PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Incidence and clearance of oral and cervicogenital HPV infection: longitudinal analysis of the MHOC cohort study
AUTHORS	Brouwer, Andrew; Campredon, Lora; Walline, Heather; Marinelli, Brittany; Goudsmit, Christine; Thomas, Trey; Delinger, Rachel; Lau, Yan; Andrus, Emily; Nair, Thankam; Carey, Thomas; Eisenberg, Marisa; Meza, Rafael

VERSION 1 – REVIEW

REVIEWER	Anak Iamaroon Chiang Mai University
REVIEW RETURNED	17-Sep-2021

GENERAL COMMENTS	1. To make the study reproducible, the authors should elaborate DNA testing technique in the Materials and Methods. 2. The authors should provide more details or confounding factors in Discussion why there was no association between oral infection and oral sex behavior in this study. 3. The authors should provide details of vaccination given in this study for example how many dosages, HPV type coverage, companies (Cervarix or Gardasil), etc. 4. Ever marijuana was associated with greater incidence of cervicogenital HPV infection. Why? 5. This study shows no association between vaccination and oral infection while most previous studies found the opposite. The hypothesis of superficial infections seems a vague explanation without molecular study support. Are there any other alternative explanations for this point? 6. Sexual orientations of the volunteers may be one of the crucial parameters of this study. Can the authors provide and use this information for further interpretation of the study?

REVIEWER	Elizabeth Machado Universidade Federal do Rio de Janeiro, Serviço de Doenças Infecciosas - Hospital Universitário
REVIEW RETURNED	21-Sep-2021

GENERAL COMMENTS	The objectives of the study are to describe the prevalence, incidence and clearance of oral and cervicogenital HPV and their relationship to sexual behaviors. A total of 325 participants who completed at least 2 visits were enrolled in the oral study and 127 were enrolled in the cervicogenital HPV study. The incidence and clearance of both infections are described but the results are hard to understand due to lack of clarity. It is stated that of the 1993 total oral tests, 148 were positive, however, the breakdown of positive tests at any point was 94 (HPV neg to pos), 107 (HPV pos to neg) and 20 that remained positive during the study, totaling 221. Could this discrepancy be

	explained? 2. Could the authors describe how many participants were positive at time of enrollment and how many had more than one episode of infection? 3. On page 8, the authors might need to correct the results of the tests. Where it states 'oral tests' might be about the cervicogenital samples. 4. On page 21, the figure describes the status of the test as 'invalid'. Please describe the meaning of 'invalid' in Methods. 5. The authors don't mention the results of the genotype tests (maybe it will be described in another paper?) but could perhaps elaborate a little about clearance in those infected with multiple types and if they noticed any difference in time of clearance among the different types.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Anak Iamaroon, Chiang Mai University

1. To make the study reproducible, the authors should elaborate DNA testing technique in the Materials and Methods.

> Thank you for letting us know that it was not clear that full DNA testing methods were separately provided in our protocol paper and in our original PCR Mass Array paper. We have updated the text accordingly.
>
> [HPV Testing]. “DNA was extracted from samples and genotyped using PCR Mass Array; technical details of sample processing are given in our protocol paper [12], and technical details of the PCR Mass Array test are given in [16].”

2. The authors should provide more details or confounding factors in Discussion why there was no association between oral infection and oral sex behavior in this study.

> We have added additional discussion of the lack of association between oral infection and oral sex behaviors.
>
> [Discussion]. “Previous literature has shown that oral HPV infection is most likely related to oral sex behaviors [23, 39, 40], so our lack of association may be due to confounding. Indeed, the association between oral sex behavior and oral HPV infection was shown to be confounded by age-cohort and race in a previous study [40].”

3. The authors should provide details of vaccination given in this study for example how many dosages, HPV type coverage, companies (Cervarix or Gardasil), etc.

> We have added additional context on the vaccination variable.
>
> [Surveys]. “Vaccination status was self-reported, and due to missingness in the number of vaccine doses variable, we classified any participant reporting at least one dose of an HPV

vaccine as vaccinated. We did not ask participants about the type of vaccine they received; given the time frame and geographic location of the study, most vaccinated participants would have received Gardasil (6, 11, 16, 18)."

4. Ever marijuana was associated with greater incidence of cervicogenital HPV infection. Why?

We have added additional discussion of ever marijuana use.

[Discussion]. "Ever marijuana use, which was associated with increased incidence of cervicogenital HPV infection, may not be a direct risk factor but instead be associated with true underlying risk factors that are difficult to measure directly. Although there is some laboratory evidence of immune modulation by cannabinoids [41], epidemiological evidence for an association between marijuana use and cervicogential HPV has been mixed [42-45], suggesting that it is indeed likely confounded with other behaviors."

5. This study shows no association between vaccination and oral infection while most previous studies found the opposite. The hypothesis of superficial infections seems a vague explanation without molecular study support. Are there any other alternative explanations for this point?

We have added additional discussion on lack of association between vaccination and oral infection.

[Discussion]. "In this analysis, HPV vaccination was associated with reduced incidence of cervicogenital HPV but not oral HPV. Previous, cross-sectional work has indicated the HPV vaccination does reduce prevalence of oral HPV [36–38]. Our longitudinal results, then, may give further credence to the hypothesis that we are detecting superficial oral infections. However, because oral HPV infections were relatively rare, we may have not had the power to detect an impact of vaccination. Cohort and age differences between our study sample and others might also explain the lack of detected association. Also, if most of the observed genotypes were not covered by the participants' vaccines (and cross-protection is likely minimal), then this result might be expected. However, of the 193 distinct detections of genotypes in oral tests, more than half (109) were type 6, 11, 16, or 18 (Table S1). In comparison, about one-fifth (36) of the 166 distinct cervicogenital detections were type 6, 11, 16, or 18. These results may suggest that vaccination had a greater impact on cervicogenital infection than on oral infection in this cohort."

6. Sexual orientations of the volunteers may be one of the crucial parameters of this study. Can the authors provide and use this information for further interpretation of the study?

We asked participants about sexual attraction rather than sexual orientation. We have added those results to Tables 1 and 2 and to the discussion.

Table 1

	Full cohort		Cervicogenital substudy cohort	
	%	n	%	n
Sexual attraction				
Only to another gender	72%	229	73%	84
Mostly to another gender	15%	46	20%	23

Equal or mostly/only to same gender	10%	33	3%	4

Table 2. †Cells with fewer than 5 participants are censored.

	Oral HPV incidence			Cervicogenital HPV incidence		
	n	Hazard ratio	95% CI	n	Hazard ratio	95% CI
Sexual attraction						
Only to another gender	229	1 (ref)		84	1 (ref)	
Mostly to another gender	46	**1.57**	(1.02, 2.43)	23	**1.53**	(1.09, 2.17)
Equal or mostly/only to same gender	33	0.92	(0.50, 1.68)	4	†	†

[Discussion]. “Incidence of both oral and cervicogenital HPV was greater in participants who indicated sexual attraction mostly but not only to another gender; this type of “heteroflexible” orientation has been previously associated with higher-risk sexual behavior and STIs [46]. There was no indication of increased incidence for participants expressing sexual attraction to multiple genders equally or mostly or only to the same gender.”

Reviewer: 2
Dr. Elizabeth Machado, Universidade Federal do Rio de Janeiro

1. The objectives of the study are to describe the prevalence, incidence and clearance of oral and cervicogenital HPV and their relationship to sexual behaviors. A total of 325 participants who completed at least 2 visits were enrolled in the oral study and 127 were enrolled in the cervicogenital HPV study. The incidence and clearance of both infections are described but the results are hard to understand due to lack of clarity. It is stated that of the 1993 total oral tests, 148 were positive, however, the breakdown of positive tests at any point was 94 (HPV neg to pos), 107 (HPV pos to neg) and 20 that remained positive during the study, totaling 221. Could this discrepancy be explained?

Yes, the confusion is understandable. However, the numbers of transitions do not directly correspond to the numbers of tests. An individual with history *negative-positive*, for example, will contribute 2 tests but only 1 transition, while an individual with history *negative-negative-positive-positive* will contribute 4 tests and 3 transitions. Each participant contributes one less transition than their total number of tests. Thus, the connection between total number of tests and the transitions depends on the specific distribution of number of tests each participant has. We have added additional clarification.

[Results]. “(Note: the numbers of transitions will not add up to the number of tests because each participant contributes one fewer transition than their number of tests, and so the correspondence between transitions and tests depends on the specific distribution of number of tests each participant has).”

2. Could the authors describe how many participants were positive at time of enrollment and how many had more than one episode of infection?

Our full baseline results are currently under review separately. We have included abbreviated results. Here we define two separate episodes of infection as two positive tests with at least one negative in between.

[Methods]. “We also separately tested the association of the detection of multiple HPV types with clearance in a model with fixed incidence.”

[Results]. “Oral HPV prevalence among first valid tests was 11% (34). …

Only 8 individuals had multiple distinct detections (i.e., two positive tests with at least one negative test in between). …

Cervicogenital HPV prevalence among first valid tests was 20% (23). …

Twenty-one individuals had multiple distinct detections of the same genotype.”

3. On page 8, the authors might need to correct the results of the tests. Where it states 'oral tests' might be about the cervicogenital samples.

Thank you for catching this error. It has been corrected.

4. On page 21, the figure describes the status of the test as 'invalid'. Please describe the meaning of 'invalid' in Methods.

Thank you for catching this oversight. We had added the following line to the methods.

[HPV Testing]. “Participants whose samples contained insufficient DNA or otherwise resulted in inconclusive test results were denoted as invalid.”

5. The authors don't mention the results of the genotype tests (maybe it will be described in another paper?) but could perhaps elaborate a little about clearance in those infected with multiple types and if they noticed any difference in time of clearance among the different types.

We have added a supplemental table (Table S1) with the number of individuals who ever tested positive and the number of total positive tests for each genotype. We have added additional results for specific genotypes (when there was statistical power, as we now describe in the methods) and when the individual has multiple genotypes.

[Methods]. “We estimated genotype-specific rates only if there were at least 25 detections and more than one observation of persistence.”
[Results: oral]. “No single genotype was detected as being persistent in an oral test more than once; accordingly, we did not estimate genotype-specific time-to-clearance for any genotypes. Time to clear one genotype was not significantly different if the participant had multiple genotypes detected (HR: 1.25, 95% CI: 0.65, 2.24).”

[Results: cervicogenital]. “We estimated genotype-specific time-to-clearance for HPV59 (85 days, 95% CI: 54–135), HPV66 (76 days; 95% CI: 56–102), and HPV90 (70 days; 95% CI: 47–104), which were all comparable. Time to clear one genotype was not significantly different if the participant had multiple genotypes detected (HR: 0.79, 95% CI: 0.33, 1.91).”

VERSION 2 – REVIEW

REVIEWER	Anak Iamaroon Chiang Mai University
REVIEW RETURNED	05-Dec-2021

GENERAL COMMENTS	The authors have well responded to all comments.