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Prevalence and Epidemiologic Profile of Oral Infection with Alpha, Beta, and Gamma Papillomaviruses in an Asian Chinese Population

Martin C. S. Wong,[1] Alexander C. Vlantis,[2] Miaoyin Liang,[1] Po Yee Wong,[3] Wendy C. S. Ho,[3] Siaw S. Boon,[3] Ryan Kin Ho Sze,[3] Colette Leung,[1] Paul K. S. Chan,[3,4] and Zigui Chen[3,4]

[1]Jockey Club School of Public Health and Primary Care, [2]Department of Otorhinolaryngology, Head and Neck Surgery, [3]Department of Microbiology, and [4]Stanley Ho Center for Emerging Infectious Diseases, Faculty of Medicine, The Chinese University of Hong Kong, People's Republic of China

Background.   Knowledge of the prevalence of and risk factors for oral human papillomavirus (HPV) infection, especially cutaneous types, is limited.

Methods.   A population-based study using next-generation sequencing consecutively recruited asymptomatic individuals aged 18–64 years from a proportional sampling of the general population of Hong Kong, according to age groups, gender, and regions of residence. We examined associations of alpha-, beta-, and gamma-HPVs from oral rinse samples with participants' sociodemographics by logistic regression models.

Results.   The prevalence of oral HPV infection among 1426 ethnic Chinese was 15.5% (95% confidence interval [CI], 13.7%–17.5%), 2.5% (95% CI, 1.8%–3.5%), 11.9% (95% CI, 10.3%–13.6%), and 2.9% (95% CI, 2.1%–3.9%) for any type, alpha-, beta-, and gamma-HPV, respectively. Prevalence of any high-risk HPV was 0.8% (95% CI, 0.4%–1.4%), and that of HPV-16 was 0.4% (95% CI, 0.2%–0.8%). HPV-8 and HPV-98 were the most common beta types detected, while HPV-4 and HPV-SD2R were the most common gamma types. Prevalence of alpha- and beta/gamma-HPV infection showed a similar pattern of increase with age, and was higher in men than women. Smoking, drinking, oral sex, and more sexual partners were associated with alpha-HPV. Teeth brushing before sleep was protective for beta/gamma-HPVs.

Discussion.   The epidemiologic factors associated with oral infection with alpha-HPVs are different from those of beta/gamma-HPVs, suggesting different modes of acquisition and persistence.

Keywords.   human papillomavirus; cutaneous; mucosal; oral infection; oropharyngeal cancer.

Oral infection with high-risk "mucosal" human papillomaviruses (HPVs) within the genus *Alphapapillomavirus* has been identified as one of the important risk factors for a subset of head and neck squamous cell carcinomas [1]. Over the last decade, oral HPV infection has become an important contributor to the global health burden [1–4]. The proportion of HPV-associated oropharyngeal cancer has increased from 16.3% during the 1984–1989 period to 71.7% during the 2000–2004 period in the United States [2, 3]. A similar increasing trend has also been observed among young adults in Australia and many European countries

[2]. However, the reported prevalence of HPV-associated oropharyngeal squamous cell carcinoma in China is much lower (range 3.5%–17%), compared to that reported from the West [5–11]. This may suggest an ethnic and/or geographic difference in the prevalence and disease attribution of oral HPV infection.

The majority of the characterized HPV types classified within the genera *Betapapillomavirus* and *Gammapapillomavirus* are referred to as "cutaneous" HPVs because of their well-recognized tropism for skin. However, recent studies have detected these "cutaneous HPVs" in mucosal sites, from cervical samples [12, 13], mouth wash samples [14, 15], and head and neck papilloma samples [16, 17]. At present, very little is known about the epidemiology and clinical course of beta- and gamma-HPV infections detected from mucosal sites.

The aim of this study was to elucidate the epidemiology of oral infection with mucosal and cutaneous HPVs in a large cohort of ethnic Chinese residing in an urban city, Hong Kong.

METHODS

Study Population and Sampling

The population of Hong Kong was estimated to be 7.23 million in mid-2014 [18]. The proportion of males was 46.2%. The territory of Hong Kong is divided into 3 geographic clusters, namely

the Hong Kong Island, Kowloon, and the New Territories, with Hong Kong Island being the most urban and the New Territories being the most rural [19]. The number of domestic households was estimated to be 2.43 million, with an average domestic household size of 2.9 persons.

Cluster sampling is a commonly used sampling strategy [20], where participants are invited to face-to-face interviews in community settings. The design of the present study was based on the proven concept of a previous study from our institution that adopted cluster randomization, which achieved a high response rate of 72.4% [21]. We consecutively recruited participants for screening from a geographically representative sample of the general population of Hong Kong, which forms the sampling frame of this population-based survey. A proportional sampling methodology was adopted according to age groups (18–24, 25–34, 35–44, 45–54, 55–64 years), gender, and the geographic regions of residence (Hong Kong Island, Kowloon, or the New Territories), based on the population figures published in the latest version of the Hong Kong Census report. All permanent residents of Hong Kong aged 18–64 years, recruited by health talks and media announcements, were eligible for this study. Subjects who were unable to give consent, had symptoms suggestive of oropharyngeal cancer, or who had a medical condition rendering them unable to participate in the study were excluded.

Self-Administered Survey

On entering the study, all subjects completed a self-administered, anonymous survey that recorded their basic demographic profiles including age, gender, education level, household income, occupation, marital status, past medical history, and lifestyle habits including cigarette smoking, alcohol consumption, and sex history. A research assistant assisted those subjects who requested help in completing the survey, for reasons such as illiteracy, by reading the question items to them.

Oral Sample Processing and HPV Genotyping

Each participant provided an oral rinse sample collected in 0.9% normal saline after completing the survey. Approximately 1 mL of the oral rinse sample was centrifuged at 5000g for 5 minutes and DNA extracted from the pellet using the QIAamp DNA Mini Kit (Qiagen). Two recently developed novel PCR-based next-generation sequencing (NGS) assays targeting the consensus regions of HPV L1 open reading frame were used to detect and identify the full spectrum of human papillomaviruses including alpha-, beta-, and gamma-HPV types (Supplementary Figure S1) [17, 22]. For each assay, a pair of unique 12-bp barcodes was introduced to the PCR amplicon by forward and reverse primers. Successful amplicons with predicted fragment sizes were pooled at approximately equal molar DNA concentrations and sequenced on an Illumina MiSeq (Illumina) using 150-bp paired-end reads. The demultiplexed paired-end Illumina short reads passing the quality filter (≥Q20 and ≥50 bp) were merged

into single reads using FLASh v1.2.11 [23] and blasted against a genomes online database (gold) papilloma virus (PV) reference database using UPARSE software [24]. Our PV reference database contains 387 fully characterized human (n = 225) and animal (n = 162) PV types, and 467 potential novel partial PV sequences. An operation taxonomic unit (OTU) count table giving the number of reads per sample per OTU was created using a 95% identity threshold with in-house developed scripts [22]. The OTU taxonomy was classified at the type level based on sequence homology to the reference database: if OTUs hitting the reference database had ≥ 90% identities to a characterized PV type, they represented known viruses; those with 60%–89% identities were regarded as "uncharacterized" types and assigned with a unique identity. An HPV type was considered positive if the reads were ≥ 50. The processing from each NGS assay was separated while data was then combined to determine the presence of HPV DNA. HPV test results were not delinked to personal identifiers as clinical follow-up would be arranged for HPV-positive subjects for medicolegal reasons. We performed some measures to minimize the potential of contamination, where we processed the DNA extraction in a physically separated room from PCR amplification and introduced dual-index in the PCR primers. For each PCR amplification, multiple negative controls and random repeats were set.

HPV Classification and Phylogenetic Analysis

Tree topology of short OTU sequences was constructed using the pplacer v1.1.alpha17 [25] by placing OTU reads on a PV reference tree to maximize phylogenetic likelihood according to a complete genome alignment. The reference tree was created using RAxML MPI v8.2.9 [26] based on a complete genome alignment containing 387 PV types. A cutoff value of maximum likelihood ≥ 0.8 was set as confident assignment of PV type into each species. In a monograph from the International Agency for Research on Cancer (IARC) [27], 12 alpha-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) were classified as "carcinogenic to humans" (Group 1) and were considered as high-risk HPV types in this study.

Statistical Analysis

The Statistical Package for Social Sciences 21.0 (Chicago, Illinois) software was used for all data entry and analysis. We examined associations of alpha-, beta-, and gamma-HPVs with participant demographic and lifestyle characteristics using conditional logistic regression models for matched risk sets to estimate ORs and 95% confidential intervals (CIs). Each epidemiological factor potentially associated with HPV infection was assessed by univariate analysis, and those with $P < .20$ were selected for multivariate analysis using binary logistic regression models, where selection of the variable was backward and stepwise. A P value of ≤ 0.05 was regarded as statistically significant. The investigators performed the study with informed

consent after approval by The Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CREC 2014.708) and in accordance with the precepts of the Helsinki Declaration.

RESULTS

Participant Characteristics

A total of 2410 subjects were invited to participate in the present study, and 1475 (61.2%) agreed to join (Supplementary Figure S2). Forty-nine subjects who were found to be ineligible, including those who were non-Chinese, were excluded. Characteristics of the 1426 subjects included for analysis are shown in Table 1. Briefly, the average age was 46.3 years (standard deviation [SD] 14.9), 47.7% were male, the majority were married or cohabited (63.6%), never smoked (79.2%), and were nondrinkers (77.7%). One-third (33.0%) reported having had oral sex, and 7.5% had had 2 or more sex partners in the past 2 years, while the majority (54.9%) reported having had only 1 sex partner during their lifetime. Furthermore, 72.4% reported teeth brushing before sleep for more than 90% of the time, 18.6% had dentures, and 30.9% suffered from gum disease in the past 3 months.

Prevalence of HPV Infection

The prevalence of oral infection with all-type, alpha-, beta-, and gamma-HPV was 15.5% (95% CI, 13.7%–17.5%), 2.5% (95% CI, 1.8%–3.5%), 11.9% (95% CI, 10.3%–13.6%), and 2.9% (95% CI, 2.1%–3.9%), respectively (Table 2). Coinfection with more than 1 HPV type was detected in 47 (3.3%) subjects. Among the 47 coinfections, 38 (2.7%) were coinfected with multiple cutaneous (beta and/or gamma) types, 8 (0.6%) were coinfected with mucosal (alpha) and cutaneous (beta and/or gamma) types, and 1 (0.1%) was coinfected with multiple alpha-HPV types. Alpha-, beta-, and gamma-HPV infections showed a similar pattern of increase in prevalence with age, and the prevalence in men was higher than in women (Figure 1).

Distribution of HPV Types and Genera

The distribution of HPV types associated with their phylogenetic and oncogenic relationship is shown in Figure 2 and Table 2. Of the 36 subjects infected with mucosal (alpha-) HPVs, 11 (0.8%, 95% CI, 0.4%–1.4%) were infected with high-risk types, and 26 (1.8%, 95% CI, 1.3%–2.7%) were infected with low-risk types. HPV-16 was detected in 5 subjects (0.4%, 95% CI, 0.2%–0.8%), while no HPV-18 was found in this series. All subjects with high-risk HPV infection were examined by an otorhinolaryngologist and none were found to have suspicious oral or oropharyngeal mucosal lesions or oropharyngeal cancer.

Beta-HPVs were the dominant cutaneous group in HPV-positive subjects, which was about 4 times more common than gamma-HPVs (prevalence of 11.9% vs 2.9%). The most common beta-HPV types were HPV-8 and HPV-98, each found in 21 subjects (1.5%, 95% CI, 1.0%–2.2%), followed by HPV-38,

Table 1.   Characteristics of Study Participants (N = 1426)

	N	%
Age (years)		
≤24[a]	268	18.8
25–34	76	5.3
35–44	115	8.1
45–54	421	29.5
55–64	539	37.8
≥ 65	7	0.5
Gender[b]		
Male	680	47.7
Female	745	52.2
Ethnicity		
Chinese	1426	100
Marital status		
Single	426	29.9
Married	895	62.8
Divorced/separated/widowed	93	6.5
Cohabit	12	0.8
Education level[b]		
Middle school or below	312	21.9
High school	423	29.7
Matriculation/diploma/postsecondary/tertiary	690	48.4
Work status[b]		
Student	261	18.3
Unemployed/part time	126	8.8
Housewife	181	12.7
Retired	173	12.1
Full time	682	47.8
Personal monthly income (HK$)[b]		
≤ 3600	452	31.7
3601–20 000	614	43.1
≥ 20 001	348	24.4
Receive social allowance	7	0.5
Smoking[b]		
Current	120	8.4
Never	1130	79.2
Exsmoker	163	11.4
Drinking[b]		
Drinker	291	20.4
Nondrinker	1108	77.7
Exdrinker	24	1.7
HPV vaccination[b]		
Yes	106	7.4
No	1316	92.3
Teeth brushing before sleep[b]		
>90%	1032	72.4
50–90	164	11.5
10–50	112	7.9
<10 or never	110	7.7
Denture wearers[b]		
Yes	267	18.7
No	1112	78.0
Not sure	37	2.6
Gum disease in the past 3 months[b]		
Yes	440	30.9
No	849	59.5
Not sure	124	8.7
Oral sex per month in the past 2 years[b]		

Table 1. Continued

	N	%
Never	937	65.7
0–1 time	266	18.7
2–3 times	127	8.9
≥ 4 times	77	5.4
Number of sexual partner in the past 2 years[b]		
0	390	27.3
1	923	64.7
2	62	4.3
≥ 3	45	3.2
Lifetime number of sexual partner[b]		
0	280	19.6
1	783	54.9
2	130	9.1
3	75	5.3
4	24	1.7
≥ 5	132	9.2

Study participants were asked to fill in a structured questionnaire to provide information on a voluntary basis, and were reassured that the information would not be linked to their personal identifiers. Questions left blank were regarded as missing for the purposes of analysis in this study.

[a]Includes 2 subjects of 16 years old, and 2 subjects of 17 years old.

[b]The numbers do not add up to 1426 due to missing data.

HPV-HIVGc36, HPV-21, HPV-22, and HPV-24 found in 16 (1.1%, 95% CI, 0.7%–1.8%), 14 (1.0%, 95% CI, 0.6%–1.6%), 13 (0.9%, 95% CI, 0.5%–1.6%), 13 (0.9%, 95% CI, 0.5%–1.6%), and 12 (0.8%, 95% CI, 0.5%–1.5%) subjects, respectively. The 2 most common gamma types were HPV-4 and HPV-SD2R, each found in 6 subjects (0.4%, 95% CI, 0.2%–0.9%). A total of 87 unique HPV types were detected in the surveyed subjects, including 22 (25.3%) alpha-HPV types, 40 (46.0%) beta-HPV types, and 25 (28.7%) gamma-HPV types (Supplementary Table S1). Among them, 3 and 6 potential novel beta-HPV (FA127, F031, SE48) and gamma-HPV types (SE80, SE54, F059, FA4, FA12, FA133) warrant further work to characterize their complete genomes.

Factors Associated With Oral HPV Infection

Univariate analysis showed that oral alpha-, beta-, and gamma-HPV infections were associated with different epidemiological risk factor profiles (Table 3, Figure 3). Smoking (crude odds ratio [cOR] 2.62, 95% CI, 1.32–5.19) and drinking (cOR 2.59, 95% CI, 1.32–5.08) were risk factors for alpha-HPV, but not for beta- or gamma-HPV infections. Furthermore, individuals reported to have had oral sex (cOR 2.56, 95% CI, 1.31–4.98) or 2 or more sexual partners (cOR 3.71, 95% CI, 1.65–8.35) in the past 2 years, or who had 4 or more sexual partners in their lifetime (cOR 3.77, 95% CI, 1.82–7.82) were associated with a higher prevalence of alpha-HPV, but not beta- or gamma-HPV infections.

A habit of teeth brushing before sleeping for more than 90% of the time was protective against beta-HPV (cOR 0.64, 95% CI, 0.46–0.90) and gamma-HPV (cOR 0.52, 95% CI, 0.28–0.97) infections. Subjects with dentures at the time of survey were associated with a marginal increase in the risk of beta-HPV (cOR 1.49, 95% CI, 1.02–2.18), but not alpha- or gamma-HPV infection.
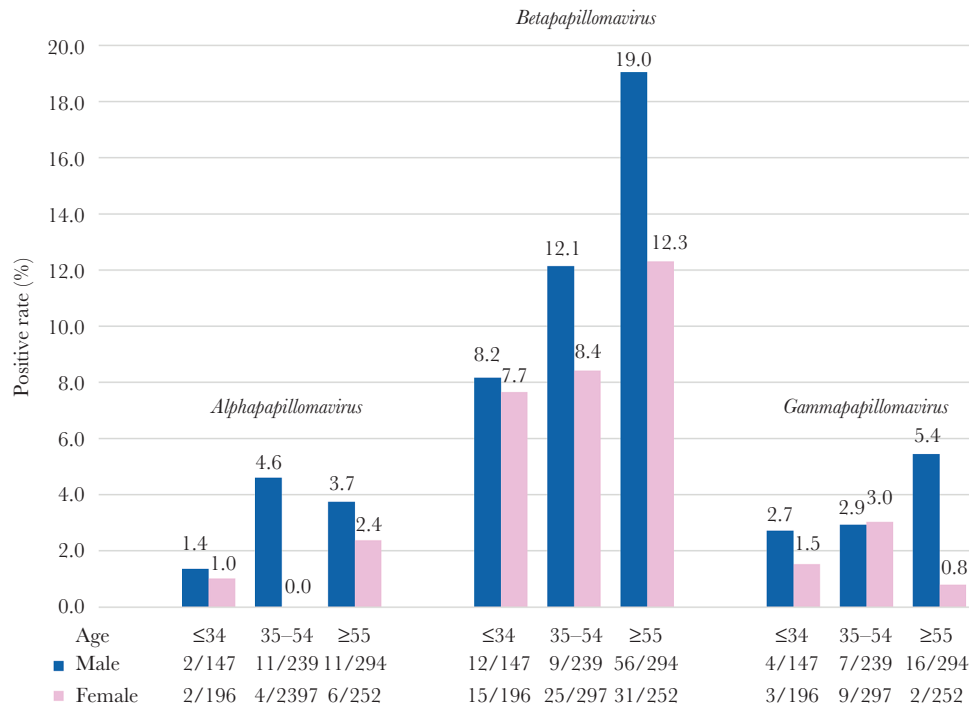
Table 2. Age- and Sex-Specific Prevalence of Oral Alpha-, Beta-, and Gamma-human Papillomavirus (HPV) Infections in 1426 Hong Kong Ethnic Chinese

	N	%
Any HPV	221	15.5
Alpha-HPV	36	2.5
Single infection	27	1.9
Coinfection with other		
Alpha-HPV	1	0.1
Beta-HPV	5	0.4
Gamma-HPV	1	0.1
Beta- and Gamma-HPV	2	0.1
Beta-HPV	169	11.9
Single infection	124	8.7
Coinfection with other		
Alpha-HPV	5	0.4
Beta-HPV	23	1.6
Gamma-HPV	15	1.1
Alpha- and Gamma-HPV	2	0.1
Gamma-HPV	41	2.9
Single infection	23	1.6
Coinfection with other		
Alpha-HPV	1	0.1
Beta-HPV	15	1.1
Gamma-HPV	0	0.0
Alpha- and Beta-HPV	2	0.1

Characteristics with a *P* value of ≤ 0·2 on univariate analysis were entered into a multivariate regression analysis to identify independent associations. Because cigarette smoking and alcohol drinking are highly correlated, they were entered independently into the multivariate regression analysis, and not simultaneously. Similarly, sexual behavior, including oral sex, number of sexual partners in the past 2 years, and lifetime partners, were entered separately into the multivariate regression analysis and not together. As a result, ex-/current smokers (adjusted odds ratio [aOR] 2.18, 95% CI, 1.08–4.41), ex-/current drinkers (aOR 2.33, 95% CI, 1.18–4.61), a positive history of oral sex in the past 2 years (aOR 2.24, 95% CI, 1.13–4.41), 2 or more sexual partners in the past 2 years (aOR 3.21, 95% CI, 1.41–7.31), and 4 or more lifetime sexual partners (aOR 2.91, 95% CI, 1.34–6.31) were found to be independently associated with an increased risk of oral alpha-HPV infection. Male gender (aOR 1.45, 95% CI, 1.04–2.03) and age ≥ 55 years (aOR 1.78, 95% CI, 1.28–2.47) were independent risk factors, whereas teeth brushing before sleep on > 90% of evenings was an independent protective factor (aOR 0.68, 95% CI, 0.48–0.96) for oral beta-HPV infection. Male gender was the only independent risk factor observed for gamma-HPV infection (aOR 2.27, 95% CI, 1.18–4.38).

DISCUSSION

To the best of our knowledge, this is the first population-based study that investigated the epidemiology of oral infection with a full spectrum of HPV types in an urbanized ethnic Chinese

Figure 1. Age- and sex-specific prevalence of oral alpha-, beta-, and gamma human papillomavirus infections.

population. While most of the published studies on oral HPV infection are limited to mucosal (alpha-) HPVs, we took advantage of the next-generation sequencing to interrogate both mucosal (alpha-) and cutaneous (beta- and gamma-) HPVs. Thus, we are able to compare the epidemiologic profile associated with oral infections of different genera of HPVs.

The prevalence rate of mucosal (alpha-) HPV infection observed in this study was lower than those reported from the Western countries. While we found the prevalence of 2.5% for alpha-HPV and 0.4% for HPV-16, the corresponding prevalence rates from a recent meta-analysis that pooled estimates from 29 studies, predominately from Western countries, 5.5% and 1.0% [28, 29]. Nevertheless, our prevalence rate for alpha-HPV was much higher than that reported from Anyang, a rural region of China (0.67%) [30].

We observed a trend of increased prevalence of oral HPV infection with age, whereas others have reported a bimodal age-related peak with one at a younger age (30–34 years) and the other at an older age (60–64 years) [28–31]. This could reflect age as a surrogate for different sets of risk factors among populations. Older subjects in our study could have lower immunity, more frequent denture use, periodontitis, gingivitis, or tooth loss. The higher prevalence of oral HPV among older people could also be due to a longer persistence of the virus, as reported in a prospective study [32]. The age-specific prevalence of oral HPV infection needs further examination in large-scale surveys.

Tobacco smoking was found to be a risk factor for alpha-HPV infection. This finding is compatible with reports from other

studies [33–36]. It has been postulated that smoking can induce inflammation and suppress humoral immunity in the oral mucosa via its oxidative components such as nicotine, carbon monoxide, and other chemicals [36]. Smoking might also promote mutagenesis that may favor the persistence of oral alpha-HPV infection [29]. Furthermore, our results showed that men were more likely to be infected, which could be attributed to a higher probability of HPV transmission through oral sex on women compared to men [37]. Another possible explanation is the hormonal differences between men and women, which may influence the persistence of oral alpha-HPV infection [38] because women have higher seroconversion rates in response to genital infection and this may protect against oral infection [39, 40].

Our observation that oral sex was significantly associated with alpha-HPV infection is compatible with findings from previous studies [30, 34, 35, 40, 41]. Nevertheless, there are studies that did not report any association between oral sex and HPV infection [33, 36, 39], which could be due to the colinearity of sexual behaviors, including other sexually associated contacts such as deep kissing that precluded associating a particular sexual behavior with HPV infection [29]. The present study did not distinguish whether subjects were oral sex providers or receivers. This could have different implications for male and female subjects, which could be examined in future studies.

Previous studies have shown that cutaneous (beta-/gamma-) HPVs are commonly found in anogenital areas and in the uterine cervix, but distinct associated risk factors were not identified [13, 40]. While we found that smoking, drinking, and

Figure 2. Distribution of human papillomavirus (HPV) types in oral rinse samples. The tree was inferred from the topology based on the complete genome alignment. The barplot on the right panel of the tree shows the number of samples positive for each type. Samples with coinfections were counted more than once. High-risk (HR) alpha-HPV, red; low-risk (LR) alpha-HPV, blue; any beta-HPV, orange; and any gamma-HPV, green.

sexual behavior were strongly associated with oral alpha-HPV, none of them was linked with beta-/gamma-HPVs suggesting that the route of transmission and factors favoring persistence were different between these 2 groups of viruses. The fact that oral cutaneous (beta-/gamma-) HPV infections were associated with a lack of teeth brushing before sleep might reflect the importance of oral hygiene in clearing these common infections, which probably do not have a strong intrinsic ability to

persist. It would be worthwhile to carry out further studies to identify types and factors associated with HPV persistence and its clinical consequence.

Some limitations of this study are now addressed. Firstly, participants who joined the study might be more health conscious than the general population, and so the generalizability of these finding will need caution. On the contrary, some participants might have joined the study due to their particular

Table 3. Univariate Analysis of Epidemiologic Risk Factors of Oral Alpha-, Beta-, and Gamma-HPV Infections

		Alpha-HPV				Beta-HPV				Gamma-HPV			
		N	%	Crude OR (95% CI)	P Value	N	%	Crude OR (95% CI)	P Value	N	%	Crude OR (95% CI)	P Value
Gender	Female (N = 745)	12	1.6	Reference	.021	71	9.5	Reference	.004	14	1.9	Reference	.018
	Male (N = 680)	24	3.5	2.24 (1.11–4.50)		98	14.4	1.60 (1.16–2.21)		27	4.0	2.16 (1.12–4.15)	
Age (years)	18–54 (N = 876)	19	2.2	Reference	.253	81	9.3	Reference	<.001	22	2.5	Reference	.473
	55–64 (N = 539)	17	3.2	1.47 (0.76–2.85)		85	15.8	1.84 (1.33–2.54)		17	3.2	1.26 (0.67–2.40)	
Marital Status	All other statuses (N = 531)	15	2.8	Reference	.578	46	8.7	Reference	.004	12	2.3	Reference	.284
	Married (N = 895)	21	2.4	0.83 (0.42–1.62)		123	13.7	1.68 (1.18–2.40)		29	3.2	1.45 (0.73–2.86)	
Education level	Middle school or below (N = 312)	8	2.6	Reference	.649	46	14.7	Reference	.186	10	3.2	Reference	.066
	High school (N = 423)	13	3.1	1.21 (0.49–2.94)		47	11.1	0.72 (0.47–1.12)		18	4.3	1.34 (0.61–2.95)	
	Matriculation or above (N = 690)	15	2.2	0.84 (0.35–2.01)		75	10.9	0.71 (0.48–1.05)		13	1.9	0.58 (0.25–1.34)	
Working status	No full-time work (N = 741)	14	1.9	Reference	.109	84	11.3	Reference	.511	16	2.2	Reference	.090
	Full-time work (N = 682)	22	3.2	1.73 (0.88–3.41)		85	12.5	1.11 (0.81–1.54)		25	3.7	1.72 (0.91–3.26)	
Personal monthly income (HK$)	10000 below (N = 658)	13	2.0	Reference	.214	71	10.8	Reference	.263	14	2.1	Reference	.113
	10001 above (N = 763)	23	3.0	1.54 (0.78–3.07)		97	12.7	1.20 (0.87–1.67)		27	3.5	1.69 (0.88–3.25)	
Smoking	Never (N = 1130)	22	2.0	Reference	.004	127	11.2	Reference	.235	29	2.6	Reference	.231
	Ex/current smokers (N = 283)	14	5.0	2.62 (1.32–5.19)		39	13.8	1.26 (0.86–1.86)		11	3.9	1.54 (0.76–3.11)	
Drinking	Nondrinker (N = 1108)	21	1.9	Reference	.004	131	11.8	Reference	.970	28	2.5	Reference	.134
	Ex/current drinker (N = 315)	15	4.8	2.59 (1.32–5.08)		37	11.8	0.99 (0.67–1.46)		13	4.1	1.66 (0.85–3.25)	
HPV vaccination	No (N = 1316)	32	2.4	Reference	.338	161	12.2	Reference	.151	39	3.0	Reference	.764
	Yes (N = 106)	4	3.8	1.57 (0.55–4.54)		8	7.6	0.59 (0.28–1.23)		2	1.9	0.63 (0.15–2.64)	
Teeth brushing before sleep	Less than 90% of the time (N = 386)	12	3.1	Reference	.404	60	15.5	Reference	.010	17	4.4	Reference	.038
	More than 90% of the time (N = 1032)	24	2.3	0.74 (0.37–1.50)		109	10.6	0.64 (0.46–0.90)		24	2.3	0.52 (0.28–0.97)	
Denture wearer	No/not sure (N = 1149)	30	2.6	Reference	.734	125	10.9	Reference	.041	29	2.5	Reference	.156
	Yes (N = 267)	6	2.3	0.86 (0.35–2.08)		41	15.4	1.49 (1.02–2.18)		11	4.1	1.66 (0.82–3.37)	
Gum disease in the past 3 months	No/not sure (N = 973)	25	2.6	Reference	.939	118	12.1	Reference	.773	32	3.3	Reference	.197
	Yes (N = 440)	11	2.5	0.97 (0.47–1.99)		51	11.6	0.95 (0.67–1.35)		9	2.1	0.61 (0.29–1.30)	
Oral sex in the past 2 years	Never (N = 937)	16	1.7	Reference	.004	111	11.9	Reference	.753	24	2.6	Reference	.497
	Any number of times (N = 470)	20	4.3	2.56 (1.31–4.98)		53	11.3	0.95 (0.67–1.34)		15	3.2	1.25 (0.65–2.41)	
Number of sexual partner in the past 2 years	0–1 (N = 1313)	28	2.1	Reference	.004	153	11.7	Reference	.892	39	3.0	Reference	.764
	≥2 (N = 107)	8	7.5	3.71 (1.65–8.35)		12	11.2	0.96 (0.51–1.79)		2	1.9	0.62 (0.15–2.61)	
Lifetime number of sexual partner	0–3 (N = 1268)	25	2.0	Reference	.001	145	11.4	Reference	.150	36	2.8	Reference	.798
	≥4 (N = 156)	11	7.1	3.77 (1.82–7.82)		24	15.4	1.41 (0.88–2.25)		5	3.2	1.13 (0.44–2.93)	

Study participants were asked to fill in a structured questionnaire to provide information on a voluntary basis, and were reassured that the information would not be linked to their personal identifiers. Questions left blank were regarded as missing for the purposes of analysis in this study.

Abbreviations: HPV, human papillomavirus; OR, odds ratio.
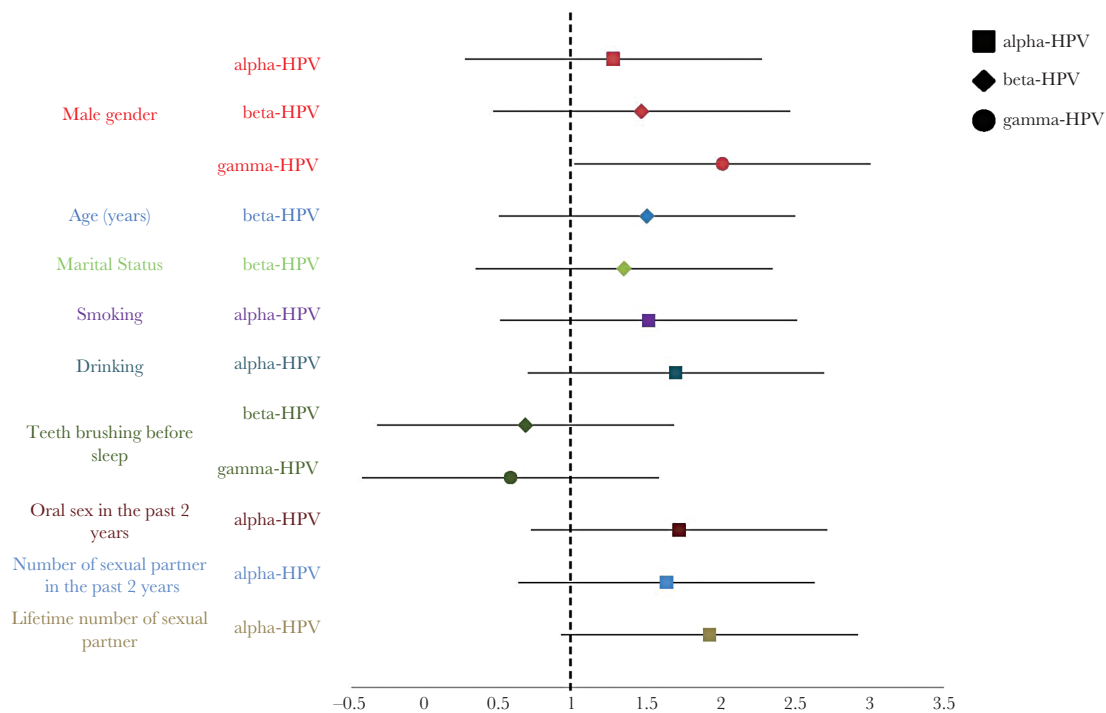
Figure 3. Epidemiologic characteristics and risk of oral infection with alpha, beta, and gamma- human papillomavirus (HPV). The central symbols represent crude odds ratio and the lines indicate the 95% confidental interval from univariate analysis.

risk factors for oral HPV infection, such as a history of oral sex that was reported by more than one-third of participants. In addition, lifestyle habits and sexual behaviors are difficult to accurately quantify, and underreporting due to social desirability biases might lead to residual confounding. Further, this is a cross-sectional study and one could not establish cause-and-effect relationship due to the possibility of reverse causality. Also, the "gold standard" collection and testing method for oral HPV is not yet widely recognized—some studies collected exfoliated cells from various sites of oral mucosa [30], while others used oral rinses and gargles [29]. Finally, the proportion of participants aged 25–34 years in the present study was relatively small, and this might reduce the reported estimate of alpha-HPV infection of the oral cavity because they are sexually more active. The lower proportion could, firstly, be explained by the fact that older participants usually report more favorable evaluation of health care than younger individuals [42]. Second, younger subjects often have better perception of their own health and lower prevalence of self-reported health problems, and hence are less motivated to participate in clinical research [43]. Third, the social exchange and benefit-cost theories stat that everyday commodities and shortage of time are among the main burdens for individuals' participation of nonincentive research [44]. Our sample-collection sessions were only open during office hours. The time-effectiveness of joining our study was lower for people of working age than that for the older population.

The findings of our study have important public health and research implications. The factors found to be independently associated with oral HPV infection could form the target for future population-based disease prevention and education programs, for example daily teeth brushing. We recommend that future prospective longitudinal studies examine the rate of acquisition, persistence, and clearance of oral HPV infection. In addition, identifying cutaneous types, if any, that tend to persist and cause mucosal disease is worthy of study.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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