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Diversity of human papillomavirus in the anal canal of men: The HIM study

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Abstract

Human papillomavirus (HPV) infections are associated with development of anogenital lesions in men. There are no reports describing the distribution of non-alpha HPV types in the anal canal of a sexually diverse men group. The HIM (*HPV in Men*) Study is a multicenter study of the natural history of HPV infection in Brazil, Mexico and USA. At baseline, 12% of anal canal specimens PCR HPV-positive were not typed by the Roche Linear Array and were considered unclassified. Our goal was characterizing HPVs among these unclassified specimens at baseline and assess associations with participant socio-demographic and behavioral characteristics. Unclassified HPVs were typed by sequencing amplified PGMY09/11 products or cloning of PGMY/GP+ nested amplicons followed by sequencing. Further analysis was conducted using FAP primers. Of men with unclassified HPV at the anal canal, most (89.1%) were men who have sex with women (MSW). Readable sequences were produced for 62.8% of unclassified specimens, of which 75.2%

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Conflict of Interest

ARG receives research funding from Merck Sharp & Dohme Corp. LLV and ARG are consultants of Merck Sharp & Dohme Corp. for HPV vaccines. None of the other authors have conflicts of interest to report.

Ethical approval

Ethical approval was given by the University of South Florida (IRB# 102660), Ludwig Institute for Cancer Research, Centro de Referência e Treinamento em Doenças Sexualmente Transmissíveis e AIDS, and Instituto Nacional de Salud Pública de México.

were characterized HPV types. A total of 18, 26, and 3 different α-, β- and γ-HPV types were detected, respectively. Compared to older men (45-70 years), α-HPVs were more commonly detected among young men (18-30 years) whereas β-HPVs were more frequent among mid-adult men (31-44 years). β-HPVs were more common among heterosexual men (85.0%) than non-heterosexual men. β2-HPV types composed all β-HPVs detected among non-heterosexual men. The high prevalence of β-HPV in the anal canal of men who do not report receptive anal sex is suggestive of other forms of transmission that do not involve penile-anal intercourse.

Keywords

human papillomavirus; cutaneous HPV; males; HIM Study; prevalence; anal canal; unclassified types

Introduction

Although anal canal cancer is an uncommon disease, incidence is increasing in developed countries [1]. Human papillomavirus (HPV) causes most cases of anal cancers (80-90%), and HPV-16 is the most prevalent type in these tumors (~80%) [2]. Among men, the incidence of HPV-associated anal cancer is highest in men who have sex with men (MSM) and in HIV-infected males [3].

HPV genome consists of a circular double-strand DNA molecule of about 8,000bp. HPV types differ by ≥10% in the complete *L1* gene sequence [4]. To date, 199 different HPV types have been fully sequenced, and nearly all cluster into three genera: Alpha [α]-, Beta [β]- and Gamma [γ]-papillomavirus. Whereas α-HPV types have been mainly isolated from mucosal and genital lesions and thus categorized as mucosal types, β- and γ-HPV types were mostly isolated from the skin and have been grouped together as cutaneous HPV [4]. β- and γ-HPV genera have more genotypes than the α-HPV genus, and partial DNA sequence information points to the existence of hundreds of putative novel HPV types of both viral genera [5-6]. Recent data describe high prevalence of cutaneous HPV at diverse anatomic sites that are different from those in which they were isolated [7-11].

The HIM Study is an ongoing, prospective anogenital HPV natural history study of over 4,000 men aged 18-70 years residing in Brazil, Mexico, and the United States. Baseline anal canal genotype-specific HPV prevalence in this population was 16.3%; another 12.4% of specimens were positive for HPV DNA but could not be classified as any of the 37 genotypes identified by the Roche Linear Array and were grouped as HPV unclassified [12]. Our aim was to characterize HPV types among anal canal HPV unclassified specimens collected at baseline, and evaluate associated socio-demographic and behavioral risk factors.

Materials and Methods

Clinical samples and study design

Men were enrolled in Brazil (São Paulo), Mexico (Cuernavaca), and the USA (Tampa) between 2005 and 2009, reported no prior diagnosis of anogenital warts or cancers, and had no recent symptoms of or treatment for a sexually transmitted infection, including HIV/

AIDS. Men completed a pre-enrollment (baseline) visit, were enrolled on completion of their second (enrollment) visit two weeks post-baseline, and afterward followed every six months for up to four years. Details of the HIM Study are described elsewhere [12-13]. This cross-sectional analysis included the 3524 men who completed their baseline visit between September 2005 and June 2009 and consented to collection of anal canal exfoliated cells. The ethics committees of participating hospitals and institutions approved all study procedures, and participants provided written informed consent to the study protocol.

At baseline men completed 88-item computer-assisted self-interview covering information about demographic characteristics, substance use, and sexual behaviors. Specimens were obtained from the genital area using Dacron swabs (Digene, Gaithersburg, MD, USA). Using a separate swab, exfoliated cells from between the anal verge and the dentate line were collected. All staff collecting anal canal samples was trained to avoid touching the swab to the perianal skin. All samples were placed in standard transport medium and stored at −80°C until HPV testing.

HPV detection

DNA extraction was conducted using the QIAamp Media MDx Kit (Qiagen, Valencia, CA, USA). Samples were tested for PCR amplification with PGMY09/11 primers and HPV genotyping was conducted using the Roche Linear Array (Roche Molecular Diagnostics, Alameda, CA, USA) that is able to discriminate 37 α-HPV types (oncogenic types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59,and 66; nononcogenic types 6, 11, 26, 40, 42, 53, 54, 55,61, 62, 64, 67–73, 81–84, IS39, CP6108) [14]. Samples that tested PCR-positive and Linear Array-negative with all specific HPV probes were considered unclassified and included in the present study.

Unclassified HPV characterization

Purified HPV DNA was initially genotyped by direct sequencing of PGMY09/11 PCR amplimers or cloning of these fragments followed by sequencing. Next, 1μl of PGMY09/11 negative products were used in a nested PCR using GP5+/6+ primers [15]; positive samples were sequenced following cloning. Finally, nested PCR negative samples were submitted to a novel amplification reaction employing FAP59/64 primers [16], and positive samples were analyzed exclusively by direct sequencing. AmpliTaq Gold polymerase (Perkin-Elmer, Foster City, CA, USA) was used in all PCRs. Purification of the amplimers using the EXO SAP-IT (GE Healthcare, Buckinghamshire, UK) preceded sequencing. Sequencing was conducted in an ABI 3130XL Genetic Analyzer (AB Applied Biosystems, CA, USA) using the BigDye Terminator v3.1 Cycle Sequencing kit (AB Applied Biosystems, CA, USA). Sequence identity was determined through comparison with the BlastN database.

Statistical analysis

Men were categorized as men who have sex with women (MSW), men who have sex with men (MSM), men having sex with both men and women (MSMW), and men who denied having any sex based on their self-reported recent (prior 3-6 months) and lifetime penetrative sexual behavior [17]. Specimens were classified in one of five categories: HPV-negative, HPV-positive for a characterized type, HPV-positive for an uncharacterized type,

untyped (i.e., inconclusive) or inadequate (human beta-globin PCR negative). Characterized HPV types were additionally classified according to genus and species. Differences in socio-demographic and behavioral risk factors considered to be associated with the presence of a specific HPV genus (α-, β-, or γ-), and species (β-1 or β-2) were evaluated using the Fisher's exact test. All statistical tests were two-sided and attained statistical significance at $\alpha=0.05$. Analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA).

Results

A total of 4,299 men attended the pre-enrollment visit of the HIM study from June 2005-December 2009. Of these, 3,524 men (82.0%) consented to collection of anal canal exfoliated cells (Figure 1). Funding restrictions allowed PCR and genotyping on only the first 1,970 specimens collected. Three persons were eliminated due to reporting HIV infection. Of the remaining 1,967 anal specimens, 1,706 (86.7%) were β-globin positive and available for analysis. Of these 1,706 valid specimens, 1,276 (74.8%) were negative for any HPV DNA, while HPV DNA was detected by PCR or Roche Linear Array genotyping in the remaining 430 specimens: 213 were PCR positive and Roche Linear array negative (unclassified specimens), 14 of which had insufficient volume of DNA leaving 199 available for sequencing. HPV unclassified specimens included 55, 71, and 73 men from the USA, Brazil and Mexico, respectively. During statistical analysis, one participant was excluded due to age inclusion criteria leaving a total of 198 participants retained in final analyses. Although slight differences were observed in participants' characteristics by country, most men with unclassified HPV were young (18-30 years, 37.9%) or mid-adult (31-44 years, 40.9%), married (45.0%), and men who have sex with women (MSW) (84.6%) (Table 1). A large proportion of men reported having had sexual intercourse with 3-9 lifetime female sexual partners (42.8%).

For unclassified HPV characterization, a broad spectrum of viral types from α-, β-, and γ-HPV genera were identified. Successful DNA sequences were obtained from 125 (62.8%) samples; 31 (24.8%) were considered uncharacterized once sequence comparison analysis indicated elevated identity to partial HPV nucleotide sequences in GenBank although no type designation has been made by now. We were able to successfully type 94 (75.2%) of the 125 sequenced specimens: 26 (27.7%), 63 (67.0%), and 5 (5.3%) belonged to the α-, β-, and γ-HPV genus, respectively (Table 2). The most common α-HPV types were HPV-74 (4.3%) and HPV-13 (3.2%), the most common β-HPV types were HPV-22 (9.6%), HPV-107 and HPV-120 (7.4% each), and the most prevalent γ-HPV types were HPV-147 and HPV-130 (2.1% each) (Figure 2). Some differences in HPV type-specific distribution were observed across countries. For example, γ-HPV types were not detected in specimens from the USA, HPV-13 was only detected in Mexico, and HPV 74 was not detected in Mexico.

Overall, 25 specimens (12.6%) were considered inadequate due to non-amplification of the human beta-globin gene indicating that DNA extracted was insufficient or degraded (Table 2). Further, 17 (8.6%) were HPV negative using all primers, suggesting spurious amplification during the previous PCR/Linear Array analysis. Direct sequencing of 32 (16.0%) FAP59/64 PCR-positive specimens was inconclusive due to overlapping peak

patterns hindering sequence analysis and thus HPV genotyping. Data from the three centers were combined for the remaining analyses that included 125 specimens for which readable DNA sequences were generated.

No risk factors were significantly associated with the detection of a specific HPV genus or species. Nevertheless, compared to older men (45-70 years), α-HPVs were more commonly detected among young men (18-30 years) whereas β-HPV types were more prevalent in mid-adult men (31-44 years) (p=0.06) (Table 3). Additionally, compared to α-HPVs detection, β-HPVs were non-significantly more prevalent among MSW than MSM (p=0.4) and among men who reported never using condom during vaginal sex within the past three months compared to those who said they always used a condom (p=0.3). Interestingly, all β-HPV detected among MSM and MSMW were from β-2 species (Table 3).

Discussion

Recently, the prevalence of anal β-HPV infection and type distribution was analyzed among Slovenian MSM of which 17% were HIV positive and β-HPV was detected in 65.2% of specimens [9]. To our knowledge this is the first report to describe the distribution of cutaneous HPVs in the anal canal of men from three countries, most of whom are MSW, and to investigate risk factors associated with β-HPV detection. We analyzed 199 anal canal samples obtained from the HIM study at the baseline visit that had been categorized as unclassified HPV specimens [12]. A total of 18, 26, and 3 different α-, β- and γ-HPV types were detected, respectively. β-HPVs were more common among heterosexual men (85.0%) than non-heterosexual men.

Unreadable sequences (and thus inconclusive typing) were generated after direct sequencing of FAP54/69 PCR products of 16% of the specimens for which overlapping peak patterns in sequencing chromatograms were obtained suggesting the presence of multiple HPV types. This is a common finding when using these primers for a broad range of β-HPVs, and it has been shown by DNA sequencing of a number of clones or by Luminex methodology that these in fact correspond to multiple infections. It has been shown not only in the normal skin and cutaneous tumors [8], but also in the oral cavity and the nasal mucosa [7, 8] that multiple detection of β-HPVs is more frequent than that of α-HPV types. Collectively these findings point toward cutaneous HPV types being a commensal component of the microbiological flora of the human skin throughout the body [18,19].

Nucleotide sequences of 31 anal canal specimens corresponded to incomplete FAP59/64 sequences previously deposited in the GenBank database (uncharacterized HPV types). In fact, more than 200 different presumed new HPV types are waiting for full genome characterization of which the majority clusters within the γ-HPV genus [4-6]. Some differences were observed regarding the prevalence of individual types among the three geographical locations. Additionally, as previously observed for the 37 α-HPV types that can be identified with the Linear Array, higher diversity of β- and γ- genotypes was detected in Brazil and Mexico as compared to the USA [20].

Overall, anal canal unclassified HPV detection at baseline visit was more common in young and middle-aged adults, married men, and MSW reporting 3-9 female lifetime sexual partners. In a previous study enclosing 222 MSW from two USA cities, overall HPV infection was 24.8%, and another 7.7% of men had unclassified infection at anal sites [21]. Unfortunately, risk factor analysis was restricted to men with HPV infections that could be characterized by the Linear Array precluding any comparisons.

In this study, α-HPVs were more common among young men (18-30 years) while middle-aged (45-70 years) men most frequently harbored β-HPV types. Further, as compared to cutaneous HPV types, α-HPV types were more commonly detected among previously unclassified specimens obtained from MSM and MSWM. In fact, for this population it has been reported that HPV infection prevalence among MSM was 4-10 times higher than among MSW for HPV types discriminated by the Linear Array while MSW and MSM had virtually the same prevalence of unclassified HPV [12]. Although small sample size may explain the lack of sufficient power to identify risk factors for β-HPV infection, we cannot exclude the possibility of other unmeasured factors being associated for anal β-HPV detection. For example, the questionnaire completed by HIM participants focused mostly on penetrative sexual practices and did not ask questions about masturbation and non-penetrative sexual activities. It is plausible that direct digital/objects contact may favor viral transmission of cutaneous HPV to the anal area. Actually, evidence of hand transmission to the anogenital region has been reported for α-HPV types [22], and α-HPV has been detected on fingers of women infected at the cervix [23]. The possibility remains that the detection of β-HPVs in the anal canal may represent deposition of virions shed from other body sites.

This study was designed to identify HPV types that were detected in the anal canal of men and grouped together as unclassified HPV types at baseline. Thus potential limitations of this study include the exclusion of specimens for which an HPV type was assigned by Linear Array, although these specimens may also co-harbor β- and/or γ-HPV types. Co-detection of α- and β-HPV types was commonly observed in the oral cavity [7] and in penile condylomas [24].

We recently reported the broad distribution of β- and γ-HPV types in genital specimens of men from the HIM Study [11,25]. We also observed among these men that β-HPV DNA is prevalent in 61.1% of external genital lesions (EGL) and that most viral DNA is detected on the normal genital skin before EGL development [26].

Although largely detected throughout the human body, the role of cutaneous HPVs in the development of lesions and skin cancer has not been determined. Some studies indicate that skin infected with β-HPV types harbor increased susceptibility to ultraviolet radiation induced DNA damage, which may precede skin cancer development. Currently however it is only accepted that HPV-5 and HPV-8 are associated with benign and malignant lesions of the cutaneous disease *epidermodysplasia verruciformis* [27]. Additionally, the mere detection of HPV DNA by PCR from healthy skin not necessarily corresponds to active viral infection.. It has been hypothesized that true infections should contain viral transcripts as an indication of viral activity. Interestingly, α- and β-HPV DNAs were detected in the oral cavity, but only α-HPV transcripts were present in cancer specimens [28].

This study provides evidence that cutaneous HPV is a common finding in the anal canal in men. At present it is not known if β-HPV types influence lesion development in the anogenital region of men; a possible role of β- and γ-HPV as co-factors in the oncogenesis of anogenital cancer may be hampered by ubiquity of these viral types in the skin surface. Larger studies with extended follow-up in MSM and MSW will contribute to this knowledge and may help to evaluate strategies for prevention of anal cancer in men.

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Abbreviations

HPV	Human papillomavirus
HIM Study	*HPV Infection in Men Study*
β-HPV	beta-HPV
α-HPV	alpha-HPV
γ-HPV	gamma-HPV
EGL	external genital lesions
MSM	men who have sex with men
MSW	men who have sex with women
MSMW	men who have sex with men and women

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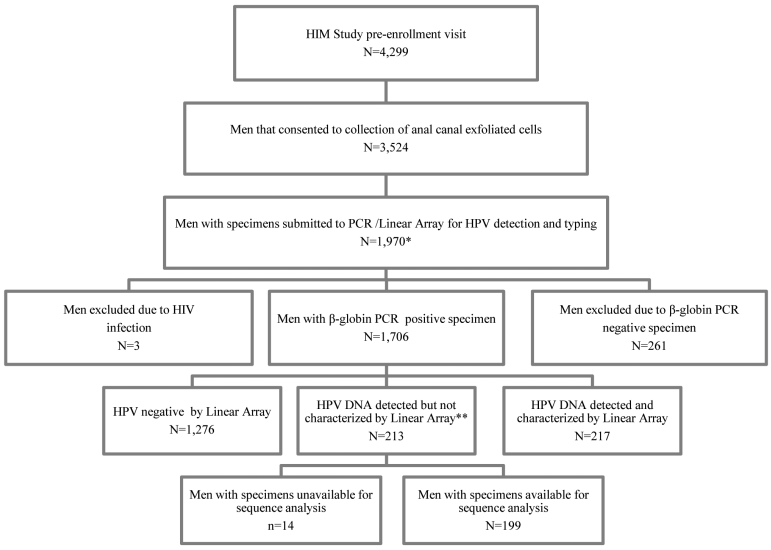

Figure 1. Diagram delineating selection of specimens from the HIM Study pre-enrollment visit included in this analysis

*First group of samples submitted to HPV detection and typing; **A positive PCR sample that did not hybridize to any specific HPV probe.

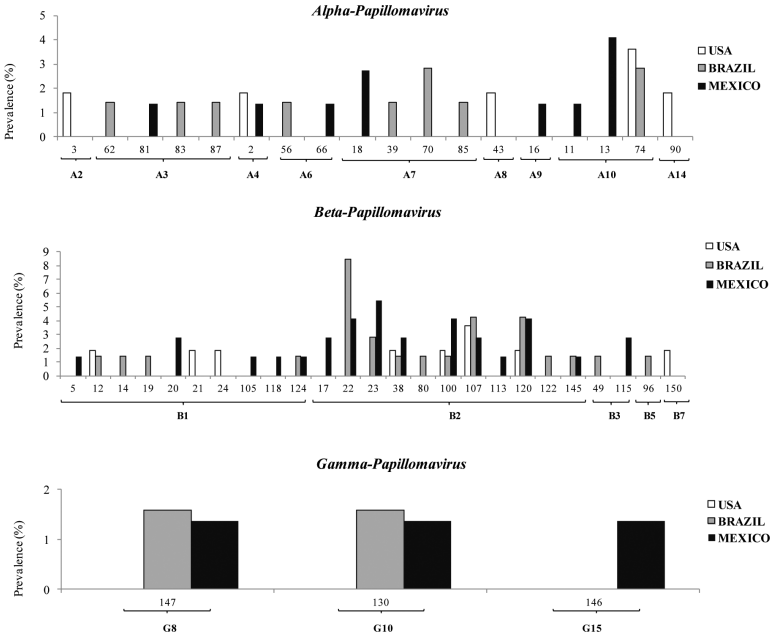

Figure 2. HPV type distribution by HPV genus and species (on x-axis) among unclassified anal canal specimens of the HIM study at baseline (n=94)

Table 1

Characteristics of HIM Study participants with unclassified HPV DNA at the anal canal.

Characteristic	USA (n=55) n (%)	Brazil (n=70) n (%)	Mexico (n=73) n (%)	Total (n= 198) n (%)	P value[a]
Age					0.0031
18-30	29 (52.6)	27 (38.6)	19 (26.0)	75 (37.9)	
31-44	13 (23.7)	34 (48.6)	34 (46.6)	81 (40.9)	
45-70	13 (23.7)	9 (12.8)	20 (27.4)	42 (21.2)	
Median	24	34	39	35	
Race					<0.001
White	36 (65.4)	44 (65.7)	0 (0.0)	80 (40.4)	
Black	12 (21.8)	19 (28.3)	0 (0.0)	31 (15.7)	
Mixed/other	7 (12.8)	4 (6.0)	73 (100.0)	84 (42.4)	
Refused/ missing				3 (1.5)	
Ethnicity					<0.001
Spanish/Hispanic/Latino	4 (9.5)	25 (36.2)	70 (100.0)	99 (50.0)	
Non-Hispanic	38 (90.5)	44 (63.8)	0 (0.0)	82 (41.4)	
Refuse/missing				17 (8.6)	
Marital Status					<0.001
Single, never married	30 (55.6)	23 (32.9)	12 (16.7)	65 (32.8)	
Married	14 (25.9)	27 (38.6)	48 (66.7)	89 (45.0)	
Cohabiting	2 (3.7)	12 (17.1)	10 (13.9)	24 (12.1)	
Divorced/separated/widow	8 (14.8)	8 (11.4)	2 (2.7)	18 (9.1)	
Refuse/missing				2 (1.0)	
Education					<0.001
Less than 12	4 (7.3)	14 (20.0)	40 (54.8)	58 (29.3)	
Completed 12 years	11 (20.0)	30 (42.8)	13 (17.8)	54 (27.3)	
13-16 years	37 (67.3)	22 (31.4)	15 (20.6)	74 (37.4)	
17 or more years	3 (5.4)	4 (5.7)	5 (6.8)	12 (6.1)	
Sexual Orientation					0.036
MSW	49 (89.1)	53 (76.8)	62 (88.6)	164 (84.6)	
MSM	2 (3.6)	4 (5.8)	0 (0.0)	6 (3.1)	
MSMW	0 (0.0)	8 (11.6)	3 (4.3)	11 (5.6)	
No Sex	4 (7.3)	4 (5.8)	5 (7.1)	13 (6.7)	
Lifetime number of female partners					0.002
No sex	7 (21.9)	6 (9.2)	5 (7.2)	18 (10.9)	
1-2	7 (21.9)	8 (12.3)	10 (14.5)	25 (15.0)	
3-9	11 (34.3)	20 (30.8)	40 (58.0)	71 (42.8)	
10-1,000	7 (21.9)	31 (47.7)	14 (20.3)	52 (31.3)	
Lifetime number of male anal sex partners					0.005
No sex	52 (94.5)	49 (72.1)	66 (91.7)	167 (85.7)	
1-2	3 (5.5)	8 (11.8)	4 (5.5)	15 (7.7)	

Characteristic	USA (n=55) n (%)	Brazil (n=70) n (%)	Mexico (n=73) n (%)	Total (n= 198) n (%)	P value[a]
3-9	0 (0.0)	5 (7.3)	1 (1.4)	6 (3.1)	
10-1,000	0 (0.0)	6 (8.8)	1 (1.4)	7 (3.5)	

MSW, men who have sex with women; MSM, men who have sex with men; MSWM, men who have sex with men and women.

[a] P value using Fisher's exact test.

Table 2

HPV type distribution of previously unclassified HPV infections, by country.

	USA (n=55)		Brazil (n=71)		Mexico (n=73)		Total (n=199)	
	n	**%**	**n**	**%**	**N**	**%**	**N**	**%**
Any HPV type[a]	21	38.2	50	70.5	54	74.0	125	62.8
Uncharacterized HPV	6	28.6	13	26.0	12	22.2	31	24.8
Characterized HPV	15	71.4	37	74.0	42	77.8	94	75.2
Alpha(α)-HPV	6	40.0	10	27.0	10	23.8	26	27.7
Beta(β)-HPV	9	60.0	25	67.6	29	69.1	63	67.0
Gamma(γ)-HPV			2	5.4	3	4.1	5	5.3
HPV negative	11	20.0	3	4.2	3	4.1	17	8.6
inadequate sample	15	27.3	3	4.2	7	9.6	25	12.6
Inconclusive[b]	8	14.5	15	21.1	9	12.3	32	16.0

[a] Characterized and uncharacterized types.

[b] Sequences could not be interpreted.

Table 3

Prevalence of Alpha- and Beta-HPVs among unclassified anal canal specimens at baseline, by age and by sexual behavior characteristics.

		Beta-species			
		ALL Beta	B1	B2	Other Beta
	ALL Alpha[a]				
	n (%)	n (%)	n (%)	n (%)	n (%)
Age[b]					
18-30	**14 (53.5)**	**18 (29.0)**	**5 (38.5)**	**11 (25.0)**	**2 (40.0)**
31-44	**7 (30.2)**	**32 (51.6)**	**6 (46.1)**	**25 (56.8)**	**1 (20.0)**
45-70	**5 (16.3)**	**12 (19.4)**	**2 (15.4)**	**8 (18.2)**	**2 (40.0)**
Sexual Orientation					
No Sex	**2 (8.4)**	**3 (5.0)**	**1 (7.7)**	**1 (2.4)**	**1 (20.0)**
MSW	**17 (70.9)**	**51 (85.0)**	**12 (92.3)**	**35 (83.3)**	**4 (80.0)**
MSM	**2 (8.4)**	**2 (3.3)**	**0 (0.0)**	**2 (4.8)**	**0 (0.0)**
MSMW	**3 (12.3)**	**4 (6.7)**	**0 (0.0)**	**4 (9.5)**	**0 (0.0)**
Lifetime number of female partners					
No sex	**3 (15.0)**	**4 (7.1)**	**1 (8.3)**	**2 (5.0)**	**1 (25.0)**
1-2	**4 (20.0)**	**8 (14.3)**	**0 (0.0)**	**8 (20.0)**	**0 (0.0)**
3-9	**5 (25.0)**	**25 (44.7)**	**6 (50.0)**	**18 (45.0)**	**1 (25.0)**
10-1,000	**8 (40.0)**	**19 (33.9)**	**5 (41.7)**	**12 (30.0)**	**2 (50.0)**
Number of female partners in the past 3 months					
None	**11 (55.0)**	**23 (42.6)**	**3 (27.3)**	**18 (45.0)**	**2 (66.7)**
1	**4 (20.0)**	**20 (35.7)**	**5 (45.4)**	**14 (35.0)**	**1 (33.3)**
≥2	**5 (25.0)**	**11 (19.7)**	**3 (27.3)**	**8 (20.0)**	**0 (0.0)**
Lifetime number of male anal sex partners					
No sex	**17 (68.0)**	**54 (87.1)**	**13 (100.0)**	**36 (81.8)**	**5 (100.0)**
1-2	**3 (12.0)**	**3 (4.8)**	**0 (0.0)**	**3 (6.8)**	**0 (0.0)**
3-9	**0 (0.0)**	**3 (4.8)**	**0 (0.0)**	**3 (6.8)**	**0 (0.0)**
10-1,000	**5 (20.0)**	**2 (3.3)**	**0 (0.0)**	**2 (4.6)**	**0 (0.0)**
Number of male anal sex partners in the past 3 months					
None	**21 (84.0)**	**60 (96.8)**	**13 (100.0)**	**42 (95.4)**	**5 (100.0)**
1	**0 (0.0)**	**1 (1.6)**	**0 (0.0)**	**1 (2.3)**	**0 (0.0)**
≥2	**4 (16.0)**	**1 (1.6)**	**0 (0.0)**	**1 (2.3)**	**0 (0.0)**
Condom use during vaginal sex in the past 3 months					
always	**3 (15.0)**	**6 (10.6)**	**1 (8.3)**	**5 (12.2)**	**0 (0.0)**
sometimes	**7 (35.0)**	**19 (33.3)**	**4 (33.3)**	**15 (36.6)**	**0 (0.0)**
never	**4 (20.0)**	**22 (38.6)**	**6 (50.0)**	**14 (34.2)**	**2 (50.0)**

		Beta-species			
		ALL Beta	B1	B2	Other Beta
	ALL Alpha[a]				
	n (%)	n (%)	n (%)	n (%)	n (%)
no recent vaginal sex	**6 (30.0)**	**10 (17.5)**	**1 (8.3)**	**7 (17.0)**	**2 (50.0)**
Condom use during anal sex in the past 3 months					
always	**3 (18.7)**	**7 (24.1)**	**2 (40.0)**	**5 (22.7)**	**0 (0.0)**
sometimes	**3 (18.7)**	**1 (3.5)**	**0 (0.0)**	**1 (4.6)**	**0 (0.0)**
never	**3 (18.7)**	**7 (24.1)**	**1 (20.0)**	**5 (22.7)**	**1 (50.0)**
no recent anal sex	**7 (43.8)**	**14 (48.3)**	**2 (40.0)**	**11 (50.0)**	**1 (50.0)**

[a] Differences in Alpha and Beta HPV types, and in beta-1 and beta-2 HPV types by exposure were assessed by Fisher exact test.

[b] All p values were >0.10 with the exception of age (p=0.06) that was different among men in which we detected Alpha versus Beta HPV types.