Author's response to reviews

Title: Human papillomavirus prevalence amongst Indigenous and non-Indigenous Australian women prior to a national HPV vaccination program

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Author's response to reviews: see over
Dear Editor,

Please find attached our reply to the three reviewer’s comments to our recently submitted research paper for consideration for publication in BMC Medicine and entitled ‘Human papillomavirus prevalence amongst Indigenous and non-Indigenous Australian women prior to a national HPV vaccination program.’

I have addressed each of these comments/queries in chronological order below, plus enclosed a “marked up version,” as well as an accepted change version of the manuscript. Our comments are italics to each reviewer comment (bolded).

Reviewer 1

Major Compulsory Revisions

This is a very interesting piece of work that shows lack of substantial difference in HPV prevalence between indigenous and non-indigenous women in Australia despite a several-fold difference in cervical cancer incidence between the two groups.

The present findings seem to agree with a pooled analyses of international HPV prevalence surveys and case-control studies carried out at IARC (Franceschi et al, Brit J Cancer, 101, 865-870, 2009) that suggested that low education level (as a proxy for socio-economic status) was associated with increased cervical cancer risk but not with higher HPV prevalence. In addition to different screening uptake, substantial part of the difference in cervical cancer risk by education level in the paper by Franceschi et al (2009)
was explained by earlier age at first sexual intercourse and first birth and by higher parity in the least educated women. The authors should consider that paper and check whether similar differences in age at first sexual intercourse and first birth and parity existed between indigenous and non-indigenous women in Australia.

- Thank you; we had already considered the higher fertility rate, young age at first intercourse and birth of Indigenous women in the second paragraph of the discussion. We have now added in the suggested reference page 35 paragraph 1 ‘....incidence and mortality observed in Indigenous women.[21,31] Franceschi et al have previously found that lower education levels (as a proxy for socioeconomic status) are correlated with cervical cancer risk but not increased HPV prevalence and may be mediated through risk factors such as earlier age at first intercourse and higher and earlier parity. [32] Regardless of the underlying reasons, the presence of other high risk HPV DNA types

To a large extent, the present findings by Garland et al, similarly to those by Franceschi et al, challenge the concept that an association of low socio-economic class or ethnic group explain to any great extent differences in cervical cancer risk.

Another aspect the authors should address is whether the recruitment modality (i.e., through community-based clinics rather than GPs) might have led to a substantial difference in the selection of indigenous and non-indigenous women (e.g., selection of non-indigenous women at higher risk for STIs).

- Thank you for this observation. We do recognize, as stated in the manuscript, that the sample is not geographically or demographically representative of the Australian population. It is indeed likely that the non Indigenous women recruited in the study, are of lower socioeconomic status than average, given that they are accessing free community health services rather than general practitioners, but that they will still be on average of higher socioeconomic status than the Indigenous women. We have added a sentence highlighting this point to paragraph four (page 36) of the discussion. ‘Non-Indigenous women attending free community health services are likely to be of lower socioeconomic status on average than other Australian women.’

In tables 1 and 2 it would be interesting to add, in addition to age at first sexual intercourse and first birth and parity, time since last smear, if the information is available.

- Unfortunately this data is not available (as outlined in the methods)

Tables 3, 7, etc: I strongly recommend to always adjust for age and ignore crude ORs.

- These have been deleted from TABLE 3 (now TABLE 2). We prefer to leave this in table 7 now TABLE 6). etc as shows the impact of age.
MOST IMPORTANT: authors should be extremely careful in over interpreting differences in the prevalence of HPV types between cancer-free indigenous and non-indigenous women in Australia as evidence of different HPV 16/18 vaccine efficacy. They should clearly state that HPV 16/18 vaccine efficacy is ultimately due the ability of the vaccine to prevent cervical cancer and, therefore, the most important thing would be to compare the distribution of HPV types in cervical cancer in the two groups.

- We believe that we have been appropriately cautious throughout the manuscript but in order to avoid any misunderstanding have changed the following line in the third paragraph of the introduction (page 6): was ‘Should types causing disease differ amongst Indigenous women,…’ now reads ‘Should the prevalence of vaccine preventable types differ amongst Indigenous women,…’

Minor Essential Revisions

Abstract: give age range CHANGE <41 TO 17-40 YEARS

- This has been done: line 1 of methods of abstract

page 12-13: please clarify the use of AMPLICOR: it seems to me that eventually both AMPLICOR-positive and AMPLICOR-negative women were genotyped using LA.

- As per the methods, page 12 all AMPLICOR positive samples were genotyped by LA. For those negative by AMPLICOR, we used an in-house assay as this has the ability to pick up more than the 13 high risk types and as previously published. As the Amplicor HPV test only detects 13 HR-HPV, in order to rule out presence of other types, all samples testing negative for HR-HPV by Amplicor HPV test, were tested using a 20 μl aliquot of extracted DNA by PGMY09/11-based HPV consensus PCR.[28] A PCR ELISA detection, as described previously, was utilized.[29] All assays utilized incorporated the amplification of β-globin gene as an internal control.

- page 9: specify if +/-5% refer to the absolute percent.

- Yes it is absolute and it has been added to clarify this point: “Page 9 ...for a Pap smear and was powered (alpha 0.05, 1- beta = 0.90) to detect an absolute difference in HPV16/18 of +/-5% or.....”

Discretionary Revisions

Omit, please, the first 3 lines of the Introduction.

- As suggested this has been omitted from the first part of the Background

By and large, I believe that there are some repetitions in the text and in tables that should be avoided for brevity sake. Table 1 and 2 may be merged. Table 4b can be eliminated.
• We have reviewed the text for repetitions or wordy comments and reduced accordingly throughout the paper. For example page 7 Background has been modified to “…12-year-old girls as an ongoing program, with a catch up program delivered through school and community providers for women up to 26 years of age which finished December 31, 2009.” to reduce words.

• Similarly in the methods page 10 words reduced to “Women were asked to allow the cervical samples collected for Pap cervical cytology to be also tested for HPV DNA. Once fully informed consent was obtained, Pap smear samples were collected in the routine clinic settings. In addition to routine pathology specimen details, information

• We have merged tables 1 and 2. We could delete 4b (now table 3b), although this is the table showing all the types by Indigenous and NI status, so would prefer to keep it.

• We have deleted Table 6 and the text (paragraph pages 25-26) that described this.

Reviewer 2

Major: I understand that there are political and social reasons that one would want to conduct heightened surveillance of Indigenous populations. Agnostically, the data do not in my opinion show an important difference in prevalence patterns between the groups. If they had been labeled Group A vs. Group B, or Country 1 vs. Country 2, I would have said that there was no important difference that require separate consideration moving forward. The last sentence of the Abstract and the Conclusion are wrong in my view. I understand that the project was conceived to study inter-group differences, and the project is excellent, but the results are fundamentally null. The few subgroup findings by age and ethnicity could easily represent chance or fluctuations of little meaning to Public Health practice regarding HPV.

• This study was undertaken because of real and important disparity in health between Indigenous and non Indigenous women in Australia. To clarify this we have modified the final sentence in the first paragraph of the introduction, to describe that Indigenous Australian women have over double the incidence of cervical cancer and four times the mortality rate. Ie

• However, there are large disparities within the total Australia population; incidence and mortality are higher for regional and remote than urban residents, and much higher (approximately two and five times higher respectively) for Indigenous (Aboriginal and Torres Strait Islanders) than other Australian women. [1]
Does length of presentation matter? This text, as it went on, struck me as a condensation of a government project report, with substantial repetition of figures and tables and text. It was good quality, just too long for the old days of journals. Perhaps that is not relevant but the article could be substantially shorter while preserving the major elements.

- As delineated above to reviewer one, we have cut text accordingly.

Minor: Poor nutrition has not been firmly linked to HPV prevalence.

- Thank you. We have deleted the reference in the discussion to the possible role of poorer diet.

I don't see why Greenlandic Inuits are relevant to Indigenous people of Australia, please explain or delete.

- We have deleted the paragraph referring to HPV in other Indigenous populations from the text to reduce the word count.

Reviewer 3

The manuscript by Garland et al., reports the prevalence and risk for HPV in women from Australia with a focus on indigenous and non-indigenous women. The study reports no difference in HPV16/18, but did detect significant differences in stratified groups with smoking and Pap test abnormalities, that were common among Indigenous women. The main problem with the manuscript is that the observations are not unexpected, there is no correlation with pathology, yet the report goes on for 39 pages.

- Please note that the manuscript is double spaced and includes double spaced tables. We have however substantially edited it in response to all 3 reviewers' comments.

The lack of data on the association of cervical pathology and HPV types is a major limitation of the study.

- Due to space limitations, these data are being presented in a separate manuscript.

Are all indigenous women genetically equivalent?

- Indigenous women are heterogeneous in the same way that all women are heterogeneous.

The age range is quite limited, is there a reason women over 40 were not screened?

- In this study we wished to focus on women at the peak age of HPV exposure as these are the proximal infections that prophylactic vaccines will be preventing.
Specific comments:

Abstract: remove term of “vaccine-preventable high risk genotypes”, since newer vaccines are on the horizon this statement will be dated soon and description of HPV16/18 is sufficient in itself.

- This has been removed as suggested.

HPV DNA detection and genotyping: The authors’ should clarify the HPV typing. Samples negative for amplicor were tested by PGMY. It later states that all positive samples for either method were genotyped using 50 ul DNA by LA? What was the “global” test for HPV-positivity for these methods? HPV types should be designated by their proper names, IS39 = HPV82 and CP6108 = HPV89.

- Please see comments above for reviewer one. This has this has already been rectified in the text in the methods. Similarly we have modified the methods on page 12 third last line .. to read.. “82 (previously known as IS39), 83, 84, and 89 (previously known as CP6108).

We look forward to receiving your feedback about the paper.

Kind regards,

Suzanne and Julia

On behalf of all authors

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