Author's response to reviews

Title: More men than women make mucosal IgA antibodies to Human Papillomavirus type 16 (HPV-16) and HPV-18: a study of oral HPV and oral HPV antibodies in a normal healthy population.

Authors:

Dianne J Marais (dmarais@curie.uct.ac.za)
Candice Sampson (csampson@curie.uct.ac.za)
Anthea Jeftha (ajeftha@uwc.ac.za)
Dherendra Dhaya (ddhaya@uwc.ac.za)
Jo-Ann Passmore (jopann@curie.uct.ac.za)
Lynnette Denny (ldenny@uctgsh1.uct.ac.za)
Edward P Rybicki (ed@science.uct.ac.za)
Eric Van De Walt (eric@science.uct.ac.za)
Lawrence X.G Stephen (lstephen@uwc.ac.za)
Anna-Lise Williamson (annalise@curie.uct.ac.za)

Version: 4 Date: 30 March 2006

Author's response to reviews: see over
The Editor,
BMC Infectious Diseases

The manuscript ID 1092259782862267 entitled: “More men than women make mucosal IgA antibodies to Human Papillomavirus type 16 (HPV-16) and HPV-18: a study of oral HPV and oral HPV antibodies in a normal healthy population” as been resubmitted after revision in accordance with the reviewer comments below.

Responses to reviewers of manuscript entitled:  
More men than women make mucosal IgA antibodies to Human Papillomavirus type 16 (HPV-16) and HPV-18: a study of oral HPV and oral HPV antibodies in a normal healthy population.

Responses in Times New Roman font, bold.

Reviewer's report 1

Reviewer: John Sellors

Reviewer's report:
General
While the subject is of interest, the ability to draw meaningful conclusions from the data presented is limited.

Response: The study aim was to test the presence of oral HPV antibodies in a normal population, including men and children to assess the acceptance of the oral sampling method for determining HPV infection. This had not been done before. This was made clear in the revised manuscript

Since the assay for oral IgA and IgG only was for antibodies to HPV types 16, 18, and 11 and the proportion of IgA that was secreted was not determined, and the presence and typing of anogenital HPV DNA was not determined, it is difficult to correlate the findings in this paper.
Response: The prevalence of HPV-16 antibodies in oral and serum samples from a group of women with HPV-associated CIN was included in the revised manuscript.

Consideration should be given to whether these findings could be reported in a short report or a letter to the editor.

Response: The inclusion of a group of women with CIN in the manuscript made this impossible.

It is a concern that, since the study recruitment was conducted in a dental clinic setting there may be potential bias introduced if the prevalence of gingivitis was higher than in the general population. It is reasonable to expect that the presence of gingivitis may increase the transudation of serum antibodies or affect the local production of IgA in some way. This would impair the ability to generalize the findings. Was the presence of gingivitis assessed in each subject and if so, did it influence the results in any way?

Response: The presence/absence of gingivitis was not recorded for this study. This was reported in the revised manuscript and was a shortcoming of the study.

-------------------------------------------------------------------------------
Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

- The date of when the study was conducted should be given.

Response: The date when the study was conducted has been included in the revised manuscript.

- Pg. 4, para 1 - not clear whether this statement on need to test prior to vaccination applies to a research setting or a public health vaccination program.

Response: The requirement to test for HPV prior to vaccination is important both in a research setting to assess the presence of the most prevalent HPV types to be included in a vaccine and to test for prior exposure to the HPV types included in a vaccine. The statement has been clarified in the manuscript.

- Pg. 5, para 1 - not clear why authors state categorically that men would have to be included in a HPV vaccination strategy.

Response: Men have to be included in vaccine strategies because men have as much genital HPV if not more than women and pass HPV to their sexual partners. This was clarified in the revised manuscript.

- Pg. 7, end of para 1 - was pre-and post - test HIV counselling done?

Response: HIV testing was anonymous and unlinked. One child and one adolescent person tested HIV positive. These facts were included in the revised manuscript.

- Pg. 9, last para - presence of HPV-13 lesion and FEH lesions in one patient does not confirm an association.
Response: Agreed. In the revised manuscript “confirming” was changed to “supporting” the association of HPV-13 and FEH.

Table 1 - the data of some of the 34 study subjects seems to be missing in this Table and the first column is not labelled.

Response: The table was corrected in the revised manuscript.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Pg 10, para 1 - the meaning of the first sentence is difficult to understand.

Response: The sentence was reconstructed in the revised manuscript.

Responses to reviewer's report 2

Reviewer: Reinhard Höpfl

Reviewer's report:
General
HPV vaccines are imminent, therefore the question posed by the authors is of great interest, since only a few epidemiologic data on oral HPV infection exist. In addition, there is need to elucidate mucosal humoral immune mechanisms against HPV, with only scarce data available in the literature. It is a conceivable approach to analyse the natural history of HPV infection by studying different age groups of patients. This could help to get some hints on the time course of this potentially ubiquitous but silent infection. The paper contains some new but incomplete scientific information and PubMed search revealed no hint for duplication. The methods applied are adequate: The number of patients is sufficient and the DNA analysis applied is diligent. Antibodies to HPV-VLPs are quite specific and can indeed answer epidemiologic questions. However, the assay with oral fluids may not yet be a reliable alternative to serum testing (Cameron et al.; Zitation 12).

Discretionary Revisions (which the author can choose to ignore).

It would be of interest to discuss the potential of crossreactivity of HPV11 antibodies with HPV 13 VLPs.

Response. HPV-11 and HPV-13 are closely related phylogenetically, both clustering in species A10, there is a potential for cross-reactivity. As no assessment has been reported for HPV-13 antibodies the relevance for this study is limited.

Minor Compulsory Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
Page 10, line 1 … population in (eliminate!) is …. 

Response: This was corrected in the revised manuscript
Page 11, line 2 … to substantiate this, almost …
The discussion is somewhat confuse and could be presented in a more structured stil.

Response: An attempt was made to correct this in the revised manuscript.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The major draw back of the study is, that there are no data shown on cervical diseases or serum antibodies in the patients.

Response: A study of this nature has been reported ie antibodies in women with cervical intraepithelial neoplasia (CIN) (Marais et al 2001 J Med Virol.). In the revised manuscript women with CIN were included as a reference group. Both oral and serum antibodies were assessed in these women.

This appears to be particularly problematic, since there is no defined control population, and also no well characterised positive or negative oral fluid for control purposes available for the test.

Response: We used the ELISA OD values from the children (aged 2-10 years) to determine the cut off for positivity for the oral fluid (and serum) antibodies as is done routinely for serum.

It is also unclear how many men of the study are homosexuals, which is important to know, since MSM are known to have increased antibody responses to HPV.

Response: This study constituted the first assessment of oral HPV responses in men. As the study was conducted at a Dental clinic the percentage of homosexuality in the participants was thought to be low and of little consequence to the results. A large study is planned with men where the demographic details of participants will be known.

Therefore the scientific output of the (in principle!) very interesting study is disappointing. Consequently, - an this is stated completely correct by the authors - Marais et al. write in their conclusion, that they believe only, and also postulate but not conclude something.

One way to bring out the best of the situation would be to analyse the existing data more profoundly, particularly the correlation of IgG to IgA.

Response: This has been done in the revised manuscript.

In addition, I propose that at least in a subgroup of patients clinical data (questionnaire, correlation with gynaecological history - Pap results) should be included.

Response: This has been done in the revised manuscript.
Even more useful if achievable would be a correlation of oral fluid antibodies with cervical antibodies and/or serum antibodies in some of the patients.

Response: This has been done in the revised manuscript.

I trust the responses and revision are satisfactory for a successful publication.

Yours sincerely,
Di Marais
Responses to reviewer's report 2

Reviewer: Reinhard Höpfl

Reviewer's report:
General
HPV vaccines are imminent, therefore the question posed by the authors is of great interest, since only a few epidemiologic data on oral HPV infection exist. In addition, there is need to elucidate mucosal humoral immune mechanisms against HPV, with only scarce data available in the literature. It is a conceivable approach to analyse the natural history of HPV infection by studying different age groups of patients. This could help to get some hints on the time course of this potentially ubiquitous but silent infection. The paper contains some new but incomplete scientific information and PubMed search revealed no hint for duplication. The methods applied are adequate: The number of patients is sufficient and the DNA analysis applied is diligent. Antibodies to HPV-VLPs are quite specific and can indeed answer epidemiologic questions. However, the assay with oral fluids may not yet be a reliable alternative to serum testing (Cameron et al.; Zitation 12).

Discretionary Revisions (which the author can choose to ignore).

It would be of interest to discuss the potential of crossreactivity of HPV11 antibodies with HPV 13 VLPs.

Response. HPV-11 and HPV-13 are closely related phylogenetically, both clustering in species A10, there is a potential for cross-reactivity. As no assessment has been reported for the prevalence HPV-13 antibodies the relevance for this study is limited and this was not included in the revised manuscript.

Minor Compulsory Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
Page 10, line 1 … population in (eliminate!) is ….

Response: This was corrected in the revised manuscript

Page 11, line 2 … to substantiate this, almost …
The discussion is somewhat confuse and could be presented in a more structured stil.

Response: An attempt was made to correct this in the revised manuscript.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The major draw back of the study is, that there are no data shown on cervical diseases or serum antibodies in the patients.
Response: A study of this nature has been reported ie antibodies in women with cervical intraepithelial neoplasia (CIN) (Marais et al 2001 J Med Virol.). In the revised manuscript women with CIN were included as a reference group. Both oral and serum antibodies were assessed in these women.

This appears to be particularly problematic, since there is no defined control population, and also no well characterised positive or negative oral fluid for control purposes available for the test.

Response: We used the ELISA OD values from the children (aged 2-10 years) to determine the cut off for positivity for the oral fluid (and serum) antibodies as is done routinely for serum.

It is also unclear how many men of the study are homosexuals, which is important to know, since MSM are known to have increased antibody responses to HPV.

Response: This study constituted the first assessment of oral HPV responses in men. As the study was conducted at a Dental clinic the percentage of homosexuality in the participants was thought to be low and of little consequence to the results. A large study is planned with men where the demographic details of participants will be known.

Therefore the scientific output of the (in principle!) very interesting study is disappointing. Consequently, - an this is stated completely correct by the authors - Marais et al. write in their conclusion, that they believe only, and also postulate but not conclude something.

One way to bring out the best of the situation would be to analyse the existing data more profoundly, particularly the correlation of IgG to IgA.

Response: This has been done in the revised manuscript.

In addition, I propose that at least in a subgroup of patients clinical data (questionnaire, correlation with gynaecological history - Pap results) should be included.

Response: This has been done in the revised manuscript.

Even more useful if achievable would be a correlation of oral fluid antibodies with cervical antibodies and/or serum antibodies in some of the patients.

Response: This has been done in the revised manuscript.