

Short communication

Prevalence of oral human papillomavirus infection among university students in Valencia, Spain

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ARTICLE INFO

Article history:

Received 19 May 2019

Received in revised form 19 August 2019

Accepted 23 August 2019

Available online 9 September 2019

Keywords:

Oral human papillomavirus

Prevalence

Persistence

Clearance

Young adult

Oropharynx

ABSTRACT

Human papillomavirus (HPV) infection contributes to the pathogenesis of oropharyngeal squamous cell carcinomas. We estimated prevalence and six-month persistence of oral HPV infections among university students ages 18–25 years living in Valencia, Spain, during the 2012–2013 academic year. Participants provided oral rinse samples; HPV-positive subjects provided a follow-up sample. The study included 543 students; 70 (12.9%) women had received HPV vaccination. Prevalence among vaccinees and non-vaccinees were 10.0% (95% CI: 4.1–19.5%) and 6.8% (95% CI: 4.7–9.4%), respectively. All HPV infections among vaccinees were non-typeable genotypes; 59.4% of non-vaccinees had high-risk genotype infections. Follow-up samples were obtained from 36 participants; one vaccinee (whose specimen was non-typeable) and seven non-vaccinees were found to be HPV positive. Among non-vaccinees, six-month persistence was 10.3% (95% CI: 2.2–27.4%); all persistent infections were with high-risk genotypes. Our results, although subject to study limitations, may support the need to implement new public health strategies.

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1. Introduction

Human papillomavirus (HPV) infection has been identified as the underlying cause of a subset of head and neck cancers. More specifically, 90% of head and neck cancers are squamous cell carcinomas, and, although 75% of oropharyngeal squamous cell carcinomas (OPSCCs) have been attributed to alcohol and tobacco consumption, HPV positivity, particularly with the high-risk HPV-16 type, has been shown to confer a 50-fold increase of the risk of OPSCCs [1,2]. Most HPV infections are cleared in six to 30 months [3–7], but increasing trends in HPV-positive OPSCCs incidence have been reported worldwide, particularly among younger cohorts, coinciding with sexual behavioral changes [3,8]. The incidence of HPV-related OPSCCs has increased among men (2.9% per year) and has generally plateaued among women (0.6% per year) [9].

Estimates of oral HPV prevalence present great variability across nations and study populations (0.6–81%) [3,4,6,7]. Little is known about prevalence differences between genders and sexual

behaviors, and about oral concurrent HPV infection with multiple genotypes, particularly among healthy individuals [3,4,8,10]. While HPV vaccination is expected to reduce oral HPV-related cancers, it is unclear whether currently available HPV vaccines will provide primary protection [11]. Thus, it is important to assess the proportion and genotype distribution of oral HPV infection, particularly among young adults, who usually present the highest prevalence of sexually transmitted infections [8]. Our aim was to estimate prevalence, genotype distribution, and six-month persistence of oral HPV infection among university students in the Valencia Region, Spain, by sex and HPV vaccination status.

2. Materials and methods

2.1. Participants and study design

A cross-sectional study, using a convenience sample, was performed among university students (18–25 years old) living in the Valencia Region, Spain, during the 2012–2013 academic year. Subjects were recruited during November 2012 in eight campuses of the University of Valencia, with 63,940 students registered. Students who agreed to participate were requested to sign an informed consent, provide an oral rinse sample, and complete a questionnaire collecting information on tobacco and alcohol

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consumption, sexual behavior, and HPV vaccination. Participants were informed about data anonymity and were requested to be followed up with a second sample collection six months later in case of oral HPV positivity in the first one, to determine persistence, clearance, and presence of new oral HPV infection.

According to our sample size calculations, 500 healthy volunteers would be needed to detect a prevalence of 10%, with a precision of $\pm 2.6\%$.

The University of Valencia collaborated in facilitating awareness of the study and provided spaces for sample and data collection. The study was approved by the Ethics Committee for Clinical Research of the Directorate General of Public Health and Center for Advanced Research (CEIC DGSP/CSISP).

2.2. Biological samples – collection and preparation

Biological samples were obtained by a 30-second gargle and rinse in 15 mL of saline serum. Fresh specimens were kept refrigerated (2–8 °C) during transportation. Samples were maintained at –20 °C until processing. Specimens were centrifuged, and the cellular pellet underwent DNA purification. DNA was isolated using the NucliSENS® EasyMAG® nucleic acid extractor (BioMérieux Clinical Diagnostics, UK) following the manufacturer's instructions. HPV detection and genotyping was carried out using the HPV SPF10 PCR-DEIA-LiPA₂₅ assay (LaboBiomedial Product, Rijswijk, The Netherlands) that detects simultaneously 25 genotypes. Samples HPV-DNA positive and LiPA₂₅ negative were denoted as non-typeable. Presence of HPV DNA was considered as oral infection and, for the sample obtained after six months, positive results were considered as persistent infection if the genotype remained the same as the first sample, or reinfection if a new genotype was found.

2.3. Statistical analysis

Participant characteristics including data on demographics, sexual behavior, and previous sexually transmitted diseases of interviewees, HPV and HPV-type specific prevalence, persistence, and re-infection rates were estimated overall, by sex, and vaccination status. A chi-square test was used for comparisons of categorical variables and student's T test for continuous variables. A *p* value ≤ 0.05 was considered significant. Stata/IC 12.1 (StataCorp LP Texas, USA) was used for all analyses.

3. Results

3.1. Study population characteristics

Of 548 students recruited, five were excluded, as their laboratory results were not interpretable. Thus, the final study population included 543 participants, 288 (53%) were female and 70 (13%) received at least one dose of HPV vaccine. The mean age was 21.1 ± 2.0 years.

According to the responses, 354 (65%) never smoked and 82 (15%) reported no weekly alcohol consumption. Most, 532 (98%) had sexual contact (intimate kissing, oral sex, and/or sexual intercourse) in their lifetime; 41 (8%) reported no sexual intercourse, and 104 (20%) had never performed oral sex. The mean age of the first sexual intercourse was 16.7 ± 1.5 and 16.6 ± 1.5 years for males and females, respectively. A total of 320 (60%) students had one sexual partner during the last year and 151 (28%) had two or more. A more detailed description of risk behavior, overall and by sex and vaccination status, is shown in [Table 1](#).

3.2. HPV prevalence and genotype distribution

Among 543 participants, 39 (7.2%; 95% CI: 5.2–9.7%) were positive for detectable oral HPV-DNA, including both typeable (20; 51.3%) and non-typeable genotypes (not included in the LiPA₂₅). A total of 13 different genotypes were identified ([Fig. 1](#)). Among the 20 individuals with typeable HPV, 14 (70%) had a single HPV-type and six (30%) had two or more. Prevalence of high-risk HPV genotypes was 3.5% (95% CI: 2.1–5.4%). HPV-18 and HPV-51 were the most common types, with a prevalence of 1.1% (95% CI, 0.4–2.4%) for each type. HPV-16 was found in two specimens (0.37%; 95% CI, 0.04–1.3%). All multiple infections included at least one high-risk type; four (67%) were in males.

Oral HPV-DNA was detected in 20/255 men (7.8%; 95% CI: 4.9–11.9%) and 19/288 females (6.6%; 95% CI: 4.0–10.1%). No statistically significant sex differences were found ($p > 0.05$). A total of ten and nine infections, respectively, included high-risk genotypes; prevalence was 3.9% (95% CI: 1.9–7.1) among males and 3.1% (95% CI: 1.4–5.8) among females.

Among 218 non-vaccinated females, 12 (5.5%; 95% CI: 2.9%–9.4%) were HPV positive; of them, nine (75%) had high-risk genotypes, prevalence was 4.1% (95% CI: 1.9–7.7). Oral HPV infections were identified in 7/70 (10%; 95% CI: 4.1–19.5%) vaccinated females; all isolated genotypes were non-typeable. No statistically significant differences in overall HPV prevalence were found by female vaccination ($p > 0.05$).

Overall prevalence and by genotype, sex and HPV vaccination status are shown in [Fig. 2](#).

3.3. Six-month persistence and new infections among HPV positive students

Six months after the first sample was taken, a second oral sample was obtained from 36/39 (92%) positive subjects (29 unvaccinated and seven vaccinated). Eight (20%) of these 36 subjects (one vaccinated female and seven unvaccinated [one male and six females]) were HPV-positive. The global clearance was 77.7% (95% CI: 60.8–89.9). The specimen from the vaccinated student was non-typeable both in the first and second sample. One unvaccinated female had both persistent and new HPV infections. Thus, there were three (8.3%; 95% CI: 1.5–22.5%) persistent infections, in one male and in two unvaccinated females, all including high-risk genotypes (HPV-18, –31, –66). Among unvaccinated females, six-month persistence of the same genotype was 10.3% (95% CI: 2.2–27.4%).

Among the five (13.9%; 95% CI: 4.7–29.5%) new oral HPV infections, two included high-risk genotypes (HPV-31, –39/68/73); six-month incidence of infection with a new genotype was 17.2% (95% CI: 5.8–35.8%).

4. Discussion

To our knowledge, this is the first study to provide estimates of oral HPV prevalence, persistence of infection and re-infection in a healthy university student population in Spain. Vaccine coverage was 71.2% in the Valencia Region and 70.5% in Spain.

Although great variability in oral HPV prevalence among healthy individuals has been observed worldwide (0.7–23%) [[6,7,11–13](#)], our results were similar to those from studies conducted in the United States and Brazil [[3,14](#)]. This variability may suggest geographical differences, but it could also be attributed to heterogeneity of the studies, with variances in sample sizes, study designs or populations observed, along with methods of specimen collection, processing and genotyping. In Spain, published studies showed oral HPV prevalence ranging from 8% to

Table 1
 Characteristics of study participants (n = 543) overall, by sex and by HPV vaccination status.

Characteristics	All n = 543	Men ¹ n = 255	Women n = 288	
			Unvaccinated n = 218	Vaccinated n = 70
Age				
Mean (years)	21.1 ± 2.0	21.1 ± 2.0	21.5 ± 1.6	20.1 ± 1.8
Smoking classification²				
Never-smoker	354 (65.4)	172 (67.7)	133 (61.3)	49 (70.0)
Former smoker	33 (6.1)	13 (5.1)	18 (8.3)	2 (2.9)
Occasional	62 (11.5)	24 (9.5)	25 (11.5)	13 (18.6)
Current	92 (17.0)	45 (17.7)	41 (18.9)	6 (8.6)
Total answer	541 (99.6)	254 (99.6)	217 (99.5)	70 (100)
Weekly alcohol consumption				
No alcohol	82 (15.2)	31 (12.2)	38 (17.6)	13 (18.6)
≤2 days/week	392 (72.6)	174 (68.5)	164 (75.9)	54 (77.1)
>2 days/week	66 (12.2)	49 (19.3)	14 (6.5)	3 (4.3)
Total answer	540 (99.4)	254 (99.6)	216 (99.1)	70 (100)
Mouthwash use				
No	201 (37.2)	102 (40.5)	72 (33.0)	27 (38.6)
Occasionally	209 (38.7)	90 (35.7)	94 (43.1)	25 (35.7)
Yes	130 (24.1)	60 (23.8)	52 (23.9)	18 (25.7)
Total answer	540 (99.4)	252 (98.8)	218 (100)	70 (100)
Marital status				
Single	189 (36)	98 (40.4)	67 (31.5)	24 (34.8)
Sporadic	56 (10.7)	38 (15.6)	12 (5.6)	6 (8.7)
Stable couple	280 (53.3)	107 (44.0)	134 (62.9)	39 (56.5)
Total answer	525 (96.7)	243 (95.3)	213 (97.7)	69 (98.6)
Age 1st sexual relationship				
No sex	41 (7.7)	17 (6.8)	18 (8.4)	6 (9.0)
<16 years	100 (18.8)	44 (17.7)	46 (21.4)	10 (14.9)
≥16 years	390 (73.5)	188 (75.5)	151 (70.2)	51 (76.1)
Total answer	531 (97.8)	249 (97.6)	215 (98.6)	67 (95.7)
Sexual orientation				
Heterosexual	503 (93.3)	235 (92.5)	199 (92.2)	69 (100)
Homosexual	20 (3.7)	13 (5.1)	7 (3.2)	0
Bisexual	16 (3.0)	6 (2.4)	10 (4.6)	0
Total answer	539 (99.3)	254 (99.6)	216 (99.1)	69 (98.6)
Circumcision				
No	–	222 (88.5)	–	–
Yes	–	29 (11.5)	–	–
Total answer	–	251 (98.4)	–	–
Previous sexual transmitted disease				
No	522 (97.2)	244 (97.6)	208 (95.9)	70 (100)
Yes	15 (2.8)	6 (2.4)	9 (4.1)	0
Total answer	537 (98.9)	250 (98.0)	217 (99.5)	70 (100)
Condom				
No sex	41 (7.6)	17 (6.7)	18 (8.3)	6 (8.6)
Yes	312 (57.9)	168 (66.4)	96 (44.4)	48 (68.6)
Occasionally	112 (20.8)	51 (20.2)	52 (24.1)	9 (12.9)
No	74 (13.7)	17 (6.7)	50 (23.2)	7 (10.0)
Total answer	539 (99.3)	253 (99.2)	216 (99.1)	70 (100)
Oral sex condom				
No oral sex	104 (19.5)	50 (20.1)	38 (17.5)	16 (23.5)
Yes	20 (3.7)	10 (4.0)	5 (2.3)	5 (7.3)
Occasionally	53 (9.9)	16 (6.4)	30 (13.8)	7 (10.3)
No	357 (66.9)	173 (69.5)	144 (66.4)	40 (58.8)
Total answer	534 (98.3)	249 (97.6)	217 (99.5)	68 (97.1)
Intimate kissing last 12 months³				
No	20 (3.8)	11 (4.4)	5 (2.3)	4 (6.0)
With one couple	246 (46.2)	107 (42.6)	106 (49.5)	33 (49.2)
With 2–10 couples	203 (38.2)	99 (39.4)	78 (36.5)	26 (38.8)
≥10 couples	63 (11.8)	34 (13.6)	25 (11.7)	4 (6.0)
Total answer	532 (97.9)	251 (98.4)	214 (98.2)	67 (95.7)
Sexual couples last 12 months				
No	64 (12)	33 (13)	23 (10.5)	8 (12.1)
1 couple	320 (59.8)	137 (54.4)	140 (64.5)	43 (65.2)
2–10 couples	145 (27.1)	79 (31.4)	52 (24.0)	14 (21.2)
≥10 couples	6 (1.1)	3 (1.2)	2 (1.0)	1 (1.5)
Total answer	535 (98.2)	252 (98.8)	217 (99.5)	66 (94.3)
Oral sex last 12 months⁴				
No	115 (21.5)	55 (22)	43 (19.8)	17 (24.6)

Table 1 (continued)

Characteristics	All n = 543	Men ¹ n = 255	Women n = 288	
			Unvaccinated n = 218	Vaccinated n = 70
1 couple	315 (58.8)	136 (54.4)	137 (63.1)	42 (60.9)
2–10 couples	99 (18.5)	57 (22.8)	32 (14.8)	10 (14.5)
≥10 couples	7 (1.2)	2 (0.8)	5 (2.3)	0
Total answer	536 (98.7)	250 (98.0)	217 (99.5)	69 (98.6)

¹ All men unvaccinated.

² Cigarette smoking status evaluated following the WHO tobacco classification.

³ Intimate kissing defined in self-questionnaire as “kissing with tongue.”

⁴ Oral sex defined in self-questionnaire as “oral-genital contact with sexual partner”.

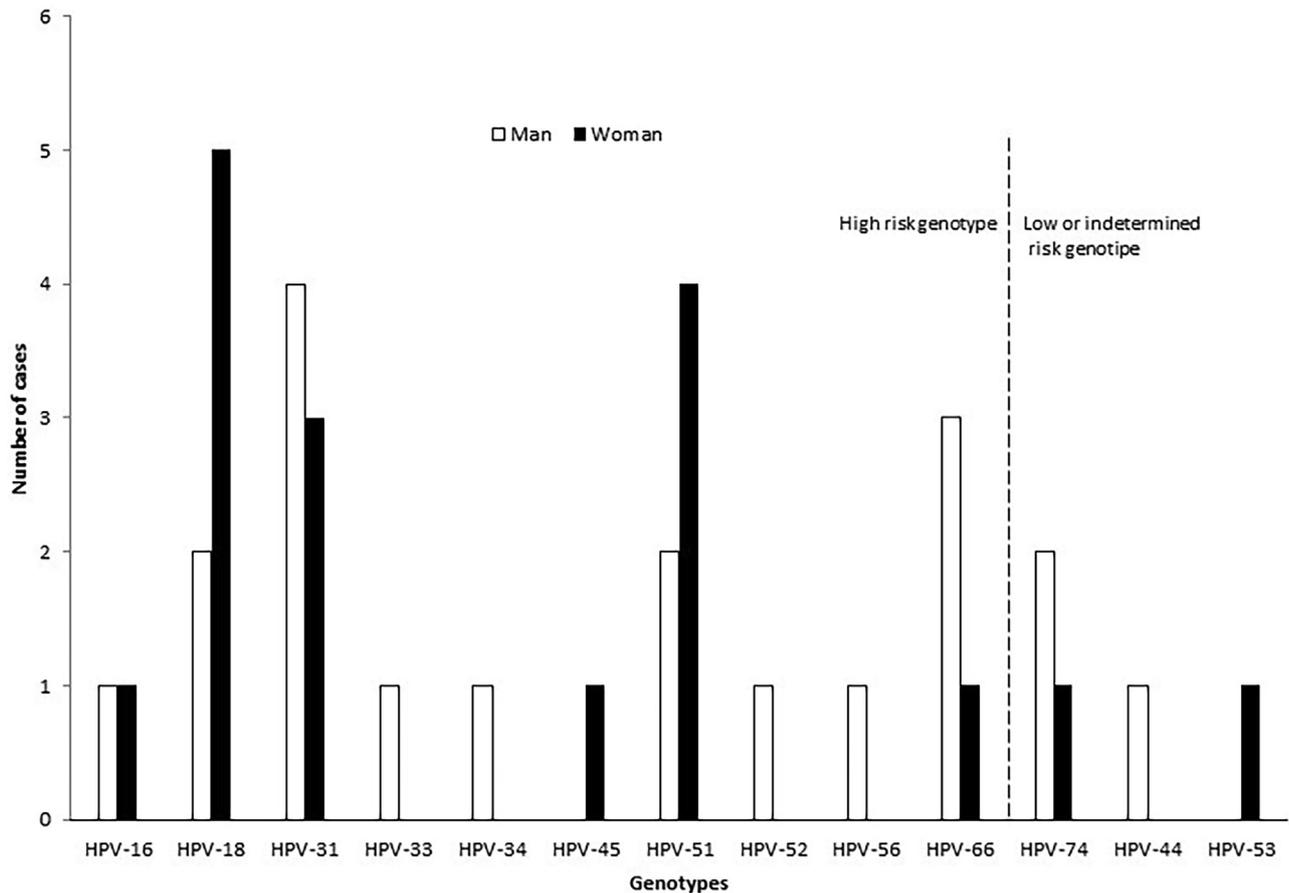


Fig. 1. HPV-genotypes identified in first sample.

23%, but these studies were performed in very different populations, including sexual workers, HIV-infected men and infants [13,15,16]. Our results showed similar prevalence for both male and female students. The most prevalent genotypes were HPV-18 and HPV-51 (1.1% [95% CI; 0.4–2.4%]) each, while prevalence of HPV-16 was 0.37% (95% CI; 0.04–1.3%). Although HPV-16 is the most common high-risk genotype in the majority of studies [3,6], our estimates are similar to those found in a recently published U.S. study [11]. Although prevalence of detectable oral HPV in students who reported having received HPV vaccination was similar to that found among those who reported not having been vaccinated; none of them had typeable genotypes. In the HPV-positive vaccinated student, HPV infection was present six months after the first sample; however, it is unknown if the infection was persistent or new, as the genotype remained non-typeable. Although our results have to be considered with caution given study limitations, including limited sample size, our findings are consistent

with results from recent studies in the United States suggesting protection of HPV vaccination against vaccine-type oral HPV infection [11].

Persistent infections have been recognized as a risk factor in oral, oropharyngeal and cervical carcinogenesis [7]. Data about genotype specific persistence in cancer-free populations are scant, and only few studies reported results of oral HPV infection persistence, incidence and/or clearance [7,17]. Low-risk HPV types seem to have a shorter clearance than high-risk ones [17]. After six months, our study found a 8.3% (95% CI, 1.5–22.5%) persistence of oral HPV infections. As found in others studies [7,17], all known persisting genotypes in our sample were high-risk. Data about oral HPV incidence in healthy populations are still sparse [7,17]. In our study, among the 36 individuals with oral HPV infections who were re-tested six months later, the re-infection rate with a new genotype was 13.9%. However, our study was not properly powered to investigate this endpoint.

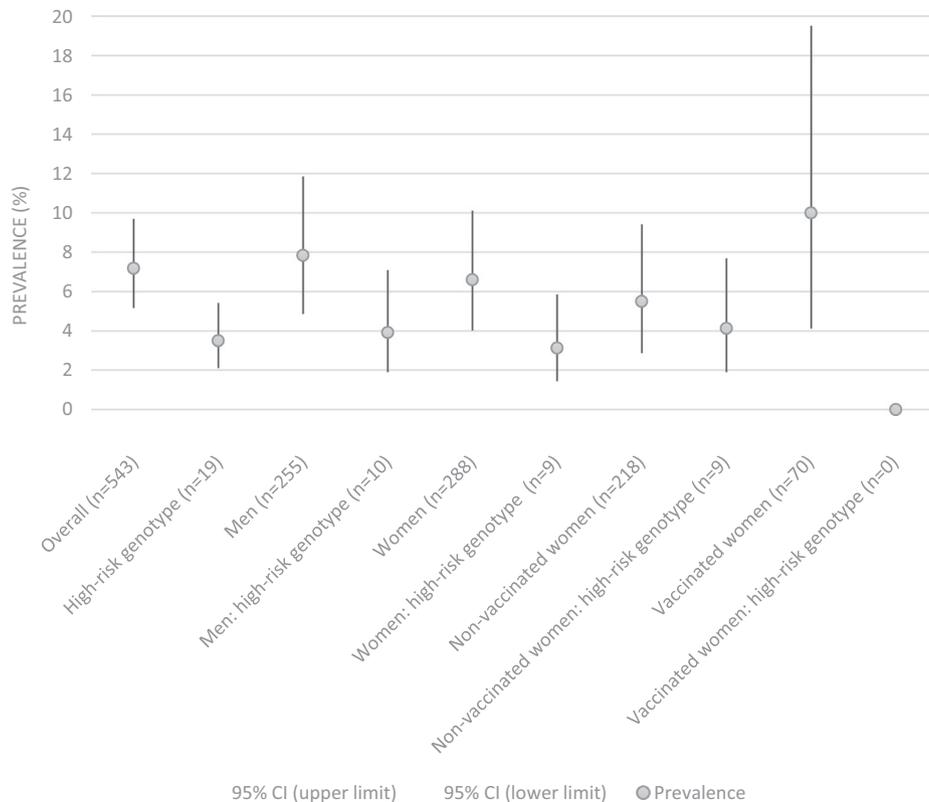


Fig. 2. HPV prevalence overall and by sex and vaccination status.

Detection rate of asymptomatic oral HPV infection is critically dependent on the use of different sampling methods, DNA extraction or HPV detection, and genotyping tests [13]. In addition, study populations may be exposed to different levels of risk according to their genetics, location or social habits, and even the level of keratinization of oral mucosa (more permeable and sensitive to oral penetration when less keratinized) might be a factor for oral HPV infection [13]. In a study conducted in men, ages 18–24 years, the overall oral HPV infection, using both gargle/rinsing and swab/cytobrush positives, was 7.5%, although the estimation was reduced to 5% when the sample technique used was only oral rinses [18]. As there is no standardized detection technique, our study was carried out using SPF10 PCR-DEIA-LipA₂₅ assay with proven high performance compared to other tests in terms of sensitivity, reproducibility and coverage of HPV types [19].

The use of a convenience sample would limit representativeness and generalizability of our results, associated with individual's decision to self-select for research participation. Nonetheless, recruitment was conducted in eight of the 18 campuses of the University during the same month, which would suggest that students who attended classes during that period had a reasonably similar probability of being contacted by interviewers. Also, vaccination status was self-reported. Previous work validated the accuracy of self-reported HPV vaccination status, with a positive predictive value of 96% [20]. Although the response rate was not formally evaluated, interviewers indicated that “most students who were approached agreed to participate.”

5. Conclusions

Our study provides, for the first time, data of oral HPV prevalence, genotype distribution, persistence of infection and six-month incidence, overall and by sex, and HPV vaccination status

among university students in Valencia, Spain. Our results, although subject to limitations, suggest that HPV vaccination might protect against vaccine-type oral HPV infection. These results may help inform public health decision-making regarding HPV vaccination strategies for healthy young individuals of both sexes. Further studies are needed to validate these findings.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: *Javier Díez-Domingo has been principal investigator in clinical trials with 4v and 9v HPV vaccine. FISABIO received research grants from MSD and GSK. All other authors have no conflicts of interest to disclose. At the time of this research, M. Sastre-Cantón and S. Perez-Vilar were employed by FISABIO-Public Health. This work was entirely done while working at FISABIO Public Health.*

Acknowledgements

We thank the University of Valencia, Alma Bracho, Lúcia Martínez-Priego and all the members of the Fisabio Vaccine Research Department who helped in this project.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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