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Frequency of Human Papillomavirus Infection, Coinfection, and Association with Different Risk Factors in Colombia

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PURPOSE: The aims of this study were to provide new insights into infection patterns of six high-risk human papillomaviruses (HR-HPV-16, -18, -31, -33, -45, and -58) and two low-risk HPV types (LR-HPV-6 and -11), their association with risk factors and coinfection.
METHODS: Cervical samples of 2110 women were tested for the presence of HPV–DNA by polymerase chain reaction. Statistical analyses were performed to determine viral-type frequencies in single and multiple infections and association between infection and different risk factors.
RESULTS: HPV-16 was the most prevalent type among the studied population, followed by HPV-31. This last viral type showed a variable distribution between the different cities evaluated. The results showed distinct type-specific distributions among regions and a high association between absence of pregnancies, cities as Girardot and Leticia, the indigenous ethnicity, and coinfection.
CONCLUSIONS: The results showed a variable distribution of HPV types according to the geographical region analyzed. In addition, data suggest that some sociodemographic-factors such as ethnicity, number of pregnancies, lifetime number of sexual partners, and geographic region were significantly associated, and our results showed little differences between single and multiple infections by HPV with regard to risk factors. Furthermore, these results provide relevant information that will allow assessing in further studies the impact that vaccination programs on these populations and the selective pressure would have on the distribution of HPV types.
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KEY WORDS: Cervical Intraepithelial Neoplasia, Colombia, Epidemiology, Papillomavirus Infections, Risk Factors.

INTRODUCTION

Cervical cancer (CC) is the second most-common type of cancer among women worldwide. Human papillomavirus (HPV) is recognized today as the main causal factor of virtually all CC cases, given that high-risk (HR)-HPV DNA has been detected in approximately 99.7% of women with CC (1). In Colombia, the annual incidence of CC is estimated to be 32.9 cases/year per every 100,000 women, and HPV infections are detected in 6.2 of every 100 women per year, with women younger than the age of 20 being the most affected population (2, 3). CC is a major public health issue in Colombia because approximately 18.1 of every 100,000 women at an average age of 46.5 years die annually as a result of this disease (3).

Previous studies conducted in women from Bogotá D.C. (Colombia) found that nearly 20%–30% of all HPV-infected women are infected with more than one HPV type, which can be either phylogenetically related or unrelated and acquired simultaneously or subsequently (4). Nevertheless, in another study performed in rural populations of Colombia, with lower socioeconomic background, the authors (5) found that the prevalence of HPV could be even greater (36%).

The aim of the present study was to establish the prevalence of the HR-HPV types 16, 18, 31, 33, 45, and 58 (which are associated with ~90% of all CC worldwide), as well as the low risk (LR)-HPV types 6 and 11 (mainly involved in anogenital warts) in women inhabiting five geographical regions of Colombia and having different socioeconomic backgrounds: Chaparral, Girardot, Engativá, Leticia, and Tumaco. These five regions comprise socially heterogeneous

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Selected Abbreviations and Acronyms

CC = cervical cancer
HPV = human papillomavirus
HR-HPV = high-risk human papillomavirus
LR-HPV = low-risk human papillomavirus
PCR = polymerase chain reaction
OR = odds ratio
CI = confidence interval

female populations with high and low risks of developing CC (6), which allows establishing a correlation between cytological findings and molecular data from an epidemiological point of view by considering different demographical features and sexually related behaviors such as age, ethnicity, geographical origin, age at first intercourse, number of full-term pregnancies, and the lifetime number of sexual partners. The study also assessed the risk factors associated with HPV infection and coinfection (i.e., infection with more than one HPV viral type).

The information gathered in this study could be relevant for HPV vaccination studies, due to the selective pressure that the introduction of new HPV vaccines may have on the distribution of HPV types, and could thus provide hints about the expected success of vaccination schedules applied to these populations, and about distribution and coinfection rates of HPV in Colombia.

MATERIALS AND METHODS

Populations

Three of the regions included in this study comprised Andean territories (i.e., Colombia's west-central region): Chaparral, which is located in the department of Tolima and has approximately 8500 inhabitants as well as low population mobility patterns; Girardot, which is the second city of importance in the department of Cundinamarca after Bogotá (Colombia's capital city), with 131,354 inhabitants and with an economy largely based on tourism; and Engativá which is a locality of Bogotá, situated on the northwestern part of the city.

Leticia was the fourth population included in this study. It is the capital of the department of Amazonas and has an average population of 39,636 inhabitants. The city is located in a jungle region at the country's southernmost edge (in the Colombian, Brazilian and Peruvian triborder), and acts as a major port on the Amazon River. Finally, the fifth region was Tumaco, a port city in the southwestern department of Nariño with 170,000 inhabitants; its coasts are bathed by the Pacific Ocean and limits with Ecuador.

These regions were selected based on the assumption that they represented people from different socioeconomical backgrounds (mainly low), culturally diverse and with different access levels to health facilities. They comprised socially heterogeneous female populations that are both at high risk of developing CC, such as Amazonas and Tolima, where mortality rates per 100,000 females per year are 5.67 and 5.55, respectively, as well as at a moderate risk (Bogotá, 3.29; Cundinamarca, 3.22; and Nariño, 3.12 per 100,000 female per year) (6).

Sociodemographic Data

A total of 2110 women were enrolled in this study. These women who were asked to take part in this study attended to their CC prevention consult between April and September of 2007 at the League against cancer, Leticia-Amazonas (n = 173), Hospital San Juan Bautista, Chaparral-Tolima (n = 174), Hospital Engativá-Bogotá (n = 921), Nuevo Hospital San Rafael, Girardot-Cundinamarca (n = 334), and Hospital San Andrés, Tumaco-Nariño (n = 508). The women filled out a questionnaire regarding sociodemographic features, sexual behaviors, and risk factors before undergoing gynecological examination (Table 1). Five women between the ages of 70 and 77 years were excluded from the statistical analysis because Colombian legislation indicates that no screening by conventional cytology is required for women older than 69 years (7).

Ethical Approval

Before undergoing gynecological examination, all female subjects provided their written informed consent to provide a cervical sample for Papanicolaou testing and polymerase chain reaction (PCR) HPV-DNA detection; informed consent of women under the age of 18 was signed by a parent or guardian. All procedures performed in this study were approved and supervised by the Ethics Committee of each institution.

Collection, Processing, and Detection of Human Papillomavirus DNA in Cervical Samples by PCR Amplification

Samples of cervical epithelium were collected with a cytobrush and kept in 95% ethanol until further analysis (8). DNA from these samples was digested in lysing buffer (10 mM Tris–HCl, pH 7.9; 0.45% Nonidet P-40; 0.45% Tween 20 and 60 μg/mL of Proteinase K; Invitrogen, Camarillo, CA), first at 60°C for 1 h and then at 95°C for 10 min. Aliquots of 2.7 μL of each processed sample were then amplified by PCR with the use of the human β-globin GH20/PC04 specific primers, to evaluate DNA integrity (9).

Two different sets of generic primers for HPV annealing in the *L1* gene were used to detect HPV-DNA. We took into account studies in which the authors reported that the use of

AEP Vol. 21, No. 3
March 2011: 204–213

TABLE 1. Demographic profile of the 1810 women with positive human β-globin amplification

Variable	Regions*					
	Leticia-Amazonas (n = 139)	Chaparral- Tolima (n = 149)	Engativá-Bogotá (n = 796)	Girardot-Cundinamarca (n = 320)	Tumaco-Nariño (n = 406)	Total (n = 1810)
Age, years†	39.7 [17–68] SD = 12.0	35.7 [15–66] SD = 10.9	39.9 [17–69] SD = 12.1	41.0 [16–69] SD = 12.2	35.8 [14–62] SD = 10.3	38.8 [14–69] SD = 11.8
Ethnicity						
White	24.6	7.1	9.7	52.7	3.9	17.0
Indigenous	25.4	1.4	0.1	0.4	1.7	2.6
Mestizo	46.4	90.0	86.3	42.5	13.8	59.2
Black	3.6	1.5	3.9	4.4	80.6	21.2
Marital status						
Single	14.1	7.0	26.6	16.8	1.4	16.7
Married	22.4	36.6	26.7	24.8	9.4	22.9
Civil union	46.3	49.4	33.0	46.3	88.5	50.2
Separated	12.7	4.9	11.0	8.6	0.0	7.7
Widowed	4.5	2.1	2.7	3.5	0.7	2.5
Age at first intercourse, years						
<15	34.1	30.2	21.9	18.2	31.7	25.0
16–17	21.8	24.6	23.1	30.3	29.9	25.9
18–19	24.9	21.2	26.0	22.2	23.8	24.4
>19	19.2	24.0	29.0	29.3	14.6	24.7
Pregnancies						
None	9.2	0.9	7.9	7.4	4.7	6.7
1–2	29.0	27.1	41.9	35.6	28.1	35.7
2–3	31.3	42.4	36.1	37.8	31.6	35.4
>4	30.5	29.6	14.1	19.2	35.6	22.2
Cytological findings						
Normal	99.3	98.7	83.4	95.3	96.0	91.0
Abnormal	0.7	1.3	16.6	4.7	4.0	9.0
Lifetime number of sexual partners						
1	39.1	45.8	41.1	52.0	39.4	42.8
2–3	42.2	45.1	46.2	42.8	53.3	46.8
>3	18.7	9.1	12.7	5.2	7.3	10.4
Contraceptive method						
None	44.7	37.6	45.0	41.9	36.8	42.1
Oral contraceptives	6.8	4.5	3.2	10.9	5.3	5.4
Surgery	31.1	39.2	24.9	33.3	39.4	31.1
Condom	4.5	3.6	6.1	5.0	1.7	4.6
Intrauterine device	3.0	14.2	15.1	8.6	8.6	11.5
Injectable contraceptives	9.9	0.9	5.7	0.3	8.2	5.3
Smoking status						
Yes	90.7	4.0	86.0	88.4	7.6	89.0
No	9.3	96.0	14.0	11.6	92.4	11.0

Values are in percentages unless otherwise noted.
*The number of women reported for each region corresponds to samples having a good DNA quality (positive human β-globin).
†Mean [range].

different primers provides greater robustness for detecting infections with multiple HPV types compared with analyses that use a single consensus primer set (10). In brief, the supernatant gathered after processing samples with the lysing buffer was subjected to PCR amplification with GP5+/GP6+, which allows detecting low viral copies (9, 10), and MY09/MY11, which has greater sensitivity for detecting more than one viral type (11). Samples testing positive with either one or both generic primer sets, as assessed by visualization of PCR products in 2% agarose gels, were considered to be infected with HPV and underwent further PCR amplification with type-specific primers annealing within the E5-E6 and E7 regions of HR-HPV-16, -18, -31, -33, -45, and -58 (12, 13), as well as with a set of primers that allow the identification of LR-HPV-6/11, although this latter primer set does not allow discriminating between these two types (14). The HR-HPV types evaluated in this study have been linked with 90% of all CCs reported in Latin America and around the globe (15). Synthetic genes encoding HPV-18, -31, -45, and -58 regions and HPV-6,

-11, -16, and -33—infected samples were used as positive controls for type-specific identifications (16). Appropriate positive and negative controls were included in each assay to rule out DNA contamination.

Statistical Analysis

The population size was estimated with the consideration that 1778 individuals yield a 95% confidence interval, equal to the sample proportion plus or minus 0.02 when the estimated proportion is 0.25 (4). On the basis of a stratum of five geographical regions, the number of samples was adjusted considering a proportional allocation, where the size depends on the number of cytologies being taken in each region. For the statistical analysis, the age of the subject, age at first intercourse, lifetime number of sexual partners, and number of full-term pregnancies were treated as categorical variables and summarized by the use of percentages with their corresponding 95% confidence intervals (CIs). Differences between proportions were evaluated by applying a χ^2 and a Fisher exact test, whereas associations between categorical variables were assessed using odds ratios (ORs) with their corresponding 95% CIs. Binomial logistic regression was performed to evaluate the relationship between risk factors and infection (taken as a binomial outcome). In addition, an ordinal logistic regression was used for estimating adjusted ORs, considering the presence of any of the three infection levels (none, single, multiple) as the main outcome variable. An ordinal logistic model was performed to assess association between variables, including interaction terms between the variables of pregnancies, lifetime number of sexual partners, ethnicity and geographic region, for this purpose, we performed a stepwise model using 0.15 as p.e and 0.2 as p.r. These statistical procedures were carried out using STATA® software, setting the level of significance to 0.05.

RESULTS

A total of 2110 cervical epithelium samples were collected from women inhabiting the five different regions analyzed in this study. Two hundred and five samples (14%) were excluded from the study because of poor DNA quality, and five samples (0.2%) were excluded because they belonged to women older than 69 years of age. The remaining 1810 samples (testing positive for the β-globin gene) were analyzed with the two generic primer sets, identifying HPV-DNA (as indicated by the positive amplification with GP5+/GP6+, MY09/11, or both) in 897 samples (49.6%) and coinfection in 581 (64.8% of HPV-positive samples) of them. A total of 702 samples (38.8%) showed positive amplification with MY09/11 and 457 (25.2%) showed positive amplification with GP5+/GP6+. PCR assays with generic primers were carried out twice at different times to avoid false-positive readings attributable to sample contamination.

Cytological findings were classified according to the Bethesda system. According to this criteria, 92 Pap smears (5.1%) were missing/unsatisfactory, 1563 (86.4%) were negative for intraepithelial lesion or malignancy, and 155 (8.5%) showed cytologic abnormalities, with this later value being slightly greater than the percentages reported for other Colombian populations (17). There were atypical squamous cells of undetermined significance in 92 of the samples with cytologic abnormalities (5.1%), low-grade squamous intraepithelial lesion in 50 (2.7%), and high-grade squamous intraepithelial lesions in 13 (0.7%) of them.

The frequency of HPV-DNA detection was 61.1% for Leticia, 54.3% for Chaparral, 46.1% for Engativá, 51.6% for Girardot, and 49.0% for Tumaco. HPV-16 was the most frequently found viral type, followed in decreasing order by HPV 31, 18, 33, 45, 58, and 6/11. Whenever the LR-HPV types 6/11 were detected, coinfection with HR-HPV types was also detected. A similar distribution of HPV frequencies was observed in Engativá, Leticia, and Girardot; however, it is worth noting that the greatest frequency of coinfection was found in the latter two populations ($p = .010$; Table 2). In Chaparral and Tumaco, HPV-18 was the second most commonly encountered type of HPV, followed in decreasing order by HPV 31, 33, 45, and 58 (Figure 1).

Significant differences were observed between the proportions of HPV infecting each population when being analyzed regarding the place of origin of the females (Figure 1). HPV-16 was more common in Leticia-Amazonas ($p = .004$) and HPV-18 in Girardot-Cundinamarca and Chaparral-Tolima ($p = .001$). HPV-31, -33, and -45 were more frequently detected in Girardot-Cundinamarca and Leticia-Amazonas ($p = .000$), whereas HPV-58 was predominantly identified in Girardot-Cundinamarca, Leticia-Amazonas, and Engativá-Bogotá ($p = .002$), and HPV-6/11 in Chaparral-Tolima ($p = .000$).

When we calculated the association between any HPV infection (PCR amplification with at least one primer set)

TABLE 2. Relative frequency of single and multiple HPV infections with the types evaluated in this study, according to the different regions

Region	Without infection n (%)*	Single infection n (%)*	Multiple infections n (%)*	Total
Leticia-Amazonas	54 (38.9)	19 (13.7)	66 (47.4)	139
Chaparral-Tolima	68 (45.6)	31 (20.9)	50 (33.5)	149
Engativá-Bogotá	429 (53.8)	153 (19.3)	214 (26.9)	796
Girardot-Cundinamarca	155 (48.4)	19 (6.0)	146 (45.6)	320
Tumaco-Nariño	207 (51.0)	94 (23.2)	105 (25.8)	406
Total	913 (50.4)	316 (20.2)	581 (29.4)	1,810

*Percentages are calculated by rows.

AEP Vol. 21, No. 3
March 2011: 204–213

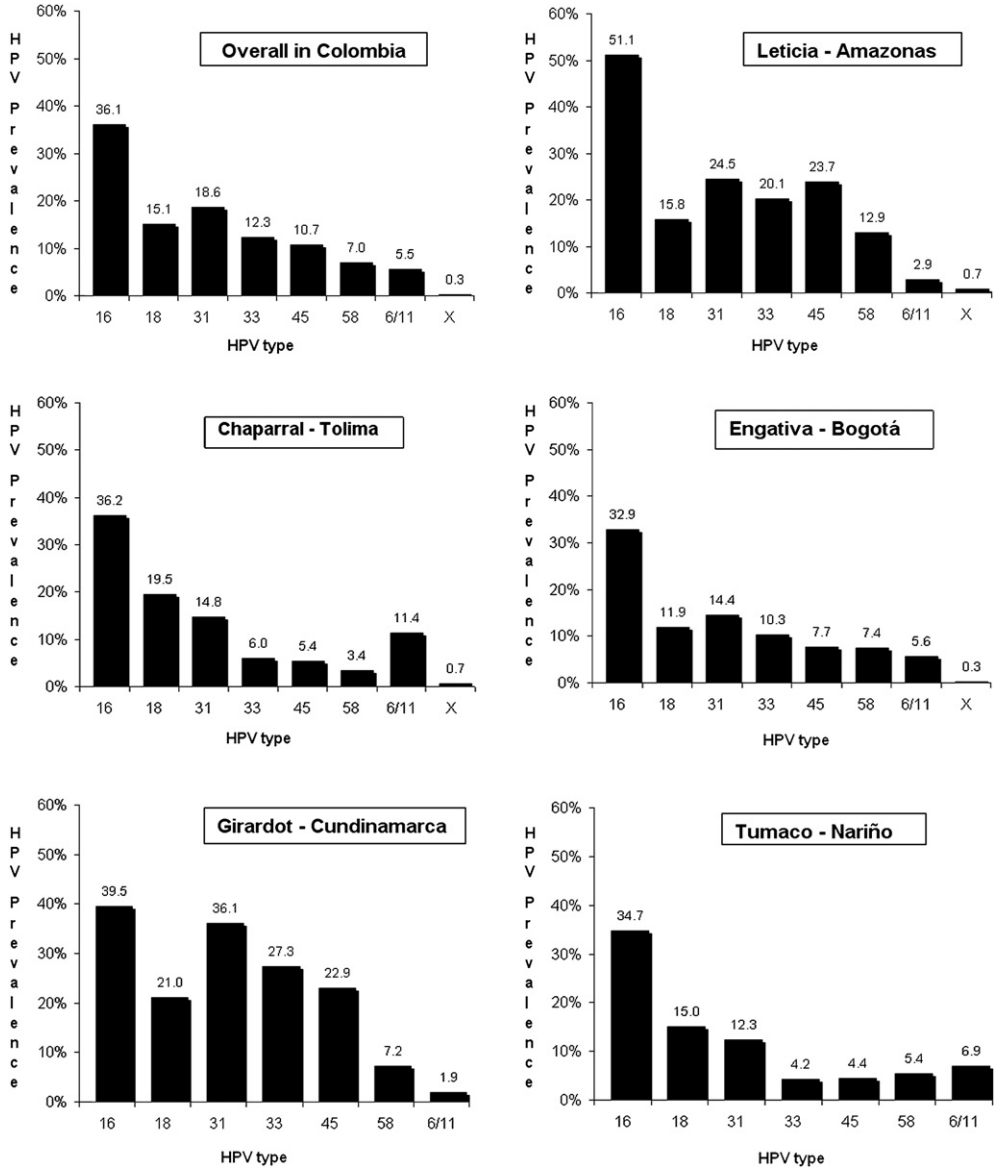

FIGURE 1. Prevalence of HPV types among 1810 Colombian women from different geographical regions.

and different factors associated with the risk of developing CC (age, contraceptive method, pregnancies, age at first intercourse, lifetime number of sexual partners, marital status, ethnicity and geographic region), the regression analysis showed that the number of pregnancies (absence of pregnancies conferred risk when compared to having had 4 or more full-term pregnancies), geographical region (the greater risk was observed in women coming from Chaparral and Leticia, compared with women coming from Engativá), and lifetime sexual partners (having had two or three sexual partners represents a lower risk compared with having had a single sexual partner) were strongly associated with any HPV infection (Table 3); these risk factors were analyzed regarding coinfection, being the results similar to those found for any infection; differences were only observed for the ethnical background (indigenous people were at greater risk compared with white people) and for geographical region, where women from Girardot or Leticia were at greater risk (Table 4).

Assessed variables remained significant when we ran the stepwise model, which included interaction terms to determine whether these had an effect in the model, as follows: pregnancies-nulliparity adjusted OR 1.56 (95% CI, 1.07–2.29), lifetime number of sexual partners (2–3) adjusted OR 0.81 (95% CI, 0.67–0.98), ethnicity-indigenous adjusted OR 2.26 (95% CI, 1.13–4.52), as well as for the

TABLE 3. Behavioral and sociodemographic factors associated with any HPV infection (single and multiple infection)

Factors	Any HPV infection n (%)	Crude ORs (95% CI)	Adjusted ORs (95% CI)
Age			
<24	115 (52.0)	1.00 (—)	
25–34	235 (50.9)	0.95 (0.69–1.32)	0.98 (0.68–1.42)
35–44	238 (47.2)	0.82 (0.60–1.13)	0.92 (0.61–1.37)
45–54	194 (48.3)	0.86 (0.62–1.43)	0.95 (0.61–1.47)
>55	95 (51.6)	0.98 (0.66–1.45)	1.04 (0.61–1.76)
Contraceptive method			
None	357 (49.1)	1.00 (—)	
Oral contraceptives	48 (51.6)	1.10 (0.71–1.69)	1.09 (0.68–1.74)
Surgery	255 (47.5)	0.93 (0.74–1.17)	0.94 (0.73–1.23)
Condom	40 (50.0)	1.03 (0.65–1.64)	1.01 (0.61–1.67)
Intrauterine device	102 (51.5)	1.09 (0.80–1.50)	1.06 (0.75–1.50)
Injectable contraceptives	51 (55.4)	1.28 (0.83–1.99)	1.20 (0.74–1.92)
Pregnancies			
None	68 (58.7)	1.47 (1.00–2.17)*	1.78 (1.04–3.04)*
1–2	305 (49.0)	0.97 (0.80–1.18)	1.14 (0.82–1.60)
3–4	309 (50.1)	1.03 (0.84–1.25)	1.18 (0.88–1.58)
>4	181 (46.8)	1.00 (—)	
Age at first intercourse			
<15	207 (47.6)	1.00 (—)	
16–17	236 (51.9)	1.20 (0.92–11.56)	1.22 (0.92–1.62)
18–19	209 (48.9)	1.06 (0.81–1.39)	1.13 (0.84–1.52)
>19	212 (48.9)	1.06 (0.81–1.39)	1.04 (0.75–1.44)
Lifetime sexual partners			
1	385 (51.7)	1.00 (—)	
2–3	376 (46.1)	0.80 (0.65–0.97)*	0.79 (0.63–0.99)*
>3	92 (51.1)	0.97 (0.70–1.35)	0.96 (0.66–1.39)
Marital status			
Single	144 (48.3)	1.00 (—)	
Married	206 (50.5)	1.09 (0.80–1.47)	1.06 (0.74–1.54)
Civil union	444 (49.5)	1.04 (0.80–1.36)	1.05 (0.76–1.46)
Separate	61 (44.2)	0.84 (0.56–1.27)	0.93 (0.59–1.47)
Widow	27 (61.3)	1.69 (0.88–3.25)	1.72 (0.82–3.59)
Ethnicity			
White	152 (50.3)	1.00 (—)	
Indigenous	30 (65.2)	1.85 (0.96–3.54)	2.03 (0.90–4.58)
Mestizo	517 (49.0)	0.95 (0.73–1.22)	1.01 (0.81–1.50)
Black	182 (48.1)	0.91 (0.67–1.24)	0.92 (0.58–1.46)
Geographic region			
Engativa	367 (46.1)	1.00 (—)	
Chaparral	81 (54.3)	1.23 (0.88–1.72)	1.66 (1.01–2.71)*
Girardot	165 (51.6)	1.01 (0.86–1.40)	1.25 (0.91–1.72)
Leticia	85 (61.1)	1.66 (1.16–2.37)*	1.82 (1.14–2.91)*
Tumaco	199 (49.0)	0.97 (0.77–1.21)	1.26 (0.83–1.93)

CI = confidence interval; HPV = human papillomavirus; OR = odds ratio.
OR adjusted for age, contraceptives method, pregnancies, age at first intercourse, number of lifetime sexual partners, marital status, ethnicity, and geographic region.
*$p < .05$.

two geographical regions: Girardot adjusted OR 1.60 (9% CI, 1.23–2.07) and Leticia adjusted OR 1.92 (95% CI, 1.28–2.88).

DISCUSSION

In this study, the frequencies of HR-HPV infection and coinfection were greater than the ones reported for other Colombian populations (2, 18). It is possible that the frequency of HPV infection was underestimated in such studies because of the use of a single primer set for detecting HPV–DNA and because the population was not as culturally diverse as the one in this study (19). Similar frequencies to the ones found here have been reported for Latin America (20–22), Africa (20), and Europe (23, 24). Moreover, other methodological aspects such as the PCR's greater sensibility for detecting viral DNA, compared with other available methods (10), and the simultaneous detection of low viral

AEP Vol. 21, No. 3
March 2011: 204–213

TABLE 4. Behavioral and sociodemographic factors associated with multiple infection

Factors	Multiple infection *n* (%)	Crude ORs (95% CI)	Adjusted ORs (95% CI)
Age			
<24	81 (70.4)	1.00 (—)	
25–34	140 (59.6)	0.90 (0.66–1.22)	0.95 (0.67–1.34)
35–44	142 (59.6)	0.77 (0.57–1.04)	0.86 (0.58–1.25)
45–54	138 (71.1)	0.90 (0.65–1.23)	0.96 (0.63–1.46)
>55	69 (72.6)	1.02 (0.70–1.48)	1.03 (0.62–1.70)
Contraceptive method			
None	226 (63.31)	1.00 (—)	
Oral contraceptives	34 (70.8)	1.17 (0.77–1.77)	1.09 (0.70–1.71)
Surgery	159 (62.3)	0.92 (0.74–1.13)	0.98 (0.76–1.25)
Condom	31 (77.5)	1.06 (0.68–1.65)	1.04 (0.65–1.66)
Intrauterine device	68 (66.6)	1.07 (0.80–1.44)	1.09 (0.78–1.52)
Injectable contraceptives	31 (60.7)	1.15 (0.77–1.71)	1.13 (0.73–1.75)
Pregnancies			
None	43 (63.27)	1.64 (1.11–2.43)*	1.67 (1.01–2.76)*
1–2	199 (65.2)	1.09 (0.85–1.39)	1.12 (0.81–1.54)
3–4	195 (63.1)	1.08 (0.85–1.38)	1.10 (0.83–1.45)
>4	120 (66.3)	1.00 (—)	
Age at first intercourse			
<15	138 (66.6)	1.00 (—)	
16–17	163 (69.0)	1.20 (0.93–1.54)	1.18 (0.90–1.55)
18–19	130 (62.2)	1.07 (0.83–1.38)	1.12 (0.84–1.48)
>19	131 (61.7)	1.07 (0.83–1.38)	1.01 (0.74–1.38)
Lifetime sexual partners			
1	268 (69.6)	1.00 (—)	
2–3	230 (61.1)	0.80 (0.66–0.97)*	0.79 (0.64–0.98)*
>3	56 (60.8)	0.93 (0.69–1.27)	0.87 (0.61–1.23)
Marital status			
Single	101 (70.1)	1.00 (—)	
Married	139 (67.4)	1.00 (0.75–1.33)	0.95 (0.67–1.35)
Civil union	271 (61.0)	0.88 (0.69–1.14)	0.93 (0.67–1.27)
Separate	37 (60.6)	0.77 (0.52–1.15)	0.81 (0.52–1.27)
Widow	18 (66.6)	1.48 (0.82–2.67)	1.51 (0.76–3.00)
Ethnicity			
White	115 (75.6)	1.00 (—)	
Indigenous	25 (83.3)	1.96 (1.07–3.59)*	2.51 (1.20–5.22)*
Mestizo	328 (63.40)	0.82 (0.64–1.05)	1.14 (0.85–1.53)
Black	100 (54.9)	0.71 (0.53–0.95)*	0.99 (0.64–1.53)
Geographic region			
Engativa	214 (58.3)	1.00 (—)	
Chaparral	50 (61.7)	1.27 (0.92–1.76)	1.45 (0.92–2.26)
Girardot	146 (88.4)	1.66 (1.29–2.15)*	1.68 (1.23–2.29)*
Leticia	66 (77.6)	2.08 (1.47–2.93)*	2.05 (1.33–3.16)*
Tumaco	105 (52.7)	0.98 (0.79–1.23)	1.10 (0.74–1.63)

CI = confidence interval; OR = odds ratio.
OR adjusted for age, contraceptives method, pregnancies, age at first intercourse, number of lifetime sexual partners, marital status, ethnicity and geographic region.
Percentages refer to the group of infected women.
*p <0.05.

loads, as well as several viral types thanks to the use of two generic primer sets (9–11), might have contributed to detect a greater viral prevalence. In addition, there are reports in literature stating that the selection of a type-specific primer can influence the results, due to preferential amplification of certain types.

HPV-16 appears to be the most prevalent type worldwide, as it is detected in approximately 50% of the cases, followed in second place by HPV-18. However, it should be noted that other authors have found a significant variation in the prevalence of other HR-HPV types among different geographical regions (25), possibly because of the influence of the biological aspects of each particular HPV type, the host immune response, as well as of each population genetics and specific environmental features (26). As expected, our study found that the most prevalent type was HPV-16, but interestingly the second most prevalent type was HPV-31 instead of HPV-18. HPV-31 has been

reported as the second most prevalent type among European females having cytological abnormalities (27, 28), which is similar to the results of this study.

The high prevalence of HPV infection found in this study does not necessarily imply a large percentage of cytological abnormalities in infected women, given that viral detection is determined by the amplification of the gene encoding the L1 protein, which is found in large amounts during active replication of HPV, and less frequently in cases of severe dysplasia due to integration of viral DNA to host cells (29, 30).

However, this study found a greater frequency of both infection and coinfection in the cities of Girardot and Leticia. The economies of these two cities rely greatly on tourism, and therefore people are more exposed to high risk sexual behaviors favoring the acquisition of multiple HPV infections and other sexually transmitted diseases (31, 32). In addition, most of the indigenous population included in this study came from the city of Leticia and showed a greater risk of presenting coinfection.

Interestingly, a HR-HPV infection rate greater than expected was here found for older women. A multivariate analysis (multiple correspondence analysis) showed that both HR-HPV infection and coinfection in this particular group were associated with an early initiation of sexual intercourse and a prolonged use of contraceptive methods (data not shown). As it has been thoroughly shown, the risk of acquiring a subsequent HPV infection increases in females already infected with HPV, being the subsequently acquired HPV types not necessarily phylogenetically related (4, 33).

Regarding socioeconomic features, indigenous ethnicity showed a high association with multiple infection, possibly attributable to cultural particularities of this ethnicity such as an early initiation of sexual activity, a reduced access to sexually transmitted disease prevention programs, or to intrinsic biological characteristics yet to be defined (34, 35). In addition, behavioral differences associated to the different ethnicities and genetic differences such as HLA allele distribution (36) cannot be ruled out as a possible explanation to the predisposition shown by the indigenous ethnicity to infection with HPV, and further studies that address this issue are required to understand better such association. Another risk factor that was found to be associated was the lifetime number of sexual partners (n = 2−3). This association has been observed in studies with Hispanic, Asian and African-American women, since it has been reported that the number of sexual partners and HPV infection vary significantly depending on the race/ethnicity (37); in addition, it has not been clearly established whether the exposure to a bigger number of viruses as the result of sexual behavior creates a competition among them in a positive or a negative manner (38).

An additional risk factor was identified when we analyzed data infection in regard to the number of pregnancies. The records showed that women with no history of previous pregnancies had a greater risk of HPV infection (single and multiple) than the ones who have had more than four full-term pregnancies. These results can be addressed from several perspectives. Because women without previous gestations are more likely to engage in sexually risky practices, such as having a large number of sexual partners. Moreover, the use of hormonal contraception methods is most frequent among women who had not given birth, which has been reported to modulate the risk of infection and disease progression, given that the virus harbors a hormone recognition fragment and therefore its behavior and distribution could be influenced by hormone changes in the female host (39).

For Latin-American populations, CC risk factors have been described as being short-termed (as age at first sexual intercourse, lifetime number of sexual partners) and long-termed (as use of oral contraceptives, high parity and cigarette smoking) (22); however, the identification of risk factor profiles in the acquisition of single or multiple infections is limited. Interestingly, our results for both types of infection did not show great differences with regard to related risk factors; this has also been observed in previous cohort studies conducted in Brazil (40) and might be attributable to the fact that all types of HPV have the same transmission vector (sexual activity) and that it has been established that coinfections depend mostly on the pre-existence of other viral types (38); according to the aforementioned, these risk factors might be contributing to a greater extent to viral persistence leading to an increased risk of developing CC than to the presence of single or multiple infections.

Additional studies are needed to provide further support to the conclusions drawn from this study, given that its cross-sectional design does not allow to fully characterize the chronology of events, such as the time elapsing between exposure to one or more than one HR-HPV and progression to significant lesions, or identifying a particular predisposition to infection by a certain HPV type when there is already another infecting HPV type.

According to our study, the frequency of all viral types varied not only among the five geographical regions, but also among the different ethnic groups herein analyzed. The results suggest that HPV infection and coinfection frequencies are strongly influenced by demographic aspects, and therefore indicate that it would be relevant to determine whether the susceptibility of a particular ethnicity is associated with intrinsic genetic features of each population, with cultural aspects and/or with particular environmental conditions that can ultimately modulate the host immune response. It would be thus important to further characterize

the risk of acquiring HPV infections in susceptible populations, in order to broaden and improve CC-prevention programs in Colombia.

In addition, because this study included a more culturally and geographically diverse population of women than other studies carried out in Colombia, the greater prevalence of HPV-31 found in this study is significant, considering that the clinical importance of this viral type has been underestimated. This viral type is considered to share a significant burden on the incidence of CC (28, 41, 42); moreover, the prevalence differences of HR-HPVs should be considered in vaccine development studies targeting Colombian populations. This is particularly relevant for anticipating the impact that immunization programs will have on these female populations, given that the currently available vaccines do not protect against all HR-HPV types (43) and that cross-immunity against other high-risk types (not included in the commercial preparations) decreases to moderate after a year of vaccination.

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