

Genital Human Papillomavirus Infection in Women Who Have Sex with Women

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Genital infection with human papillomavirus (HPV), as determined by polymerase chain reaction detection of HPV DNA and prevalence of HPV-6 and -16 serum antibodies, was investigated in 149 women who were sexually active with women. By use of HPV L1 consensus primers and hybridization to types 6/11, 16, 18, 31/33/35/39, and 45 and a generic probe, HPV DNA was detected in 30% of subjects; of these, 20% had type 31/33/35/39, 18% had type 16, and 2% had type 6/11. Of 21 subjects reporting no prior sex with men, HPV DNA was detected in 19% and squamous intraepithelial lesions in 14%. By capture ELISA with HPV-6 and -16 L1 capsids, 47% of subjects were seropositive for HPV-16 and 62% for HPV-6. Current smoking was associated with detectable HPV DNA. Genital HPV infection and squamous intraepithelial lesions are common among women who are sexually active with women and occur among those who have not had sex with men.

An estimated 2.3 million women in the United States describe themselves as lesbian [1], and the number of women who have sex with women (WSW) during their lifetime is still higher [1–5]. The National Health and Social Life Survey, a probability sample of 3432 US adults aged 18–59 years, found that 4.3% of women surveyed reported same-gender sex since puberty [2], similar to findings reported in other studies [3, 4]. Despite case reports of cervical neoplasia among WSW who report no history of sex with men [6, 7], the prevalences of human papillomavirus (HPV) infection and of cervical neoplasia have not been carefully studied in WSW. While potential for transmission of HPV between female sex partners exists, most WSW (53%–99%) have had sex with men, and many (21%–30%) continue to do so [1, 2]. Acquisition of chronic viral sexually transmitted disease (STD) from male partners presumably occurs with a frequency similar to that in heterosexual women.

We sought to define the type-specific prevalence of genital

HPV as detected by a polymerase chain reaction (PCR) assay and to measure serologic evidence of prior infection with HPV-6 and -16 in a self-referred sample of WSW. Because some research suggests that WSW receive routine Pap smear screening less frequently than do heterosexual women of similar age [8–10], we also examined the frequency of routine Pap smear screening in our subjects.

Methods

Beginning in June 1995, subjects were recruited through advertisements posted in community gathering places (restaurants, bookstores, clubs, bars), newspaper and magazine articles, and electronic bulletin boards and by referral from clinics providing care to WSW. Because self-identification as lesbian may not predict actual participation in same-sex behavior or its frequency [11], we oriented recruitment materials to WSW rather than lesbians. Neither current STD symptomatology nor prior STD history were mentioned in recruitment materials. Women who reported having sex with another woman in the preceding year were eligible for inclusion.

At study entry, a detailed medical and sexual history was obtained by use of a standardized questionnaire. Pelvic examination was done, with collection of cervical specimens for Pap smear and cervical, vaginal, and vulvar specimens for HPV DNA testing by PCR. Colposcopic examination of the cervix was done. All subjects were interviewed and examined in the same clinic by one of two investigators (K.S. or J.M.M.). All Pap smears were read by the same cytopathologist (N.B.K.) using the Bethesda classification system [12].

PCR was done in a single laboratory as previously described [13]. Briefly, swab samples that were collected in specimen transport medium (Digene, Silver Spring, MD) for HPV DNA testing were digested with proteinase K, and the DNA was ethanol-precipitated and suspended in Tris-EDTA buffer. PCR amplification of HPV

Received 23 February 1998; revised 2 July 1998.

Presented in part: XI International Conference on AIDS, Vancouver, Canada, July 1996; 18th Conference of the National Lesbian and Gay Health Association, Seattle, July 1996; XII International Society for STD Research Meeting, Seville, Spain, October 1997.

Informed consent was obtained from all subjects who participated in this study, and the study was approved by the Human Subjects Review Committee of the University of Washington.

Financial support: NIH (Training Grant AI-07140 to J.M.M., AI-38382, CA-34493).

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The Journal of Infectious Diseases 1998;178:1604–9

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0022-1899/98/7806-0009\$02.00

Table 1. Characteristics of subjects in study of HPV infection in women who have sex with women (*n* = 149).

Characteristic	No. of subjects (%)
Age, years ^a	
<25	19 (12.8)
25–30	39 (26.2)
31–39	66 (44.3)
>39	25 (16.8)
Race	
Caucasian	137 (91.9)
Black	3 (2.0)
Asian/Pacific islander	4 (2.7)
Hispanic	3 (2.0)
Native American	1 (0.01)
Other	1 (0.01)
Mean monthly income, \$	1707
SD	1494
Cigarette smoking	
Current	28 (18.8)
Past	51 (34.2)
Never	70 (47.0)
Alcohol use, drinks ^b /week	
None	109 (73.2)
1–14	39 (26.2)
>14	1 (0.01)
Yearly income, \$	
<14,400	75 (50.3)
>14,400	74 (49.7)
Recruitment source	
Advertisements	73 (49.0)
Community organizations	66 (44.3)
Community clinics	10 (6.7)
History of genital infections	
Vulvar warts	16 (10.7)
Yeast vaginitis	115 (77.2)
Trichomoniasis	20 (13.4)
Bacterial vaginosis	43 (28.9)
Genital herpes	14 (9.4)
Chlamydial infection	16 (10.7)
Gonorrhea	6 (4.0)
History of abnormal Pap smear ^c	
HGSIL	5 (3.4)
LGSIL	10 (6.7)
ASCUS	21 (14.1)
Abnormal, unspecified	5 (3.4)
No abnormal Pap smear	106 (71.1)
No prior Pap done	2 (1.3)
Prior pregnancy	40 (26.8)
Hormonal contraceptive use	
Current	10 (6.7)
Ever	72 (48.3)
Never	77 (51.7)
Sexual history with male partner(s)	
Sex with male, ever	128 (85.9)
Sex with male, prior year	35 (23.5)
Receptive oral sex, ever	30 (20.0)
Rectal intercourse, ever	44 (29.5)
Male partner with genital warts, ever	22 (14.8)
No. of male partners, median	
Lifetime	7
Prior year	0
Prior 30 days	0
Sexual history with female partner(s)	
Female partner with genital warts, ever	23 (15.4)
Sexual practices, prior year	
Oral-vulvar sex received	147 (98.7)
Oral-vulvar sex given	146 (98.0)
Oral-anal sex received	57 (38.3)
Oral-anal sex given	57 (38.3)
Digital-vaginal sex received	147 (98.7)

Table 1. (Continued).

Characteristic	No. of subjects (%)
Sexual history with female partner(s)	
Sexual practices, prior year	
Digital-vaginal sex given	147 (98.7)
Digital-anal sex received	96 (64.4)
Digital-anal sex given	98 (65.8)
Use of insertive sex toys	86 (57.3)
No. of female partners, median	
Lifetime	7
Prior year	1
Prior 30 days	1

^a Mean ± SD, 32 ± 8; range, 19–53.

^b Defined as beer (35.4 mL), wine (11.8 mL), or liquor (4.5 mL).

^c HGSIL, high-grade squamous intraepithelial lesion; LGSIL, low-grade intraepithelial lesion; ASCUS, atypical squamous cells of uncertain significance.

DNA was done using HPV L1 consensus primers MY09/MY11. HPV amplicons were identified by dot blot hybridization done as previously described [13, 14] using a biotin-labeled HPV generic probe and oligonucleotide probes specific for nine HPV types in five hybridization probe mixes. The five probe mixes included types 6 and 11; types 31, 33, 35, and 39; type 16; type 18; and type 45. HPV types not identified by any of these probes were not further characterized. Seroreactivity was assessed to HPV-6 and -16 by an ELISA in which HPV-6 and -16 L1 capsids generated by recombinant vaccinia viruses were captured by type-specific monoclonal antibodies to conformational epitopes, as previously described [15, 16].

Statistical analysis was done with SPSS software (SPSS, Chicago). Direct comparisons of proportions were made using Pearson's χ^2 test or, if the expected cell frequencies were <5, Fisher's exact method. Continuous variables were compared between groups by Student's *t* test, and the Mantel-Haenszel χ^2 test for trend was used. In univariate analysis, variables used to assess the contribution of each subject's sexual history with men included lifetime number of male sex partners, time in years to last sex with a male partner, and a combined risk index that included both of these measures. To describe independent predictors of the presence of HPV infection, multivariate analysis with logistic regression was done; HPV DNA detected by PCR at any of three genital sites (cervix, vagina, or vulva) was the dependent variable. Variables that were significant (*P* < .05) in univariate analysis or whose inclusion was judged to have important effects on the overall model were included. Time to last sex with a male partner was used as a measure of experience with male partners because this characteristic was most strongly associated with detection of HPV DNA in univariate analysis.

Results

Study population. A total of 149 subjects were recruited, most of whom (93.3%) responded to posted advertisements in community venues or were referred by community organizations. Study participants were predominantly Caucasian and <40 years of age (mean, 32) (table 1). None had genitourinary complaints at study entry. Eleven percent reported a history of

vulvar or cervical warts, and 28% reported a prior abnormal Pap smear. Seven (33%) of 21 women who had never had sex with a male partner reported having had fewer than two routine Pap smears in the preceding 5 years, compared with only 15 (12%) of 128 women who had had sex with male partners during that time ($P = .026$).

Most subjects (86%) reported having had sex with a male partner, with 24% of them reporting this behavior in the preceding year (table 1). The majority (59%) had had 1 female partner during the prior year, 20% had had 2, and 21% had had ≥ 3 . The median number of lifetime male partners was 7, as was the median lifetime number of female partners. Twenty-seven percent of subjects had been pregnant at least once, and 48% had used oral contraceptives for birth control. Almost all subjects reported receiving and giving oral-vaginal and digital-vaginal sex with their female partners during the last year, and many also reported oral-anal or digital-anal sex during this time period (38% and 66%, respectively).

Detection of HPV DNA and Pap smear abnormalities. HPV DNA was detected by PCR in genital tract specimens of 45 subjects (30%) (table 2). None of the women in whom HPV DNA was detected gave a history of genital warts, and only 2 had had a male sex partner that they knew or suspected to have genital warts. Among all subjects with detectable HPV DNA, 29 (69%) had unclassified types of HPV DNA only, 9 (21%) had type 31/33/35/39, 8 (19%) had type 16, and 1 (2%) had type 6/11. Twenty-eight (62%) of these women had HPV DNA detected in the specimens obtained from the cervix, 26 (58%) from the vagina, and 32 (71%) from the vulva; 24 (53%) had HPV DNA in specimens from more than one site. Among the 41 women with HPV DNA who reported prior sex with men, 21 (51%) had not had sex with a male partner in >1 year

(range, 1–18 years; median, 2). In their sexual practices with female partners, women who had detectable HPV DNA did not differ from those who had no detectable HPV DNA. With male partners, women with detectable HPV DNA were more likely to report a history of receptive oral sex ($P = .05$).

Thirteen women (6% of all subjects) had abnormal Pap smears (table 2). Three of these women reported no history of sex with men, and 3 reported having had female sex partners with genital warts. Squamous intraepithelial lesions (SIL) were detected in 2 (10%) of 21 women who had never had sex with men, in 2 (2%) of 93 women who had had sex with a man >1 year previously, and in 2 (6%) of 35 women who reported sex with men during the last year (not significant). HPV DNA was detected in 5 of the 6 women with SIL; type 31/33/35/39 was found in both cases of SIL that occurred in women without a history of sex with men. Of the subjects with SIL who had never had sex with men, 1 woman, with high-grade SIL, had a Pap smear 3 months before study entry that was read as consistent with HPV infection; the other woman, with low-grade SIL, had last had a Pap smear 4 years before study entry (reported to her as normal).

In univariate analysis, factors associated with an increased likelihood of detecting HPV DNA included age 25–30 years relative to age >30 years, current cigarette smoking, history of abnormal Pap smear, >10 lifetime male sex partners, and ≤ 2 years since last sex with a male partner (table 3). By use of a combined risk index that incorporated both time to last sex with a male partner and number of lifetime male partners, subjects in the highest two risk strata (i.e., time to last sex with male ≤ 2 years, regardless of lifetime number of male partners) were about five times more likely than women who had never had sex with men to have detectable HPV DNA ($P < .001$, χ^2

Table 2. HPV and Pap smear findings categorized by subjects' sexual history.

Study finding	Sex with women only, lifetime ($n = 21$)	Sex with men >1 year ago ($n = 93$)	Sex with men and women, past year ($n = 35$)	All subjects ($n = 49$)
Any HPV DNA detected by PCR ^a	4 (19.0) ^b	21 (22.6) ^b	20 (57.1)	45 (30.2)
HPV DNA type				
16	1 (4.8)	3 (3.2)	4 (11.4)	8 (5.4)
31/33/35/39	3 (14.3)	3 (3.2)	3 (8.6)	9 (6.0)
6/11	0	0	1 (2.9)	1 (0.7)
Unclassified	1 (4.8) ^b	14 (15.1)	14 (40.0)	29 (19.5)
Pap smear result ^c at study visit				
HGSIL	1 (4.8)	0	0	1 (0.01)
LGSIL	1 (4.8)	2 (2.2)	2 (5.7)	5 (3.4)
ASCUS	1 (4.8)	5 (5.2)	1 (2.6)	7 (4.7)
Genital warts on examination	0	1 (1.1)	0	1 (0.1)
Antibodies to HPV-16 ^d	5 (26.3)	46 (53.5)	11 (39.3)	62 (46.6)
Antibodies to HPV-6 ^d	8 (42.1)	58 (67.4)	17 (60.7)	83 (62.4)
No antibodies to HPV-16 or HPV-6 ^d	11 (57.9)	22 (25.6)	8 (28.6)	41 (30.8)

NOTE. Data are no. (%).

^a HPV positivity by polymerase chain reaction (PCR) assay of ≥ 1 sites (cervix, vagina, and/or vulva).

^b $P \leq .01$ vs. women reporting sex with men and women in prior year.

^c HGSIL, high-grade squamous intraepithelial lesion; LGSIL, low-grade squamous intraepithelial lesion; ASCUS, atypical squamous cells of uncertain significance.

^d Measured on first 133 subjects (19 without prior sex with men, 86 reporting sex with men >1 year previously, and 28 reporting sex with men in past year); %s refer to these nos.

Table 3. Univariate relationships between detection of HPV DNA and patient characteristics (*n* = 149).

Characteristic	No. HPV-positive (% of subgroup)	Univariate odds ratio (95% confidence interval)
Age, years		
<25	9 (47.4)	2.1 (0.8–5.4)
25–30	15 (38.5)	2.9 (1.2–7.1)
>30	21 (23.1)	Referent
Race		
Caucasian	41 (29.9)	1.1 (0.5–2.6)
Non-Caucasian	4 (33.3)	Referent
Cigarette use		
Current	13 (46.4)	2.5 (1.0–6.3)
Past	14 (27.5)	1.1 (0.5–2.5)
Never	18 (25.7)	Referent
History of abnormal Pap smear		
Yes	17 (41.5)	1.7 (1.0–2.7)
No	26 (24.8)	Referent
Oral contraceptive use		
Ever	26 (36.1)	1.5 (0.9–2.4)
Never	19 (24.7)	Referent
Use of insertive sex toy		
Ever	29 (33.7)	1.3 (0.8–2.2)
Never	16 (25.4)	Referent
Lifetime no. of male partners		
>10	23 (41.8)	2.4 (1.0–5.7)
6–10	8 (27.6)	1.2 (0.4–3.6)
≤5	14 (21.5)	Referent
Lifetime no. of female partners		
>10	14 (27.5)	0.7 (0.3–1.7)
6–10	12 (28.6)	0.8 (0.3–1.0)
≤5	19 (33.9)	Referent
Time to last sex with male ^a		
≤2 years	24 (53.3)	1.7 (1.2–2.4)
>2 years	17 (20.5)	Referent
Combined male risk index		
Contact ≤2 years, ≥10 SP ^b	16 (53.3)	4.9 (1.3–27.0)
Contact ≤2 years, <10 SP	8 (53.3)	4.9 (1.1–21.5)
Contact >2 years, ≥10 SP	8 (25.8)	1.5 (0.4–5.7)
Contact <2 years, <10 SP	9 (17.3)	0.9 (0.2–3.3)
Never sex with male	4 (19.0)	Referent

^a Comparison excludes women reporting no prior sex with males.

^b SP = male sex partners.

test for trend). Characteristics that were not significantly associated with the presence of HPV DNA included annual income, history of a partner of either sex with genital warts, history of nongenital warts, or use of insertive sex toys in the preceding year.

In multivariate analysis, current but not prior cigarette smoking was an independent predictor of the presence of HPV DNA (odds ratio, 3.4; 95% confidence interval, 1.2–9.6) (table 4). Time to last sex with a male partner was also significantly associated with increased risk (*P* = .002). Since the index variable for sexual exposure to males suggested that recent contact with a male partner rather than report of >10 male partners was the important risk factor, only time since last contact was included in the multivariate model. Age, ever having used oral contraceptives, and ever having used insertive sex toys were not independently associated with the presence of HPV DNA.

HPV serology. Among 133 subjects tested, 92 (69%) had antibodies to HPV-16 or HPV-6: 62 (47%) had antibodies to

HPV-16, 83 (62%) had antibodies to HPV-6 (table 2), and 54 (40%) had antibodies to both. Among 19 subjects reporting never having had sex with a man and who had serology results available, 5 (26%) had antibodies to HPV-16, and 8 (42%) had antibodies to HPV-6. Antibodies to HPV-6 or -16 were detected in 8 (42%) of 19 women reporting no prior sex with men and in 84 (74%) of 114 women reporting prior sex with men (*P* = .16). Subjects who were seropositive to either HPV-6 or HPV-16 were older than seronegative subjects (mean age, 34 vs. 30 years; *P* = .01). Seropositive subjects did not differ significantly from seronegative subjects in time to last sex with a male partner, lifetime number of male or female partners, or detection of HPV DNA.

Discussion

By use of type-specific DNA probes to nine common genital HPV types (6, 11, 16, 18, 31, 33, 35, 39, and 45) and a universal probe to detect infection by other related types, genital infection with HPV was detected in 30% of WSW. We also found that SIL associated with HPV type 31/33/35/39 occurred in WSW who had never had sex with men. While the presence of HPV DNA by PCR was strongly associated with more recent sex with men and higher lifetime number of male partners, HPV DNA was detected among women who reported no prior sex with men or sex with men many years earlier (up to 18 years). We also found, as have other investigators [17], that current cigarette smoking was associated with an increased risk of HPV DNA detection. The prevalence of SIL was similar to that reported for heterosexual women [18]. Serologic evidence of prior infection with HPV-6 and -16 was common and did not differ significantly among subjects by reported history of sex with male partners. These data suggest that HPV is sexually transmitted between women. Our data also confirm previous

Table 4. Multivariate relationships between detection of HPV DNA and patient characteristics.

Characteristic	Multivariate odds ratio (95% confidence interval)
Age, years	
≤30	1.7 (0.8–3.8)
>30	Referent
Cigarette use	
Current	3.4 (1.2–9.6)
Past	1.2 (0.5–2.9)
Never	Referent
Oral contraceptive use	
Ever	1.4 (0.6–3.4)
Never	Referent
Use of insertive sex toy	
Ever	1.5 (0.7–3.4)
Never	Referent
Time to last sex with male ^a	
≤2 years	3.6 (0.9–14.3)
>2 years	0.8 (0.2–3.1)
No history of sex with male	Referent

^a Overall, *P* = .002.

observations that most WSW have had sex with men. Among our subjects, sex with men in the prior year was common, as were sexual practices between female partners that possibly could transmit HPV (e.g., digital-vaginal sex, digital-anal sex, oral sex, and use of insertive sex toys). Report of anal sex with male partners was also common, highlighting the potential for anal HPV infection in WSW. Finally, WSW who had never had sex with men received less frequent routine Pap smears.

The few studies that have reported a low prevalence of sexually transmitted disease among WSW [2, 8, 19] did not use newer diagnostic tests or provided minimal information on sexual behaviors. Transmission of human immunodeficiency virus and hepatitis A virus [20–24] have been reported to occur between female sex partners. HPV-16 was found in a woman with cervical intraepithelial neoplasia (CIN-2) who had never had sex with a male partner [7]. Although 3% of 608 female university students reported sex with another woman in a study in which PCR for HPV DNA was used, the prevalence of HPV among WSW was not reported [17]. Among 27 WSW evaluated at an STD clinic in London, all but 3 had prior heterosexual contact; 6 women (17%), of whom 2 denied prior sex with men, had genital warts. Of 25 evaluable women, 4 (16%) had SIL and 6 (24%) had inflammatory Pap smears [25]. Among 148 WSW evaluated in San Francisco in the early 1980s, 3% had SIL, none of whom had had sex with men in the preceding 2 years [8]. A case-control study of 241 WSW and 241 matched heterosexual controls attending an STD clinic found that the only 2 subjects with Pap smears showing high-grade SIL had not had sex with a male partner for at least 15 years [26].

While results from our study of WSW and results from studies of heterosexual women may not be directly comparable because of differences in subject recruitment and laboratory assays, some findings deserve comment. Our finding of increasing genital HPV prevalence as detected by PCR with more recent sex with men or higher cumulative number of male sex partners is in agreement with the results of a study of young Swedish women, in whom the only independent risk for the presence of HPV was lifetime number of male sex partners [27]. Further, a study of 604 university students found that in addition to female subjects' lifetime number of male partners, male partners' reported number of lifetime partners was also a significant predictor of HPV DNA detection by PCR [17].

A high proportion of the HPV detected in our subjects was not typeable by the probes we used. Among 184 heterosexual female STD clinic clients tested for HPV DNA by PCR with the same nine probes in our laboratory, the overall prevalence of HPV was 47%, with 8% identified as type 31, 33, 35, or 39; 15% as type 16, 18, or 45; 7% as type 6 or 11; and 20% as unclassified types (Koutsky LA, unpublished data). When additional DNA probes have been used in other populations, the percentage of unclassified types (by PCR) has ranged from 13% to 21% in female university students [14, 17].

Our data suggest that routine Pap smear screening practices

among WSW may be influenced by reported history of prior sex with men. This finding is in accord with those of other investigators. In one study, the mean interval between routine Pap smears among WSW was longer than for age-matched heterosexual women attending the same clinic (21 vs. 8 months) [10]. Twenty-three percent of the 1925 respondents in the National Lesbian Health Care Survey reported having had their last Pap smear >2 years previously; 5% of all respondents and 23% of women aged 17–24 years had never had a Pap smear [28]. Possible reasons for reduced use of health care by WSW include the perception of alienating behavior on the part of health care providers [9, 28, 30–33] and lack of health care coverage [29, 34]. Self-perception of low risk for STD and cervical cancer and reduced need for birth control among WSW may also contribute to fewer visits to providers that perform Pap smears [35].

The seroprevalence of HPV-6 and -16 in our subjects is somewhat high relative to assessments in other populations and approaches that documented in STD clinic clients [15]. Possible explanations for this are the older mean age of our subjects (32 years) compared with STD clinic populations and the relatively high lifetime number of sex partners reported by the subjects seropositive for either HPV type (median number of male partners, 7; median number of female partners, 8). The finding of antibodies to HPV-16 and HPV-6 among women reporting sex with only women supports the hypothesis that HPV is transmitted between female sex partners.

Our study has important limitations in addition to its small sample size. Subjects were self-referred; therefore, they may not be representative of all WSW or self-identified lesbians. Most of our subjects were Caucasian, and median income was relatively high. Finally, we did not obtain specific information on the timing of recent sex practices with female partners and so could not relate such practices to the detection of HPV. However, given that the presence of HPV on fomites has been reported [36], and HPV-16 has been documented to cause periungual carcinoma in immunosuppressed patients [37], transmission by hands or sex toys are possibilities to be investigated in future studies.

The high prevalence of genital HPV infection and of SIL and the suboptimal Pap smear screening observed in our study among WSW reporting no prior sex with men support the need for investigation in a larger number of women. Such information will help clarify messages provided to WSW about their risk of cervical cancer and need for Pap smear screening and protective sexual practices. In the meantime, recommendations for routine Pap smear screening in WSW should not differ from those for heterosexual women.

Acknowledgment

We thank Alison Starling for assistance in data management.

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