

Human papillomavirus cervical infection in Guarani Indians from the rainforest of Misiones, Argentina

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KEYWORDS

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Summary *Objective:* To evaluate the prevalence of human papillomavirus (HPV) cervical infection in women from the South American Guarani Indian tribe located in the rain forest of Misiones, north-eastern Argentina; a region with a high incidence of cervical carcinoma.

Methods: A cross-sectional cytological and HPV screening of sexually active Guarani women from nine Indian settlements was conducted. Demographic data, information about sexual behavior, and gynaecological history were recorded. Fresh cervical specimens from 239 patients were collected, of which 207 were included in this study. Cytology and microbiological detection were carried out by the Papanicolaou and Gram stain methods, respectively. HPV detection and typing were analyzed by PCR and RFLP.

Results: Pap smears in 96% of all patients showed an inflammatory pattern. A possible etiologic agent was found in 58% of cases: 52% *Trichomonas vaginalis*, 35% *Gardnerella vaginalis* and 13% *Candida sp.* Seven cases had cytological changes compatible with Low Grade Intraepithelial Lesion (LGSIL), one with High Grade Intraepithelial Lesion (HGSIL) and one in situ cervical cancer. The prevalence for generic HPV infection was 64% (133/207). Genotyping gave a 26% prevalence for HPV types 16/18, 13% for types 6/11 and 30% for other types, with nine mixed infections.

Conclusion: This work reports for the first time the prevalence of cervical HPV infection in Guarani women. Nearly all Guarani women had some grade of cervical disease. Generic HPV infection prevalence was elevated (64%), with predominance

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of high risk types 16/18. A large variety of viral types was detected, including high to intermediate risk types not found previously in the region.

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Introduction

International studies have shown that cervical cancer is the second most common cancer in women worldwide, with a variable prevalence according to the implementation of successful public health screening and treatment programmes.¹

This prevalence varies from less than 5 cases in 100,000 women in developed nations to more than 40 in 100,000 in underdeveloped countries. Numerous clinical, epidemiological, and molecular investigations have been performed on Human Papillomavirus (HPV) infection in cervical cancers and associated lesions of the low female genital tract. Particular types of HPV, designated as high risk types, have been detected in more than 95% of invasive cervical neoplasias with variable percentages in different lesions. This fact supports the concept that cervical infection by high risk HPV types is necessary but not sufficient for progression to cervical neoplasia.^{2–6}

Over the years, studies examining risk factors in HPV infection have not been entirely consistent.^{7,8} Factors contributing to this have been suggested to include differences in study populations and also the different detection methods used. It is, therefore, important to concentrate on particular characteristics of the study population such as geographic location, demographic growth rate, cultural particularities and regional human displacement in the area under analysis, as well as overall HPV prevalence, presence of specific HPV types and sexual habits.⁹

In Argentina there are still no general epidemiological data to indicate the overall prevalence of HPV infection and its association with cervical neoplasia. Nevertheless, different regional studies have shown that cervical cancer incidence varies widely, with values ranging from 10 cases in 100,000 women living in urban areas to nearly 40 in 100,000 in peripheral regions.^{10–12}

The Province of Misiones is located at the north-eastern tip of Argentina, sharing international borders with Paraguay and Brazil. It possess the last remnant of rainforest in the region. Misiones is considered a region with both a high incidence of cervical carcinoma and HPV genital infection in the urban population.¹³

This area is experiencing an intense population migration along the entire territory due to ecologi-

cal and economical factors. This new situation has changed several cultural habits dramatically. Large numbers of families leave the countryside and move to the suburbs of the major cities where there is insufficient healthcare. Furthermore, economic variations between countries, such as exchange rate fluctuations, cause international migration. These regional differences affect public health in several ways, sexually transmitted diseases being one of them.

The Guaraní Indian population inhabits the rainforest of Misiones with a population of around 1700 concentrated in more than 40 small communities. Guaraní women begin sexual activity early, after menarche; they have multiple sexual partners and are multiparous. Some of their partners have already had intercourse with white women of neighboring towns. Until the last decade, the Indian social units had been self-sustaining, avoiding direct exchanges with the white population of urban areas. However, the recent aforementioned economical and ecological factors have generated an important change in the Indian–white relationship, compelling male Indians to integrate with the rural working force in running white agricultural establishments. This abrupt social change has had a direct impact on the Indian community health status, bringing a significant increase in sexually transmitted and respiratory diseases.

This situation led us to conduct the first reported cervical HPV infection screening study among sexually active Guaraní women resident in this region, as representative of a particular community beginning to interact with the urban population.

Patients and methods

Study population

A community-based, horizontal, cervical HPV infection screening was conducted among 239 sexually active Guaraní women, aged 12–64 (median age 15), from nine Indian settlements located deep in the rainforest in the region of Misiones, Argentina. The group represented 93% of the local Indian female population.

All women underwent a general gynecological examination, and a questionnaire was completed

on their sexual and reproductive habits, including any history of sexually transmitted diseases. During these visits, material for cytological and virological analysis was taken. To minimize the bias in sample collection and to ensure a sufficient amount of exfoliated cells, sampling was performed in non-pregnant women and only if the last menstruation had occurred at least one week before. All participants gave their informed consent for inclusion in this study, either personally or through their guardian, assisted by trained health and social workers. All procedures were approved by the University of Misiones Bioethical Committee and in accordance with the Helsinki Declaration.

Samples

Endo- and ectocervical cells were collected with wooden spatulas for PAP smears and Gram stain, and with cytobrush for HPV-DNA analysis. The latter were immediately dipped in sterile PBS, pH 7.4 at 5 °C and transported to the laboratory on ice within 48 hours for virology processing.

Processing

Cytological diagnosis

A direct Gram stain and standard PAP processing with the Bethesda classification system were used for cytological diagnosis.¹⁴

All the smears were screened and reviewed independently by two of the authors (WN and JG) who had no prior information on patient HPV status. The final diagnosis was based on the worst morphologic picture.

HPV-DNA analysis

The polymerase chain reaction (PCR) was used for detecting HPV-DNA in cervical scrapes. Collected cervical cells were lysed with proteinase K and SDS in a final reaction volume of 500 µl of crude cellular suspension. Nucleic acid was extracted by ethanol precipitation. As a control of sample sufficiency, the primer set PCO4 and GH20 was employed to amplify a 268 bp DNA fragment of the human beta-globin gene.¹⁵ For each assay, 100 ng of total DNA were used as a template for HPV-DNA detection by PCR. The amplification was performed with the GP5+ and GP6+ consensus primers, targeting a region of approximately 140 bp in the highly conserved L1 ORF of the viral genome.¹⁶ CaSki and HeLa cell DNAs were used as positive controls; negative control included no template DNA and was incorporated after the tenth specimen in each test. The amplification products were analyzed on 1.5% agarose gels, visualized with ethidium bro-

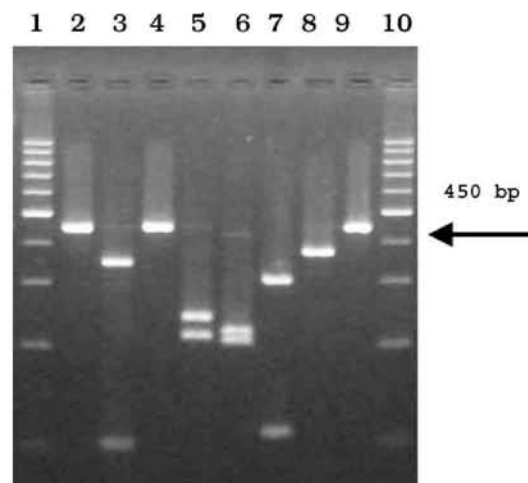


Figure 1 Representative RFLP pattern. Submarine 3% agarose gel electrophoresis of restriction fragment length polymorphism from a representative HPV L1 amplicon obtained with the MY09/11 primer set; Lanes 1 and 10: Molecular weight standard ladder (100 bp); Lane 2: *Bam* HI, Lane 3: *Hae* III, Lane 4: *Hinf* I, Lane 5: *Dde* I, Lane 6: *Pst* I, Lane 7: *Rsa* III, Lane 8: *Sau* III digested products, respectively; Lane 9: Undigested MY09/11 amplicon; RFLP Pattern compatible with HPV type 58.

mid staining under UV light exposure and finally photographed.

Typing of HPV-DNA positive samples was performed by restriction digestion of PCR products (RFLP analysis) according to Bernard et al.¹⁷ Briefly, aliquots of the PCR products obtained using the degenerate consensus primers MY09-MY11, targeting a region of approximately 450 bp in length in the L1 ORF of the viral genome, were mixed with 10 µl of seven different restriction enzymes (*Bam* HI, *Hae* III, *Dde* I, *Pst* I, *Hinf* I, *Sau* III, and *Rsa* I) in separate reactions. Digestion products were separated by electrophoresis in 3% agarose gel and the pattern obtained compared with published data (Figure 1).

Clinical treatments

After cytological diagnosis, all women showing signs of vaginitis were treated at home with metronidazole as a single oral dose of 2 g a day for seven days and a local application of clotrimazole for three days, provided by agents of the Public Health Ministry. Patients with low grade intraepithelial lesions (LGSIL) were scheduled for a colposcopic follow-up at six-month intervals. Patients with high grade intraepithelial lesions (HGSIL) and in situ carcinoma were surgically treated by cold knife cone excision at the Central Provincial Hospital and proper specimens were sent for histopathological evaluation.

Table 1 Study population: age and pregnancy distributions.

Age range	Percentage
12–14	9
15–19	21
20–24	20
25–29	11
30–34	9
35–39	7
40–44	6
45–49	5
50–54	4
55–59	5
60–64	3
Pregnancies	Percentage
0	15
1	16
2	23
3	22
4	15
>4	9

Results

A total of 239 women were originally involved in this study. Interviews were successfully completed for 228 women during the accrual period. The cervical specimens from 12 women could not be evaluated because of problems in transport or material quality. An additional set of nine specimens failed to be amplified for beta-globin. This analysis therefore refers to the remaining 207 women.

The population under analysis had a median age of 15, 61% being younger than 30. This particular age distribution is common among Guarani Indians mainly due to high mortality rates for those above 30–35 years being caused by respiratory tract infections. The mean age at first intercourse was 13 years old, first pregnancy at 15. Polygamy reached 80%. The illiteracy rate reached 87% as is usual in their semi-closed society. Participation in this study represented the first medical visit for 93% of the women. There was no statistical correlation between any of these particular population characteristics and HPV infection or cervical disease. Age and pregnancy distributions are presented in Table 1.

Cytological diagnosis

Pap smears in 96% (199/207) of all patients showed an inflammatory pattern. Main infections were due to *Trichomonas vaginalis* in 30% (62/207), *Gardnerella vaginalis* in 20% (42/207) and *Candida* sp. in 8% (16/207) of patients.

Table 2 Distribution of HPV types in the Guarani Indian population as determined by PCR-RFLP.^a

High risk types ^c	<i>n</i>	Percentage ^b
HPV 16	41	30.8
HPV 18	13	9.8
HPV 58	7	5.3
HPV 31	6	4.5
HPV 45	5	3.8
HPV 35	3	2.3
HPV 68	2	1.5
HPV 39	1	0.8
HPV 59	1	0.8
Total	79	59.4
Low risk types ^c	<i>n</i>	Percentage ^b
HPV 6	16	12.0
HPV 11	10	7.5
HPV 53	6	4.5
HPV 56	4	3.0
CP8304	4	3.0
MM8	3	2.3
HPV 61	2	1.5
HPV 69	2	1.5
HPV 55	1	0.8
HPV 66	1	0.8
HPV 72	1	0.8
HPV 73	2	1.5
CP8061	1	0.8
ISO39	1	0.8
Total	54	39.8
Undetermined	10	7.5

^a Including mixed infections.
^b Over the infected population.
^c According to^{30,32}.

Seven cases (3%) had morphological changes compatible with LGSIL, one with HGSIL and, in a 47 year old Indian, in situ cervical cancer.

HPV–DNA analysis

HPV–DNA was found in 133 of the total suitable samples analyzed, giving a HPV infection prevalence of 64% in this population.

Viral genotyping gave a 26% (54/207) prevalence for types 16/18, 13% (26/207) for types 6/11 and 30% for other types (62/207), with 4% (9/207) mixed infections. The viral distribution is presented in Table 2.

Discussion

Few studies can be found regarding epidemiological surveys of cervical dysplasia and HPV cervi-

cal infection in American Indian populations,^{18–21} particularly in South American tribes.^{22–24}

This situation may be attributed to, amongst other things, difficulties in accessing settlements, cultural barriers and funding. Nevertheless, the migration, social exchange and specific sexual habits of these communities can have an important impact on public health, particularly with regard to the interaction with inhabitants of nearby regions.

These results from generic and type-specific HPV cervical infection analysis performed for the first time on 207 Guarani Indian women, are presented as part of an ongoing study into an epidemiological HPV survey of the region. Approximately 93% of the sexually active and accessible female Indian population of this area have been studied. The study group was representative of the Guarani population inhabiting the rainforest of Misiones, Argentina, characterized by early sexual initiation, early pregnancy, multiparity, polygamy, and illiteracy.

Although cytology showed 96% (199/207) of inflammatory changes were mainly due to bacterial and/or parasitic infections, in view of their hygienic habits and almost no access to healthcare this situation can be considered as 'normal' for this particular population. Participation in this study enabled direct pharmacological treatment of these infections, establishing one of the first contacts between this population with so called 'white medicine'.

Cervical lesions were present in 4% of women, LGSIL in 7 patients, HGSIL in one patient, and one in situ carcinoma. All were treated at the central hospital and lead to a full recovery. Taking into account that the studied population comprised 93% of Guarani women of the region, this represents a very high prevalence of lesions.

Cervical carcinoma is the leading cause of death by cancer in women in the province of Misiones, with an elevated incidence of approximately 35 cases per 100,000. This value is particularly high compared to other regions in the country where data are available (10 cases per 100,000 on average).¹³ The Public Health Ministry therefore considers cervical carcinoma as an endemic pathology for this region which impacts highly on public health.

The Guarani population showed an elevated HPV cervical infection prevalence of 64% (133/207), only comparable to that reported by Ter Meulen et al. for women in Tanzania (59%, age range 15–70 years, mean age 31).²⁵

Demographic factors, including specific location, sexual behavior patterns, polygamy and no effective healthcare could play a key role in sustaining HPV reservoirs. In view of this, several health organizations have carried out multinational studies

in order to characterize the epidemiology of HPV in Latin American, Asian and African countries with the aim of identifying prevention strategies.^{2,26,27}

Stressing this concept, we recall the results obtained in 1998 from a case-control study carried out by our group in white females representative of regional urban population.^{11,13} HPV infection status and associated risk factors for cervical lesions were analyzed, detecting an elevated number of HPV-infected white women (43%, 85/199) with no cytological or colposcopic sign of viral infection. A comparative high generic HPV prevalence value in cytologically normal women (43.4%) had only been found previously in Greenland by Kjaer et al.⁸ Similar studies carried out in high incidence areas for cervical cancer in South America, such as in Recife, Brazil²⁸ and Colombia²⁹ have reported lower HPV infection prevalences (13% and 9%, respectively).

In this work, a population residing in the region of Misiones showed a remarkably high prevalence of HPV cervical infection. A possible explanation may be the young age of the Guarani women under analysis, a particularity of the demographics of this tribe where 61% subjects were younger than 30 years old (median age 15). This finding is in accordance with the general view that younger rather than older women are more likely to contract genital HPV infection, and that most of them are transient.⁹ Nevertheless, it is not clear whether the presence of HPV-DNA in women with no cervical lesions indicates a recent infection or predicts future abnormalities.

When viral genotyping was carried out, types 16/18 (considered carcinogenic to humans (Group 1) by the International Agency for Research on Cancer⁹) were found in 26% and types 6/11 (low-risk types) in 13% of patients respectively. These prevalences are comparable with that of the white urban population inhabiting the region (24% and 11% respectively).¹³

The presence of a large variety of viral types in the Guarani Indian women was noteworthy (24 different viral types). This is in contrast to the six types found in the white urban population.¹³ The distribution includes types found almost exclusively in this region (39, 59, ISO39),² and some still undetermined, that are in process of typing (Table 2).

Furthermore, when analyzing the phylogenetic relationship³⁰ of all HPVs affecting the Guarani population, a strong representation of certain groups is clearly noticed, bringing homogeneity to the apparent diverse presence of more than 20 viral types (Table 3). High risk and related types (A7 and A9 groups) count for 56% (79/142) of typified positive samples, while most of the uncommon types,

Table 3 Phylogenetic relationships of mucosal HPV detected in Guaraní women.

Phylogenetic group ^a	Viral type	Infected samples	Total	Percentage ^b
A9 (high risk)	16	41	57	40%
	58	7		
	31	6		
	35	3		
A10 (low risk)	6	16	27	19%
	11	10		
	55	1		
A7 (high risk)	18	13	22	15%
	45	5		
	68	2		
	39	1		
A6 (low risk)	53	6	11	8%
	56	4		
	66	1		
A3 (low risk)	CP8304	4	10	7%
	MM8	3		
	61	2		
	72	1		
A5 (low risk)	69	2	3	2%
	ISO39	1		

^a The phylogenetic tree of the HPV family includes 108 sequences computed for the L1 consensus primer region using the neighbor joining method;³⁰ The A supergroup comprises mucosal/genital isolates, the high risk types members being to the A7 and A9 groups.
^b Relative to 130 virus genotyped in this study, HPV 73 and CP8061 have not been assigned to any definitive A group yet.

representing the 17% of total typed viruses, were clustered into three groups (A6, A3 and A5).

Knowing that genomes of papillomaviruses evolve at very slow rates, it is believed that HPV types have existed in nearly identical molecular form since the beginning of the humankind.³¹ Determining the distribution of the viral type in particular communities is of special epidemiological interest as it constitutes a potential infective reservoir susceptible to viral spreading once external contact begin to be established.

Having a significant number of Guaraní women infected with various types of HPV, many of them with known carcinogenic potential not usually found in the urban population, is an important risk factor to be considered when implementing regional public health policy programmes.

Another particular characteristic of the study population is its seasonal migration between set-

tlements located in north-eastern Argentina and southern Paraguay. This behavior is a consequence of the nomadic behavior of the Guaraní tribe and its agricultural practices. During this transit, contact with the white population is established, exposing people to different sanitary conditions and hygienic standards, making the transmission of infectious diseases more likely.

In summary, the detection of an elevated HPV cervical infection prevalence in Guaraní women and the presence of HPV viral types not found previously in the urban population constitutes a potential infective reservoir that can spread once contact becomes more frequent due to the economic and ecological changes affecting the region.

This study has contributed to the implementation of a PAP screening program and the promotion of sexual health education within the Guaraní Indian community. These actions may help in decreasing cervical disease and cancer cases in this population.

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