Author's response to reviews

Title: Comparing human papillomavirus prevalences in women with normal cytology or invasive cervical cancer to rank genotypes according to their oncogenic potential: A meta-analysis of observational studies

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Author's response to reviews: see over
Dear Dr Harris,

Please find enclosed our revised manuscript (MS: 3121319289071894) entitled, Comparing human papillomavirus prevalences in women with normal cytology or invasive cervical cancer to rank genotypes according to their oncogenic potential: A meta-analysis of observational studies, by E Bernard, M Pons-Salort, M Favre, I Heard, E Delarocque-Astagneau, D Guillemot et ACM Thiébaut, that we are resubmitting for publication as a Research Article in *BMC Infectious Diseases*.

We sincerely thank the Reviewers for their pertinent comments that have helped us revise our manuscript to improve its clarity and, hence, its message.

Below you will find our detailed point-by-point responses to each comment.

Thank you in advance for considering our revised paper and we look forward to hearing from you soon.

Yours sincerely,

Anne THIÉBAUT
Reviewer #1 (Tricia Luhn)

Major compulsory:

It is unclear why there are no North American studies included in this meta-analysis. Based on the inclusion criteria listed (unvaccinated women, prevalence data for at least one genotype other than 16 & 18, 20+ cases and 20+ controls, and data presented for cases and controls separately), there are at least two large epidemiologic studies in the US that were not included, specifically SUCCEED and KNPC. These studies should be included in the analysis, however, if they were excluded for specific reasons, that should be explicitly addressed in the discussion where the authors mention the lack of North American Studies. Although cervical cancer is declining in the US, there are still several epidemiologic studies that include cancers as well as controls and therefore the statement that they were not included due to the majority of the cancer burden residing elsewhere in the world doesn’t seem to be reflected in published literature. The lack of these two studies in particular also leaves the question of what other studies may have been excluded or missed and how that would affect the results.

We are well-aware that epidemiologic studies were conducted in North America. However, none of them fulfilled our selection criteria to be included in the meta-analysis. Specifically, the cross-sectional Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED) recruited women “referred to colposcopy for abnormal cytology” and, thus lacked control women with normal cytology and, although the HPV Persistence and Progression (PaP) Cohort at Kaiser Permanente Northern California (KPNC) did include women with normal cytology, it was just starting and had not yet reported the prevalences of HPV genotypes among them and case women with invasive cervical cancer at the time we performed the meta-analyses.

We think our selection algorithm did not miss important studies in the field, especially after checking cross-references. Indeed, publications from both studies belonged to the 757 potentially eligible references identified through the Medline database search but were discarded after screening of their titles and abstracts (manuscript Figure 1):


It can be expected that invasive cancer cases will develop in the coming years as the PaP cohort ages. We indeed found a publication from KPNC that was published after our search in March 2011 [Castle et al. Human papillomavirus (HPV) genotypes in women with cervical precancer and cancer at Kaiser Permanente Northern California. Cancer Epidemiol Biomarkers Prev. 2011, 20:946-953. doi: 10.1158/1055-9965.EPI-10-1267] and would now be eligible.

Similarly, we have indeed heard of the McGill-Concordia Cohort in Canada [Richardson H. The natural history and epidemiology of cervical human papillomavirus infections in Montreal
University students. PhD thesis, McGill University, 2003] but, to the best of our knowledge, no publication reporting prevalences of HPV genotypes among women with normal cytology and a sufficient number of women with invasive cervical cancer has been published to date.

To take the Reviewer’s comment into account, we added references to SUCCEED (Wang et al., 2009) and KPNC (Castle et al., 2011) in the Discussion on page 12, paragraph 3, lines 3-6:

That choice rendered the several large investigations conducted in North America ineligible [66,67], which is consistent with 85% of ICC cases occurring in developing countries [1], and HPV-vaccine trials being conducted more frequently in Asia-Pacific, Europe or Latin America, than North America [68,69].

Moreover, we rewrote the following sentences in the Results on page 6, last paragraph, lines 1-5:

The Medline and Embase database searches provided, respectively, 757 and 182 references, while additional searches identified 11 studies, yielding, after deleting 55 duplicates, a total of 895 references (Figure 1). Among them, 794 were excluded based on their titles and abstracts. The full texts of the remaining 101 references were read and the 27 studies fully satisfying the inclusion criteria were finally retained.

The authors should include some discussion about the how the results relate to the biology of HPV infections in cervical cancer, especially the differences in the alpha 5 and alpha 7 types and their detection in cervical specimens.

Our results do not suggest a clear picture of how the oncogenic potentials of HPV genotypes may be related to the species to which they belong. Thus, among genotypes of the α-7 species, HPV-18 and -45 ranked first, -39 and -59 at the end of the first 10, and -68 and -70 at the bottom after the non-oncogenic HPV-6 and -11 of the α-10 species. Among genotypes of the α-5 species, published data on third-ranked HPV-69 were scarce, while HPV-82 and -51 tended to rank close to HPV-11.

Notably, genotypes of the same species are not necessarily classified in the same carcinogenicity categories according to IARC current classification (Bouvard et al., Lancet Oncol. 2009, manuscript ref. 8). Thus, among α-7 species genotypes, HPV-18, -39, -45 and -59 are classified as carcinogenic to humans (Group 1), while HPV-68 stands alone in a separate category with limited evidence in humans but strong mechanistic evidence for cervical cancer (Group 2A), and HPV-70 is among those with limited evidence in humans for cervical cancer (Group 2B). Among α-5 species genotypes, HPV-51 belongs to Group 1, while HPV-26, -69, and -82 belong to Group 2B.

To take the Reviewer’s comment into account, we added the following in the Discussion:

- Page 9, paragraph 4, lines 3-5: ...the genotypes with the highest ORs and relatively narrow CIs, namely HPV-18, -31, -33, -39, -45, -52, -58 and -59, all belong to the α-7
and -9 species and are IARC-classified as carcinogenic to humans with sufficient evidence...

- Page 9, paragraph 4, lines 6-7: ...HPV-18 (α-7) ranked closest to, but distinct from, HPV-16 (α-9)...

- Page 9, paragraph 5, lines 2-3: The same was observed for HPV-68 (α-7), currently IARC-classified as probably carcinogenic...

- Page 10, paragraph 2, lines 2-4: Our meta-analytic assessment of the oncogenic potentials of HPV-69 and -82 (both α-5 species), -30 (α-6), -67 (α-9), and -34 and -73 (α-11) was based on small numbers of cases...

- Page 10, paragraph 3, lines 1-2: Conversely, little to no mechanistic evidence supports that HPV-6 and -11 (both α-10 species), which commonly cause benign genital warts, can contribute to carcinogenesis...

- Page 11, first paragraph, lines 6-7: ...HPV-39 and -59 (both α-7), about four times less prevalent than HPV-52 (α-9), had higher oncogenic potentials...

- Page 14, last paragraph, lines 5-6: ...although HPV-39 and -59 belong to the same α-7 species as HPV-18, they are not, at present, included in a future nonavalent anti-HPV vaccine...

Minor essential:

Several paragraphs in the discussion could benefit from careful editing.

The manuscript has been corrected by a native English-speaking American scientist. Changes are highlighted in yellow.
Reviewer #2 (Lauren Wilson)

The paper by Bernard et al. is a meta-analysis of HPV genotype distribution drawn from case-control studies of invasive cervical cancer that included some HPV genotyping. The study design is interesting, and approached this question in a fairly unique way. Their ranking of oncogenic potential of HPV genotypes is relatively consistent with existing literature, but does suggest that some HPV genotypes that are considered only potentially carcinogenic should be examined more carefully and included in more genotyping assays for further study. These are interesting findings worthy of publication.

Discretionary Revisions

1. I understand why the authors chose to use only ICC as their endpoint in terms of investigating carcinogenic potential instead of pre-cancerous lesions. However, it may be worthwhile to point out in the discussion that in terms of clinical management, precancerous lesions are still currently the major endpoint of interest as we have no good way of predicting which lesions will invade and which will regress.

In response to the Reviewer’s suggestion, we added a sentence in the Discussion page 13, end of paragraph 2:

Moreover, clinical management guidelines also recommend the excision of precancerous lesions, and will continue to do so as long as whether these would regress or progress cannot be foreseen [73].

2. There were a few things that were slightly unclear to me in the analysis that might be useful to explain a bit more in the methods. If HPV16-positive women are serving as the reference group but no accounting is done for multiple infections, if a women is infected with both 16 and 18 is she included both in the reference group and the 18 comparison group? I was not sure whether the analysis was being conducted on an infection-level, where the authors were basically looking at whether an infection was occurring in a case or a control, or whether the analysis was being conducted on a woman-level which would not include the same woman in both comparison groups. I may just have missed this but I found it confusing.

As stated in the Methods on page 5, first paragraph, lines 8-9: For multiple infections with ≥2 HPV genotypes, no weighting was used and each HPV genotype was counted equally.

That approach implies, as the Reviewer correctly inferred, that the analyses were performed at the infection level, not the individual level. Thus, if a woman was infected with both HPV-16 and -18, she was counted twice (once among HPV-16 infections and once among HPV-18 infections).

To make this clearer, we rewrote the following sentence in the Methods page 5, last paragraph, lines 4-7:

For each study and each available genotype, an odds ratio (OR) and its 95% confidence interval (CI) were computed from the reported numbers of case and control infections, considering HPV-16 infections as the reference group.
Reviewer #3 (Peng Guan)

This article is of major public health interest for the prevention of invasive cervical cancer. The methodology is acceptable to address the issues, and it would be better that the following things could be explained or modified.

Minor:

1. As different HPV typing methods have different typing coverage, then uncharacterized HPV X varied in the included studies. The authors can first describe the distribution of HPV X in the studies, and exclude it in the ranking.

We fully agree with the Reviewer that HPV-X covers a variety of genotypes, depending on the chip used for HPV-typing. In the table below, we list the studies reporting positive numbers of HPV-X infections among cases or controls to further emphasize the variability across studies.

<table>
<thead>
<tr>
<th>First author, year of publication</th>
<th>Number of detectable HPV genotypes</th>
<th>Numbers of HPV-X among Cases' infections</th>
<th>Controls' infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alibegashvili, 2011</td>
<td>44</td>
<td>0</td>
<td>2/104 (1.9%)</td>
</tr>
<tr>
<td>Andersson, 2005</td>
<td>–</td>
<td>1/45 (2.2%)</td>
<td>4/24 (16.7%)</td>
</tr>
<tr>
<td>Asato, 2004</td>
<td>–</td>
<td>15/311 (4.8%)</td>
<td>53/333 (15.9%)</td>
</tr>
<tr>
<td>Baay, 2001</td>
<td>–</td>
<td>1/102 (1.0%)</td>
<td>5/31 (16.1%)</td>
</tr>
<tr>
<td>Bardin, 2008</td>
<td>44</td>
<td>0</td>
<td>3/181 (1.7%)</td>
</tr>
<tr>
<td>Castellsagué, 2008</td>
<td>25</td>
<td>5/342 (1.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Chaouki, 1998</td>
<td>33</td>
<td>9/132 (6.8%)</td>
<td>8/36 (22.2%)</td>
</tr>
<tr>
<td>Cho, 2003</td>
<td>22</td>
<td>2/45 (4.4%)</td>
<td>15/178 (8.4%)</td>
</tr>
<tr>
<td>Ferrera, 1999</td>
<td>–</td>
<td>4/88 (4.5%)</td>
<td>42/173 (24.3%)</td>
</tr>
<tr>
<td>Hammouda, 2011</td>
<td>44</td>
<td>0</td>
<td>2/45 (4.4%)</td>
</tr>
<tr>
<td>Herrero, 2005</td>
<td>–</td>
<td>3/47 (6.4%)</td>
<td>239/2280 (10.5%)</td>
</tr>
<tr>
<td>Illades-Aguiar, 2010</td>
<td>–</td>
<td>0</td>
<td>830/1251 (66.3%)</td>
</tr>
<tr>
<td>Keita, 2009</td>
<td>44</td>
<td>2/68 (2.9%)</td>
<td>30/502 (6.0%)</td>
</tr>
<tr>
<td>Maehama, 2005</td>
<td>6</td>
<td>134/330 (40.6%)</td>
<td>368/434 (84.8%)</td>
</tr>
<tr>
<td>Sasagawa, 2001</td>
<td>–</td>
<td>8/89 (9.0%)</td>
<td>23/173 (13.3%)</td>
</tr>
<tr>
<td>Sherpa, 2010</td>
<td>44</td>
<td>0</td>
<td>1/89 (1.1%)</td>
</tr>
</tbody>
</table>

To take the Reviewer’s comment into account, we made the following changes in the manuscript:

- We deleted HPV-X in the Results on page 8 (twice) and added a specific sentence page 7, end of paragraph 2: The overall HPV-X prevalence was 7.6% but this value represents different numbers of genotypes from one study to another.

- In the Results on page 8, we deleted HPV-X related to heterogeneity analyses (three times) and rewrote the sentence page 8, paragraph 2, lines 1-2: Cochran’s Q-test suggested heterogeneity for six HPV genotypes: HPV-31, -33, -45, -51, -58 and -74; and the I² statistic for four among them: HPV-31, -33, -58 and -74 (Table 2).
We deleted HPV-X and its corresponding footnote in Table 2.

We deleted HPV-X on the abscissa of Figure 2 and its corresponding legend.

We deleted its corresponding forest plot in Additional File 2.

We deleted its corresponding funnel plot in Additional File 3.

We deleted HPV-X and its corresponding footnote in the Table in Additional File 4.

2. It is not clear that when considering potential sources of heterogeneity due to geographical area, why ‘Asia versus all other continents’.

We chose to compare Asia to the other continents for several reasons. First, it has been suggested that the distribution of HPV genotypes may differ in Asia, with especially HPV-58 being more prevalent in East Asia than in more Western countries (manuscript ref. 25). Second, we thought that using fewer subgroups would limit the loss of statistical power had we compared all four continents (Africa, Asia, Europe, and South America). Moreover, almost half of the studies (12 out of 27) had been conducted in Asia.

To respond to the Reviewer’s comment, we added the following reference: [18. Guan et al. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer. 2012, 31:2349-59. doi: 10.1002/ijc.27485] to further justify the choice of Asia versus all other continents in the Methods page 6, paragraph 2, line 8. We also added this reference in the Discussion on page 12, paragraph 3, line 7.