Journal of Infection 90 (2025) 106397

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Viruses and Viral Diseases

Ureaplasma parvum serovar 6 may be a novel element in the progression of HPV infection to CIN: A cross-sectional study of 7058 women

Yingxuan Zhang [1], Rongdan Chen [1], Zuyi Zhou [1], Wei Qing, Cancan Qi, Jinxia Ou, Hongwei Zhou [*], Muxuan Chen [*], CALM 2004 Study Group [2]

Microbiome Medicine Centre, Department of Laboratory Medicine, ZhuJiang Hospital, Southern Medical University, Guangzhou, Guangdong, China

ARTICLE INFO

Article history:
Accepted 22 December 2024
Available online 26 December 2024

Keywords:
Ureaplasma parvum serovar
Human papillomavirus
Cervical intraepithelial neoplasia
Pathogenic variations

SUMMARY

Background: *Ureaplasma parvum* (*U. parvum*) is generally regarded as innocuous, and studies focusing on variations in pathogenicity among *U. parvum* serovars are inadequate. We elucidated the variations in the pathogenicity of *U. parvum* serovars in promoting human papillomavirus (HPV) infection and cervical intraepithelial neoplasia (CIN).

Methods: This cross-sectional study used baseline data from a Chinese multicenter prospective cohort of women of childbearing age undergoing routine cervical cancer screening. We employed multivariate logistic regression analysis to estimate the pathogenic effects of specific *U. parvum* serovars on HPV infection and CIN. Causal mediation analysis was performed to ascertain the direct effects of specific *U. parvum* serovars on CIN and their indirect implications via HPV infection.

Findings: The final data analysis encompassed 7058 participants. Upon adjusting for confounding factors, a positive association was observed between *U. parvum* serovars 1, 3, and 6 and HPV infection (OR 1.53, 95%CI 1.15–2.03; OR 1.31, 95%CI 1.06–1.64; OR 2.34, 95%CI 1.90–2.87); however, only participants with *U. parvum* serovar 6 showed an increased risk of CIN (OR 1.90, 95%CI 1.19–3.02). No substantial correlation was observed between *U. parvum* serovar 14 and HPV or CIN incidence. HPV infection potentially mediates the influence of *U. parvum* serovar 6 on CIN, with a mediation proportion of 76.66%.

Interpretations: Our findings suggest that different *U. parvum* serovars vary in pathogenicity regarding HPV and CIN. Early detection of specific *U. parvum* serovars, such as *U. parvum* serovar 6, in HPV-infected individuals may enable early intervention therapies and reduce the risk of CIN development.

Introduction

Ureaplasma spp. has been formally classified into 2 distinct species, *Ureaplasma urealyticum* (*U. urealyticum*) and *Ureaplasma parvum* (*U. parvum*).[1] Both species have been associated with human infections.[2,3] *Ureaplasma* spp. have been associated with a variety of adverse outcomes, including spontaneous abortion and premature birth.[4] Given the high detection rate of *U. parvum* in asymptomatic women, it may be a regular component of the female genital tract's flora.[1,5–7] The European guideline editorial board of sexually transmitted infections (STIs) issued a statement suggesting that routine testing and treatment for *U. parvum* may not provide significant benefits.[8] *U. parvum* can be further divided into four serovars, specifically serovars 1, 3, 6, and 14, based on its genetic variations.[9,10] Initial findings suggest *U. parvum* may be largely harmless; however, it is crucial to understand that the pathogenicity of its four identified serovars may differ.

High-risk genotypes of human papillomavirus (HPV) are known to be the primary cause of cervical cancer and its precursor lesions, cervical intraepithelial neoplasia (CIN).[11] However, studies investigating the connection between *U. parvum* and HPV infections have yielded inconclusive results, leaving the nature of their relationship ambiguous. Notably, these investigations failed to account for the confounding effects of other genitourinary pathogens and the pathogenic heterogeneity between *U. parvum* serovars.[12,13] Research evaluating the correlation between various serovars of *U. parvum*, HPV infection, and the incidence of CIN is currently insufficient.

In our study, we used the baseline data from the Chinese Association for cLinical Microbiome 2004 (CALM 2004) project, a

* Correspondence to: ZhuJiang Hospital, Southern Medical University, 253 Gongye Middle Avenue, Haizhu District, Guangzhou, Guangdong, 510280, China.
E-mail addresses: biodegradation@gmail.com (H. Zhou), muxuanchen@126.com (M. Chen).
[1] These authors contributed equally.
[2] The investigators of the CALM 2004 Study Group are completely listed in Table S1

https://doi.org/10.1016/j.jinf.2024.106397

multicenter prospective cohort study that comprised women in China who had undergone routine cervical cancer screening. Our primary objective was to investigate the correlation between varying serovars of *U. parvum*, HPV infection, and CIN.

Methods

Study population

For this cross-sectional study, we utilized the baseline data from the CALM 2004 project (2020.11–2022.10). CALM 2004, an ongoing prospective cohort study of 42 centers aimed at investigating the microecology of the reproductive tract in Chinese women of reproductive age and identifying key factors influencing the regression of HPV infection. The study design has been previously described.[14] Table S2 outlines the inclusion, exclusion, and elimination criteria for the CALM 2004 project. We recorded basic information and conducted genital tract pathogen tests on sexually experienced women aged 18–50 years, who were normally screened for cervical cancer at the outset of study.

Prior to initiation, the study was registered (https://clinicaltrials.gov, NCT04694495) and received approval from the ZhuJiang Hospital of Southern Medical University (NO.2020-KY-071–01) and all participating subcenters.

Sample collection

We collected two main types of samples - vaginal secretions and cervical exfoliated cells. A sterile disposable swab was used to gather vaginal secretion samples from the posterior fornix while individuals were in the lithotomy position. These samples were mainly used to evaluate Nugent and Donders scores. Cervical exfoliated cells were collected with a specialized cervical cytology brush (Hybribio, Guangdong, China) for the identification of HPV types, detection of various STIs, and ThinPrep Cytologic Tests. All samples were collected by qualified clinicians.

Ascertainment of exposures and outcomes

Detection of *U. parvum* serovars was performed using STIs detection kit (Hybribio, Guangdong, China), adhering to the manufacturer's instructions.[15,16] A specific multi-banded antigen (MBA) based nested polymerase chain reaction (PCR) for serotyping *U. parvum* and *U. urealyticum*. The specific probe was used to differentiate *U. parvum* serovar 1, 3, 6, or 14. Table S3 lists the primer and probe sequences for each serovar. Initially, 0.5–1.0 mL of cell preservation solution, containing cervical cells, was centrifuged at 7000 *g* for 5 min with the supernatant subsequently discarded. Subsequently, 0.5 mL of cell preservation solution was added, the cells were centrifuged at 7000 *g* for 1 min, and the supernatant was disposed of, ensuring minimal moisture residue. Each sample was subsequently treated with 50 μL of cell lysate, and the cell resuspension was violently agitated and boiled for 10 min. The supernatant was preserved after centrifugation at 7000 *g* for 10 min. A volume of 3.0 μL of the extracted DNA was employed as the template for the PCR amplification. DNA fragments from *U. parvum* were subsequently amplified by PCR. The amplified DNA was hybridized with *U. parvum* serovars using specific probes before the flow-through hybridization of amplification products with nylon membranes tagged with probes on a flow-through hybridization platform (Hybribio, Guangdong, China). The results were analyzed using a chemical color development technique.

HPV genotypes were identified using the HPV GenoArray diagnostic kit (Hybribio, Guangdong, China) in accordance with the manufacturer's instructions, as previously described.[17] The results were determined using a chemical color development technique. High-risk HPV infection was characterized by any infection with HPV 16, 18, 31, 33, 35, 39, 45, 51, 53, 56, 58, 59 or 68,[18] whereas low-risk HPV infection corresponded to any infection with HPV 63, 66, 6, 11, 42, 43, 44 or CP8304.[19]

In adherence to the American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines,[20] a colposcopy biopsy was recommended upon detection of either HPV16 or 18. Additional cytology was conducted for the 12 other types of high-risk HPV genotypes. If the findings showed atypical squamous cells of undetermined significance (ASC-US) or higher degree, an additional referral for a colposcopy biopsy was issued. The diagnosis of CIN was based on the results from the colposcopy histopathological biopsy.

Assessment of covariates

The baseline questionnaire collected self-reported data on socioeconomic variables such as age, body mass index (BMI), ethnicity, educational level, methods of contraception, number of sexual partners, and counts of pregnancies and deliveries. Ethnicity was classified into either Han Chinese or other minority groups. Educational level was segmented into below undergraduate level, and undergraduate level or above. The four categories of contraception included none, intrauterine device, oral contraceptive, and condom use. The number of sexual partners was divided into two categories: 1 or ≥2. Past pregnancies and births were categorized into four distinct groups, which included 0 (never had a child or birth), 1 (one pregnancy or birth), 2 (two pregnancies or births), or ≥3 (three or more pregnancies or births).

In addition to the above, screenings for other reproductive tract infections were conducted. Test for *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *U. urealyticum*, *Mycoplasma hominis* (*M. hominis*), Mycoplasma genitalium (*M. genitalium*), Herpes simplex virus type 2 (HSV-2), and *Chlamydia trachomatis* (*C. trachomatis*) was performed using similar methods as those for the *U. parvum* serovars, employing the STIs detection kit (Hybribio, Guangdong, China). *Trichomonas vaginalis* (*T. vaginalis*) infections were diagnosed by wet-mount microscopy.[21] Fungi were identified by the wet mount microscopy and culture method,[21] with positivity defined as detection by both methods. Bacterial vaginosis (BV) was diagnosed using Nugent Scores, a gram stain grading system developed by Nugent,[22] with a score of ≥7 defined as positive for BV.[23,24] We also evaluated and diagnosed of aerobic vaginitis (AV) using the Donders score,[25] with a Donders score of ≥3, indicating a positive result for AV.[26]

Statistical analyses

All data were analyzed with R (http://www.R-project.org; version 4.2.0) and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc. Boston, MA). We characterized continuous data using mean ± standard deviation and categorical data using frequencies and percentages. Chi-squared tests with two tails were implemented to compare proportions, whereas Student's t-tests analyzed continuous variables. We performed three logistic regression models to assess the relationship between *U. parvum* serovars, HPV infection, and CIN. In Model 1, no covariate adjustments were performed. Model 2 incorporated adjustments based on demographic factors such as age, BMI, and ethnicity. Consequently, Model 3 was adjusted for demographic factors, including Model 2 plus education level, methods of contraception, number of sexual partners, pregnancies and deliveries, and other genital pathogens that were found to be associated with HPV and CIN based on the univariate analysis ($P < 0.1$).

Additionally, for variables demonstrating significant associations with both HPV and CIN, a causal mediation analysis was conducted to ascertain the relationship between *U. parvum* serovars, HPV, and CIN.[27]

Several sensitivity analyses were conducted to evaluate the strength and reliability of our findings. To ensure that the relationship between *U. parvum* serovars, HPV and CIN was not confounded by other STIs, we repeated logistics regression analysis on primary results among participants who were STIs-free, excluding exposure and outcome. A multiple-imputation (MI) analysis, based on five replications and a chained equation approach method in the R analytical MI procedure,[28] was further evaluated to determine whether the inclusion of indicator variables for missing data introduced bias into our findings. Primary analyses were stratified by age (18–30, 30–40 and 40–50 years) and BMI (≤18.5, 18.5–23 and ≥23). A p-value of < 0.05 was used to determine statistical significance.

Results

Participant characteristic

Fig. 1 shows the study design. A total of 7072 women met the inclusion and exclusion criteria and were thus enrolled in the CALM 2004 study. Fourteen participants were disqualified owing to inadequate samples, leaving a cohort of 7058 women for final data analysis. Table 1 presents the baseline demographic information of the study. *U. parvum* serovar 1, 3, and 6 were identified in 560 (7.9%), 1242 (17.6%), and 1132 (16.0%) participants respectively. However, only 129 (1.8%) women tested positive for *U. parvum* serovar 14. Individuals who were *U. parvum* serovar 1 and 6-positive were typically older than those who tested negative. Women who used condoms as a contraceptive method were less likely to test positive for *U. parvum* serovars. Furthermore, nulliparous women exhibited a higher likelihood of detecting *U. parvum* serovars 3.

Furthermore, regarding the co-infection of *U. parvum* serovars with other genital tract pathogens (Table 2), *U. urealyticum* was less likely to co-infect with *U. parvum* serovars 3 and 6, whereas *M. hominis* showed a higher likelihood of co-infecting with *U. parvum* serovars 1, 3 and 6. HSV-2 and *C. trachomatis* were more frequently co-infected with *U. parvum* serovars 3 and 14. *U. parvum* serovar 6 frequently co-occurred with BV, as indicated by the higher Nugent scores in individuals infected with *U. parvum* serovar 6 than those of uninfected individuals. Co-infections among *U. parvum* serovars were uncommon. Specifically, the probability of *U. parvum* serovars 1, 3 and 6 co-infecting with each other was much lower than the probability of individual infections. Conversely, women who were *U. parvum* serovar 14-positive displayed a higher likelihood of being infected with *U. parvum* serovars 1, 3, and 6 than those who tested negative.

Independent effects of U. parvum serovars on HPV infection and CIN

Table S4 presents the results of the univariate analysis related to HPV infection and CIN. The association between *U. parvum* serovars and both HPV infection and CIN was evaluated using three separate multivariate logistic regression models (Table 3). Adjusting for the presence of other pathogens revealed a significant increase in the likelihood of HPV infection apparent in *U. parvum* serovars 1, 3, and 6-positive females, relative to their negative counterparts (Model 3, OR 1.53, 95% CI, 1.15–2.03; 1.31, 95%CI 1.06–1.64; 2.34, 1.90–2.97 respectively). The same tendency was observed in high-risk HPV. Only *U. parvum* serovar 6 was positively associated with low-risk HPV when other STIs confounders were adjusted (Model 3, OR 2.49, 95%CI, 1.79–3.46). Exposure to *U. parvum* serovars 1, 3, and 14 infections failed to demonstrate a significant association with the development of CIN, in contrast to non-exposure. *U. parvum* serovar 6-positive participants exhibited a 0.90-fold increased likelihood of CIN development (Model 3, OR 1.90, 95%CI, 1.19–3.02), as compared to *U. parvum* serovar 6 negative individuals, after controlling for other genital tract pathogens.

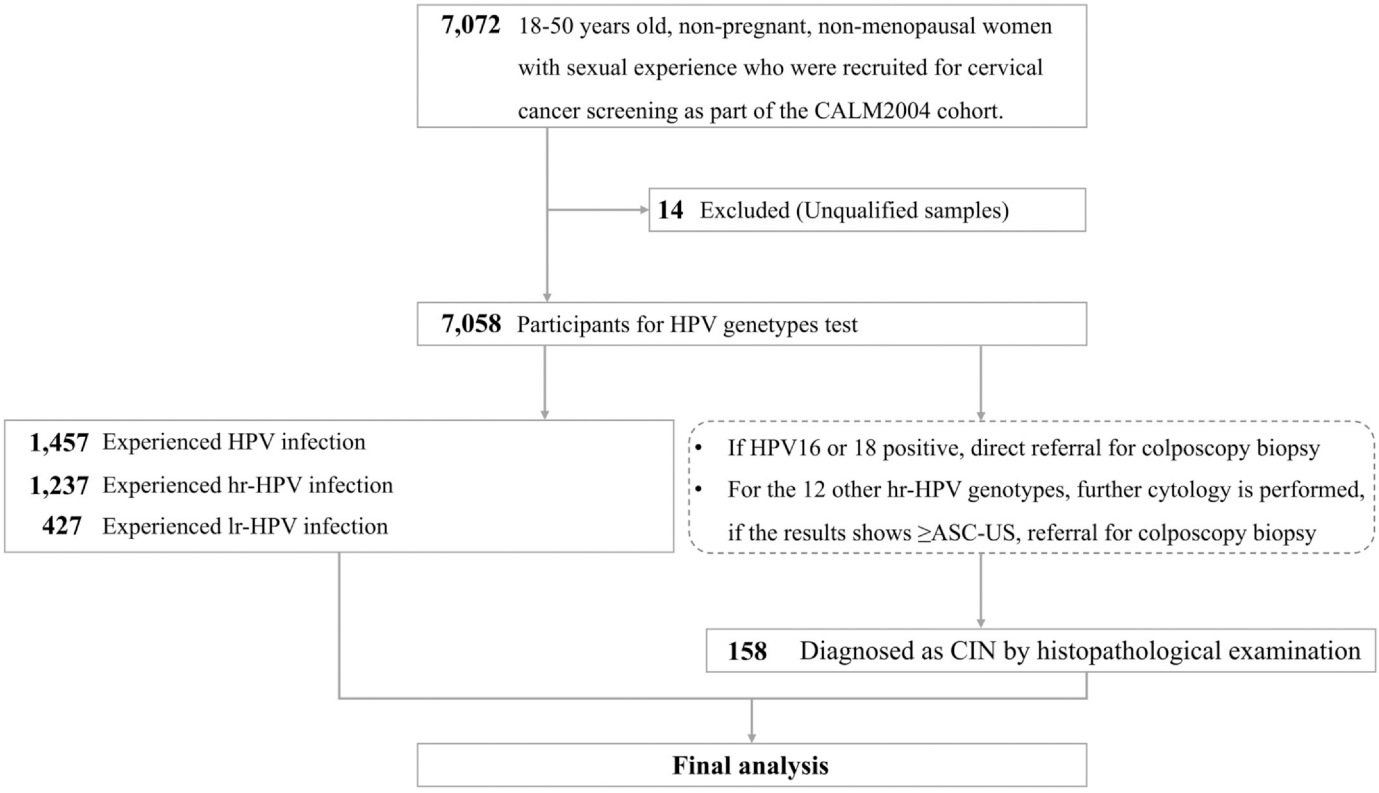

Fig. 1. Flow chart of study design. Abbreviations: HPV, Human papillomavirus; hr-HPV, high-risk HPV; lr-HPV, low-risk HPV; CIN, Cervical intraepithelial neoplasia.

Table 1
Characteristics of participants.

Characteristic	*U. parvum* serovar 1			*U. parvum* serovar 3			*U. parvum* serovar 6			*U. parvum* serovar 14		
	Negative (n=6498)	Positive (n=560)	*P*	Negative (n=5816)	Positive (n=1242)	*P*	Negative (n=5926)	Positive (n=1132)	*P*	Negative (n=6929)	Positive (n=129)	*P*
Age, mean (SD), year	36.11 ± 7.21	36.92 ± 7.47	**0.011**	36.13 ± 7.22	36.36 ± 7.29	0.324	36.10 ± 7.26	36.57 ± 7.09	**0.042**	36.18 ± 7.25	35.72 ± 6.42	0.473
BMI, mean (SD), kg/m^2	22.05 ± 3.07	22.37 ± 3.09	**0.030**	22.08 ± 3.06	22.07 ± 3.13	0.915	22.09 ± 3.07	22.03 ± 3.09	0.596	22.07 ± 3.07	22.82 ± 3.14	**0.036**
Ethnicity, No. (%)			0.798			0.534			0.168			0.190
Han	5136 (93.96%)	444 (93.67%)		4613 (94.03%)	967 (93.52%)		4695 (94.13%)	885 (92.96%)		5515 (93.98%)	65 (90.28%)	
Minorities	330 (6.04%)	30 (6.33%)		293 (5.97%)	67 (6.48%)		293 (5.87%)	67 (7.04%)		353 (6.02%)	7 (9.72%)	
Education level, No. (%)			0.186			0.104			0.055			0.055
Below undergraduate	2934 (63.27%)	267 (66.58%)		2624 (63.03%)	577 (65.94%)		2661 (62.97%)	540 (66.50%)		3153 (63.39%)	48 (75.00%)	
Undergraduate or above	1703 (36.73%)	134 (33.42%)		1539 (36.97%)	298 (34.06%)		1565 (37.03%)	272 (33.50%)		1821 (36.61%)	16 (25.00%)	
Methods of contraception, No. (%)			**0.001**			**0.031**			**< 0.001**			0.130
None	818 (17.63%)	84 (21.27%)		722 (17.41%)	180 (20.25%)		729 (17.11%)	173 (22.29%)		892 (17.97%)	10 (14.08%)	
IUD	516 (11.12%)	51 (12.91%)		462 (11.14%)	105 (11.81%)		470 (11.03%)	97 (12.50%)		553 (11.14%)	14 (19.72%)	
Oral contraceptives	108 (2.33%)	19 (4.81%)		97 (2.34%)	30 (3.37%)		103 (2.42%)	24 (3.09%)		126 (2.54%)	1 (1.41%)	
Condom	3199 (68.93%)	241 (61.01%)		2866 (69.11%)	574 (64.57%)		2958 (69.44%)	482 (62.11%)		3394 (68.36%)	46 (64.79%)	
Number of sexual partners, No. (%)			0.718			**0.032**			0.862			0.121
1	5418 (96.87%)	450 (96.57%)		4865 (97.07%)	1003 (95.80%)		4943 (96.86%)	925 (96.76%)		5795 (96.81%)	73 (100.00%)	
≥2	175 (3.13%)	16 (3.43%)		147 (2.93%)	44 (4.20%)		160 (3.14%)	31 (3.24%)		191 (3.19%)	0 (0.00%)	
Number of pregnancies, No. (%)			0.174			**0.003**			**0.028**			0.611
0	726 (12.94%)	63 (13.46%)		622 (12.39%)	167 (15.81%)		666 (13.06%)	123 (12.58%)		782 (13.04%)	7 (8.97%)	
1	1285 (22.91%)	100 (21.37%)		1165 (23.20%)	220 (20.83%)		1173 (23.00%)	212 (21.68%)		1369 (22.82%)	16 (20.51%)	
2	1493 (26.62%)	108 (23.08%)		1349 (26.87%)	252 (23.86%)		1369 (26.85%)	232 (23.72%)		1577 (26.29%)	24 (30.77%)	
≥3	2105 (37.53%)	197 (42.09%)		1885 (37.54%)	417 (39.49%)		1891 (37.09%)	411 (42.02%)		2271 (37.86%)	31 (39.74%)	
Number of deliveries, No. (%)			0.331			**< 0.001**			0.287			0.129
0	937 (16.93%)	87 (18.67%)		808 (16.29%)	216 (20.71%)		844 (16.75%)	180 (18.71%)		1016 (17.14%)	8 (10.81%)	
1	2473 (44.67%)	188 (40.34%)		2189 (44.14%)	472 (45.25%)		2229 (44.23%)	432 (44.91%)		2620 (44.20%)	41 (55.41%)	
2	1864 (33.67%)	166 (35.62%)		1711 (34.50%)	319 (30.58%)		1727 (34.27%)	303 (31.50%)		2006 (33.84%)	24 (32.43%)	
≥3	262 (4.73%)	25 (5.36%)		251 (5.06%)	36 (3.45%)		240 (4.76%)	47 (4.89%)		286 (4.82%)	1 (1.35%)	

Data were presented as mean ± SD and n (%).
Text in bold indicates statistical significance ($P < 0.05$).
Abbreviations: *U. parvum*, *Ureaplasma parvum*; SD, Standard deviation; BMI, Body mass index; IUD, Intrauterine device.

Table 2
Microbiological features of participants.

Characteristic	U. parvum serovar 1			U. parvum serovar 3			U. parvum serovar 6			*U. parvum* serovar 14		
	Negative (n=6498)	Positive (n=560)	*P*	Negative (n=5816)	Positive (n=1242)	*P*	Negative (n=5926)	Positive (n=1132)	*P*	Negative (n=6929)	Positive (n=129)	*P*
N. gonorrhoeae, No. (%)			0.732			0.777			0.274			**0.006**
Negative	6480 (99.72%)	558 (99.64%)		5800 (99.72%)	1238 (99.68%)		5911 (99.75%)	1127 (99.56%)		6911 (99.74%)	127 (98.45%)	
Positive	18 (0.28%)	2 (0.36%)		16 (0.28%)	4 (0.32%)		15 (0.25%)	5 (0.44%)		18 (0.26%)	2 (1.55%)	
U. urealyticum, No. (%)			0.104			**0.001**			**0.021**			0.619
Negative	5849 (90.01%)	516 (92.14%)		5214 (89.65%)	1151 (92.67%)		5323 (89.82%)	1042 (92.05%)		6247 (90.16%)	118 (91.47%)	
Positive	649 (9.99%)	44 (7.86%)		602 (10.35%)	91 (7.33%)		603 (10.18%)	90 (7.95%)		682 (9.84%)	11 (8.53%)	
M. hominis, No. (%)			**< 0.001**			**0.015**			**< 0.001**			0.318
Negative	6015 (92.57%)	496 (88.57%)		5386 (92.61%)	1125 (90.58%)		5496 (92.74%)	1015 (89.66%)		6395 (92.29%)	116 (89.92%)	
Positive	483 (7.43%)	64 (11.43%)		430 (7.39%)	117 (9.42%)		430 (7.26%)	117 (10.34%)		534 (7.71%)	13 (10.08%)	
M. genitalium, No. (%)			0.090			0.910			0.569			0.599
Negative	6432 (98.98%)	550 (98.21%)		5753 (98.92%)	1229 (98.95%)		5864 (98.95%)	1118 (98.76%)		6855 (98.93%)	127 (98.45%)	
Positive	66 (1.02%)	10 (1.79%)		63 (1.08%)	13 (1.05%)		62 (1.05%)	14 (1.24%)		74 (1.07%)	2 (1.55%)	
HSV-2, No. (%)			0.189			**0.004**			0.280			**0.005**
Negative	6423 (98.85%)	550 (98.21%)		5756 (98.97%)	1217 (97.99%)		5851 (98.73%)	1122 (99.12%)		6849 (98.85%)	124 (96.12%)	
Positive	75 (1.15%)	10 (1.79%)		60 (1.03%)	25 (2.01%)		75 (1.27%)	10 (0.88%)		80 (1.15%)	5 (3.88%)	
C. trachomatis, No. (%)			0.340			**< 0.001**			0.605			**0.044**
Negative	6228 (95.84%)	532 (95.00%)		5594 (96.18%)	1166 (93.88%)		5679 (95.83%)	1081 (95.49%)		6641 (95.84%)	119 (92.25%)	
Positive	270 (4.16%)	28 (5.00%)		222 (3.82%)	76 (6.12%)		247 (4.17%)	51 (4.51%)		288 (4.16%)	10 (7.75%)	
T. vaginalis, No. (%)			0.234			**0.003**			0.133			0.892
Negative	6228 (98.89%)	532 (99.44%)		5569 (98.76%)	1191 (99.75%)		5674 (98.85%)	1086 (99.36%)		6654 (98.93%)	106 (99.07%)	
Positive	70 (1.11%)	3 (0.56%)		70 (1.24%)	3 (0.25%)		66 (1.15%)	7 (0.64%)		72 (1.07%)	1 (0.93%)	
Fungus, No. (%)			0.626			0.633			0.094			0.620
Negative	5572 (90.40%)	478 (91.05%)		5001 (90.37%)	1049 (90.82%)		5072 (90.18%)	978 (91.83%)		5961 (90.47%)	89 (89.00%)	
Positive	592 (9.60%)	47 (8.95%)		533 (9.63%)	106 (9.18%)		552 (9.82%)	87 (8.17%)		628 (9.53%)	11 (11.00%)	
Nugent score, No. (%)			0.511			0.095			**< 0.001**			0.616
≤3	4867 (77.22%)	424 (79.40%)		4357 (77.22%)	934 (78.16%)		4461 (77.66%)	830 (75.94%)		5204 (77.33%)	87 (81.31%)	
4–6	1095 (17.37%)	84 (15.73%)		993 (17.60%)	186 (15.56%)		1003 (17.46%)	176 (16.10%)		1164 (17.30%)	15 (14.02%)	
≥7	341 (5.41%)	26 (4.87%)		292 (5.18%)	75 (6.28%)		280 (4.87%)	87 (7.96%)		362 (5.38%)	5 (4.67%)	
Donders score, No. (%)			0.196			0.554			0.214			0.687
< 3	5313 (86.35%)	463 (88.36%)		4768 (86.39%)	1008 (87.05%)		4870 (86.73%)	906 (85.31%)		5689 (86.49%)	87 (87.88%)	
≥3	840 (13.65%)	61 (11.64%)		751 (13.61%)	150 (12.95%)		745 (13.27%)	156 (14.69%)		889 (13.51%)	12 (12.12%)	
U. parvum serovar 1			-			**< 0.001**			**< 0.001**			**< 0.001**
Negative	-	-		5315 (91.39%)	1183 (95.25%)		5400 (91.12%)	1098 (97.00%)		6410 (92.51%)	88 (68.22%)	
Positive	-	-		501 (8.61%)	59 (4.75%)		526 (8.88%)	34 (3.00%)		519 (7.49%)	41 (31.78%)	
U. parvum serovar 3			**< 0.001**			-			**< 0.001**			**< 0.001**
Negative	5315 (81.79%)	501 (89.46%)		-	-		4783 (80.71%)	1033 (91.25%)		5789 (83.55%)	27 (20.93%)	
Positive	1183 (18.21%)	59 (10.54%)		-	-		1143 (19.29%)	99 (8.75%)		1140 (16.45%)	102 (79.07%)	
U. parvum serovar 6			**< 0.001**			**< 0.001**			-			**< 0.001**
Negative	5400 (83.10%)	526 (93.93%)		4783 (82.24%)	1143 (92.03%)		-	-		5839 (84.27%)	87 (67.44%)	
Positive	1098 (16.90%)	34 (6.07%)		1033 (17.76%)	99 (7.97%)		-	-		1090 (15.73%)	42 (32.56%)	
U. parvum serovar 14			**< 0.001**			**< 0.001**			**< 0.001**			-
Negative	6410 (98.65%)	519 (92.68%)		5789 (99.54%)	1140 (91.79%)		5839 (98.53%)	1090 (96.29%)		-	-	
Positive	88 (1.35%)	41 (7.32%)		27 (0.46%)	102 (8.21%)		87 (1.47%)	42 (3.71%)		-	-	

Data were presented as n (%).
Text in bold indicates statistical significance ($P < 0.05$).
Abbreviations: *U. parvum*, *Ureaplasma parvum*; HPV, Human papillomavirus; hr-HPV, high-risk HPV; lr-HPV, low-risk HPV; CIN, Cervical intraepithelial neoplasia; *N. gonorrhoeae*, *Neisseria gonorrhoeae*; *U. urealyticum*, *Ureaplasma urealyticum*; *M. hominis*, Mycoplasma hominis; *M. genitalium*, *Mycoplasma genitalium*; HSV-2, Herpes simplex virus type 2; *C. trachomatis*, *Chlamydia trachomatis*; *T. vaginalis*, *Trichomonas vaginalis*.

Table 3
Association of *U. parvum* serovars with HPV and CIN.

	HPV	lr-HPV	hr-HPV	CIN
U. parvum serovar 1				
Model 1[a]	**1.33 (1.08, 1.62)**	1.21 (0.85, 1.71)	**1.33 (1.07, 1.64)**	1.42 (0.83, 2.41)
Model 2[b]	1.22 (0.97, 1.53)	1.22 (0.84, 1.78)	1.21 (0.95, 1.54)	1.37 (0.78, 2.42)
Model 3[c]	**1.53 (1.15, 2.03)**	1.39 (0.86, 2.25)	**1.50 (1.11, 2.03)**	1.62 (0.86, 3.05)
U. parvum serovar 3				
Model 1[a]	**1.36 (1.18, 1.57)**	**1.49 (1.18, 1.89)**	**1.35 (1.16, 1.58)**	1.10 (0.72, 1.68)
Model 2[b]	**1.30 (1.10, 1.53)**	**1.37 (1.05, 1.79)**	**1.35 (1.14, 1.61)**	0.92 (0.57, 1.47)
Model 3[c]	**1.31 (1.06, 1.64)**	1.37 (0.97, 1.95)	**1.30 (1.03, 1.65)**	0.71 (0.38, 1.30)
U. parvum serovar 6				
Model 1[a]	**2.12 (1.84, 2.44)**	**2.24 (1.79, 2.82)**	**2.07 (1.79, 2.41)**	**2.17 (1.49, 3.15)**
Model 2[b]	**2.21 (1.88, 2.58)**	**2.16 (1.67, 2.80)**	**2.19 (1.85, 2.59)**	**2.23 (1.50, 3.32)**
Model 3[c]	**2.34 (1.90, 2.87)**	**2.49 (1.79, 3.46)**	**2.31 (1.85, 2.87)**	**1.90 (1.19, 3.02)**
U. parvum serovar 14				
Model 1[a]	**2.09 (1.45, 3.02)**	**2.39 (1.37, 4.18)**	**1.91 (1.28, 2.85)**	**2.48 (1.06, 5.83)**
Model 2[b]	1.46 (0.84, 2.53)	0.78 (0.24, 2.53)	1.70 (0.98, 2.94)	2.42 (0.85, 6.87)
Model 3[c]	1.08 (0.53, 2.18)	0.84 (0.22, 3.17)	1.31 (0.65, 2.65)	2.47 (0.81, 7.49)

Data were presented as OR (95%CI).
Text in bold indicates statistical significance ($P < 0.05$).
Abbreviations: HPV, Human papillomavirus; hr-HPV, high-risk HPV; lr-HPV, low-risk HPV; CIN, Cervical intraepithelial neoplasia; *U. parvum*, *Ureaplasma parvum*.
[a] Model 1: Logistic regression with no adjustment.
[b] Model 2: Logistic regression with adjustment for age, BMI, ethnicity.
[c] Model 3: Logistic regression with adjustment for age, BMI, ethnicity, education level, methods of contraception, number of sexual partners, number of pregnancies, number of deliveries, and other genital pathogens that were found to be associated with HPV and CIN based on the univariate analysis ($P < 0.1$).

Causal mediation analyses

A mediation analysis was carried out to further elucidate the extent of interaction between *U. parvum* serovar 6, HPV infection, and CIN development (Fig. 2). Only a minor proportion of CIN cases can be directly attributed to *U. parvum* serovar 6, yet approximately 76.66% of CIN cases were mediated by *U. parvum* serovar 6 via HPV infection. The direct effect value of *U. parvum* serovar 6 on CIN development was 0.010 ($P = 0.062$), whereas the effect value mediated through HPV was 0.031 ($P = 0.018$). When HPV was considered as exposure and *U. parvum* serovar 6 as the mediator, the mediation proportion for *U. parvum* serovar 6 was approximately 0% (Fig. S1).

Sensitivity analyses

Table S5 presents the results of the sensitivity analysis regarding the association between *U. parvum* serovars and HPV and CIN among participants who were without STIs, excluding *U. parvum* serovars and HPV (STIs-free). We observed similar trends to the population-wide analyses of *U. parvum* serovar 6 on HPV infection and CIN (Model3, OR 2.20, 95%CI 1.62–2.98; 2.05, 95%CI 1.02–4.12 respectively). Further causal mediation analyses among STIs-free participants show that approximately 89.37% of CIN cases were influenced by *U. parvum* serovar 6 through HPV (Fig. S2). Alternative analytic strategies, including the multiple imputation of missing variables, yielded consistent results with population-wide analyses. *U. parvum* serovar 6-positive participants exhibited a 1.27-fold greater risk of HPV infection (Model 3, OR 2.27, 95%CI, 1.95–2.63), and a 0.94-fold increased likelihood of CIN development (Model 3, OR 1.94, 95%CI, 1.30–2.88). However, after MI, it was determined that *U. parvum* serovar 14 exhibited a positive correlation with the implementation of CIN (Model 3, OR 2.62, 95%CI, 1.04–6.62) (Table S6).

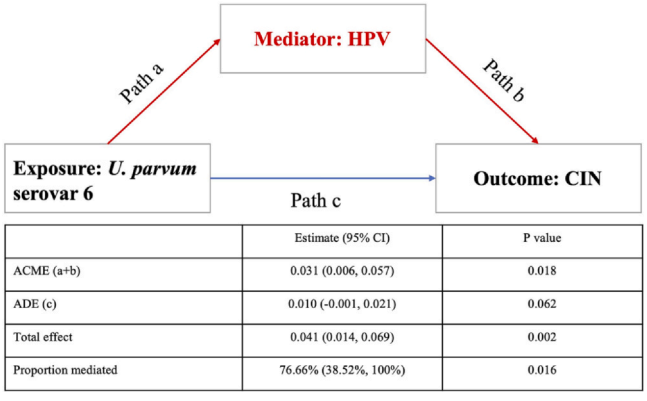

Fig. 2. Causal mediation analysis. *U. parvum* serovar 6 was regarded as exposure and HPV as the mediator to explore the direct effect (path c) and indirect effect (path a+b) of *U. parvum* serovar 6 on CIN. Adjusted for age, BMI and ethnicity. ACME, Average causal mediation effects; ADE, Average direct effects.

Subgroup analyses

We further investigated the effect of *U. parvum* serovars infection in subgroup defined by age and BMI. *U. parvum* serovar 6 has a significant positive correlation with HPV infection across all age groups. However, it only presents a risk factor for CIN in women between the ages of 18–30 and 40–50 (OR 3.16, 95%CI, 1.09–12.00; OR 2.49, 95%CI, 1.00–6.18 respectively). The impact of *U. parvum* serovar 6 on HPV was comparable among all BMI groups; however, only participants with a BMI ≤18.5 exhibited a positive correlation between *U. parvum* serovar 6 and CIN (Tables S7 and S8).

Discussion

In our multicenter cross-sectional study, we observed that *U. parvum* serovars 1, 3, and 6 were each associated with HPV infection. However, there was no significant correlation between *U. parvum* serovar 14 and HPV infection was observed after adjusted other STIs. In further exploring associations with CIN, only *U. parvum* serovar 6 was identified as an independent risk factor for CIN development. In addition, we examined the relationship among *U. parvum* serovar 6, HPV, and CIN. Notably, *U. parvum* serovar 6 seemed to influence CIN development more frequently through an HPV infection pathway. Positive associations of *U. parvum* serovar 6 with both HPV and CIN were identified from STIs-free women in which missing data were imputed by MI.

To date, few studies have investigated the relationship between *Ureaplasma* spp. and HPV infections, and these generally indicate no correlation between both infections.[29,30] However, a limited number of studies have further examined the correlation between *U. parvum* and HPV infections, and considerable variation in the findings

exists.[31–33] Previous studies, however, have notable limitations, including small sample sizes, their inability to distinguish among different *U. parvum* serovars, and the inadequate adjustment for confounding effects from other reproductive tract pathogens. We have improved the confidence of our findings by employing a larger sample size and considering actual instances of co-infection with other STIs.

The differences in pathogenicity of diverse *U. parvum* serovars continue to be a topic of ongoing debate and uncertainty. Most current international standards or consensus minimally emphasize the importance of detecting and treating *U. parvum* infections. According to the 2021 recommendations for STIs treatment by the Center for Disease Control and Prevention, no evidence currently substantiates a relationship between *U. parvum* and the development of cervicitis.[34] Likewise, the European STIs Guidelines Editorial Board concurred that systematic screening and treatment of *U. parvum* infections are unwarranted.[8] These statements emphasized *U. parvum* as a whole, rather than distinguishing between serovars. We observed that the heterogeneity in the pathogenicity of different *U. parvum* serovars indicated a potential need for serovar-specific testing. Erica L. Plummer's research on the association of *U. parvum* with clinical signs and symptoms in infertile women revealed no significant correlations.[6] However, specific *U. parvum* serovars were associated with diverse clinical symptoms and signs. For instance, *U. parvum* serovar 3/14 was significantly associated with symptomatic patients, whereas *U. parvum* serovar 6 was related more to asymptomatic women.[35,36] A separate investigation established a correlation between *U. parvum* serovar 6 and preterm birth, irrespective of co-infection with *Candida*.[37] These findings suggest that certain pathogenic serovars of *U. parvum* may not manifest clinical symptoms and signs, complicating the detection and diagnosis of this subtle infection. Our study identified a 1.01-fold increased risk of CIN in individuals infected with *U. parvum* serovar 6 compared to uninfected ones. However, *U. parvum* serovar 6 may not provoke obvious clinical symptoms and signs, thereby necessitating screening for *U. parvum* serovar 6 to identify high-risk CIN individuals within populations with HPV infections.

HPV infection is widely recognized as a key prerequisite for the development of CIN.[38,39] Considering the links among *U. parvum* serovars, HPV and CIN development, we performed a mediation effect analysis. This revealed that *U. parvum* serovar 6 might contribute to CIN development by mediating HPV infection, potentially introducing a novel pathophysiological aspect of CIN. Currently, no studies have elucidated the mechanism by which *U. parvum* serovar 6 causes cervical disease, to the best of our knowledge. In a mouse model of ascending *U. parvum* infection, cervical injury facilitates intrauterine *U. parvum* infection, upregulates pro-inflammatory cytokines, and increases preterm birth rates,[40] which may provide insight to the mechanisms between *U. parvum* serovar 6, HPV and CIN. In addition, it is reported that the diversity of MBA variable domains, the organism's ability to alter their sizes, and the transition between these domains may indicate that distinct MBAs, upon recognition by TLRs, could possess varying capacities to activate the innate immune system.[41,42] These mechanisms could also be associated with variations in local microbial communities within the cervicovaginal niche and inflammatory marker production.[43–45,46] Further experimental research is required to elucidate the potential mechanisms.

Our study possesses both strengths and limitations. Our study, to the best of our knowledge, is the first multi-center study investigating the pathogenicity of *U. parvum* serovars. The use of logistic regression models facilitated the evaluation of genital tract pathogens and HPV infection, while accounting for other genital tract pathogens. Additionally, we probed the relationship between *U. parvum* serovars, HPV and CIN, uncovering further pathways implicated in CIN development. Despite its strengths, our study had several limitations. First, the cross-sectional nature of our study precludes establishing of a causal link between *U. parvum* serovars, HPV, and CIN. Second, we cannot discount the impact of unobserved confounding variables on study findings and conclusions. Finally, our study focuses on the Chinese population, therefore, extrapolating its findings to other races or nations may be unfeasible. It is important to note that the use of varying commercial assay lists of *Ureaplasma* across different studies may make it complicated in comparing our results with other research.

Conclusion

In this multicenter cross-sectional study, we explored the association between *U. parvum* serovars, HPV, and CIN and evidenced that all *U. parvum* serovars, excluding *U. parvum* serovar 14, were independent risk factors for HPV infection, specifically high-risk HPV infection. Notably, individuals infected with *U. parvum* serovar 6 were at a substantially elevated risk for CIN, a risk potentially mediated by HPV infection. These findings indicate the necessity for routine screening and treatment of *U. parvum* serovars in individuals with HPV infections, especially with certain serovars such as *U. parvum* serovar 6 that may not exhibit clinical symptoms. This emphasizes the requirement for heightened vigilance regarding latent infections to detect individuals at high risk of CIN and to facilitate early intervention.

Funding

The study was supported by the National Natural Science Foundation of China (82205157 (Y.Z.), 81925026 (H.Z.) and 82302610 (C.Q.)), the Guangdong Basic and Applied Basic Research Foundation (2021B1515230007 (H.Z.)), and the China Postdoctoral Science Foundation (2022M721538 (Y.Z.)).

Author contributions

Hongwei Zhou and Muxuan Chen conceived and implemented the research. Zuyi Zhou and Rongdan Chen were responsible for data collection. Jinxia Ou contributed to the data assessment. Yingxuan Zhang, Wei Qing and Cancan Qi conducted statistical analysis. Yingxuan Zhang authored the initial version. Yingxuan Zhang, Rongdan Chen, and Zuyi Zhou reviewed and edited the manuscripts. All writers reviewed and endorsed the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank all participants, staff and collaborators who participated in the CALM 2004 project. We gratefully acknowledge the financial support of the National Natural Science Foundation of China, the Guangdong Basic and Applied Basic Research Foundation and the China Postdoctoral Science Foundation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106397.

References

1. Kletzel HH, Rotem R, Barg M, Michaeli J, Reichman O. *Ureaplasma urealyticum: the role as a pathogen in women's health, a systematic review. Curr Infect Dis Rep* 2018;**20**:33. https://doi.org/10.1007/s11908-018-0640-y
2. Schelonka RL, Waites KB. *Ureaplasma infection and neonatal lung disease. Semin Perinatol* 2007;**31**:2–9. https://doi.org/10.1053/j.semperi.2007.01.001
3. Gwee A, Curtis N. *Ureaplasma—are you sitting comfortably? J Infect* 2014;**68**(1):S19–23. https://doi.org/10.1016/j.jinf.2013.09.027
4. Sprong KE, Mabenge M, Wright CA, Govender S. *Ureaplasma species and preterm birth: current perspectives. Crit Rev Microbiol* 2020;**46**:169–81. https://doi.org/10.1080/1040841X.2020.1736986
5. Robertson JA, Stemke GW, Davis JW, Harasawa R, Thirkell D, Kong F, et al. *Proposal of Ureaplasma parvum sp. nov. and emended description of Ureaplasma urealyticum (Shepard et al. 1974) Robertson et al. 2001. Int J Syst Evol Microbiol* 2002;**52**:587–97. https://doi.org/10.1099/00207713-52-2-587
6. Plummer EL, Vodstrcil LA, Bodiyabadu K, Murray GL, Doyle M, Latimer RL, et al. *Are Mycoplasma hominis, Ureaplasma urealyticum and Ureaplasma parvum associated with specific genital symptoms and clinical signs in nonpregnant women? Clin Infect Dis* 2021;**73**:659–68. https://doi.org/10.1093/cid/ciab061
7. Cm W, Le M, Ms L, Mr G, Nl J, Sg A, et al. *Ureaplasma urealyticum is associated with nongonococcal urethritis among men with fewer lifetime sexual partners: a case-control study. J Infect Dis* 2011;**204**:1274–82. https://doi.org/10.1093/infdis/jir517
8. Horner P, Donders G, Cusini M, Gomberg M, Jensen JS, Unemo M. *Should we be testing for urogenital Mycoplasma hominis, Ureaplasma parvum and Ureaplasma urealyticum in men and women? – a position statement from the European STI Guidelines Editorial Board. J Eur Acad Dermatol Venereol* 2018;**32**:1845–51. https://doi.org/10.1111/jdv.15146
9. Stellrecht KA, Woron AM, Mishrik NG, Venezia RA. *Comparison of multiplex PCR assay with culture for detection of genital mycoplasmas. J Clin Microbiol* 2004;**42**:1528–33. https://doi.org/10.1128/JCM.42.4.1528-1533.2004
10. kong F, James G, Ma Z, Gordon S, Bin W, Gilbert GL. *Phylogenetic analysis of Ureaplasma urealyticum—support for the establishment of a new species, Ureaplasma parvum. Int J Syst Bacteriol* 1999;**49**(Pt 4):1879–89. https://doi.org/10.1099/00207713-49-4-1879
11. zur Hausen H. *Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer* 2002;**2**:342–50. https://doi.org/10.1038/nrc798
12. Magaña-Contreras M, Contreras-Paredes A, Chavez-Blanco A, Lizano M, De la Cruz-Hernandez E. *Prevalence of sexually transmitted pathogens associated with HPV infection in cervical samples in a Mexican population. J Med Virol* 2015;**87**:2098–105. https://doi.org/10.1002/jmv.24278
13. Ye H, Song T, Zeng X, Li L, Hou M, Xi M. *Association between genital mycoplasmas infection and human papillomavirus infection, abnormal cervical cytopathology, and cervical cancer: a systematic review and meta-analysis. Arch Gynecol Obstet* 2018;**297**:1377–87. https://doi.org/10.1007/s00404-018-4733-5
14. Zhou Z, Hou Y, Qing W, Shi Y, Zhang Y, Chen R, et al. *The association of HPV infection and vaginal microbiota of reproductive women in China: a multicenter cohort study protocol. Med Microecol* 2023;**15**:100072. https://doi.org/10.1016/j.medmic.2022.100072
15. Bai S, Li Y, Wan Y, Guo T, Jin Q, Liu R, et al. *Sexually transmitted infections and semen quality from subfertile men with and without leukocytospermia. Reprod Biol Endocrinol* 2021;**19**:92. https://doi.org/10.1186/s12958-021-00769-2
16. Zhao N, Li KT, Gao Y-Y, Xu J-J, Huang D-S. *Mycoplasma genitalium and Mycoplasma hominis are prevalent and correlated with HIV risk in MSM: a cross-sectional study in Shenyang, China. BMC Infect Dis* 2019;**19**:494. https://doi.org/10.1186/s12879-019-4138-5
17. Low HC, Silver MI, Brown BJ, Leng CY, Blas MM, Gravitt PE, et al. *Comparison of Hybribio GenoArray and Roche human papillomavirus (HPV) linear array for HPV genotyping in anal swab samples. J Clin Microbiol* 2015;**53**:550–6. https://doi.org/10.1128/JCM.02274-14
18. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al. *2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis* 2013;**17**:S1–27. https://doi.org/10.1097/LGT.0b013e318287d329
19. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. *Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med* 2003;**348**:518–27. https://doi.org/10.1056/NEJMoa021641
20. Huh WK, Ault KA, Chelmow D, Davey DD, Goulart RA, Garcia FAR, et al. *Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Obstet Gynecol* 2015;**125**:330–7.
21. Workowski KA, Bolan GA. Centers for Disease Control and Prevention. *Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep* 2015;**64**:1–137.
22. Nugent RP, Krohn MA, Hillier SL. *Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol* 1991;**29**:297–301. https://doi.org/10.1128/jcm.29.2.297-301.1991
23. *Vaginitis in nonpregnant patients: ACOG Practice Bulletin, Number 215. Obstet Gynecol* 2020;**135**:e1–17. https://doi.org/10.1097/AOG.0000000000003604
24. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. *Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med* 1983;**74**:14–22. https://doi.org/10.1016/0002-9343(83)91112-9
25. Tempera G, Bonfiglio G, Cammarata E, Corsello S, Cianci A. *Microbiological/clinical characteristics and validation of topical therapy with kanamycin in aerobic vaginitis: a pilot study. Int J Antimicrob Agents* 2004;**24**:85–8. https://doi.org/10.1016/j.ijantimicag.2003.12.013
26. Donders GGG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Spitz B. *Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. BJOG* 2002;**109**:34–43. https://doi.org/10.1111/j.1471-0528.2002.00432.x
27. Imai K, Keele L, Tingley D. *A general approach to causal mediation analysis. Psychol Methods* 2010;**15**:309–34. https://doi.org/10.1037/a0020761
28. Su Y-S, Gelman A, Hill J, Yajima M. *Multiple imputation with diagnostics (mi) in R: opening windows into the black box. J Stat Softw* 2011;**45**:1–31. https://doi.org/10.18637/jss.v045.i02
29. Denks K, Spaeth EL, Jõers K, Randoja R, Talpsep T, Ustav M, et al. *Coinfection of Chlamydia trachomatis, Ureaplasma urealyticum and human papillomavirus among patients attending STD clinics in Estonia. Scand J Infect Dis* 2007;**39**:714–8. https://doi.org/10.1080/00365540701199824
30. Zhang D, Li T, Chen L, Zhang X, Zhao G, Liu Z. *Epidemiological investigation of the relationship between common lower genital tract infections and high-risk human papillomavirus infections among women in Beijing, China. PLoS One* 2017;**12**:e0178033. https://doi.org/10.1371/journal.pone.0178033
31. Zanotta N, Campisciano G, Morassut S, Castro-Silva E, Luksa V, Zito G, et al. *Emerging role for Ureaplasma parvum serovar 3: active infection in women with silent high-risk human papillomavirus and in women with idiopathic infertility. J Cell Physiol* 2019;**234**:17905–11. https://doi.org/10.1002/jcp.28423
32. Xie L, Li Q, Dong X, Kong Q, Duan Y, Chen X, et al. *Investigation of the association between ten pathogens causing sexually transmitted diseases and high-risk human papilloma virus infection in Shanghai. Mol Clin Oncol* 2021;**15**:132. https://doi.org/10.3892/mco.2021.2294
33. Wang L, Zhu L, Li H, Ma N, Huang H, Zhang X, et al. *Association between asymptomatic sexually transmitted infections and high-risk human papillomavirus in cervical lesions. J Int Med Res* 2019;**47**. https://doi.org/10.1177/0300060519865633
34. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, et al. *Sexually transmitted infections treatment guidelines, 2021.* 2021;**70**:192.
35. De Francesco MA, Negrini R, Pinsi G, Peroni L, Manca N. *Detection of Ureaplasma biovars and polymerase chain reaction-based subtyping of Ureaplasma parvum in women with or without symptoms of genital infections. Eur J Clin Microbiol Infect Dis* 2009;**28**:641–6. https://doi.org/10.1007/s10096-008-0687-z
36. Kim MS, Lee DH, Kim TJ, Oh JJ, Rhee SR, Park DS, et al. *The role of Ureaplasma parvum serovar-3 or serovar-14 infection in female patients with chronic micturition urethral pain and recurrent microscopic hematuria. Transl Androl Urol* 2021;**10**:96–108. https://doi.org/10.21037/tau-20-920
37. Payne MS, Ireland DJ, Watts R, Nathan EA, Furfaro LL, Kemp MW, et al. *Ureaplasma parvum genotype, combined vaginal colonisation with Candida albicans, and spontaneous preterm birth in an Australian cohort of pregnant women. BMC Pregnancy Childbirth* 2016;**16**:312. https://doi.org/10.1186/s12884-016-1110-x
38. Woodman CBJ, Collins SI, Young LS. *The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer* 2007;**7**:11–22. https://doi.org/10.1038/nrc2050
39. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. *Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol* 1999;**189**:12–9. https://doi.org/10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F
40. Pavlidis I, Spiller OB, Sammut Demarco G, MacPherson H, Howie SEM, Norman JE, et al. *Cervical epithelial damage promotes Ureaplasma parvum ascending infection, intrauterine inflammation and preterm birth induction in mice. Nat Commun* 2020;**11**:199. https://doi.org/10.1038/s41467-019-14089-y
41. Waites KB, Schelonka RL, Xiao L, Grigsby PL, Novy MJ. *Congenital and opportunistic infections: Ureaplasma species and Mycoplasma hominis. Semin Fetal Neonatal Med* 2009;**14**:190–9. https://doi.org/10.1016/j.siny.2008.11.009
42. Paralanov V, Lu J, Duffy LB, Crabb DM, Shrivastava S, Methé BA, et al. *Comparative genome analysis of 19 Ureaplasma urealyticum and Ureaplasma parvumstrains. BMC Microbiol* 2012;**12**:88. https://doi.org/10.1186/1471-2180-12-88
43. Abou Chacra L, Fenollar F, Diop K. *Bacterial vaginosis: what do we currently know? Front Cell Infect Microbiol* 2021;**11**:672429. https://doi.org/10.3389/fcimb.2021.672429
44. Guleria C, Suri V, Kapoor R, Minz RW, Aggarwal R. *Human papillomavirus 16 infection alters the Toll-like receptors and downstream signaling cascade: a plausible early event in cervical squamous cell carcinoma development. Gynecol Oncol* 2019;**155**:151–60. https://doi.org/10.1016/j.ygyno.2019.07.023
45. Belleti R, Marcolino LD, Novak J, Ferreira CST, do Nascimento Bolpetti A, da Silva Pinto GV, et al. *Cervicovaginal loads of Gardnerella spp. are increased in immunocompetent women with persistent high-risk human papillomavirus infection. J Med Microbiol* 2022;**71**. https://doi.org/10.1099/jmm.0.001527