Reviewer's report

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

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Reviewer: Elizabeth R Unger

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To address the difficult issue of vertical transmission of HPV, the authors conducted a well-designed cohort study of 82 HPV-positive women, 87 HPV negative women and their offspring. The study provides additional evidence that the risk of vertical transmission is low and that HPV persistence in infants is rare. The data is important and will be used by the HPV community. There are several questions the authors should address.

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.

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).

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. 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. Does sex of offspring have impact on success of sampling or likelihood of HPV DNA detection?

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?]

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? Were all women attending the prenatal clinic offered enrollment and did all consent?

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.

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?

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 declare that I have no competing interests.