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## Original article

# Cervical human papillomavirus infection and persistence: a clinic-based study in the countryside from South Brazil



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## ABSTRACT

Human papillomavirus (HPV) infection is common in sexually active women and viral persistence may cause intraepithelial lesions and eventually progress to cervical cancer (CC). The present study aimed to investigate epidemiological factors related to HPV infection and to evaluate viral persistence and CC precursor lesions frequencies in women from a city in the countryside of South Brazil. Three hundred women were recruited from a primary public health care clinic. The patients were interviewed and underwent sampling with cervical brushes for HPV-DNA detection/typing by a PCR-based assay and cytological analysis by Pap smear test. HPV was detected in 47 (15.7%) women. HPV infection was significantly associated with young age (<30 years) and low socio-economic status. Seventeen (5.7%) women presented cytological abnormalities, three of them with precursor CC intraepithelial lesions. A subgroup of 79 women had been previously analyzed and thirteen (16.4%) were persistently infected, two with precursor CC intraepithelial lesions and high-risk HPV types infection (both of them without cervical abnormalities in the first exam). In conclusion, HPV infection was associated with young age (<30 years) and low family income; viral persistence was low (16.4%) but related to CC precursor lesions; and HPV-DNA high risk types detection would help to screen CC in the population.

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## Introduction

Human papillomavirus (HPV) is one of the most common causes of sexually transmitted diseases in the worldwide.

It has the ability to infect epithelial and may resist asymptomatic or cause a variety of diseases, including cancer.<sup>1</sup> HPV infection is usually transient and most people eliminate the virus from the body with the effective action of the immune system after 5.1–15.4 months.<sup>2</sup> However, HPV

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persistence can cause benign lesions, known as warts (in different parts of the body), or low/high grade intra-epithelial lesions (LSIL/HSIL) that can progress to cancer, mainly in the uterine cervix.<sup>3</sup> Further, HPV persistence and consequently cervical cancer (CC) depends on other factors, such as age, high parity, smoking, long-term use of contraceptives, sexual behavior and co-infection with other sexually transmitted infectious agents.<sup>4</sup>

HPV prevalence ranges from 13.7% to 54.3% according to the studied population and geographic area in Brazil reviewed by.<sup>5</sup> However, main epidemiological studies have been performed in the capitals and metropolitan cities. These studies demonstrated that HPV infection was associated with multiple sexual partners<sup>6</sup>; young age, more lifetime sex partners and abnormal vaginal flora<sup>7</sup> and non-stable sexual partners.<sup>8</sup> HPV persistence also presented frequencies as different as 19.2% in Porto Alegre (Rio Grande do Sul state) and 59.6% in Ouro Preto (Minas Gerais state) in two studies performed in primary public health care clinics from Brazil.<sup>9,10</sup>

This study aimed to investigate epidemiological aspects associated with HPV infection and to evaluate HPV persistence in women from the city of Cruz Alta and surrounding small localities. This countryside region is located in the North of the Rio Grande do Sul (the southern most state in Brazil) and it is more than 300 km away from the respective capital city (Porto Alegre).

## Methodology

### Study population and sample collection

A cross-sectional study was conducted with 300 women who accepted to participate in the study while attending for CC screening in a primary public health care clinic (Center for Women and Children) in the city of Cruz Alta (Rio Grande do Sul State, Brazil) from January 2012 to April 2013. Epidemiological informations (socio-demographic, behavioral, and clinical) were obtained from a standardized individual questionnaire that was administered by a trained interviewer in a private room. The research project was approved by the Research Ethics Committee of the University of Cruz Alta (Protocol No. 078.0.417-09).

After each participant gave informed consent, cervical samples were collected from all participants for HPV-DNA testing and cytological analysis. Clinical samples were collected by scraping the ectocervix and endocervix of each patient with an endocervical brush, smeared on a glass slide (that was fixed immediately with polyethylene glycol for cytological examination) and after stored in a buffer solution (EDTA pH 8.0 0.01 M, SDS 0.03 M), and stored at  $-20^{\circ}\text{C}$  until analysis.

Women also enrolled in a previous report<sup>11</sup> or with two visits in this study (minimum interval of twelve months) were identified to evaluate HPV persistence. A total of 79 (26.3%) women attended these criteria (57 evaluated in the previous study and 22 analyzed twice in this study) and composed a subgroup to investigate HPV persistence.

The clinical management of the patients was in accordance with the "Brazilian classification for cervical reports and recommended procedures: recommendations for health professionals".<sup>12</sup> This protocol do not establish HPV testing in the routine screening, so results of HPV types were not used in the management of the patients.

### HPV-DNA detection and typing

HPV-DNA testing was performed by nested polymerase chain reaction (nested-PCR) and restriction fragment length polymorphism (RFLP) as previously described.<sup>13</sup> Samples presenting insufficient DNA for HPV typing were classified as inconclusive. Results were interpreted by two independent analysts and HPV types were classified into high-risk (HR) and low-risk (LR).<sup>14</sup>

### Cytological analysis

The cytological analysis was performed by conventional Pap smear test evaluated by two independent cytologists (conflicting results were submitted to a third evaluation). Cell abnormalities were classified according to the Bethesda System 2001.<sup>15</sup> Basically, it classifies the modified cells into nine categories (five to squamous and four to glandular cells): (1) squamous cell carcinoma, (2) high-grade squamous intraepithelial lesion (HSIL), (3) low-grade squamous intraepithelial lesion (LSIL), (4) atypical squamous cells of undetermined significance (ASC-US), (5) atypical squamous cells that cannot exclude HSIL (ASC-H), (6) adenocarcinoma, (7) endocervical adenocarcinoma in situ, (8) atypical glandular cells (AG), and (9) atypical glandular cells not otherwise specified (AG-NOS). Normal cells were defined as negative for intraepithelial lesion and malignancy (NILM).

### HPV persistence

Women with two evaluations were classified into four categories according to the HPV infection status: (1) persistent infection: HPV-DNA positive in both assessments; (2) conversion: HPV-DNA negative in the first visit and HPV-DNA positive in the follow-up; (3) elimination (clearance): HPV-DNA positive only in the first evaluation; (4) without HPV infection: HPV-DNA negative in both visits.

### Statistical analysis

Data analysis was conducted using the SPSS version 17.0 software (SPSS Inc., USA). Association between HPV infection status and other variables was determined with the chi-square test. Multivariate models were conducted using a modified Poisson regression<sup>16</sup> to test the independent associations of HPV infection with socio-demographic, behavioral and clinical characteristics. Associations that presented values of  $p$  between 0.05 and 0.15 in bivariate analysis were regarded as having borderline significance and were included in the modeling of confounding factors. All  $p$  values presented are two-tailed and the values of  $p < 0.05$  were considered statistically significant.

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