Epidemiology of *Chlamydia trachomatis* endocervical infection in a previously unscreened population in Rome, Italy, 2000 to 2009

V Marcone (valentinamarcone@libero.it)[1], N Recine[2], C Gallinelli[1], R Nicosia[1], M Lichtner[3], A M Degener[4], F Chiarini[1], E Calzolari[2], V Vullo[1]

1. Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy
2. Department of Obstetric and Gynaecological Sciences and Urologic Sciences, Sapienza University, Rome, Italy
3. Department of Infectious Diseases, Sapienza University, Polo Pontino, Rome, Italy
4. Department of Molecular Medicine, Sapienza University, Rome, Italy

Citation style for this article:
Marcone V, Recine N, Gallinelli C, Nicosia R, Lichtner M, Degener AM, Chiarini F, Calzolari E, Vullo V. Epidemiology of *Chlamydia trachomatis* endocervical infection in a previously unscreened population in Rome, Italy, 2000 to 2009 . Euro Surveill. 2012;17(25):pii=20203. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20203

Article submitted on 07 October 2011 / published on 21 June 2012

As reliable data on *Chlamydia trachomatis* infection in Italy are lacking and as there is no Italian screening policy, epidemiological analyses are needed to optimise effective strategies for surveillance of the infection in the country. We collected data from 6,969 sexually active women aged 15 to 55 years who underwent testing for endocervical *C. trachomatis* infection at the Cervico-Vaginal Pathology Unit in the Department of Gynaecology and Obstetrics of Sapienza University in Rome between 2000 and 2009. The mean prevalence of *C. trachomatis* endocervical infection during this period was 5.2%. Prevalence over time did not show a linear trend. Univariate analysis demonstrated a significant association of infection with multiple lifetime sexual partners, younger age (<40 years), never having been pregnant, smoking, use of oral contraceptives, and human papillomavirus and *Trichomonas vaginalis* infections. Multivariate stepwise logistic regression showed that *T. vaginalis* infection, age under 20 years and more than one lifetime sexual partner remained significantly associated with *C. trachomatis* infection in the final model. Prevalence of *C. trachomatis* in this study was high, even among women aged 25–39 years (5.1%): our data would suggest that a *C. trachomatis* screening policy in Italy is warranted, which could lead to a more extensive testing strategy.

Introduction

Chlamydia trachomatis endocervical/urethral infection, caused by serotypes D to K is the most common bacterial, treatable sexually transmitted infection worldwide [1,2]. As up to 80% of cases are asymptomatic, *C. trachomatis* can be spread unknowingly and remains largely undiagnosed [1,2]. The prevalence of the infection in Europe varies according to the population, setting, country, resource allocation for surveillance and prevention and national reporting system, if there is one. A systematic review of *C. trachomatis* infection among asymptomatic unscreened European women showed that the prevalence ranged from 1.7% (among women aged 15–40 years in the United Kingdom in the mid-1990s) to 17% (among women aged 15–55 years in France in the late 1980s) and was more than 5% in the majority of the countries examined [3,4]. More recently the European Centre for Disease Prevention and Control (ECDC) described surveys from seven countries, estimating a population prevalence of 1.4–3.0% in people aged 18–44 years [5]. They also reported that overall trends over time across Europe appeared to be increasing, from 1990 to 2009, although data were not available from Bulgaria, Czech Republic, France, Germany, Italy, Liechtenstein and Portugal [6]. Moreover, the organisation of the control of *C. trachomatis* infection varied widely, with many countries having no organised activities until 2009 [7].

Pelvic inflammatory disease, tubal sterility or infertility, newborn eye infection or pneumonia and, although controversial, sperm pathology, male sterility and spontaneous abortion or preterm labour, are well-known complications of untreated *C. trachomatis* infection [8-14].

Since treating complications is costly in both psychosocial and financial terms, and is often unsuccessful [15], screening is critical for the early detection and treatment of uncomplicated *C. trachomatis* infection, the control of the overall prevalence of the infection in the population and thus the reduction of transmission and finally for the reduction of treatment costs.

C. trachomatis screening programmes exist in only two European countries (England and the Netherlands) and in the United States: they are opportunistic or pro-active and are mostly directed at young women aged under 25 years [7,16]. Sweden, although lacking

nationally organised screening programmes, is the first country in the world to offer testing for *C. trachomatis* infection, treatment and partner notification – all free of charge – throughout the country. It is also the first to have a national diagnostic and reporting system [5]. In these four countries, after substantial decreases in complication rates of *C. trachomatis* infection at the end of the 1980s and early 1990s, further decreases in pelvic inflammatory disease and ectopic pregnancy rates after 2000 were observed [7,16-20].

Unfortunately, reliable and recent data concerning *C. trachomatis* control in Italy are lacking, except for those in studies such as that of the Italian MEGIC Group (Multicentre Epidemiology Group for Investigation of Chlamydia trachomatis) that reported a prevalence of *C. trachomatis* infection of 3.9% among 1,321 asymptomatic women [21] or that of the STD Surveillance Working Group, which described 809 female incident cases from mainly dermatology and venereology departments and a few gynaecological departments between 1991 and 1996 [22].

There is no screening policy for *C. trachomatis* infection in Italy. A national women's health report released in 2008 suggested for the first time that women should be tested for *C. trachomatis* when they have their first cervical smear test [23]. In order to understand if a screening strategy would be appropriate, the prevalence of the infection needs to be ascertained and there needs to be a preliminary analysis of the epidemiological variables in the population at risk, as well as a surveillance network. No existing epidemiological model can be applied to a different population without analysis and adjustment. New, larger epidemiological analyses are therefore needed in Italy to plan specific and effective strategies for the surveillance and screening of *C. trachomatis* infection in the country.

The purpose of this study was to investigate the prevalence of *C. trachomatis* endocervical infection and its determinants in a large population of sexually active women aged 15–55 years attending an outpatient service of a cervico-vaginal pathology unit in Rome over a 10-year period.

Methods

Patient population

Between January 2000 and December 2009, a total of 7,620 women (aged 13–58 years) attending the outpatient service of the Cervico-Vaginal Pathology Unit in the Department of Gynaecology and Obstetrics of Sapienza University in Rome were examined for genitourinary symptoms or routine gynaecological examination.

A team of gynaecologists collected socio-demographic and behavioural data, as well as clinical data, for each woman during this time, using our model of clinical record taking for sexually transmitted infections – a structured questionnaire. The data were archived as digital files.

The self-administered, structured, paper questionnaire comprised 25 questions on socio-demographic characteristics, sexual behaviour, reproductive history, and tobacco, alcohol and drug use.

Testing for *C. trachomatis* infection, along with testing for human papillomavirus (HPV) and N. gonorrhoeae infection and vaginal wet mount examination, was offered to all sexually active women presenting to the Unit.

Women who refused to be tested for *C. trachomatis* and/or to answer the questionnaire and/or were not sexually active were excluded from the study (n=651).

According to these criteria, a total of 6,969 sexually active women aged 15–55 years who were tested for cervical *C. trachomatis* infection were enrolled. The women were categorised as symptomatic if they presented with either dysuria or pelvic pain or both (symptoms typical of *C. trachomatis* infection). Women not exhibiting either of these symptoms were classified as asymptomatic. They were then further categorised according to whether they were seeking care for family planning, infertility routine gynaecological examination or matters related to pregnancy.

All participating women gave written informed consent. The research was carried out in compliance with the Declaration of Helsinki [24] and was approved by the local ethics committee (reference number 148/11, 2022). Data were stored and managed according to Italian privacy rules [25].

Examinations performed

On a scheduled visit, during the gynaecological examination, an unmoistened sterile speculum was inserted into vagina, so that vaginal walls, fornices and cervix could be evaluated for any erythema and colour and viscosity of any discharge. The pH of the vaginal walls was measured using colorimetric paper. For wet mount examinations, vaginal fluor samples were collected from lateral fornices by a wooden Ayre's spatula, mixed first with saline and then with 10% potassium hydroxide, on two different slides, and immediately observed under a phase contrast microscope [26].

A 'whiff test' using 10% potassium hydroxide was performed for each sample in order to detect abnormal amine production by anaerobes [27].

Wet mount examination allowed the vaginal microflora (predominance of lactobacillary morphotypes) to be assessed and *Trichomonas vaginalis* to be detected (in order to investigate coexisting sexually transmitted infections). In addition, we also looked for bacterial vaginosis-associated clue cells, aerobic

vaginitis-associated pleomorphic bacteria, yeasts and white blood cells.

Samples were taken from the endocervix for detection of *C. trachomatis* and from the ecto-endocervix for detection of HPV DNA, as described below.

Detection of microorganisms

Chlamydia trachomatis

Endocervical swabs were tested for the presence of *C. trachomatis* using the BD ProbeTec ET System (Becton, Dickinson and Company, United States). These assays amplify *C. trachomatis* DNA in separate wells and monitor inhibition of amplification for each specimen using strand displacement amplification and detection by fluorescent energy transfer probes, producing a method-other-than-acceleration (MOTA) score for each specimen The original algorithm involved retesting specimens with MOTA scores between 2000 and 9999. A negative repeat result (MOTA score ‹2000) was considered indeterminate [28].

Human papillomavirus

DNA was extracted from cervical samples using QIAampTissue Kit (Qiagen, Italy) and then genotyped by sequencing a 450-base pair fragment amplified from the L1 region of HPV DNA [29]. Sequence homology was determined using BLAST and ClustalW programs.

Neisseria gonorrhoeae

Identification of *N. gonorrhoeae* was carried out by growth on media selective for pathogenic *Neisseria* species (Oxoid) incubated for up to 48 hours in 5–10% CO_2 at 35–37 °C. Colonies obtained were identified by API NH (bioMérieux) [30].

Statistical analysis

The chi-square test was used to analyse contingency tables; the t-test was used to compare means and odds ratios (ORs), with 95% confidence intervals (CIs), in order to measure the strength of association between *C. trachomatis* infection and behavioural and clinical characteristics and age.

We used the Cochran–Armitage test to assess the possibility of a linear trend in the observed patterns for number of lifetime sexual partners and increasing age.

Statistical tests were considered significant if p was 0.05 or less. A stepwise backward logistic regression analysis, entering the variables significantly associated with *C. trachomatis* infection, was used to assess the effect of more than one variable at a time and to identify possible confounding factors in the range of test values under consideration. Statistical analysis was performed using SPSS version 18.0.

Results

A total of 366 (5.2%) of the 6,969 women sexually active women enrolled in the study tested positive for *C. trachomatis* endocervical infection (Table 1).

Prevalence of *C. trachomatis* infection by year is shown in the Figure: the p value for the chi-square statistic was not statistically significant (p=0.938) (the chi-square test for the resulting 2×10 contingency table tested the null hypothesis of no association against the alternative hypothesis of an association of some sort). Thus prevalence and time appeared not to be associated and were not expected to have a linear correlation over the study period.

A total of 4,620 (66%) of the women were asymptomatic for *C. trachomatis* infection: 256 (5.5%) of them tested positive. This prevalence was slightly higher than that in the 2,349 symptomatic women (4.7%), but the difference was not statistically significant (p=0.1289). Of the 366 women who were positive for *C. trachomatis* infection, 256 (70%) were asymptomatic.

Prevalence was also slightly higher among women without clinical signs of infection (238/4,328; 5.5% compared with those with signs (128/2,641; 4.8%), but this difference was also not statistically significant (p=0.2362).

Univariate analysis of sexual and reproductive history and of age (Tables 1 and 2) highlighted a significant association of *C. trachomatis* infection with age under 40 years, having never been pregnant, smoking, use of oral contraceptives and multiple lifetime sexual partners: women with two to four partners had a slightly higher risk of infection (in comparison with women who had had one partner); women with five to nine partners had double the risk; having had more than nine partners was linked to a threefold higher risk. The p value for the Cochran–Armitage test (p‹0.0001) suggested an underlying positive linear trend between number of lifetime sexual partners and prevalence of infection.

Comparison of the prevalence of *C. trachomatis* infection in stratified age groups with that in women over 49 years of age showed that teenage women aged 15–19 years had the highest increased risk of infection (OR: 4.55 (95% CI: 1.90–10.89); p=0.0002) and that the odds ratios for the remaining strata declined with increasing age. The p value for the Cochran–Armitage test (p‹0.0001) suggested an underlying negative linear trend between age and prevalence of infection.

Further univariate analysis showed that the prevalence of the infection was similar (no statistical significance) whatever the reason for seeking care (Table 2). Condom use was not found to be associated with *C. trachomatis* infection.

The frequency of *C. trachomatis* infection was significantly higher among patients who were also infected

with HPV (OR: 5.50 (95% CI: 4.39–6.89)) and *T. vaginalis* (OR: 4.97 (95% CI: 2.57–9.59)) (Table 3).

Multivariate stepwise logistic regression analysis shows that after backwards elimination, *T. vaginalis* infection (OR: 3.23 (95% CI: 1.61–6.46); p=0.001), age 15–19 years (OR: 2.33 (95% CI: 1.02–5.31); p=0.04) and more than one lifetime sexual partner (OR: 1.50 (95% CI: 1.21–1.87); p=0.000) remained significantly associated with *C. trachomatis* infection in the final model.

We found no cases of gonorrhoea among the first thousand patients referred to the clinic and systematically screened. We then tested *C. trachomatis*-positive cases only, if they showed symptoms or signs of cervicitis: none were positive for *N. gonorrhoeae*.

Discussion

To the best of our knowledge, this is the first study reporting on the epidemiology of *C. trachomatis* infection in Italy in a large sample of a diverse group of women over a long period of time. The mean prevalence of the infection was high (5.2 %) and showed no linear trend over time. The prevalence in asymptomatic women was higher than that observed in 1990 by the MEGIC group (5.5% vs 3.9%, respectively) [21]. In symptomatic women and in those seeking care for infertility the prevalence in our study (4.7% and 4.9% respectively) was similar to that reported by the same group (5.0% and 5.4%, respectively) [21]. These findings may reflect the lack of control and screening activities in Italy.

We also found a high prevalence of *C. trachomatis* infection in pregnant women (5.3%), i.e. those seeking obstetric care (Table 2) which has not been described in Italy and suggests we should consider screening in pregnancy according to CDC guidelines [16]. This strategy could also reduce the rate of obstetric complications due to *C. trachomatis* infection.

Two of the variables independently associated with *C. trachomatis* infection in our study, younger age and multiple lifetime sexual partners (particularly more

Table 1

Univariate analysis of age and sexual and reproductive history of women tested for *Chlamydia trachomatis* infection, Cervico-Vaginal Pathology Unit, Sapienza University, Rome, Italy, 2000–2009 (n=6,969)

Characteristic	Tested for *C. trachomatis* endocervical infection			Odds ratio[a] (95% CI)	P value (t-test statistic)[b]
	Number positive (%)[a]	Number negative[a]	**Total[a]**		
Mean age in years					
15–19	9 (10.8)	74	**83**	4.55 (1.90–10.89)	0.0002
20–24	71 (7.8)	835	**906**	3.18 (1.78–5.70)	0.0000
25–29	86 (5.6)	1,441	**1,527**	2.23 (1.26–3.96)	0.0049
30–34	84 (5.2)	1,519	**1,603**	2.07 (1.17–3.68)	0.0113
35–39	61 (5.1)	1,125	**1,186**	2.03 (1.12–3.66)	0.0166
40–44	29 (4.2)	656	**685**	1.65 (0.87–3.16)	0.1242[c]
45–49	12 (2.7)	429	**441**	1.05 (0.48–2.29)	0.9084[c]
≥50–55	14 (2.6)	524	**538**	1 Reference	–
Mean age per category	32.0 years	34.4 years	**33.2 years**	Difference (those positive vs those negative): −2.4	0.001 (t=−4.610)
Number of lifetime sexual partners					
1	89 (3.4)	2,508	**2,597**	1 Reference	–
2	71 (5.6)	1,191	**1,262**	1.68 (1.22–2.31)	0.0013
3	57 (5.1)	1,063	**1,120**	1.51 (1.08–2.12)	0.0167
4	41 (5.5)	702	**743**	1.65 (1.13–2.40)	0.0094
5–9	54 (7.9)	626	**680**	2.43 (1.71–3.45)	0.0000
≥10	54 (9.5)	513	**567**	2.97 (2.09–4.21)	0.0000
Mean number of lifetime sexual partners per category	2.9	1.7	**2.3**	Difference (those positive vs those negative): 1.2	0.02 (t=2.518)
Ever been pregnant					
Yes	115 (3.8)	2,896	**3,011**	1 Reference	–
No	251 (6.3)	3,707	**3,958**	1.71 (1.36–2.14)	0.0000
Total	**366 (5.2)**	**6,603**	**6,969**	–	–

CI: confidence interval.

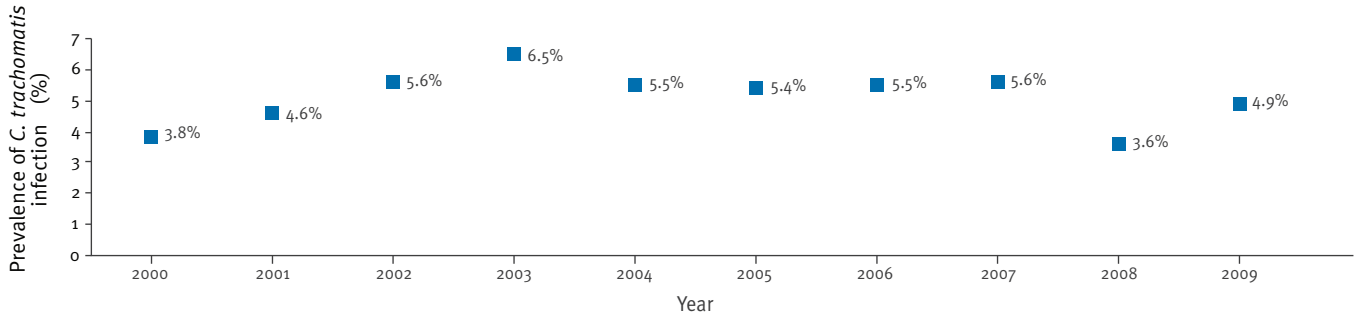
[a] Unless otherwise indicated.

[b] Where relevant. The t-test compares the mean values for women who tested positive for *C. trachomatis* and those who were negative.

[c] Not statistically significant.

Figure

Prevalence of *Chlamydia trachomatis* infection in women tested at the Cervico-Vaginal Pathology Unit, Sapienza University, Rome, Italy, 2000–2009 (n=6,969)



The overall chi-square statistic was 6.255 (the chi-square test for the resulting 2×10 contingency table tested the null hypothesis of no association against the alternative hypothesis of an association of some sort). The p value for the chi-square statistic (p=0.938) was not statistically significant.

Table 2

Univariate analysis of reasons for seeking care, clinical features, contraceptive use and smoker status of 6,969 women attending as outpatients the Cervico-Vaginal Pathology Unit, Sapienza University, Rome, Italy, 2000–2009

Characteristic	Tested for *Chlamydia trachomatis* infection			Odds ratio (95% CI)	P value
	Number positive (%)	Number negative	**Total**		
Reason for seeking care					
Gynaecological	207 (5.3)	3,666	**3,873**	1 Reference	–
Infertility	68 (4.9)	1,331	**1,399**	0.90 (0.68–1.20)	0.4852[a]
Obstetrics	50 (5.3)	889	**939**	1.00 (0.73–1.37)	0.9806[a]
Family planning	41 (5.4)	717	**758**	1.01 (0.72–1.43)	0.9427[a]
Symptoms of *C. trachomatis* infection[c]					
Yes	110 (4.7)	2,239	**2,349**	1.19 (0.95–1.50)	0.1289[a]
No	256 (5.5)	4,364	**4,620**	1 Reference	–
Signs of *C. trachomatis* infection[d]					
Yes	128 (4.8)	2,513	**2,641**	1.14 (0.92–1.42)	0.2362[a]
No	238 (5.5)	4,090	**4,328**	1 Reference	–
Contraceptive use					
None	269 (5.1)	5,025	**5,294**	1 Reference	–
Oral contraceptives	43 (7.3)	546	**589**	1.47 (1.05–2.05)	0.0226
Intrauterine device	20 (5.1)	372	**392**	1.00 (0.63–1,60)	0.9856[a]
Condoms	34 (4.9)	660	**694**	0.96 (0.67–1.39)	0.8370[a]
Smoker					
Yes	120 (6.1)	1,838	**1,958**	1.26 (1.01–1.58)	0.0402
No	246 (4.9)	4,765	**5,011**	1 Reference	–
Total	**366 (5.2)**	**6,603**	**6,969**	–	–

CI: confidence interval.

[a] Not statistically significant.

[b] Dysuria or pelvic pain.

[c] Cervical erythema, inflammation or discharge.

than five), have also been highlighted by research groups worldwide in various populations [7,16,31]. We found that the highest prevalence of infection (10.8%) was associated with a nearly fivefold increased risk of infection (as an independent factor, it showed a two-fold increased risk) in women aged 15–19 years.

Before 2008, *C. trachomatis* control activities in Italy consisted of case management in dermatovenereology clinics with Chlamydia testing for symptomatic people only [7]. C trachomatis testing is currently recommended for women at the time of their first cervical smear test, which takes place when women are 25 years of age in Italy. To the best of our knowledge, no report on the uptake and results of this testing recommendation is yet available. However, our data suggest that women aged under 25 years, and in particular those under 20 years, would be the core population of a good testing policy and a hypothetical *C. trachomatis* screening programme, as in other screening programmes worldwide [7,16]. Thus, the current Italian policy could be ineffective. The high prevalence of infection observed until the age of 40 years – which is a novel aspect of our findings – could also lead to a more extensive testing strategy. Although being aged 25–39 years was not an independent risk factor for infection, our data suggest that older women should also be tested.

Furthermore, as prevalence in women with signs or symptoms of infection did not differ statistically from that in women with no signs or symptoms in this study, case management appears to be an insufficient Chlamydia control activity.

The prevalence of infection among women seeking care for family planning was also high (5.4%): despite the low number of women in our study who sought advice for family planning, given the high number of women who usually attend this type of service and their young age, we suggest that family planning clinics could be sentinel for *Chlamydia* surveillance or an appropriate setting for *Chlamydia* opportunistic screening.

Our data also show that having HPV or *T. vaginalis* infection was associated with a fivefold higher risk of *C. trachomatis* coinfection, as expected in groups at higher risk as a result of age and behaviour [32,33]. In our logistic regression, HPV was not significantly associated with *C. trachomatis* infection, suggesting that age and multiple partners could be possible confounding factors, while *T. vaginalis* infection was an independent risk factor for *C. trachomatis* infection. It is possible that severe inflammation of the cervix due to *T. vaginalis* infection may make the cervix more susceptible to *C. trachomatis* infection. It could therefore be suggested that patients diagnosed with *T. vaginalis* infection should be tested for *C. trachomatis* or even given treatment for *C. trachomatis* infection without being tested, as proposed by Lo et al. [33].

Data on *N. gonorrhoeae* and *C. trachomatis* coinfection in Italy are limited, but our findings on *N. gonorrhoeae* seem to be consistent with those reported in 1998 by a dermatovenereology network, which found that fewer than 1% the infections in 44,438 individuals with sexually transmitted infections were *N. gonorrhoeae* cervical infections [22].

We also found a statistical association of *C. trachomatis* infection with absence of previous pregnancies, use of oral contraceptives and smoking. However, as they were not shown to be statistically associated with infection in the logistic regression final model, age, having multiple lifetime sexual partners and *T. vaginalis* infection are likely to be confounders, in contrast to the findings of others [34-36].

The lack of statistical association between *C. trachomatis* infection and condom use (as a protective factor) is unexpected, given the findings of others [21,37]. This could be considered a result of incorrect condom use and lack of health education. It could also be that some of the women were not entirely truthful when providing details of the type of contraception they used. There are probably some methodological limitations in the epidemiological study of condom effectiveness in

Table 3

Univariate analysis of other sexually transmitted infections in 6,969 women attending as outpatients the Cervico-Vaginal Pathology Unit, Sapienza University, Rome, Italy, 2000–2009

Other sexually transmitted organisms detected	Tested for *Chlamydia trachomatis* infection			Odds ratio (95% CI)	P value
	Number positive (%)	Number negative	**Total**		
Trichomonas vaginalis or HPV[a]	145 (16.9)	714	**859**	5.41 (4.33–6.77)	0.0000
Trichomonas vaginalis	11 (15.7)	59	**70**	4.97 (2.57–9.59)	0.0000
HPV	142 (17.1)	688	**830**	5.50 (4.39–6.89)	0.0000
Neither *Trichomonas vaginalis* nor HPV	221 (3.6)	5,889	**6,110**	1 Reference	–
Total	**366 (5.2)**	**6,603**	**6,969**	–	–

CI: confidence interval; HPV: human papillomavirus.

[a] Women coinfected with *T. vaginalis* and HPV (n=41) are not included.

preventing *C. trachomatis* infection, as has been highlighted by Warner et al. [37].

A new *C. trachomatis* variant was detected in 2006 following an unexpected 25% decrease in the number of infections in a Swedish county [38,39]. As we used the Becton Dickinson ProbeTec – which detects the new variant – the presence or absence of the variant in Italy has no impact on our prevalence data. However, as no data are available on the type and distribution of *C. trachomatis* diagnostic methods used in Italy, nor on whether this variant is present among Italian women, surveillance is also needed to provide such information.

In conclusion, the prevalence and determinants of *C. trachomatis* infection observed in this study seem to highlight the need for a focus on control activities in Italy, with special attention to standardisation of diagnostic tests and women aged under 25 years, who would be the core population of a screening programme.

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