Published in final edited form as:

Int J STD AIDS. 2010 February ; 21(2): 105–109. doi:10.1258/ijsa.2009.008436.

Prevalence, distribution and correlates of endocervical human papillomavirus types in Brazilian women

S A Lippman, PhD MPH^{*,†}, M C A Sucupira, PhD[‡], H E Jones, MPH^{*,§}, C G Luppi, PhD MD^{**}, J Palefsky, MD^{††}, J H H M van de Wijert, MPH PhD^{*,††}, R L S Oliveira, PhD MD^{**}, and R S Diaz, PhD[‡]

^{*}Population Council, New York, NY [†]Division of Epidemiology, University of California, Berkeley, CA, USA [‡]Escola Paulista de Medicina, Disciplina de Doenças Infecciosas, Laboratório de Retrovirologia, São Paulo, Brazil [§]Division of Family Planning and Preventive Services, Columbia University Medical Center, New York, NY, USA ^{**}Centro de Estudos, Augusto Leopoldo Ayrosa Galvão, Department of Social Medicine, Santa Casa Medical School, São Paulo, Brazil ^{††}Department of Medicine, University of California, San Francisco, CA, USA ^{‡‡}Academic Medical Center, University of Amsterdam, Center for Poverty-related Communicable Diseases, Amsterdam, Netherlands

Summary

We determined the prevalence, distribution and correlates of human papillomavirus (HPV) types in 386 mixed-income, sexually active women in São Paulo, Brazil. Endocervical samples were tested for HPV DNA with L1 primers MY09 and MY11; negative and indeterminate samples were retested using GP 5+/6+ consensus primers. HPV was detected in 35% of all women; high-risk/probable high-risk types in 20%; low-risk types in 7%; and an indeterminate type in 10%. Twenty-five HPV types were found overall: 17 (probable) high-risk types and eight low-risk types. Approximately one-third (29%) of women with HPV infection were positive for type 16 or 18 and 36% were positive for types 6, 11, 16 or 18. The presence of (probable) high-risk HPV was associated with younger age, more lifetime sex partners and abnormal vaginal flora. Additional studies mapping the distribution of HPV types worldwide are necessary to prepare for vaccination programmes and direct future vaccine development.

Keywords

Brazil; human papillomavirus; HPV type; sexually transmitted infections

Introduction

Human papillomavirus (HPV) is estimated to cause 500,000 new cases of cervical cancer every year, with an estimated 260,000 deaths due to cervical cancer in 2005.¹ The World Health Organization estimates that 80% of cancers caused by HPV occur in developing countries.¹ In Brazil, cervical cancer is the second most common cancer among women, with 18,000 to 19,000 new cases annually.² Regional incidence varies from 18 to 24 per 100,000 women.² With the recent successes of the first HPV vaccine trials,^{3–5} there is renewed hope that vaccination efforts can stem cervical cancer where screening efforts have failed, particularly

in low-resource settings where the burden of disease is greatest. Two HPV vaccines are currently being introduced in various countries: Gardasil (Merck & Co, Inc, Whitehouse Station, NJ, USA), which is approved by the United States Food and Drug Administration, and Cervarix (GlaxoSmithKline Biologicals, Rixensart, Belgium), which is approved in the USA, Brazil, Europe, Japan and Australia. Both include protection against HPV types 16 and 18, estimated to cause 70% of cervical cancers globally;⁶ Gardasil also includes protection for types 6 and 11, responsible for 90% of genital warts.⁵

Because the prevalence of HPV types differs by region,⁷ investigators have conducted a series of pooled and meta-analyses to establish global and regional patterns of HPV distribution and distribution of associated clinical disease.^{8–10} Research has also been conducted in women without abnormal cytology or cervical cancer,^{11,12} including population-based samples from various South American countries.^{11,13,14} These data can be used to estimate the proportion of the population who would be protected by the currently available HPV vaccines and to establish which HPV types are most prevalent across regions as pharmaceutical companies consider adding new types to vaccines in the pipeline.

As part of a study to test the acceptability and feasibility of home- and clinic-based self-sampling and self-testing for sexually transmitted infections (STIs), women in a mixed-income neighbourhood in central São Paulo, Brazil, underwent provider collection of an endocervical specimen. The specimens were tested and typed for HPV. We report on the prevalence, distribution and correlates of HPV in these women.

Methods

Study recruitment and collection procedures

From April to November 2004, 818 women were recruited from the primary care clinic Centro de Saúde Escola Dr Alexandre Vranjac, Barra Funda, and from the clinic's catchment community to participate in a home-based STI screening study (see reference¹⁵ for details). Eligibility criteria included being 18–40 years old, self-reporting literacy (to follow specimen collection and testing instructions) and lack of genital ulcers and/or urgent gynaecological complaints at the time of recruitment. Eligible women who chose to participate underwent informed consent procedures, responded to a questionnaire, received STI/HIV risk reduction counselling and were randomized to a home collection ($n = 410$) or a clinic-based specimen collection ($n = 408$) group. Both groups self-collected vaginal swabs for polymerase chain reaction (PCR) testing of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) using COBAS AMPLICOR CT NG PCR (Roche Molecular Diagnostics, Pleasanton, CA, USA) and *Trichomonas vaginalis* (TV) using an in-house PCR test adapted from a previously validated assay.¹⁶ However, only women randomized to clinic-based screening underwent speculum exam, which included wet mount for detection of bacterial vaginosis (BV) and vaginal yeast, and clinician collection of an endocervical specimen for storage and future HPV testing. Women testing positive for high-risk HPV were invited to the clinic for counselling and follow-up.

We used the HPV classification proposed by Munoz *et al.*⁷ supplemented by phylogenetic classification as indicated by de Villiers *et al.*¹⁷ for types not classified in our primary source. HPV types classified as high risk include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82. We considered types 53, 66, 67, 69 and 70 to be probable high-risk types and pooled probable high-risk and high-risk types for the purposes of these analyses. Types classified as low risk include 6, 11, 40, 42, 43, 44, 54, 57, 61, 62, 64, 72 and 81. Participants were offered a Pap smear, HIV test and syphilis test as per routine clinic procedures; 57% of participants agreed to both Pap smear collection and sharing results with the research team. The study protocol, including testing of stored samples for HPV, was approved by the ethical committee

at the Irmandade Santa Casa de Misericórdia de São Paulo, the Brazilian National Ethics Committee and the Population Council's Institutional Review Board.

Laboratory procedures

Endocervical samples were collected by trained auxiliary nurses using Dacron swabs, which were dry-transported in their original tubes to the study laboratory. The samples were washed in Roche STM-transport medium and stored at -70°C until HPV detection and typing at the Retrovirology Laboratory, Escola Paulista de Medicina in São Paulo, Brazil.

DNA was isolated using the GFX™ Genomic Blood DNA Purification Kit (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions. To determine whether samples yielded sufficient DNA, endocervical specimens were PCR-amplified using beta-globin genomic primers.¹⁸ The PCR products were analysed by electrophoresis on a 2% agarose gel at 110 V for one hour at room temperature, followed by incubation in ethidium bromide, and visualized by transillumination. HPV screening was performed using MY09/11 primers and typing was performed by restriction fragment length polymorphism (RFLP), as previously described.¹⁹ Typing results were interpreted by two independent experienced laboratory analysts. Samples that were negative by MY09/11 PCR or with indeterminate typing by RFLP were subjected to a nested PCR using primers GP5+/6+.²⁰ Amplified products were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (ABI Prism® 3100 Genetic Analyser, Applied Biosystems, Inc, Foster City, CA, USA). Nucleotide sequences were edited by Sequencher software (Gene Code Corp., Ann Arbor, MI, USA), and DNA sequences were subjected to on-line BLAST analysis (<http://0-www.ncbi.nlm.nih.gov/ilsprod.lib.neu.edu/BLAST/>). The use of multiple PCR systems has been recommended to maximize HPV detection.^{21,22}

Additionally, 6% of HPV-negative samples and 12% of HPV-positive samples were typed at the University of California, San Francisco, for external quality control. A crude DNA preparation was made from the cervical specimen, subjected to proteinase K lyses (Invitrogen Life Technologies, Carlsbad, CA, USA) and eluted in 25 μL TE. PCR was performed using MY09/MY11 consensus HPV L1 primers as well as primers for amplification of the human beta-globin as an indicator of specimen adequacy.²³ Following PCR, specimens were probed with a biotin-labelled HPV L1 consensus probe mixture. A separate membrane was probed with a biotin-labelled probe to the human beta-globin gene. Specimens were also typed by hybridizing to 29 different HPV types as well as 10 additional types together in a probe mixture.

Data analysis

All data were double-entered; analysis was performed using STATA version 8.2.²⁴ The distribution of HPV types in the population is presented by frequency and proportions. Association between sociodemographic characteristics, behavioural variables and clinical presentation by HPV status was assessed using χ^2 statistics and the non-parametric test for trend across ordered groups.

Results

HPV results were obtained for 386 women: 408 women were randomized to undergo endocervical swab collection, 14 (3%) did not present to the clinic to provide a sample, one sample could not be found in the laboratory and seven samples (<2%) were negative for beta-globin. External quality control procedures demonstrated 81% agreement with respect to the presence or absence of HPV, similar to inter-laboratory agreement reported previously,²⁵ and 80% agreement with respect to typing.

HPV was detected in 135 participants (35%): 78 women (20%) tested positive for at least one high-risk type, 25 women (6.5%) for at least one low-risk type, with six women (2%) testing positive for both low- and high-risk types, and HPV type could not be determined in 38 women (10%) (Table 1). More than one type of HPV was detected in 13 of 135 women positive for HPV (10%). A total of 25 HPV types were identified, including 17 classified as (probable) high risk and eight as low risk. Every multiple infection included at least one high-risk type. The most common high-risk types identified were 16, 18, 31, 53, 58, 33 and 35, with types 16 and 18 comprising 17% and 13% of the HPV-positive samples, respectively. The most common low-risk types were 11, 61, 54 and 6. Ten percent of all participants had either type 16 or 18, and 12% had type 6, 11, 16 or 18, the types included in the quadrivalent vaccine. Of women with HPV, 39 (29%) had type 16 or 18 (2 women were positive for both 16 and 18), 7% had type 6 or 11, and 35.6% had any of the four types covered by the quadrivalent vaccine.

Median age, education level and number of lifetime partners for the overall sample were 27 years, 10 years and three partners, respectively, making this sample slightly more educated than the female adult population of São Paulo (Table 2).²⁶ Just over 12% of participants were diagnosed with chlamydia, gonorrhoea or trichomonas, and 54% with bacterial vaginosis or vaginal yeast at the time of sample collection. Younger age, more lifetime partners and the presence of abnormal vaginal flora (defined as BV or yeast) were associated with testing positive for high-risk HPV (Table 2). All three variables remained significant in a multivariable model for having high-risk HPV (data not shown). Analysis of correlates of specific HPV types had limited statistical power, but demonstrated a possible trend for younger age being associated with types 31 ($P = 0.04$) and 11 ($P = 0.1$) but not with the most prevalent types 16 and 18 ($P = 0.24$ combined).

Cytology results were available for 218 women with HPV test results, of which 209 cytology results were normal, six women had ASCUS/AGUS (atypical squamous cells/atypical glandular cells of undetermined significance) and three had LSIL (low-grade squamous intraepithelial lesion) (Table 3). Women with high-risk HPV types were significantly more likely to present with abnormal cytology than those with no or low-risk HPV (Table 3). However, only 12% of women with a high-risk type and 8% of women with any HPV type presented with abnormal cytology. Of 209 women with normal cytology, 73 (35%) were positive for HPV and 38 (18%) were positive for a high-risk type. Only three of nine women with abnormal cytology had an HPV type that is included in the currently available vaccines.

Discussion

About a third of women (35%) in this population from central São Paulo, Brazil, had at least one HPV type. Twenty-five types of HPV were identified, including 17 classified as high or probable high risk and eight as low risk. The most common high-risk types identified were 16, 18, 31, 53, 58, 33 and 35, and the most common low-risk types were 11, 61, 54 and 6.

These results are comparable to findings from other large studies in Latin America.^{27,28} In the Latin American Screening Study (LAMS), HPV prevalence among women aged 40 and under in São Paulo was estimated at 19.7%.²⁷ When we restrict our analysis to the same 13 HPV types that were investigated using Hybrid Capture II in the LAMS study, our prevalence was 16.3%. A study among women presenting for routine gynaecological care at clinics in five Brazilian cities, with testing for the same HPV types as in our study, found an HPV prevalence of 38% among 15–25-year-olds,²⁸ which is comparable to the 41% prevalence in our 18–24-year-old group. Our prevalence estimate is higher than in some studies among Brazilian women;^{13,29,30} however, these studies included women over 40 and more limited diagnostic tests.

The bulk of HPV type distribution research, including that conducted in Brazil, has been implemented in populations with cervical cancer or abnormal cytology, priority populations in determining the types for inclusion in vaccines. Pooled analyses and meta-analyses carried out by IARC investigators and colleagues have demonstrated that world patterns match regional HPV type distribution, with a few exceptions. A meta-analysis of HPV in cases of cervical cancer and high-grade squamous intraepithelial lesions (HSIL) worldwide demonstrated that the 10 most common HPV types in women with cervical cancer in South America were 16, 18, 31, 45, 52, 33, 35, 39, 58 and 59, and in women with HSIL types 16, 58, 18, 51, 6, 31, 33, 11, 45 and 35.¹⁰ All these types, with the exception of 51 and 59, were found in our study population of Brazilian women without cervical cancer or HSIL. A pooled analysis of HPV types in women with cervical cancer from 25 countries found 30 types, of which 16, 18, 31, 45, 33, 35, 52 and 58 were the eight most common in Central and South America.⁹ Type 53 (type 53 appears to be unassociated with cancer, but is found in LSIL and HSIL;⁸ type 53 was classified as low risk in the five-city Brazilian study²⁸) was common in our study and in other studies with cytologically normal samples,¹¹ but not in either the meta-analysis or the pooled analysis of cervical cancer and HSIL. The five-city study among healthy women in Brazil found that the most prevalent oncogenic types from São Paulo included 16, 31, 52, 66, 58, 18, 51, 56, 53 and 35.²⁸ These high-risk types were also common in our study, with the exception of 51 and 56.

There is some evidence that HPV types 31 and 39 are more prevalent, and type 18 less prevalent, in South/Central America than in other continents,⁹ making the estimated HPV 16/18-positive fraction in cervical cancer lower in South/Central America than in other regions.^{8,9} In our population of women without cervical cancer or HSIL, 29% of participants with HPV (10% of all women) had HPV type 16 or 18 (preventable by both vaccines), and 35.6% of those with HPV (12.4% of all women) had HPV type 6, 11, 16 or 18 (preventable by the quadrivalent vaccine). There is evidence that both vaccines provide some cross-infection against HSIL caused by other HPV types, including HPV 31, which is common in South America and in our sample; as a result vaccine coverage may be underestimated.³¹

In our study, both younger age and having more lifetime partners were associated with testing positive for HPV and for having an oncogenic HPV type, findings that are consistent with Brazilian literature^{28,29} and international literature.^{32–34} The presence of abnormal vaginal flora (BV or yeast) was also associated with HPV. This association has been reported in the literature,^{35,36} though not consistently,³⁷ and directionality remains unclear, meriting further research.

Our study is clinic-based, which may limit its generalizability. However, over 30% of participants were recruited outside of the clinic environment and, among those recruited from within the clinic population, only 10% presented at the clinic for a gynaecological health concern; none of whom were seeking care related to HPV. Anyone requiring urgent care was excluded from the study. We were unable to type 38 samples (28% of HPV-positive samples) in this study. While this figure is comparable to the proportion of uncharacterized HPV types found among healthy subjects in studies with similar diagnostic methods,⁷ the large number of indeterminate HPV types may lead to an underestimation of the prevalence of some HPV types in our population.

Given that the current HPV vaccines do not protect from all oncogenic types of HPV, vaccination cannot yet replace cervical cancer screening programmes and, therefore, may not be cost-effective in countries where screening programmes have achieved high coverage and quality treatment is readily available. In developing countries where cervical cancer screening and treatment programmes are non-existent, have low coverage or are of poor quality, HPV vaccination programmes may provide the best opportunity to prevent mortality due to cervical

cancer. However, cost-effectiveness will depend to a large extent on the length of vaccine efficacy, currently evaluated for only up to five years.³⁸ Both cost and the logistical difficulties of implementing a delivery structure for a three-dose vaccination schedule are major barriers to HPV vaccine implementation. One study concluded that vaccination in Brazil is cost-effective;³⁹ however, HPV vaccines are currently only available in private clinics and have not yet been included in Brazil's national immunization programme because of the need for continued cytology screening, the cost of vaccination (approximately US\$300,³⁹ although prices may fall substantially when negotiated for the public sector) and logistical challenges of implementation in a large and diverse country where universal coverage must be guaranteed prior to implementation.

In the longer term, current vaccines could be expanded to include additional high-risk HPV types, thereby potentially reducing the need for continued cervical cancer screening. The distribution of HPV types varies by region, and this should be taken into account by vaccine developers. Meanwhile, surveillance of HPV types around the world should continue in populations with and without cytological abnormalities.¹²

Acknowledgments

We thank Maria da Costa at UCSF for performing quality control, Michelle Camargo, Giana Rabello Mota and Ana Carolina Denadai Sanchez Rosa for assistance in HPV testing, and Dr Helio Hehl Caiaffa at the Santa Casa Hospital for STI testing. We thank the study coordinator Adriana A Pinho as well as Jardelina Nascimento Santos and Melissa Enriquez for extraction of cytology results. Data collection for this study was funded by the Office of Population and Reproductive Health, Bureau for Global Health, US Agency for International Development, under the terms of Award No. HRN-A-00-99-00010 with additional funding received from the William and Flora Hewlett Foundation. The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development or the Hewlett Foundation. The first author support from the Fogarty AIDS International Training and received Research Program (AITRP) (Grant 1 D43 TW00003) at the School of Public Health, University of California, Berkeley.

References

1. World Health Organization. Human Papillomavirus and HPV Vaccines: Technical Information for Policy-makers and Health Professionals. Geneva: WHO; 2007.
2. Ministério da Saúde, Instituto Nacional de Câncer. Coordenação de Prevenção e Vigilância de Câncer. Estimativa 2008: Incidência de Câncer no Brasil. Rio de Janeiro: Instituto Nacional de Câncer – INCA; 2007.
3. Joura EA, Leodolter S, Hernandez-Avila M, et al. Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11, 16, and 18) L1 virus-like-particle vaccine against high-grade vulval and vaginal lesions: a combined analysis of three randomised clinical trials. *Lancet* 2007;369:1693–702. [PubMed: 17512854]
4. Paavonen J, Jenkins D, Bosch FX, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet* 2007;369:2161–70. [PubMed: 17602732]
5. Villa LL. Overview of the clinical development and results of a quadrivalent HPV (types 6, 11, 16, 18) vaccine. *Int J Infect Dis* 2007;11(Suppl 2):S17–25. [PubMed: 18162241]
6. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer* 2003;88:63–73. [PubMed: 12556961]
7. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;348:518–27. [PubMed: 12571259]
8. Clifford G, Franceschi S, Diaz M, Munoz N, Villa LL. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine* 2006;24(Suppl 3):S26–34.
9. Munoz N, Bosch FX, Castellsague X, et al. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *Int J Cancer* 2004;111:278–85. [PubMed: 15197783]

10. Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer* 2007;121:621–32. [PubMed: 17405118]
11. de Sanjose S, Diaz M, Castellsague X, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis* 2007;7:453–9. [PubMed: 17597569]
12. Clifford GM, Gallus S, Herrero R, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *Lancet* 2005;366:991–8. [PubMed: 16168781]
13. Oliveira FA, Pflieger V, Lang K, et al. Sexually transmitted infections, bacterial vaginosis, and candidiasis in women of reproductive age in rural Northeast Brazil: a population-based study. *Mem Inst Oswaldo Cruz* 2007;102:751–6. [PubMed: 17924006]
14. Vaccarella S, Herrero R, Dai M, et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. *Cancer Epidemiol Biomarkers Prev* 2006;15:2148–53. [PubMed: 17119039]
15. Lippman SA, Jones HE, Luppi CG, Pinho AA, Veras MA, van de Wijert JH. Home-based self-sampling and self-testing for sexually transmitted infections: acceptable and feasible alternatives to provider-based screening in low-income women in Sao Paulo, Brazil. *Sex Transm Dis* 2007;34:421–8. [PubMed: 17091118]
16. Madico G, Quinn TC, Rompalo A, McKee KT Jr, Gaydos CA. Diagnosis of *Trichomonas vaginalis* infection by PCR using vaginal swab samples. *J Clin Microbiol* 1998;36:3205–10. [PubMed: 9774566]
17. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology* 2004;324:17–27. [PubMed: 15183049]
18. Souza IE, Nicholson D, Matthey S, et al. Rapid epidemiologic characterization of cytomegalovirus strains from pediatric bone marrow transplant patients. *Infect Control Hosp Epidemiol* 1995;16:399–404. [PubMed: 7673645]
19. Bernard HU, Chan SY, Manos MM, et al. Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. *J Infect Dis* 1994;170:1077–85. [PubMed: 7963696]
20. de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol* 1995;76(Pt 4):1057–62. [PubMed: 9049358]
21. Qu W, Jiang G, Cruz Y, et al. PCR detection of human papillomavirus: comparison between MY09/MY11 and GP5+/GP6+ primer systems. *J Clin Microbiol* 1997;35:1304–10. [PubMed: 9163434]
22. Smits HL, Bollen LJ, Tjong AHSP, et al. Intermethod variation in detection of human papillomavirus DNA in cervical smears. *J Clin Microbiol* 1995;33:2631–6. [PubMed: 8567896]
23. Palefsky JM, Holly EA, Ralston ML, Jay N. Prevalence and risk factors for human papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. *J Infect Dis* 1998;177:361–7. [PubMed: 9466522]
24. StataCorp. Stata Statistical Software: Release 8.0. College Station, TX: Stata Corporation; 2003.
25. Palefsky JM, Minkoff H, Kalish LA, et al. Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. *J Natl Cancer Inst* 1999;91:226–36. [PubMed: 10037100]
26. Instituto Brasileiro de Geografia e Estatística (IBGE). Censo Demográfico 2000. [January 2008]. See <http://www.ibge.gov.br/home/estatistica/populacao/censo2000/>
27. Longatto-Filho A, Erzen M, Branca M, et al. Human papillomavirus testing as an optional screening tool in low-resource settings of Latin America: experience from the Latin American Screening study. *Int J Gynecol Cancer* 2006;16:955–62. [PubMed: 16803469]
28. Souza, EPd. Doctoral Dissertation. UNICAMP; Campinas, Brasil: 2004. Epidemiologia da infecção genital por HPV e anormalidades na citologia cervical em mulheres jovens brasileiras.

29. Nonnenmacher B, Breitenbach V, Villa LL, Prola JC, Bozzetti MC. Genital human papillomavirus infection identification by molecular biology among asymptomatic women. *Rev Saude Publica* 2002;36:95–100. [PubMed: 11887236]
30. Trottier H, Mahmud S, Costa MC, et al. Human papillomavirus infections with multiple types and risk of cervical neoplasia. *Cancer Epidemiol Biomarkers Prev* 2006;15:1274–80. [PubMed: 16835323]
31. Bonanni P, Borealini S, Bechini A. Efficacy, duration of immunity and cross protection after HPV vaccination: a review of the evidence. *Vaccine* 2009;27(suppl 1):A46–53. [PubMed: 19480962]
32. Vaccarella S, Franceschi S, Herrero R, et al. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. *Cancer Epidemiol Biomarkers Prev* 2006;15:326–33. [PubMed: 16492924]
33. Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. *JAMA* 2007;297:813–9. [PubMed: 17327523]
34. Figueroa JP, Ward E, Luthi TE, Vermund SH, Brathwaite AR, Burk RD. Prevalence of human papillomavirus among STD clinic attenders in Jamaica: association of younger age and increased sexual activity. *Sex Transm Dis* 1995;22:114–8. [PubMed: 7624812]
35. Mao C, Hughes JP, Kiviat N, et al. Clinical findings among young women with genital human papillomavirus infection. *Am J Obstet Gynecol* 2003;188:677–84. [PubMed: 12634640]
36. Watts DH, Fazzari M, Minkoff H, et al. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. *J Infect Dis* 2005;191:1129–39. [PubMed: 15747249]
37. Allsworth JE, Lewis VA, Peipert JF. Viral sexually transmitted infections and bacterial vaginosis: 2001–2004 National Health and Nutrition Examination Survey data. *Sex Transm Dis* 2008;35:791–6. [PubMed: 18607314]
38. Stanley M, Villa LL. Monitoring HPV vaccination. *Vaccine* 2008;26(Suppl 1):A24–7. [PubMed: 18642459]
39. Goldie SJ, Kim JJ, Kobus K, et al. Cost-effectiveness of HPV 16, 18 vaccination in Brazil. *Vaccine* 2007;25:6257–70. [PubMed: 17606315]

Table 1
Human papillomavirus (HPV) type distribution by single and multiple infections among 386 women in São Paulo, Brazil

HPV type	Single (N = 122) n	Multiple (N = 13) n	All HPV infections (N = 135) n (%)	All women (N = 386) %
High risk				
16	17	6	23 (17.0)	6.0
18	12	6	18 (13.3)	4.7
31	2	4	6 (4.4)	1.6
33	4	1	5 (3.7)	1.3
35	2	2	4 (3.0)	1.0
39	1	0	1 (0.7)	0.3
45	2	1	3 (2.2)	0.8
52	3	0	3 (2.2)	0.8
53	6	0	6 (4.4)	1.6
56	1	0	1 (0.7)	0.3
58	5	1	6 (4.4)	1.6
66	3	0	3 (2.2)	0.8
67	1	0	1 (0.7)	0.3
68	1	0	1 (0.7)	0.3
69	1	0	1 (0.7)	0.3
70	3	0	3 (2.2)	0.8
82	1	0	1 (0.7)	0.3
Total high risk*	65 (83.3)	13 (16.7)	78 (57.8)	20.2
Low risk				
6	3	1	4 (3.0)	1.0
11	6	0	6 (4.4)	1.6
54	2	3	5 (3.7)	1.3
57	1	0	1 (0.7)	0.3
61	5	1	6 (4.4)	1.6
62	1	0	1 (0.7)	0.3
64	0	1	1 (0.7)	0.3
81	1	0	1 (0.7)	0.3
Total low risk†	19 (76.0)	6 (24.0)	25 (18.5)	6.5
Indeterminate	38 (100)		38 (28.1)	9.8

* Number (percent) of single and multiple infections over all women infected with high-risk types

† Number (percent) of single and multiple infections over all women infected with low-risk types

Table 2
Sociodemographic, behavioural and clinical characteristics by the presence of high risk, low risk and any positive subtype of HPV

Characteristics	Overall (<i>N</i> = 386) <i>n</i> (%)	High-risk HPV (<i>N</i> = 78)* <i>n</i> (%)	Only low-risk HPV (<i>N</i> = 19) <i>n</i> (%)	Any HPV (<i>N</i> = 135) [†] <i>n</i> (%)
Age (years)				
18–24	149 (38.6)	33 (42.3) [‡]	7 (36.8)	55 (40.7)
25–29	94 (24.4)	24 (30.8)	4 (21.1)	38 (28.2)
30–34	68 (17.6)	13 (16.7)	4 (21.1)	25 (18.5)
35–40	75 (19.4)	8 (10.2)	4 (21.1)	17 (12.6)
Education				
Incomplete primary	125 (32.5)	28 (35.9)	8 (42.1)	47 (34.8)
Some secondary	83 (21.5)	13 (16.7)	0 (0)	22 (16.3)
Some university/technical	177 (46.0)	37 (47.4)	11 (57.9)	66 (48.9)
Marital status				
Single	117 (30.4)	27 (34.6)	8 (42.1)	41 (30.4)
Married/cohabitating	233 (60.5)	45 (57.7)	9 (47.4)	82 (60.7)
Divorced/widowed	35 (9.1)	6 (7.7)	2 (10.5)	12 (8.9)
Parity				
0	118 (30.6)	27 (34.6)	5 (26.3)	47 (34.8)
1	102 (26.4)	18 (23.1)	6 (31.6)	37 (27.4)
2	97 (25.1)	18 (23.1)	5 (26.3)	29 (21.5)
≥3	69 (17.9)	15 (19.2)	3 (15.8)	22 (16.3)
Number of lifetime partners				
1	91 (23.7)	9 (11.5) ^{§‡}	3 (15.8)	23 (17.2) [‡]
2	61 (15.9)	15 (19.2)	3 (15.8)	23 (17.2)
3–4	106 (27.6)	23 (29.5)	6 (31.6)	38 (28.4)
≥5	126 (32.8)	31 (39.8)	7 (36.8)	50 (37.3)
Age at sexual debut				
≤15	117 (30.5)	26 (33.8)	5 (26.3)	47 (35.1)
16–17	106 (27.6)	26 (33.8)	7 (36.8)	37 (27.6)
≥18	161 (41.9)	25 (32.4)	7 (36.8)	50 (37.3)
Concurrent STI**	48 (12.5)	11 (14.7)	4 (18.2)	18 (13.4)
Concurrent vaginal infection ^{††}	209 (54.2)	53 (68.0) [§]	12 (63.2)	85 (63.0)

STI, sexually transmitted infection; HPV, human papillomavirus

* Includes six people with high/low-risk type co-infection

[†] Includes HPV positive with indeterminate types (*n* = 38)

[‡] Test for trend significant *P* < 0.05 for HPV category versus no HPV

[§] χ^2 significant at *P* < 0.05 for HPV category versus no HPV

** *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* diagnosed by polymerase chain reaction

^{††} Bacterial vaginosis or yeast diagnosed on clinical exam

Table 3
Cytology findings by the presence of high- and low-risk HPV

Cytology	Total (N = 218) n (%)	High-risk HPV (N = 43) n (%)	Only low-risk HPV (N = 11) n (%)	Any HPV (N = 79)[†] n (%)
Normal	209 (95.9)	38 (88.4) [*]	11 (100)	73 (92.4)
ASCUS/AGUS	6 (2.8)	3 (7.0) [*]	0 (0.0)	4 [‡] (5.1)
LSIL	3 (1.4)	2 (4.7) [*]	0 (0.0)	2 ^δ (2.5)

HPV= human papillomavirus; ASCUS/AGUS = atypical squamous cells/atypical glandular cells of undetermined significance; LSIL= low-grade squamous intraepithelial lesion

^{*} $P < 0.05$ comparing high-risk HPV to no HPV and no + low-risk HPV

[†] Includes HPV positive with indeterminate type ($n = 25$)

[‡] Infection with types 16, 18, 33 and indeterminate

^δ Infection with types 18 and 35