their study did not clinically measure BV status. A limitation of the study was that BV was not clinically diagnosed in these women.

We agree with the reviewer that a major limitation of this study was that we did not have information on BV diagnosis, and could therefore not assess the link between high vaginal pH, BV, and HPV and/or C. trachomatis infections. To be more conservative, we changed the language to the following: “The absence of protective Lactobacilli and an increased vaginal pH has been shown to increase susceptibility for sexually transmitted infections, and thus could possibly be associated with elevated risk of HPV acquisition”.

6. Discussion pg 12 line 270 – In the discussion the authors have identified a limitation of their study is the lack of male sexual behavior data which may help explain their findings. In fact all sexual behavior including gender of partners, condom use, and number of partners could be associated with vaginal pH and HPV infection independently. Please elaborate on this further in the discussion. The authors may also wish to speculate how sexual behaviors might influence vaginal pH and/or HPV infection.

We identified the lack of both female and male sexual behavior data as a limitation in our study. We are not saying that vaginal pH is influenced by sexual behavior, rather we are saying that we were not able to explore whether such confounding exists. If acceptable, we would prefer not to speculate on mechanisms, which might take us too far beyond the data.

7. Discussion pg 13 line 279 – The authors briefly mention the significance of their findings and how knowing vaginal pH may be beneficial when identifying HPV infection and the development of cancer. The significance of the study should be discussed in more detail. How might knowing a woman’s vaginal pH be used clinically?

We agree that we might have overstepped based on this epidemiologic observation, and have added the following sentence to the Discussion section: “If increased vaginal pH is found to precede an HPV infection, this might elevate the clinical significance of measuring vaginal pH. However, utility of this or any biomarker must be firmly established before introduction into clinical practice.”

Minor Revisions

1. Abstract pg 3 line 52 – The conclusions in the abstract could be more specific. The authors could expand on what they mean by ‘...certain age groups’ and also comment on the overall significance of their findings or what future research is needed.

We have revised the conclusions section in the abstract to provide more specific information on the age groups, and included a sentence on the importance of our findings and what future research is needed.
2. Methods pg 6 line 118 – The sentence reads ‘For the current analysis focused...’ please change ‘For’ to ‘As’

*We have changed “For” to “As” in this sentence.*

3. Methods pg 5 paragraph 1 – The number of women approached and the number of eligible participants would be clearer to understand if supported by a consort diagram.

*We have included a consort diagram for clarity of study participant information.*

4. Methods pg 9 line 181 – The explanation as to why the dichotomous variable of pH was split to <5.0 and >5.0 may be confusing for some readers given the description of pH of >4.5 as abnormal in the following sentence.

*We agree with the reviewer and we modified the explanation to read: “When appropriate to increase statistical power and stability of estimates, we included vaginal pH as a dichotomous variable (pH <5.0 and pH ≥5.0)”.*

5. Results pg 9 line 192 – The sentence reads ‘The majority of women younger than age 45...’ the word ‘age’ can be removed.

*We have removed the word “age”.*

6. Results pg 9 line 198 – The sentence reads, ‘The prevalence of HPV was highest at age 25-35 years...’ please change to ‘...in the 25-34 age group...’

*We changed the sentence to read “The prevalence of HPV was highest in the 25-34 year age group...”*

7. Results pg 10 line 200 – In general, the paragraphs describing the results refer to the table at the beginning of the sentence. I would suggest starting with a description of the findings and then referring to the table as is done for table 3 in the third paragraph

*We have changed the formatting so that each paragraph starts with a description of the findings and then refers to the table in parentheses within the text.*

8. Results pg 10 line 201 – The sentence reads ‘...with about a 10-20% increased risk for a vaginal pH ≥5.0.’ What the increased risk is referring to should be inserted.

*We have modified this sentence to read,”...with about a 10-20% increased risk of HPV detection for a vaginal pH ≥5...”*

9. Results pg 10 line 245 – The authors report that there was no association between HSIL and elevated pH. However there are no data present in the tables reflecting this. Please either include the data table or state that data is not shown.

*We have stated “data not shown.”*
10. Discussion pg 12 line 264 – In the discussion the authors write that their findings in post-menopausal women may be because of a ‘link between the decline in circulating estrogens...’ Please elaborate on this further in your discussion to include how estrogens are known to be involved in regulation of vaginal pH.

We have included the following sentence with a reference to support it: “The vaginal pH is known to increase after menopause, as circulating estrogens decline and cause a depletion of glycogen and glucose metabolism.”

Reviewer #2

Major Revisions

1. The description of the study population in the Methods section is hard to follow...the vast majority of this paragraph can be summarized with a flow chart and make it easier for the reader to follow.

_Per both the reviewers’ suggestions, we have included a consort diagram to explain where the 9094 participants came from._

2. The statistical analysis component of the Methods section is far too long compared to the rest of the methods described that are concise and to the point. Remove any justification of the methodology used. Include information regarding the statistical program used with their version number.

_We have removed sentences pertaining to the justification of our methodology. We have included the information regarding the statistical program, STATA 11.0, in this section._

3. This study employed a method able to detect 18 different HPV types/groups. However there is no mention in the Results section of the distribution of HPV types detected in this population. It would be of interest to show the association between HPV type and vaginal pH and age, in particular high risk (carcinogenic) versus low risk types.

_We performed analysis on carcinogenic HPV types (data attached for consideration). We added a few sentences regarding these results, but as they were not remarkably different, we would suggest not including these data in the manuscript._

4. This paper reports a high level of multiple HPV types detected. Which multiple HPV types were detected and did this vary within the population? Did they all possess high risk types?

_In other manuscripts, we have analyzed the patterns of multiple type infections in this cohort, and found no tendency for particular types to pair (Vaccarella S). Both high-risk and low-risk types are found in_
combination. HPV types tend to act individually. We did not include these points in the manuscript but are happy to do so if the Editor wishes.

5. It would be of interest to make a distinction between persistent and sporadic detection of HPV based on the HPV type detected in these individuals over time.

*We agree this would be of some interest, but the complexity of that analysis is beyond the scope of this manuscript.*

**Minor Revisions**

1. It is acceptable in bacterial nomenclature to abbreviate a genus and its associated species to C. trachomatis (italicized), in the case of Chlamydia trachomatis. In addition, use the proper format for Lactobacillus (italicized) species.

*We have abbreviated Chlamydia trachomatis as “C. trachomatis” throughout the text. In addition, we italicized the species name, Lactobacillus.*

2. In the Methods section more information is required detailing the detection of *C. trachomatis* DNA. Please add information regarding the targets this assay will detect and the fact it is a real-time PCR assay coupled with DNA Enzyme ImmunoAssay. Did you quantify the bacterial load or determine serovars? It would be of value to include what effects vaginal pH has upon detectable *C. trachomatis* load.

*We added the following information regarding the *C. trachomatis* assay: *C. trachomatis* DNA was detected in enrollment samples using a *C. trachomatis* real-time PCR-DEIA assay (Labo Biomedical Products BV, Rijswijk, the Netherlands). After the PCR, *C. trachomatis* DNA was detected by *C. trachomatis*–specific probe hybridization in a DNA enzyme immunoassay that used 10 μL of the PCR product. The mixture of probes present in the *C. trachomatis* DNA enzyme immunoassay can recognize all *C. trachomatis* serovars and genovariants that have been deposited in GenBank.*

3. Please include p-values in Tables 2 and 3

*We have added a column to include the p-values for Tables 2 and 3.*

4. In the Results section concerning Table 2, how can a woman possess more than one HPV type if she is HPV negative?

*Analyses were conducted among all women, we did not restrict to the HPV positives.*

**Discretionary Revisions**

1. In the Conclusions section, the shared region of the bacterial ribosome used in metagenomic studies is the 16S ribosomal RNA or 16s rRNA.

*We changed “16s” to “16S ribosomal RNA”*