PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	TIMING OF TWO VERSUS THREE DOSES OF QUADRIVALENT HPV VACCINE AND ASSOCIATED EFFECTIVENESS AGAINST CONDYLOMA IN SWEDEN: A NATIONWIDE COHORT STUDY.
AUTHORS	Lamb, Favelle; Herweijer, Eva; Ploner, Alexander; Uhnoo, Ingrid; Sundström, Karin; Sparén, Pär; Arnheim-Dahlstrom, Lisen

VERSION 1 - REVIEW

REVIEWER	Eugenio Ventimiglia Division of Experimental Oncology/Unit of Urology; URI; IRCCS Ospedale San Raffaele
REVIEW RETURNED	28-Nov-2016

GENERAL COMMENTS	In this register based cohort study, the authors investigate whether optimal timing of two doses of qHPV vaccine could confer the same level of protection against condyloma as a standard three dose-schedule. The paper is well written and the study question appropriately addressed. However, some considerations need to be addressed before the manuscript can be considered suitable for publication: - What is the expected coverage of the Swedish HPV Vaccination Register (i.e., is it possible to have an estimate of the proportion of included subjects?) - It is not clear the how long was the follow-up in the 2-doses and 3-doses group. Please report it (as median and IQR, not as mean-range) - The two doses administration seems to be much more represented in the 17-19 years group compared to the ≤16 years group. Please explain how this does not represent a limitation to your results - If possible, it would be interesting to see baseline differences in between the 2-3 doses scheme groups (e.g., geographical, socioeconomic).

REVIEWER	Kate Cuschieri Royal Infirmary of Edinburgh NHS Lothian United Kingdom
REVIEW RETURNED	19-Dec-2016

GENERAL COMMENTS	Two dose schedules of prophylactic HPV vaccine have been introduced at the programme level in various settings. Justification for this approach was largely based on data which suggested that antibody levels generated by a 2 dose "prime-boost" strategy generated levels consistent with those where clinical efficacy had

	been demonstrated in the past. Determination of actual, clinical efficacy in programmes where two doses have been introduced is important to further validate this assumption. The m/s which uses genital warts as an "early" outcome of success is therefore welcome. There are some issues with the m/s that would benefit from attention The first line of the intro states ."...typically require multiple does to confer sufficient immune response". As we do not know what a "sufficient" response for protection is (as is alluded to later in the m/s) - the authors should consider rephrasing this The authors are realistic about the limitations of the study, one key limitation being that with the various stratifications for age/dosing, the exclusions and the timeframe of the extract the case numbers within certain, stratified groups are very small. Apologies if I missed this, but are the authors committed to repeat the analysis to see if their results are consistent over time? The data on the 8m+ (between two doses) being less protective than the 4-7 month group was somewhat surprising to me and I wonder how much of this may be driven by power? The data on the 8m+ group is insufficiently discussed. My understanding is that policy makers - at least in certain settings - set a minimum of 6 months between doses but are less proscriptive about the upper end...If there is an issue with postponing the second dose then this should be discussed further. Line 15 – page 9 = typographical error The authors discuss the underestimation "in the registers for the true number of condyloma cases " and suggest that this "would be either non-differential with regards to vaccination exposure, or conservative in impact, based on the apparent health-seeking behavior of women who are vaccinated" I am not sure why it would necessarily be non-differential with regards to vaccine exposure. I would suggest that this point needs teased out.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Q1. What is the expected coverage of the Swedish HPV Vaccination Register (i.e., is it possible to have an estimate of the proportion of included subjects?)

A1: The coverage of the Swedish HPV vaccination register was 80-85% between 2006 and 2010. We thank reviewer 1 for highlighting the absence of this information in the Manuscript. We have corrected for this on page 20 with the quoted text below.

“Another potential limitation is that SVEVAC was a voluntary register for the period 2006-2010, with only 80-85% coverage. To avoid an underestimation of vaccination exposure, we complemented missing data using the Prescribed Drug Register. This method has been used previously in a study by Herweijer et al, who found unique vaccination dose dates for 99.6% of the vaccinated girls and women in the cohort. (reference 13)”

Q2. It is not clear the how long was the follow-up in the 2-doses and 3-doses group. Please report it (as median and IQR, not as mean-range)

A2. We have changed the reporting of the follow up time from mean and range to Median and IQR as suggested by reviewer 1. This information can be seen on page 10

“The median time in follow up was 259 days [interquartile range = 186 - 1271 days]”

Q3. The two doses administration seems to be much more represented in the 17-19 years group compared to the ≤16 years group. Please explain how this does not represent a limitation to your results
- If possible, it would be interesting to see baseline differences in between the 2-3 doses scheme groups (e.g., geographical, socioeconomic).

A3. We assume that the issue of overrepresentation of two doses among the older subjects is based on our Table 1, which indeed shows that relatively speaking, many more subjects contribute two dose risk time among the older cohort (ca. 50,000 for two doses vs ca. 40,000 for three doses) than for the younger cohort (ca. 224,000 vs 156,000). However, these numbers do not represent two separate classes of subjects that can be easily compared in terms of their baseline characteristics: as stated in the Methods on p.8, we have modelled vaccination status as a time-varying exposure, so that every subject who contributes risk time after three doses necessarily has contributed risk time for two doses previously.

Consequently, the number of subjects who did not receive a third dose during the study period is reflected by the difference in subjects contributing risk time to two and three doses, e.g. for the older group ca. 50,000-40,000 = 10,000 subjects. Note however the crucial caveat “during the study period”: a subject who received their second dose just a few months prior to the end of study may well have got their third shot just after that period, and only differs administratively, not systematically, from other three dose subjects. The large overhang of ca. 224,000-156,000 = 68,000 subjects with only two doses does not reflect a more slovenly approach to getting vaccinated in the younger cohort, but the fact that the Swedish school vaccination program started during the last year of our study period, so that many girls had not yet been exposed to their third shot (note that this is also what drives the large shift from mean to median follow-up time above in A3).

In summary, the number of subjects in Table 1 is not indicative of different behavior in terms of vaccine uptake, but only serves as a measure of underlying available information, and by implication, precision of the derived estimates.

That said, we share the reviewer’s concern about socioeconomic status and geographic location as potential confounders for the relationship between number of doses and subsequent condyloma. For the former, we have performed sensitivity analyses that included mother’s and father’s highest education level, nearest to the date of entry, as proxy for socioeconomic status and have included a supplementary table as part of the resubmission (supplementary table 1) as we do not believe that it is a confounder in our study. We have also amended the manuscript accordingly to account for the addition of the sensitivity analysis on page 7, 9, 15 and 21.

In terms of geographical information, we agree with reviewer 1 that this would be interesting but it is not something that we were able to do here.

Reviewer: 2

Q1. There are some issues with the m/s that would benefit from attention
The first line of the intro states."...typically require multiple does to confer sufficient immune response". As we do not know what a "sufficient" response for protection is (as is alluded to later in the m/s) - the authors should consider rephrasing this

A1. This has been addressed this in the manuscript on page 5. We removed the word “sufficient” from the first line of the introduction so it now reads:

“Human Papillomavirus (HPV) vaccines are subunit vaccines containing virus-like particles (VLPs), and typically require multiple doses to confer an immune response”

Q2. The authors are realistic about the limitations of the study, one key limitation being that with the various stratifications for age/dosing, the exclusions and the timeframe of the extract the case numbers within certain, stratified groups are very small. Apologies if I missed this, but are the authors committed to repeat the analysis to see if their results are consistent over time?

A2. We agree that a repeat analysis with longer follow-up time would be ideal. However, we do not have access to data with longer follow-up time.

Q3. The data on the 8m+ (between two doses) being less protective than the 4-7 month group was somewhat surprising to me and I wonder how much of this may be driven by power?

A3. Power may be an issue in terms of significance, but not in terms of the effect size and while the power is low for the 8+ month group it is not low enough that the significance of the findings are in error. We have discussed this issue at length and we think that because the 8+ month group had fewer individuals the excess risk we see may be due to confounding that we have not been able to address, i.e. there may be some (unknown) underlying reasons for both having long time to dose 3 and high incidence/high exposure. The increased risk in the 8+ group would then possibly be caused by selection.

Q4. The data on the 8m+ group is insufficiently discussed. My understanding is that policy makers - at least in certain settings - set a minimum of 6 months between doses but are less proscriptive about the upper end...If there is an issue with postponing the second dose then this should be discussed further.

A4. It is true that there is a minimum recommendation of 6 months between doses for a two dose schedule. However, we don’t really know what the maximum time between the two doses can be before the effectiveness is affected and this is probably why it is less proscriptive about the upper end. We cannot really elucidate here regarding that however, merely report what our data shows. We have however, included a paragraph on page 22 of the manuscript that discusses the need for further studies and our finding from previous question.

“The finding that the 8+ months between doses was less protective that the 4-7 month group was unexpected as for one-dose priming schedules it is often better with a longer interval between doses. Since this is an observational study, we cannot exclude that our finding was due to an unmeasured confounding factor however, with some (unknown) underlying reason why these girls had a longer time to dose three and high incidence/exposure. While we can only speculate about this higher risk in the 8+ month group, it has highlighted the need for further studies with a longer follow up time investigating the upper time limit between doses and vaccine effectiveness.”

Q6. Line 15 – page 9 = typographical error

The authors discuss the underestimation "in the registers for the true number of condyloma cases " and suggest that this "would be either non-differential with regards to vaccination exposure, or conservative in impact, based on the apparent health-seeking behavior of women who are vaccinated" I am not sure why it would necessarily be non-differential with regards to vaccine

exposure. I would suggest that this point needs teased out.

A6. We have amended that section of the manuscript (page 20) so it now reads:

“A limitation of our study is that a small proportion of patients will neither seek hospital care for condyloma nor receive prescription for treatment, and thus will not be included in the registers, resulting in an underestimation of the true number of condyloma cases. (reference 12) However, we expect this to be negligible in our study, as a) vaccinated women have been found to have higher screening uptake than unvaccinated women and can thus also be assumed not to be less prone to access healthcare (reference 30) b) it would only inflate the estimated effect of the two-dose regimens if the subjects less willing to complete the three-dose regimens would be substantially more likely to see healthcare for genital warts than those who complete three doses.”

VERSION 2 – REVIEW

REVIEWER	Eugenio Ventimiglia IRCCS Ospedale San Raffaele Italy
REVIEW RETURNED	03-Feb-2017

GENERAL COMMENTS	The Authors have replied properly to all my concerns.

REVIEWER	Kate Cuschieri Scottish HPV Reference Laboratory NHS Lothian UK
REVIEW RETURNED	20-Feb-2017

GENERAL COMMENTS	The authors have provided considered responses to the reviewer comments. Generally all have been addressed adequately other than (page 20, line 16) "b) it would only inflate the estimated effect of the two-dose regimens if the subjects less willing to complete the three-dose regimens would be substantially more likely to see healthcare for genital warts than those who complete three doses.” ...where the English and syntax need to be checked and amended to improve clarity,

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

Q1. The authors have provided considered responses to the reviewer comments. Generally all have been addressed adequately other than (page 20, line 16)

"b) it would only inflate the estimated effect of the two-dose regimens if the subjects less willing to complete the three-dose regimens would be substantially more likely to see healthcare for genital warts than those who complete three doses.”

...where the English and syntax need to be checked and amended to improve clarity,

A1: We thank reviewer 2 for bringing this to our attention. We have addressed this in the manuscript on page 20 so it now reads:

“b) the estimated effect of the two-dose schedule would only be inflated if girls less willing to complete the three-dose schedule would have been more likely to seek healthcare for condyloma than those going on to complete three doses.”

We hope that this has clarified the text sufficiently.