Author’s response to reviews

Title: Prevalence of HPV high and low risk types in cervical samples of the Italian general population: a population based study.

Authors:

Paolo Giorgi Rossi (giorgirossi@asplazio.it)
Simonetta Bisanzi (s.bisanzi@ispo.toscana.it)
Irene Paanini (i.paanini@ispo.toscana.it)
Angela Di Iasi (moscati.anatpat@aslcaserta2.it)
Claudio Angeloni (claudio.angeloni@asltermo.it)
Aurora Scalisi (screegin@ausl3.ct.it)
Rosalba Macis (centrodonnacagliari@asl8cagliari.it)
Maria T Pini (matinf.dip59@asl2.napoli.it)
Francesco Chini (chini@asplazio.it)
Francesca M Carozzi (f.carozzi@ispo.toscana.it)
HPV prevalence Italian Working Group (camilloni@asplazio.it)

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Author’s response to reviews: see over
Answers to the editor

Ethics - Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm), and any experimental research on animals must follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

Informed consent must also be documented. Manuscripts may be rejected if the editorial office considers that the research has not been carried out within an ethical framework, e.g. if the severity of the experimental procedure is not justified by the value of the knowledge gained.

RE: We added a paragraph about the approval of our ethics committee and documentation about informed consent.

Further consideration of your manuscript is conditional on improvement of the English used - please bear in mind that as we are a free-access publisher, we cannot bear the costs of copyediting English ourselves. Please ensure particular attention is paid to the abstract.

RE: We engaged an English speaking editor to revise the paper.

Please also highlight (with 'tracked changes'/coloured/underlines/highlighted text) all changes made when revising the manuscript to make it easier for the Editors to give you a prompt decision on your manuscript.

RE: All changes made on the manuscript are in typed in red.
Answers to reviewers

Reviewer 1

RE: The final version of the manuscript has been edited by an English-speaking editor experienced in scientific writing.

Reviewer 2

With the introduction of the new HPV vaccine and the access to more sophisticated technology, manuscripts like this are important to know the importance of HPV infection, their characteristics and their evolution, and then develop strategies to prevent their consequences. In this way, the manuscript recollected a lot of women to reassert their results from different places in centre and south Italy.

In general, I think that the authors has a good material, but it is not always very well balanced, and it need a revision.

RE: We thank the reviewer for the encouraging comment.

Introduction and objective

According to the discussion and the specific section (Systematic review), I think that a second objective should be added: “compare the data with other Italian studies”.

RE: We thank the reviewer for this suggestion. We added this objective in the last sentence of the introduction.

Material and methods

In Population, the population (total women) covered in the areas tested should be added.

RE: We added the reference population in the participating centres.

In Samples collection, the reference or composition of the media to collect the samples must be included.

RE: Commercially available transport media were used in our study. We added the required references in the sample collection paragraph.

In HPV testing, the reference of HCII is needed. The abbreviations must be included after a first description, and always the same (i.e. hr or HR).

RE: We added a reference for the HCII test. Furthermore we specified the abbreviations used in the text and made sure only one type of abbreviation for each item was present throughout the manuscript (for example HR for high risk HPV types).

RE: We added the required information and checked the indicated annealing temperature was the one actually used in our experiments. The primer set volume used is recommended in the Digene amplification protocol for HPV DNA.
Hybridization methods is too large, particularly in revelation system, when below they don’t explain PCR condition for typing HPV-6 or HPV-11. A better balance could be possible without information loss.

**RE:** We re-wrote the hybridization methods, trying to better balance between hybridization and PCR protocols. We specified the method for PCR amplification of HPV 6 and 11.

In Systematic review, like I mentioned above they must explain why they include this section. I think that could be another objective.

**RE:** The objectives have been modified. Now the systematic review is more coherent with the aim of the paper.

Results

Don’t start a sentences with a number.

**RE:** We changed the first sentence of the paragraph.

In this section for better comprehension is necessary to include the most amount of data, and not only use percentage.

**RE:** Numbers (numerator/denominator) have been added in the text.

Also, percentage in figure 1 must be 100% in Y axe, or at least same value (i.e. 25%).

**RE:** We changed the y axe using a uniform scale (0-25%).

In this figure, is significative the slope in section 1a in any case?. The value of p must be included in text.

**RE:** We made a test for trend for both curves and we added the resulting p value in text.

In Typing, there is a mistake in “293 displayed infection by lrHPV”. It will be 293 hrHPV.

**RE:** OK, thanks, it was a type mismatch.

I suppose that the difference between 350 women with hrHPV, and 380 hrHPV positive samples are due to mix infection.

**RE:** No, the difference is due to the fact that in 8 cases the age of women undergoing the test fell out of the selected range (25 – 64) and 22 additional cases were excluded because HPV tests were performed as follow-up analyses. We changed the text and reported the typing flow only for the 350 cases included in the study.

In this section, table 3 can not precede to table 1 and 2. I suggest to change the order (because it is not completely correct to talk of mix infection before to describe genotypes found), or simply change the numbers of tables.

**RE:** OK, we changed the order in the presentation of co-infections.

The legend in table 1 could be better “Distribution of HPV types by age and centre”.

**RE:** OK, we changed the order in the presentation of co-infections.
In this table, it can have a confusion between line “All age” and “25-64 years” according the numbers: a line of separation would be good.

**RE:** OK, we changed the legend of Table 1 and we changed the format for the 25-64 age range.

Why the type of the 3 remaining cases of CINI are not included?.

**RE:** OK, we reported the types for all 17 CIN2.

How can the authors explain types found by hybridization from HCII positive, which are not included in this method?.

**RE:** We adopted a typing strategy that was able to identify cross hybridization of HCII with types not included in the 13 HR types of the HCII HR probe. Furthermore, co-infections with types included and not included in the HCII probes may be detected.

Why they can not type 100 samples?

**RE:** Actually only 12 HR HPV samples were not typed, while 23 samples were HR HCII positive but HPV PCR negative. For LR HPV samples, we searched for HPV types 6 and 11 only, so we classified the samples HPV6 and 11 negative as positive for others LR HPV types.

Discussion
In discussion, the authors include reference to table 4 and figure 2 that they must be related in Results.

**RE:** We added the reference to Table 4 and Figure 2 in the last paragraph of the results.

An explanation or suggestion why the prevalence is similar in screening programs than in studies with a population attending gynaecological clinics or high-risk populations is needed, moreover when lesions in this second group are higher.

**RE:** Actually the prevalence differs between population-based studies and gynaecological clinic-based ones: it is lower in the first case. We added a sentence about this in the discussion, explaining that this finding is not surprising, given the higher prevalence of lesions in women undergoing follow-up analyses upon previous abnormal Pap test, colposcopy or treatment.

The explanation that cancer is lower in south Italy because less HPV test are made could be incomplete, especially when HPV-test coverage is extremely lower. And the idea that are increasing now by sexual behaviour is not completely true. I think that the fact of low coverage of HPV-test underestimate the infection. Indeed, the numbers of cases of cancer are very similar. The authors must explain better this point, because social situation, facilities of screening and more sensitive methods could influence in the data.

**RE:** We are sorry, we were not clear in exposing our hypothesis: despite a well documented lower coverage of Pap test in southern Italy, the incidence of cervical cancer has been historically lower. This was mostly explained by a lower prevalence of HPV compared to northern Italy. Actually, our study does not confirm the previously reported lower incidence. We suppose we are observing a transition period from low to high prevalence in the south; this is also confirmed by the slope of the prevalence curve in southern Italy. The change in sexual behaviours is only one possible explanation for the observed changes.
RE: With regard to cervical cancer screening coverage, the relevant period for actual incidence is from 1980s to the early years of the XIX century. Then, the HPV test had a virtually null diffusion as primary screening test, particularly in southern Italy. Unfortunately, the National Health Interview only collected data on Pap test usage until 2005.

I miss a paragraph about types of HPV found, and differences in methods, like written above in last two sentences of results.

RE: We added a sentence on the comparison with previous studies.

Conclusions
Except last sentence, conclusions are not true conclusions of the work, but suggestions, and they must be included in discussion.

RE: We agree with the reviewer, actually the only true conclusion is that an epidemic of cervical cancer may occur in the south if screening coverage does not improve. We tried to rewrite the conclusions, but if we do not report the first two sentences summarising background and main results, the conclusion is too apodictic and it is not self-explaining. We prefer to leave all the sentences in the conclusions, but we separated the true conclusion from the background sentences.

Abstract
According all comments made before, I think that objective and results are incomplete, for instance to make first conclusions.

RE: We changed the aims of the study.

The last two paragraph in results are conclusions (without p), I expected numbers: “the prevalence in Sardinia was X%, in ... Y% .... (p<0.00005)”.

RE: We added the percentages for the Sardinia results. We also dropped the second part of the sentence from the abstract.

The second conclusion is a suggestion.

RE: We dropped part of the second sentence of the conclusions.