



**SEROPREVALENCE OF HPV-16 IN A POPULATION-BASED
SUB-SAMPLE OF HISPANIC ADULTS**

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Complete List of Authors:	<p>Ortiz, Ana; University of Puerto Rico Comprehensive Cancer Center, Cancer Control and Population Sciences Program; Graduate School of Public Health, University of Puerto Rico, Biostatistic and Epidemiology Department</p> <p>Unger, Elizabeth; Centers for Diseases Control and Prevention, Chronic Viral Diseases Branch</p> <p>Muñoz, Cristina; Graduate School of Public Health, University of Puerto Rico, Biostatistic and Epidemiology Department</p> <p>Panicker, Gitika; Centers for Diseases Control and Prevention, Chronic Viral Diseases Branch</p> <p>Tortolero-Luna, Guillermo; University of Puerto Rico Comprehensive Cancer Center, Cancer Control and Population Sciences Program</p> <p>Soto-Salgado, Marievelisse; University of Puerto Rico Medical Sciences Campus, UPR-MDACC Partnership for Excellence in Cancer Research Program</p> <p>Otero, Yomayra; University of Puerto Rico Medical Sciences Campus, UPR-MDACC Partnership for Excellence in Cancer Research Program</p> <p>Suarez, Erick; Graduate School of Public Health, University of Puerto Rico, Biostatistic and Epidemiology Department</p> <p>Perez, Cynthia; Graduate School of Public Health, University of Puerto Rico, Biostatistic and Epidemiology Department</p>
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7 **HISPANIC ADULTS**
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11 Ortiz AP^{1,2}, Unger ER³, Muñoz C², Panicker G³, Tortolero-Luna G¹, Soto-Salgado M⁴, Otero
12
13 Y⁴, Suárez E², Pérez CM².
14
15

16
17
18 **Affiliations:** ¹Cancer Control and Population Sciences Program, University of Puerto Rico
19
20 Comprehensive Cancer Center; ²Biostatistics and Epidemiology Department, Graduate School of
21
22 Public Health, University of Puerto Rico; ³Chronic Viral Diseases Branch, Centers for Disease
23
24 Control and Prevention, Atlanta, GA; ⁴UPR-MDACC Partnership for Excellence in Cancer
25
26 Research Program, Medical Sciences Campus, University of Puerto Rico.
27
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32
33 **Correspondence to:** Ana Patricia Ortiz, PhD, MPH
34

35
36 University of Puerto Rico Comprehensive Cancer Center, PMB 711, 89 De Diego Ave. Suite
37
38 105, San Juan, PR, 00927-6346

39
40 Phone (787) 772-8300 x-1204/ Fax (787) 758-2557 / E-mail: ana.ortiz7@upr.edu
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Abstract

Background: Persistent HPV infection is associated with cancer of the cervix, anus, vulva, vagina, penis, mouth and oropharynx. Although HPV is a public health problem worldwide, population-based data on HPV seroprevalence for Puerto Rico (PR) is unavailable. HPV-16 is the most common HPV type detected in most regions of the world. This study aimed to estimate the prevalence and correlates of seropositivity to HPV-16 in a sub-sample of adults in PR.

Methods: Cross-sectional study in which the last 450 consecutive adults aged 21-64 years who participated in an island-wide population-based study (n=1,654) in PR conducted between 2005-2008 provided serum samples. The samples were tested by ELISA for HPV-16 viral-like particle-specific IgG. Information on socio-demographic, health and lifestyle characteristics was collected. Logistic regression modeling was used to estimate the prevalence odds ratio (POR) to assess factors associated to HPV-16 seropositivity.

Results: Prevalence of seropositivity to HPV-16 was 11.3%. Seroprevalence was higher in women (15.8%) than men (5.6%) (p=0.001). After adjusting for age and sex, ever smokers (POR: 2.06, 95% CI=1.08-3.92) and participants with at least five lifetime sexual partners (POR: 2.91, 95% CI=1.24-6.81) were more likely to be HPV-16 seropositive.

Conclusions: HPV-16 seropositivity is similar to that reported in the US (10.4%) for NHANES 2003-2004 participants, although different assays were used in these studies. Our results highlight the need to further understand the burden of HPV and HPV-related malignancies in PR.

Strengths and limitations of this study:

- This study is the first to evaluate HPV-16 seroprevalence and correlates in Puerto Rico and provides insights of the epidemiology of HPV infection in this population.
- Prevalence of HPV-16 seropositivity among 450 adults aged 21-64 years in Puerto Rico was 11.3%; prevalence was higher for women (15.8%) than for men (5.6%).
- Women, smokers and participants with ≥ 5 lifetime sexual partners were more likely to be HPV-16 seropositive; highlighting population sub-groups with higher odds of sero-conversion.
- HPV-16 seropositivity in our study (2005-2008) is similar to that reported in the US NHANES 2003-2004 (10.4%), although different assays were used.

INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide. Persistent infection with certain types of HPV has been established as a necessary cause for cervical cancer[1, 2] and has been associated with cancer of the anus, vulva, vagina, penis, and oropharynx.[3,4] Approximately 5.2% of all cancers worldwide are attributable to HPV infection[3] Moreover, the economic burden of the HPV infection is high, and second only to the cost of Human Immunodeficiency Virus (HIV) infection.[5] Currently, there are two HPV vaccines licensed for use worldwide that have proven to be effective in preventing HPV infection and progressive disease in people that were previously HPV naïve.[6] With widespread use, the vaccines could provide a cost-effective prevention strategy.

Antibody response to HPV infection is considered a key determinant of protective immunity and may play a role as a predictor of HPV-associated cervical neoplasia.[2,7] The protective antibody response is mainly type-specific and directed against conformational epitopes of the major capsid protein L1,[8] while antibodies to E6 and E7 oncoproteins may be markers of invasive cervical cancer, with tumor stage and mass determining the magnitude of the response.[9] Seroconversion occurs several months after detection of HPV-DNA infection; approximately only 60% of women with an incident of HPV-DNA infection seroconvert within 18 months after detection. No differences in median time of seroconversion are observed by HPV type, although antibody responses to high-risk HPV types have been found to persist longer.[1, 10] While HPV DNA testing detects current infection, serological testing serves as a useful epidemiologic research tool to measure lifetime exposure to HPV infection because antibodies may persist even after the virus has cleared. Although HPV-16 is the most common HPV type detected in most regions of the world,[11] no population-based sero-epidemiological

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3 studies of HPV-16 infection have been conducted in Puerto Rico (PR). An estimate of natural
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5 HPV infection would not only help understand the burden of the disease, but also provide the
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7 necessary baseline data for HPV vaccine implementation and monitoring in PR. Hence, the
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9 purpose of this pilot study was to estimate prevalence of HPV-16 IgG responses and factors
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11 associated with HPV-16 seropositivity in a cross-section of adults in PR.
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MATERIALS AND METHODS

Study Design and Population

An island-wide population-based cross-sectional household survey aimed at estimating the seroprevalence of hepatitis C and other viral infections (hepatitis A, hepatitis B, HIV and Herpes Simplex type 2) was performed in PR (n=1,654) between 2005 and 2008. Detailed descriptions of the study sampling design and data collection procedures have been previously published.[12] In brief, a cluster sampling design for household surveys using the census tracts of PR was employed, and one individual aged 21-64 years from each selected household was randomly selected to participate in the study. Participants underwent a personal interview and an audio computer-assisted self-interview (ACASI) using QDS (Nova Research Co., Washington D.C.), and provided a sample of blood for serologic testing. All study procedures were reviewed and approved by the Institutional Review Board of the University of Puerto Rico Medical Sciences Campus. For the current analysis we used residual serum from a sub-sample of the last 450 consecutive adults aged 21 to 64 years who participated in the study and agreed to participate in HPV testing.

Serologic Testing

Antibody testing for HPV-16 was performed at the Human Papillomavirus Laboratory at the Centers for Disease and Control in Atlanta. Virus-like particles (VLPs) were produced by expression of an HPV-16 L1 recombinant baculovirus in insect cells.[13] HPV-16 specific IgG antibody was detected using a VLP-based direct enzyme-linked immunosorbent assay (ELISA) as described by Karem et.al[13] but with a few modifications. Microtiter plates were coated overnight at 4°C with HPV-16 VLP diluted to 0.5µg/ml in PBS. Sera (both reference and test samples) were serially-diluted at 1:10, 1:31.6 and 1:100 in 1X TBST with 10% goat serum, 10%

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3 Super-Block, and 10% insect cell lysate. An optimized concentration of goat anti-human IgG
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5 conjugated to alkaline phosphatase (EMD biosciences) diluted in 1X TBST with 10% goat
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7 serum, 10% Super-Block was used as the secondary antibody.
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10 Sample antibody titers (IU/ml) were calculated using the parallel line method (PLL)
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12 against a reference sample calibrated to the International Standard 16 (NIBSC 05/134) with
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14 known titer of 10 IU/ml.[14] A pooled serum, negative for antibodies to HPV-16, 18, 6 and 11
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16 as tested by an alternate assay, competitive Luminex assay[15] was used to establish cut-off
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18 value. The cut-off for positive results was set at values greater than or equal to the median
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20 antibody titer of the negative control plus two standard deviations (1.97 IU/ml). To determine
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22 differences by gender in the median titers of HPV a Mann-Whitney-Wilcoxon test was used.
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29 **Study variables**

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31 Demographic characteristics included age (21-34, 35-50, 51-64 years), education (<12 vs. ≥12
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33 years), annual family income (<\$20,000 vs. ≥ \$20,000), health insurance coverage (private,
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35 government-sponsored, none) and marital status (single, married/living together,
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37 divorced/separated/widowed). Sexual practices (yes/no) included lifetime history of vaginal,
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39 anal, and oral sex as well as age of sexual initiation (≤18 vs. >18 years), and number of lifetime
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41 sexual partners (0-1, 2-4, ≥5). History of smoking (yes / no) was also assessed.
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Statistical Analysis

Summary measures (mean±SD or median and 25th and 75th percentiles) and frequency distributions were performed to characterize the demographic, clinical, and lifestyle characteristics of study participants. Contingency tables were generated to assess the associations of these covariates with HPV-16 seropositivity. Multiple logistic regression models were fitted to estimate the prevalence odds ratios (POR) with 95% confidence intervals (CI) for HPV-16 seropositivity.[16] Variables statistically associated with HPV-16 seropositivity in age- and sex-adjusted logistic regression models ($p<0.05$) were included in the multivariate model. All data were evaluated using Stata for Windows release 11.0 (Stata Corporation, College Station, Texas). No significant interaction terms were found in the multivariate logistic regression models evaluated ($p>0.05$).

RESULTS

Sociodemographic, Clinical, and Lifestyle Characteristics

The characteristics of the sub-sample of 450 adults are shown in Table 1. Three participants were HIV positive (0.99%; data not shown). Although a comparison between the study sub-sample and the parent study population showed no significant differences in the clinical and lifestyle characteristics studied, a higher proportion of participants of the sub-sample reported less than 12 years of education (30.7% vs. 23.2%), a government-based health insurance (51.1% vs. 41.9%), and an annual family income below \$20,000 (72.6% vs. 63.8%) ($p<0.05$) (data not shown).

Seroprevalence of HPV-16

Overall, 11.3% of participants were seropositive to HPV-16. Seroprevalence among women (15.8%) was higher as compared to men (5.6%) ($p=0.001$) (Table 1). Median titers (IU per ml) of HPV positive women (8.39, 2.68-16.01) were higher than those for men (3.32, 3.04-7.13) (p -value <0.0001). Although no significant differences ($p>0.05$) in prevalence were observed across age groups in men or women, the seroprevalence was higher in younger women (Figure 1). The mean age of HPV-16 seropositive individuals was lower (38.5 ± 12.7) than among those HPV-seronegative (41.9 ± 12.2); this result was marginally significant ($p=0.06$). In bivariate analysis, no significant differences in seropositivity were observed by education level, household income, marital status, health care coverage, place of birth, sexual practices and smoking status ($p>0.05$) (Table 1). After adjusting for age and sex, ever smokers (POR: 2.06, 95% CI=1.08-3.92) and those reporting at least five lifetime sexual partners (POR: 2.91, 95% CI=1.24-6.81) were more likely to be HPV-16 seropositive (Table 2). Nonetheless, these associations attenuated to non-significance in the multivariable model. Only sex remained significantly associated with HPV-16 seropositivity in multivariate analysis, with women being more likely to be seropositive as compared to men (POR: 4.16, 95% CI=1.91-9.03) (Table 2).

DISCUSSION

The first HPV vaccine, which includes HPV-16, was approved by the Food and Drug Administration for use in the US and PR in 2006 for females only (aged 9-26 years) and later in 2008 for men. Our estimate of HPV-16 seropositivity for this sub-sample of adults in PR (11.3%) from 2005-2008 is comparable to those reported in the US (10.4%) for persons aged 14-59 years participating in the NHANES 2003-2004.[17] Our findings are also comparable to those

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3 reported in studies worldwide. A study in the Netherlands during 2006-2007 among persons aged
4 ≥ 14 years found an HPV-16 seroprevalence of 11.3%,[11] whereas a study in England among
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6 persons aged 10-49 years reported a seroprevalence of 14.7%.[18] Also, consistent with the
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8 study by Dunne et al.,[19] the prevalence of HPV seroreactivity in our study was higher among
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10 women (15.8%) than men (5.6%), with 4-fold increased odds of infection. In a study performed
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12 by Stone et al.,[7] HPV-16 seroreactivity was over two-times higher in women (17.9%) than in
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14 men (7.9%), and constant across all age and racial/ethnic groups evaluated in the study.
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16 Moreover, several studies have shown this sex difference in antibody response in all HPV
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18 vaccine types (6, 11, 16 and 18).[17,18] Sex differences in HPV-16 seroreactivity are also
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20 observed among high-risk populations. Women attending STD clinics had higher prevalence of
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22 HPV-16 seropositivity (30.2%) than men (18.7%), supporting that there are biological reasons
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24 for men and women differing in serologic responses.[20] Potential explanations proposed by
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26 Thompson and colleagues include that men may be: 1) not as susceptible to HPV-16 infection, 2)
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28 more able to clear the infection spontaneously without developing a systemic antibody response,
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30 and 3) less likely to get infected with HPV-16 given that their sexual exposure frequently
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32 involves keratinized epithelium (penis) rather than mucosal epithelium (cervix).[20]
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41 In concordance with the literature, HPV-16 seropositivity was also associated with
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43 lifetime number of sexual partners; a strong predictor of HPV seropositivity for both males and
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45 females.[2, 7, 10, 21] In a study performed in Costa Rica, it was found that women with at least
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47 three lifetime sexual partners had a two-fold increase in the detection of HPV-16 antibodies
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49 compared to women with one lifetime sexual partner.[22] Similarly, results from the HPV
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51 Infection in Men (HIM) Study showed that men with multiple lifetime male partners (≥ 11
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53 partners) were more likely to be seropositive to HPV-16 (OR=7.74; 95% CI: 3.96-15.12).[10]
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3 We observed a strong association between smoking and HPV-16 seropositivity, with smokers
4 being more likely to be positive for HPV-16 antibodies in this sample. Smoking habits have
5 been identified as a risk factor for HPV infection and seropositivity, but these findings are
6 inconsistent.[17, 23, 24] Unlike previous studies showing a decline in seropositivity among those
7 aged 50+ years, we did not observe a significant association between age and HPV-16
8 seropositivity.[4, 17, 21] This could be due to the small numbers of seropositive persons in this
9 study.
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20 Study limitations include the lack of HPV vaccination data of participants and the sample
21 size; only 51 samples were HPV positive, limiting the power of our study to detect significant
22 associations with HPV serology. Future studies should evaluate HPV seroprevalence using a
23 larger population-based sample; the research team is currently executing a population-based
24 study among women living in the San Juan metropolitan area, which will soon produce data in
25 this area. All HPV serology assays are limited by the lack of commercial reagents and difficulties
26 in comparing results between different platforms. We reported on results in terms of IU to help
27 in inter-study comparisons.
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39 This study is the first to evaluate the prevalence and correlates of HPV seroprevalence in
40 PR. With the introduction of the two prophylactic HPV vaccines, it is important to establish
41 baseline population HPV prevalence and better identify population subgroups at high risk for
42 HPV-related cancers in the population before the uptake of the vaccine continues to increase.
43 This is particularly important in PR, where population-based estimates of vaccine uptake
44 continue to be low among children and adults (<5% among women aged 16-26 years).[25]
45 Knowledge of the burden of infection prior to the expansion of these programs will allow a better
46 assessment and understanding of the short-term and long-term effectiveness of this primary
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3 prevention strategy for anogenital lesions (cervix, vagina, vulva and anus). Thus, these initial
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5 findings provide a much needed insight on the epidemiology of HPV infection in PR and will
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7 permit the development of future HPV-related research.
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Table 1. Characteristics of study participants by HPV-16 serostatus (n=450)

Characteristics	Total Study Population N (%)	HPV-16 negative (n=399, 88.7%) n (%)	HPV-16 positive (n=51, 11.3%) n (%)	p-value ^a
<i>Age group in years</i>				0.11
21-34	153 (34.0)	129 (32.3)	24 (47.0)	
35-50	180 (40.0)	164 (41.1)	16 (31.4)	
51-64	117 (26.0)	106 (26.6)	11 (21.6)	
Mean±SD	40.9 ±12.3	41.9±12.2	38.5±12.7	0.06 ^b
<i>Sex</i>				0.001
Female	253 (56.2)	213 (53.4)	40 (78.4)	
Male	197 (43.8)	186 (46.6)	11 (21.6)	
<i>Education in years</i>				>0.10
<12	138 (30.7)	123 (30.8)	15 (29.4)	
≥12	312 (69.3)	276 (69.2)	36 (70.6)	
<i>Annual family income (n=424)</i>				>0.10
< \$20,000	308 (72.6)	274 (72.9)	34 (70.8)	
≥ \$20,000	312 (69.3)	102 (27.1)	14 (29.2)	
<i>Marital status</i>				>0.10
Never married	77 (17.1)	67 (16.8)	10 (19.6)	
Married/Cohabiting	258 (57.3)	230 (57.6)	28 (54.9)	
Divorced/Separated/Widowed	115 (25.6)	102 (25.6)	13 (25.5)	
<i>Health care coverage</i>				>0.10
None	38 (8.4)	37 (9.3)	1 (1.9)	
Government-administered	230 (51.1)	201 (50.4)	29 (56.9)	
Private	182 (40.4)	161 (40.3)	21 (41.2)	
<i>Place of birth</i>				>0.10
Puerto Rico	414 (92.0)	365 (91.5)	49 (96.1)	
United States	36 (8.0)	34 (8.5)	2 (3.9)	
<i>Seropositive status to HSV-2 (n=440)</i>				>0.10
Positive	110 (25.0)	96 (24.6)	14 (28.0)	
Negative	330 (75.0)	294 (75.4)	36 (72.0)	
<i>Ever had sex (n=445)</i>				>0.10
Yes	431 (96.9)	382 (96.7)	49 (98.0)	
No	14 (3.1)	13 (3.3)	1 (2.0)	
<i>Age at first sexual intercourse (n=427)</i>				>0.10
< 18	228 (53.4)	200 (52.8)	28 (58.3)	
≥ 18	199 (46.6)	179 (47.2)	20 (41.7)	
<i>Number of lifetime sex partners</i>				>0.10

(n=442)				
0-1	100 (23.6)	91 (24.3)	9 (18.8)	
2-4	152 (35.9)	137 (36.5)	15 (31.2)	
≥ 5	171 (40.4)	147 (39.2)	24 (50.0)	
<i>Anal Sex</i>				>0.10
Ever	262 (58.2)	234 (58.7)	28 (54.9)	
Never	188 (41.8)	165 (41.3)	23 (45.1)	
<i>Oral Sex</i>				>0.10
Ever	333 (74.0)	295 (73.9)	38 (74.5)	
Never	117 (26.0)	104 (26.1)	13 (25.5)	
<i>History of smoking</i>				>0.10
Yes	253 (56.2)	219 (54.9)	34 (66.7)	
No	197 (43.8)	180 (45.1)	17 (33.3)	

a. p-values from Chi-square statistics.

b. p-value from t-test.

Table 2. Magnitude of the association (POR) between of HPV-16 and different characteristics

Characteristics	Crude POR	Age-and-sex-adjusted POR (95% CI)	Multivariate-adjusted POR ^a (95% CI)
<i>Age group in years</i>			
51-64	1.0	---	1.00
35-50	0.96 (0.43-2.12)	---	0.99 (0.42-2.32)
21-34	1.81 (0.87-3.79)	---	1.52 (0.67-3.47)
<i>Sex</i>			
Male	1.0	---	1.00
Female	3.18 (1.58-6.37)	---	4.16 (1.91-9.03)
<i>Number of lifetime sex partners</i>			
0-1	1.0	1.0	1.00
2-4	1.11 (0.46-2.64)	1.18 (0.48-2.87)	1.09 (0.44-2.68)
≥ 5	1.65 (0.73-3.71)	2.78 (1.15-6.73)	2.36 (0.94-5.90)
<i>History of smoking</i>			
No	1.0	1.0	1.00
Yes	1.64 (0.89-3.04)	2.06 (1.08, 3.92)	1.58 (0.79-3.16)

a. Additionally adjusted by number of lifetime sexual partners and history of smoking; no significant interaction terms detected in this logistic regression model ($p > 0.05$).

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13 the funding agency.
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16
17 design; monitoring of data collection and quality, analysis and interpretation of data; drafting the
18
19 article and revising based on reviewer comments. Tortolero-Luna G contributed to the concept
20
21 and design and interpretation of data and revised the article for important intellectual content.
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23 Muñoz C, Soto-Salgado M and Otero Y contributed to the concept, data analysis and
24
25 interpretation of data; drafting the article and revising based on reviewer comments. Panicker G
26
27 and Unger ER contributed to the design, HPV laboratory analyses, and interpretation of findings
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29 and revised the article for important intellectual content.
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6 International Papillomavirus Conference; 2012 November 30-December 6; San Juan, PR.
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STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*
Checklist for cohort, case-control, and cross-sectional studies (combined)

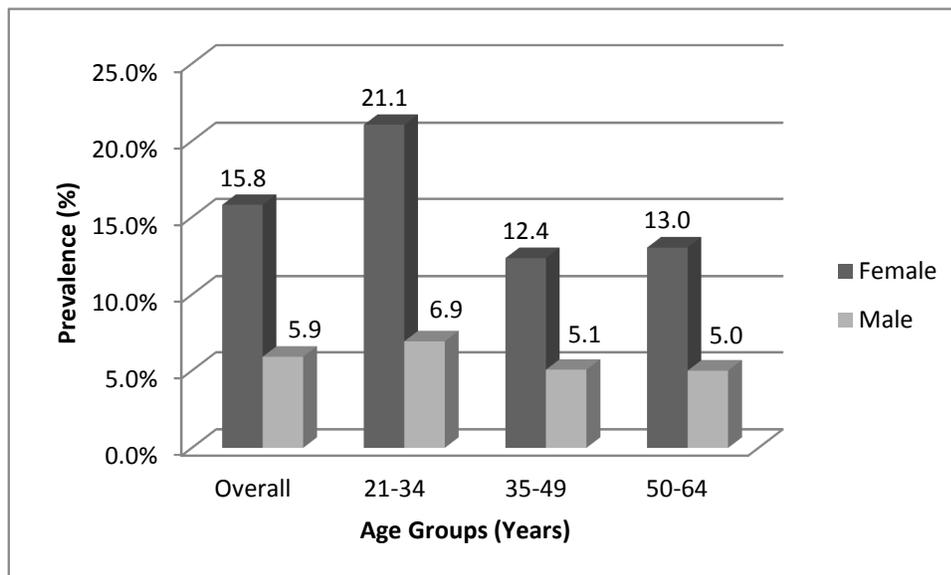
Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any pre-specified hypotheses	3-4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	

		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	7
		(e) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7-8
		(b) Give reasons for non-participation at each stage	7-8
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7-8,12
		(b) Indicate number of participants with missing data for each variable of interest	7-8
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	7-8
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-8,14
		(b) Report category boundaries when continuous variables were categorized	7-8
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	7-8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	8-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8-11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

Figure 1. Seroprevalence of HPV-16 by age and sex (n=450).





Cross-sectional study of HPV-16 infection in a population-based sub-sample of Hispanic adults.

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Manuscripts

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3 **CROSS-SECTIONAL STUDY OF HPV-16 INFECTION IN A POPULATION-BASED**
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5 **SUB-SAMPLE OF HISPANIC ADULTS**
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10 Ortiz AP^{1,2}, Unger ER³, Muñoz C², Panicker G³, Tortolero-Luna G¹, Soto-Salgado M⁴, Otero
11 Y⁴, Suárez E², Pérez CM².
12
13
14

15
16
17 **Affiliations:** ¹Cancer Control and Population Sciences Program, University of Puerto Rico
18 Comprehensive Cancer Center; ²Biostatistics and Epidemiology Department, Graduate School of
19 Public Health, University of Puerto Rico; ³Chronic Viral Diseases Branch, Centers for Disease
20 Control and Prevention, Atlanta, GA; ⁴UPR-MDACC Partnership for Excellence in Cancer
21 Research, School of Medicine, Medical Sciences Campus, University of Puerto Rico.
22
23
24
25
26
27
28
29
30
31

32 **Correspondence to:** Ana Patricia Ortiz, PhD, MPH
33
34 University of Puerto Rico Comprehensive Cancer Center, PMB 711, 89 De Diego Ave. Suite
35 105, San Juan, PR, 00927-6346
36
37 Phone (787) 772-8300 x-1204/ Fax (787) 758-2557 / E-mail: ana.ortiz7@upr.edu
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54 **Word count (abstract to discussion):** 2,402 words
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Abstract

Background: Persistent HPV infection is associated with cancer of the cervix, anus, vulva, vagina, penis, mouth and oropharynx. Although HPV is a public health problem worldwide, population-based data on HPV seroprevalence for Puerto Rico (PR) is unavailable. HPV-16 is the most common HPV type detected in most regions of the world. **Objective:** This study aimed to estimate the prevalence and correlates of seropositivity to HPV-16 in a sub-sample of adults in PR. **Methods:** Cross-sectional study in which the last 450 consecutive adults aged 21-64 years who participated in an island-wide population-based study (n=1,654) in PR conducted between 2005-2008 provided serum samples. The samples were tested by ELISA for HPV-16 viral-like particle -specific IgG. Information on socio-demographic, health and lifestyle characteristics was collected. Logistic regression modeling was used to estimate the prevalence odds ratio (POR) to assess factors associated to HPV-16 seropositivity. **Results:** Prevalence of seropositivity to HPV-16 was 11.3%. Seroprevalence was higher in women (15.8%) than men (5.6%) (p=0.001). After adjusting for age and sex, ever smokers (POR: 2.06, 95% CI=1.08-3.92) and participants with at least five lifetime sexual partners (POR: 2.91, 95% CI=1.24-6.81) were more likely to be HPV-16 seropositive. **Conclusions:** HPV-16 seropositivity is similar to that reported in the US (10.4%) for NHANES 2003-2004 participants, although different assays were used in these studies. Our results highlight the need to further understand the burden of HPV and HPV-related malignancies in PR.

Strengths and limitations of this study:

- This study is the first to provide initial insights of the epidemiology of HPV infection by assessing HPV-16 seroprevalence and its correlates in Puerto Rico.
- Although different laboratory assays were used, our estimate of HPV-16 seropositivity for this sub-sample of adults in Puerto Rico (11.3%) from 2005-2008 is comparable to that reported in the US (10.4%) for persons aged 14-59 years participating in the NHANES 2003-2004. Study limitations include the lack of HPV vaccination data of study participants and the modestly sized sample of HPV-16 seropositive individuals, limiting the power of our study to detect significant associations with HPV serology.

INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide. Persistent infection with certain types of HPV has been established as a necessary cause for cervical cancer[1, 2] and has been associated with cancer of the anus, vulva, vagina, penis, and oropharynx.[3,4] Approximately 5.2% of all cancers worldwide are attributable to HPV infection[3] Moreover, the economic burden of the HPV infection is high, and second only to the cost of Human Immunodeficiency Virus (HIV) infection.[5] Currently, there are two HPV vaccines licensed for use worldwide that have proven to be effective in preventing HPV infection and progressive disease in people that were previously HPV naïve.[6] With widespread use, the vaccines could provide a cost-effective prevention strategy.

Antibody response to HPV infection is considered a key determinant of protective immunity and may play a role as a predictor of HPV-associated cervical neoplasia.[2,7] The protective antibody response is mainly type-specific and directed against conformational epitopes of the major capsid protein L1,[8] while antibodies to E6 and E7 oncoproteins may be markers of invasive cervical cancer, with tumor stage and mass determining the magnitude of the response.[9] Seroconversion occurs several months after detection of HPV-DNA infection; approximately only 60% of women with an incident of HPV-DNA infection seroconvert within 18 months after detection. No differences in median time of seroconversion are observed by HPV type, although antibody responses to high-risk HPV types have been found to persist longer.[1, 10] While HPV DNA testing detects current infection, serological testing serves as a useful epidemiologic research tool to measure lifetime exposure to HPV infection because antibodies may persist even after the virus has cleared. Although HPV-16 is the most common HPV type detected in most regions of the world,[11] no population-based sero-epidemiological

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3 studies of HPV-16 infection have been conducted in Puerto Rico (PR). An estimate of natural
4
5 HPV infection would not only help understand the burden of the disease, but also provide the
6
7 necessary baseline data for HPV vaccine implementation and monitoring in PR. Hence, the
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9 purpose of this pilot study was to estimate prevalence of HPV-16 IgG responses and factors
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11 associated with HPV-16 seropositivity in a cross-section of adults in PR.
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MATERIALS AND METHODS

Study Design and Population

An island-wide population-based cross-sectional household survey aimed at estimating the seroprevalence of hepatitis C and other viral infections (hepatitis A, hepatitis B, HIV and Herpes Simplex type 2) was performed in PR (n=1,654) between 2005 and 2008. Detailed descriptions of the study sampling design and data collection procedures have been previously published.[12] In brief, a cluster sampling design for household surveys using the census tracts of PR was employed, and one individual aged 21-64 years from each selected household was randomly selected to participate in the study. Participants underwent a personal interview and an audio computer-assisted self-interview (ACASI) using QDS (Nova Research Co., Washington D.C.), and provided a sample of blood for serologic testing. All study procedures were reviewed and approved by the Institutional Review Board of the University of Puerto Rico Medical Sciences Campus. For the current analysis we used residual serum from a sub-sample of the last 450 consecutive adults aged 21 to 64 years, recruited between February 2007 and January 2008, who participated in the study and agreed to participate in HPV testing.

Serologic Testing

Antibody testing for HPV-16 was performed at the Human Papillomavirus Laboratory at the Centers for Disease and Control in Atlanta. Virus-like particles (VLPs) were produced by expression of an HPV-16 L1 recombinant baculovirus in insect cells.[13] HPV-16 specific IgG antibody was detected using a VLP-based direct enzyme-linked immunosorbent assay (ELISA) as described by Karem et.al[13] but with a few modifications. Microtiter plates were coated overnight at 4°C with HPV-16 VLP diluted to 0.5µg/ml in PBS. Sera (both reference and test samples) were serially-diluted at 1:10, 1:31.6 and 1:100 in 1X TBST with 10% goat serum, 10%

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3 Super-Block, and 10% insect cell lysate. An optimized concentration of goat anti-human IgG
4 conjugated to alkaline phosphatase (EMD biosciences) diluted in 1X TBST with 10% goat
5 serum, 10% Super-Block was used as the secondary antibody.
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10 Sample antibody titers (IU/ml) were calculated using the parallel line method (PLL)
11 against a reference sample calibrated to the International Standard 16 (NIBSC 05/134) with
12 known titer of 10 IU/ml.[14] A pooled serum, negative for antibodies to HPV-16, 18, 6 and 11
13 as tested by an alternate assay, competitive Luminex assay[15] was used to establish cut-off
14 value. The cut-off for positive results was set at values greater than or equal to the median
15 antibody titer of the negative control plus two standard deviations (1.97 IU/ml).
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27 **Study variables**

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29 Demographic characteristics under study included age group (21-34, 35-50, 51-64 years),
30 education (<12 vs. \geq 12 years), annual family income (<\$20,000 vs. \geq \$20,000), health insurance
31 coverage (private, government-sponsored, none) and marital status (single, married/living
32 together, divorced/separated/widowed). Sexual practices (yes/no) included lifetime history of
33 vaginal, anal, and oral sex as well as age of sexual initiation (\leq 18 vs. >18 years), and number of
34 lifetime sexual partners (0-1, 2-4, \geq 5). In addition, we calculated the sexual exposure period by
35 subtracting the participant's age at the first sexual intercourse from the participant's age at the
36 time of interview. Then, the number of sexual partners was normalized to the sexual exposure
37 period. Smoking status was assessed by a question asking participants if they have ever smoked
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52 **Statistical Analysis**

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3 To characterize the demographic, clinical, and lifestyle characteristics of study participants,
4 summary measures for continuous variables (mean±SD or median (25th and 75th percentiles)) and
5 frequency distributions for categorical variables were computed. Differences between HPV-16
6 seropositivity groups were assessed using Student's Mann-Whitney test for continuous variables,
7 and Chi-square test for independence or Fisher's exact test, when appropriate, for categorical
8 variables. Multivariable logistic regression models were fitted to estimate the prevalence odds
9 ratios (POR) with 95% confidence intervals (CI) for HPV-16 seropositivity [16]. Variables
10 statistically associated with HPV-16 seropositivity in age- and sex-adjusted logistic regression
11 models ($p < 0.05$) were included in the multivariable model. All data were evaluated using Stata
12 for Windows release 11.0 (Stata Corporation, College Station, Texas). No significant interaction
13 terms were found in the multivariable logistic regression models evaluated ($p > 0.05$).
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31 RESULTS

32 Sociodemographic, Clinical, and Lifestyle Characteristics

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34 The characteristics of the sub-sample of 450 adults are shown in Table 1. Three participants
35 were HIV positive (0.99%; data not shown). Although a comparison between the study sub-
36 sample and the parent study population showed no significant differences in the clinical and
37 lifestyle characteristics studied, a higher proportion of participants of the sub-sample reported
38 less than 12 years of education (30.7% vs. 23.2%), a government-based health insurance (51.1%
39 vs. 41.9%), and an annual family income below \$20,000 (72.6% vs. 63.8%) ($p < 0.05$) (data not
40 shown).
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52 Seroprevalence of HPV-16

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54 Overall, 11.3% of participants were seropositive to HPV-16. Seroprevalence among women
55 (15.8%) was higher as compared to men (5.6%) ($p = 0.001$) (Table 1). Median titers (IU per ml)
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3 for the whole sample were 6.07 (25th and 75th percentiles: 2.78, 13.8); these titers were
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5 significantly higher in HPV positive women (median: 8.39, 25th and 75th percentiles: 2.68, 16.01)
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7 than in HPV positive men (median: 3.32, 25th and 75th percentiles: 3.04, 7.13) (p-value<0.0001).
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9 Although no significant differences (p>0.05) in prevalence were observed across age groups in
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11 men or women, the seroprevalence was higher in younger women (Figure 1). The mean age of
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13 HPV-16 seropositive individuals was lower (38.5±12.7) than among those HPV-seronegative
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15 (41.9±12.2); this result was marginally significant (p=0.06). In bivariate analysis, no significant
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17 differences in seropositivity were observed by education level, household income, marital status,
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19 health care coverage, place of birth, sexual practices and smoking status (p>0.05) (Table 1).
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21 After adjusting for age and sex, ever smokers (POR: 2.06, 95% CI=1.08-3.92) and those
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23 reporting at least five lifetime sexual partners (POR: 2.91, 95% CI=1.24-6.81) were more likely
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25 to be HPV-16 seropositive (Table 2). Nonetheless, these associations attenuated to non-
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27 significance in the multivariable model. Only sex remained significantly associated with HPV-16
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29 seropositivity in multivariate analysis, with women being more likely to be seropositive as
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31 compared to men (POR: 4.16, 95% CI=1.91-9.03) (Table 2).
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41 DISCUSSION

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43 The first HPV vaccine, which includes HPV-16, was approved by the Food and Drug
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45 Administration for use in the US and PR in 2006 for females only (aged 9-26 years) and later in
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47 2008 for men. Our estimate of HPV-16 seropositivity for this sub-sample of adults in PR
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49 (11.3%) from 2005-2008 is comparable to those reported in the US (10.4%) for persons aged 14-
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51 59 years participating in the NHANES 2003-2004.[17] Our findings are also comparable to those
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53 reported in studies worldwide. A study in the Netherlands during 2006-2007 among persons aged
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≥ 14 years found an HPV-16 seroprevalence of 11.3%,[11] whereas a study in England among persons aged 10-49 years reported a seroprevalence of 14.7%.[18] Also, consistent with the study by Dunne et al.,[19] the prevalence of HPV seroreactivity in our study was higher among women (15.8%) than men (5.6%), with 4-fold increased odds of infection. In a study performed by Stone et al.,[7] HPV-16 seroreactivity was over two-times higher in women (17.9%) than in men (7.9%), and constant across all age and racial/ethnic groups evaluated in the study. Moreover, several studies have shown this sex difference in antibody response in all HPV vaccine types (6, 11, 16 and 18).[17,18] Sex differences in HPV-16 seroreactivity are also observed among high-risk populations. Women attending STD clinics had higher prevalence of HPV-16 seropositivity (30.2%) than men (18.7%), supporting that there are biological reasons for men and women differing in serologic responses.[20] Potential explanations proposed by Thompson and colleagues include that men may be: 1) not as susceptible to HPV-16 infection, 2) more able to clear the infection spontaneously without developing a systemic antibody response, and 3) less likely to get infected with HPV-16 given that their sexual exposure frequently involves keratinized epithelium (penis) rather than mucosal epithelium (cervix).[20]

In concordance with the literature, HPV-16 seropositivity was also associated with lifetime number of sexual partners; a strong predictor of HPV seropositivity for both males and females.[2, 7, 10, 21] In a study performed in Costa Rica, it was found that women with at least three lifetime sexual partners had a two-fold increase in the detection of HPV-16 antibodies compared to women with one lifetime sexual partner.[22] Similarly, results from the HPV Infection in Men (HIM) Study showed that men with multiple lifetime male partners (≥11 partners) were more likely to be seropositive to HPV-16 (OR=7.74; 95% CI: 3.96-15.12).[10] We observed a strong association between smoking and HPV-16 seropositivity, with smokers

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3 being more likely to be positive for HPV-16 antibodies in this sample. Smoking habits have
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5 been identified as a risk factor for HPV infection and seropositivity, but these findings are
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7 inconsistent.[17, 23, 24] Unlike previous studies showing a decline in seropositivity among those
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9 aged 50+ years, we did not observe a significant association between age and HPV-16
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11 seropositivity.[4, 17, 21] This could be due to the small numbers of seropositive persons in this
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13 study.
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17 Study limitations include the lack of HPV vaccination data of participants and the sample
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19 size; only 51 samples were HPV positive, limiting the power of our study to detect significant
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21 associations with HPV serology. Future studies should evaluate HPV seroprevalence using a
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23 larger population-based sample; the research team is currently executing a population-based
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25 study among women living in the San Juan metropolitan area, which will soon produce data in
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27 this area. All HPV serology assays are limited by the lack of commercial reagents and difficulties
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29 in comparing results between different platforms. We reported on results in terms of IU to help
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31 in inter-study comparisons.
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37 This study is the first to evaluate the prevalence and correlates of HPV seroprevalence in
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39 PR. With the introduction of the two prophylactic HPV vaccines, it is important to establish
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41 baseline population HPV prevalence and better identify population subgroups at high risk for
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43 HPV-related cancers in the population before the uptake of the vaccine continues to increase.
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45 This is particularly important in PR, where population-based estimates of vaccine uptake
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47 continue to be low among children and adults (<5% among women aged 16-26 years).[25]
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49 Knowledge of the burden of infection prior to the expansion of these programs will allow a better
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51 assessment and understanding of the short-term and long-term effectiveness of this primary
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53 prevention strategy for anogenital lesions (cervix, vagina, vulva and anus). Thus, these initial
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findings provide a much needed insight on the epidemiology of HPV infection in PR and will permit the development of future HPV-related research.

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Table 1. Characteristics of study participants by HPV-16 serostatus (n=450)

Characteristics	Total Study Population	HPV-16 negative (n=399, 88.7%)	HPV-16 positive (n=51, 1.3%)	p-value

	N (%)	n (%)	n (%)	
<i>Age group in years</i>				0.111 ^a
21-34	153 (34.0)	129 (32.3)	24 (47.0)	
35-50	180 (40.0)	164 (41.1)	16 (31.4)	
51-64	117 (26.0)	106 (26.6)	11 (21.6)	
Mean ± SD	40.9 ±12.3	41.9±12.2	38.5±12.7	0.058 ^c
<i>Sex</i>				0.001 ^a
Female	253 (56.2)	213 (53.4)	40 (78.4)	
Male	197 (43.8)	186 (46.6)	11 (21.6)	
<i>Education in years</i>				0.836 ^a
<12	138 (30.7)	123 (30.8)	15 (29.4)	
≥12	312 (69.3)	276 (69.2)	36 (70.6)	
<i>Annual family income (n=424)</i>				0.765 ^a
< \$20,000	308 (72.6)	274 (72.9)	34 (70.8)	
≥ \$20,000	312 (69.3)	102 (27.1)	14 (29.2)	
<i>Marital status</i>				0.874 ^a
Never married	77 (17.1)	67 (16.8)	10 (19.6)	
Married/Cohabiting	258 (57.3)	230 (57.6)	28 (54.9)	
Divorced/Separated/Widowed	115 (25.6)	102 (25.6)	13 (25.5)	
<i>Health care coverage</i>				0.202 ^b
None	38 (8.4)	37 (9.3)	1 (1.9)	
Government-administered	230 (51.1)	201 (50.4)	29 (56.9)	
Private	182 (40.4)	161 (40.3)	21 (41.2)	
<i>Place of birth</i>				0.408 ^b
Puerto Rico	414 (92.0)	365 (91.5)	49 (96.1)	
United States	36 (8.0)	34 (8.5)	2 (3.9)	
<i>Seropositive status to HSV-2 (n=440)</i>				0.603 ^a
Positive	110 (25.0)	96 (24.6)	14 (28.0)	
Negative	330 (75.0)	294 (75.4)	36 (72.0)	
<i>Ever had sex (n=445)</i>				0.999 ^b
Yes	431 (96.9)	382 (96.7)	49 (98.0)	
No	14 (3.1)	13 (3.3)	1 (2.0)	
<i>Age at first sexual intercourse (n=427)</i>				0.467 ^a
< 18	228 (53.4)	200 (52.8)	28 (58.3)	
≥ 18	199 (46.6)	179 (47.2)	20 (41.7)	
<i>Number of lifetime sex partners (n=442)</i>				0.349 ^a
0-1	100 (23.6)	91 (24.3)	9 (18.8)	
2-4	152 (35.9)	137 (36.5)	15 (31.2)	
≥ 5	171 (40.4)	147 (39.2)	24 (50.0)	
<i>Number of sexual partners normalized to the sexual exposure period</i>	0.18 (0.08, 0.45)	0.18 (0.08, 0.40)	0.20 (0.09, 0.75)	0.112 ^c

Median (25 th and 75 th percentiles)				
<i>Anal Sex</i>				0.610 ^a
Ever	262 (58.2)	234 (58.7)	28 (54.9)	
Never	188 (41.8)	165 (41.3)	23 (45.1)	
<i>Oral Sex</i>				0.930 ^a
Ever	333 (74.0)	295 (73.9)	38 (74.5)	
Never	117 (26.0)	104 (26.1)	13 (25.5)	
<i>History of smoking</i>				0.110 ^a
Yes	253 (56.2)	219 (54.9)	34 (66.7)	
No	197 (43.8)	180 (45.1)	17 (33.3)	

a. p-values from Chi-square test.

b. p-values from Fisher's exact test.

c. p-value from Student's Mann-Whitney test.

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Table 2. Magnitude of the association (POR) between of HPV-16 and different characteristics

Characteristics	Crude POR	Age-and-sex-adjusted POR (95% CI)	Multivariate-adjusted POR ^a (95% CI)
<i>Age group in years</i>			
51-64	1.0	---	1.00
35-50	0.96 (0.43-2.12)	---	0.99 (0.42-2.32)
21-34	1.81 (0.87-3.79)	---	1.52 (0.67-3.47)
<i>Sex</i>			
Male	1.0	---	1.00
Female	3.18 (1.58-6.37)	---	4.16 (1.91-9.03)
<i>Number of lifetime sex partners</i>			
0-1	1.0	1.0	1.00
2-4	1.11 (0.46-2.64)	1.18 (0.48-2.87)	1.09 (0.44-2.68)
≥ 5	1.65 (0.73-3.71)	2.78 (1.15-6.73)	2.36 (0.94-5.90)
<i>History of smoking</i>			
No	1.0	1.0	1.00
Yes	1.64 (0.89-3.04)	2.06 (1.08, 3.92)	1.58 (0.79-3.16)

a. Additionally adjusted by number of lifetime sexual partners and history of smoking; no significant interaction terms detected in this logistic regression model (p=0.740).

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10 **Data sharing:** No additional data available.
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19 based on reviewer comments. GTL contributed to the concept and design and interpretation of
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21 data and revised the article for important intellectual content. CM, MSS and YO contributed to
22
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27 interpretation of findings and revised the article for important intellectual content.
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37 **Figure legend**

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40 **Figure 1. Seroprevalence of HPV-16 by age and sex (n=450).**
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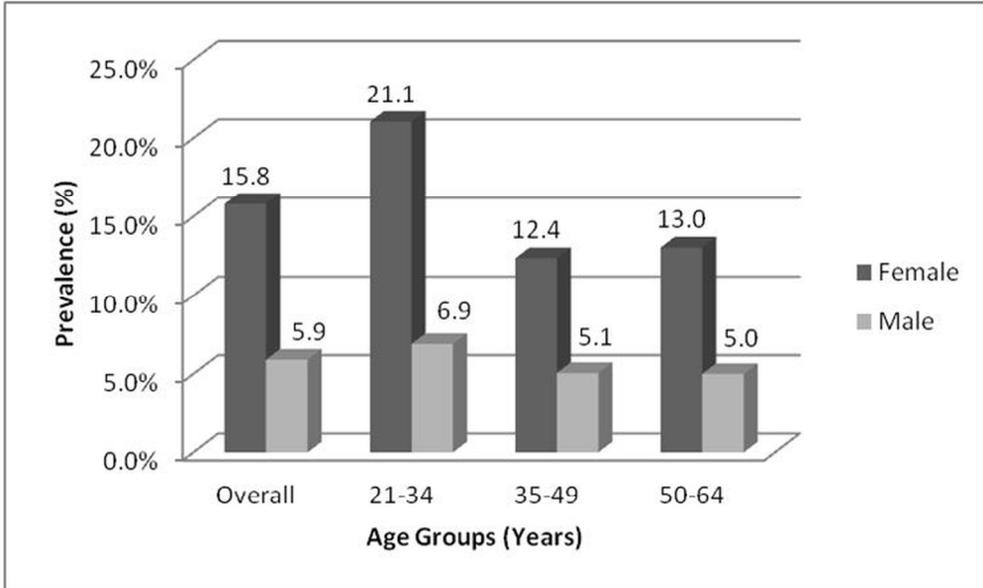
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9 **CROSS-SECTIONAL STUDY SEROPREVALENCE OF HPV-16 INFECTION -IN A**
10 **POPULATION-BASED SUB-SAMPLE OF HISPANIC ADULTS**
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14 Ortiz AP ^{1,2}, Unger ER³, Muñoz C², Panicker G³, Tortolero-Luna G¹, Soto-Salgado M⁴, Otero
15 Y⁴, Suárez E², Pérez CM².
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20 **Affiliations:** ¹Cancer Control and Population Sciences Program, University of Puerto Rico
21 Comprehensive Cancer Center; ²Biostatistics and Epidemiology Department, Graduate School of
22 Public Health, University of Puerto Rico; ³Chronic Viral Diseases Branch, Centers for Disease
23 Control and Prevention, Atlanta, GA; ⁴UPR-MDACC Partnership for Excellence in Cancer
24 Research, School of Medicine, Program, Medical Sciences Campus, University of Puerto Rico.
25
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27
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30
31 **Correspondence to:** Ana Patricia Ortiz, PhD, MPH
32

33 University of Puerto Rico Comprehensive Cancer Center, PMB 711, 89 De Diego Ave. Suite
34 105, San Juan, PR, 00927-6346
35

36
37 Phone (787) 772-8300 x-1204/ Fax (787) 758-2557 / E-mail: ana.ortiz7@upr.edu
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Abstract

Background: Persistent HPV infection is associated with cancer of the cervix, anus, vulva, vagina, penis, mouth and oropharynx. Although HPV is a public health problem worldwide, population-based data on HPV seroprevalence for Puerto Rico (PR) is unavailable. HPV-16 is the most common HPV type detected in most regions of the world. **Objective:** This study aimed to estimate the prevalence and correlates of seropositivity to HPV-16 in a sub-sample of adults in PR. **Methods:** Cross-sectional study in which the last 450 consecutive adults aged 21-64 years who participated in an island-wide population-based study (n=1,654) in PR conducted between 2005-2008 provided serum samples. The samples were tested by ELISA for HPV-16 viral-like particle -specific IgG. Information on socio-demographic, health and lifestyle characteristics was collected. Logistic regression modeling was used to estimate the prevalence odds ratio (POR) to assess factors associated to HPV-16 seropositivity. **Results:** Prevalence of seropositivity to HPV-16 was 11.3%. Seroprevalence was higher in women (15.8%) than men (5.6%) (p=0.001). After adjusting for age and sex, ever smokers (POR: 2.06, 95% CI=1.08-3.92) and participants with at least five lifetime sexual partners (POR: 2.91, 95% CI=1.24-6.81) were more likely to be HPV-16 seropositive. **Conclusions:** HPV-16 seropositivity is similar to that reported in the US (10.4%) for NHANES 2003-2004 participants, although different assays were used in these studies. Our results highlight the need to further understand the burden of HPV and HPV-related malignancies in PR.

Strengths and limitations of this study:

- This study is the first to provide initial insights of the epidemiology of HPV infection by assessing evaluate HPV-16 seroprevalence and its correlates in Puerto Rico and provides insights of the epidemiology of HPV infection in this population.
- ~~Prevalence of HPV-16 seropositivity among 450 adults aged 21-64 years in Puerto Rico was 11.3%; prevalence was higher for women (15.8%) than for men (5.6%).~~
- ~~Women, smokers and participants with ≥ 5 lifetime sexual partners were more likely to be HPV-16 seropositive; highlighting population sub-groups with higher odds of seroconversion.~~
- Although different laboratory assays were used, our estimate of HPV-16 seropositivity for this sub-sample of adults in Puerto Rico (11.3%) from 2005-2008 is comparable to that reported in the US (10.4%) for persons aged 14-59 years participating in the NHANES 2003-2004. HPV-16 seropositivity in our study (2005-2008) is similar to that reported in the US NHANES 2003-2004 (10.4%), although different assays were used.
- Study limitations include the lack of HPV vaccination data of study participants and the modestly sized sample sample size; only of HPV-16 seropositive 51 samples individuals were HPV positive, limiting the power of our study to detect significant associations with HPV serology.

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INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide. Persistent infection with certain types of HPV has been established as a necessary cause for cervical cancer[1, 2] and has been associated with cancer of the anus, vulva, vagina, penis, and oropharynx.[3,4] Approximately 5.2% of all cancers worldwide are attributable to HPV infection[3] Moreover, the economic burden of the HPV infection is high, and second only to the cost of Human Immunodeficiency Virus (HIV) infection.[5] Currently, there are two HPV vaccines licensed for use worldwide that have proven to be effective in preventing HPV infection and progressive disease in people that were previously HPV naïve.[6] With widespread use, the vaccines could provide a cost-effective prevention strategy.

Antibody response to HPV infection is considered a key determinant of protective immunity and may play a role as a predictor of HPV-associated cervical neoplasia.[2,7] The protective antibody response is mainly type-specific and directed against conformational epitopes of the major capsid protein L1,[8] while antibodies to E6 and E7 oncoproteins may be markers of invasive cervical cancer, with tumor stage and mass determining the magnitude of the response.[9] Seroconversion occurs several months after detection of HPV-DNA infection; approximately only 60% of women with an incident of HPV-DNA infection seroconvert within 18 months after detection. No differences in median time of seroconversion are observed by HPV type, although antibody responses to high-risk HPV types have been found to persist longer.[1, 10] While HPV DNA testing detects current infection, serological testing serves as a useful epidemiologic research tool to measure lifetime exposure to HPV infection because antibodies may persist even after the virus has cleared. Although HPV-16 is the most common HPV type detected in most regions of the world,[11] no population-based sero-epidemiological

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9 studies of HPV-16 infection have been conducted in Puerto Rico (PR). An estimate of natural
10 HPV infection would not only help understand the burden of the disease, but also provide the
11 necessary baseline data for HPV vaccine implementation and monitoring in PR. Hence, the
12 purpose of this pilot study was to estimate prevalence of HPV-16 IgG responses and factors
13 associated with HPV-16 seropositivity in a cross-section of adults in PR.
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MATERIALS AND METHODS

Study Design and Population

An island-wide population-based cross-sectional household survey aimed at estimating the seroprevalence of hepatitis C and other viral infections (hepatitis A, hepatitis B, HIV and Herpes Simplex type 2) was performed in PR (n=1,654) between 2005 and 2008. Detailed descriptions of the study sampling design and data collection procedures have been previously published.[12] In brief, a cluster sampling design for household surveys using the census tracts of PR was employed, and one individual aged 21-64 years from each selected household was randomly selected to participate in the study. Participants underwent a personal interview and an audio computer-assisted self-interview (ACASI) using QDS (Nova Research Co., Washington D.C.), and provided a sample of blood for serologic testing. All study procedures were reviewed and approved by the Institutional Review Board of the University of Puerto Rico Medical Sciences Campus. For the current analysis we used residual serum from a sub-sample of the last 450 consecutive adults aged 21 to 64 years, recruited between February, 2007 and to January, 2008, who participated in the study and agreed to participate in HPV testing.

Serologic Testing

Antibody testing for HPV-16 was performed at the Human Papillomavirus Laboratory at the Centers for Disease and Control in Atlanta. Virus-like particles (VLPs) were produced by expression of an HPV-16 L1 recombinant baculovirus in insect cells.[13] HPV-16 specific IgG antibody was detected using a VLP-based direct enzyme-linked immunosorbent assay (ELISA) as described by Karem et.al[13] but with a few modifications. Microtiter plates were coated overnight at 4°C with HPV-16 VLP diluted to 0.5µg/ml in PBS. Sera (both reference and test samples) were serially-diluted at 1:10, 1:31.6 and 1:100 in 1X TBST with 10% goat serum, 10%

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9 Super-Block, and 10% insect cell lysate. An optimized concentration of goat anti-human IgG
10 conjugated to alkaline phosphatase (EMD biosciences) diluted in 1X TBST with 10% goat
11 serum, 10% Super-Block was used as the secondary antibody.
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14 Sample antibody titers (IU/ml) were calculated using the parallel line method (PLL)
15 against a reference sample calibrated to the International Standard 16 (NIBSC 05/134) with
16 known titer of 10 IU/ml.[14] A pooled serum, negative for antibodies to HPV-16, 18, 6 and 11
17 as tested by an alternate assay, competitive Luminex assay[15] was used to establish cut-off
18 value. The cut-off for positive results was set at values greater than or equal to the median
19 antibody titer of the negative control plus two standard deviations (1.97 IU/ml). ~~To determine~~
20 ~~differences by gender in the median titers of HPV a Mann-Whitney-Wilcoxon test was used.~~
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28 29 **Study variables**

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31 Demographic characteristics under study included age group (21-34, 35-50, 51-64 years),
32 education (<12 vs. ≥12 years), annual family income (<\$20,000 vs. ≥ \$20,000), health insurance
33 coverage (private, government-sponsored, none) and marital status (single, married/living
34 together, divorced/separated/widowed). Sexual practices (yes/no) included lifetime history of
35 vaginal, anal, and oral sex as well as age of sexual initiation (≤18 vs. >18 years), and number of
36 lifetime sexual partners (0-1, 2-4, ≥5). In addition, we calculated the sexual exposure period by
37 subtracting the participant's age at the first sexual intercourse from the participant's age at the
38 time of interview-. Then, the number of sexual partners was normalized to the sexual exposure
39 period. Smoking status was assessed by a question asking participants if they have ever smoked
40 in their lifetime.
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Statistical Analysis

To characterize the demographic, clinical, and lifestyle characteristics of study participants, summary measures for continuous variables (mean±SD or median (and 25th and 75th percentiles)) and frequency distributions for categorical variables were computed. Differences between HPV-16 seropositivity groups were assessed using Student's Mann-Whitney test for continuous variables, and Chi-square test for independence or Fisher's exact test, when appropriate, for categorical variables. Multivariable logistic regression models were fitted to estimate the prevalence odds ratios (POR) with 95% confidence intervals (CI) for HPV-16 seropositivity [16]. Variables statistically associated with HPV-16 seropositivity in age- and sex-adjusted logistic regression models ($p < 0.05$) were included in the multivariable model. All data were evaluated using Stata for Windows release 11.0 (Stata Corporation, College Station, Texas). No significant interaction terms were found in the multivariable logistic regression models evaluated ($p > 0.05$).

RESULTS

Sociodemographic, Clinical, and Lifestyle Characteristics

The characteristics of the sub-sample of 450 adults are shown in Table 1. Three participants were HIV positive (0.99%; data not shown). Although a comparison between the study sub-sample and the parent study population showed no significant differences in the clinical and lifestyle characteristics studied, a higher proportion of participants of the sub-sample reported less than 12 years of education (30.7% vs. 23.2%), a government-based health insurance (51.1% vs. 41.9%), and an annual family income below \$20,000 (72.6% vs. 63.8%) ($p < 0.05$) (data not shown).

Seroprevalence of HPV-16

Overall, 11.3% of participants were seropositive to HPV-16. Seroprevalence among women (15.8%) was higher as compared to men (5.6%) ($p=0.001$) (Table 1). Median titers (IU per ml) for the whole sample were 6.07 (25th and 75th percentiles: 2.78, 13.8); these titers were significantly higher in HPV positive women (median: 8.39, 25th and 75th percentiles: 2.68, 16.01) ~~were higher than those for~~ HPV positive men (median: 3.32, 25th and 75th percentiles: 3.04, 7.13) ($p\text{-value}<0.0001$). Although no significant differences ($p>0.05$) in prevalence were observed across age groups in men or women, the seroprevalence was higher in younger women (Figure 1). The mean age of HPV-16 seropositive individuals was lower (38.5 ± 12.7) than among those HPV-seronegative (41.9 ± 12.2); this result was marginally significant ($p=0.06$). In bivariate analysis, no significant differences in seropositivity were observed by education level, household income, marital status, health care coverage, place of birth, sexual practices and smoking status ($p>0.05$) (Table 1). After adjusting for age and sex, ever smokers (POR: 2.06, 95% CI=1.08-3.92) and those reporting at least five lifetime sexual partners (POR: 2.91, 95% CI=1.24-6.81) were more likely to be HPV-16 seropositive (Table 2). Nonetheless, these associations attenuated to non-significance in the multivariable model. Only sex remained significantly associated with HPV-16 seropositivity in multivariate analysis, with women being more likely to be seropositive as compared to men (POR: 4.16, 95% CI=1.91-9.03) (Table 2).

DISCUSSION

The first HPV vaccine, which includes HPV-16, was approved by the Food and Drug Administration for use in the US and PR in 2006 for females only (aged 9-26 years) and later in 2008 for men. Our estimate of HPV-16 seropositivity for this sub-sample of adults in PR

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9 (11.3%) from 2005-2008 is comparable to those reported in the US (10.4%) for persons aged 14-
10 59 years participating in the NHANES 2003-2004.[17] Our findings are also comparable to those
11 reported in studies worldwide. A study in the Netherlands during 2006-2007 among persons aged
12 ≥ 14 years found an HPV-16 seroprevalence of 11.3%,[11] whereas a study in England among
13 persons aged 10-49 years reported a seroprevalence of 14.7%.[18] Also, consistent with the
14 study by Dunne et al.,[19] the prevalence of HPV seroreactivity in our study was higher among
15 women (15.8%) than men (5.6%), with 4-fold increased odds of infection. In a study performed
16 by Stone et al.,[7] HPV-16 seroreactivity was over two-times higher in women (17.9%) than in
17 men (7.9%), and constant across all age and racial/ethnic groups evaluated in the study.
18 Moreover, several studies have shown this sex difference in antibody response in all HPV
19 vaccine types (6, 11, 16 and 18).[17,18] Sex differences in HPV-16 seroreactivity are also
20 observed among high-risk populations. Women attending STD clinics had higher prevalence of
21 HPV-16 seropositivity (30.2%) than men (18.7%), supporting that there are biological reasons
22 for men and women differing in serologic responses.[20] Potential explanations proposed by
23 Thompson and colleagues include that men may be: 1) not as susceptible to HPV-16 infection, 2)
24 more able to clear the infection spontaneously without developing a systemic antibody response,
25 and 3) less likely to get infected with HPV-16 given that their sexual exposure frequently
26 involves keratinized epithelium (penis) rather than mucosal epithelium (cervix).[20]
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43 In concordance with the literature, HPV-16 seropositivity was also associated with
44 lifetime number of sexual partners; a strong predictor of HPV seropositivity for both males and
45 females.[2, 7, 10, 21] In a study performed in Costa Rica, it was found that women with at least
46 three lifetime sexual partners had a two-fold increase in the detection of HPV-16 antibodies
47 compared to women with one lifetime sexual partner.[22] Similarly, results from the HPV
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9 Infection in Men (HIM) Study showed that men with multiple lifetime male partners (≥ 11
10 partners) were more likely to be seropositive to HPV-16 (OR=7.74; 95% CI: 3.96-15.12).[10]
11 We observed a strong association between smoking and HPV-16 seropositivity, with smokers
12 being more likely to be positive for HPV-16 antibodies in this sample. Smoking habits have
13 been identified as a risk factor for HPV infection and seropositivity, but these findings are
14 inconsistent.[17, 23, 24] Unlike previous studies showing a decline in seropositivity among those
15 aged 50+ years, we did not observe a significant association between age and HPV-16
16 seropositivity.[4, 17, 21] This could be due to the small numbers of seropositive persons in this
17 study.
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26 Study limitations include the lack of HPV vaccination data of participants and the sample
27 size; only 51 samples were HPV positive, limiting the power of our study to detect significant
28 associations with HPV serology. Future studies should evaluate HPV seroprevalence using a
29 larger population-based sample; the research team is currently executing a population-based
30 study among women living in the San Juan metropolitan area, which will soon produce data in
31 this area. All HPV serology assays are limited by the lack of commercial reagents and difficulties
32 in comparing results between different platforms. We reported on results in terms of IU to help
33 in inter-study comparisons.
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41 This study is the first to evaluate the prevalence and correlates of HPV seroprevalence in
42 PR. With the introduction of the two prophylactic HPV vaccines, it is important to establish
43 baseline population HPV prevalence and better identify population subgroups at high risk for
44 HPV-related cancers in the population before the uptake of the vaccine continues to increase.
45 This is particularly important in PR, where population-based estimates of vaccine uptake
46 continue to be low among children and adults (<5% among women aged 16-26 years).[25]
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Knowledge of the burden of infection prior to the expansion of these programs will allow a better assessment and understanding of the short-term and long-term effectiveness of this primary prevention strategy for anogenital lesions (cervix, vagina, vulva and anus). Thus, these initial findings provide a much needed insight on the epidemiology of HPV infection in PR and will permit the development of future HPV-related research.

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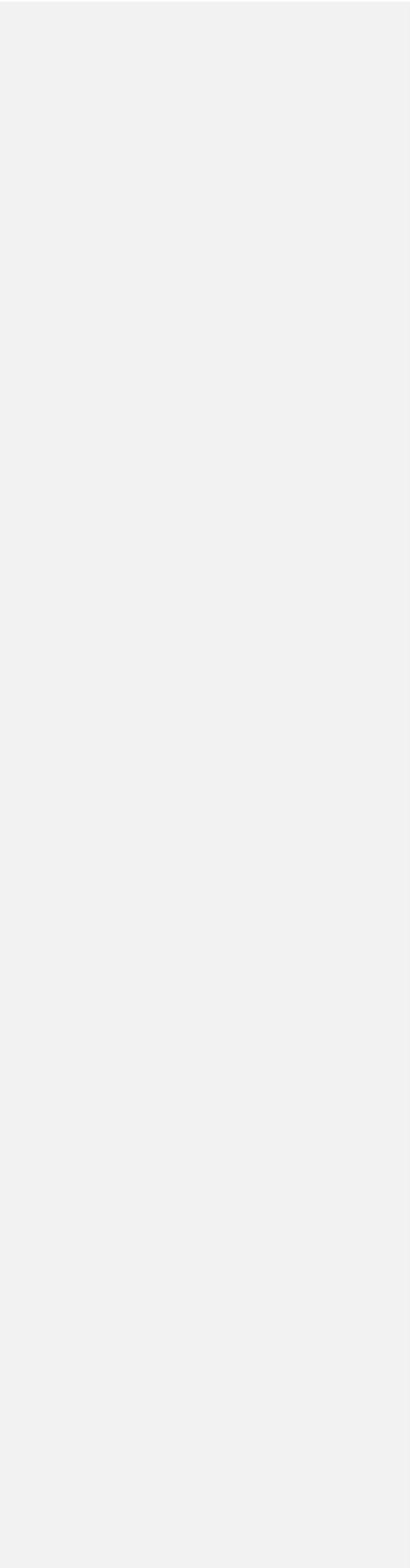


Table 1. Characteristics of study participants by HPV-16 serostatus (n=450)

Characteristics	Total Study Population N (%)	HPV-16 negative (n=399, 88.7%) n (%)	HPV-16 positive (n=51, 1.3%) n (%)	p-value ^a
<i>Age group in years</i>				0.111 ^a
21-34	153 (34.0)	129 (32.3)	24 (47.0)	
35-50	180 (40.0)	164 (41.1)	16 (31.4)	
51-64	117 (26.0)	106 (26.6)	11 (21.6)	
Mean \pm SD	40.9 \pm 12.3	41.9 \pm 12.2	38.5 \pm 12.7	0.0658 ^c
<i>Sex</i>				0.001 ^a
Female	253 (56.2)	213 (53.4)	40 (78.4)	
Male	197 (43.8)	186 (46.6)	11 (21.6)	
<i>Education in years</i>				$\geq 0.83640^a$
<12	138 (30.7)	123 (30.8)	15 (29.4)	
≥ 12	312 (69.3)	276 (69.2)	36 (70.6)	
<i>Annual family income (n=424)</i>				$\geq 0.76540^a$
< \$20,000	308 (72.6)	274 (72.9)	34 (70.8)	
\geq \$20,000	312 (69.3)	102 (27.1)	14 (29.2)	
<i>Marital status</i>				$\geq 0.87440^a$
Never married	77 (17.1)	67 (16.8)	10 (19.6)	
Married/Cohabiting	258 (57.3)	230 (57.6)	28 (54.9)	
Divorced/Separated/Widowed	115 (25.6)	102 (25.6)	13 (25.5)	
<i>Health care coverage</i>				$\geq 0.20240^b$
None	38 (8.4)	37 (9.3)	1 (1.9)	
Government-administered	230 (51.1)	201 (50.4)	29 (56.9)	
Private	182 (40.4)	161 (40.3)	21 (41.2)	
<i>Place of birth</i>				$\geq 0.40840^b$
Puerto Rico	414 (92.0)	365 (91.5)	49 (96.1)	
United States	36 (8.0)	34 (8.5)	2 (3.9)	
<i>Seropositive status to HSV-2 (n=440)</i>				$\geq 0.60340^a$
Positive	110 (25.0)	96 (24.6)	14 (28.0)	
Negative	330 (75.0)	294 (75.4)	36 (72.0)	
<i>Ever had sex (n=445)</i>				$\geq 0.99940^b$
Yes	431 (96.9)	382 (96.7)	49 (98.0)	
No	14 (3.1)	13 (3.3)	1 (2.0)	
<i>Age at first sexual intercourse (n=427)</i>				$\geq 0.46740^a$
< 18	228 (53.4)	200 (52.8)	28 (58.3)	
≥ 18	199 (46.6)	179 (47.2)	20 (41.7)	
<i>Number of lifetime sex partners (n=442)</i>				$\geq 0.34940^a$

0-1	100 (23.6)	91 (24.3)	9 (18.8)	
2-4	152 (35.9)	137 (36.5)	15 (31.2)	
≥ 5	171 (40.4)	147 (39.2)	24 (50.0)	
<i>Number of sexual partners normalized to the sexual exposure period</i>	<u>0.18</u> (0.08, 0.45)	<u>0.18</u> (0.08, 0.40)	<u>0.20</u> (0.09, 0.75)	<u>0.112^c</u>
<i>Median (25th and 75th percentiles)</i>				
<i>Anal Sex</i>				<u>>0.61040^a</u>
Ever	262 (58.2)	234 (58.7)	28 (54.9)	
Never	188 (41.8)	165 (41.3)	23 (45.1)	
<i>Oral Sex</i>				<u>>0.93040^a</u>
Ever	333 (74.0)	295 (73.9)	38 (74.5)	
Never	117 (26.0)	104 (26.1)	13 (25.5)	
<i>History of smoking</i>				<u>>0.1100^a</u>
Yes	253 (56.2)	219 (54.9)	34 (66.7)	
No	197 (43.8)	180 (45.1)	17 (33.3)	

a. p-values from Chi-square test.

b. p-values from Fisher's exact test.

c. p-value from Student's Mann-Whitney test.

Table 2. Magnitude of the association (POR) between of HPV-16 and different characteristics

Characteristics	Crude POR	Age-and-sex-adjusted POR (95% CI)	Multivariate-adjusted POR ^a (95% CI)
<i>Age group in years</i>			
51-64	1.0	---	1.00
35-50	0.96 (0.43-2.12)	---	0.99 (0.42-2.32)
21-34	1.81 (0.87-3.79)	---	1.52 (0.67-3.47)
<i>Sex</i>			
Male	1.0	---	1.00
Female	3.18 (1.58-6.37)	---	4.16 (1.91-9.03)
<i>Number of lifetime sex partners</i>			
0-1	1.0	1.0	1.00
2-4	1.11 (0.46-2.64)	1.18 (0.48-2.87)	1.09 (0.44-2.68)
≥ 5	1.65 (0.73-3.71)	2.78 (1.15-6.73)	2.36 (0.94-5.90)
<i>History of smoking</i>			
No	1.0	1.0	1.00
Yes	1.64 (0.89-3.04)	2.06 (1.08, 3.92)	1.58 (0.79-3.16)

a. Additionally adjusted by number of lifetime sexual partners and history of smoking; no significant interaction terms detected in this logistic regression model ($p=0.740>0.05$).

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19 the funding agency.
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14 **Data sharing:** There is no additional data available.
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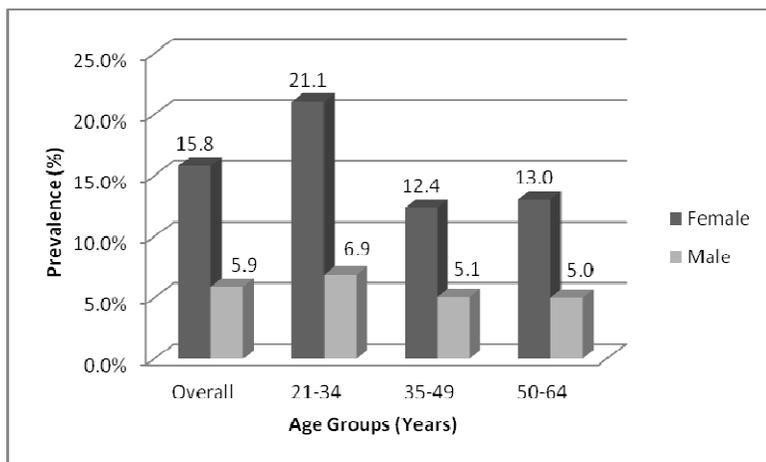
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Figure 1. Seroprevalence of HPV-16 by age and sex (n=450).



STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*
Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any pre-specified hypotheses	4-5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	6-7
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	8
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	

		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	8
		(e) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8-9
		(b) Give reasons for non-participation at each stage	8-9
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9, 13-14
		(b) Indicate number of participants with missing data for each variable of interest	8-9
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	8-9, 13-14
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-9, 15
		(b) Report category boundaries when continuous variables were categorized	8-9, 13-14
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8-9, 15
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	3, 9-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	9-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16-17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.