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Epidemiology of high-risk human papillomavirus among women of the north-central region of Ecuador: a retrospective study

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Abstract

Background High-risk human papillomavirus (HR-HPV) infection is the major risk factor for cervical cancer, which remains a significant concern, particularly in low and middle-income countries due to the lack of effective treatment and preventive measures. Examining the prevalence and distribution of HR-HPV is key to designing or reformulating policies aimed at eliminating HPV-associated cancers.

Methods A retrospective analysis was conducted using HR-HPV genotyping data from 9,524 women aged ≥18 years of the north-central region of Ecuador, who underwent cervical sampling either in cervical cancer prevention campaigns or during gynecological outpatient clinics, between January 2015 and August 2022. Samples were tested for HR-HPV using the cobas® 4800 HPV Test. Infection rates and genotype distributions were assessed across years, age groups, and provinces. Multivariable logistic regression was conducted to identify independent factors associated with HR-HPV infections. Potential association profiles among genotypes were explored through Multiple correspondence analysis.

Results The overall prevalence of HR-HPV was 26.87% [95% CI: 25.98, 27.77]. In particular, HPV 16 was found in 8.64% [95% CI: 8.08, 9.22] of the participants, HPV 18 in 2.85% [95% CI: 2.52, 3.20], and Other HR-HPV in 19.41% [95% CI: 18.62, 20.22]. Fluctuating prevalence trends during the study period were determined, with Other HR-HPV as the only category with a positive trend (aAPC: 3.44% [95% CI: 0.89, 6.05]; $p < 0.01$). The age-specific infection rate of HR-HPV showed a bimodal distribution, with the younger women (18–24 years old) harboring the highest rate. A second peak was identified in the postmenopausal group of 65–69 years old. Relative to Pichincha, Imbabura exhibited 50% higher odds ratio of HPV 16 infection ($p < 0.001$), while Santo Domingo de los Tsáchilas had 27% and 30% lower odds of Overall HR-HPV and Other HR-HPV infections, respectively ($p < 0.01$).

Conclusions This study represents the largest-scale analysis on the epidemiology of HR-HPV in Ecuador. Given the high prevalence and positive trend of Other HR-HPV, our findings underscore the need for multivalent HPV vaccines.

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National screening strategies may consider screening of women over the age of 65 years when clinically indicated based on the patient history or physician judgment.

Clinical trial number Not applicable.

Keywords High-risk human papillomavirus, Prevalence, Genotype, Cervical cancer, Ecuador

Background

Human papillomavirus (HPV) is the name of a group of small, non-enveloped viruses in the family *Papillomaviridae*, and it stands as one of the most common infections of the reproductive tract across the globe [1]. It is assumed that over 90% of the HPV-infected individuals eventually clear the infection within two years [2]. To date, about 200 HPV types have been approved by the International Committee on Taxonomy of Viruses [3], which can be classified into low-risk HPV (LR-HPV) and high-risk HPV (HR-HPV), depending on their carcinogenic potential [4]. LR-HPV genotypes may cause benign lesions such as low-grade cervical cell abnormalities, anogenital warts, and recurrent papillomas of the upper aerodigestive tract. On the other hand, persistent infection with HR-HPV genotypes is strongly associated with high-grade cervical lesions, comprising 99.7% of cervical cancer cases [5]. HR-HPV can also cause cancers of the anogenital region—including the anus, cervix, penis, vagina, and vulva—as well as the oropharynx. [6]. A recent comprehensive study conducted in the USA found that HPV was responsible for the highest proportion of cancer cases and deaths attributable to any carcinogenic infection [7]. This underscores the necessity of studying this virus within specific populations to effectively observe and analyze the occurrence of HR-HPV and cervical cancer.

The 14 most clinically relevant HR-HPV genotypes are 16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51, 56, 66, and 68. Specifically, HPV 16 and 18 account for the most cervical cancers, spanning from 67.2% in Africa up to 76.6% in Oceania [8]. Furthermore, these two genotypes are linked to more than 90% of noncervical cancers caused by HPV [9]. Along with HPV 16 and HPV 18, HPV 45 exhibits a predominant contribution in the pathogenesis of adenocarcinoma [10]. Genotypes 16 and 31 carried the highest risk for cervical intraepithelial neoplasia grade 2 or higher (≥CIN2) (11.6% and 12.1%, respectively) and for grade 3 or higher (≥CIN3) (8.1% and 7.5%, respectively) [11].

Although cervical cancer is a highly preventable disease, it remains one of the most prevalent cancers worldwide. According to the World Health Organization (WHO), the incidence and mortality rate due to this disease were higher in 2022 compared to 2018 (660,000 versus 570,000 new cases and 350,000 versus 311,000 deaths) [12, 13]. Cervical cancer is a leading cause of cancer-related morbidity and mortality among women living in low-income countries, where 90% of deaths from this disease occur [14, 15]. According to GLOBOCAN 2022, cervical cancer ranked fifth among new cancers in Ecuador (5.8%), which was higher than the global ranking of eighth (3.3%) [16, 17]. During the same period, bordering countries Peru and Colombia exhibited a rate of 6.6% and 3.9%, respectively [18, 19]. On the other hand, the significant lower rates in developed countries such as Germany (0.75%) [20], USA (0.59%) [21], and Netherlands (0.57%) [22], unveil a star contrast in public health management compared to some developing nations. Regarding the presence of carcinogenic genotypes HPV 16 and HPV 18 in Ecuadorian women with cytological abnormalities, reported prevalence based on studies conducted between 2008 and 2016 was 34.5% in low-grade cervical lesions, 37.6% in high-grade cervical lesions, and 37.9% in cervical cancer [23].

Changes in prevalence as well as variability of specific genotypes of HPV is well-documented among different geographical regions and populations, highlighting the role of localized epidemiological research to accurately evaluate the disease burden and support evidence-based policymaking [24, 25]. Some studies have aimed to understand the presence of this virus in Ecuador; however, they remain scarce and are often limited by modest sample sizes. Moreover, there is an insufficient and heterogeneous epidemiological view because most of the available data has been generated in the South or coastal region of the country [26–30]. Our study intends to contribute to a comprehensive epidemiological understanding of HPV in the north-central region by presenting large-scale data collected over eight years from women attending routine check-ups or outpatient appointments.

Evidence supports that nucleic acids screening of HPV using clinically validated tests on cervical cell samples has better accuracy and reproducibility than cervical cytology, as well as a greater sensitivity in detecting ≥CIN2 and ≥CIN3, ultimately diminishing the incidence and mortality due to cervical cancer [31]. In addition, the implementation of HPV screening has allowed clinicians to recognize and stratify risk before the manifestation of cellular abnormalities [32]. Hence, the transition of primary cervical cancer screening from cytology to HPV-based tests is an increasingly occurring event around the globe [33]. Depending on the specialist and patient,

HPV/Pap co-test using the same sample could be the preferred option as complementary screening.

The cobas® 4800 HPV Test is one of the FDA-approved HPV DNA tests that fully match all requirements for In Vitro Diagnostic (IVD) use in cervical cancer screening [33]. The process can be split into two stages: total DNA extraction and real-time polymerase chain reaction (PCR) targeting the polymorphic L1 region of 14 HR-HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) as well as the human β-globin gene as internal control.

Ecuador is a developing South American country comprising 24 provinces. According to the most recent population census of 2022, with a total population of 16,938,986 people, 51.28% are women [34]. Imbabura, Pichincha, Santo Domingo de los Tsáchilas, and Tungurahua are provinces located in the north-central region and are home to 2.80%, 18.41%, 2.91%, and 3.37% of the Ecuadorian female population, respectively. To our knowledge, large-scale research on the epidemiology of HPV covering a diverse age range from youth to elderly adults is limited and outdated. The objective of this study was to determine the prevalence and genotype distribution of HR-HPV among women of the north-central region of Ecuador between 2015 and 2022, using the cobas® 4800 HPV Test.

Material and methods

Study population

A retrospective study was performed using data from women who underwent HR-HPV genotyping, either through mobile health units as part of cervical cancer prevention campaigns or at gynecological outpatient clinics at Hospital Oncológico SOLCA–Núcleo de Quito and Hospital Oncológico SOLCA–Tungurahua, located in the four aforementioned provinces, from January 2015 to August 2022. Accordingly, no sample size calculation was conducted. Inclusion criteria were: 1) women aged 18 years or older; 2) no sexual intercourse in the past 48 hours; 3) no suppositories, creams, or vaginal douches in the past 48 hours; 4) no coursing a menstrual period. Exclusion criteria was pregnancy.

Sample collection and HR-HPV genotyping

Procedures were conducted using the cobas® 4800 HPV System, following rigorous adherence to the instructions provided by the manufacturer. Cervical specimens were collected by gynecological practitioners using a Cervex-Brush® (Rovers Medical Devices BV, Oss, The Netherlands) which was placed into a Roche cell collection medium (Roche Diagnostics, Mannheim, Germany), and stored at 22 °C for up to 15 days. Before each run, samples were vortexed and big clumps removed given the risk of pipetting obstruction during sample preparation using the cobas x 480. The plate containing the extracted DNA and other reagents for amplification and detection was loaded into the cobas z 480. Importantly, while HPV 16 and HPV 18 are detected individually, the other 12 HR-HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) cannot be distinguished because their probes have the same fluorescent dye.

Statistical analysis

The first HR-HPV genotyping result from each woman was considered to guarantee that participants were included only once in the study. All data processing and statistical analyses were carried out using the R Statistical Software (v. 4.4.0, R Core Team 2024) [35]. Normality distribution in the continuous variable age was assessed using the Lilliefors test, Anderson-Darling test, and Q-Q plot. Prevalence of HR-HPV and corresponding 95% confidence intervals (CI) were calculated relative to years, age groups (18–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59, 60–64, 65–69, ≥70 years), provinces, and coinfection proportions. The age differences between participants with positive and negative results were explored using an independent Wilcoxon rank-sum test. Pearson's chi-square test was applied to evaluate the difference among prevalence rates of HR-HPV. A single infection was defined as the infection caused by one HPV genotype, while a coinfection involved the infection of more than one HPV genotype.

Using Poisson regression models with a log link function, adjusted annual percentage changes (aAPCs) and their 95% CI were calculated to summarize trends in HR-HPV infections during the study period. A multivariable binary logistic regression analysis was conducted to examine the association between HR-HPV infection status and the explanatory variables age group, province of residence, and year of study period. Adjusted odd ratios and their 95% CI were calculated to quantify the strength of associations. Multiple correspondence analysis (MCA) was applied to explore potential associations between infections by HPV 16, HPV 18, and Other HR-HPV. Province was incorporated as supplementary variable to allow for complementary interpretation of the resulting profiles. Coinfection proportions were calculated as the number of individuals with coinfections divided by the number of individuals infected with at least one genotype. A two-sided p-value of less than 0.05 was considered significant.

Results

HR-HPV prevalence

A total of 9,524 women aged 18 to 88 years (median: 42, [IQR: 34–50]) were examined. The median age of participants with a positive result was significantly lower than those with a negative result, in the eight years (Fig. 1,

Fig. 1 Violin plots represent the age distribution of participants who tested negative and positive to HR-HPV, by study year. Medians are marked by white dots and interquartile ranges are represented by boxplots. Significant *p*-values with star notation: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

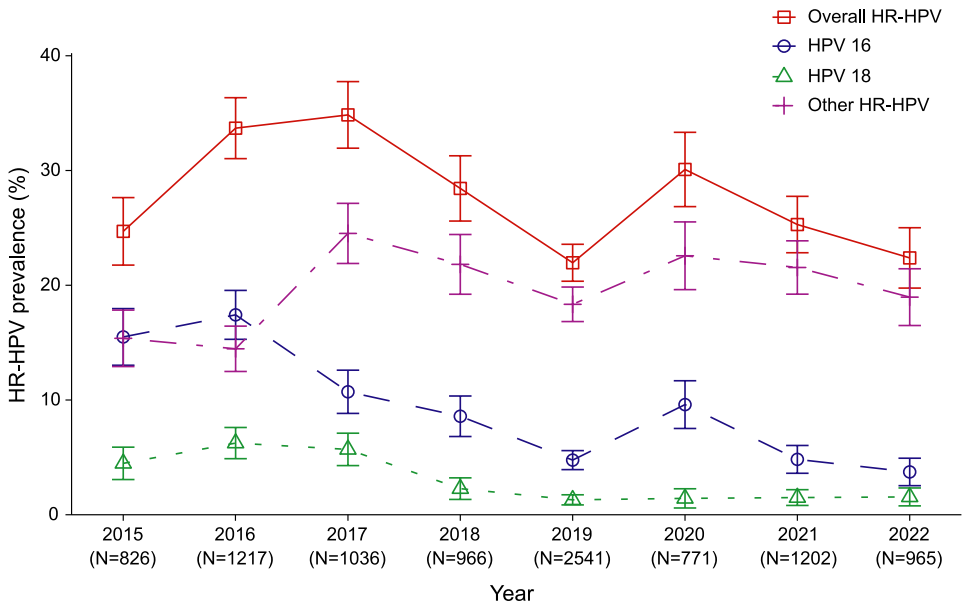

Fig. 2 Prevalence trends of HR-HPV from 2015 to 2022. HR-HPV: high-risk human papillomavirus; overall HR-HPV: any of the 14 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68); other HR-HPV: any of the 12 HR-HPV genotypes not individually differentiated (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Error bars represent 95% confidence intervals

Supplementary Table S1). The overall prevalence of any 14 HR-HPV genotypes was 26.87% [95% CI: 25.98, 27.77]. The infection rates of genotypes HPV 16, HPV 18, and Other HR-HPV group were 8.64% [95% CI: 8.08, 9.22], 2.85% [95% CI: 2.52, 3.20], and 19.41% [95% CI: 18.62, 20.22], respectively.

The overall prevalence of HR-HPV infections was 24.70% [95% CI: 21.76, 27.64] in 2015, reached 34.85% [95% CI: 31.94, 37.75] in 2017, decreased to 21.96% [95% CI: 20.35, 23.57] in 2019, and then rose again up to 30.09% [95% CI: 26.85, 33.33] in 2020, before declining to 22.38% [95% CI: 19.75, 25.01] in 2022 (Fig. 2, Supplementary Table S2). Similar fluctuations were observed for HPV 16 infections, peaking in 2016 at 17.42% [95% CI: 15.29, 19.55] and reaching 3.73% [95% CI: 2.53, 4.93] in 2022. On the other hand, the trend of infections with

Other HR-HPV started at 15.38% [95% CI: 12.92, 17.84], then peaked in 2017 at 24.52% [95% CI: 21.90, 27.14], and decreased to 18.96% [95% CI: 16.49, 21.44] in 2022. Compared to HPV 16 and the group Other HR-HPV, HPV 18 prevalence was the lowest during the eight years, respectively, and exhibited almost a linear trend since 2019, when it decreased to 1.30% [95% CI: 0.86, 1.74]. For each category, the HR-HPV rates were significantly different ($p < 0.001$).

During the course of the study, only the infection with Other HR-HPV showed a positive trend (aAPC for Other HR-HPV: 3.44% [95% CI: 0.89, 6.05], $p < 0.01$), while the aAPC for overall HR-HPV infection was −4.19% [95% CI: −6.23, −2.11], $p < 0.001$; for HPV 16 infection, −21.30% [95% CI: −24.48, −17.99], $p < 0.001$; and for HPV 18 infection, −22.03% [95% CI: −27.42, −16.24], $p < 0.001$.

Participants were categorized into 11 age groups, and their distributions are presented in Supplementary Table S3. Across these categories, the overall prevalence of HR-HPV infections varied significantly ($p < 0.001$), displaying a "U-shaped" pattern (Fig. 3). The 18–24 years age group exhibited the highest prevalence (48.48% [95% CI: 43.56, 53.41]), and was used as the reference in multiple logistic regression. Regarding infections by genotypes, significant differences were found for HPV 16 ($p < 0.001$), HPV 18 ($p < 0.05$), and Other HR-HPV ($p < 0.001$). The infections caused by Other HR-HPV exhibited their maximum and minimum rates in women aged 18–24 years (39.65% [95% CI: 34.83, 44.46]) and 55–59 years (13.05% [95% CI: 10.56, 15.54]), respectively. For HPV 16 infections, whereas the highest rate was found in women aged 25–29 years (14.32% [95% CI: 11.95, 16.69]), the lowest rate was determined in the group 40–44 years old (5.76% [95% CI: 4.59, 6.93]). The differences among infection rates of HPV 18 were small but significant. Similar to the other trends, a peak of infection was revealed in the group 65–69 years (Fig. 3).

HR-HPV distribution by geography

Supplementary Table S4 provides a summary of the epidemiological distribution of HR-HPV in the north-central region of Ecuador during the eight-year study period. The overall crude infection rates of HR-HPV across the studied provinces varied significantly ($p < 0.01$), with the highest and lowest crude prevalence rates found in women from Pichincha (28.00% [95% CI: 26.78, 29.22]) and Santo Domingo de los Tsáchilas (21.81% [95% CI: 18.94, 24.69]), respectively. Significant differences in positive rates of HPV 16 ($p < 0.001$) and HPV 18 ($p < 0.001$) were determined. Notably, infections with Other HR-HPV did not differ significantly ($p = 0.053$).

HR-HPV genotypes association and infection type

An MCA was conducted to graphically explore possible relationships between the studied HR-HPV genotypes. However, none of the three HR-HPV categories participated in the formation of association profiles (Fig. 4A). This aligns with the finding that coinfections proportions

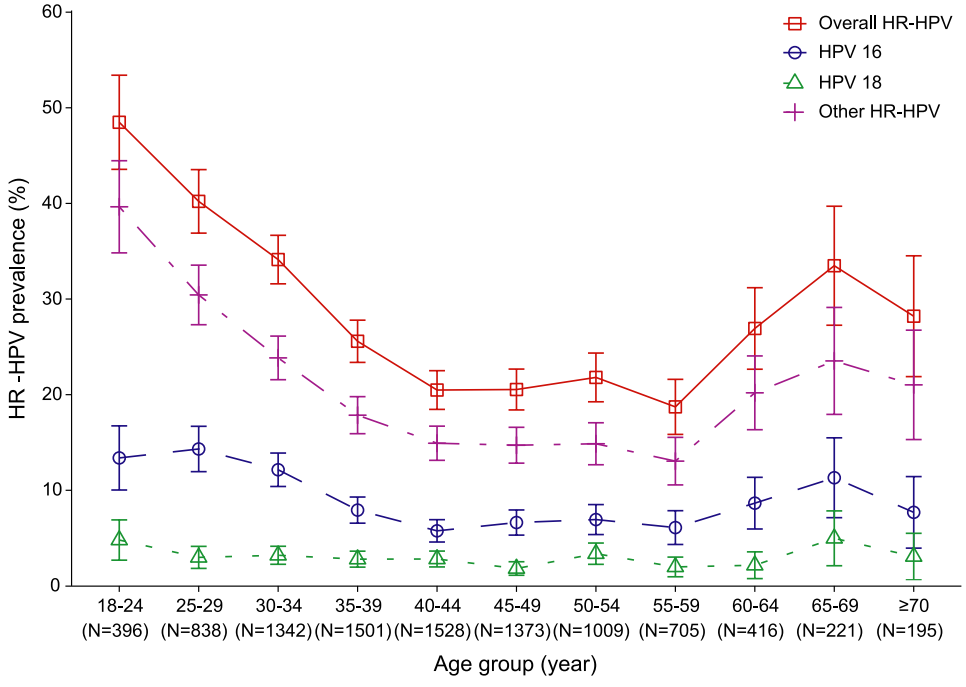

Fig. 3 Prevalence of HR-HPV across age groups. HR-HPV: high-risk human papillomavirus; overall HR-HPV: any of the 14 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68); other HR-HPV: any of the 12 HR-HPV genotypes not individually differentiated (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Error bars represent 95% confidence intervals

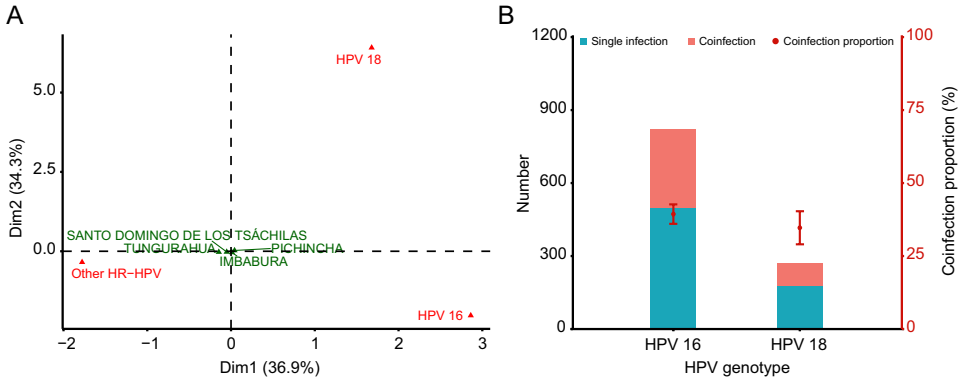

Fig. 4 (**A**) multiple correspondence analysis of the studied HR-HPV genotypes, with province as illustrative variable. The percentage on each axis refers to the variance explained by each dimension. (**B**) distribution of HPV 16 and HPV 18 by infection type. Error bars represent 95% confidence intervals in coinfection proportion

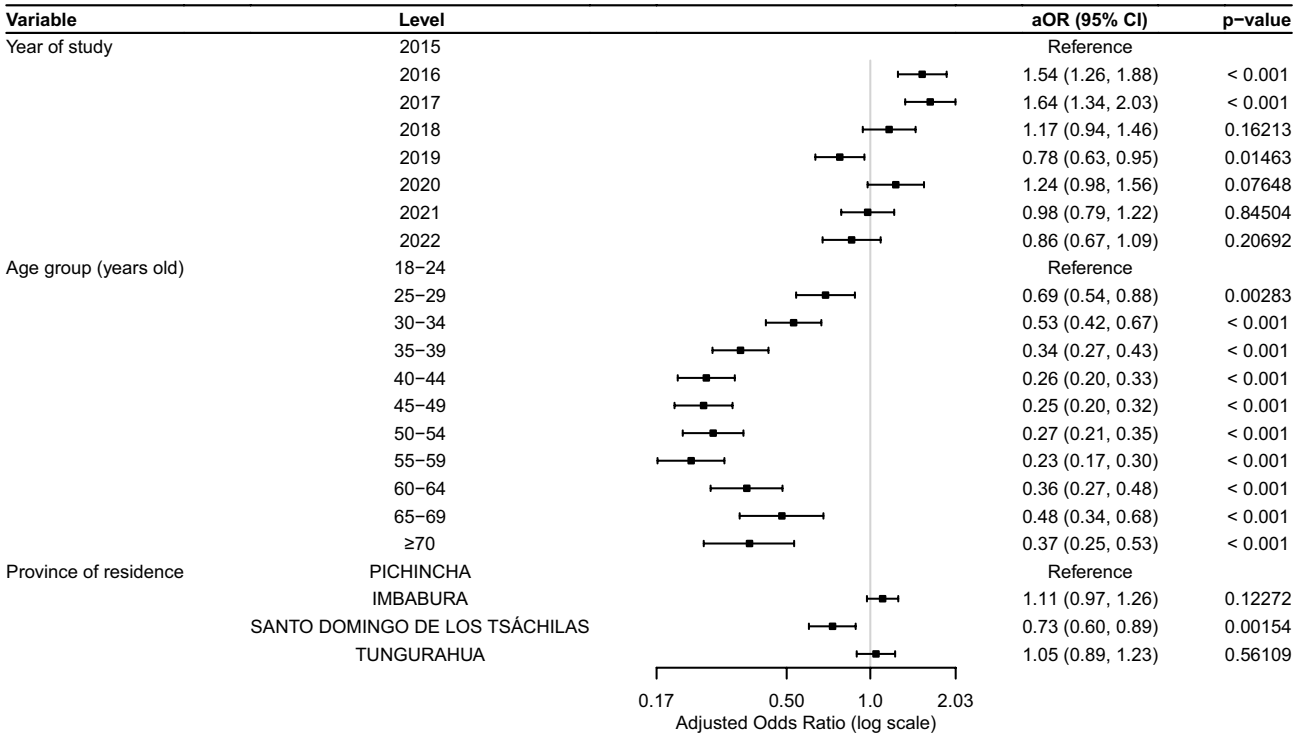

Fig. 5 Forest plot of multivariable logistic regression analysis of factors associated to overall HR-HPV infection in women form the north-central region of Ecuador

involving HPV 16 (39.37% [95% CI: 36.03, 42.71]) and HPV18 (34.69% [95% CI: 29.02, 40.35]) were not statistically different ($p = 0.1923$) (Fig. 4B). In general, whereas single infections caused by these two genotypes exhibited a frequency of 7.10% [95% CI: 6.58, 7.61], the rate of coinfections involving at least two of the categories HPV 16, HPV18, and Other HR-HPV was 3.81% [95% CI: 3.43, 4.20]. Additionally, no province was linked to any HR-HPV (Fig. 4A).

Factors associated with HR-HPV infection

To further evaluate whether the observed differences in HR-HPV prevalence by year of study, age group, and province of residence remained significant after adjusting for potential confounders, a multivariable logistic regression including these variables was performed. The three studied variables were identified as independent factors significantly associated with overall HR-HPV infection (Fig. 5). This analysis revealed that women in the youngest age group had significantly higher aORs of overall HR-HPV infection compared to the other age groups.

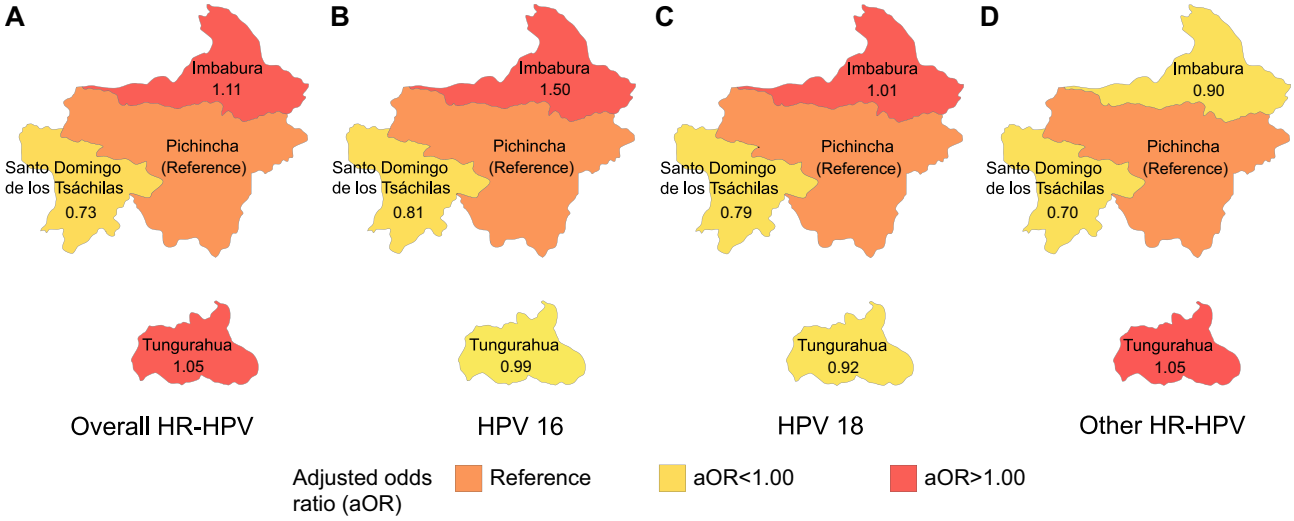

Fig. 6 Map of the studied provinces, color-coded according to adjusted odds ratios for HR-HPV infection among women. Pichincha was used as reference

Regarding geographic variations, Fig. 6 depicts aORs for HR-HPV infections across the provinces where participants resided. When compared to Pichincha, no statistically significant differences were observed in the aORs for overall HR-HPV as well as Other HR-HPV infections for women from Imbabura and Tungurahua. In contrast, women from Santo Domingo de los Tsáchilas exhibited significantly lower aOR than those from Pichincha (Fig. 5, Supplementary Figure S1). Although the crude OR suggested a lower likelihood of HPV 16 infections in Imbabura compared to Pichincha (crude OR = 0.83 [95% CI: 0.70, 0.99], $p < 0.05$) (Supplementary Table 4), the adjusted OR was significantly higher (aOR = 1.50 [95% CI: 1.22, 1.84], $p < 0.001$) (Fig. 6B, Supplementary Figure S2). This discrepancy suggests that the observed crude prevalence may be confounded by covariates. As for HPV 18 infections, there were no significant differences in the aORs between Pichincha and the other three provinces (Supplementary Figure S3).

Discussion

From an epidemiological perspective, examining the prevalence and temporal trends of risk factors is part of a comprehensive plan to design strategies aimed at diminishing cervical cancer mortality. The primary objective of this retrospective study was to report the prevalence of HR-HPV among 9,524 women of the north-central region of Ecuador, based on the analyses carried out in the Molecular Biology Laboratory at Hospital SOLCA-Núcleo de Quito between 2015 and 2022.

The prevalence of HPV differs noticeably across global geographic areas [8]. For instance, the HPV and HR-HPV infection rates among 302 women aged ≥18 years attending to an oncological hospital in Santa Elena (a southwest province of Ecuador), between September 2005 and January 2006, were 24.17% and 14.57%, respectively [36]. In Guayas (a border province to Santa Elena) the prevalence of HPV and HR-HPV among 1,581 females aged 20 to 70 years were 43.58% and 25.23%, respectively [29]. In Azuay (a south-central province of Ecuador), a randomly selected sample from 500 women aged 17 to 50 years revealed a HR-HPV prevalence of 20.80% [28]. The overall prevalence of HR-HPV presented in our study (26.87% [95% CI: 25.98, 27.77]) is higher than those reported for Santa Elena and Azuay, but similar to Guayas. This prevalence is also markedly lower than the HR-HPV infection rate found among 291 women aged 14 to 75 years from two rural communities of a northwestern province of Ecuador (54.64%) [37]. A cross-sectional study analyzed endocervical samples from 396 sexually active indigenous women (median age: 31 years, [IQR: 24, 37]) from three provinces in the southern region of Ecuador (Cañar, Loja, and Morona Santiago), finding a HR-HPV prevalence of 23.48% [38]. The infection rate reported in our study with a sample including both sexually and non-sexually active women from rural and urban areas was slightly higher.

The presence of HPV 16 and HPV 18 have been explored worldwide. Compared to the global statistics, the infection rate of HPV16/18 in South America is higher among women with normal cytology (5.8% [95% CI: 5.4, 6.3]), low-grade cervical lesions (35.6% [95% CI: 33.6, 37.6]), and high-grade cervical lesions (56.3% [95% CI: 54.4, 58.2]) [8]. In addition, HPV 16 is the most prevalent high-risk genotype among females around the world [8]. The infection rates of HPV 16 and HPV 18 determined in our analysis are lower compared to the rates reported in 2018 from a sample of 400 women aged 10–70 years from Guayaquil (capital of Guayas, Ecuador) (HPV 16: 13.92%; HPV 18: 3.16%) [30]. The same research group, using a similar methodology and larger

sample size collected from 2008 to 2013, found the positive rate of HPV 18 to be 3.16% [29]. This low sustained infection pattern observed in Guayas resembled the HPV 18 prevalence trend determined in the north-central region of Ecuador since 2019, which implies that this infection persisted steadily at a sustained rate. Similar to our data, the studies conducted by Cabrera V et al. [28] and Ortiz Segarra et al. [38] found that the prevalence of HPV 16 was higher than that corresponding to HPV 18.

Fluctuating trends were observed for infections with HPV 16 and Other HR-HPV from 2017 to 2022, and the respective local maximums were lower or comparable to previous peaks. These oscillating patterns over time may indicate that the studied population did not benefit from the protective effects of the HPV vaccines, a consequence of their introduction into Ecuador in 2014. Despite general patient absenteeism in ambulatory visits due to the COVID-19 pandemic, as reported by gynecologists, the number of HR-HPV genotyping tests in 2021 and 2022 was not significantly different compared to previous years ($p = 1.0$).

The progressive decline of HPV 16 and HPV 18 prevalence rates since 2016 might be attributed to both the decrease of symptomatic gynecological outpatients and the increase of asymptomatic women enrolling in external health screening programs organized in urban and rural areas. In fact, after the HPV genotyping test was implemented in 2014, the marketing department of Hospital Oncológico SOLCA-Núcleo de Quito has gradually promoted the HPV test and HPV/Pap co-test through outreach campaigns in private and public institutions. Moreover, the increased adoption of the HPV-based test as the primary method for cervical cancer screening among specialists has led to a higher proportion of asymptomatic participants, although this might be linked to the rise of Other HR-HPV (Fig. 2).

The infection rate of HR-HPV also varied across age groups (Fig. 3, Supplementary Table S3). Unlike regions such as Oceania, the infection pattern is similar in the Americas, Africa, Asia, and Europe. The highest prevalence of HPV is reached in youth after the onset of sexual activity (< 25 years old) [8]. After decreasing until the age group 55–64 (45–54 in Africa) years, it increases slightly. The prevalence patterns of overall HR-HPV as well as HPV 16 and Other HR-HPV observed in our study mirrors bimodal distributions reported in other regions, such as China [39]. Interestingly, while the age group younger than 25 years old presented the highest prevalence rates of overall HR-HPV and Other HR-HPV, infections with HPV 16 showed similar prevalence rates across the age groups of 18–24, 25–29, and 30–34 years. Compared to other age groups, the high prevalence rates observed in young groups might be attributed to the exposure after sexual activity initiation. Indeed, two independent studies found that the risk of testing positive for cervical HPV DNA after first intercourse with a first male sex partner increased from 28.5% in the first year to almost 50% by three years [40, 41].

A second peak occurring in postmenopausal women and older men is frequently reported for both HR-HPV and LR-HPV [39, 42, 43], although its prominence and age group varies according to the studied population. Specifically, the increased rate in postmenopausal women may be due to: i) reactivation of latent infections exploiting the host immune senescence; ii) newly acquired infections due to changes in sexual behaviors; or iii) a cohort effect reflecting in elderly generations significant exposures experienced throughout lifetime [44]. Given the large dataset and cohort comprising participants from diverse provinces and urban/rural background, the second peak in our study may be associated to HPV reactivation and/or new infections.

Age range, test type, and intervals for cervical cancer screening vary worldwide. Most of these international guidelines including the American Cancer Society (ACS, 2020), France, Italy, and Australia recommend starting at age of 25 years [45]. In Ecuador, similar to the US Preventive Services Task Force (USPSTF, 2018), the strategy promotes starting the screening at 21 years with Pap test every three years until 65 years; those aged 30–65 years can follow either HPV or HPV/Pap co-test every five years [46]. According to the National Survey on Health and Nutrition carried out in Ecuador in 2018, 35.4% and 65.4% of women in the age group 20–24 years and 25–29 years, respectively, received screening with Pap smear, while 80–90% of women above 30 years old underwent this test [47]. Based on the STEPS Ecuador 2018 Survey, only 67.7% of women between 30 and 49 years old received a Pap or HPV test [48]. Taking together and considering the high prevalence in young groups, cervical screening should be monitored to guarantee the achievement of the screening pillar in the 90–70-90 program of the WHO, which proposes to test 70% of women by 35 and again by 45 years using a high-performance test similar to or better than HPV test. Regarding the end of the screening, some of the international guidelines including the ACS and USPSTF make explicit that it can be stopped at 65 years if previous tests were consistently normal and not at high risk of cervical cancer [45]. Meanwhile, the Australian Program targets women until 74 years old [45]. Given the HR-HPV prevalence peak in the postmenopausal group (65–69 years old) found in the present report, we argue that cervical cancer screening of women over 65 years old in this region might be warranted based on the patient history or clinical judgment.

Our study cohort consisted of female participants from four provinces located in the north-central region of Ecuador. Research on HPV infections in women

presenting normal and abnormal cytology is limited in this area of Ecuador. The overall prevalence of HR-HPV determined in women from Santo Domingo de los Tsáchilas (21.81% [95% CI: 18.94–24.69]) was lower to that found recently by an independent study analyzing cervical samples from pregnant women of this province (24.1%) [49]. Participants from Imbabura had 50% higher odds of HPV 16 infection than those from Pichincha (Supplementary Fig. 2). In contrast, the aORs for women from the other provinces did not indicate statistically significant differences in the association of HPV 18 infection compared to those from Pichincha. It is worth nothing that residual confounding cannot be ruled out and future research should incorporate additional factors—such as sexual behavior, healthcare accessibility, screening practices, among others—that may contribute to the observed regional disparities. This approach could help discern whether the adjusted odds reflect true epidemiological differences or are influenced by unmeasured characteristics.

The association between HPV coinfections and occurrence or progression of cervical lesions needs further research [50, 51]. No association profiles were observed among the studied HR-HPV categories. Similarly, Del Río-Ospina et al. [52] did not find HPV 18 to be associated with HPV 16, HPV 31, HPV 33, HPV 45 or HPV 58. The coinfection proportions of HPV 16 and HPV 18 reported in our study cohort were not statistically different. Compared to a study carried out in China from 2014 to 2018 [53], the HPV 16 coinfection ratio was similar (HPV 16: 39.37% versus 40.23%), and the HPV 18 coinfection ratio was lower (HPV 18: 34.69% versus 46.67%). Given the clinical significance of HR-HPV other than 16 and 18, the Hospital Oncológico SOLCA-Núcleo de Quito switched to extended genotyping since November 2022. Therefore, future research can explore more detailed distributions and associations of HPV genotypes.

The prevalence of HPV in Latin America was estimated to be around twice higher the worldwide average [54]. HPV immunization programs have been implemented in this region, with Panama as the pioneer starting in 2008 [55]. The Ministry of Public Health (MSP) of Ecuador started the HPV vaccination program in 2014 for women aged 9-14 years, with 800,000 being immunized up to early 2017; and the program was expanded to include boys aged 9 years in 2024, estimating to immunize 595,000 children [56, 57]. Therefore, the impact of immunization may be further explored through epidemiological surveillance of HPV prevalence, as well as the incidence and trends of cervical precancerous lesions and HPV-associated cancers in both women and men, comparing vaccinated and unvaccinated groups.

Besides the bivalent and quadrivalent vaccines, Ecuador also included the Gardasil 9-valent vaccine [58], which is effective not only against HPV 16, HPV 18, the low-risk HPV 6 and HPV 11, but also five additional HR-HPV (HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58) responsible for 20% of cervical cancers [59]. Given the high prevalence of Other HR-HPV reported in this study, these vaccination efforts will substantially contribute to controlling and reducing the prevalence of several HPV genotypes, thereby decreasing not only the cervical cancer incidence and mortality, but also that of other HPV-associated cancers. However, it is imperative to strengthen the screening and treatment/management measures to achieve by 2030 the triple-intervention approach established by the WHO in 2020 [60]. This strategy, aimed at eliminating cervical cancer within the next century, comprises: i) 90% of girls by age 15 years fully vaccinated with HPV vaccine, ii) 70% of women screened with a high-performance test by age 35 years and repeat the test by age 45 years, iii) 90% of women with cervical disease get treatment or management.

To the best of our knowledge, our research comprises the largest-scale study in terms of population and geographic area, over a timeline of eight years in Ecuador, providing a better understanding on the prevalence and distribution of HR-HPV in the north-central region. In addition, similar to other studies, we used a real-time PCR IVD kit, which followed the standard clinical validation and verification. The results and interpretations of this study should be considered in the framework of the following limitations. First, all participants either took part in cervical cancer prevention campaigns or attended gynecological outpatient services at Hospital Oncológico SOLCA–Núcleo de Quito and Hospital Oncológico SOLCA–Tungurahua. Second, the Pap test results from samples analyzed in our study varied from normal up to high-grade squamous intraepithelial lesions; and during the first years, a large segment of the outpatients possessed gynecological disorders. Third, the cost of the test may restrict access for low-income women. Therefore, the findings may not represent all women from urban/rural areas in the studied provinces.

Future directions may include expanding the analysis to understudied regions, such as the Amazon and other low-resource settings, as well as incorporating additional sociodemographic factors, healthcare access, and ethnic groups. Furthermore, including results of extended genotyping along with Pap smear—often preferred by physicians for risk stratification—will provide a more detailed picture of cervical disease status.

In conclusion, this study presents the prevalence and genotype distribution of HR-HPV in women of the north-central region of Ecuador, between 2015 and 2022. The age-group prevalence rates of overall HR-HPV as well as HPV 16 and Other HR-HPV showed clear bimodal distributions, with one peak at young women (18–24 years

for overall HR-HPV and Other HR-HPV; 25–29 years for HPV 16) and a second peak at the postmenopausal women group 65–69 years. These findings are significant by contributing to cervical cancer screening strategies of elderly women, in addition to supporting the vaccination of young women years before sexual activity initiation. Since the HR-HPV prevalence was characterized by fluctuating behavior during the eight-year study period, it warrants monitoring. The infection rate of genotype 16 was higher than genotype 18, as reported in other regions around the globe.

Abbreviations

CIN	Cervical intraepithelial neoplasia grade
HPV	Human papillomavirus
HR-HPV	High-risk human papillomavirus
IQR	Interquartile range
IVD	In vitro diagnostic
LR-HPV	Low-risk human papillomavirus
PCR	Polymerase chain reaction
95% CI	95% confidence interval

Supplementary Information

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Supplementary Material 1

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Author contributions
FM-Y: Conceptualization, methodology, data curation, formal analysis, writing-original draft, writing-review and editing. DLP.: Methodology, data curation, writing-review and editing. OR: Project administration, methodology, data curation. BL: Project administration, resources. MFGA: Methodology, writing-review and editing. TR: Methodology, writing-review and editing. SS: Methodology, data curation. MDS: Project administration, resources. All authors reviewed the manuscript.

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Data availability
The data that supports the findings of this study are available from the Research Department of Hospital SOLCA-Núcleo de Quito upon request and approval of Hospital SOLCA-Núcleo de Quito and Hospital SOLCA-Tungurahua.

Declarations

Ethical approval
Ethical guidelines for research as defined by the Declaration of Helsinki were considered and the research was approved by the Human Research Ethics Committee of Hospital Oncológico SOLCA – Núcleo de Quito (assigned code: OBS.24.226). Anonymized raw data was retrieved from REDCap via the application interface [61].

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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