Author’s response to reviews

Title: Human Papillomavirus (HPV) infection in pregnant women and mother-to-child transmission of genital HPV genotypes: a prospective study in Spain

Authors:

Xavier Castellsagué (xcastellsague@iconcologia.net)
Teresa Drudis (tdrudis@gmail.com)
Maria Paz Cañadas (pazjordi@gmail.com)
Anna Goncé (AGONCE@clinic.ub.es)
Ramón Ros (rros@hmartorell.es)
José M. Pérez (jmperez@clinic.ub.es)
M. Jesús Quintana (MJQuintana@santpau.es)
Jesús Muñoz (jesus@iconcologia.net)
Ginesa Albero (g.albero@iconcologia.net)
Silvia de Sanjosé (s.sanjose@iconcologia.net)
F. Xavier Bosch (x.bosch@iconcologia.net)

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Author’s response to reviews: see over
Dear Editors of *BMC Infectious Diseases,*

I am pleased to re-submit for the consideration of the editors of *BMC Infectious Diseases* the revised original manuscript referred above. We have substantially re-written the manuscript and performed further analyses to incorporate or address all the reviewers’ comments as detailed below.

We really appreciate all the comments and suggestions received from the reviewers which all together have greatly improved the quality of the manuscript. I hope that the manuscript will be now acceptable for publication in your journal.

Looking forward to a positive and prompt response,

Sincerely,

Xavier Castellsagué, MD, MPH, Ph.D.
Institut Català d’Oncologia (ICO)
Barcelona, Catalonia, Spain

December 19, 2008
Reply to reviewer’s comments:

Reviewer 1:

1. Table 4 is very difficult to understand and it is the key data table in the paper. And, there is a high percentage of missing test results among the HPV positives to assess type concordance. For each comparison, I would rather see more details about the paired results: infant positive/mother positive, complete concordance; infant positive/mother positive, partial concordance; infant positive/mother positive, no concordance; infant positive/mother negative; infant positive/mother missing; and so forth.

We have re-designed Table 4 and added as suggested the HPV type details of the mother/child HPV positive pairs with concordant and discordant HPV types. We have changed the title and the labels to improve the understanding and interpretation of the contents of the table.

We agree with the reviewer that there are many HPV X samples that make it difficult to interpret type-specific concordance. We admit and briefly discuss this limitation in the discussion.

2. Table 1 should present the two populations, the HPV+ recruited from the prevalence survey and “random sample” of the population.

Unfortunately this is not possible by study design. The HPV screening survey was only conducted to identify or screen for HPV-positive pregnant women to be potentially recruited for the prospective cohort study to assess vertical and perinatal HPV transmission, as in the initial cohort we ended up with too few HPV-positive pregnant women (Spain is a low risk country for both HPV and cervical cancer). Thus, socio-demographic or reproductive data for the HPV-negative screened women were not collected and cannot be presented. We don’t think this is an important limitation as it is clearly stated that the HPV screening survey was carried out just as a source of subjects for the prospective study and to assess HPV prevalence and type-specific distribution in an unselected population of pregnant women.

The overall study design used is now explained in more detail as well as the different components of the recruitment: (1) the initial cohort study that finally recruited too few HPV-positive women. (2) the large HPV screening survey among unselected pregnant women to recruit more HPV-positive women and estimate HPV statistics in pregnancy, and (3) the final prospective cohort study including all women form the initial cohort plus the HPV-positive women from the HPV screening survey.

3a. Table 2 can be simplified by eliminating the cytology. It is not really relevant here.

The cytology results have been removed as suggested by the reviewer. Since the sample is not representative of the general population the distribution of the cytology results is not relevant.

3b. I would like to know if the overall distribution of HPV types (among HPV+) were (statistically) the same between mothers and infants.

As shown below the overall distribution of HPV types among mothers at pregnancy was not statistically significantly different from that among infants at any time (p=0.80). This sentence has been added to the Results section “Mother to child HPV transmission”.

<table>
<thead>
<tr>
<th></th>
<th>At pregnancy</th>
<th>Children at any time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>HPV X</td>
<td>19</td>
<td>28.8%</td>
</tr>
<tr>
<td>6/11</td>
<td>10</td>
<td>15.2%</td>
</tr>
<tr>
<td>16</td>
<td>17</td>
<td>25.8%</td>
</tr>
</tbody>
</table>
4a. I really do not see the rationale for Table 3, the multivariable model for HPV in the mothers. We think that Table 3 is informative as there are no published data on the correlates of HPV infection among pregnant women in the literature and less in Spain. Thus, it provides for the first time in a population of pregnant women in Spain HPV positivity estimates by a number of factors of interest. Also, given the statistically significant associations found with known risk factors for HPV, it also provides reassurance about the internal validity of the study. It is indeed informative and of interest to many Spanish and non Spanish readers as it contributes HPV data by risk factors that were not available before.

4b. The outcome of interest is HPV in the infants. It is more relevant to do a multivariate model for the infants based on the mothers’ characteristics and the details about birthing. We agree with the reviewer that it would be of interest to explore determinants of HPV DNA detection in children. Thus, we’ve added a new table (new Table 4) with the results of these analyses. This has greatly improved the contents and interest of the paper and has allowed us the estimation of formal age-adjusted estimates of associations between HPV status and HPV persistence in the mother and the excess risk of HPV infection in the child. Many thanks for this useful comment.

5. I think that it would useful to present a cumulative incidence of infection among infants by mothers’ baseline status and overall status i.e., any HPV or not. This has been explored as shown below. However, given the limited number of HPV positive samples in children and the similarity of the curves and the lack of statistical significance we don’t think it is very useful to include the graph in the paper.

Kaplan Meier (% HPV-free infants) stratified by mother’s HPV status at pregnancy (Log Rank p value, 0.66)
Kaplan Meier (% HPV-free in infants) stratified by mother’s HPV status at pregnancy and/or port-partum visit (Log Rank p value, 0.34)

6. For key prevalences, the authors should present binomial 95% confidence intervals. Confidence intervals have been added to the key prevalences in Table 2

7a. The authors should also present the concordance of oral and genital HPV in the infants. HPV status concordance between oral and genital samples was 93%. Of the 26 infants that tested HPV positive at any point during follow-up, a valid PCR result from both the oral and the genital sites was obtained in 26 pairs. In 24 of these oral-genital pairs the HPV status was discordant, in one pair the detected types were concordant for HPV 11 and in the other pair the types were not concordant. This has been added in the results section (“HPV positivity in infants”).

7b. Did the distribution of HPV types detected from the infants change in time, which would speak to the horizontal transmission? As shown below there was no statistical evidence that the distribution of HPV types detected from the infants changed over time. A sentence explaining this has been added in the results section (“HPV persistence in mothers and infants”)

<table>
<thead>
<tr>
<th>HPV distribution in children: 0-6 days vs 6 weeks vs 3-24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>HPV X</td>
</tr>
<tr>
<td>6/11</td>
</tr>
<tr>
<td>31</td>
</tr>
</tbody>
</table>
8. Of those who tested positive but untyped (HPVX), how often were women repeatedly HPVX positive? This would say something about whether these are true positive results for types amplified but not detected vs. false positives. Of the 19 mothers that tested HPV X at pregnancy, only 2 were HPV X persistent at the post-partum visit, 9 became negative, 3 became HPV-16 positive, and in the remaining 5, no PCR result was available at the post-partum visit. Numbers are too small to draw any conclusion. Nevertheless, these results have been added in the Results section (“HPV persistence in mothers and infants”)

9. Minor Essential Revisions:
All minor revisions suggested by the reviewer have been incorporated.
Reviewer 2:

Reviewer’s report:

Abstract:
It is essential that the final sample size used to estimate HPV prevalence among pregnant women and transmission (mother-child pairs) be written in the abstract. The overall description of the study design components used has been re-written and explained in more detail in the Methods. As requested, the final sample sizes for both the HPV prevalence survey and the prospective cohort study have been included in the abstract.

Also, ideally the timing of the HPV evaluation pre- and post-partum should be included in the abstract.
This has also been added in the abstract

Methods:
It is difficult to follow the study design features of the study as written due to inconsistencies in reporting sample sizes in different sections of the manuscript. A paragraph that briefly describes and tracks the sample sizes would be most useful as an introduction. For example, the abstract lists that 943 pregnant women were screened for HPV prevalence but the Methods state that the first 115 women were excluded from prevalence analyses due to the fact that they were selected based on a high risk profile. The authors then state that all women from the pilot (n=115) were entered into the prospective part of the study but do not report the prevalence of HPV in this group or state why they chose this design element.

See reply to point 2 of Reviewer 1 above. The study design used is now explained in more detail to better understand the various components of the project. In brief: (1) the initial cohort study among selected pregnant women which unfortunately yielded too few HPV-positive pregnant women to appropriately assess HPV vertical transmission, (2) the large HPV screening survey among unselected consecutive pregnant women to recruit more HPV-positive women for the cohort (HPV data from the survey was also used to estimate HPV and type-specific distribution in pregnancy), and (3) the final prospective cohort study including all women from the initial cohort study plus the HPV-positive women form the HPV screening survey.

It would be useful to have a time frame for what is referred to as the “first or second obstetric visit”. When do these visits usually occur in the pregnancy – e.g., week 8, 12, 15? Was a full questionnaire administered to women at this time? When was the “enrollment” visit to the prospective cohort study? As written it looks indistinguishable from the HPV prevalence screening portion of the study. State when in the pregnancy this enrollment visit occurred.

The time frame of the study is now specified in the Methods section. The obstetric visit was at mean pregnancy time of 32.1 weeks, rank 30 to 34 weeks for the initial cohort, and 31 weeks, rank 29 to 33 weeks, for the HPV screening survey. The enrollment date was the date of the pregnancy visit in which the cervical sample for HPV DNA testing was collected. However, the actual enrollment visit in which the questionnaire and clinical data was retrospectively collected occurred weeks after the date of sample collection as we had to wait for the HPV result to actually recruit the potential woman and then schedule the visit at her earliest convenience. The time between date of cervical sample collection and date of actual clinic visit ranged from 2 to 12 weeks. This has been added under section “Prospective cohort study”.

Clearly state what the final sample size of mother-child pairs was for the prospective study and state the distribution of HPV positivity among the 26 pairs that were dropped.
The final numbers are now clearly stated in the abstract and in the Results section under “Baseline characteristics of subjects in the cohort.”

The following paragraph has been added:

“Of the initial 169 recruited women with a known HPV-DNA status 26 mother-infant pairs (16 from the initial cohort and 10 from the HPV screening survey) were excluded from the prospective cohort study because no adequate sample from the child could be obtained for HPV testing neither at birth nor at any of the subsequent follow-up visits. Of the 26 excluded pairs, 16 (6 from the initial cohort and 10 from the HPV screening survey) were HPV positive. The distribution of HPV types in these 16 excluded HPV-positive women didn’t statistically differ from the 66 HPV positive women finally included in the cohort (p=0.23). Thus, the final prospective cohort study included 143 mother-infant pairs with a valid PCR result.”

The following table summarizes these results:

<table>
<thead>
<tr>
<th>Mothers' HPV-DNA prevalence at pregnancy</th>
<th>N=143 (final sample)</th>
<th>N=26 (excluded)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive/Tested</td>
<td>66/143  46.2%</td>
<td>16/26 61.5%</td>
</tr>
<tr>
<td>Type distribution among HPV positive:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV 6,11</td>
<td>10 15.2  4 25.0</td>
<td></td>
</tr>
<tr>
<td>HPV 16</td>
<td>17 25.8  3 18.8</td>
<td></td>
</tr>
<tr>
<td>HPV 18</td>
<td>4 6.1  1 6.3</td>
<td></td>
</tr>
<tr>
<td>HPV 31</td>
<td>1 1.5  1 6.3</td>
<td></td>
</tr>
<tr>
<td>HPV 33</td>
<td>1 1.5  1 6.3</td>
<td></td>
</tr>
<tr>
<td>HPV 39</td>
<td>1 1.5  1 6.3</td>
<td></td>
</tr>
<tr>
<td>HPV 16 + other types</td>
<td>7 10.6  0 0</td>
<td></td>
</tr>
<tr>
<td>HPV 31 + other types</td>
<td>5 7.6  0 0</td>
<td></td>
</tr>
<tr>
<td>HPV 33 + other types</td>
<td>1 1.5  0 0</td>
<td></td>
</tr>
<tr>
<td>HPV 18 + other types</td>
<td>0 0  1 6.3</td>
<td></td>
</tr>
<tr>
<td>HPV X</td>
<td>19 28.8  4 25.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>66 100.0 16 100.0</td>
<td></td>
</tr>
</tbody>
</table>

Fisher’s exact test 0.2272

Adding a timeline (table or figure) of sampling mothers and children would be most helpful to clearly identify the time points and concordance of sampling between mother-child pairs. For example, were mothers sampled at each of the same time points that the children were sampled?

As explained now better in the Methods section mothers were sampled only at pregnancy and at the 6-week post-partum visit. Thus, only at the 6-week post-partum visit both mothers and infants were concomitantly sampled. Table 5 (old Table 4) shows now the type-specific results of each of the mother-infant pairs and the time period of the HPV testing.

There is a significant problem with the approach taken to examine factors associated with HPV prevalence in pregnant women. The authors specifically highlight their concern in including the 115 high risk pregnant women in an analysis of prevalence but then include all these women and HPV positive women from the general screened population for an analysis to determine factors associated with HPV infection in pregnancy. By design this group of 143 women are a biased sample. It would have been more appropriate to start with an examination of HPV prevalence and type distribution in the screened pregnant study population (n=828), describe the type distribution in this group and then go on to describe factors independently associated with infection. The number of pregnant women approached for this part of the study, the number consented, and enrolled, and the timeframe in which
this occurred need to be reported. Tables 1 and 2 would then present the screened pregnant population characteristics and associations with HPV. The remaining tables would then focus on the 143 mother-child pairs.

The overall study design is now better explained making emphasis on the justification for the different components used: the initial cohort, the HPV screening survey to include more HPV-positive pregnant women, and the final prospective cohort study.

As explained before to reviewer 1, we don’t have risk-factor data for the HPV-negative women screened, so we cannot assess HPV determinants in this group. We believe that the fact that a fraction of women in the cohort are highly selected is not a problem to assess determinants of infections, as this approach is similar to the standard case-control study design in which “cases” are “HPV-positive pregnant women” and “controls” “HPV-negative pregnant women”. This case-control design is efficient in a low-risk population such that in Spain, as it concentrates efforts in recruiting women with the disease of interest, HPV positivity in our study, and selects controls which are generally similar to the cases from the socio-demographic and hospital catchment area perspective. We don’t think this is an important limitation that may introduce substantial bias. The only bias would be in the inclusion of all pregnant women, those form the initial cohort plus those from the screening survey, in the estimation of HPV prevalence in pregnancy. But the final prevalence estimate that we provide for pregnant women is computed only among those in the HPV screening survey, thus not including the highly selected women from the initial cohort study.

A second related but separate part of the study is the prospective evaluation of transmission. A more careful description of how positive and negative pregnant women were selected for this part of the study, the sample size, exclusions, etc. should be reported. This is now explained in detail in the re-written “Methods” section.

Were pregnant women told their HPV status prior to cohort enrollment?
Only HPV-positive women were told their HPV status.

Results:
As mentioned above the presentation of sample size is inconsistent. In the first paragraph 54 HPV positive women were reported from the 828 screened. In the next sentence a total of 82 HPV positives are reported with no description of where the remaining 28 HPV positive women were derived from.
Again, the section has been re-written and all the numbers are now consistent throughout the paper sections.

Caution should be taken in reporting HPV prevalence estimates from a population of pregnant women specifically selected on HPV status. See comments above. I strongly suggest this analysis be replaced with one derived from the screened pregnant population of 828 women. Similarly, determinants of HPV status is more appropriately estimated form the screened pregnant population.
See comments above. In brief, risk factor data is only available for the women recruited in the prospective cohort study. Women in the HPV screening survey did not undergo interview/questionnaire unless they were HPV-positive and were included in the prospective cohort study. The HPV prevalence estimates for HPV in pregnant women that we provide are based on women included in the HPV screening survey and do not include the highly selected women of the initial cohort study.

Please add the data for genotype specific concordance to Table 4.
New Table 5 (old Table 4) includes now the type-specific concordance among mother-child pairs.
Discussion:
Please add a limitations paragraph to the manuscript, especially for the transmission component of the study reported. For example how does acquisition of HPV post-partum influence HPV status of the child – can this be measured and assessed? What data are needed to more clearly distinguish vertical from horizontal transmission? Are there any available data to explain the HPV positivity among children born to HPV negative women? Also, add in citations to support statements regarding ease of transmission of genital compared with cutaneous infections and horizontal transmission.
These issues are now addressed in the discussion.
Reviewer 3:

Major compulsory revisions:

1. As there are two time-points for HPV detection in the mothers, was classification as “HPV-positive” or “HPV-negative” made solely on the pre-natal visit? The number or absence of “incident” or new infections noted at the post-partum visit should be stated.

The classification of mother’s HPV status was made solely on the pre-natal HPV screening visit.

The correlation between HPV status at pregnancy and at the post-partum visit is explained in a new paragraph that has been added:

“A total of 118 women had valid PCR results both at pregnancy and at the post-partum visit. Among women HPV-positive at pregnancy HPV persistence up to the post-partum visit was 46.2% (24/52). New infections at the post-partum visit among HPV-negative women at pregnancy occurred in only one woman (1.5%, 1/66). Thus, there was a strong association between HPV status at pregnancy and HPV status at the post-partum visit (p<0.0001). Type-specific concordance among HPV-positive women between pregnancy and post-partum visit was 100% (17/17, after excluding 7 women with HPV X at either visit)”

2. If I follow the study design correctly, all HPV negative women were drawn from the high-risk pilot group. This group was indeed high risk, as 28/115 = 24.4% were HPV positive compared to the unselected group (6.5%). The authors should address this as another possible explanation for HPV transmission noted from HPV negative mothers (i.e. increased likelihood of HPV exposure increases risk of false negative results).

This is correct. All HPV-negative women included in the final prospective study came from the initial cohort study (i.e. none came from the HPV screening survey). However, not all women from the initial cohort were high-risk women as explained now in the text (“A total of 115 consenting pregnant women were included and classified according to their risk of HPV exposure into high- (n=73) and low-risk (n=42) groups”). Nevertheless, we agree with the reviewer that increased HPV exposure may increase risk of false negative results which might somehow explain HPV transmission among HPV-negative mothers. We have added the following paragraph in the Discussion:

“In assessing HPV positivity in children born to HPV-negative mothers we can not rule out that these mothers were false HPV negatives at pregnancy. We need to take into account that 52% of the HPV-negative mothers came from the high-risk group of women included in the initial cohort study. Thus, increased HPV exposure may increase risk of false negative results which might somehow partially explain HPV transmission among HPV-negative mothers.”

3. The authors note that HPV detected in the mother at the post-partum visit is a stronger correlate of HPV detection in the child than HPV detected during pregnancy. They argue that this is evidence for horizontal transmission. However it should also be considered that time interval between the pre-natal visit and birth is longer, allowing more uncertainty about the “true” HPV status of the mother at delivery. It would be interesting to know if transmission rates were different for mothers with HPV persistence and type-specific persistence.

Thank you for this interesting comment. The association between HPV persistence in the mothers and HPV status in the child has been explored and reported in new Table 4 and in the following paragraph that has been added in “Results” under “Mother to child HPV transmission”:

“As shown in detail in Table 4, mothers testing HPV positive both at the pregnancy visit and at the post-partum visit, were more likely than mothers testing negative at both visits to have HPV positive children at any time during follow-up (29.2% versus 15.4%). However, the increased likelihood didn’t reach statistical significance”
In addition, was there any difference in transmission based on whether mothers were tested at first or second prenatal visit. While the numbers may be too small for a meaningful analysis, it would be helpful to know the time (in weeks or months) of when prenatal sample was obtained.

There was only one pre-natal visit in which a cervical sample was collected. As detailed now in "Methods", the sample was collected at the visit that occurred at mean pregnancy time of 32.1 weeks (rank 30 to 34 weeks) in the initial cohort and at mean pregnancy time of 31 weeks (rank 29 to 33 weeks) for the HPV screening survey.

Does sex of offspring have impact on success of sampling or likelihood of HPV DNA detection?
We explored these relationships and as indicated in the footnote of new Table 4, child’s sex was not associated with HPV DNA detection, neither it was with child’s yield of appropriate oral or genital samples.

4. The biological sampling of both mothers and infants is key to interpretation of the data and should be more fully described. For the mothers, a cervical sample was obtained. What was the collection device, what media and volume was used for collection, how was sample stored prior to extraction? For the infants, a dry swab was used to sample mouth and genital region. What kind of dry swab? What sampling method was employed and was it taught and performed reproducibly (i.e. what specific site or sites was rubbed, for how long and with what force)? For both samples, what volume was extracted, what extraction method was used, what was the final volume of the extract and what volume of extract was added to the 50 microliter PCR? These details are important for others seeking to reproduce these results and should be included to remind others of the importance and attention that must be paid to the biologic samples. A 15% (26/169) loss of participants due to inadequate infant samples further attests to the importance of sampling. [It appears that different personnel may have sampled the infants at birth and at the 6-week visit; are the authors certain that this did not impact difference in HPV detection?]

The “Methods” section has been re-written and detailed information on these issues are now provided in the new text.

5. What was the time-frame of the pilot study compared to the consecutive enrollment study, and what was the total time-frame for enrollment?
The dates have now been included for the initial cohort study, which was started in 1995, and the HPV screening survey carried out between 1997 and February 2000.

Were all women attending the prenatal clinic offered enrollment and did all consent?
All women were offered enrollment and refusals were not registered but replaced by the next attending and consenting woman. We don’t think this may introduce much bias for the analyses about determinants of HPV infection or transmission estimates (except for the false negative issue addressed above). Selection of HPV-positive and HPV-negative woman follows a case-control design in which efforts are made to recruit and concentrate on the cases, i.e. HPV positive women, a rare event in Spain since it is a low-risk country for HPV and cervical cancer.

6. The MY09/MY11 primer system has been largely superseded by the PGMY primer system because of improved reproducibility of primer synthesis and improved detection of mucosal HPVs. This deserves a comment. In addition, both primer systems detect cutaneous types with poor efficiency, so it is unlikely that a significant proportion of “HPV X” is attributable to cutaneous HPVs.
This has been addressed in the discussion in the following paragraph:
“Thus the high percentage of samples classified as HPV X could be true rare genital HPV types, cutaneous types (unlikely because of the poor efficiency of the primer system in detecting cutaneous types) or still untyped HPVs. Furthermore the MY09/MY11 primer system has been improved by the PGMY primer system in terms of improved reproducibility of primer synthesis as well as detection of mucosal HPVs. Unfortunately, very few samples remained available for re-testing with the newer PGMY system. This limitation may also have resulted in an underestimation of the true underlying type-specific concordance”.

Minor essential revisions
1. The statement “active measures to reduce the risk of cross-contamination were implemented at the clinic and at the laboratory where the PCR was performed” (last sentence of HPV detection methods) should really be expanded. What specific measures were used in the clinic? What were used in the lab? Was a water blank taken through extraction process with each set of samples and tested to control for contamination during extraction? Were water blanks used for each set of amplifications? What steps were taken if contamination was noted?

This has been also addressed in the Methods section.
METHODS
HPV-DNA detection and genotyping
"Samples hybridizing with the generic probe were also tested with probes for HPV 6, 11, 16, 18, 31, 33 and 39 specific probes." ? combined - which primers were used?
The type-specific testing was done individually for each specific type. We used the consensus primers MY09/MY11, and the same PCR products positive with the generic probe hybridization were also tested with HPV 6, 11, 16, 18, 31, 33 and 39 specific probes to asses type-specific positivity.

"Specimens were processed blindly to child or mother status and active measures to reduce the risk of cross contamination were implemented at the clinic and at the laboratory where the PCR was performed. " Since the authors conclude that there was a considerable percentage of horizontal spread, it would be of interest if they elaborate on "..active measures.." and if they followed up if the women were at all taking them.
The protocol to avoid contamination in the clinic was applied throughout the study with the same trained personnel (nurses, pediatrician and gynecologists). There was no evidence of contamination. The active measures to reduce contamination are now detailed in the text under section "Cervical samples and follow-up":
"Particular care was taken with sample collection procedures to prevent cross-contamination between subjects and different anatomical sites by using disposable equipment and changing bed lining and gloves for each woman."

RESULTS
Baseline characteristics of subjects in the cohort "Table 2 summarizes the cytological results in the mother and the HPV results in mothers and infants at different timepoints during follow-up. Of the 143 pregnant women included in the cohort, 46.2% tested positive for HPV-DNA during pregnancy.

HPV persistence in mothers and infants " Comment on discrepancies between HPV test and Pap smear and severity of cytological abnormality and HPV vertical transmission rate. Was there any association found between mothers' cytological status at pregnancy and children's HPV status at any of the visits combined.
As sown in Table 3, there was a strong association between cytology results in the mother and HPV positivity in the mother. Thus pregnant women with LSIL or higher abnormalities were 3.15 times more likely to be HPV positive than women with ASCUS or a normal cytology result.
As shown in new Table 4, cytological status at pregnancy was not associated with HPV positivity in the child at any point during follow-up.
These results are now included in Table 3 and new Table 4, as suggested by the reviewers.

DISCUSSION
"First, we found that up to 16.9% of children born to HPV-negative mothers had HPV infections in the first 24 months of life. This percentage is only slightly higher, and not statistically significantly different, than that observed in infants born to HPV positive mothers (19.7%)."
This error has been now amended.
Add a comment on the impact of your data regarding percent of horizontal transmission on the potential usefulness of elective caesarian section as an attempt to prevent neonatal HPV transmission.

As shown in new Table 4 and below, type of delivery was not associated with HPV positivity in the infant at any point during follow-up. Thus, there is no evidence from this study that performing cesarean section reduces the risk of HPV infection in the new-born.

<table>
<thead>
<tr>
<th>Type of delivery</th>
<th>HPV Positive/ Tested</th>
<th>HPV-positive children (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eutocic</td>
<td>12/86</td>
<td>14.0</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td>Instrumental</td>
<td>10/38</td>
<td>26.3</td>
<td>2.20 (0.85-5.68)</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>4/19</td>
<td>21.1</td>
<td>1.60 (0.45-5.75)</td>
</tr>
</tbody>
</table>

Chi-square p-value 0.24