PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Oral human papillomavirus infection in England and associated risk factors; a case control study
AUTHORS	Hearnden, Vanessa; Murdoch, Craig; d'Apice, Katy; Duthie, Susan; Hayward, Nicholas; Powers, Hilary

VERSION 1 – REVIEW

REVIEWER	Rachael Dodd University of Sydney, Australia
REVIEW RETURNED	02-Mar-2018

GENERAL COMMENTS	I believe the manuscript to be much improved, however, I still find some need for improvement. I would also have appreciated seeing the tracked changes from the first submission. 1) Please keep the language consistent throughout when referring to the collection of samples. You use 'buccal cell', 'oral epithelial cell' – please clarify the differences if there are some. Sentence added to clarify what buccal cells are can be found in the abstract. Samples were collected from the buccal region of the oral cavity and therefore these cells are buccal cells (but also oral epithelial cells). I can't see the sentence that has been added to explain what buccal cells are? It would be beneficial for you to say how these are collected, as you have for 'oral rinse and gargle sample'. ABSTRACT 1) I think this could be improved by a bit of further clarification about 'buccal cells'. I am not familiar with this term and I am sure many other readers would also not be. See above I don't agree that this has been addressed in the abstract, or elsewhere in the manuscript. 2) You have already stated the study is in Sheffield, so probably no need to be so vague in the conclusions by saying 'a northern city in England' METHODS 1) Sampling and questionnaire – please provide examples of the questions asked relating to smoking status, alcohol consumption and sexual history. Were these validated measures? Questionnaires were adapted from the head and neck 5000 study (https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-14-973) A full copy of the questionnaire can be made available as a supplementary figure if required. I think it would be beneficial for the readers to be able to see what

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	questions participants were asked. It would be beneficial to include some examples in the text, as well as in supplementary material. RESULTS 1) Table 1 – you state you have omitted 20 samples from the table, yet all 700 adults are included in the first column. I have added a sentence to clarify these 20 were omitted from the left 3 columns where samples were categorised by HPV status. I think this would be better as a footnote to the table. 2) I would like to see some reporting of odds ratios in the text. 3) The paragraph which gives results of the blood folate and buccal cell folate needs to be put into context – what are normal levels? DISCUSSION 1) You have introduced information about the HPV vaccination into the discussion, but you don't discuss how participants in the study who had been vaccinated could have impacted the findings. 2) On page 10, how are 'oral sexual experience and number of oral sexual partners' different? 3) On page 10, line 22, you repeat 'number of' 4) Limitations – what was the demographics of the city where this took place, can you generalise? I have added a paragraph in the methods to describe the demographics of Sheffield. You don't acknowledge in your limitations about the generalisability of the findings from Sheffield, to elsewhere in the country, or the world. 5) You don't discuss in the strengths and limitations whether the measures were validated – if they were or were not, please state as limitation/strength. The HPV detection method used is clinically validated and was performed in a clinical laboratory. This detection method has been used for the majority of previously reported papers on oral HPV prevalence. Folate analysis was carried out using a well-established method in human biomonitoring. In excess of 99% of plasma folate samples fell within the QA cut off for intra-individual variation in replicate samples of 10% across the study, with in excess of 90% of buccal cell samples falling within the QA cut off of 15% Questionnaire was not validated, this is a limitation which we acknowledge. You don't acknowledge the questionnaire not being validated as a limitation in your discussion.

REVIEWER	Karolin Hijazi University of Aberdeen, UK
REVIEW RETURNED	10-Apr-2018

GENERAL COMMENTS	The authors aim to assess the prevalence and associated risk factors of oral high-risk HPV infection in a mixed cohort recruited in Sheffield – they confirm smoking and sexual behaviour as risk factors for oral HPV infection in keeping with previous reports. They also explore HPV association of oral epithelial cell and blood folate concentration and conclude that no association exists. The study may represent a meaningful addition to the literature given the limited number of high-quality UK reports in this important field. The study has clear limitations which the authors go to great lengths to highlight. Further need for clarity and/or revision is required in regards to several aspects of study design:

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	• The sample size is small comparatively with population based studies powered to examine the prevalence of oral high risk HPV. Studies based on similarly sized cohorts, including the HOPSCOTCH study quoted throughout the study, have acknowledged the lack of power to examine prevalence of HPV infection in relation to risk association. Further detail and clarity should be provided in regards to the power calculation. • The authors should explain the rationale for recruiting participants from different settings. • Medical profiles of participants should be reported as well as acknowledgement of factors relating to medical status known to affect individual's susceptibility to HPV infection, e.g. immune status, co-infections. • It is mentioned that exclusion criteria included oral lesions – authors should clarify what is intended and make specific mention of which oral conditions were excluded. • As repeatedly acknowledged by the authors the validity of the case-control analysis to examine depletion of folate as a risk factor is questionable due to the small sample size. One wonders about the value of this part of the study particularly given the inability to adjust for important risk factors of HPV infection. Other comments: • The study dates should be added. Reference to UKCRN is surprising given replacement with CPMS. • Line 12, pg 4 – correct referencing error.

VERSION 1 – AUTHOR RESPONSE

Reviewers' Comments to Author:

Reviewer: 1
Reviewer Name: Rachael Dodd
Institution and Country: University of Sydney, Australia
Competing Interests: None declared

I believe the manuscript to be much improved, however, I still find some need for improvement. I would also have appreciated seeing the tracked changes from the first submission.

1) Please keep the language consistent throughout when referring to the collection of samples. You use 'buccal cell', 'oral epithelial cell' – please clarify the differences if there are some.

All reference to these cells is now "oral mucosal buccal epithelial cell" for consistency and clarity.

I can't see the sentence that has been added to explain what buccal cells are? It would be beneficial for you to say how these are collected, as you have for 'oral rinse and gargle sample'.

A detailed description of how oral mucosal buccal epithelial cell were collected can be found on page 6

ABSTRACT
1) I think this could be improved by a bit of further clarification about 'buccal cells'. I am not familiar

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with this term and I am sure many other readers would also not be.
I don't agree that this has been addressed in the abstract, or elsewhere in the manuscript.

We hope the description added to the methods section explains this sufficiently.

2) You have already stated the study is in Sheffield, so probably no need to be so vague in the conclusions by saying 'a northern city in England'

Amended to say 'Sheffield in the north of England'.

METHODS
1) Sampling and questionnaire – please provide examples of the questions asked relating to smoking status, alcohol consumption and sexual history. Were these validated measures?

Questionnaires were adapted from the Head and Neck 5000 study (https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-14-973)
A full copy of the questionnaire used will be made available as supplementary figure 1.
These questionnaires were not validated, a limitation that has been acknowledged on page 3 and 12

I think it would be beneficial for the readers to be able to see what questions participants were asked. It would be beneficial to include some examples in the text, as well as in supplementary material.

A pdf of the questionnaire has been uploaded to be included as supplementary information. Two example questions have also been included in the main text on page 5.

RESULTS
1) Table 1 – you state you have omitted 20 samples from the table, yet all 700 adults are included in the first column.

We have added a sentence to clarify that the 20 sample with insufficient cellular material were omitted from the left 3 columns where samples were categorised by HPV status.

I think this would be better as a footnote to the table.

This can now be found in the footnote

2) I would like to see some reporting of odds ratios in the text.

These have now been included throughout the results section where appropriate.

3) The paragraph which gives results of the blood folate and buccal cell folate needs to be put into context – what are normal levels?

The whole blood folate concentrations reported in this paper cannot readily be compared with average values for the UK population because the analytical methods used are different. Additionally, we report whole blood folate whilst the National Diet and Nutrition Survey (NDNS) reports red blood cell folate. There has been intense debate in the scientific community over the past decades,

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regarding the preferred methods for measuring folate status, and the setting of thresholds; this debate is ongoing, evidence by the very recent supplementary information published for the NDNS (NDNS folate Supplementary Report. PHE 2018). Oral mucosal buccal epithelial cell folate is relatively rarely measured and there are no nationally representative values against which to compare.

DISCUSSION

1) You have introduced information about the HPV vaccination into the discussion, but you don't discuss how participants in the study who had been vaccinated could have impacted the findings.

None of our study participants had been vaccinated against HPV as this was an exclusion criteria (see "sampling and questionnaire" section).

2) On page 10, how are 'oral sexual experience and number of oral sexual partners' different?

Oral sexual experience relates to factors other than just number of partners, eg. frequency of oral sex and other sexual behaviour which could give rise to transmission from genital to mouth (toys, hands etc.).

3) On page 10, line 22, you repeat 'number of'

Now amended.

4) Limitations – what was the demographics of the city where this took place, can you generalise?

We have added a paragraph in the methods to describe the demographics of Sheffield.

You don't acknowledge in your limitations about the generalisability of the findings from Sheffield, to elsewhere in the country, or the world.
A sentence to acknowledge the study limitations has been added on page 10.

5) You don't discuss in the strengths and limitations whether the measures were validated – if they were or were not, please state as limitation/strength.

The HPV detection method used is clinically validated and was performed in a clinical laboratory. This detection method has been used for the majority of previously reported papers on oral HPV prevalence. Folate analysis was carried out using a well-established method in human biomonitoring. In excess of 99% of plasma folate samples fell within the QA cut off for intra-individual variation in replicate samples of 10% across the study, with in excess of 90% of samples falling within the QA cut off of 15%

You don't acknowledge the questionnaire not being validated as a limitation in your discussion.

Questionnaire was not validated; this is a limitation that we now acknowledge on pages 3 and 12.

Reviewer: 2
Reviewer Name: Karolin Hijazi

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Institution and Country: University of Aberdeen, UK
Competing Interests: none declared

The authors aim to assess the prevalence and associated risk factors of oral high-risk HPV infection in a mixed cohort recruited in Sheffield – they confirm smoking and sexual behaviour as risk factors for oral HPV infection in keeping with previous reports. They also explore HPV association of oral epithelial cell and blood folate concentration and conclude that no association exists.
The study may represent a meaningful addition to the literature given the limited number of high-quality UK reports in this important field.

We would like to thank the reviewer for their positive comments.

The study has clear limitations which the authors go to great lengths to highlight. Further need for clarity and/or revision is required in regards to several aspects of study design:

- The sample size is small comparatively with population based studies powered to examine the prevalence of oral high risk HPV. Studies based on similarly sized cohorts, including the HOPSCOTCH study quoted throughout the study, have acknowledged the lack of power to examine prevalence of HPV infection in relation to risk association. Further detail and clarity should be provided in regards to the power calculation.

At the time this study was designed and conceived (2012) there was very limited data available on the prevalence of oral HR-HPV in European populations. This was the first investigation to examine the relationship between methyl donor status and oral HPV infection.

The sample size for the case-control study was based on our data from a nested case-control study of cervical cell folate concentration in women with HR-HPV infection that persists (cases) and those in whom the virus is cleared (controls) over 6 months[12]. We detected an almost two-fold difference in mean folate concentration in cervical cells from cases vs controls (mean (SD) 5.4, ±4.83 ng folate/ mg protein in cases versus 2.8, ±2.70 controls). We calculated that 50 cases with 150 controls would allow us to detect a 45 % difference between cases (oral HR-HPV positive) and controls (oral HR-HPV negative) in buccal cell folate, with a power of 80% and P < 0.05.

Reported prevalence values for oral HPV are extremely variable and there were few data from the UK at the time of the study. Jalal et al (1992)[13] reported a 44% prevalence of a high risk HPV in oral epithelial cells in a UK sample; Gillison et al[8] reported an age dependent prevalence of between 4.2 and 11.4% oral HPV infection and an average of 3.7% with HR-HPV. A sample size of 700 was selected, assuming a prevalence of 7% in the population sampled.

- The authors should explain the rationale for recruiting participants from different settings.

Participants were recruited from a variety of settings to capture a more diverse study population.

- Medical profiles of participants should be reported as well as acknowledgement of factors relating to medical status known to affect individual's susceptibility to HPV infection, e.g. immune status, co-infections.

This information was not collected therefore cannot be included in this manuscript.

- It is mentioned that exclusion criteria included oral lesions – authors should clarify what is intended and make specific mention of which oral conditions were excluded.

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A sentence has been added on page 5 to explain that any patients with an oral lesion that could cause pain or discomfort during cell collection was excluded.

- As repeatedly acknowledged by the authors the validity of the case-control analysis to examine depletion of folate as a risk factor is questionable due to the small sample size. One wonders about the value of this part of the study particularly given the inability to adjust for important risk factors of HPV infection.

We are mindful of the limitation of the folate aspect of the study, given the very small sample size, and we do state this as a limitation (page 12). However, the putative importance of folate in determining susceptibility to HPV infection, and ability to clear, was an important part of the rationale of conducting the study, and we feel it important that the reader recognizes this possible causal link between folate status and HPV infection.

Other comments:

- The study dates should be added. Reference to UKCRN is surprising given replacement with CPMS.

At the time the study was conducted UKCRN was the registration body.

- Line 12, pg 4 – correct referencing error.

Thank you, this has been amended.

FORMATTING AMENDMENTS (if any)
Required amendments will be listed here; please include these changes in your revised version:
- Please include Figure legends at the end of your main manuscript.

These have been added on page 15, thank you for spotting this omission.

VERSION 2 – REVIEW

REVIEWER	Rachael Dodd The University of Sydney, Australia
REVIEW RETURNED	05-Jun-2018

GENERAL COMMENTS	The manuscript is much improved, well done.

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