Human Papillomavirus (HPV) vaccine effectiveness and potential herd immunity for reducing oncogenic oropharyngeal HPV16 prevalence in the UK

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Human papillomavirus (HPV) vaccine effectiveness and potential herd immunity for reducing oncogenic oropharyngeal HPV16 prevalence in the UK; a cross-sectional study

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Key Words

Head and neck cancer, vaccination, oropharyngeal cancer, cancer prevention, clinical trial.

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Key Points

- HPV-related oropharyngeal cancers are rapidly increasing.
- This study shows that vaccinating girls in a national programme against HPV reduces oropharyngeal oncogenic HPV16 infection.
- The data also show low oral HPV 16 prevalence in unvaccinated boys, suggesting potential herd immunity.

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Abstract

Background

Oropharyngeal cancer incidence is rapidly rising due to human papillomavirus (HPV) 16 infection. The dearth of data on effectiveness of national girl-only vaccination program in preventing oral HPV infection and the potential herd immunity effect on unvaccinated boys has resulted in considerable controversy regarding the need to vaccinate boys, especially in countries with high vaccination coverage of girls.

Methods

Subjects aged 0-65 years undergoing tonsillectomy for non-malignant indications were recruited in 6 UK hospitals. Oral samples were collected in following order: oral rinse, tongue base and pharyngeal wall brushes, then tonsil tissue (tonsillectomy). Vaccination data was obtained from regional health authorities. All samples were centrally tested for HPV-DNA by PCR amplification. (NCT01330147).

Results

Of 940 subjects, 243 girls and 69 boys were aged 12-24; median age 18.6 years. 189 (78%) girls and no boys received HPV vaccination. Overall, oropharyngeal-HPV16 prevalence in vaccinated girls was significantly lower than unvaccinated girls (0.5% vs 5.6%, p=0.04). In contrast, prevalence of any oropharyngeal-HPV type was similar in vaccinated and unvaccinated girls (19% vs 20%, p=0.76). Oropharyngeal-HPV16 prevalence in (unvaccinated) boys was similar to vaccinated girls (0% vs 0.5%, p>0.99), and lower than unvaccinated girls (0% vs 5.6%, p=0.08).

Conclusions

Our findings indicate that the UK girl-only national vaccination program is associated with significant reductions in oropharyngeal-HPV16 infections in children and young adults. This is also the first data to suggest potential herd immunity from girl-only vaccination against oropharyngeal HPV infection in contemporaneously-aged boys.

Introduction

Infection with human papillomaviruses (HPV) can cause oropharyngeal cancers, as well as cervical, anal, penile, and vulvovaginal cancers, and genital warts. HPV is the main cause for the increasing incidence of oropharyngeal cancers in the USA and many Western European countries[1-5], and affects three times as many men than women. HPV16 has been identified as the primary type causing these cancers[4, 5]. Three HPV vaccines are now licensed in many countries worldwide; the HPV-16/18 AS04-adjuvanted vaccine (AS04-HPV-16/18v, *Cervarix*, GSK) and the four- (4vHPVv) and nine-valent (9vHPVv) Sulfate d'hydroxyphosphate d'aluminium-adjuvanted vaccines (*Gardasil*, Merck). These vaccines have been shown to prevent anogenital HPV16/18 infection and high-grade cervical and anogenital lesions[6-11]. The AS04-HPV-16/18 vaccine targets two types of HPV that together cause more than 70% of cervical cancer (HPV16 and 18) and has also shown cross-protection against HPV types 31, 33, and 45, the next most common HPV types in cervical cancer[12-15]. As well as HPV16 and 18, the 4vHPV vaccine targets HPV6 and 11, which cause over 86% of genital warts[16]. The 9vHPV vaccine (against HPV-6/11/16/18/31/33/45/52/58) has also been recently approved in many countries[17].

HPV vaccination was first introduced in the UK in September 2008, with AS04-HPV-16/18v offered to all girls aged 12-13 years (UK Year 8) as well as all girls aged 14-17 as part of a time-limited catch-up program, with a switch to 4vHPV vaccine in September 2012. HPV vaccination in UK girls has had high uptake with 77% of 12-13 year-olds and 49% of 14-17 year-olds in the "catch-up" cohort having received all three doses[18].

In addition to trial data demonstrating that HPV vaccination effectively reduces cervical HPV infection and precancerous lesions, there have now been several studies showing population effects of national vaccination program. A systematic review and meta-analysis and several studies of the impact of national immunization program have shown considerable reductions in the risk of cervical HPV16/18 and HPV31/33/45 infections, anogenital warts, and cervical abnormalities (including invasive HPV-associated cancers) among women vaccinated before 20 years of age[15, 19-24].

To date, the effect of vaccination on oral HPV infection has not been well explored. Secondary analysis of a randomized controlled trial assessing AS04-HPV-16/18 vaccine efficacy on cervical HPV in Costa Rica[25] demonstrated that vaccination was associated with a 93% (95% CI 63% - 100%) decrease in the prevalence of oral HPV16/18 in adult women four years after vaccination. More recently, evidence has been reported supporting reduced HPV6/11/16/18 oral prevalence rates in vaccinated compared to unvaccinated 18-33 year old subjects in the USA (0.11% vs 1.61 %, p=0.08)[26]. Importantly, all studies have been carried out using oral rinse, and there have been no studies examining HPV prevalence using oral rinse and tonsil tissue together, or the effect of the vaccine on HPV prevalence in tonsil tissue (the primary site of oropharyngeal cancer). In addition, there have been no studies evaluating the efficacy of vaccination programs on oral HPV prevalence in children, or studying protection of boys from oral HPV infection by the potential herd effect from a national girl-only vaccination program.

To address that, this study aimed to assess the effect of HPV vaccination on HPV prevalence in tonsillar tissue and oral exfoliated cells among girls and young adult women in the UK undergoing voluntary tonsillectomy for non-malignant indications, and to compare levels of infection to those of unvaccinated, contemporaneous young males of the same age.

Methods

Study Design

This paper uses data collected in the Oromouth study (NCT01330147), a cohort of 940 patients (340 males, 600 females) aged 0-65 years old undergoing tonsillectomy for non-malignant indications. Subjects were enrolled across 6 hospitals in the U.K. from 2013-2015. To assess vaccine effectiveness, we concentrated the analysis on female subjects aged 12-24 at enrollment who could have been vaccinated under the national UK HPV vaccination program, and on contemporaneous males of the same age. The West Midlands – Solihull National Health Service Research Ethics Committee approved this study (approval no 11/WM/0283) and all patients or parent(s)/legal guardian(s) gave written informed consent.

Data Collection

Oral samples were collected in the following order: oral mucosal transudate (using Oracol S10 devices- Malvern Medical Developments) followed by a 60 second, sterile-saline oral rinse and gargle, then an oropharyngeal brush of the base of tongue (using Orcellex brushes; Rovers, The Netherlands), then an oropharyngeal brush of the posterior pharyngeal wall, and finally, all left and right tonsil tissue by tonsillectomy. Further details on collection and processing of all samples are provided in supplementary methods and figure S1. Urine, blood and nail brush samples were also collected pre-operatively (results not reported here). Samples were collected using pre-defined protocols by research nurses and surgeons who were trained before embarking on the study.

A standardized survey was completed by participants (sample shown in Figure S2, Supplementary Material). The survey included detailed demographic information, vaccination and clinical history, and for subjects 16 years and older sexual, smoking, and drinking behaviors. To avoid feelings of embarrassment and under-reporting by patients, surveys forms had unique identifiers only, with no names, and were submitted in closed envelopes deposited in locked ballot-type boxes, only to be opened by researchers who were independent and did not know the clinical teams.

Data on vaccination was obtained from the regional health authorities that provided information on which patients received vaccination through the school program and the catch-up program, and how many doses they received.

A study log was maintained to record those approached to be part of the Oromouth study and to record reasons for lack of consent. A total of 1356 individuals were approached, of which 71.6% consented. The main reasons for not gaining consent were patients refusing (38.9%) and parents declining (21.5%). Of this cohort, 30 patients were part of a pilot study and were therefore not included in the analysis for the main study.

Processing and HPV testing of samples

All samples were tested centrally for the presence of HPV DNA by PCR amplification using the HPV SPF_{10} PCR-DEIA (DNA enzyme immunoassay)-$LiPA_{25}$ (Line probe assay) version 1 (Laboratory Biomedical Products, Rijswijk, The Netherlands). Briefly, this broad-spectrum PCR-based HPV DNA testing system uses SPF_{10} primers to amplify and a DNA enzyme immunoassay to detect at least 57 HPV genotypes and the $LiPA_{25}$ line probe assay to genotype 25 carcinogenic

and non-carcinogenic HPVs in all samples (HPV types 6, 11, 16, 18, 31, 33 to 35, 39, 40, 42 to 45, 51 to 54, 56, 58, 59, 66, 68, 70, and 74)[27, 28]. To increase the specificity of type-specific detection of HPV using the SPF_{10} DEIA system, all specimens that were SPF_{10} PCR/DEIA-positive were tested with the E6-based multiplex type-specific system (MPTS123) that uses xMAP technology (Luminex, Austin, TX, USA)[29]. The HPV types detected by the MPTS123 assay are HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 6, and 11). See Supplementary materials for details.

Oropharyngeal HPV positivity was defined as HPV DNA detection in any of the collected oral samples (oral rinse, either of the oral brushes, or the tonsillar tissue samples) regardless of type. Oncogenic, or high-risk HPV (HR-HPV) was defined as types 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, or 59 based upon previous work.[30]

Risk of bias mitigation

Consecutive patients were recruited to avoid bias. Samples were analyzed at laboratories in a blinded fashion, with no knowledge of patient characteristics or behaviors. Questionnaires were collected and analyzed in a pseudo-anonymized manner, as described above.

Statistical Analysis

In this pre-specified analysis of secondary outcome measures, demographic characteristics, risk factors, and sample-specific HPV prevalence for girls and boys aged 12-24 years were compared by vaccination status and tested for differences using Pearson’s chi-squared tests or Fisher’s

Exact test. The following HPV type-specific outcomes for prevalence were compared between differences by vaccinated and unvaccinated subjects and by sample type: HPV16, HPV16/18, HPV31/33/45, any oncogenic HPV, and any HPV. To explore previously found cross-protective effects of *Cervarix* (AS04-HPV-16/18v) vaccination[12-14] with HPV types 31, 33, and/or 45, positivity to these types was considered as a separate outcome. Logistic regressions were performed for each of the outcomes to test the association between vaccination and prevalence of HPV after controlling for age. Because behavioral factors were collected for subjects aged 16 and above, there were insufficient vaccinated patient numbers to undertake multiple logistic regressions to adjust for behavioral factors.

Results

Of the 940 subjects in the study, there were 243 girls and 69 boys aged 12-24, with a median age of 18.6 years (Interquartile range 16.3-20.7) and 19.1 years (IQR=15.0-21.0) respectively. Of the girls, 189 (78%) received HPV vaccination. None of the boys were vaccinated. Girls who were vaccinated were more likely than unvaccinated girls to be white (90% vs 76%, p=0.03) and <20 years old at enrollment (70% vs. 54%, p=0.01), but were similar in terms of enrollment center, year enrolled, and sexual behavior. 89% of those vaccinated received the AS04-HPV-16/18 vaccine (Table 1).

Effect of vaccination on HPV prevalence

HPV prevalence was compared in vaccinated and unvaccinated girls, by HPV type and by sample type (Figure 1, Table 2). Overall oropharyngeal HPV16 prevalence was significantly lower in vaccinated than unvaccinated girls (0.5% vs 5.6%, p=0.04). Prevalence of oropharyngeal HPV16 appeared lower among vaccinated than unvaccinated girls in both the routine and catch-up vaccination cohorts (Table S1). Prevalence of oropharyngeal HPV16 and/or 18 together (1.1% vs 5.6%, p=0.07) also appeared to be reduced (Figure 1). All four participants who had oropharyngeal HPV16 infections had HPV16 detected in tonsillar tissue. Only one of these participants with tonsillar HPV16 had HPV16 detected in an oral rinse sample. Of the four participants with oropharyngeal HPV 16 infections, three were unvaccinated and one was vaccinated participant. The vaccinated participant was a girl who was 20 years old when she enrolled in the study in 2015, reported receiving 3 doses of AS04-HPV-16/18v, had 8 oral sex partners, and was a current smoker. One (vaccinated) participant had an oropharyngeal HPV18 infection detected in an oral brush sample.

Oropharyngeal prevalence of HPV31, 33, and/or 45 was 0% in vaccinated girls compared to 1.9% (1 case) in unvaccinated girls (p=0.22). Prevalence of any type of oropharyngeal HPV (19% vs 20%, p=0.76) or any oncogenic HPV type (7.4% vs. 7.4%, p>0.99) was similar in vaccinated and unvaccinated girls. Adjustment for age did not change results materially (Table S2).

Next, HPV prevalence among unvaccinated boys 12-24 years of age was compared to that among unvaccinated and vaccinated girls of the same ages. There were no oropharyngeal HPV16 or HPV18 infections detected among boys. Indeed, oropharyngeal HPV16 prevalence in boys appears to be similar to vaccinated girls (0% vs0.5%, p>0.99), and lower than unvaccinated girls (0% vs 5.6%, p=0.08) (Figure 1, Table 2). Among 84 older males in the study, aged 25 to 56, prevalence of oropharyngeal HPV16 (7.1%, p=0.03), and of combined oropharyngeal HPV16 and/or HPV18 infections (8.3%, p=0.02), were significantly higher than that observed among the 12-24 year old boys (Figure 2, Table S3).

Effect of vaccination by sample type

When considering each sample type separately, HPV16 prevalence in tonsillar tissue samples was significantly lower in vaccinated than unvaccinated women aged 12-24 years (HPV16: 0.5% vs 5.6%, p=0.04). Only one non-HPV16 type was detected in tonsillar samples in this age group, an HPV6 infection in a participant aged 17 years who received 3 doses of AS04-HPV-16/18v. When considering HPV16 in oral rinse samples alone, smaller differences were seen between vaccinated girls aged 12-24 years old, compared to unvaccinated ones (0% vs 1.9%, p=0.44) (Table 2). HPV detection in oropharyngeal brushes was low, with no HPV16 being detected.

Discussion

Our findings are the first to indicate that routine vaccination against HPV, as part of a national program, is associated with reductions in oropharyngeal HPV16 infections (the primary HPV type linked to oropharyngeal cancers) in children and young adults. Specifically, vaccination reduces the prevalence of tonsillar HPV infections, which is the commonest site of oropharyngeal cancer and for which data has hitherto been lacking. This data are consistent with data *in adults* from post-hoc analyses of the GSK HPV-040 study[31]; with a randomized controlled trial in Costa Rica[25] and with recent data from the USA[32]. The differences in oropharyngeal HPV16 infection shown within this relatively small study population suggests that the population impact of the UK vaccination program on oropharyngeal HPV is likely to be substantial.

Importantly, our data also demonstrate low HPV16 prevalence amongst unvaccinated boys aged 12-24 years old. Boys' prevalence rates were similar to rates in vaccinated girls, and considerably lower than in unvaccinated girls and males aged 25 and over, despite boys reporting significantly more sexual activity (ever had sex) and more sexual partners than vaccinated girls. This effect was also demonstrated despite a likely reduction in prevalence rates in unvaccinated girls due to the potential herd effect from vaccinated girls, as demonstrated for cervical infections in Scotland, England and the Netherlands[15, 21, 23, 24, 33]. Previously, the only evidence of any potential herd immunity in males from the UK girls vaccination program was a reported 62% reduction in genital warts in heterosexual boys and young men in England since 2009[34]. Our data may be one of the first indications of a potential herd immunity effect from the girls-only vaccination program on oropharyngeal HPV infection in contemporaneously-aged boys. If confirmed in larger population based studies, these new findings could carry important

implications for the decision to extend national HPV vaccination programs to include boys, where there is high coverage of girls.

No previous study has had the opportunity to *prospectively* test tonsillar tissue for HPV in vaccinated and unvaccinated individuals. The few studies available were undertaken retrospectively on formalin fixed tissue samples from historic cohorts and have reported rates of 0-1%[35-37]. By including tonsillar samples in our combined oropharyngeal HPV outcome, we were able to detect HPV in participants with greater sensitivity than by oral rinse alone. We were therefore able to find HPV in considerably more subjects, enabling us to detect a compelling difference in HPV16 prevalence between the vaccinated and unvaccinated groups in the tissue expected to be most relevant for disease. These results suggest that current estimates of oral HPV16 prevalence rates, based predominantly on oral rinse samples, may be an under-estimate of the true prevalence. It should be noted that more HPV16 was identified in tonsils than oral rinse samples, whereas HPV subtypes overall were identified much more commonly in oral rinse than tonsil samples. This may reflect a predilection of HPV16 to tonsils, compared to other HPV subtypes.

Our study had limitations in that there were a small number of people with infection, especially for non-HPV16 oncogenic types, which limited the analyses and adjustments that could be undertaken. There was only one HPV18 case (in a vaccinated girl) and only one HPV31/33/45 infection detected in our study (in an unvaccinated girl), so we could not make reliable conclusions for non-HPV16 oncogenic infections or adequately evaluate the cross-protective effects that have been found in previous studies[12-14]. However, these are rare causes of HPV-

related oropharyngeal cancer. Furthermore, only participants aged 16 and older at enrollment completed the risk behavior survey, and we therefore could not adjust for these factors in our overall analysis without severely truncating our dataset. This means that residual confounding could remain in the estimates from the logistic regression. However, when restricting analyses to those who completed the survey and adjusting for behavioral risk factors, the results were of a similar magnitude to those displayed by the whole sample (Table S2). Furthermore, we undertook multiple analysis of secondary outcomes, with no control for multiplicity of inferences, which should be kept in mind when interpreting these results. Despite these limitations above, our results demonstrated convincing differences. Finally, more girls aged 12-24 were recruited compared to boys. This reflects a lower willingness of boys to agree to participate in the study. This may introduce biases, albeit the prevalence of overall HPV and importantly all (sexually transmitted) high risk HPV infections was the same in girls and boys of the same age (data not shown) suggesting that the differences seen in HPV16prevalence were not due to recruitment bias.

While the UK vaccination program was designed to prevent cervical cancers in women, the secondary effects of preventing oropharyngeal HPV infection are important to consider. With a rising public health focus on preventing HPV-positive oropharyngeal cancers due to their increasing incidence,[38] the effective reduction in oropharyngeal HPV16 prevalence in vaccinated adolescents and young adults seen in our study means that national vaccination programs could considerably reduce the incidence of oropharyngeal HPV cancers. Our study also demonstrated reduced oropharyngeal HPV16 prevalence in the vaccinated groups of both the

routine and catch-up vaccine programs. As with cervical cancer, however, longitudinal data are necessary to fully establish the effectiveness of vaccination for preventing oropharyngeal cancers.

In summary, our results are one of the first to show that a girl-only vaccination program protects against oncogenic oropharyngeal HPV16 infection in girls and young women, and may also confer protection on contemporaneously-aged unvaccinated boys through potential herd immunity. This suggests that oropharyngeal HPV prevalence may be reduced by girl-only national HPV vaccination programs with high coverage.

Trademarks

Cervarix is a trade mark owned by or licensed to the GSK group of companies.

Gardasil is a trade mark of Merck & Co, Inc.

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Contributorship:

Hisham Mehanna conceived, designed, conducted and interpreted the study and wrote the manuscript. Jennifer Bryant, Rachel Spruce, Nikolaos Batis, Oladejo Olaleye, Jaspreet Babrah and June Jones conducted the study, interpreted results and wrote the manuscript. Sylvia Taylor and Dominique Rosillon participated in the study design, analysis/interpretation of the data and writing the manuscript. Gypsyamber D'souza and Tyler Bryant analysed the data and wrote the manuscript. Anco Molijn, Linda Struijk and Alex Vorsters participated in the design of the sampling procedures, laboratory testing and interpretation of the results and writing of the manuscript.

Data Sharing: More data on HPV antibody status and urine HPV infections and on behavioral survey are available on request from authors, and is being prepared for manuscript submission.

Conflicts of Interest

Sylvia Taylor and Dominique Rosillon are employees of the GSK group of companies and hold shares in the GSK group of companies. Hisham Mehanna has research grants and advisory consultancy fees from Astra Zeneca and Merck, Sharpe & Dohlme, and previous grants from the GSK group of companies, unrelated to this study or research area. All other authors declare no competing interest. No authors have relationships or activities that could appear to influence the submitted work.

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Tables

Table 1: Description of boys and girls ages 12-24 in study population, with data on girls by HPV vaccination history.

	Girls			Boys	
	Received HPV Vaccine		P-value Unvaccinated vs vaccinated girls		P-value Boys vs vaccinated girls
Participant Characteristic	**No (n = 54)**	**Yes (n = 189)**		**Number (%) (n=69)**	
Age, in years			0.01		0.02
12-15	16 (29.6%)	41 (21.7%)		21 (30.4%)	
16-19	13 (24.1%)	92 (48.7%)		20 (29.0%)	
20-24	25 (46.3%)	56 (29.6%)		28 (40.6%)	
Ethnicity			0.03		0.38
White	41 (75.9%)	171 (90.5%)		59 (85.5%)	
Black or Black British Mixed	2 (3.7%)	4 (2.1%)		5 (7.3%)	
Asian or British Asian	5 (9.3%)	5 (2.7%)		2 (2.9%)	
Mixed or Other Ethnic Group	6 (11.1%)	9 (4.8%)		3 (4.4%)	
Centre Enrolled			0.35		0.78
Worcester Royal Hospital	1 (1.9%)	6 (3.2%)		2 (2.9%)	
University Hospital Coventry and Warwickshire	27 (50.0%)	66 (34.9%)		31 (44.9%)	
University Hospital Birmingham	13 (24.1%)	63 (33.3%)		20 (29.0%)	
New Cross Hospital Wolverhampton	2 (3.7%)	4 (2.1%)		1 (1.5%)	
Kidderminister General Hospital	1 (1.8%)	10 (5.3%)		4 (5.8%)	
Birmingham Heartlands Hospital	10 (18.5%)	40 (21.2%)		11 (15.9%)	

Year enrolled			0.60		0.16
2013	17 (31.5%)	66 (34.9%)		23 (33.3%)	
2014	23 (42.6%)	86 (45.5%)		25 (36.2%)	
2015	14 (25.9%)	37 (19.6%)		21 (30.4%)	
SURVEY AMONG THOSE ≥16 YEARS ONLY					
Age at First Sex, in years mean (SD)	16.2 (1.7)	15.9 (1.5)	0.24	16.2 (1.3)	0.12
Ever had Sex			0.31		0.57
No	1 (2.9%)	14 (10.3%)		3 (6.5%)	
Yes	34 (97.1%)	122 (89.7%)		43 (93.5%)	
Ever had Oral Sex			0.08		0.09
No	2 (6.5%)	25 (19.7%)		3 (7.1%)	
Yes	29 (93.5%)	102 (80.3%)		39 (92.9%)	
Number of oral sex partners in lifetime			0.09		0.02
0	3 (10.7%)	26 (21.1%)		7 (46.7%)	
1	8 (28.6%)	24 (19.5%)		1 (6.7%)	
2-5	16 (57.1%)	51 (41.5%)		2 (13.3%)	
6 or more	1 (3.6%)	22 (17.9%)		5 (33.3%)	

455 **Table 2:** Difference in HPV prevalence among 69 unvaccinated boys, 189 girls vaccinated with
456 any HPV vaccine and 54 unvaccinated girls aged 12-24 years old at enrollment, by sample type
457 and among select HPV types.

	Girls		Un-vaccinated vs vaccinated girls	Boys	Boys vs. vaccinated girls	Boys vs. non-vaccinated girls
HPV Type and Sample Type	**Not Vaccinated (N = 54)**	**Yes Vaccinated[a] (N = 189[b])**	**P-value**	**(N=69)**	**P-value**	**P-value**
HPV 16						
Oropharyngeal (overall)	**3 (5.6%)**	**1 (0.5%)**	**0.04**	0 (0%)	**>0.99**	**0.08**
Oral Rinse	1 (1.9%)	0 (0.0%)	0.22	0 (0%)	--	0.44
Oral Brush (either sample)	0 (0.0%)	0 (0.0%)	--	0 (0%)	--	--
Tonsil	**3 (5.6%)**	**1 (0.5%)**	**0.04**	0 (0%)	**>0.99**	**0.08**
HPV 16 or 18						
Oropharyngeal (overall)	3 (5.6%)	2 (1.1%)	0.07	0 (0%)	>0.99	0.08
Oral Rinse	1 (1.9%)	0 (0.0%)	0.22	0 (0%)	--	0.44
Oral Brush (either sample)	0 (0.0%)	1 (0.5%)	>0.99	0 (0%)	>0.99	--
Tonsil	**3 (5.6%)**	**1 (0.5%)**	**0.04**	0 (0%)	>0.99	**0.08**
HPV 31 or 33 or 45						
Oropharyngeal (overall)	1 (1.9%)	0 (0.0%)	0.22	1 (1.5%)	0.27	>0.99
Oral Rinse	1 (1.9%)	0 (0.0%)	0.22	0 (0%)	--	0.44
Oral Brush (either sample)	0 (0.0%)	0 (0.0%)	--	1 (1.5%)	0.27	>0.99
Tonsil	0 (0.0%)	0 (0.0%)	--	0 (0%)	--	--
Any Oncogenic Type						

Oropharyngeal (overall)	4 (7.4%)	14 (7.4%)	>0.99	2 (2.9%)	0.25	0.40
Oral Rinse	2 (3.7%)	12 (6.4%)	0.74	1 (1.5%)	0.20	0.58
Oral Brush (either sample)	0 (0.0%)	2 (1.1%)	>0.99	1 (1.5%)	>0.99	>0.99
Tonsil	**3 (5.6%)**	**1 (0.5%)**	**0.04**	0 (0%)	>0.99	**0.08**
Any type of HPV						
Oropharyngeal (overall)	11 (20.4%)	35 (18.5%)	0.76	12 (17.4%)	0.84	0.67
Oral Rinse	8 (14.8%)	28 (14.8%)	>0.99	9 (13.2%)	0.72	0.77
Oral Brush (either sample)	1 (1.9%)	8 (4.2%)	0.69	3 (4.4%)	>0.99	0.63
Tonsil	3 (5.6%)	2 (1.1%)	0.07	1 (1.5%)	>0.99	0.32

[a]HPV16 was detected in the tonsil sample of 1 person who was vaccinated with AS04-HPV16/18v (with 3 doses), reported having 8 lifetime oral sex partners, current smoker, and was enrolled in 2015 when she was 20 years old. Only 1 HPV18 infection was detected in any oral sample, it was in a AS04-HPV16/18v vaccinated participant who received all 3 doses, reported never performing oral sex or any other sexual activity, never smoker, and was enrolled in 2013 at age of 17.

[b]Two vaccinated subjects did not have tonsil samples (tonsillar data for vaccinated subjects shown is among 187 subjects). Three vaccinated subjects and one unvaccinated subject did not have oral rinse samples (oral rinse data for vaccinated and unvaccinated subjects shown is 186 and 53, respectively).

Figures

Figure 1: Oropharyngeal HPV prevalence in unvaccinated girls, vaccinated girls, and boys aged 12-14 years by vaccination status and HPV type. P values represent comparisons to unvaccinated girls using Pearson's chi-squared tests or Fisher's Exact test.

Figure 2: Oropharyngeal HPV prevalence in males 12-24 years of age and males over 24 years old and by HPV type. P values represent comparisons to males 12-24 years old using Pearson's chi-squared tests or Fisher's Exact test.