

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

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

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29 **Key Words**

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

31 **Running title:** HPV vaccination and oral HPV prevalence

32 **Key Points**

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

38

39 **Word count:** Abstract: 239 Manuscript: 2721

40 **Abstract**

41 **Background**

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

47 **Methods**

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

53 **Results**

54 Of 940 subjects, 243 girls and 69 boys were aged 12-24; median age 18.6 years. 189 (78%) girls
55 and no boys received HPV vaccination. Overall, oropharyngeal-HPV16 prevalence in vaccinated
56 girls was significantly lower than unvaccinated girls (0.5% vs 5.6%, $p=0.04$). In contrast,
57 prevalence of any oropharyngeal-HPV type was similar in vaccinated and unvaccinated girls
58 (19% vs 20%, $p=0.76$). Oropharyngeal-HPV16 prevalence in (unvaccinated) boys was similar to
59 vaccinated girls (0% vs 0.5%, $p>0.99$), and lower than unvaccinated girls (0% vs 5.6%, $p=0.08$).

60 **Conclusions**

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

65

66

67 **Introduction**

68 Infection with human papillomaviruses (HPV) can cause oropharyngeal cancers, as well as
69 cervical, anal, penile, and vulvovaginal cancers, and genital warts. HPV is the main cause for the
70 increasing incidence of oropharyngeal cancers in the USA and many Western European
71 countries[1-5], and affects three times as many men than women. HPV16 has been identified as
72 the primary type causing these cancers[4, 5]. Three HPV vaccines are now licensed in many
73 countries worldwide; the HPV-16/18 AS04-adjuvanted vaccine (AS04-HPV-16/18v, *Cervarix*,
74 GSK) and the four- (4vHPVv) and nine-valent (9vHPVv) Sulfate d'hydroxyphosphate
75 d'aluminium-adjuvanted vaccines (*Gardasil*, Merck). These vaccines have been shown to prevent
76 anogenital HPV16/18 infection and high-grade cervical and anogenital lesions[6-11]. The AS04-
77 HPV-16/18 vaccine targets two types of HPV that together cause more than 70% of cervical
78 cancer (HPV16 and 18) and has also shown cross-protection against HPV types 31, 33, and 45,
79 the next most common HPV types in cervical cancer[12-15]. As well as HPV16 and 18, the
80 4vHPV vaccine targets HPV6 and 11, which cause over 86% of genital warts[16]. The 9vHPV
81 vaccine (against HPV-6/11/16/18/31/33/45/52/58) has also been recently approved in many
82 countries[17].

83
84 HPV vaccination was first introduced in the UK in September 2008, with AS04-HPV-16/18v
85 offered to all girls aged 12-13 years (UK Year 8) as well as all girls aged 14-17 as part of a time-
86 limited catch-up program, with a switch to 4vHPV vaccine in September 2012. HPV vaccination
87 in UK girls has had high uptake with 77% of 12-13 year-olds and 49% of 14-17 year-olds in the
88 “catch-up” cohort having received all three doses[18].

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

96 To date, the effect of vaccination on oral HPV infection has not been well explored. Secondary
97 analysis of a randomized controlled trial assessing AS04-HPV-16/18 vaccine efficacy on cervical
98 HPV in Costa Rica[25] demonstrated that vaccination was associated with a 93% (95% CI 63% -
99 100%) decrease in the prevalence of oral HPV16/18 in adult women four years after vaccination.
100 More recently, evidence has been reported supporting reduced HPV6/11/16/18 oral prevalence
101 rates in vaccinated compared to unvaccinated 18-33 year old subjects in the USA (0.11% vs 1.61
102 %, $p=0.08$)[26]. Importantly, all studies have been carried out using oral rinse, and there have
103 been no studies examining HPV prevalence using oral rinse and tonsil tissue together, or the
104 effect of the vaccine on HPV prevalence in tonsil tissue (the primary site of oropharyngeal
105 cancer). In addition, there have been no studies evaluating the efficacy of vaccination programs
106 on oral HPV prevalence in children, or studying protection of boys from oral HPV infection by
107 the potential herd effect from a national girl-only vaccination program.

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

112 **Methods**

113 *Study Design*

114 This paper uses data collected in the Oromouth study (NCT01330147), a cohort of 940 patients
115 (340 males, 600 females) aged 0-65 years old undergoing tonsillectomy for non-malignant
116 indications. Subjects were enrolled across 6 hospitals in the U.K. from 2013-2015. To assess
117 vaccine effectiveness, we concentrated the analysis on female subjects aged 12-24 at enrollment
118 who could have been vaccinated under the national UK HPV vaccination program, and on
119 contemporaneous males of the same age. The West Midlands – Solihull National Health Service
120 Research Ethics Committee approved this study (approval no 11/WM/0283) and all patients or
121 parent(s)/legal guardian(s) gave written informed consent.

122

123 *Data Collection*

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

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

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

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

148

149 *Processing and HPV testing of samples*

150 All samples were tested centrally for the presence of HPV DNA by PCR amplification using the
151 HPV SPF₁₀ PCR-DEIA (DNA enzyme immunoassay)-LiPA₂₅ (Line probe assay) version 1
152 (Laboratory Biomedical Products, Rijswijk, The Netherlands). Briefly, this broad-spectrum PCR-
153 based HPV DNA testing system uses SPF₁₀ primers to amplify and a DNA enzyme immunoassay
154 to detect at least 57 HPV genotypes and the LiPA₂₅ line probe assay to genotype 25 carcinogenic

155 and non-carcinogenic HPVs in all samples (HPV types 6, 11, 16, 18, 31, 33 to 35, 39, 40, 42 to
156 45, 51 to 54, 56, 58, 59, 66, 68, 70, and 74)[27, 28]. To increase the specificity of type-specific
157 detection of HPV using the SPF₁₀ DEIA system, all specimens that were SPF₁₀ PCR/DEIA-
158 positive were tested with the E6-based multiplex type-specific system (MPTS123) that uses
159 xMAP technology (Luminex, Austin, TX, USA)[29]. The HPV types detected by the MPTS123
160 assay are HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 6, and 11). See
161 Supplementary materials for details.

162 Oropharyngeal HPV positivity was defined as HPV DNA detection in any of the collected oral
163 samples (oral rinse, either of the oral brushes, or the tonsillar tissue samples) regardless of type.
164 Oncogenic, or high-risk HPV (HR-HPV) was defined as types 16, 18, 31, 33, 35, 39, 45, 51, 52,
165 58, or 59 based upon previous work.[30]

166

167 *Risk of bias mitigation*

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

171

172 *Statistical Analysis*

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

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

185

186 **Results**

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

194 *Effect of vaccination on HPV prevalence*

195 HPV prevalence was compared in vaccinated and unvaccinated girls, by HPV type and by sample
196 type (Figure 1, Table 2). Overall oropharyngeal HPV16 prevalence was significantly lower in
197 vaccinated than unvaccinated girls (0.5% vs 5.6%, $p=0.04$). Prevalence of oropharyngeal HPV16
198 appeared lower among vaccinated than unvaccinated girls in both the routine and catch-up
199 vaccination cohorts (Table S1). Prevalence of oropharyngeal HPV16 and/or 18 together (1.1% vs
200 5.6%, $p=0.07$) also appeared to be reduced (Figure 1). All four participants who had
201 oropharyngeal HPV16 infections had HPV16 detected in tonsillar tissue. Only one of these
202 participants with tonsillar HPV16 had HPV16 detected in an oral rinse sample. Of the four
203 participants with oropharyngeal HPV 16 infections, three were unvaccinated and one was
204 vaccinated participant. The vaccinated participant was a girl who was 20 years old when she
205 enrolled in the study in 2015, reported receiving 3 doses of AS04-HPV-16/18v, had 8 oral sex
206 partners, and was a current smoker. One (vaccinated) participant had an oropharyngeal HPV18
207 infection detected in an oral brush sample.

208 Oropharyngeal prevalence of HPV31, 33, and/or 45 was 0% in vaccinated girls compared to
209 1.9% (1 case) in unvaccinated girls ($p=0.22$). Prevalence of any type of oropharyngeal HPV
210 (19% vs 20%, $p=0.76$) or any oncogenic HPV type (7.4% vs. 7.4%, $p>0.99$) was similar in
211 vaccinated and unvaccinated girls. Adjustment for age did not change results materially (Table
212 S2).

213 Next, HPV prevalence among unvaccinated boys 12-24 years of age was compared to that among
214 unvaccinated and vaccinated girls of the same ages. There were no oropharyngeal HPV16 or
215 HPV18 infections detected among boys. Indeed, oropharyngeal HPV16 prevalence in boys
216 appears to be similar to vaccinated girls (0% vs 0.5%, $p>0.99$), and lower than unvaccinated girls
217 (0% vs 5.6%, $p=0.08$) (Figure 1, Table 2). Among 84 older males in the study, aged 25 to 56,
218 prevalence of oropharyngeal HPV16 (7.1%, $p=0.03$), and of combined oropharyngeal HPV16
219 and/or HPV18 infections (8.3%, $p=0.02$), were significantly higher than that observed among the
220 12-24 year old boys (Figure 2, Table S3).

221

222 *Effect of vaccination by sample type*

223 When considering each sample type separately, HPV16 prevalence in tonsillar tissue samples was
224 significantly lower in vaccinated than unvaccinated women aged 12-24 years (HPV16: 0.5% vs
225 5.6%, $p=0.04$). Only one non-HPV16 type was detected in tonsillar samples in this age group, an
226 HPV6 infection in a participant aged 17 years who received 3 doses of AS04-HPV-16/18v. When
227 considering HPV16 in oral rinse samples alone, smaller differences were seen between
228 vaccinated girls aged 12-24 years old, compared to unvaccinated ones (0% vs 1.9%, $p=0.44$)
229 (Table 2). HPV detection in oropharyngeal brushes was low, with no HPV16 being detected.

230

231 **Discussion**

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

241 Importantly, our data also demonstrate low HPV16 prevalence amongst unvaccinated boys aged
242 12-24 years old. Boys' prevalence rates were similar to rates in vaccinated girls, and considerably
243 lower than in unvaccinated girls and males aged 25 and over, despite boys reporting significantly
244 more sexual activity (ever had sex) and more sexual partners than vaccinated girls. This effect
245 was also demonstrated despite a likely reduction in prevalence rates in unvaccinated girls due to
246 the potential herd effect from vaccinated girls, as demonstrated for cervical infections in
247 Scotland, England and the Netherlands[15, 21, 23, 24, 33]. Previously, the only evidence of any
248 potential herd immunity in males from the UK girls vaccination program was a reported 62%
249 reduction in genital warts in heterosexual boys and young men in England since 2009[34]. Our
250 data may be one of the first indications of a potential herd immunity effect from the girls-only
251 vaccination program on oropharyngeal HPV infection in contemporaneously-aged boys. If
252 confirmed in larger population based studies, these new findings could carry important

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

255
256 No previous study has had the opportunity to *prospectively* test tonsillar tissue for HPV in
257 vaccinated and unvaccinated individuals. The few studies available were undertaken
258 retrospectively on formalin fixed tissue samples from historic cohorts and have reported rates of
259 0-1%[35-37]. By including tonsillar samples in our combined oropharyngeal HPV outcome, we
260 were able to detect HPV in participants with greater sensitivity than by oral rinse alone. We were
261 therefore able to find HPV in considerably more subjects, enabling us to detect a compelling
262 difference in HPV16 prevalence between the vaccinated and unvaccinated groups in the tissue
263 expected to be most relevant for disease. These results suggest that current estimates of oral
264 HPV16 prevalence rates, based predominantly on oral rinse samples, may be an under-estimate of
265 the true prevalence. It should be noted that more HPV16 was identified in tonsils than oral rinse
266 samples, whereas HPV subtypes overall were identified much more commonly in oral rinse than
267 tonsil samples. This may reflect a predilection of HPV16 to tonsils, compared to other HPV
268 subtypes.

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

275 related oropharyngeal cancer. Furthermore, only participants aged 16 and older at enrollment
276 completed the risk behavior survey, and we therefore could not adjust for these factors in our
277 overall analysis without severely truncating our dataset. This means that residual confounding
278 could remain in the estimates from the logistic regression. However, when restricting analyses to
279 those who completed the survey and adjusting for behavioral risk factors, the results were of a
280 similar magnitude to those displayed by the whole sample (Table S2). Furthermore, we
281 undertook multiple analysis of secondary outcomes, with no control for multiplicity of
282 inferences, which should be kept in mind when interpreting these results. Despite these
283 limitations above, our results demonstrated convincing differences. Finally, more girls aged 12-
284 24 were recruited compared to boys. This reflects a lower willingness of boys to agree to
285 participate in the study. This may introduce biases, albeit the prevalence of overall HPV and
286 importantly all (sexually transmitted) high risk HPV infections was the same in girls and boys of
287 the same age (data not shown) suggesting that the differences seen in HPV16 prevalence were not
288 due to recruitment bias.

289

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

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

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

304 **Trademarks**

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

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

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317 **Contributorship:**

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

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

328 **Conflicts of Interest**

329 Sylvia Taylor and Dominique Rosillon are employees of the GSK group of companies and hold
330 shares in the GSK group of companies. Hisham Mehanna has research grants and advisory
331 consultancy fees from Astra Zeneca and Merck, Sharpe & Dohlme, and previous grants from the
332 GSK group of companies, unrelated to this study or research area. All other authors declare no
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334 submitted work.

335

336

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447 **Tables**

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

Participant Characteristic	Girls		Boys	
	Received HPV Vaccine		P-value	P-value
	No (n = 54)	Yes (n = 189)	Unvaccinated vs vaccinated girls	Boys vs vaccinated girls
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%)
Kidderminster 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%)	

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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.

HPV Type and Sample Type	Girls		Un- vaccinated vs vaccinated girls		Boys vs. vaccinated girls	Boys vs. non- vaccinated girls
	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

458 ^aHPV16 was detected in the tonsil sample of 1 person who was vaccinated with AS04-
459 HPV16/18v (with 3 doses), reported having 8 lifetime oral sex partners, current smoker, and was
460 enrolled in 2015 when she was 20 years old. Only 1 HPV18 infection was detected in any oral
461 sample, it was in a AS04-HPV16/18v vaccinated participant who received all 3 doses, reported
462 never performing oral sex or any other sexual activity, never smoker, and was enrolled in 2013 at
463 age of 17.

464 ^bTwo vaccinated subjects did not have tonsil samples (tonsillar data for vaccinated subjects
465 shown is among 187 subjects). Three vaccinated subjects and one unvaccinated subject did not
466 have oral rinse samples (oral rinse data for vaccinated and unvaccinated subjects shown is 186
467 and 53, respectively).

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470 **Figures**

471 **Figure 1:** Oropharyngeal HPV prevalence in unvaccinated girls, vaccinated girls, and boys aged
472 12-14 years by vaccination status and HPV type. P values represent comparisons to unvaccinated
473 girls using Pearson's chi-squared tests or Fisher's Exact test.

474

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