Author's response to reviews

Title: Prevalence and type distribution of Human Papillomavirus infection in women from North Sardinia, Italy.

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Author's response to reviews: see over
Dr. Ibrahim Abubakar  
Associate Editor  
BMC Public Health  
RE: Submission of revised manuscript for “Prevalence and type distribution of Human Papillomavirus infection in women from North Sardinia, Italy.”  

(Manuscript ID:1231465792559758)  

Dear Dr Abubakar,  

On behalf of the Authors, I would like to submit a revised version of the manuscript on the “Prevalence and type distribution of Human Papillomavirus infection in women from North Sardinia, Italy.” for publication in BMC Public Health.  

We addressed the comments of the reviewers (Dr Giovanni Gabutti and Kate Soldan), including your editorial requirements. All the point-by-point responses are displayed in two easy-to-read tables.  

As stated when we initially submitted the manuscript for peer review, all the Authors substantially contributed to the design and implementation of the study as well as to the drafting and to the revision of the manuscript, and declare that they have no competing interests.  

The responsibility for the contents of this manuscript rests entirely with the Authors.  

Thank you for considering this paper for publication.  

Sincerely,  

Andrea Piana
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<th>Comments by reviewer GG</th>
<th>Response to reviewer/Revised text</th>
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<tr>
<td><strong>1</strong> Abstract: page 2 line 21. The percentage related to HPV31 should be added.</td>
<td>We thank the reviewer. The requested percentage has been added up (i.e., <strong>10.5%</strong>).</td>
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<td><strong>2</strong> Background: page 4 line 9 the types possibly carcinogenic to humans should be added in brackets.</td>
<td>As kindly requested by the reviewer, the quoted line was edited as follows: “...carcinogenic (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59), probably carcinogenic (type 68) and possibly carcinogenic (types 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85 and 97) to humans (4).”</td>
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<td><strong>3</strong> Methods: page 5 line 9. A sentence on the Ethical Committee approval should be added.</td>
<td>We followed the kind suggestion of both the reviewer and of the AE; a statement on the Ethical Committee approval is now included in the Methods section of the manuscript, quoting the Ethical Committee which gave its authorization to the implementation of the study and the reference number. “.Personal data have been treated in compliance with the Italian Law Decree No. 196/2003, article 24 (Code for the protection of personal data). This study, for which a written consent from each enrolled patient or her relatives was obtained, was formerly approved by the Ethical Committee of the Azienda Sanitaria Locale n°1 of Sassari (PN-132) on June 18, 2007.” Moreover, this statement, as requested by the Editor, has been also reported in the Acknowledgements section: “Written consent for publication in compliance with the Italian Law Decree No. 196/2003, article 24 (Code for the protection of personal data) was obtained from the patients or their relatives.”</td>
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<td><strong>4</strong> Results and Discussion: page 9 line 18 The possibility of HPV replacement following vaccination should be discussed in more detail (does this possibility exist? what is already reported in literature on this topic? etc.)</td>
<td>Following reviewer’s suggestion, comments on replacement were edited as follows: “…The successful nationwide implementation of the HPV vaccination program in Italy will probably change the frequency of the most prevalent genotypes, decreasing the prevalence of the current genotypes and increasing the possibility of HPV replacement following vaccination, as already described for several bacterial infections (Lipsitch 1997). Even if the probability of occurrence of that condition was deemed low until now (Garnet et al 2000), it was recently observed a competitive advantage of some HPV genotypes over other genital HPV types in the unvaccinated population (Merikukka et al. 2011). Therefore, HPV genotype prevalence should be monitored for type replacement before, during and after mass vaccination.”</td>
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<td><strong>1</strong> Describe the selection biases applying to women eligible to enter the study, and the participation rate (and biases) amongst the eligible women. Without this it is impossible to interpret any comparisons to other studies. Similarly, when referring to other studies that have supposedly sampled the &quot;Italian&quot; population, please specify whether women sampled were undergoing cervical screening, and the conditions of participation that may have affected HPV prevalence.</td>
<td>We thank the reviewer for having raised this relevant methodological issue; it allows us to clarify the methodological features behind our study. In Sardinia, Italy, the average coverage of PAP-test in women aged 25-64 years is 49.3%; almost one fourth (i.e., 24.9%) of them are evaluated on a voluntary basis (references 13-14). During the study period we consecutively enrolled women aged between 15 and 54 years admitted to public and private outpatient settings located in the town of Sassari, Northern area of Sardinia, where cervical screening, unlike other Italian geographical areas, was performed on a voluntary basis and not as part of a prevention program. The female population aged 15-54 in Sassari in 2006 was 37,353; consequently, the eligible population (i.e., 24.9% of the total population) was 9,301. On the basis of the prevalences of HPV-positivity and of HPV-16, estimated in an Italian city, (8.8% and 2.87%, respectively; Ronco et al., 2005), we computed the sample size: <strong>Eligible population size: 9,301</strong></td>
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Hypothesized % frequency of outcome factor in the population: 2.87%
Confidence limits as % of 100 (absolute +/- %): 5%
Sample Size = 43

Therefore, we decided to enroll at least 50 individuals in every planned age group (i.e., 15-24, 25-34, 35-44, 45-54 years), as described in the Methods section. Every eligible admitted woman, given the chance to be tested for HPV free of charge, accepted to be enrolled in the study and signed a written informed consent.

Revise literature review in background. Many of the refs cited are not appropriate, e.g. refs 1 and 2 are not the source for the facts in the text, refs 3 and 4 do not give same list of HR types are in text (suggest use IARC monograph 90, and replace HPV68 in current list of 13 types with HPV66).

We thank the reviewer for her suggestion, which allowed us to modify and update the references of the Introduction section. We deleted references from1 to 4, which were replaced by the following ones:


Abstract and main text makes much of the "striking findings" of high frequency of co-infection with HPV 16 and 51, but does not show (with appropriate statistical analysis) that this is a more common occurrence than would be expected given the individual frequencies of HPV 16 and HPV 51. Please add analysis and qualify findings re frequency of co-infections.

Following the kind suggestion of the reviewer, we computed the observed and the expected frequencies of HPV co-infection. Statistical comparison was performed using the test of proportion. The following sentence was added up in the Results section in order to clarify that point: "...Overall, the prevalence of the two most frequent types (i.e., HPV16 and 51) was 19.8% and 13.8%, respectively. The observed proportion of HP16/51 co-infection was 9.7%, whereas the expected one was 2.7% (p<0.001). No HPV16/18 co-infection was detected..."

The specificity of the INNO-LiPA positive results for some of the most prevalent HR-HPV genotypes, such as HPV 51, was further confirmed by means of genotype specific "in-house" Real-time PCR assays, which were based on the detection of different DNA regions within the HPV genome (sequences from genes E1/E2 and E6/E7 as described in references 19,20 and 21 as compared to the conserved L1 region detected by INNO-LiPA). In particular, HPV 51 specificity of the INNO-LiPA was determined by E6/E7 primers and probe described in reference no. 21.

As kindly suggested by the reviewer, the manuscript was amended to clearly indicate that the identification of the most prevalent genotypes was further confirmed using "in-house" Real-time PCR assays:

Methods: "...Samples found to be positive for HR-HPV genotypes 16, 18, 31, 45, 51 and 52 by INNO-LiPA were further confirmed by previously described "in-house" Real-Time quantitative TaqMan PCR assays (19, 20, 21)...."

Results: "...The specificity of INNO-LiPA positive samples for HR-HPV genotypes 16, 18, 31, 45, 51 and 52 was further confirmed by previously described available "in-house" Real-Time quantitative TaqMan PCR assays (19, 20, 21). All re-tested positive samples were confirmed to harbor the same HR-HPV types detected by INNO-LiPA, indicating a high specificity in genotype detection..."."
Moreover, as kindly requested, we pointed out the weakness of our study in terms of statistical power, including a line which states the wideness of confidence intervals: “... However, the precision of our study, as displayed by the wide confidence intervals, may be limited by the small sample size. Therefore, for some low prevalent genotypes the statistical power was low and the possibility that the true prevalence may be different than the observed one cannot be excluded...”.

Thanks for that question: it was felt necessary to confirm INNO-LiPA results with genotypic-specific independent molecular assays as well as to obtain some preliminary indications on HPV viral loads for the most prevalent oncogenic HPV types.

In order to clarify the reasons for re-testing only some of the HPV positive samples and to provide some preliminary (considering the relatively small number of positive samples tested by the quantitative assays) results on viral loads for the more prevalent HR-HPV types, the following paragraph was added to the Results section:

“...The specificity of INNO-LiPA positive samples for HR-HPV genotypes 16, 18, 31, 45, 51 and 52 was further confirmed by previously described available “in-house” Real-Time quantitative TaqMan PCR assays (19, 20, 21). All re-tested positive samples were confirmed to harbour the same HR-HPV types detected by INNO-LiPA, indicating a very good specificity in genotype detection of this assay. Furthermore, overall viral loads detected by the quantitative “in-house” Real-Time PCR assays for the studied HR-HPVs were found to range from 1 to 34,750 copies/10^4 cells with median values for the two most prevalent HPV genotypes 16 and 51 being 962 and 2,055 copies/10^4 cells, respectively...”.

Minor essential revisions

1. Correct and improve the use of English language throughout.

Thanks for that suggestion. The text was evaluated and edited by an English native speaker.

2. State expected proportions used in power calculations.

Thanks for your comment. In the Methods section we added up the following statement: “... on the basis of the female population aged 15-54 in Sassari in 2006 (N=37,353 women), the eligible (i.e.; 24.9% of the total referring to specialized centers on a voluntary basis) population was 9,301 women. Since the prevalences of HPV-positivity and of HPV-16, estimated in an Italian city, (8.8% and 2.87%, respectively; Ronco et al., 2005), the overall sample size for any HPV infection was 318 individuals and the sample size for each planned age group (i.e., 15-24, 25-34, 35-44, 45-54 years) for the most frequent HPV type (i.e., 2.87% for HPV16) was 43. Therefore, at least 50 individuals were expected to be enrolled in every group...”.

3. Please give reason for re-testing samples found positive for HPV 16,18,31, 45,51 and 52 but not other types?

Samples were re-tested only for the indicated HR-HPV genotypes as “in-house” assays have been set up and are being currently used in our laboratories only for those HPV types. Moreover, confirmatory re-testing for only the indicated genotypes was felt to be appropriate as it included the most prevalent observed HR genotypes in our
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<th>4</th>
<th>Split results and discussion.</th>
<th>In order to better clarify the reasons for retesting only these genotypes the manuscript was amended in both the Methods and the Results sections, as previously described.</th>
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<td>5</td>
<td>State whether ”result“ about no statistically significant association between HPV 16, 18 and 51 and abnormal cytology is meaningful or the study lacked power to assess this association?</td>
<td>Thanks for your question: we modified the statement on the association between HPV types adding the following sentence up: “………, but such association was not confirmed with HPV 16, 18 and 51 infections, probably due to the low statistical power of the study, that was set up using the estimated prevalence of HPV-16 infection.”</td>
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<td>6</td>
<td>Comments on page 9 touch on the use of HPV testing as a screening test. Please review whether these comments are justified by the study’s data and necessary to include or would be better removed from the discussion of these data.</td>
<td>As kindly requested, we deleted the section dealing with HPV testing as a screening test.</td>
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<td>7</td>
<td>Table 1. Please show characteristics for positives vs negatives, with chi-2 results.</td>
<td>Thanks for your suggestion. Characteristics of HPV-negatives were added up in the table no. 1. Moreover, statistical comparisons between groups (HPV+ vs. HPV-) were performed by strata (Mann-Whitney test between medians, t-test between means and z-test between proportions as described in the Methods section) and significant results reported as “*=P&lt;0.05” or “**=P&lt;0.01”. The row No 9 “Median (IQR) No of partners since first sexual intercourse” was deleted because pleonastic, being the same variable described in the following rows.</td>
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<td>8</td>
<td>Table 2. Also throughout paper and in Fig 1. Given that the relevance of these data is largely related to HPV 16/18 vaccination, please include data for HPV 18 prevalence specifically, or at least for HPV 16 and 18 combined (i.e. for directly vaccine preventable HR-HPV).</td>
<td>As kindly suggested, we modified table no. 2 and figure no. 1 adding a new column on HPV-18 up, whose prevalence was 1.9%; co-infections with HPV-16 were not detected.</td>
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**Discretionary Revisions**

<p>|   | 1 | Abstract background: check basis for HPV associated with cancers of the skin. To my knowledge, this is not established (certainly not to the same extent) as for genital tract and oropharynx. IARC Monograph 90 concludes “limited evidence” for HPV carcinogenicity in | Thanks for that comment. We followed the suggestion. |</p>
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<td>2</td>
<td><strong>Background:</strong> review whether &quot;recently&quot; is correct for association of HPV and cervical cancer.</td>
<td>Thanks for that comment. We modified the text accordingly.</td>
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<td>3</td>
<td>To better understand the bias in the participants it is necessary to know the findings of previous PAP tests within past 3 years. How many of these women were returning for repeat screening due to previous abnormal cytology (i.e. likely biased to high HPV prevalence)?</td>
<td>Thanks for having given us the opportunity to clarify this point. We actually do not know this information because its collection was not planned in the design of the study. However, we believe that it was not so influential since there is no significant difference between positive and negative women related to the compliance to the PAP test in the last 3 years (see Tab. 1). This shortcoming was also inserted in the Conclusions section where we describe the weakness of the study: “...Moreover, we know that the results of the study may be limited because of the characteristics of the screening adopted in Northern Sardinia so far. In fact, a voluntary based access instead of a systematic one may have selected those patients with higher prevalence of symptoms and it may explain the higher HPV prevalence observed. However, the potential selection bias might be not so influential, since no significant differences between positive and negative women were detected in terms of compliance to the PAP test in the last 3 years, as reported in Tab. 1.” However, Therefore, in spite of this such a this study should be able to give a reliable was performed in order to have a picture of the situation in the local population, particularly regarding the epidemiological HPV pattern, at the beginning of the vaccination campaign of the local population.”</td>
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