

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

Mehanna, Hesham; Bryant, Tyler S; Babrah, Jaspreet; Louie, Karly; Bryant, Jennifer; Spruce, Rachel; Batis, Nikolaos; Olaleye, Oladejo; Jones, June; Struijk, Linda; Molijn, Anco; Vorsters, Alex; Rosillon, Dominique; Taylor, Sylvia; D'Souza, Gypsyamber

DOI:

[10.1093/cid/ciy1081](https://doi.org/10.1093/cid/ciy1081)

License:

Other (please specify with Rights Statement)

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Mehanna, H, Bryant, TS, Babrah, J, Louie, K, Bryant, J, Spruce, R, Batis, N, Olaleye, O, Jones, J, Struijk, L, Molijn, A, Vorsters, A, Rosillon, D, Taylor, S & D'Souza, G 2018, 'Human Papillomavirus (HPV) vaccine effectiveness and potential herd immunity for reducing oncogenic oropharyngeal HPV16 prevalence in the UK: a cross-sectional study', *Clinical Infectious Diseases*. <https://doi.org/10.1093/cid/ciy1081>

[Link to publication on Research at Birmingham portal](#)

**Publisher Rights Statement:**

Checked for eligibility: 17/12/2018

This is the accepted manuscript for a forthcoming publication in *Clinical Infectious Diseases*.

**General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

**Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

Download date: 18. Jan. 2021

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

3 **Authors**

4 Hisham Mehanna<sup>1</sup>, Tyler S Bryant<sup>2</sup>, Jaspreet Babrah<sup>3</sup>, Karly Louie<sup>4</sup>, Jennifer L Bryant<sup>1</sup>, Rachel J  
5 Spruce<sup>1</sup>, Nikolaos Batis<sup>1</sup>, Oladejo Olaleye<sup>1</sup>, June Jones<sup>1</sup>, Linda Struijk<sup>5</sup>, Anco Molijn<sup>5</sup>, Alex  
6 Vorsters<sup>6</sup>, Dominique Rosillon<sup>7</sup>, Sylvia Taylor<sup>7</sup>, Gypsyamber D'Souza<sup>2</sup>

7  
8 <sup>1</sup>H Mehanna, Chair of Head and Neck Surgery and Director; J Bryant, Post-doctoral Research  
9 Fellow; N Batis, Post-doctoral Research Fellow; R Spruce, Post-doctoral Research Fellow; O  
10 Olaleye, Clinical Research Fellow and Specialty Registrar in Otolaryngology, Head and Neck  
11 Surgery; and J Jones, Senior Research Nurse, the Institute of Head & Neck Studies and  
12 Education, College of Medical and Dental Sciences, University of Birmingham, Birmingham,  
13 B15 2TT, UK.

14 <sup>2</sup>G D'Souza, Associate Professor, and TS Bryant, Medical Student, Johns Hopkins Bloomberg  
15 School of Public Health, Department of Epidemiology, Baltimore, MD 21205, USA

16 <sup>3</sup>J Babrah, Senior Trials Coordinator, Cancer Research UK Clinical Trials Unit, University of  
17 Birmingham, Birmingham, B15 2TT, UK.

18 <sup>4</sup>K Louie, Senior Epidemiologist and Statistician, Centre for Cancer Prevention, Wolfson  
19 Institute of Preventive Medicine, Queen Mary University of London, London, EC1M 6BQ, UK.

20 <sup>5</sup>L Struijk, Project Leader, and AC Molijn, Director of Operations, DDL Diagnostic Laboratory,  
21 2288 ER, Rijswijk, The Netherlands.

22 <sup>6</sup>A Vorsters, Senior Project Coordinator/Researcher, Centre for the Evaluation of Vaccination,  
23 Vaccine and Infectious Disease Institute, University of Antwerp, 2610 Antwerp, Belgium.

24 <sup>7</sup>D Rosillon, Biostatistician in Epidemiology, and S Taylor, Senior Manager, Clinical and  
25 Epidemiology Research Development, GSK, 1300 Wavre, Belgium.

## 26 **Corresponding Author**

27 Prof Hisham Mehanna

28 Email: h.mehanna@bham.ac.uk      Web: www.inhanse.org

## 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 SPF<sub>10</sub> PCR-DEIA (DNA enzyme immunoassay)-LiPA<sub>25</sub> (Line probe assay) version 1  
152 (Laboratory Biomedical Products, Rijswijk, The Netherlands). Briefly, this broad-spectrum PCR-  
153 based HPV DNA testing system uses SPF<sub>10</sub> primers to amplify and a DNA enzyme immunoassay  
154 to detect at least 57 HPV genotypes and the LiPA<sub>25</sub> 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 SPF<sub>10</sub> DEIA system, all specimens that were SPF<sub>10</sub> 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.

#### 307 **Acknowledgements**

308 The authors thank all study participants and their families and all clinical study site personnel  
309 who contributed to the conduct of this trial. The authors also thank the following for their help in  
310 sample testing: Dimitrie Grégoire, Dominique Gilson, Stéphanie Maerlan, Nathalie Houard, Jean-  
311 Marc Delroisse, Serge Durviaux and Thierry Pascal; Pam Kalodimos, Corinne Willame, Monique  
312 Dodet, Edwin Kolp for their help in study coordination; Sylviane Poncelet and Martin Ryser for  
313 manuscript review, Sarah Welby for her review and input to the study and manuscript and  
314 Gemma Jones for manuscript preparation.

315 **Funding:** This work was supported by GlaxoSmithKline Biologicals SA with an unrestricted  
316 research grant.

#### 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  
333 competing interest. No authors have relationships or activities that could appear to influence the  
334 submitted work.

335

336

REFERENCES

- 337
- 338
- 339 1. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and  
340 oropharyngeal Cancer. *New England Journal of Medicine* **2007**; 356(19): 1944-56.
- 341 2. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck  
342 squamous cell carcinomas worldwide: A systematic review. *Cancer Epidemiology Biomarkers &*  
343 *Prevention* **2005**; 14(2): 467.
- 344 3. Saraiya M, Unger ER, Thompson TD, et al. US assessment of HPV types in cancers: implications  
345 for current and 9-valent HPV vaccines. *JNCI Journal of the National Cancer Institute* **2015**; 107(6):  
346 djv086.
- 347 4. Mehanna H, Beech T, Nicholson T, et al. Prevalence of human papillomavirus in oropharyngeal  
348 and nonoropharyngeal head and neck cancer--systematic review and meta-analysis of trends by  
349 time and region. *Head & neck* **2013**; 35(5): 747-55.
- 350 5. Mehanna H, Franklin N, Compton N, et al. Geographic variation in human papillomavirus-related  
351 oropharyngeal cancer: Data from 4 multinational randomized trials. *Head & neck* **2016**; 38 Suppl  
352 1: E1863-9.
- 353 6. Paavonen J, Naud P, Salmerón J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-  
354 adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types  
355 (PATRICIA): final analysis of a double-blind, randomised study in young women. *The Lancet* **2009**;  
356 374(9686): 301-14.
- 357 7. FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-  
358 grade cervical lesions. *New England Journal of Medicine* **2007**; 356(19): 1915-27.

- 359 8. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human  
360 papillomavirus to prevent anogenital diseases. *New England Journal of Medicine* **2007**; 356(19):  
361 1928-43.
- 362 9. Palefsky JM, Giuliano AR, Goldstone S, et al. HPV vaccine against anal HPV infection and anal  
363 intraepithelial neoplasia. *New England Journal of Medicine* **2011**; 365(17): 1576-85.
- 364 10. Swedish KA, Factor SH, Goldstone SE. Prevention of recurrent high-grade anal neoplasia with  
365 quadrivalent human papillomavirus vaccination of men who have sex with men: a nonconcurrent  
366 cohort study. *Clinical Infectious Diseases* **2012**; 54(7): 891-8.
- 367 11. Harper DM. Impact of vaccination with Cervarix™ on subsequent HPV-16/18 infection and  
368 cervical disease in women 15–25 years of age. *Gynecologic Oncology* **2008**; 110(3): S11-S7.
- 369 12. Kavanagh K, Pollock KGJ, Potts A, et al. Introduction and sustained high coverage of the HPV  
370 bivalent vaccine leads to a reduction in prevalence of HPV 16/18 and closely related HPV types.  
371 *British Journal of Cancer* **2014**; 110(11): 2804-11.
- 372 13. Wheeler CM, Castellsagué X, Garland SM, et al. Cross-protective efficacy of HPV-16/18 AS04-  
373 adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic  
374 HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *The*  
375 *Lancet Oncology* **2012**; 13(1): 100-10.
- 376 14. Einstein MH, Baron M, Levin MJ, et al. Comparison of the immunogenicity of the human  
377 papillomavirus (HPV)-16/18 vaccine and the HPV-6/11/16/18 vaccine for oncogenic non-vaccine  
378 types HPV-31 and HPV-45 in healthy women aged 18–45 years. *Human Vaccines* **2011**; 7(12):  
379 1359-73.
- 380 15. Kavanagh K, Pollock KG, Cuschieri K, et al. Changes in the prevalence of human papillomavirus  
381 following a national bivalent human papillomavirus vaccination programme in Scotland: a 7-year  
382 cross-sectional study. *Lancet Infect Dis* **2017**; (12): 1293-302.

- 383 16. Garland SM, Steben M, Singhs HL, et al. Natural history of genital warts: analysis of the placebo  
384 arm of 2 randomized phase III trials of a quadrivalent human papillomavirus (Types 6, 11, 16, and  
385 18) vaccine. *The Journal of Infectious Diseases* **2009**; 199(6): 805-14.
- 386 17. European Medicines Agency. Gardasil 9: EPAR Summary for the public. **2015**:1-4.
- 387 18. Public Health England. Human papillomavirus (HPV) vaccine coverage in England, 2008/09 to  
388 2013/14: A review of the full Six years of the three-dose schedule. London: Public Health  
389 England. **2015**.
- 390 19. Drolet M, Bénard É, Boily M-C, et al. Population-level impact and herd effects following human  
391 papillomavirus vaccination programmes: a systematic review and meta-analysis. *The Lancet*  
392 *Infectious diseases* **2015**; 15(5): 565-80.
- 393 20. Mesher D, Panwar K, Thomas SL, Beddows S, Soldan K. Continuing reductions in HPV 16/18 in a  
394 population with high coverage of bivalent HPV vaccination in England: an ongoing cross-sectional  
395 study. *BMJ Open* **2016**; 6(2): e009915.
- 396 21. Mesher D, Panwar K, Thomas SL, et al. The Impact of the National HPV Vaccination Program in  
397 England Using the Bivalent HPV Vaccine: Surveillance of Type-Specific HPV in Young Females,  
398 2010-2016. *J Infect Dis* **2018**; 218(6): 911-21.
- 399 22. Pollock KG, Kavanagh K, Potts A, et al. Reduction of low- and high-grade cervical abnormalities  
400 associated with high uptake of the HPV bivalent vaccine in Scotland. *Br J Cancer* **2014**; 111(9):  
401 1824-30.
- 402 23. Donken R, King AJ, Bogaards JA, Woestenbergh PJ, Meijer C, de Melker HE. High Effectiveness of  
403 the Bivalent Human Papillomavirus (HPV) Vaccine Against Incident and Persistent HPV Infections  
404 up to 6 Years After Vaccination in Young Dutch Women. *J Infect Dis* **2018**; 217(10): 1579-89.
- 405 24. Luostarinen T, Apter D, Dillner J, et al. Vaccination protects against invasive HPV-associated  
406 cancers. *Int J Cancer* **2018**; 142(10): 2186-7.

- 407 25. Herrero R, Quint W, Hildesheim A, et al. Reduced prevalence of oral human papillomavirus (HPV)  
408 4 years after bivalent HPV vaccination in a randomized clinical trial in Costa Rica. *PLoS One* **2013**;  
409 8(7): e68329.
- 410 26. Chaturvedi AK, Graubard BI, Broutian T, et al. Effect of Prophylactic Human Papillomavirus (HPV)  
411 Vaccination on Oral HPV Infections Among Young Adults in the United States. *J Clin Oncol* **2018**;  
412 36(3): 262-7.
- 413 27. Kleter B, van Doorn LJ, Schrauwen L, et al. Development and clinical evaluation of a highly  
414 sensitive PCR-reverse hybridization line probe assay for detection and identification of  
415 anogenital human papillomavirus. *Journal of clinical microbiology* **1999**; 37(8): 2508-17.
- 416 28. Kleter B, van Doorn L-J, ter Schegget J, et al. Novel short-fragment PCR assay for highly sensitive  
417 broad-spectrum detection of anogenital human papillomaviruses. *The American Journal of*  
418 *Pathology* **1998**; 153(6): 1731-9.
- 419 29. van Alewijk D, Kleter B, Vent M, et al. A human papilloma virus testing algorithm comprising a  
420 combination of the L1 broad-spectrum SPF10 PCR assay and a novel E6 high-risk multiplex type-  
421 specific genotyping PCR assay. *Journal of clinical microbiology* **2013**; 51(4): 1171-8.
- 422 30. Castle PE. The evolving definition of carcinogenic human papillomavirus. *Infectious Agents and*  
423 *Cancer* **2009**; 4(1): 7.
- 424 31. Lehtinen M, Eriksson T, Natunen K, Damaso S, Bi D, Struyf F. HN03-03 efficacy of AS04-  
425 adjuvanted HPV-16/18 vaccine in reducing oropharyngeal HPV infections in adolescent girls-  
426 results from a community-randomized trial. *EUROGIN*. Amsterdam, **2017**.
- 427 32. Sonawane K, Suk R, Chiao EY, et al. Oral human papillomavirus infection: differences in  
428 prevalence between sexes and concordance with genital human papillomavirus infection,  
429 NHANES 2011 to 2014. *Ann Intern Med* **2017**; 167(10): 714-24.

- 430 33. Cameron RL, Kavanagh K, Pan J, et al. Human papillomavirus prevalence and herd immunity after  
431 introduction of vaccination program, Scotland, 2009-2013. *Emerging infectious diseases* **2016**;  
432 22(1): 56-64.
- 433 34. England PH. Sexually transmitted infections and chlamydia screening in England, 2016: Public  
434 Health England, **2017** 9 June 2017.
- 435 35. Ernster JA, Sciotto CG, O'Brien MM, Robinson LJ, Willson T. Prevalence of oncogenic human  
436 papillomavirus 16 and 18 in the palatine tonsils of the general adult population. *Arch Otolaryngol*  
437 **2009**; 135(6): 554-7.
- 438 36. Klingenberg B, Hafkamp HC, Haesevoets A, et al. p16INK4A overexpression is frequently detected  
439 in tumour - free tonsil tissue without association with HPV. *Histopathology* **2010**; 56(7): 957-67.
- 440 37. Bekker JB, Evans MF, Threlkeld KJ, Rajendran V, Adamson CS, Cooper K. Screening for HPV in  
441 clinically benign tonsillectomy specimens. *Modern Pathol* **2012**; 25: 305-.
- 442 38. Taberna M, Mena M, Pavon MA, Alemany L, Gillison ML, Mesia R. Human papillomavirus related  
443 oropharyngeal cancer. *Annals of oncology : official journal of the European Society for Medical*  
444 *Oncology* **2017**; 28(10): 2386-98.
- 445
- 446

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
<b>Age, in years</b>			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%)
<b>Ethnicity</b>			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%)
<b>Centre Enrolled</b>			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%)



<b>Year enrolled</b>			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%)	
<b>SURVEY AMONG THOSE ≥16 YEARS ONLY</b>					
<b>Age at First Sex, in years mean (SD)</b>	16.2 (1.7)	15.9 (1.5)	0.24	16.2 (1.3)	0.12
<b>Ever had Sex</b>			0.31		0.57
No	1 (2.9%)	14 (10.3%)		3 (6.5%)	
Yes	34 (97.1%)	122 (89.7%)		43 (93.5%)	
<b>Ever had Oral Sex</b>			0.08		0.09
No	2 (6.5%)	25 (19.7%)		3 (7.1%)	
Yes	29 (93.5%)	102 (80.3%)		39 (92.9%)	
<b>Number of oral sex partners in lifetime</b>			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%)	

450

451

452

453

454

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 <sup>a</sup> (N = 189 <sup>b</sup> )	P-value	(N=69)	P-value	P-value
<b>HPV 16</b>						
<b>Oropharyngeal (overall)</b>	<b>3 (5.6%)</b>	<b>1 (0.5%)</b>	<b>0.04</b>	0 (0%)	<b>&gt;0.99</b>	<b>0.08</b>
<b>Oral Rinse</b>	1 (1.9%)	0 (0.0%)	0.22	0 (0%)	--	0.44
<b>Oral Brush (either sample)</b>	0 (0.0%)	0 (0.0%)	--	0 (0%)	--	--
<b>Tonsil</b>	<b>3 (5.6%)</b>	<b>1 (0.5%)</b>	<b>0.04</b>	0 (0%)	<b>&gt;0.99</b>	<b>0.08</b>
<b>HPV 16 or 18</b>						
<b>Oropharyngeal (overall)</b>	3 (5.6%)	2 (1.1%)	0.07	0 (0%)	>0.99	0.08
<b>Oral Rinse</b>	1 (1.9%)	0 (0.0%)	0.22	0 (0%)	--	0.44
<b>Oral Brush (either sample)</b>	0 (0.0%)	1 (0.5%)	>0.99	0 (0%)	>0.99	--
<b>Tonsil</b>	<b>3 (5.6%)</b>	<b>1 (0.5%)</b>	<b>0.04</b>	0 (0%)	>0.99	<b>0.08</b>
<b>HPV 31 or 33 or 45</b>						
<b>Oropharyngeal (overall)</b>	1 (1.9%)	0 (0.0%)	0.22	1 (1.5%)	0.27	>0.99
<b>Oral Rinse</b>	1 (1.9%)	0 (0.0%)	0.22	0 (0%)	--	0.44
<b>Oral Brush (either sample)</b>	0 (0.0%)	0 (0.0%)	--	1 (1.5%)	0.27	>0.99
<b>Tonsil</b>	0 (0.0%)	0 (0.0%)	--	0 (0%)	--	--
<b>Any Oncogenic Type</b>						

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

458 <sup>a</sup>HPV16 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 <sup>b</sup>Two 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).

468

469

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.