Human Papillomavirus Prevalence and Type Distribution in Male Anogenital Sites and Semen

Carrie M. Nielson,[1] Roberto Flores,[2] Robin B. Harris,[1] Martha Abrahamsen,[2] Mary R. Papenfuss,[2] Eileen F. Dunne,[3] Lauri E. Markowitz,[3] and Anna R. Giuliano[2]

[1]Arizona Cancer Center and Mel and Enid Zuckerman College of Public Health, Tucson, Arizona; [2]H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida; and [3]Division of STD Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract

Background: **Human papillomavirus (HPV) is sexually transmitted and causes cervical cancer. Although HPV can infect men and women, little is known about infection in men. Specifically, the prevalence of type-specific HPV infection and the distribution of infections by anogenital anatomic site in men are incompletely characterized.**
Methods: **We tested 463 men ages 18 to 40 years for HPV at the glans/corona, penile shaft, scrotum, urethra, perianal area, anal canal, and in a semen sample. Eligible men acknowledged no history of genital warts and had sexual intercourse with a woman within the past year. HPV testing by PCR and reverse line blot genotyping for 37 types was conducted on each of the specimens from the seven sampling sites.**
Results: **When HPV results from any sampling site were considered, 237 (51.2%) men were positive for at least one oncogenic or nononcogenic HPV type, and another 66 (14.3%) men were positive for an unclassified HPV type. The types with the highest prevalence were HPV-16 (11.4%) and 84 (10.6%). External genital samples (glans/corona, shaft, and scrotum) were more likely than anal samples to contain oncogenic HPV (25.1% versus 5.0%). HPV-positive penile shaft and glans/corona samples were also more likely to be infected with multiple HPV types than other sites.**
Conclusions: **More complete anogenital sampling and sensitive detection for 37 HPV types resulted in a higher HPV prevalence in primarily asymptomatic men than reported previously. The penile shaft was the site most likely to be HPV positive and harbored the greatest proportion of multiple type and oncogenic infections. These results have implications for research of HPV among men and transmission between partners.** (Cancer Epidemiol Biomarkers Prev 2007;16(6):1107–14)

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection (1) and is the necessary cause of cervical cancer. Approximately 60 HPV genotypes are known to infect the genital tract, at least 13 of which are considered ''high risk'' or oncogenic (2, 3). Although infection is most often asymptomatic and transient, oncogenic genotypes of HPV are strongly associated with development of cervical cancer and are associated with other anogenital cancers in both men and women to varying degrees (4). Nononcogenic HPV types cause genital warts and other benign lesions (5).

Several studies have provided estimates of HPV prevalence among men; however, these estimates range widely from 0% to 73% (6). Significant barriers to the synthesis and interpretation of these studies are the inconsistency of sampling techniques used, the variety and combinations of sites and specimens sampled, and the testing for different HPV types. Few studies have assessed multiple specimens from different anatomic sites. Recent studies among university students or sexually transmitted disease (STD) clinic attendees in the United States found HPV in 28% to 43% of men (7-9). Studies with similar testing strategies in male partners of women with HPV-related cervical abnormalities reported prevalences of ~70% (10, 11).

Comparisons of the proportion of HPV-positive results among the various sampling sites and specimens have been reported recently; however, the interpretation of these studies is limited by small sample sizes and differences in methods of collection or anatomic sites and specimens (e.g., urine or semen; refs. 8, 9, 11-20). Consistently, the prepuce has the highest proportion of HPV-positive samples. The shaft, perhaps because of its greater surface area, also usually yields more HPV-positive samples than, for example, the urethra, glans/corona, or scrotum samples (6).

To further the understanding of male HPV infection and the prevention of infection in men, as well as transmission to women, it is necessary to assess HPV infection at several different anatomic sites of the male genital tract. In the current study, genital HPV infection in primarily asymptomatic men was determined in six anatomic sites and semen. HPV type-specific prevalence estimates were compared across sample types.

Materials and Methods

Study Design. A cross-sectional study of HPV infection was conducted in Tucson, Arizona, from 2003 to 2006 and Tampa, Florida, in 2005. Men were eligible to join the study if they (*a*) were between 18 and 40 years old, (*b*) had sexual intercourse with a woman within the past year, (*c*) acknowledged no previous diagnosis of genital warts or penile or anal cancer, (*d*) had no current penile discharge or pain during urination, and (*e*) had no current diagnosis of a STD. Primary methods of recruitment were through advertisements in city and university newspapers, flyers in public places, and in-person

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Requests for reprints: Anna R. Giuliano, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, MRC 2067D Tampa, FL 33612. Phone: 813-903-6820; Fax: 813-745-1328. E-mail: giuliano@moffitt.usf.edu

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recruitment at the local air force base and the county health department STD clinic.

All participants gave informed consent, and all procedures were approved by the University of Arizona Human Subjects Protection Program, Centers for Disease Control and Prevention Institutional Review Board, United States Department of Defense, and the University of South Florida Institutional Review Board.

Participants completed a self-administered, scannable questionnaire that included questions on demographic factors (race, ethnicity, age, income, occupation, education, country of origin, and length of U.S. residency), alcohol and tobacco use, age at first sexual intercourse, lifetime number of partners, frequency of sexual intercourse, ever having had sex with a man or having been diagnosed with a STD, and condom use in the past 3 months.

Clinical Sampling. Men were asked (*a*) not to have sex 24 h before the clinical visit to avoid detecting HPV from partners and (*b*) not to wash the genitals the morning of the visit. Men who indicated that they had not complied with these instructions were rescheduled for a later sampling visit. The study clinician examined each participant's genital, abdominal, and anal areas and recorded the number and location of any lesions or warts. These were sampled by rubbing with a saline-wetted Dacron swab and stored as described below. The clinician also recorded the presence and location of any erythema, abrasions, rashes, inflammation, discharge, or piercings in the same regions and whether the participant was circumcised. The study clinician used a calcium alginate or Dacron urethral swab to sample the urethral epithelium. The swab was inserted ~2 cm into the urethra and rotated 360 degrees while removing it. The clinician sampled the other anogenital sites by rubbing separate saline-wetted Dacron swabs over the entire surface of the (*a*) glans penis/coronal sulcus, (*b*) penile shaft (including prepuce, if present), (*c*) scrotum, and (*d*) perianal area. Another saline-wetted Dacron swab was inserted into the anal canal up to the anal verge. Each of these six swabs was placed in a separate collection tube filled with 250 μL (urethral swab) or 350 μL (all other swabs) specimen transport medium (Digene Corp.). Men were instructed to collect a semen sample by masturbation 12 to 36 h before the clinical sampling visit, not to touch the inside of the cup, and to refrigerate the sample until the clinical visit. Collection tubes were labeled with the participant's study identification number, date of collection, and specimen type and were stored at −20°C. Samples were collected by one of five study clinicians: 74% of men were sampled by the primary clinician in Tucson; 21% by the primary clinician in Tampa; and 5% by three additional clinicians in Tucson.

To improve recruitment, the urethral sample was made optional midway through the recruitment period. In addition, a preliminary analysis revealed that the urethral and semen samples did not significantly contribute to overall HPV prevalence and were eliminated in the 3rd year of the study. The anal canal sample was added after the study began; therefore, this sample was not provided by the first 58 men.

HPV DNA Detection and Genotyping. HPV testing of swabbed cellular material and semen was conducted using PCR for amplification of a fragment of the *L1* gene (21). DNA extraction was done using the QIAamp DNA Mini kit (Qiagen) according to the instructions of the manufacturer. Briefly, 200 μL aliquots of clinical material were digested with 20 μL proteinase K for 1 h at 65°C followed by 200 μL lysis buffer. DNA was eluted with 50 μL of 10 mmol/L Tris-EDTA buffer (pH 7.5) at 60°C. DNA was stored at −20°C until use.

Specimens were tested for the presence of HPV by amplifying 5 μL of the DNA extracts with the PGMY09/11 *L1* consensus primer system (21) and AmpliTaq Gold polymerase (Perkin-Elmer). Each 50 μL amplification contained 1× PCR buffer II; 2.5 mmol/L $MgCl_2$; 200 μmol/L (each) dCTP, dGTP, and dATP; 600 μmol/L dUTP; 7.5 units AmpliTaq Gold; 1 μmol/L PGMY09; 1 μmol/L PGMY11; 2.5 nmol/L B_PC04; 2.5 nmol/L B_GH20; and 5 μL of the template. For eventual inclusion of uracil-*N*-glycosylase to prevent product carryover, dTTP was replaced with dUTP. To determine specimen adequacy, the GH20/PC04 human *β-globin* target was coamplified using the B_PCO4 and B_GH20 primers along with HPV consensus primer amplification. For every PCR plate, a negative control (H_2O) and a positive control (CaSki Cells DNA) were run to control for possible contamination and accuracy. The samples were amplified using Perkin-Elmer GeneAmp PCR System 9700. The following amplification profile was used: 95°C hot start for 9 min, 95°C denaturation for 1 min, 55°C annealing for 1 min, and 72°C extension for 1 min for 40 cycles; followed by a 5-min terminal extension at 72°C; and a hold step at 4°C. As a first step in conducting analysis for HPV positivity, all specimens were analyzed after PCR using agarose gel electrophoresis. Briefly, 5 μL PCR product was mixed with 5 μL 2× DNA loading buffer and loaded onto a 2% agarose gel and run in LAE buffer at 100 V for 50 min at room temperature. DNA bands were visualized by UV light, and the presence of HPV was determined to be positive if a band of 450 bp appeared on a gel, corresponding to a defined sequence of nucleotides within the polymorphic *L1* region of the HPV genome. Likewise, positivity for *β-globin* on an agarose gel was determined by the presence of two bands, one corresponding to a size of 150 bp (high *β-globin* band) and a second band of 80 bp (low *β-globin* band).

HPV genotyping was conducted using the reverse line blot method (22) on all samples, regardless of HPV PCR result. This detection method uses the HPV *L1* consensus PCR products labeled with biotin to detect 37 HPV types. The HPV genotype strip contains 39 probe lines, detecting 37 individual HPV genotypes and two concentrations of the *β-globin* control probe (Roche Molecular Diagnostics). The PCR products labeled with biotin were denatured and added to the probe strip in a hybridization buffer. After strips were washed, a streptavidin-horseradish peroxidase conjugate was added to facilitate detection of the various HPV types. After final wash, buffer was removed by vacuum aspiration, and strips were rinsed in 0.1 mol/L sodium citrate. Color development was activated by incubation in a mixture of hydrogen peroxide in sodium citrate buffer and tetramethylbenzidine in dimethylformamide for 5 min on a rotating platform (70 RPM). Developed strips were interpreted and photographed for future reference. Strip interpretation was done with a labeled overlay, with lines indicating the position of each probe relative to the reference mark.

Definition of Outcomes. The oncogenic HPV types associated with cervical dysplasia and cancer included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 (3). The other (non-oncogenic) types detected with the line blot methodology of Roche were 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108. The presence of any HPV DNA was defined as a positive result by PCR or by genotyping. *β-globin*–negative samples that were positive for HPV by PCR and/or genotyping were included as HPV positive. The presence of HPV DNA in the absence of *β-globin* can be explained by DNA degradation that has occurred in these postapoptotic desquamated cells. The absence of any HPV DNA was defined as a negative result by PCR and genotyping in a sample positive for human *β-globin*. Samples without detection of *β-globin* or HPV were deemed inadequate for evaluation and treated as missing. If the sample was positive for HPV by PCR but none of the 37 types was found in genotyping, the HPV type was classified as ''other type/unclassified.''

Statistical Analysis. To determine the overall prevalence of HPV, a man was considered to have HPV infection if any of his samples were positive for any HPV type or for an unknown type. Similarly, the prevalence of oncogenic, nononcogenic, unclassified, and multiple types was calculated, using the same denominator.

To determine whether there were differences in infection depending on anatomic region or specimen examined, the proportion HPV positive and binomial exact 95% confidence intervals were calculated for oncogenic types, nononcogenic types, unclassified, and multiple types. Furthermore, the proportions of HPV types 16, 18, 6, and 11 and those HPV types that occurred in 5% or more of men were tested across anatomic sites and semen. Two-sample tests of proportions were carried out to compare each site/specimen with the penile shaft. Analyses were conducted using Intercooled Stata 9.1 for Windows (Stata Corp.).

Results

Of 1,674 men who were approached for recruitment, 768 completed the screening questions and were eligible. Of those eligible, 546 (71.1%) attended the first, enrollment visit, at which time they consented to the study and picked up a specimen cup for the semen sample, to be collected at home; 493 (64.2%) completed the second, sample collection visit. Samples from the first 30 men were used for optimization of HPV testing and were not included in the prevalence analysis. In total, results from 463 men are included in these analyses.

Demographic characteristics of study participants are presented in Table 1. Men were between the ages of 18 and 40 years, with 49.9% under age 25 years. Most (77.5%) were recruited from Tucson, and 22.5% were recruited from Tampa. Most participants were white in both Tucson (72.7%) and Tampa (60.6%). Approximately 5.3% were black in Tucson, and 13.5% were black in Tampa; Hispanic ethnicity was reported by 17.8% in Tucson and 14.4% in Tampa. Other racial groups were represented by 9 or fewer participants in each city. Most participants (70.6%) were single, never married, and 73.7% had completed at least some college education. The majority (61.4%) of men reported more than five female sexual partners in their lifetime, and 84.0% of men were circumcised. Only 6.4% of men who responded to a question about anal sex with other men indicated they had ever done so, and 20.7% of men reported ever having had a STD. Although men were excluded during screening for eligibility if they acknowledged a history of genital warts, 18 (3.9%) men had warts detected at the clinical visit and were not excluded from the study.

All 463 participants provided at least one sample that was adequate (tested positive for *β-globin*) for HPV DNA testing. Between 93.7% and 95.7% of the external genital site samples and anal samples were adequate, whereas 83.7% of urethral samples and 99.7% of semen were adequate. All 463 men provided penile shaft, glans/corona, scrotum, and perianal swabs; 405 provided the anal canal swab; 344 men provided a semen sample; and 331 provided a urethral swab.

When all HPV results from any anogenital site or semen sample were considered, 237 (51.2%) of men had one or more specific oncogenic or nononcogenic HPV type detected. An additional 66 men had only an unclassified HPV type detected, bringing the total to 303 (65.4%) men who were HPV positive for at least one HPV type (Table 2). By PCR analysis alone, 245 (52.9%) men were HPV positive at any sample. In 58 men, PCR results were negative but genotyping results were positive, increasing the number of positive men by either method to 303 (65.4%). This difference is attributed to the increased sensitivity of genotyping, which is based on DNA hybridization (Southern blot), over gel electrophoresis. Among all men, 29.2% had at least one oncogenic type detected in at least one sample, and 36.3% had one or more nononcogenic types detected. An additional 14.3% had positive HPV results by PCR but not by genotyping, indicating the presence of an unclassified HPV type or a HPV type other than the 37 types detected by our genotyping protocol. More than one HPV type was found from 27.2% of men (Table 2). There were no observable trends in prevalence by age group (data not shown).

Table 1. Characteristics of men in the HPV Detection in Men study (*N* = 463)

	n (%)
Residence	
Tucson, Arizona	359 (77.5)
Tampa, Florida	104 (22.5)
Age (y)	
18-19	50 (10.8)
20-24	181 (39.1)
25-29	90 (19.4)
30-34	63 (13.6)
35-40	79 (17.1)
Race	
White	324 (70.0)
Black	33 (7.1)
Asian/Pacific Islander	19 (4.1)
American Indian/Alaska Native	9 (1.9)
Other/unknown/refused	78 (16.9)
Ethnicity	
Hispanic	79 (17.1)
Non-Hispanic	376 (81.2)
Unknown/refused	8 (1.7)
Marital status	
Single, never married	327 (70.6)
Married	59 (12.7)
Cohabiting	29 (6.3)
Divorced/separated	35 (7.5)
Unknown/refused	13 (2.8)
Education (y)	
<12	117 (25.3)
12-16	240 (51.8)
≥17	101 (21.8)
Unknown/refused	5 (1.1)
Lifetime no. female sex partners	
1-5	158 (34.1)
6-10	92 (19.9)
11-20	105 (22.7)
21 or more	87 (18.8)
Unknown/refused	21 (4.5)
Circumcised (clinician observed)	
Yes	389 (84.0)
No	74 (16.0)
Ever had anal sex with a man	
Yes	16 (3.5)
No	234 (50.5)
Unknown/refused*	213 (46.0)
Ever been diagnosed with an STD	
Yes	96 (20.7)
No	354 (76.5)
Unknown/refused	13 (2.8)

*Due to Institutional Review Board restrictions, this question was not asked to all men.

Site-Specific HPV Type Distribution among HPV-Positive Samples. Table 3 shows type-specific infections by anatomic site. HPV-16 was the most common type detected overall (11.4%). Almost equally as prevalent (10.6%) was HPV type 84. Other oncogenic types, found in 4.5% to 6.0% of men, were types 39, 51, 52, and 59. The prevalence of each remaining oncogenic type was 2.2% or less. After type 84, the most common nononcogenic types were HPV types 62 (6.9%) and CP6108 (8.9%). Only 4.3% to 4.8% of men had HPV types 6, 53, and 68; 3.2% or fewer had the remaining types detected.

Table 2. Prevalence of HPV infection by any site, external genital sites, and anal sites, HPV Detection in Men study (*N* = 463)

	Infection at any site		External genital sites*		Anal sites	
	HPV-positive men, *n* (% of all men)	95% CI for percentage of HPV-positive men	HPV-positive men, *n* (% of all men)	95% CI for percentage of HPV-positive men	HPV-positive men, *n* (% of all men)	95% CI for percentage of HPV-positive men
Any HPV type	303 (65.4)	60.9-69.8	265 (57.2)	52.6-61.8	110 (23.8)	20.0-27.9
At least one oncogenic type†	135 (29.2)	25.1-33.5	116 (25.1)	21.2-29.3	23 (5.0)	3.2-7.4
Oncogenic type(s) only‡	49 (10.6)	7.9-13.7	45 (9.7)	7.2-12.8	14 (3.0)	1.7-5.0
Oncogenic and nononcogenic types‡	86 (18.6)	15.1-22.4	71 (15.3)	12.2-18.9	9 (1.9)	0.8-3.7
At least one nononcogenic or unclassified type†	168 (36.3)	31.9-40.8	149 (32.2)	27.9-36.6	87 (18.8)	15.3-22.7
Nononcogenic type(s) only‡	102 (22.0)	18.3-26.1	95 (20.5)	16.9-24.5	33 (7.1)	5.0-9.9
Unclassified type only‡	66 (14.3)	11.2-17.8	54 (11.7)	8.9-14.9	54 (11.7)	8.9-14.9
Multiple HPV types	126 (27.2)	23.2-31.5	108 (23.3)	19.5-27.4	14 (3.0)	1.7-5.0

Abbreviation: 95% CI, 95% confidence interval.
*Includes glans penis/coronal sulcus, penile shaft, and scrotum.
†The *n* and % in these two rows sum to the top row (categories are complete and mutually exclusive).
‡The *n* and % in these four rows sum to the top row (categories are complete and mutually exclusive).

HPV Type Distribution among HPV-Positive Samples. Among HPV-positive shaft samples, 45.0% had an oncogenic HPV type detected, 64.2% had a nononcogenic HPV type detected, 18.8% had an unclassified HPV type, and 38.5% had multiple HPV type infections (Table 4). When sites of infection were compared, HPV-positive perianal area and anal canal had significantly lower proportions of oncogenic, nononcogenic, and multiple HPV type infection than the shaft and significantly higher proportions of unclassified HPV types. Multiple HPV-type infections were also significantly more likely to occur in the shaft or glans/corona than in the scrotum, anal sites, or urethra (Table 4). HPV-positive shaft samples had a higher proportion of HPV-16 than other anatomic sites and a lower proportion than semen. However, the differences in proportions were not statistically significant (Table 4), and when only genotyped samples are considered, 16.8% of HPV-positive shaft samples are positive for HPV-16, and 13.3% of HPV-positive anal canal samples are positive for HPV-16.

Multiple HPV Types. Approximately one quarter of all men (27.4%), and 41.9% of HPV-positive men, tested positive for multiple HPV types. Most men who had multiple type infection (74.0%) had two or three types detected; however, an additional 33 men tested positive for 4 to 9 different types simultaneously (Table 3). Within a single site, multiple type infection was more likely to occur in the shaft or glans/corona samples (38.5% and 36.3% of HPV-positive samples, respectively) than in the scrotum (21.6%), perianal area (14.1%), or anal canal (11.9%; Table 4). Only two urethral and three semen samples were positive for more than one HPV type. Most (73.6%) of HPV-16–positive men had multiple infections, comprising 30.7% of all men with multiple infections (data not shown).

Discussion

The current study detected an overall HPV prevalence of 65.4% if unclassified types are included and 51.2% if only the 37 specific HPV types tested for are included, both of which are higher estimates than reported previously for asymptomatic men recruited from the general population. Oncogenic HPV infection occurred in 29.2% of men, and nononcogenic infection occurred in 36.3% of men. External genital samples were more likely than anal samples to contain oncogenic or nononcogenic HPV; however, unclassified types were equally as prevalent in both regions.

Generalizability of these results is limited by the self-selection of men into the study. We cannot rule out the possibility that, because men were able to self-refer, a proportion of men joined the study to be tested for HPV because of female partners who had told them of a positive result on a Pap smear or HPV test: 107 (23%) men in the study reported having had a partner who had ever had an abnormal Pap smear. This proportion is similar to that reported by women age 21 years and older who responded to the National Health Interview Survey in the United States, where 20% reported ever having had an abnormal Pap smear (23). The majority (61.4%) of men in this study reported having had more than five female sex partners in their lifetime. This might contribute to the higher prevalence found in this study than in two recent studies that also sampled the entire penis and scrotum and reported HPV prevalence of 41.3% to 44.6% (8, 24). Approximately half (48.5%) of the men in one study reported six or fewer partners (8), and 50.7% of the men in the other study reported fewer than three partners (24). Although men who were recruited from STD clinics may have been more likely to have behaviors related to HPV acquisition and may have contributed to our high HPV prevalence estimate, we believe that our exclusion criteria limit the number of ''high-risk'' men that participated. However, many STDs are asymptomatic in men. The median age of men in this study is 25.1 years, and men over age 40 years were excluded. Including only relatively young men in our study may have resulted in a population sample at higher risk for HPV and other STDs than the general population.

The higher HPV prevalence reported in this study may also be due to the sampling and HPV testing methods unique to this study. First, we collected the largest number of specimens from different anatomic sites and specimens ever reported in men. A recent study conducted in North America among 136 18- to 63-year-old men from the general population sampled five genital sites and measured 37 HPV types, yielding a prevalence of ~42% (8). Although our methods were similar, we added two anal sampling sites, limited our study to men 18 to 40 years old, and genotyped all samples, regardless of PCR result.

Second, we tested each of six anatomic samples and semen individually for HPV DNA. This practice had molecular and statistical influence on the increased likelihood of HPV positivity. Because the PCR could be inhibited by proteins in the sample, individual testing of anatomic sites allowed us a greater chance to detect HPV if it was present in one sample but absent in others than if we had tested pooled sites or

Table 3. Prevalence of type-specific HPV infection by anatomic site or specimen, HPV Detection in Men study (*N* = 463)

	Infection at any site (*n* = 463)	Penile shaft (*n* = 449)	Glans/corona (*n* = 444)	Scrotum (*n* = 441)
	n (%)	*n* (%)	*n* (%)	*n* (%)
Any HPV	303 (65.4)	224 (49.9)	159 (35.8)	151 (34.2)
Oncogenic types				
16	53 (11.4)	41 (9.1)	18 (4.1)	22 (5.0)
18	9 (1.9)	4 (0.9)	3 (0.7)	1 (0.2)
31	8 (1.7)	5 (1.1)	6 (1.4)	4 (0.9)
39	21 (4.5)	16 (3.6)	8 (1.8)	6 (1.4)
45	10 (2.2)	6 (1.4)	4 (0.9)	3 (0.7)
51	28 (6.0)	21 (4.7)	17 (3.9)	13 (3.0)
52	23 (5.0)	16 (3.6)	9 (2.0)	9 (2.1)
56	7 (1.5)	2 (0.5)	2 (0.5)	0 (0.0)
59	21 (4.5)	14 (3.2)	13 (2.9)	11 (2.5)
66	10 (2.2)	5 (1.1)	5 (1.1)	0 (0.0)
Nononcogenic types				
6	22 (4.8)	15 (3.4)	6 (1.4)	8 (1.8)
11	2 (0.4)	2 (0.5)	0 (0.0)	0 (0.0)
42	13 (2.8)	8 (1.8)	8 (1.8)	3 (0.7)
53	20 (4.3)	17 (3.8)	11 (2.5)	7 (1.6)
54	10 (2.2)	6 (1.4)	4 (0.9)	2 (0.5)
55	15 (3.2)	10 (2.3)	8 (1.8)	5 (1.1)
61	14 (3.0)	10 (2.3)	7 (1.6)	3 (0.7)
62	32 (6.9)	22 (5.0)	16 (3.6)	12 (2.7)
67	10 (2.2)	5 (1.1)	6 (1.4)	1 (0.2)
68	20 (4.3)	8 (1.8)	6 (1.4)	11 (2.5)
72	6 (1.3)	4 (0.9)	2 (0.5)	2 (0.5)
73	11 (2.4)	10 (2.3)	8 (1.8)	1 (0.2)
81	9 (1.9)	4 (0.9)	5 (1.1)	1 (0.2)
83	11 (2.4)	8 (1.8)	7 (1.6)	2 (0.5)
84	49 (10.6)	36 (8.1)	26 (5.9)	15 (3.4)
CP6108	41 (8.9)	30 (6.7)	19 (4.3)	21 (4.8)

	Perianal area (*n* = 436)	Anal canal (*n* = 386)	Urethra (*n* = 278)	Semen (*n* = 337)
	n (%)	*n* (%)	*n* (%)	*n* (%)
Any HPV	87 (20.0)	68 (17.6)	28 (10.1)	18 (5.3)
Oncogenic types				
16	8 (1.8)	6 (1.5)	3 (1.1)	5 (1.5)
18	2 (0.5)	4 (1.0)	0 (0.0)	0 (0.0)
31	1 (0.2)	1 (0.3)	0 (0.0)	0 (0.0)
39	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)
45	1 (0.2)	1 (0.3)	1 (0.4)	0 (0.0)
51	3 (0.7)	1 (0.3)	0 (0.0)	1 (0.3)
52	1 (0.2)	2 (0.5)	2 (0.7)	3 (0.9)
56	1 (0.2)	0 (0.0)	2 (0.7)	0 (0.0)
59	3 (0.7)	2 (0.5)	2 (0.7)	3 (0.9)
66	2 (0.5)	2 (0.5)	0 (0.0)	0 (0.0)
Nononcogenic types				
6	1 (0.2)	3 (0.8)	2 (0.7)	0 (0.0)
11	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
42	1 (0.2)	1 (0.3)	0 (0.0)	0 (0.0)
53	3 (0.7)	1 (0.3)	1 (0.4)	0 (0.0)
54	1 (0.2)	1 (0.3)	1 (0.4)	0 (0.0)
55	3 (0.7)	2 (0.5)	1 (0.4)	0 (0.0)
61	3 (0.7)	1 (0.3)	1 (0.4)	0 (0.0)
62	4 (0.9)	3 (0.8)	2 (0.7)	1 (0.3)
67	2 (0.5)	3 (0.8)	2 (0.7)	0 (0.0)
68	9 (2.1)	5 (1.3)	3 (1.1)	0 (0.0)
72	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
73	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)
81	2 (0.5)	1 (0.3)	1 (0.4)	0 (0.0)
83	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
84	3 (0.7)	5 (1.3)	0 (0.0)	3 (0.9)
CP6108	8 (1.8)	6 (1.5)	4 (1.4)	2 (0.6)

	Any site, *n* (%)	Penile shaft, *n* (%)	Glans/corona, *n* (%)	Scrotum, *n* (%)	Perianal area, *n* (%)	Anal canal, *n* (%)	Urethra, *n* (%)	Semen, *n* (%)
Other/unclassified*	66 (14.3)	41 (9.1)	22 (5.0)	38 (8.6)	40 (9.2)	29 (7.5)	3 (1.1)	2 (0.6)
Multiple	127 (27.4)	84 (18.7)	57 (12.8)	32 (7.3)	12 (2.8)	8 (2.1)	2 (0.7)	3 (0.9)
2 types	58 (12.5)	41 (9.1)	35 (7.9)	20 (4.5)	9 (2.1)	5 (1.3)	1 (0.3)	3 (0.9)
3 types	36 (7.8)	27 (6.0)	10 (2.3)	8 (1.8)	2 (0.5)	1 (0.3)	0 (0.0)	0 (0.0)
4 types	17 (3.7)	8 (1.8)	7 (1.6)	2 (0.5)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)
5 types	8 (1.7)	5 (1.1)	4 (0.9)	1 (0.2)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)
6 types	5 (1.1)	2 (0.4)	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.3)	0 (0.0)	0 (0.0)
≥7 types	3 (0.6)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

NOTE: *n* listed in column headers is the number of adequate samples: *β–globin* or HPV positive by PCR, genotyping or both. Due to infection with multiple HPV types, the percentage of infections exceeds 100%. Oncogenic HPV types 33, 35, and 58 and nononcogenic HPV types 26, 40, 64, 69, 70, 71, 82, and IS39 were detected in fewer than five men.
*HPV positive by PCR but not by genotyping.

Table 4. Proportion of specific oncogenic and nononcogenic HPV types by site or specimen, among samples with HPV infection, HPV Detection in Men study (*N* = 463)

	Site of sample						
	Penile shaft (*n* = 218)	Glans/corona (*n* = 157)	Scrotum (*n* = 148)	Perianal area (*n* = 85)	Anal canal (*n* = 67)	Urethra (*n* = 27)	Semen (*n* = 18)
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Oncogenic	98 (45.0)	65 (41.4)	57 (38.5)	18 (21.2)*	17 (25.4)*	10 (37.0)	11 (61.1)
16	40 (18.4)	18 (11.5)	20 (13.5)	8 (9.4)	6 (9.0)	3 (11.1)	5 (27.8)
18	4 (1.8)	3 (1.9)	1 (0.7)	2 (2.4)	4 (6.0)	0	0
51	21 (9.6)	17 (10.8)	13 (8.8)	3 (3.5)	1 (1.5) †	0	1 (5.6)
52	16 (7.3)	9 (5.7)	9 (6.1)	1 (1.2) †	2 (3.0)	2 (7.4)	3 (16.7)
Nononcogenic	140 (64.2)	93 (66.2)	76 (51.4) †	35 (41.2) ‡	28 (41.8)*	17 (63.0)	6 (33.3)*
6	15 (6.9)	6 (3.8)	8 (5.4)	1 (1.2) †	3 (4.5)	2 (7.4)	0
11	2 (0.9)	0	0	0	0	0	0
62	22 (10.1)	16 (10.2)	12 (8.1)	4 (4.7)	3 (4.5)	2 (7.4)	1 (5.6)
84	36 (16.5)	26 (16.6)	15 (10.1)	3 (3.5)*	5 (7.5)	0 †	3 (16.7)
CP6108	28 (12.8)	18 (11.5)	19 (12.8)	8 (9.4)	6 (9.0)	4 (14.8)	2 (11.1)
Other type/unclassified	41 (18.8)	22 (14.0)	37 (25.0) ‡	41 (48.2) ‡	29 (43.3) ‡	3 (11.1)	2 (11.1)
Multiple	84 (38.5)	57 (36.3)	32 (21.6) ‡	12 (14.1) ‡	8 (11.9) ‡	2 (7.4)*	3 (16.7)

NOTE: *n* listed in column headers are those samples that were HPV positive and *β-globin* positive by genotyping.
*Test of difference from shaft proportion, $P < 0.01$.
† Test of difference from shaft proportion, $P < 0.05$.
‡ Test of difference from shaft proportion, $P < 0.001$.

samples. Studies that combined anatomic sites or samples might have increased the concentration of PCR inhibitors and/or diluted the targeted HPV DNA because it is likely that not all sites would have contained HPV (25). In the current analysis, the threshold for classifying a man as HPV positive was detection of HPV in one of seven samples. The larger number of contributed samples in this study increased the likelihood of detecting HPV and therefore of classifying the participant as HPV positive. That is, if a man is positive for HPV at one but not all sites, his chance of being classified as HPV positive increases as more sites are sampled. Other recent studies have tested for HPV in multiple anatomic sites individually and reported some of the highest prevalences in men (15.8-70.0%; refs. 8, 11, 17-19). However, lack of comparability of sampling sites included in these studies and in others wherein samples were pooled from different anatomic sites makes direct comparison difficult.

Third, our HPV DNA testing methods allowed for detection of a greater number of HPV types than many earlier studies. HPV PCR and typing methods used in recent studies have allowed the detection of more HPV types than studies conducted in the 1990s. Among 34 studies published between 1991 and 2006, those published before 2000 used methods for detection of an average of 5.9 HPV types, whereas those published in 2000 or later had the capacity to detect an average of 12.4 HPV types (6). The use of sensitive, standardized genotyping methods to detect at least 27 HPV types can account for higher prevalence estimates. Most of the recent studies reported prevalence estimates >33% (only the South Korea estimate is lower, at 8.7%; refs. 8, 9, 24, 26-29).

Fourth, our system of genotyping based on hybridization and signal amplification on all samples rather than only genotyping samples that were HPV positive by PCR could account for higher HPV prevalence. Genotyping PCR-positive samples only would have resulted in classifying 220 (30.5%) of all HPV-positive samples as negative, resulting in a decrease of the prevalence estimate to 52.9%. The testing algorithm used in this study also allowed for the identification of 15 samples that were positive for HPV but *β-globin* negative. In many recent studies that used a PCR-based method for HPV DNA detection followed by reverse line blot genotyping, it was unclear whether researchers genotyped all samples or only those that were PCR positive. However, in several other studies, the authors do state that only PCR-positive samples were genotyped (7-9, 27, 30, 31). The HPV prevalence among men in these six studies ranges from 8.7% to 42.8%; we might assume that these underestimate the results that would have been obtained if PCR-negative samples had also been genotyped.

The oncogenic HPV types with the highest prevalence were types 16 (11.4%), 51 (6.0%), and 52 (5.0%). Worldwide, HPV type 16 was detected in 53.5% of HPV-positive cervical cancer cases, whereas HPV type 51 occurred in only 1.1% and HPV type 52 in 2.5% of HPV-positive cervical cancer cases (32). Among nononcogenic types, HPV types 84 (10.6%) and CP6108 (8.9%) were more commonly found than either HPV type 6 (4.8%) or 11 (0.4%). It may be of interest to further test for other HPV types, as studies of anal HPV infection among men who have sex with men have reported the presence of HPV types that were not included in our detection method [e.g., 2, 13, 34, 57, and W13B (33) and Pap155, Pap291, and AE2 (34)]. HPV types 16, 45, and 51 have been reported as the most prevalent in women in the United States (35). Although we also found types 16 and 51 to be among the most common types, with 17% of HPV-positive men having HPV-16 and 9% of HPV-positive men having HPV-51, these proportions were not as high as found in women (over 20% for HPV-16 and ~12% for HPV-51; ref. 35).

No statistically significant site- or specimen-specific differences in the proportion of HPV-positive samples containing HPV types 6, 11, 16, or 18 were identified. However, HPV-16 positive samples comprised a somewhat higher proportion of HPV-positive shaft samples (18.4%) than samples from other anatomic sites (9.0-13.5%) if any HPV type, including unclassified types, is included. Shaft and glans/corona samples were significantly more likely to have multiple type HPV infections than other sites (38.5% of shaft and 36.3% of glans/corona versus 7.4-21.6% of other sites). These data emphasize the importance of the penile shaft and glans/corona as sites of HPV infection in men, as well as being sites with the most potential for HPV transmission during sexual intercourse.

A limitation of the HPV detection method used is that the Roche kit is optimized for sensitivity but not for specificity. We cannot rule out the possibility of false positives based solely on PCR due to unspecific amplification and the fact that we did not sequence the unclassified types. In particular, because the anal canal and perianal areas were not cleaned before

sampling, these samples might be expected to contain contaminating DNA that hybridizes nonspecifically to the PGMY 09/11 primers. Indeed, we see the highest proportion of unclassified types in the anal samples. However, we have determined the PCR and genotyping methods we used to be sufficiently replicable across laboratories for detecting HPV infection in men using genital exfoliated skin cell samples (36).

This analysis of HPV type-specific differences among anatomic sites and semen was limited by the few samples that were positive for any single HPV type. For example, the proportion of HPV-positive penile shaft samples that were positive for HPV-16 (18.4%) is more than double that for the anal canal (9.0%); however, this difference did not reach statistical significance, perhaps due to small numbers of positive samples at each site.

We conclude that HPV prevalence in this sample of primarily asymptomatic men is higher than has been estimated previously for men who were not partners of women with cervical intraepithelial neoplasia. Complete sampling of the anogenital epithelium, HPV testing of each sample individually, the use of HPV assays that can detect 37 genital HPV types, and the practice of genotyping all samples have allowed us to detect HPV in men that may have otherwise been missed. With the exception of men living in Asian countries, who seem to have a much lower prevalence of HPV (27, 37-42), and men who are partners of women with HPV-related cervical disease, who may have a higher prevalence (11, 29, 43-45), we expect the majority of average-risk men to be positive for some type of anogenital HPV and nearly one third of men to harbor at least 1 of the 13 oncogenic HPV types. Due to the cross-sectional nature of this study, it is not possible to assess whether these infections are primarily transient. Future research about the incidence and persistence of HPV infection using complete anogenital sampling, including anal sites, is of interest. Further research assessing HPV transmission among heterosexual partners from these various anatomic sites will also be of importance in the prevention of HPV infection.

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Carrie M. Nielson, Roberto Flores, Robin B. Harris, et al.

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