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Urogenital *Chlamydia trachomatis* Serovars in Men and Women with a Symptomatic or Asymptomatic Infection: an Association with Clinical Manifestations?

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To determine whether certain *Chlamydia trachomatis* serovars are preferentially associated with a symptomatic or an asymptomatic course of infection, *C. trachomatis* serovar distributions were analyzed in symptomatically and asymptomatically infected persons. Furthermore, a possible association between *C. trachomatis* serovars and specific clinical symptoms was investigated. *C. trachomatis*-positive urine specimens from 219 asymptomatically infected men and women were obtained from population-based screening programs in Amsterdam. Two hundred twenty-one *C. trachomatis*-positive cervical and urethral swabs from symptomatically and asymptomatically infected men and women were obtained from several hospital-based departments. Serovars were determined using PCR-based genotyping, i.e., restriction fragment length polymorphism analysis of the nested-PCR-amplified *omp1* gene. The most prevalent *C. trachomatis* serovars, D, E, and F, showed no association with either a symptomatic or asymptomatic course of infection. The most prominent differences found were (i) the association of serovar Ga with symptoms in men ($P = 0.0027$), specifically, dysuria ($P < 0.0001$), and (ii) detection of serovar Ia more often in asymptomatically infected people (men and women) ($P = 0.035$). Furthermore, in women, serovar K was associated with vaginal discharge ($P = 0.002$) and serovar variants were found only in women ($P = 0.045$).

Chlamydia trachomatis infections are one of the leading causes of sexually transmitted diseases (STDs) in the United States and Europe. Urogenital infections with *C. trachomatis* in women have a clinical course varying from asymptomatic *C. trachomatis* infections to ascending infections leading to pelvic inflammatory disease associated with late ectopic pregnancy and tubal infertility (15). In men, urogenital *C. trachomatis* infection causes urethritis. Ascending infections (epididymitis) are rarely seen. *C. trachomatis* is an obligate intracellular bacterium, and so far, 19 different serovars have been identified. In addition to these 19 serovars, numerous variants have been characterized (6, 10, 21). Serovars A, B, Ba, and C infect mainly the conjunctiva; serovars D, Da, E, F, G, Ga, H, I, Ia, J, and K are predominantly isolated from the urogenital tract; and serovars L1, L2, L2a, and L3 can be found in the inguinal lymph nodes. Conventional serotyping is performed after *C. trachomatis* culture using polyclonal and monoclonal antibodies against the major outer membrane protein of *C. trachomatis* (1, 24, 25). The recently developed method of direct PCR-based restriction fragment length polymorphism (RFLP) analysis (genotyping of the *omp1* gene, which encodes the MOMP) of cervical and urethral swabs has partially replaced the laborious and less sensitive serotyping technique (8, 14, 21, 27, 28). An increasing number of isolates have been typed worldwide (2, 26, 31, 32, 35), transmission between sexual partners has been studied, and geographical variation in the distribution of serovars has been found (9, 13, 23). Some studies have addressed the possible relationship between particular serovars and clinical manifestations (31, 32). These studies obtained contradictory results both for specific serovars, e.g., F and G, and for lower versus upper genital tract infections (4, 7, 11, 36). Furthermore, only a few studies have been published comparing the *C. trachomatis* serovar distribution in asymptomatically infected persons, which comprise 70% of the *C. trachomatis* infections in women and 50% of those in men, with the serovar distribution in symptomatically infected persons (12).

Recently, commercially available DNA amplification systems were introduced. They can be used successfully for detection of *C. trachomatis* in urine specimens from asymptomatically infected persons (16, 22, 29). Therefore, we have developed *C. trachomatis* typing of urine specimens to determine serovar distribution in asymptomatically infected men and women (20). Comparison of symptomatic and asymptomatic *C. trachomatis* infections at the serovar level could provide a better understanding of the pathogenesis and epidemiology of urogenital *C. trachomatis* infections.

The objective of this study was to compare *C. trachomatis* serovar distributions between symptomatically and asymptomatically infected subjects to investigate a relationship between *C. trachomatis* serovars and the clinical course of infection. Furthermore, a possible association between particular serovars and the occurrence of clinical symptoms was studied.

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TABLE 1. Distribution of *C. trachomatis* genogroups, serovars, and variants in men and women

Serovar	No. (%) of:			
	Women		Men	
	Asymptomatic	Symptomatic	Asymptomatic	Symptomatic
Group B				
D	13 (5.7)	8 (8.0)	1 (1.5)	2 (4.8)
D−	11 (4.8)	9 (9.0)	4 (6.1)	3 (7.1)
E	94 (40.9)	36 (36.0)	32 (48.5)	15 (35.7)
Subtotal	118 (51.4)	53 (53.0)	39 (56.1)	20 (47.6)
Intermediate group				
F	54 (23.5)	18 (18.0)	10 (15.2)	11 (26.2)
G	13 (5.7)	7 (7.0)	5 (7.6)	1 (2.4)
Ga	3 (1.3)	1 (1.0)	0 (0.0)	6 (14.3)
Subtotal	70 (30.5)	26 (26.0)	15 (22.8)	18 (43.1)
Group C				
H	5 (2.2)	4 (4.0)	6 (7.6)	1 (2.4)
I	3 (1.3)	2 (2.0)	1 (1.5)	2 (4.8)
Ia	6 (2.6)	0 (0.0)	3 (4.5)	0 (0.0)
J	12 (5.2)	5 (5.0)	2 (3.0)	0 (0.0)
K	6 (2.6)	7 (7.0)	3 (4.5)	1 (2.4)
Subtotal	32 (13.9)	18 (18.0)	15 (21.1)	4 (9.6)
Variants	10 (4.3)	3 (3.0)	0 (0.0)	0 (0.0)
Total	230 (52.5)	100 (22.8)	66 (15.1)	42 (9.6)

MATERIALS AND METHODS

Specimen collection. Two different *C. trachomatis*-infected populations were used to collect 440 clinical specimens for this study: an asymptomatic population of patients of general practitioners (GPs) participating in a screening program (GP based) and a hospital outpatient-based population (hospital based).

(i) Asymptomatically infected population. Asymptomatically *C. trachomatis*-infected persons (n = 219; 55 men and 164 women) were identified in a *C. trachomatis* screening program in Amsterdam based on urine specimens which were prospectively collected by GPs between May 1996 and October 1997 (30, 33). The screening program included only participants between 15 and 40 years old.

(ii) Hospital outpatient-based *C. trachomatis*-infected population. Included were female cervical and male urethral swab specimens from *C. trachomatis*-infected persons (n = 250; 63 men and 187 women) collected between 1995 and 1999 at an outpatient clinical, an STD clinic, and a regional public health laboratory and between 1995 and 1998 at departments of gynecology and medical microbiology. During the clinical examination, material was collected for *C. trachomatis* detection and participants were questioned about the reason for their visit. Symptomatically infected patients were defined as those presenting with one or more genitourinary clinical symptoms (men, urethral discharge and dysuria; women, abnormal vaginal discharge, spotting, postcoital bleeding, dysuria, lower abdominal pain, dysmenorrhoea, and dyspareunia). Asymptomatically infected patients were defined as persons who did not contact a physician for urogenital complaints but contacted a physician for a variety of other reasons, including a *C. trachomatis*-positive partner or ex-partner, pregnancy control, and *C. trachomatis* testing before intrauterine device insertion.

Twenty-nine symptomatically infected patients (10 men and 19 women) were excluded due to *C. trachomatis*-related oligo-, mono-, or reactive arthritis, multiple infection with other microorganisms (concurrent gonorrhea, *Candida albicans*, genital herpes, primary or secondary syphilis, trichomoniasis, and bacterial vaginosis), missing patient files, eye-related *C. trachomatis* infections, if the reason for presenting to a physician was unknown, and if the reason for physician contact was to exclude STDs because of risky behavior. From the remaining 221 persons, 11 men and 66 women were asymptomatically infected and 42 men and 102 women were symptomatically infected with *C. trachomatis*.

***C. trachomatis* detection and typing.** *C. trachomatis* was detected either by in-house PCR, by LCx (Abbott Laboratories, Chicago, Ill.), or by COBAS AMPLICOR (Roche Diagnostic Systems, Basel, Switzerland). Amplification was performed as described previously (18, 19) or in accordance with the instructions of the manufacturers.

From the original specimens (cervical and urethral swabs and urine specimens) and/or the remaining buffer-sample solutions (from LCs and AMPLICOR), DNA was isolated for typing by using a filter tube-based DNA isolation method (High Pure PCR Template Preparation Kit; Boehringer Mannheim, Mannheim, Germany) as described previously (20).

C. trachomatis typing was performed by amplification of the *omp1* gene (1.1 kb) in a nested PCR using primers NLO and NRO and primers sero1A and sero2A as described previously for cervical and urethral swabs (8, 14, 21) and urine specimens (20). The PCR product was checked on an agarose gel for length. Subsequently, 10 μl of the PCR product was digested using different restriction enzymes. Serovars and variants (21) were identified by their RFLP patterns after polyacrylamide gel electrophoresis.

Statistical analysis. Both patient characteristics (age and gender) and clinical symptoms were analyzed for associations with individual serovars or genogroups. For the latter, *Chlamydia* serovars were classified in the following three groups (referred to as genogroups) based on their nucleotide relatedness and serological reactivity in previous studies (38): the B complex, comprising serovars B, Ba, D, Da, D−, E, L1, and L2; the intermediate genogroup or F/G group, comprising serovars F, G, and Ga; and the C complex, comprising serovars C, H, I, I′, Ia, J, K, and L3. The serovar variants (unidentified PCR-based RFLP patterns) were defined as group 4. For statistical analysis, the chi-square test or the two-tailed Fisher exact test was used. A P value of 0.05 was considered statistically significant.

RESULTS

***C. trachomatis* serovars and asymptomatic versus symptomatic infection.** The distribution of serovars and its relationship with either a symptomatic or an asymptomatic course of infection are shown in Table 1. The *C. trachomatis* serovar distribution in asymptomatically infected persons is for both GP-based and hospital-based persons, since no statistically significant differences were found between these two asymptomatic populations with regard to *C. trachomatis* serovar distribution (data not shown). In both men and women with *C. trachomatis* infection of the urogenital tract, serovars E (39 and 44%, respectively), F (19 and 22%), and D/D− (9 and 12%) were the most common. These serovars accounted for 72 and 74% of the infections in men and women, respectively. The most prominent difference between symptomatic and asymptomatic men was the detection of serovar Ga in 14.3% of the symptomatically infected men versus 0% in the asymptomatically infected men (P = 0.0027). This also resulted in more symptomatically infected (43%) than asymptomatically infected (23%) men in the intermediate genogroup (F-G/Ga)

TABLE 2. Statistically significant differences[a] between *C. trachomatis* infections in men and women with respect to serovar, genogroup, gender, age, symptomatic or asymptomatic course of infection, and clinical symptoms

Variable(s) compared	Patient group, no./total (%)[b]	*P* value
Serovar, serogroup		
Ga	M; Symp, 6/42 (14.3) vs Asymp, 0/66 (0)	0.0027
Ga	F + M; Symp, 7/142 (4.9) vs Asymp, 3/296 (1.0)	0.016[d]
Ia	F + M; Symp, 0/142 (0) vs Asymp, 9/296 (3.0)	0.035
Intermediate	M; Symp, 18/42 (43) vs Asymp, 15/66 (23)	0.033[d]
Specific symptoms of infection		
K	F; Vag. dis., 6/38 (10.3) vs No vag. dis., 7/274 (2.6)	0.002
Ga	M; Dysuria, 6/24 (25) vs No dysuria 0/84 (0)	<0.0001
CT, gender		
Ga	Symp M, 6/42 (14.3) vs Symp F, 1/100 (1.0)	0.0028
Variants	F, 13/330 (3.9) vs M, 0/108 (0)	0.045
Age, 15–19-yr-old	Symp F, 15/97 (15.5) vs Asymp F, 13/225 (5.8)	0.0084

[a] All other associations between *C. trachomatis* serovars or genogroups and a symptomatic or asymptomatic course of infection were not statistically significant.

[b] M, male; F, female; Symp, symptomatic; Asymp, asymptomatic; Vag. dis., vaginal discharge.

($P = 0.033$; Table 1). Furthermore, serovar Ia comprised 3% of the *C. trachomatis* serovars and was found only in asymptomatically infected people. The statistically significant differences in serovar distribution are summarized in Table 2.

Serovars and specific clinical symptoms. Clinical symptoms were analyzed for possible associations with particular *C. trachomatis* serovars for both men and women. Serovar K was associated with vaginal discharge in women ($P = 0.002$), and serovar Ga was associated with dysuria in men ($P < 0.001$). For the possible relationship between serovars or genogroups and the number of clinical symptoms per subject, no statistically significant associations were found. However, a trend was found for both men and women with the lowest average number of symptoms per subject for group C (women versus men: genogroup B, 2.55 [125/53] versus 1.25 [25/20]; intermediate genogroup, 2.62 [68/26] versus 1.50 [27/18]; genogroup C, 2.22 [40/18] versus 1.00 [4/4]; variants, 1.67 [5/3] versus 0 [0/0]).

***C. trachomatis* serovars and gender.** Serovar Ga was found more frequently in symptomatically infected men (14.3%) than in symptomatically infected women (1%) ($P = 0.0028$). Furthermore, variants (defined as unrecognizable PCR-based RFLP patterns after polyacrylamide gel electrophoresis) were found only in women ($P = 0.045$).

Multiple *C. trachomatis* infections. Two symptomatically infected women had multiple *C. trachomatis* infections (serovars E and J and serovars F and A). Since the group with multiple infections could not be analyzed separately due to its small size, it was excluded from further analysis.

Serovars and age. The mean ages of the patients included (between 15 and 40 years old) were 29.0 ± 7.2 years for men and 26.4 ± 6.1 years for women. When 5-year age groups between 15 and 40 years of age (15 to 19, 20 to 24, 25 to 29, 30 to 34, and 35 to 39 years old) were analyzed, women 15 to 19 years old were significantly more often symptomatically infected (15.5%) than asymptomatically infected (5.8%) ($P = 0.0084$; Table 2).

DISCUSSION

For the most prominent *C. trachomatis* serovars (D/D−, E, and F), which comprised 73% of the *C. trachomatis* serovars, no associations were found between serovar, the presence or absence of clinical symptoms, specific clinical symptoms, and gender. However, differences were found for the less frequent *C. trachomatis* serovars Ga, Ia, and K: serovar Ga was associated with dysuria in men, and serovar Ia was detected only in asymptomatically infected men and women. Furthermore, serovar K was associated with the specific clinical symptom abnormal vaginal discharge in women.

Our serovar distribution, with serovars D (D and D−), E, and F the most frequently observed serovars (73%), is in agreement with other studies worldwide and in The Netherlands (31, 32, 34).

In this study, we found associations between *C. trachomatis* serovars and the clinical course of infection (asymptomatic versus symptomatic): serovar Ga was associated with a symptomatic infection in men ($P = 0.0027$). In contrast, Lan et al. (12) found that serovar G (no subdivision into G and Ga was made) seemed to be associated with symptomatic infection in women. Furthermore, serovar Ia was associated with asymptomatically infected men and women ($P = 0.035$). Also, in an other study, serovar I (no subdivision into I and Ia was made) was associated with asymptomatic infections (12). However, the association of serovar D with asymptomatic infections reported in that study could not be confirmed in this study.

In the present study, the association between serovars and specific clinical symptoms was also investigated. We found that serovar Ga was associated with dysuria in men ($P < 0.0001$). These results are in agreement with two previously published studies in which an association was found between serovar G and symptoms in men, although no subdivision into G and Ga was made (4, 12). Furthermore, serovar F was found to be associated with urethritis in that study (4). However, two other studies showed that serovars F and G (no subdivision made) were not associated with symptoms of urethral discharge and dysuria (31, 32) and fewer signs of inflammation among men infected with serovars G and Ga were observed (32). Abnormal vaginal discharge was associated with serovar K in women ($P = 0.002$) in our study. Van Duynhoven et al. (32) reported that infections with serovars D/D−, H, and K seemed to be associated with inflammation, as shown by the presence of 10 or more leukocytes, although the association was not statistically significant. On the other hand, van de Laar et al. (31) reported that serovars from complex C exhibited somewhat less discharge but this was not significant. Also, in our study, persons infected with the serovars in complex C reported fewer symptoms but this was also not statistically significant.

Most studies investigating the association between *C. trachomatis* serovars and clinical symptoms of infection show contradictory results. This can be explained in part by geographical variations and differences in study size and population composition (1, 28). However, one should be aware that the differences found could be related to specific strains that cannot be distinguished at the serovar level. Perhaps genomic comparisons of serovars and strains could resolve the contradictory results found in the literature. Finally, host variation might also

play an important role in the clinical course of infection and should be studied in more detail.

When *C. trachomatis* and gender are compared, three items are worth mentioning. Firstly, in this study, 13 *C. trachomatis* variants (5.6%) with an unrecognizable RFLP pattern were identified. These were all from women ($P = 0.046$). The percentage of variants found in our study is comparable to the 6.5% variants reported in an earlier study by our group (21). However, the true percentage of variants might be much higher, up to 35% for serovar E, if the complete *omp1* gene were sequenced for all isolates (6). Interestingly, 10 out of 13 variants were isolated from asymptomatically infected women. Characterization of our variants by sequencing is in progress to see if specific variants are associated with the clinical course of infection. Secondly, both multiple infections were detected in two (2%) symptomatically infected women (these patients were excluded from further analysis). Other studies reported rates between 1 and 10% (3, 4, 5). However, a recent study detected multiple infections in 50% of men and 57% of women (17). Also, nucleotide sequencing of the *omp1* gene showed higher rates, up to 15% (7, 37). Thirdly, there was an association between symptomatic *C. trachomatis* infections and age in women 15 to 19 years old. This preliminary association should be investigated in more detail.

The differences in *C. trachomatis* serovar distribution between asymptomatically infected women (mostly urine specimens [71%]) and symptomatically infected women (only cervical scrapes) could be biased by the different sampling sites used. However, this is unlikely, since identical serovars were found in cervical and urethral specimens from women as described in an earlier study by our group (20).

In conclusion, for the most prevalent *C. trachomatis* serovars, D, E, and F (73% of the serovars), no association was found with either a symptomatic or an asymptomatic course of infection. However, the less frequently occurring *C. trachomatis* serovars Ga and K were associated with specific clinical symptoms and serovar Ia was associated with asymptomatic *C. trachomatis* infections. It might be worthwhile to further investigate these serovars in connection with the presence or absence of clinical symptoms, possibly by investigation of virulence genes in these specific serovars.

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