MAJOR ARTICLE

Prevalence of and Risk Factors for Oral Human Papillomavirus Among Young Women in Costa Rica

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Background. Little is known about the epidemiology of oral human papillomavirus (HPV) in Latin America.

Methods. Women (N = 5838) aged 22–29 in the control and vaccine arms of an HPV-16/18 vaccine trial in Costa Rica had oral, cervical, and anal specimens collected. Samples were tested for alpha mucosal HPV types (SPF_{10}/$LiPA_{25}$ version 1); a subset of oral samples (n = 500) was tested for cutaneous HPV types in the genera alpha, beta, gamma, mu, and nu.

Results. In the control arm (n = 2926), 1.9% of women had an oral alpha mucosal HPV detected, 1.3% had carcinogenic HPV, and 0.4% had HPV-16; similar patterns for non-16/18 HPV types were observed in the vaccine arm. Independent risk factors for any oral alpha mucosal HPV among women in the control arm included marital status (adjusted odds ratio [AOR], 3.2; 95% confidence interval [CI], 1.8–5.7 for single compared to married/living as married), number of sexual partners (AOR, 2.4; 95% CI, 1.0–6.1 for ≥4 partners compared to 0–1 partners), chronic sinusitis (AOR, 3.1; 95% CI, 1.5–6.7), and cervical HPV infection (AOR, 2.6; 95% CI, 1.4–4.6). Detection of beta HPV was common (18.6%) and not associated with sexual activity.

Conclusions. Unlike cutaneous HPV types, alpha mucosal HPV types were uncommon in the oral region and were predominately associated with sexual behavior.

Clinical Trials Registration. NCT00128661.

Keywords. human papillomavirus vaccine; HPV; oropharynx cancer; Costa Rica; Guanacaste; oral HPV DNA.

Studies have reported an increased incidence of oropharyngeal cancer predominantly among younger birth cohorts in developed countries [1–8]. This upsurge has been attributed to an increase in human papillomavirus (HPV)–driven oropharyngeal cancers [7], primarily due to HPV-16 infection, which accounts for approximately 90% of HPV-positive oropharyngeal cancers [9, 10]. Detection of oral HPV-16 DNA in oral rinse/gargle samples was associated with a substantial increase in the odds of prevalent oropharyngeal cancer [10]. However, little is known about the epidemiology of oral HPV infection.

To our knowledge, only 3 large studies (≥1000 participants), based predominantly in the United States, have examined the epidemiology of oral HPV in healthy individuals [11–13]. Prevalence of any type of oral HPV ranged from approximately 2% to 7%; reported risk factors included increasing age, male sex, higher number of sexual partners, and tobacco use [11–13]. Additionally, these studies focused on detection of oral mucosal HPV

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types from the genus *Alphapapillomavirus*. Yet, recent evidence suggests that the oral region of healthy individuals also contains a high prevalence of cutaneous HPV types (predominantly *Betapapillomavirus* and *Gammapapillomavirus*), which have previously been thought to uniquely infect keratinized epithelium and are associated with skin lesions [14–18].

Few studies have reported the prevalence of oral HPV in resource-poor regions. In Latin America, only 5 small studies (<250 participants) [19–23] and 1 large study (males only, approximately 1000 participants) [13] examined oral HPV epidemiology: the reported prevalence estimates varied greatly between studies and few specifically reported the prevalence of oral HPV-16 or associated risk factors. As such, the aim of the present study was to estimate the prevalence of oral HPV for both mucosal and cutaneous types in a young healthy population of women in Costa Rica, a Latin American country with no reported data on oral HPV prevalence, and to characterize the risk factors for oral HPV in this population.

METHODS

Subject Participants and Study Design

Women included in the present evaluation are participants in both arms of a double-blind, controlled, randomized, phase 3 study of the efficacy of an HPV-16/18 virus-like particle vaccine conducted in Costa Rica (Costa Rica Vaccine Trial). This trial was initially designed to evaluate vaccine efficacy against persistent cervical HPV-16/18 infection and precancerous lesions, as previously described [24]. Inclusion criteria for the trial were age 18–25 years (inclusive), generally healthy as determined by medical history and a physical examination, not pregnant/breastfeeding, using contraception during the vaccination period, and willing to provide informed consent. The trial was reviewed and approved by human subjects review committees of INCIENSA (Instituto Costarricense de Investigacion y Enseñanza en Nutrición y Salud) in Costa Rica and the National Cancer Institute in the United States. All subjects provided written informed consent. The study protocol is available at http://proyectoguanacaste.org.

At enrollment, a pelvic examination was performed on sexually experienced women, with collection of exfoliated cervical cells for liquid-based cytology and HPV DNA testing, and blood for HPV-16/18 serology. Women were blindly randomized to receive the HPV bivalent vaccine (Cervarix, GSK Biologicals) or a control vaccine (modified Havrix, GSK Biologicals).

At annual follow-up visits, clinicians collected cervical cells for cytology and HPV testing from sexually experienced women. Women with low-grade cytologic abnormalities were evaluated semiannually and those with high-grade or persistent low-grade abnormalities were referred for colposcopy and treatment as needed [24].

At the 4-year annual study visit, a questionnaire regarding the woman's education, marital status, sexual (including oral and anal sex) and reproductive history, use of contraceptives, and smoking habits was administered. All women were asked to provide an oral sample, and all sexually experienced women were asked to provide cervical and anal specimens. A complete medical history and physical exam were conducted as part of the process of assessment of adverse events. Information regarding medical conditions, including oral and respiratory conditions, was collected by study clinicians at each follow-up visit. If a condition was reported as present at the time of the study visit, it was confirmed by physical exam. If hospitalization occurred, a doctor reviewed the hospital chart. All medical conditions that affect the oral cavity and upper respiratory tract identified or reported at any point were included in this analysis. Examples of oral/respiratory medical conditions that were reported included hemorrhage of the respiratory passages, cough, nausea and vomiting, acute tonsillitis, chronic sinusitis, and chronic diseases of the tonsils, adenoids and peritonsillar abscess.

Specimen Collection

Oral specimens were collected using a 30-second rinse and gargle with Scope (Procter & Gamble); this method of specimen collection [25] was chosen based on previous reports that (1) a single mouthwash sample provides large amounts of high molecular weight DNA [26] and (2) optimal specimen collection time is around 30 seconds [27]. Specimens were kept between 2°C and 8°C until same-day processing at the local laboratory. The samples were concentrated by centrifugation (3000*g* for 10 minutes) to obtain a pellet that was washed with 10 mL saline solution to remove residual mouthwash, recentrifuged, and then resuspended in 1 mL of saline solution and frozen in liquid nitrogen tanks until testing. Anal specimens were collected by inserting a dry swab 3–4 cm into the anal canal, rotating once, and then removing the swab while rotation continued using gentle pressure against the wall of the anal canal. The swab was placed in 1 mL of PreservCyt and frozen immediately in liquid nitrogen. Cervical specimens were collected during the pelvic exam (see above).

HPV DNA Testing

DNA was extracted via the MagNAPure LC DNA isolation procedure (Roche Diagnostics); 10 μL of extracted DNA was used for each polymerase chain reaction (PCR) reaction; optimization of the oral HPV extractions and testing methods as well as data on quality control have been previously described [28]. All extracted DNA samples were tested for HPV DNA by PCR amplification using the HPV SPF_{10} PCR DNA enzyme immunoassay (DEIA) line probe assay ($LiPA_{25}$) version 1 system (Labo Biomedical Products). This broad-spectrum PCR-based HPV DNA testing system uses SPF_{10} primers to amplify and a DEIA to detect at least 57 HPV genotypes and the $LiPA_{25}$ to genotype HPVs. To increase the sensitivity of type-specific detection of

HPV-16 and 18 using the SPF_{10} DEIA system, all oral specimens that were SPF_{10} PCR/DEIA-positive, as well as a convenience sample of one-third of all samples (as part of quality control), were tested for the presence of HPV-16 or 18 using type-specific primers detected by the TS16 and TS18 DEIA system; for cervix and anal samples, all specimens positive for HPV DNA using SPF_{10} DEIA but negative for HPV-16 or HPV-18 by $LiPA_{25}$ were additionally tested with type-specific primers/probes [29].

A convenience sample of 500 women across both arms was tested for cutaneous HPV. Cutaneous HPV types were detected with the skin (beta) papillomavirus prototype research assay (Diassay BV) via PCR detection and a reverse-hybridization assay for typing [30]. Cutaneous HPV types in the alpha, gamma, mu, and nu genera were detected by a broad-spectrum PCR multiplex genotyping assay [31].

Statistical Analysis

A sample was considered positive for *any alpha mucosal HPV* if any of the 25 alpha mucosal types were detected (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74); was considered positive for a *carcinogenic alpha mucosal HPV* if any of the 12 carcinogenic types were detected (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59); and was considered positive for a *noncarcinogenic alpha mucosal HPV* if any of the noncarcinogenic HPV types were detected (6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 68/73, 70, and 74). A sample was considered positive for a *beta HPV* if any of the 25 beta cutaneous HPV types were detected (5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93, and 96) and was considered positive for *other cutaneous HPV* if any of the 23 nonmucosal alpha/gamma/mu/nu cutaneous HPV types were detected (1, 2, 3, 4, 7, 10, 27, 28, 29, 40, 41, 43, 48, 50, 57, 60, 63, 65, 77, 88, 91, 94, and 95). A sample that tested positive by DEIA PCR, but without positive genotyping for any of the above-mentioned types, was labeled as *uncharacterized HPV*. The prevalence of oral HPV was described by the above HPV categorizations among all women and then by arm (note that stratification by vaccine arm was only necessary in the evaluation of alpha mucosal HPV types due to vaccine protection [28]; for other genera, HPV was evaluated across both arms). Analyses for grouped HPV infections (any HPV, carcinogenic, beta HPV, etc) were conducted at the woman level; for analyses by individual HPV type, a woman could contribute data to each HPV type.

Determinants for oral HPV were evaluated by logistic regression analysis. Variables evaluated in univariate modeling included age, education, marital status, smoking status, age at first vaginal intercourse, lifetime number of sexual partners, total number of years sexually active, lifetime number of oral sex partners, age at first oral sex, lifetime number of anal sex partners, and any anal HPV DNA positivity and any cervical HPV DNA positivity (each at the contemporaneous study visit as oral specimen collection). For the oral sex variables, women were asked specifically if they had given oral sex (defined as a woman's mouth on her partner's genitals). The potential associations between oral HPV and oral/respiratory medical conditions (detailed above) were also evaluated.

To evaluate the independent determinants of oral HPV, risk factors that were significantly associated with oral HPV detection in the univariate analysis or considered important based on the literature (ie, smoking, oral sexual behavior), were included in multivariate models as adjusted odds ratios (AORs). For all analyses, $P < .05$ (2-tailed) was considered statistically significant.

RESULTS

Participant Characteristics

Of the 7466 women who were randomized, 6351 attended the 4-year study visit (3180 vaccine and 3171 control arm) and were invited to provide an oral specimen. A total of 5838 (91.9%) provided an oral specimen and 511 (268 vaccine and 243 control arm) refused. Women who provided an oral specimen were significantly more likely to report a personal history of vaginal, oral, and anal sex and to be positive for cervical HPV-16/18 DNA at enrollment than those who refused [28]. Among the women who provided an oral sample, median age was 26 years, 62.1% were married, 80.7% were never smokers, and the median number of lifetime sexual partners was 2. Oral sex was common (62.2% reported ever having given oral sex); the median age at first oral sex was 20 and the median number of lifetime oral sex partners was 1. The most commonly reported oral/respiratory medical condition was chronic sinusitis (5.6%); 3.7% of women had had acute tonsillitis. The subset whose oral sample was additionally tested for cutaneous HPV types appeared representative of the full cohort (Table 1).

Oral HPV Prevalence and Type Distribution

In the control arm, overall prevalence of alpha mucosal HPV types in the oral region was 1.9% (95% confidence interval [CI], 1.4–2.4; 55 HPV infections detected; Table 2). The prevalence of carcinogenic and noncarcinogenic HPV types was 1.3% (95% CI, .9–1.7) and 0.8% (95% CI, .5–1.1), respectively, the total of which exceeds the overall prevalence (1.9%) due to detection of multiple infections. The most common carcinogenic HPV types in the control arm were HPV-16 (0.4% [95% CI, .2–.7]), HPV-51 (0.3% [95% CI, .2–.6]), and HPV-52 (0.2% [95% CI, .1–.5]). The most common noncarcinogenic types were HPV-66 (0.2% [95% CI, .1–.4]) and HPV-44 (0.2% [95% CI, .1–.4]). Single oral HPVs were detected in 1.5% (95% CI, 1.1–2.1) of participants, and 0.4% (95% CI, .2–.6) were infected with ≥2 HPV types; HPV-51 was the most common coinfection, followed by HPV-16. In the

Table 1 Participant characteristics of the overall cohort of 5,838 young adult women from Costa Rica who provided an oral specimen for HPV DNA testing and the subset of 500 women who were additionally tested for the presence of cutaneous HPV types

Characteristics	Full Oral Cohort n = 5838 n (%)	Subset n = 500 n (%)
Age (years)		
Median (IQR)	26 (24–28)	26 (24–28)
22–23	1325 (22.7)	94 (18.8)
24–25	1557 (26.7)	136 (27.2)
26–27	1377 (23.6)	135 (27.0)
≥28	1579 (27.0)	135 (27.0)
Education level		
≤6 y	1563 (26.8)	136 (27.2)
7–9 y	1089 (18.7)	98 (19.6)
≥10 y*	1611 (27.5)	139 (27.8)
University	1575 (27.0)	127 (25.4)
Marital status		
Married or living as married*	3623 (62.1)	319 (63.8)
Single	1856 (31.8)	156 (31.2)
Divorced / Separated / Widowed	359 (6.1)	25 (5.0)
Smoking status		
Never*	4708 (80.7)	417 (83.4)
Former**	742 (12.7)	62 (12.4)
Current	388 (6.6)	21 (4.2)
Age at first vaginal intercourse (years)		
Median (IQR)	17 (15–19)	17 (15–19)
Virgin	324 (5.5)	25 (5.0)
≥19	1516 (26.0)	130 (26.0)
17–18	1678 (28.7)	138 (27.6)
≤16*	2320 (39.8)	207 (41.4)
Total number of years sexually active		
Median (IQR)	9 (6–11)	9 (6–11)
Virgin	324 (5.5)	25 (5.0)
0–7 y*	2143 (36.8)	169 (33.8)
8–10 y	1716 (29.4)	157 (31.4)
≥11 y	1655 (28.3)	149 (29.8)
Lifetime number of sexual partners		
Median (IQR)	2 (1–4)	3 (1–4)
0–1	1812 (31.0)	155 (31.0)
2–3	2006 (34.4)	159 (31.8)
≥4	2020 (34.6)	186 (37.2)
Number of male sexual partners since last visit		
Virgin	324 (5.5)	25 (5.0)
0–1*	4497 (77.0)	385 (77.0)
≥2	1017 (17.5)	90 (18.0)
Age at first oral sex (years)		
Median (IQR)	20 (18–23)	21 (19–24)
Never had oral sex*	2205 (37.8)	210 (42.0)
≥21	1579 (27.0)	151 (30.2)
≤20**	2054 (35.2)	139 (27.8)
Lifetime number of oral sex partners		
Median (IQR)	1 (0–2)	1 (0–1)
0–1*	4323 (74.0)	385 (77.0)
≥2	1515 (26.0)	115 (23.0)
Use of oral contraceptives		
Never	771 (13.2)	62 (12.4)
Virgin	324 (5.5)	25 (5.0)
In the past*	2549 (43.7)	203 (40.6)
Current (past month)	2194 (37.6)	210 (42.0)
Lifetime number of anal sex partners		
0*	4782 (81.9)	418 (83.6)
1**	879 (15.1)	69 (13.8)
≥2	177 (3.0)	13 (2.6)
Acute tonsillitis		
No	5624 (96.3)	476 (95.2)
Yes	214 (3.7)	24 (4.8)
Chronic sinusitis		
No	5511 (94.4)	478 (95.6)
Yes	327 (5.6)	22 (4.4)
Chronic diseases of tonsils and adenoids; peritonsillar abscess		
No	5794 (99.2)	498 (99.6)
Yes	44 (0.8)	2 (0.4)
Cervical HPV status at 4-yr study visit[a]		
Negative*,[b]	3885 (66.5)	321 (64.2)
Positive	1953 (33.5)	179 (35.8)
Anal HPV status at 4-yr study visit[a]		
Refused specimen collection	1328 (22.7)	113 (22.6)
Negative[b]	3225 (55.3)	276 (55.2)
Positive	1285 (22.0)	1174 (22.2)

Missing values accounted for less than 1 percent of the full cohort; yet when a value was missing: * includes women with a missing value, those who refused to respond, and those who responded "don't know" for this variable; ** includes women who did not have missing values for a related variable above, yet had a missing value, refused to respond or responded "don't know" for this variable.

Abbreviations: y, year; IQR, interquartile range.

[a] Any HPV infection detected (carcinogenic and/or non-carcinogenic).

[b] Included women that did not have a specimen collected for HPV testing because they were virgins.

vaccine arm, prevalence of HPV-16 and -18 at the 4-year visit was lower than in the control group (as previously reported [28]).

Vaccination status did not affect detection of cutaneous HPV types: the overall prevalence of any cutaneous type in the control and vaccine arms were 21.5% (95% CI, 16.6–27.2) and 21.7% (95% CI, 16.7–27.2; $P = .98$), respectively; therefore, results from both arms were pooled and are presented in Table 3. Of all the cutaneous types tested, beta HPV types were

Table 2. Prevalence of Human Papillomavirus (HPV) Alpha Mucosal Types Among the 5838 Young Adult Women From Costa Rica Who Provided an Oral Specimen for HPV DNA Testing, Stratified by Vaccine Arm

	Control Arm (n = 2926)		Vaccine Arm (n = 2912)		Overall (n = 5838)	
Test Result	No. of HPVs Detected	Prevalence, % of All Women (95% CI)	No. of HPVs Detected	Prevalence, % of All Women (95% CI)	No. of HPVs Detected	Prevalence, % of All Women (95% CI)
Any	55	1.9 (1.4–2.4)	46	1.6 (1.2–2.1)	101	1.7 (1.4–2.1)
Carcinogenic[a]	37	1.3 (.9–1.7)	20	0.7 (.4–1.1)	57	1.0 (.7–1.3)
16	12	0.4 (.2–.7)	1	0.03 (.0–.2)	13	0.2 (.1–.4)
18	4	0.1 (.0–.3)	0	0	4	0.1 (.0–.2)
31	5	0.2 (.1–.4)	3	0.1 (.0–.3)	8	0.1 (.1–.3)
35	0	0	1	0.03 (.0–.2)	1	0.02 (.0–.1)
39	1	0.03 (.0–.2)	3	0.1 (.0–.3)	4	0.1 (.0–.2)
51	10	0.3 (.2–.6)	7	0.2 (.1–.5)	17	0.3 (.2–.5)
52	7	0.2 (.1–.5)	3	0.1 (.0–.3)	10	0.2 (.1–.3)
56	4	0.1 (.0–.3)	2	0.1 (.0–.2)	6	0.1 (.0–.2)
58	1	0.03 (.0–.2)	0	0	1	0.02 (.0–.1)
Noncarcinogenic[b]	22	0.8 (.5–1.1)	28	1.0 (.6–1.4)	50	0.9 (.6–1.1)
6	4	0.1 (.0–.3)	3	0.1 (.0–.3)	7	0.1 (.0–.2)
11	0	0	1	0.03 (.0–.2)	1	0.02 (.0–.1)
34	0	0	1	0.03 (.0–.2)	1	0.02 (.0–.1)
44	5	0.2 (.1–.4)	4	0.1 (.0–.4)	9	0.2 (.1–.3)
53	4	0.1 (.0–.3)	4	0.1 (.0–.4)	8	0.1 (.1–.3)
54	0	0	3	0.1 (.0–.3)	3	0.1 (.0–.2)
66	6	0.2 (.1–.4)	7	0.2 (.1–.5)	13	0.2 (.1–.4)
70	2	0.1 (.0–.2)	2	0.1 (.0–.2)	4	0.1 (.0–.2)
74	4	0.1 (.0–.3)	5	0.2 (.1–.4)	9	0.2 (.1–.3)
Single	45	1.5 (1.1–2.1)	42	1.4 (1.0–1.9)	87	1.5 (1.2–1.8)
Multiple	10	0.4 (.2–.6)	4	0.1 (.0–.4)	14	0.2 (.1–.4)
Uncharacterized	102	3.5 (2.9–4.2)	110	3.8 (3.1–4.5)	212	3.6 (3.2–4.1)

Abbreviations: CI, confidence interval; HPV, human papillomavirus.

[a] The following carcinogenic types were tested for but not detected: 33, 45, and 59.

[b] The following noncarcinogenic types were tested for but not detected: 40, 42, 43, and 68/73.

the most common. Overall prevalence of beta HPV was 18.6% (95% CI, 15.3–22.3). The most common beta types were HPV-24 (3.8% [95% CI, 2.3–5.9]), HPV-38 (3.0% [95% CI, 1.7–4.9]), and HPV-5 (2.2% [95% CI, 1.1–3.9]). Because of their low individual prevalence, other cutaneous HPV types from the genera alpha, gamma, mu, and nu were grouped; their combined prevalence was 4.0% (95% CI, 2.5–6.1); of these, the most common type detected was HPV-1 (1.4% [95% CI, .6–2.9]). Single cutaneous HPVs were detected in 15.2% (95% CI, 12.2–18.7) of participants, whereas 6.4% (95% CI, 4.4–8.9) had multiple cutaneous HPV types detected.

Risk Factor Analysis

Because the HPV vaccine was shown to protect against oral HPV-16/18 [28], the evaluation of risk factors for alpha mucosal HPV was restricted to the control arm. The following variables were significantly associated with detection of any oral alpha mucosal HPV: marital status, former and current smoking, lifetime number of sexual partners, lifetime oral sex partners, chronic sinusitis, and positive cervical and anal HPV DNA status at the 4-year study visit (Table 4).

In the multivariate analysis, independent risk factors for detection of any oral alpha mucosal HPV included marital status (AOR, 3.2 [95% CI, 1.8–5.7] for single compared to those married/living as married), lifetime number of sexual partners (AOR, 2.4 [95% CI, 1.0–6.1] for those with ≥4 partners compared to 0–1 partners; *P* for trend across categories 0.03), chronic sinusitis (AOR, 3.1 [95% CI, 1.5–6.7]), and positive cervical HPV DNA status at the 4-year study visit (AOR, 2.6 [95% CI, 1.4–4.6]) (Table 4). Following adjustment, having given oral sex to >1 partner was no longer significantly associated with oral alpha mucosal HPV detection; its inclusion in the final multivariate model did not affect the point estimates of the other variables. Finally, the suggestive association of smoking

Table 3. Prevalence of Cutaneous Human Papillomavirus (HPV) Types Among a Subset of 500 Young Adult Women From Costa Rica Who Provided an Oral Specimen for HPV DNA Testing

Test Result	Overall (n = 500)	
	No. of HPVs Detected	Prevalence, % of All Women (95% CI)
Any	108	21.6 (18.1–25.5)
Any *Betapapillomavirus*[a]	93	18.6 (15.3–22.3)
5	11	2.2 (1.1–3.9)
8	9	1.8 (.8–3.4)
9	4	0.8 (.2–2.0)
14	4	0.8 (.2–2.0)
15	5	1.0 (.3–2.3)
17	7	1.4 (.6–2.9)
19	4	0.8 (.2–2.0)
20	5	1.0 (.3–2.3)
21	1	0.2 (.0–1.1)
22	4	0.8 (.2–2.0)
23	10	2.0 (1.0–3.6)
24	19	3.8 (2.3–5.9)
25	1	0.2 (.0–1.1)
36	3	0.6 (.1–1.7)
37	1	0.2 (.0–1.1)
38	15	3.0 (1.7–4.9)
49	3	0.6 (.1–1.7)
75	3	0.6 (.1–1.7)
76	1	0.2 (.0–1.1)
80	11	2.2 (1.1–3.9)
92	2	0.4 (.1–1.4)
93	7	1.4 (.6–2.9)
96	6	1.2 (.4–2.6)
Other cutaneous HPV types[b]	20	4.0 (2.5–6.1)
1[c]	7	1.4 (.6–2.9)
2[d]	5	1.0 (.3–2.3)
4[e]	5	1.0 (.3–2.3)
7[d]	1	0.2 (.0–1.1)
48[e]	1	0.2 (.0–1.1)
63[c]	1	0.2 (.0–1.1)
Single[f]	76	15.2 (12.2–18.7)
Multiple[f]	32	6.4 (4.4–8.9)
Uncharacterized[f]	74	14.8 (11.8–18.2)

Abbreviations: CI, confidence interval; HPV, human papillomavirus.

[a] The following beta types were tested for but not detected: 12 and 47.

[b] The following other cutaneous HPV types were tested for but not detected: 3, 10, 27, 28, 29, 40, 41, 43, 50, 57, 60, 65, 77, 88, 91, 94, 95.

[c] Mu HPV type.

[d] Alpha HPV type.

[e] Gamma HPV type.

[f] Any cutaneous HPV type.

with oral HPV in the univariate analysis lost significance in the multivariate analysis. Stratified analysis confirmed that the effect of smoking was explained by lifetime number of sexual partners, as there was little evidence for a smoking effect within strata of lifetime number of partners (data not shown).

Among the 106 women in the control group who reported acute tonsillitis and the 17 women with chronic disease of the tonsils, adenoids, or peritonsillar abscess, none had detectable oral alpha mucosal HPV. Of the women who had a tonsillectomy (as reported at enrollment and confirmed by physical exam; n = 20), all were negative for oral alpha mucosal HPV infection. No significant associations were observed for clinical conditions other than chronic sinusitis.

A risk factor analysis evaluating exposures that may increase the odds of cutaneous HPV within the oral region was conducted; however, no significant behavioral risk factors were identified (Supplementary Table), suggesting that detection of these types was not associated with sexual activity. When considering the subset of women with both oral alpha and beta HPV test results in the control arm (n = 246), detection of oral alpha mucosal HPV (n = 8 HPVs) significantly increased the odds of detection of beta cutaneous HPV within the oral region (n = 4 of the 8; OR, 4.8 [95% CI, 1.2–20.0]); no such association was observed for the other cutaneous HPV types.

DISCUSSION

Our analysis, nested within the Costa Rica Vaccine Trial, provides information regarding the epidemiology of oral HPV in a Latin American population of healthy, young adult women. Both mucosal and cutaneous HPV types were detected within the oral region. Detection of oral mucosal HPV types was relatively uncommon; overall prevalence of alpha HPV types among women who did not receive the HPV vaccine was 1.9%. Carcinogenic HPV types comprised the majority of detectable alpha mucosal HPV with HPV-16 being the most common. Independent risk factors for any oral alpha mucosal HPV included marital status, lifetime number of sexual partners, chronic sinusitis, and positive cervical HPV status at the 4-year study visit. Cutaneous HPV types were 10 times more common within the oral region than mucosal HPV types (21.6%). Beta HPV types comprised the vast majority of detectable cutaneous HPV (18.6%) compared to all other cutaneous HPV types combined (4.0%). Unlike the mucosal HPV types, detection of cutaneous HPV types in the oral region appeared independent of sexual activity. It is important to note, however, that detection of HPV using DNA-based methods does not allow us to distinguish active replicative infection (which is better assessed by markers of viral activity) from detection of virions deposited from recent sexual activity or other HPV-infected areas of one's body.

The observed prevalence of oral HPV-16 in our population of 22- to 29-year-old women in Costa Rica (0.4%) was comparable to other published reports from the United States, where prevalence estimates for oral HPV-16 in studies of men and women across a broad age range were between 0.2% and 1.0% [11, 12].

Table 4. Univariate and Multivariate Analyses of Risk Factors for Detection of Any Oral Alpha Mucosal Human Papillomavirus Types Among Young Adult Women From Costa Rica (Control Arm Only)

	Control Arm (n = 2926)			
Characteristic	No. of Women	HPV Positivity, No. (%) of Women	OR (95% CI)	AOR (95% CI)
Age, y				
22–23	671	12 (1.8)	Ref	
24–25	763	10 (1.3)	0.7 (.3–1.7)	
26–27	730	19 (2.6)	1.5 (.7–3.0)	
≥28	762	14 (1.8)	1.0 (.5–2.2)	
P for trend			.52	
Education level				
≤6 y	797	16 (2.0)	Ref	
7–9 y	535	7 (1.3)	0.6 (.3–1.6)	
≥10 y*	814	15 (1.8)	0.9 (.5–1.9)	
University	780	17 (2.2)	1.1 (.5–2.2)	
P for trend			.69	
Marital status				
Married or living as married*	**1858**	**20 (1.1)**	**Ref**	**Ref**
Single	**885**	**33 (3.7)**	**3.6 (2.0–6.2)**	**3.2 (1.8–5.7)**
Divorced/separated/widowed	**183**	**2 (1.1)**	**1.0 (.2–4.4)**	**0.7 (.2–3.1)**
Smoking status				
Never*	**2363**	**37 (1.6)**	**Ref**	Ref
Former**	**357**	**11 (3.1)**	**2.0 (1.0–4.0)**	1.4 (.7–2.9)
Current	**206**	**7 (3.4)**	**2.2 (1.0–5.0)**	1.2 (.5–2.8)
P for trend			**.02**	.54
Age at first vaginal intercourse, y				
Sexually naive	156	1 (0.6)	0.3 (.0–2.4)	
≥19	788	16 (2.0)	Ref	
17–18	834	13 (1.6)	0.8 (.4–1.6)	
≤16*	1148	25 (2.2)	1.1 (.6–2.0)	
P for trend			.38	
Lifetime No. of sexual partners				
0–1	**929**	**8 (0.9)**	**Ref**	**Ref**
2–3	**999**	**15 (1.5)**	**1.8 (.7–4.2)**	**1.4 (.6–3.4)**
≥4	**998**	**32 (3.2)**	**3.8 (1.7–8.3)**	**2.4 (1.0–6.1)**
P for trend			**<.001**	**.03**
Total No. of years sexually active				
Sexually naive	156	1 (0.6)	0.4 (.0–2.7)	
0–7*	1089	19 (1.7)	Ref	
8–10	900	19 (2.1)	1.2 (.6–2.3)	
≥11	781	16 (2.0)	1.2 (.6–2.3)	
P for trend			.31	
Lifetime No. of oral sex partners*				
0–1	**2155**	**33 (1.5)**	**Ref**	Ref
≥2	**771**	**22 (2.9)**	**1.9 (1.1–3.3)**	0.8 (.4–1.6)
P for trend			**.02**	.59
Age at first oral sex, y				
Never had oral sex*	1125	20 (1.8)	Ref	
≥21	782	14 (1.8)	1.0 (.5–2.0)	
≤20**	1019	21 (2.1)	1.2 (.6–2.2)	
P for trend			.63	

Table 4 continued.

Characteristic	Control Arm (n = 2926)			
	No. of Women	HPV Positivity, No. (%) of Women	OR (95% CI)	AOR (95% CI)
Lifetime No. of anal sex partners*				
0	2416	44 (1.8)	Ref	
1**	415	10 (2.4)	1.3 (.7–2.7)	
≥2	95	1 (1.1)	0.6 (.1–4.2)	
P for trend			.86	
Chronic sinusitis				
No	**2769**	**46 (1.7)**	**Ref**	**Ref**
Yes	**157**	**9 (5.7)**	**3.6 (1.7–7.5)**	**3.1 (1.5–6.7)**
Cervical HPV status at 4-year study visit[a]				
Negative*,[b]	**1912**	**20 (1.0)**	**Ref**	**Ref**
Positive	**1014**	**35 (3.5)**	**3.4 (1.9–5.9)**	**2.6 (1.4–4.6)**
Anal HPV status at 4-year study visit[a,c]				
Negative[b]	**1428**	**18 (1.3)**	**Ref**	
Positive	**664**	**17 (2.6)**	**2.1 (1.1–4.0)**	

ORs were adjusted for categorical age as well as all the variables included in the table. Statistically significant findings are bolded.

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.

Missing values accounted for <1% of the full cohort; *includes women with a missing value, those who refused to respond, and those who responded "don't know" for this variable; **Includes women who did not have missing values for a related variable above, yet had a missing value, refused to respond or responded "don't know" for this variable.

[a] Any HPV infection detected (carcinogenic and/or noncarcinogenic).

[b] Included women who did not have a specimen collected for HPV testing because they were sexually naive.

[c] Total was <2926 due to 678 women who refused anal specimen collection.

Likewise, risk factors for oral HPV observed within our population also mirrored those previously reported in US-based studies. As expected, sexual behavior was an important risk factor for oral alpha mucosal HPV, consistent with what is known for HPV epidemiology at other anatomical sites [11, 12]. Detection of oral alpha mucosal HPV within our population was independently associated with having a relatively high lifetime number of sexual partners. Being unmarried further increased the risk of oral alpha mucosal HPV after controlling for number of sexual partners. Apart from the expected misclassification of sexual behavior reporting, this could suggest the presence of an additional factor not captured by our questionnaire (eg, open-mouth kissing [11]). Cervical HPV positivity, a marker of sexual behavior, was also a strong risk factor for detection of oral alpha mucosal HPV. Finally, although oral sex and smoking have been consistently reported as risk factors for oral HPV detection [12, 13], after adjustment for sexual behavior, the associations observed in our univariate analyses were no longer significant. In our population, prevalence of these exposures was relatively low (>2 lifetime oral sexual partners, 26%; and current smoking, 6.6%), thus compromising our ability to fully evaluate these exposures. Due to the reported influence of age, smoking, and sexual behaviors on oral HPV prevalence [12], results from our study of younger women who are mainly nonsmokers with few sexual partners may not be generalizable to older women with different behavior/risk profiles.

Chronic sinusitis was a newly identified risk factor for oral alpha mucosal HPV detection. Chronic sinusitis is more likely to occur among women with reduced immune responses who in turn may be more susceptible to viral infections [32]. An alternative explanation may be that sinusitis compromises the integrity of the oral epithelium, a necessary step in cervical HPV infection [33]. Additionally, sinusitis may flush HPV virions/infected cells from the nasal cavity into the back of the throat, although this would be expected to apply to cutaneous types as well (assuming detection represented true infection) and no association with cutaneous types was observed.

Cutaneous HPV types are highly prevalent in human skin and hair follicles of healthy individuals [34–38]. Although multiple asymptomatic infections are common, clinical manifestations of cutaneous HPV infection include common, plantar, planar, and genital warts as well as epidermodysplasia verruciformis [39]. To date, 3 studies reported detection of cutaneous HPV types within the oral region of healthy individuals [14, 17, 18]. As in our study, beta HPV types were the predominant cutaneous HPV type detected; however, the prevalence of beta types detected within our population was notably lower (18.6% vs 64% among older HIV-negative males) [14]. Given current

knowledge of their biology, these cutaneous HPV types are not believed to be carcinogenic in healthy individuals. Although the interplay between mucosal and cutaneous HPV is unknown, our superficial analysis suggested they may not be independent. Therefore, if detection of cutaneous types within the oral region represents true infection, the presence of cutaneous HPV infections may affect the acquisition and/or clearance of mucosal HPVs.

Using data from our large, community-based study in Costa Rica, we extend the reports of low prevalence of oral alpha mucosal HPV in developed countries to a resource-poor country, and confirm its association with sexual behavior. Future work will evaluate the proportion of the oral HPVs that are detected over time and are therefore more likely to represent true persistent oral infections. As persistent oral HPV-16 infection is the putative precursor to HPV-associated oropharyngeal cancer, prevention is needed and will hopefully be accomplished through widespread implementation of the prophylactic HPV-16/18 vaccine.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. D. R. L. and J. S. report that they are named inventors on US government–owned HPV vaccine patents that are licensed to GSK and Merck and for which the NCI receives licensing fees, and are entitled to limited royalties as specified by federal law. All other authors report no potential conflicts.

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