

Prevalence of Penile Human Papillomavirus DNA in Husbands of Women with and without Cervical Neoplasia: A Study in Spain and Colombia

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To investigate the role of men in cervical cancer, 816 husbands of women enrolled in four case-control studies of cervical neoplasia in populations at high (Colombia) and low (Spain) risk for cervical cancer were interviewed. Exfoliated cells from the penis were obtained and analyzed by polymerase chain reaction for the presence of human papillomavirus (HPV) DNA. Penile HPV DNA prevalences were higher in husbands of women with cervical neoplasia than in husbands of controls. Husbands of controls in Colombia had a 5-fold higher penile HPV DNA prevalence than the corresponding husbands in Spain. Strong dose-response relationships were found between penile HPV DNA prevalence and all sexual behavior-related variables in Spain but not in Colombia. Sexual promiscuity is the most important risk factor for penile HPV infections. Differences in HPV DNA prevalence in the male populations of Spain and Colombia are consistent with their 8-fold difference in cervical cancer incidences.

The natural history of human papillomavirus (HPV) infection is still not fully understood. It is now recognized that sexually transmitted HPV infections are etiologically related to invasive carcinoma of the cervix [1–5] and that cervical cancer is essentially a long-term sequel of a sexually transmitted disease [6–10]. Extensive research has been carried out on the infection in women. However, to date, HPV infections in men have not received much attention, for two reasons. First, HPV infections do not have as serious consequences for men as they have for women. Second, in contrast to the ease with which cervical scrapes containing abundant mucosal epithelial cells are obtained for the detection of HPV DNA in women, no practical sampling technique has been established to easily identify HPV infection in the penis; relatively few cells are recovered by swabbing the urethra or the glans penis. Despite these difficulties, clinical and virologic studies have defined the spectrum of HPV-associated disease in the male genital tract [11–20], and varying degrees of concordance between HPV infections in men and in their female sex partners have

been reported in many investigations [11, 13, 14, 20–25]. However, studies using polymerase chain reaction (PCR)-based detection of HPV DNA in penises of healthy men or in sex partners of healthy women are few [16, 18, 25, 26].

We had an opportunity to examine the role of men in cervical cancer in the context of large, population-based, case-control studies of cervical intraepithelial neoplasia (CIN) grade III and invasive cervical cancer in Spain and Colombia. Spain has one of the lowest (6/100,000 women) and Colombia one of the highest (48/100,000 women) incidences of cervical cancer in the world [27]. In these studies, biologic specimens, as well as information about exposure to risk factors for cervical cancer, were obtained from patients with cervical cancer and their controls as well as from the husbands of both case-patients and controls. Virologic investigations of female participants of these studies have been reported previously [28]. HPV infections have been found to be the central etiologic factor in cervical cancer in both Spain and Colombia [2, 3, 29, 30]. The associations between husbands' sexual behavior, penile HPV and cervical cancer have also been published recently [31, 32].

This study presents further detailed results on the virologic examination of penile cell samples in a series of 816 men in Spain and Colombia. More specifically, we report overall and type-specific HPV DNA distributions in husbands of women with (case-patients) and without (controls) cervical neoplasia, male-female overall and type-specific HPV concordance, and HPV DNA prevalence by selected sexual behavior characteristics of the couple.

Methods

Sources of subjects. Eligible subjects of the study were current husbands of women recruited in four concurrent case-control stud-

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Informed consent was obtained from women enrolled in the case-control studies on cervical cancer and from their respective husbands. All protocols were cleared by the International Agency for Research on Cancer (IARC, Lyon, France) and the ethical committees of the participating institutions.

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ies of cervical neoplasia (CIN III/in situ and invasive carcinoma) carried out in Spain and in Cali, Colombia. Current husbands were defined as men who had regular sexual intercourse with the index woman for at least the past 6 months, irrespective of the existence of a formal marriage or a common house.

The design of the case-control studies has been detailed elsewhere [2, 3, 28–30]. In brief, the field work was done from June 1985 through December 1987 in nine provinces in Spain and in the city of Cali, Colombia. Case-patients for the CIN III studies included women in the study areas with a histologic diagnosis of severe dysplasia, CIN III, or in situ cancer. Controls (1/case) were selected from the same screening clinics and pathology laboratories from which the case-patients were identified. Eligible controls had to have same age (± 5 years) as case-patients and a cytologic diagnosis of normal or inflammation (Pap grade II) on the same date as the case-patients.

Cases for the studies on invasive cancer included all incident, histologically confirmed, invasive squamous-cell carcinomas of the cervix identified among residents in the study areas. Their controls (1/case) were selected from an age-stratified random sample of the same general population from which the case-patients were identified.

The study subjects were interviewed in detail regarding sexual behavior by specially trained male interviewers using a structured questionnaire in nearby health facilities and only exceptionally at home. The average time to complete the interview was 25 min.

Collection of specimens. Penile cell samples were collected by the interviewers by swabbing the intrameatal and distal urethra, the external surface of the glans, and the coronal sulcus of the penis with wet thin cotton-tipped swabs. Cervical cell samples in case-patients and controls were collected by cervical scraping. For all samples, a smear was prepared for cytology reading and the remaining cells were eluted in PBS, pelleted by low-speed centrifugation (500 g for 10 min), and stored in the field at -20°C or -70°C (depending on the center) and at -70°C , first at Lyon and later at Baltimore.

Serum samples were also obtained and analyzed for antibodies to herpes simplex virus type II (by ELISA), *Chlamydia trachomatis* (by indirect immunofluorescence), *Neisseria gonorrhoeae* (by indirect hemagglutination), and *Treponema pallidum* (by ELISA). The details of the different techniques and antigens used as well as the criteria for positivity have been described elsewhere [33].

HPV DNA detection. Both cervical and penile specimens were tested by ViraPap (Digene Diagnostics, Silver Spring, MD) in 1990–1991. The data on the cervical specimens have been previously published [28], and the protocol used to process the male specimens for ViraPap is the same as the one used for cervical specimens. Briefly, 50% of each specimen was digested, placed on filters, and hybridized with the probe pool containing individual probes of seven HPV types, HPV-6, -11, -16, -18, -31, -33, and -35. The hybridized filters were autoradiographed and the signal intensity interpreted as follows: 0, no signal; 1, less than the intensity of the reference low positive signal; 2, equal to the intensity of the low positive control; 3, greater than the intensity of the low positive control but less than that of the reference of the high positive control; 4, equal to the intensity of the high positive control; 5, greater than the intensity of the high positive control. Specimens were characterized as negative if they were scored 0 or 1 and positive if they were scored ≥ 2 .

The cervical specimens were tested in 1990–1991 by L1 consensus primers MY09/MY11 PCR as described previously [28]. The penile specimens were tested in 1993–1994, also with L1 consensus primers MY09/MY11 PCR, but with modifications, most of which have been described by Hildesheim et al. [34]. Primer HMB01 was added to MY09/MY11 to facilitate amplification of HPV-51; the generic probe (previously a mixture of HPV-16 and HPV-18) was enlarged to also include HPV-51 and HPV-66; 25 type-specific HPV probes were used, compared with 7 probes previously; and HPV identification was accomplished in dot-blot format with biotinylated probes, rather than in a Southern format with radiolabeled probes used for the cervical specimens. Specimens that hybridized with the generic probe but with none of the type-specific probes were categorized as “HPV-untyped.” This heterogeneous group may contain sequences of known HPVs not included in the probe panel, sequences of HPVs in the probe panel that were not adequately amplified, sequences of as-yet-unknown HPVs, and in some instances, non-HPV sequences.

Primers for a fragment of the β -globin gene served as an internal control to assess the sufficiency of each specimen for amplification. All specimens were prepared and analyzed in one laboratory (K.V.S.) following standard procedures. Positive controls showed that the assay detected <25 copies of HPV-16 per reaction. None of the negative controls (1 normal human kidney tissue fragment/25 study specimens analyzed) revealed HPV DNA, suggesting that laboratory contamination was not common. PCR assays were performed blindly as to the case-control status and country of origin of the subjects.

Statistical analysis. The uncorrected χ^2 statistic and, when appropriate, the Fisher’s exact test were used to test for differences between proportions. Tests for linear trends were calculated to assess the significance of the increasing or decreasing HPV DNA prevalence across the values of a given variable.

For all analyses, an α value of .05 was used to establish statistical significance. Unless otherwise indicated, all *P* values are derived from two-tailed tests.

Results

Penile cell samples were obtained from 816 (73.6%) of the 1105 participating husbands of the 1415 women with a husband. The overall husbands’ participation rate was thus 78.1% (1105/1415), being somewhat higher in Spain than in Colombia and in the CIN III studies than in the invasive carcinoma studies. All collected samples were tested by ViraPap, and 776 (95.1%) were tested by PCR. Of these, 595 (76.7%) were positive for β -globin or HPV DNA and were included in the analysis. In the remaining 181 (23.3%) specimens, neither β -globin nor HPV could be amplified, and they were considered inadequate and excluded from the analysis. In 30 penile specimens analyzed for the amount of cellular DNA, the median value was $\sim 0.3 \mu\text{g}$, which was about $<10\%$ of the median amount of cellular DNA obtained from the cervical specimens.

Penile HPV prevalence. Table 1 summarizes penile HPV DNA prevalences by country and the corresponding case-control status of the wife. HPV prevalences by ViraPap for any of the groups of men were low and fell within a narrow range

Table 1. Penile HPV DNA prevalences as determined by ViraPap and by polymerase chain reaction (PCR) in husbands of women with (case-patients) and without (controls) cervical neoplasia in Spain and Colombia.

HPV DNA test	Spain, husbands of		Colombia, husbands of	
	Case-patients	Controls	Case-patients	Controls
Mean age	44.7	45.4	45.2	46.0
ViraPap				
Total tested	237	232	151	196
Total not tested*	69	95	59	66
Negative: 0	231	223	142	188
1	4	4	4	2
Positive: 2	2	1	3	3
3	—	2	2	1
4	—	2	—	2
Total positive	2	5	5	6
% positive	0.84	2.16	3.31	3.06
<i>P</i> [†]		.24		.89
PCR				
Total tested	223	217	148	188
Total not tested*	83	110	62	74
Negative	151	165	81	107
Positive	32	6	28	25
Inadequate [‡]	40	46	39	56
% HPA DNA positive [§]	17.49	3.51	25.69	18.94
<i>P</i>		.00002		.21

NOTE. Data are no. of subjects unless indicated.

* Subjects who did not provide specimens.

† For within-country case-control comparison of HPV prevalences using ViraPap. Between-country comparison of HPV DNA prevalences: *P* = .12 among husbands of case-patients; *P* = .56 among husbands of controls.

‡ Includes specimens in which neither HPV DNA nor β-globin could be adequately amplified.

§ Excludes “inadequate” specimens.

|| For within-country case-control comparison of HPV prevalences using PCR. Between-country comparison of HPV DNA prevalences: *P* = .09 among husbands of case-patients; *P* = .00001 among husbands of controls.

(0.8%–3.3%). HPV DNA prevalences between husbands of case-patients and husbands of controls were not significantly different in either country. Similarly, intercountry differences of HPV DNA prevalences in husbands of case-patients and in husbands of controls were not statistically significant. Specimens with the signal intensity of 1, which may reflect low-level positivity, were distributed similarly across comparison groups.

HPV DNA prevalences by PCR showed a completely different pattern. In both countries, HPV DNA prevalences were higher in husbands of case-patients than in husbands of controls. The differences were highly significant in Spain (17.5% in husbands of case-patients vs. 3.5% in husbands of controls; *P* = .00002) but not in Colombia (25.7% in husbands of case-patients vs. 18.9% in husbands of controls; *P* = .21).

Penile HPV DNA prevalences were higher in Colombia than in Spain for both case-patients and controls. Husbands of controls in Colombia had a highly significant 5-fold-higher HPV DNA prevalence than the corresponding prevalence in Spain (18.9% in Colombia vs. 3.5% in Spain; *P* = .00001). Husbands of case-patients in Colombia also had a higher HPV DNA prevalence than did those in Spain, although the difference was

only marginally significant (25.7% in Colombia vs. 17.5% in Spain; *P* = .09).

The performance of ViraPap in relation to the more sensitive PCR in detecting HPV DNA was assessed in the 592 penile cell samples that were tested by both ViraPap and PCR. The ViraPap test detected 8 of the 90 samples that were HPV-positive by PCR (sensitivity = 8.9%). The specificity of ViraPap in relation to PCR (HPV-negative subjects by ViraPap among HPV-negative subjects by PCR) was 98.4% (494/502). The positive predictive value (HPV-positive subjects by PCR among HPV-positive subjects by ViraPap) was 50% (8/16). The corresponding negative predictive value (HPV-negative subjects by PCR among HPV-negative subjects by ViraPap) was 85.8% (494/576). Since only 16 samples tested positive by ViraPap, we did not explore further its performance by case-control status or country.

Figure 1 presents, for each country, PCR-based HPV DNA prevalences by age and case-control status of the wife. In Spain, for any age group, husbands of case-patients had a higher HPV DNA prevalence than did husbands of controls. In contrast, in Colombia, these differences were observed in some but not all age groups (figure 1). Penile HPV DNA prevalences did not

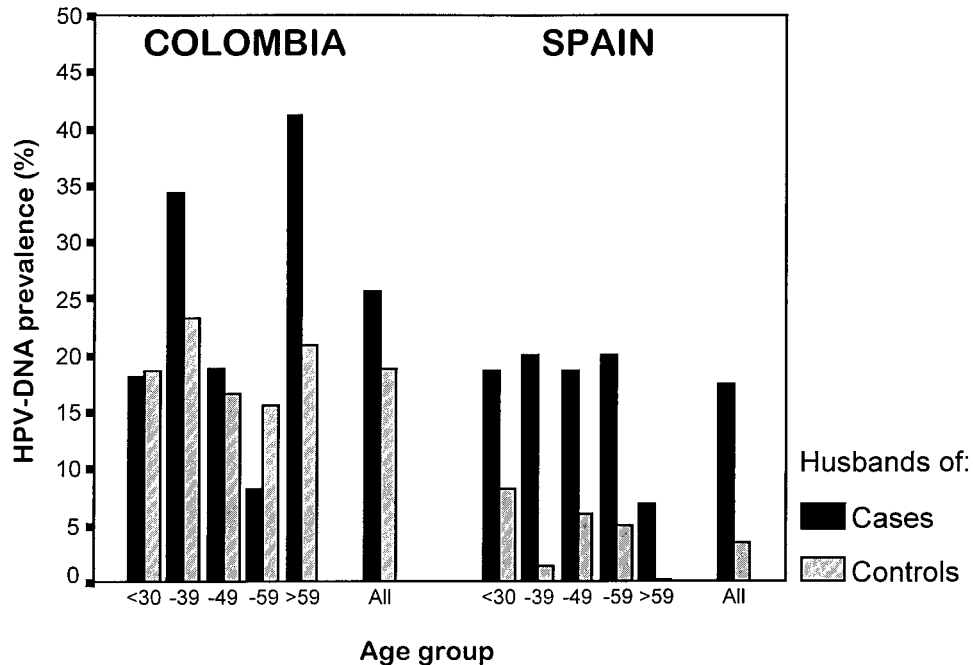


Figure 1. Penile HPV DNA prevalences by age in husbands of women with cervical neoplasia (cases) and without (controls). Trend tests of HPV DNA prevalences with age: $P = .26$ for husbands of case-patients in Spain; $P = .71$ for husbands of controls in Spain; $P = .71$ for husbands of case-patients in Colombia; $P = .81$ for husbands of controls in Colombia.

show any particular trend with age (see P values for trend tests in figure 1 legend).

Penile HPV type distribution. Sixteen specific HPV types were identified by PCR in the 595 cell specimens collected.

The penile type-specific HPV distribution by country and the case-control status of the wife is shown in table 2. Seven subjects had multiple infections: 6 subjects had double infections (HPV-31 and -66, -16 and -53, -16 and -31, -31 and -56, and 2 with HPV-33 and -6/-11). One subject had a triple infection (HPV-16, -51, and -53). Thus, the 91 HPV-positive men contributed a total of 99 HPV infections. No specimens were positive for HPV-18, -26, -40, -55, -68, PAP291, W13B, and PAP238A. HPV-16 was the most frequent type identified, accounting for 19.2% of the positive specimens.

Although the numbers were too small for a detailed comparison, there were no obvious differences in the distribution of other HPV types between husbands of case-patients and controls. Twenty-six (26.3%) of the 99 isolates tested positive by the generic probe but could not be identified by any of the 25 type-specific probes; these were labeled as "HPV-untyped." Most of these ($n = 23$) had low-intensity signals with the generic probe. While the percentage of isolates categorized as HPV-untyped showed no significant case-control differences in either country (table 2), the overall percentage in Colombia was significantly higher than the corresponding one in Spain (38.2% in Colombia vs. 11.4% in Spain; $P = .003$).

HPV concordance in couples. There were 431 couples in whom both members were adequately examined for the presence of HPV DNA in both cervical and penile specimens. Overall HPV status concordance (presence of any HPV type,

including untyped HPVs) was observed in 66.4% (286/431) of the couples. As might be anticipated, HPV-negative couples were far more frequent in the control groups (92% in Spain and 71% in Colombia) than in the case-patient groups (22.5% in Spain and 36.5% in Colombia). Conversely, HPV-positive couples were more frequent in the case-patient groups (11% in Spain and 21% in Colombia) than in the control groups (0 in Spain and 2% in Colombia). The overall HPV prevalence of couples with both members infected was higher in Colombia than in Spain (9.0% and 4.9%, respectively).

There were 27 couples, 25 among the case-patient groups and 2 among the control groups, in whom both members tested positive for HPV DNA. The HPV type-specific concordance between members is shown in table 3 for the 32 HPV infections detected in the 27 couples. The overall type-specific concordance after excluding the 10 specimens categorized as HPV-untyped was 31.8% (7/22). The 7 concordant couples were among the case-patient couples, and they were all concordant for HPV-16.

Penile HPV prevalence and couples' sexual behavior characteristics. Table 4 shows HPV DNA prevalences in all male subjects according to age and selected sexual behavior-related variables of both partners. In the combined analyses, significant and strong dose-response relationships were found with all sexual behavior-related characteristics of the couple. Thus, penile HPV DNA prevalence increased with an earlier age at first sexual intercourse and with the number of sex partners of any kind, prostitutes and nonprostitutes, and those reported both during and before marriage. However, these strong associations were evident only in Spain. In Colombia, no associations

Table 2. Penile HPV type distribution in husbands of women with (case-patients) and without (controls) cervical neoplasia in Spain and Colombia.

HPV type	Spain, husbands of		Colombia, husbands of	
	Case-patients	Controls	Case-patients	Controls
Low-risk types				
6/11	—	2	2	2
53	4	—	1	3
54	—	—	—	1
PAP155	—	—	2	—
Subtotal, no. (%)	4 (10.5)	2 (33.3)	5 (16.7)	6 (24.0)
<i>P</i> *		.18		.50
Cancer-associated types				
16	9	1	6	3
31	4	1	2	1
33	3	1	2	—
35	—	—	1	—
39	1	—	—	—
45	—	—	—	1
51	5	—	—	2
52	1	—	—	—
56	1	—	—	—
58	2	1	2	—
59	1	—	—	—
66	2	—	1	2
Subtotal, no. (%)	29 (76.3)	4 (66.7)	14 (46.7)	9 (36.0)
<i>P</i> *		.47		.42
HPV-untyped, no. (%) [†]	5 (13.2)	0	11 (36.7)	10 (40.0)
<i>P</i> *		0.46		0.80
All types [‡]	38	6	30	25

NOTE. Data are no. of subjects unless indicated.

* For within-country case-control comparison of HPV prevalences.

† Unidentified HPV (i.e., positive by generic probe and not identified by 25 type-specific probes used). Overall between-country comparison of “HPV-untyped” prevalences: 38.2% in Colombia, 11.4% in Spain, *P* = .003.

‡ Totals do not add up to those in table 1 because 7 subjects had multiple infections and were counted more than once.

were found between HPV DNA prevalence in men and the variables included in table 4. Compared with those in Spain, HPV DNA prevalences in Colombia were systematically higher and homogenous across all levels of sexual behavior variables.

Figure 2 further shows, in a pooled analysis of the 2 studies in each country, that penile HPV prevalence increased with

increasing number of sex partners of the husband, that the increase was observed in husbands of both monogamous and nonmonogamous women, and that for each category of number of sex partners of the husband, penile HPV prevalences were systematically higher in husbands of nonmonogamous women than in husbands of monogamous women.

Table 3. Type-specific HPV concordance among HPV-positive couples.

HPV type in women	HPV type in men									Total
	6/11	16	31	33	51	53	58	66	Untyped	
16	1	7	2	2	2	1	1		6	22
31	1			1						2
33		1			1	1			1	4
35								1		1
Untyped		1					1		1	3
Total	2	9	2	3	3	2	2	1	8	32

NOTE. Multiple infections were included more than once (27-HPV positive couples contributed total of 32 infections). Data are no. of subjects.

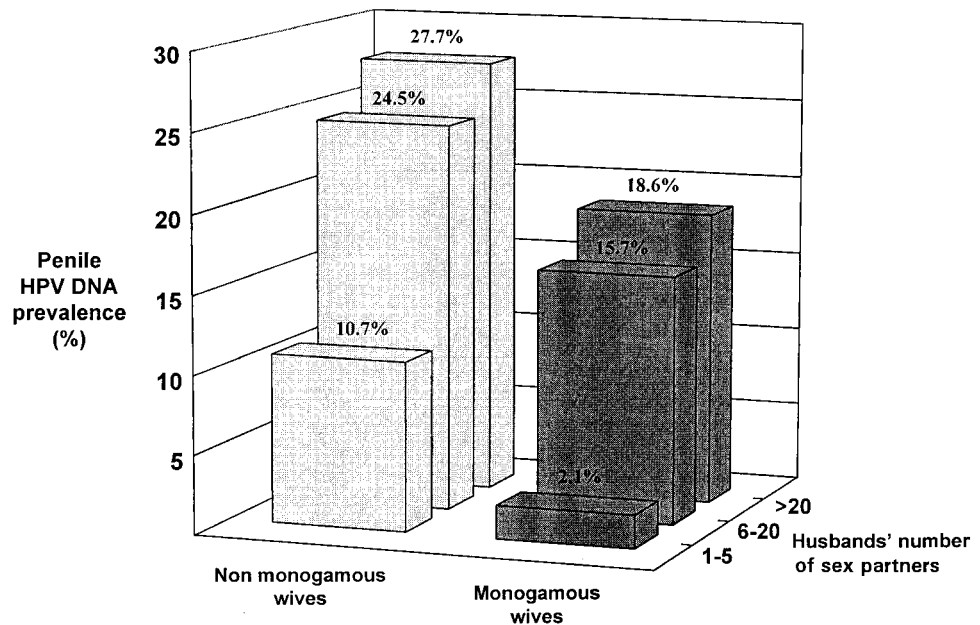
Table 4. Penile HPV DNA prevalence in men according to their sexual behavior characteristics.

Variables	Spain		Colombia		All	
	No. positive/ no. tested	% positive	No. positive/ no. tested	% positive	No. positive/ no. tested	% positive
Age						
≤29	4/28	14.3	5/27	18.5	9/55	16.4
10–39	13/128	10.2	18/62	29.0	31/190	16.3
40–49	11/92	12.0	12/67	17.9	23/159	14.5
≥50	10/106	9.4	18/85	21.2	28/191	14.7
<i>P</i>		.66		.58		.62
No. of sex partners						
Lifetime						
1–5	5/158	3.2	1/12	8.3	6/170	3.5
6–10	8/49	16.3	9/26	34.6	17/75	22.7
11–20	8/55	14.5	9/57	15.8	17/112	15.2
≥21	17/91	18.9	32/132	24.2	49/223	22.0
<i>P</i>		<.0001		.51		<.0001
During marriage						
1	15/190	7.9	24/91	26.4	39/281	13.9
2–5	8/103	7.8	13/61	21.3	21/164	12.8
6–10	5/30	16.7	6/41	14.6	11/71	15.5
11–20	7/18	38.9	5/26	19.2	12/44	27.3
≥21	3/13	23.1	5/22	22.7	8/35	22.9
<i>P</i>		<.0001		.61		.02
Before marriage						
1	4/96	4.2	1/4	25.0	5/100	5.0
2–5	4/81	4.9	5/27	18.5	9/108	8.3
6–10	8/51	15.7	5/32	15.6	13/83	15.7
11–20	7/41	17.1	11/48	22.9	18/89	20.2
≥21	15/84	17.9	29/116	25.0	44/200	22.0
<i>P</i>		.001		.30		<.0001
Wives' lifetime no. of sex partners						
1	27/284	9.5	24/142	16.9	51/426	12.0
2–5	7/58	12.1	27/88	30.1	34/146	23.3
>5	4/12	33.3	2/11	18.9	6/23	26.1
<i>P</i>		.03		.53		.001
Contacts with prostitutes						
Lifetime						
0	10/138	7.2	14/47	29.8	24/185	13.0
1–5	8/96	8.3	12/61	19.7	20/157	12.7
6–20	12/68	17.6	7/56	12.5	19/124	15.3
≥21	8/51	15.7	19/65	29.2	27/116	23.3
<i>P</i>		.02		.94		.02
During marriage						
0	23/268	8.6	38/158	24.0	61/426	14.3
1–9	9/61	14.7	9/61	14.7	18/122	14.7
≥10	6/25	24.0	6/22	27.3	12/47	25.5
<i>P</i>		.02		.69		.04
Age at 1st sexual intercourse						
≥21	6/94	6.4	3/9	33.3	9/103	8.7
16–20	26/219	11.9	22/102	21.6	48/321	14.9
≤15	6/39	15.4	28/130	21.5	34/169	20.1
<i>P</i>		.09		.66		.01
Oral/anal sex						
Never	16/183	8.7	20/100	20.0	36/283	12.7
Ever	22/170	12.9	33/141	23.4	55/311	17.7
<i>P</i> *		.14		.32		.06
Antibody to <i>Chlamydia trachomatis</i>						
No	28/287	9.8	37/167	22.2	65/454	14.3
Yes	8/44	18.2	12/43	27.9	20/87	23.0
<i>P</i> *		.08		.27		.03

NOTE. Includes husbands of both case-patients and controls.

* Fisher's exact test (1-tailed); all other comparisons are by test for trend.

Figure 2. Penile HPV DNA prevalences by number of sex partners of husbands of monogamous and nonmonogamous women. Results are combined for all 4 studies and for both countries.



Discussion

In this study, we examined the descriptive epidemiology of penile HPV infections according to a variety of sexual behavior characteristics of the couple and in particular of the man. The fact that male sexual practices (i.e., number of partners, number of prostitutes, age at first sexual intercourse) varied greatly between the two male populations enabled us to estimate penile HPV prevalences in a wide range of promiscuity levels. Since we also obtained cervical specimens of the women, we were able to assess PCR-based HPV DNA concordance within couples.

HPV prevalences determined by ViraPap in penile cell samples were low and within a narrow range. The small number of epithelial cells obtained in a penile swab, a lack of knowledge about the optimal site for sampling, and the low sensitivity of the assay probably contributed to the low HPV prevalences obtained by ViraPap. Consistent with previous evidence [13, 35], these data clearly show that non-amplification-based assays are unsuitable for study of penile scrapings.

Husbands of case-patients had a higher HPV DNA prevalence than husbands of controls in both countries, although the difference was statistically significant only in Spain. The higher prevalence in husbands of case-patients is not unexpected because the risk factors for HPV infection (e.g., number of sex partners and prostitutes) are more prevalent in partners of women with cervical neoplasia than in partners of women without cervical neoplasia [29, 30]. Also, since cervical cancer is an HPV-related disease, husbands of case-patients would have a greater contact with HPV-infected women than would husbands of controls.

The main finding of these analyses is that while the HPV DNA prevalence in husbands of case-patients in Colombia and in Spain was similar (1.5-fold higher in Colombia than in Spain), the prevalence in husbands of controls was 5-fold higher in Colombia (18.9%) than in Spain (3.5%). This observation is an important finding, since the HPV DNA prevalence among husbands of controls included in the study is probably the best estimate of the rate of HPV infections in the male populations of the two countries. These data suggest that the 8-fold-higher cervical cancer incidence in Colombia compared with that in Spain could be at least partially explained by the 5-fold difference in the background rate of HPV infection in the male population.

The higher HPV DNA prevalence in the male Colombian population compared with that in Spain is consistent with several other previously reported findings of the study: The male population in Colombia reported a 3.3-fold-larger mean lifetime number of sex partners and a 1.6-fold-larger mean lifetime number of prostitutes than the male population in Spain [36]; Colombian female controls had a 2.7-fold higher prevalence of HPV DNA than Spanish female controls (13.0% vs. 4.9% [36]); Colombian husbands of controls had a higher prevalence of antibodies to other STDs, such as *C. trachomatis*, than did their Spanish counterparts (15.2% and 7.9%, respectively [31, 32]); and Colombian males had a higher prevalence of self-reported genital warts than did males in Spain (6.5% vs. 2.1% [31, 32]).

One would expect, as suggested by studies of HPV infections in the cervixes of healthy women, age-specific penile HPV prevalences to be highest during the ages of highest sexual activity (between 16 and 35 years), with a constant decline

thereafter [37]. In our study, this question could best be examined in husbands of controls. In Spain, there were too few HPV-positive specimens (6) for an analysis of age-specific HPV prevalences. When the case and control data were pooled, we observed a decline in penile HPV prevalences by age, but this decline did not reach statistical significance and thus could not be distinguished from expected random variations of the trend. In Colombia, no age-specific HPV trend was seen among controls or when pooling the data. In contrast, a study of asymptomatic men in Germany reported a decline in prevalence by age, but with a second peak beyond 75 years of age [38]. The lower percentage of younger men in our study (24% of the men tested by PCR were ≤ 35 years compared with 67% in the German study) may explain the discrepancy between the two studies.

In previous studies, PCR-based genital HPV DNA prevalences in healthy men have ranged from 9% to 24% [16, 18, 25, 26] depending on the population chosen, the type of sample collected, the number of anatomic sites included in the sampling, and the PCR technique used. Therefore, comparisons of HPV DNA prevalences in healthy men are difficult to interpret across studies. In our study, the same protocol, including collection and processing of specimens, questionnaire data, and PCR technique, was uniformly applied to the four male populations in Spain and Colombia. Thus, it is unlikely that methodologic issues account for the differences observed in HPV DNA prevalences in these men.

The specimens were screened with 25 type-specific probes, of which 18 types were shown to be prevalent in the penis. The numbers of HPV DNA-positive specimens in individual groups were too small to compare type-specific prevalences in the two countries or between partners of case-patients and partners of controls in each country. After use of 25 type-specific probes, a significant number of positive samples, higher in Colombia than in Spain, tested positive by the generic probe only.

The correlation of HPV results of males with the HPV results for their wives revealed little evidence of shared concordant infections. Data from previous studies show that PCR-based HPV DNA concordance varies between 53% and 68% [20, 24, 25]. HPV DNA status concordance in our study was 66.4%. The estimated type-specific HPV concordance among positive couples in our study (31.8%) was found to be lower than PCR-based estimates from other studies that reported concordances of 50% [24] and 65% [20]. A possible reason that may partly account for this lower concordance is that the penile and cervical specimens were not tested under identical conditions. Also, for a fraction of couples, our study may be sampling the irrelevant partner or detecting an irrelevant HPV infection in the penis.

We found that HPV DNA prevalences were significantly related to the sexual behavior characteristics of the couple, although the associations were significant only in Spain. The fact that in Colombia the prevalence of penile HPV DNA was

not associated with these variables is consistent with the hypothesis that background rates of HPV infection in the male population of Colombia are relatively high (i.e., higher than the corresponding prevalences in Spain) and homogeneous across all promiscuity levels. This hampers the identification of an association of each sexual behavior-related variable with penile HPV infection. However, this is not contrary to the concept that global promiscuity in the population at large is related to the risk of HPV infections and consequently to the risk of cervical cancer.

In conclusion, the 5-fold difference in penile HPV DNA prevalences in the male populations of Colombia and Spain is consistent with the 8-fold difference in cervical cancer incidences between the two countries. Strong and statistically significant dose-response relationships were found between penile HPV DNA prevalence and all sexual behavior-related variables of the couples in Spain but not in Colombia, where penile HPV prevalences were higher and of similar magnitude across all levels of the sexual behavior variables. These data support the hypothesis that sexual promiscuity is the most important risk factor for penile HPV infections, which are in turn related to cervical carcinogenesis in their female sex partners.

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