**Author’s response to reviews**

**Title:** Monitoring vaccine and non-vaccine HPV type prevalence in the post-vaccination era in women living in the Basilicata region, Italy

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Philippa Harris
Editorial Manager
BMC Infectious Diseases

Re: Resubmission of original research manuscript

Dear Dr. Harris,

Thank you for allowing us to resubmit our manuscript, Monitoring vaccine and non-vaccine HPV type prevalence in the post-vaccination era in women living in the Basilicata region, Italy. We have substantially revised the manuscript based on the reviewers’ feedback and believe that it is timely and relevant to the readership of BMC Infectious Diseases.

As requested, we have updated the manuscript as requested by the reviewers as outlined below. Please see the detailed responses following this letter and the track changes within the manuscript.

Thank you in advance for reviewing this manuscript again, and we hope for a positive response regarding publication. Please do not hesitate to contact me if you have any questions or need anything further.

Sincerely,

Géraldine Dominiak-Felden, MD, MSc
Editor Comments:

1. Please include email addresses for all authors on the title page

Response: email addresses have been added for all authors on the title page.

Reviewer reports:

Vladimir Gilca (Reviewer 1): INFD-D-16-01694

- This study presents data on the HPV types prevalence a few years after a vaccination program and a catch-up was implemented.
- The manuscript might be improved by the use of a more academic approach and less use of some speculative words and generalities.

Response: We have revised the manuscript to use less speculative words and generalities.

- The authors state that the «… study aimed to describe the effect of the introduction of qHPV vaccination program on vaccine and non-vaccine HPV types prevalence…". Due to the cross-sectional design of this study (only one time-point measurement) it seems more appropriate to use "… to assess vaccine and non-vaccine HPV prevalence 5-7 years post-vaccination program implementation in vaccinated and unvaccinated women…".

Response: We changed the text as indicated in the Abstract and Background.

- Taking into consideration the low participation rate of women 18-24 y.o and the unknowns regarding the proportion of 25-50 y.o attending cervical cancer screening, the use of the wording "population-based study" is questionable.

Response: We use the term “population-based” because we invited women 18-24 years directly from the list of resident women in the area. Therefore, we believe that this term is appropriate. We do recognize that the low participation rate of 18- to 24-year-olds is a limitation of the study and we have mentioned this in the Discussion. In addition, regarding women 25-50 years, we added data on adherence to cervical cancer screening in the Background section (lines 121-122).
- It is unclear what is the number of vaccinated/unvaccinated women by age group. What was the study power to detect statistically significant differences among study groups (i.e. prevalence of different HPV genotypes)?

Response: We have added a new table (Table 1 in the revised version) reporting on the number of vaccinated and unvaccinated women by age group. We added in the Discussion section (line 368-371) the calculation for the minimal significant difference that we could detect with our sample size: “With a sample size equal to 1314 women (41% unvaccinated and 59% vaccinated) and a power of 80%, we were able to show a statistically significant reduction of the prevalence equal to 2.8% for HPV16/18 and 4.6% for HR-HPV types between unvaccinated and vaccinated women.”

- In results and discussion sections some comparisons and statements are made like "lower prevalence" or "higher prevalence" but it is unclear if these differences were statistically significant. According to the data presented in the Figure 1 the only statistically significant difference is for HPV 16 prevalence in vaccinated and unvaccinated women.

Response: We agree that this was confusing and have stated statistically significant differences as appropriate throughout the manuscript.

- Results and Discussion sections might be shortened by concentrating on the main study results and avoiding insignificant ones (i.e. HPV 51 in vaccinated vs unvaccinated (1.4% vs. 1.0%, p=0.66) or HPV39 (1.7% vs 1.7%, p=1.0).

Response: As advised, we have deleted the paragraph that discusses HPV 39, 51, and 58 in the Results Section and Discussion.

- The sections "Non-vaccine type prevalence" and "Type-replacement" might be reduced to 2-3 sentences plus the tables.

Response: We have deleted text in both sections.

- Some minor suggestions: line 99 delete or explain "… is also well organized…”; line 188 delete "significant”.

Response: We have deleted the sentence on line 99 and deleted “significant” from line 188.
- In the abstract the "efficient HPV vaccine registry" might be changed for "regional HPV vaccine registry"; in the abstract conclusions the word "large" might be deleted.

Response: We did not see “efficient HPV vaccine registry,” but we deleted “large” from the abstract.

- The attendance of a university outside of Matera seems relatively weak for explaining the low participation rates in 18-24 y.o.

Response: We recognize that this explanation may seem weak, but we feel that it is one of the most plausible, in light of the fact that during summer and Christmas holidays we observed an increased attendance rate for 18- to 24-year-olds compared with the other months. We believe that this is linked to the homecoming of female students and offsite workers.

- Line 363: what exactly is the meaning of "non-prevalent HPV types"?

Response: We have made changes to the text to clarify this point.

- Line 390: with a vaccine uptake of 59% I would suggest avoiding "well-implemented vaccination".

Response: “well-implemented” was deleted.

- Authors may want to discuss the possibility of a herd immunity in younger age groups. Such a protection would diminish the capacity to detect differences between vaccinated and unvaccinated young women.

Response: We feel that discussion of herd immunity is not relevant to our results for two main reasons: (1) We investigated vaccination for the female birth cohort (11, 14, 17, and 24 years) only and not for the entire population, so a herd immunity effect is very unlikely; (2) the HPV prevalence data for unvaccinated women in our sample are highly comparable to other Italian studies that have investigated HPV prevalence.

- Line 404: I would suggest avoiding "with a complete vaccination schedule" and to keep "are vaccinated before sexual debut". Authors clearly mention they did not assess the effectiveness of incomplete vaccination schedule.
Response: We have made this change.

- Line 457: "very important" might be changed for "important".
Response: We have made this change.

- Line 459: to avoid a potential misunderstanding the wording "has a direct effect on more oncogenic…” might be changed for "…vaccine which protects against additional five oncogenic HPV types"
Response: We have made this change.

- Lines 463-465 should be revised. The wording "significantly reduces" should be avoided because the study did not measure the reduction of the HPV prevalence. Avoid also qualificatives like "good preliminary…". A more precise terminology is preferable.
Response: Because our data showed a significant reduction in prevalence of HPV vaccine types among vaccinated women compared with unvaccinated, we prefer to maintain the word “significantly.” However, we have added at the end of the sentence “compared with the prevalence in unvaccinated women” to clarify this point.

- Conclusions would profit if based exclusively on study results and less on what will happen in the future.
Response: We have substantially modified the Conclusions section.

Dorothy Machalek (Reviewer 2):
The manuscript by Carozzi et al. evaluated the effect of HPV vaccination on the prevalence of vaccine and non-vaccine targeted HPV types among age eligible (18-30 year old) Italian women,
in the post-vaccine era. The article also provides information on the age-specific prevalence of HPV among a broader cohort of women up to the age of 50 years. Overall the reported reductions in vaccine-targeted HPV genotypes among vaccinated women contribute important data on the population impact of the vaccination program in Italy. My comments are as follows:

Major

1. The manuscript could be improved by a clearer focus on the primary analysis comparing vaccinated and unvaccinated women in the 18-30 year age group, rather than describing everything without clear indication of relevance. Please reconsider including supplementary tables 1 and tables 2, in the main body of the manuscript, with Tables 1 to 3 moved to the supplementary section.

Response: Based on this suggestion, we moved Tables 1 to 3 in the Supplementary Appendix and we focused the analysis on the comparison among vaccinated and not vaccinated women, including moving the Supplementary Tables 1 and 2 to the body of the manuscript. Moreover, text in the Results section has been modified to address the new tables.

2. Further to the above, my main point of critique is in the merging of data from two sampling frames to present HPV prevalence rates across the entire 18-50 year old cohort (estimates presented in Tables 2 and 3). The potential bias of this on the reported estimates of HPV prevalence overall, is not acknowledged in the manuscript. The key issue is that women recruited from the community are likely to be significantly different in terms of HPV risk factors, to those recruited as they present for routine screening. The percentage testing positive measured from populations accessing testing (i.e. cervical cancer screening) cannot be extrapolated to the other populations as individuals tested tend to have different risks of infection than those who have not been tested. The authors try to account for this by comparing demographic characteristics of the study population to those of the general Italian population using ISTAT data, reporting little differences. However, I am not fully convinced that this justifies the approach.

Response: The aim of the present paper was to compare vaccinated versus not vaccinated women in terms of prevalence of HPV infection (and type-specific infection). Thus, the fact that women attending cervical cancer screening could differ from the general population in terms of HPV risk factors does not bias the validity of the comparison of the relative risk of HPV prevalence between vaccinate vs not vaccinated women because they were collected from the same population.
3. The authors stated a very high participation rate (>98%) among women presenting for screening. What proportion of women access screening in the region? It would be valuable to add that information in the introduction.

Response: In Basilicata, the adherence rate of cervical cancer screening was, on average, 57% for 2013-2014 (Ronco G, personal communication). We added this information to the Background section (lines 121-122).

4. The way the data is organised in Tables 4 and 5 is a little unclear. The primary analysis includes the full cohort of women eligible for the vaccine (i.e. women aged 18-30 years), while the analysis among 18-24 year olds is a sensitivity analysis among women recruited from the community only. Is that correct? If so, this needs to be clarified in the methods. The authors could consider moving the results of the sensitivity analysis to the supplementary table.

Response: Based on the referee’s suggestion, we have modified the referenced tables (corresponding to Tables 3 and 4 of the revised version), but we prefer to maintain both tables in the body of the manuscript. In the revised version, Table 3 reports prevalence rates and odds ratios for vaccinated and unvaccinated 18- to 30-year-olds, whereas Table 4 reports the same data for women naïve for vaccination at 18-24 years old. Data on vaccinated women at all three doses were deleted from the tables and we added into the text the following sentence: “All analyses were repeated considering as vaccinated only women who completed all three doses before the consent date; similar results were found (data not shown).” We appreciate the suggestion and think that the tables are more clear and readable now.

5. The multivariable analysis presented in Table 5 includes all factors that were significantly associated (p<0.1) with HPV infection. The models should also be adjusted for sociodemographic characteristics that varied between vaccinated and unvaccinated women (those in the supplementary tables). Furthermore, results of both unadjusted and adjusted analyses should be included.

Response: Based on the referee’s suggestion, we have added marital status to the multivariate logistic model. The other sociodemographic characteristics (e.g., country of birth, education level, and occupational status) were not significantly associated with HPV infection in the univariate analyses: P = 0.78, P = 0.47 and P = 0.14, respectively. Among women 18-30 years (Table 3), we feel that crude odds ratios may be misleading due to the different age distribution among vaccinated and not vaccinated women, and odds ratios adjusted only for age were quite
similar to the odds ratios adjusted for all covariates. Thus, we prefer to leave out the unadjusted odds ratios.

Minor

Line 218: please add the proportion with a high school diploma or higher that was expected in the younger age group (i.e. study: 87%; ISTAT: ?%)

Response: We have added this information to the manuscript.

All tables where relevant: Please add numerators and 95% confidence intervals for all prevalence estimates.

Response: We have added numerator values for the brackets related to prevalence rates. We feel that adding 95% confidence interval to all prevalence rates would make tables cumbersome and less readable.