

Differences in clinical manifestations of genital chlamydial infections related to serovars

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Objectives: To study the association of serovars of *Chlamydia trachomatis* with clinical manifestations of genital tract infection and socio-demographic characteristics.

Methods: In 1986-88 the *C trachomatis* isolates from 159 heterosexual men and 116 women attending a sexually transmitted disease (STD) clinic were collected and typed accordingly. A medical history was recorded, a physical examination took place and samples were taken for laboratory diagnostics.

Results: Serovars E, F and D were the most common for both men (75%) and women (67%). Men infected with serovars of the C-complex had more often a history of STD ($p = 0.06$). The opposite was demonstrated in women ($p = 0.07$). In addition, women younger than 18 years at first intercourse were more often infected with C-complex serovars ($p = 0.05$). For men, the serovars F/G less often produced symptoms of urethral discharge ($p = 0.01$) than the serovars of the B-complex and C-complex and were less often associated with the presence of 10 or more leukocytes in a Gram-stained smear ($p = 0.04$).

Conclusions: In this study, infections with serovars F and G caused less obvious symptoms and signs of inflammation in men; in women no differences were found in the clinical manifestation of infections with different serovars.

(*Genitourin Med* 1996;72:261-265)

Keywords: *Chlamydia trachomatis*; clinical manifestations; serovars; epidemiology

Introduction

Chlamydia trachomatis (CT) infections are considered to be one of the most prevalent sexually transmitted infections today, causing severe sequelae, especially in women. Infection of the genital tract may cause a broad spectrum of clinical manifestations.¹ Strain-specific differences in virulence may account for such diverse clinical outcomes of a chlamydial infection. Currently, 18 different serotypes of *C trachomatis* have been identified.^{2-4,5} The serovars A, B, Ba and C are mainly isolated in trachoma, although B and Ba are detected in genital specimens as well.⁶ The serovars L1, L2, L3 cause human lymphogranuloma venereum. The most common serovars in genital infections are D, E and F; the serovars G, H, I, J and K are found less often.^{1,2,6-14} Geographical and temporal variation in the distribution of serovars exist.^{6,9,15,16} Because of the technical difficulty in serotyping large numbers of isolates, the association between clinical manifestations and chlamydial serovars could not be studied easily. Recently, the polymerase chain reaction (PCR) has been applied to differentiate chlamydial isolates into serovars and enables larger studies. Using both a panel of serovar-specific monoclonal antibodies and PCR genotyping we studied the prevalence of chlamydial serovars in a population attending a sexually transmitted diseases (STD) clinic. The association of serovars with symptoms, clinical manifestations, socio-demographic and behavioural characteristics was also determined.

Population and methods

Specimen collection A sample of 1173 male and 648 female visitors of the STD clinic in Amsterdam participated in this study between September 1986 and December 1988. Patients who were willing to sign informed consent and who had not been using antibiotics in the six weeks prior to their visit were included in the study. Participants were questioned on demographic characteristics, past and current sexual history, and genitourinary complaints. During the clinical examination, material was collected for laboratory tests on the presence of *C trachomatis* and other STD as previously described in this journal.¹⁷ The specimens for diagnosis of infection with *C trachomatis* were taken from several sites (women: urethral, cervical and rectal; men: urethral and rectal only with a history of homosexual contact; in both men and women: pharyngeal only when there was a history of oral sex).

Typing of clinical isolates After collection, the swab samples for cell culture were placed in 2 ml of chlamydial transport 4-SP medium at 4°C and transported to the laboratory of the Public Health Service within four hours. Material was stored at -70°C until cultured. Monolayers of HeLa 229 cells were used for the isolation of *C trachomatis*. All monolayers were pretreated with DEAE-dextran. No further passages were performed. After two or three days the cells were fixed and stained with fluorescein-labelled monoclonal antibodies to assess the presence of *C trachomatis*.^{18,19} All

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Accepted for publication
17 May 1996

positive cultures were confirmed at the Laboratory of Virology of the National Institute of Public Health and Environmental Protection. Subsequently, each isolate was passaged serially until more than 75% of the cells were infected. They were stored at -70°C until further processing.^{19,20} The first half of the isolates was typed using monoclonal antibodies as previously described.²⁰ The remaining isolates were genotyped by restriction fragment polymorphism analysis of amplified *omp 1* gene by PCR directly performed on the stored isolates.²¹⁻²³ Both techniques proved to be in complete agreement as was evaluated in a subset ($n = 93$) of the isolates described in this study.²³ The chlamydial isolates were classified in three groups on the basis of their genetic relatedness and serological cross-reactivity.^{2,24} The B-complex contains the serovars B, Ba, D, E, L1 and L2; the intermediate F/G group contains the serovars F and G; the C-complex contains serovars A, C, H, I, J, K and L3.

Statistical analysis Univariate analyses were carried out to identify predictors for infection due to different chlamydial serovars by using the χ^2 test and the two-tailed Fisher exact test. The analyses were done for the three serogroups for heterosexual men and women separately. The chlamydial isolates were evaluated per site of infection and the corresponding genital manifestation. In comparing the clinical signs and symptoms, the chlamydia patients with concurrent STD like gonorrhoea, genital herpes, bacterial vaginosis and trichomoniasis were excluded from the analysis. Variables associated with a chlamydial serovar or group of serovars ($p \leq 0.05$) were analysed by logistic regression analysis.

Results

Prevalence of chlamydial isolates The prevalence of *C trachomatis* infections in the STD population was 14.5% in men (170/1173) and 21.5% in women (139/648). The isolates from 11 men and 23 women could not be propagated; originating from 159 men and 116 women, 160 and 212 isolates were available for typing. For 97% (154/159) of the *C trachomatis* infected men the isolates were retrieved from the urethral site only, in five men from the rectum as well. In 36% (42/116) of the *C trachomatis* infected women the isolates could be obtained for typing from one site (cervix (39), urethra (3)); in 46% (53/116) women from two sites (cervix with urethra (41) or rectum (10) or pharynx (2)), in 17% (20/116) from three sites (cervix, urethra with rectum (18) or pharynx (2)) and in 1% (1/116) from all four sites. Infection with mixed serovars was detected in six (5%) women (five cervix/urethra; one cervix/urethra/rectum) and one (1%) man (urethra/rectum).

Distribution of serovars The serovars E (33%), F (21%) and D (16%) were the most common serovars in urogenital tract infection due to *C trachomatis* accounting for 75% and 67% of the infections in men and women, respectively. The distribution of serovars per

Table 1 Distribution of chlamydia serovars in women and men

Serogroup	Serovar	Females no (%)	Males no (%)
No infected individuals		116	159
No isolates		212	160
B-complex	Ba	1 (0)	1 (0)
	D	29 (14)	28 (18)
	E	63 (30)	61 (38)
F/G group	F	48 (23)	31 (20)
	G	26 (12)	12 (7)
C-complex	H	16 (8)	8 (5)
	I	4 (2)	—
	J	16 (8)	17 (10)
	K	9 (4)	2 (1)

gender is shown in table 1. For further analysis the serovars are grouped together in the B-complex, the F/G-group, and the C-complex. Subsequently, three women and one man were excluded because they had multiple infections with serovars from different subgroups. There were no differences in the distribution of serogroups between men and women ($p = 0.16$).

The association of serovars with demographics and clinical manifestations Various demographic and behavioural characteristics were analysed for a possible association with the infecting serogroup. Men and women in the three groups were comparable with respect to age, nationality, sexual preference, the number of sex partners (in last month, in last six months, lifetime), having had anal or orogenital sex in the past six months, and concurrent gonorrhoea; for women the current use of oral contraception and working as a prostitute were also equally distributed among the three groups. A history of STD was slightly more frequent among men in the C-complex ($p = 0.06$) and less frequent among women in the C-complex ($p = 0.07$). In addition, a sexarache of 18 years or younger was more frequent among women in the C-complex ($p = 0.05$).

Table 2 shows the association between the clinical manifestation, laboratory data and the serogroups. For men, the analysis was restricted to urethral infections in heterosexual men ($n = 119$). For women, the cervical ($n = 78$) and urethral ($n = 54$) isolates were studied in relation to site-specific manifestations separately. Coinfections with gonorrhoea, genital herpes, bacterial vaginosis and trichomoniasis were excluded from these analyses. Men infected with serovars F/G less often reported urethral discharge as compared with the other serogroups ($p = 0.01$). No difference was observed between the serovar F or G (33% vs 37%). The same was found (but not significant) for dysuria. Significantly less F/G infections showed 10 or more leukocytes in a Gram-stained smear from the urethra in comparison with infections from the B and C complex ($p = 0.04$). This could not be explained by recent micturition (< 2 hours) as no differences were observed between the serogroups. In a multivariate regression model including urethral discharge and the number of leukocytes the odds ratio (OR) remained significant; OR discharge = 0.27 (95% CI 0.11-0.66); OR leukocytes = 0.23 (95% CI 0.06-0.84). Thus, serovars F and G produce

Table 2 Clinical signs/symptoms in relation to chlamydial serovars for men and women attending an STD clinic, in absolute numbers and in percentages per serogroup

Characteristic	No (%) with characteristic			P
	B-complex	C-complex	F/G group	
Heterosexual men: urethral isolates (n = 119)				
<i>Symptoms</i>				
dysuria	35 (49)	8 (50)	10 (31)	ns
urethral discharge	45 (63)	11 (69)	11 (34)	0.01
<i>Signs of infection</i>				
discharge upon examination	54 (76)	15 (94)	24 (75)	ns
> 10 leukocytes urethral smear	65 (94)	15 (94)	24 (78)	0.04
> 10 leukocytes urethral smear or urine	71 (100)	16 (100)	31 (97)	ns
Women: cervical isolates (n = 78)				
<i>Symptoms</i>				
vaginal discharge	15 (45)	5 (29)	11 (39)	ns
burning/scratching	9 (27)	3 (18)	6 (21)	ns
lower abdominal pain	5 (15)	3 (18)	1 (4)	ns
"smelly" discharge	5 (15)	1 (6)	5 (18)	ns
<i>Signs of infection</i>				
swabtest*	10 (30)	1 (6)	7 (25)	ns
(muco)purulent exudate	21 (64)	13 (81)	16 (59)	ns
induced cervical bleeding	17 (52)	3 (18)	8 (29)	0.02
ectopy cervix	25 (76)	12 (71)	21 (75)	ns
> 10 leukocytes cervical smear	25 (78)	14 (82)	24 (86)	ns
Women: urethral isolates (n = 54)				
<i>Symptoms</i>				
dysuria	0 (0)	1 (8)	1 (5)	ns
frequent urination	0 (0)	1 (8)	2 (10)	ns
<i>Signs of infection</i>				
> 10 leukocytes urethral smear	8 (38)	9 (62)	8 (42)	ns

*Swabtest is the visualisation of yellow mucopurulent endocervical secretions on a white swab (Brunham RC, Paavonen J, Stevens CE, *et al.* Mucopurulent cervicitis—the ignored counterpart in women of urethritis in men. *N Engl J Med* 1994;311:1–6).

significantly less obvious symptoms and signs of inflammation in men than other serovars.

Symptoms of vaginal discharge were reported by 40% of the infected women. Those infected with C-complex serovars exhibited somewhat less vaginal (smelly) discharge, though no significant association was found. Easily induced cervical bleeding was found slightly less frequent among women infected with C-complex serovars ($p = 0.07$). Women with urethral infections rarely reported symptoms; because of small numbers no significant differences were apparent. Overall, women in the three serogroups were comparable in terms of symptoms, clinical manifestation and number of leukocytes on Gram staining.

Discussion

The serotyping of chlamydial isolates enabled us to examine the assumption that the diverse clinical outcomes of chlamydial infection are influenced by the intrinsic virulence of the infecting strain. Our results indicate that infections in men caused by serovars F and G are associated less often with symptoms of urethral discharge and a lower number of leukocytes counts in the urethral smear than those caused by serovars of the B-complex or C-complex. For men, the serovars F and G are correlated with leukocytes counts of less than ten per field in a Gram-stained smear of the urethra. If the number of leukocytes was evaluated also in the first catch urine, the differences between the serogroups disappeared. Our results differ from the observations of Batteiger *et al* with respect to urethritis in men as a relatively high rate of serovar F was found to be associated with urethritis.¹⁰ However, the main conclusion of that study was that no serovar was strongly associated with acute inflammatory response.¹⁰

For women no significant association of serovar with genital manifestations was found. This may be due to the absence of symptoms in 45% of the infected women and the large heterogeneity in clinical presentation causing low numbers per variable. In a comparable study among 155 women attending an STD clinic by Workowski and colleagues, the serovars F/G were found to exhibit significantly fewer signs of infection, including induced cervical bleeding, visible mucopus and mucopurulent endocervical discharge.¹⁴ In addition, serovar F produced significantly less signs of infections than serovar E.¹⁴ Our results agree with observations of Batteiger *et al* that cervicitis was found equally among infection with different serovars.¹⁰ For women no differences in serovars were found for urethral or cervical leukocytes counts. On the contrary, Workowski *et al* found that the F/G group in women is correlated with lower leukocytes counts on endocervical Gram staining.¹⁴ In another study fewer F/G serovars were found among infections with 0–3 leukocytes and more F/G with 10 or more leukocytes.¹⁰ Again, no consistent pattern appears from these studies regarding the association between leukocytes and serovars. The observed discrepancy between the results of these studies cannot be explained entirely by methodological differences because all chlamydial isolates were identified by cell culture and subsequent serotyping. In other studies no relationship were found between clinical manifestations and different serovars.^{8,9,12,25} For homosexual men a correlation was found between severe proctitis and lymphogranuloma venereum serovars in the rectum.²⁶ The value of the observations that serovars F/G are associated with less symptoms than the other serogroups in heterosexual men (this study) or women¹⁴ need to be confirmed consistently in time and geographic area before the association can be accepted.

Table 3 The distribution of serovars of genital infections due to *Chlamydia trachomatis* as reported in several studies

Setting	Percentage of <i>C trachomatis</i> serovars											ref
	*	†	B/Ba	D	E	F	G	H	I/Ia	J	K	
STD clinic, Netherlands	1	374	0.5	15.5	33.1	21.4	10.2	6.4	1.1	8.8	2.9	our study
STD clinic, Netherlands	1	190	0.5	17.9	23.7	20.5	2.1	11.1	3.2	6.3	14.7	15
STD clinic, Germany	1	56	—	28.6	35.7	26.8	3.6	—	—	—	5.4	11
STD clinic, USA	1	224	1.3	23.0	26.0	18.0	3.1	6.3	12.0	3.1	7.1	10
STD clinic, USA	1	1515	0.3	14.6	32.0	20.3	1.7	1.9	9.4	10.4	5.7	27
Clinical isolates, France	1	53	5.6	5.6	62.3	9.4	1.9	3.8	—	5.6	1.9	28
Hospital, Finland	1	54	2.0	5.9	25.5	9.8	3.9	5.9	5.9	7.8	7.8	8
?, France	1	203	1.0	5.9	51.7	17.3	8.4	3.9	0.5	3.9	2.9	13
Various clinics, Sweden	1	1040	1.0	13.0	38.4	24.0	3.8	2.0	0.8	4.5	7.9	29
STD clinic, USA	3	314	—	18.0	32.0	18.0	3.0	2.0	5.0	—	4.0	9
STD clinic, USA	3	99	—	13.0	35.0	26.0	1.0	1.0	5.0	10.0	6.0	14
Gynaecology, Sweden	3	424	0.2	13.0	40.0	25.0	5.0	4.0	0.7	6.0	6.0	12
STD clinic, internal care	4	493	3.5	14.2	10.6	16.6	3.9	6.3	7.1	3.3	5.9	6
STD clinic, USA	2	150	—	53.3	6.7	13.3	13.0	—	—	—	—	9

*Type of population: 1 heterosexual man and woman; 2 homosexual men only; 3 women only; 4 men, women and infants.

†Number of strains serotyped.

Additional serovars (not in table):

ref 9 (rectal) :CJ 12.0

ref 6 :ED 19.9%; BED 1.8%; GF 4.1%; CJ 2%

ref 8 :ED 25.5%

ref 13 :C 1%; Dv 2.9%.

The distribution of *C trachomatis* serovars in genital tract infections was determined in several other studies (table 3). Our results (with serovars E, F and D as the most prevalent) are in agreement with a predominance of the serovars E (range: 23–62%), F (range: 10–27%) and D (range: 6–29%) among heterosexual populations. These findings indicate that a large proportion of the genital infections is caused by a small number of serovars and that geographical variance exists.^{6 9–11 13–15 27–29} To improve the epidemiological insight in transmission patterns, a more thorough discrimination within the groups of serovars by using different microbiological techniques is required.

Infection with multiple serovars at different anatomical sites was detected in six women (5%), which is similar to the percentage found by Workowski *et al*¹⁴ but higher than the 2% (of 352 isolates) found by Barnes *et al*.³⁰ In the study by Barnes *et al*, multiple serovars were detected in respectively 1.4% and 10% of the cervical isolates from women attending an STD clinic and from women detained in jail, which could be due to exposure to various serovars through multiple sex partners.³⁰ In a previous Dutch study mixed serovars were found in two highly sexually active individuals (1%) with multiple partners in the month preceding testing.¹⁵ In our study no relationship of mixed infections with the number of partners was found.

In conclusion, in our study the serovars F and G caused significantly fewer symptoms of urethral discharge in men, but no differences were found in the clinical manifestation in women for different serovars. At present, various studies have demonstrated that no differences have been consistently observed for the different serovars of *C trachomatis* in causing genital infection in heterosexual men and women.¹² The high rate of asymptomatic infection, the heterogeneity in clinical presentation and, as a consequence, the small number of each subgroup may counteract (or hide) the specific associations. This indicates the need for larger epidemiological and clinical studies. Because it cannot be ruled out that by

cell culture a selective growth of particular *C trachomatis* serovars might occur, future studies could be initiated using PCR genotyping of serovars which has been successfully applied in clinical specimens.²² Furthermore, to evaluate the pathogenic potential of the different *C trachomatis* strains and the observed differences in clinical signs/symptoms a more advanced technique such as fingerprinting is required.

We thank the medical staff of the STD clinic in Amsterdam for the cooperation in the study and Anke de Vries and Renate Derksen of the National Institute of Public Health and Environmental Protection for the serotyping of *C trachomatis* isolates.

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