

CHLAMYDIA

Analysis of rectal *Chlamydia trachomatis* serovar distribution including L2 (lymphogranuloma venereum) at the Erasmus MC STI clinic, Rotterdam

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Objectives: Compared to urogenital infections, little is known of serovar distribution in rectal chlamydial infection. The aim of this study was to explore possible relations between demographics, sexual behaviour, clinical manifestations, rectal symptoms, and chlamydial serovars including L2 (lymphogranuloma venereum).

Methods: Genotyping was done prospectively in all rectal chlamydial infections since the outbreak of proctitis caused by lymphogranuloma venereum in February 2003. 33 (15.1%) rectal *Chlamydia trachomatis* infections from the years 2001 and 2002 were genotyped retrospectively.

Results: Of all 219 rectal chlamydial infections, detected in the period July 2001 to August 2005, a total of 149 (68.0%) were successfully genotyped including 21 (14.1%) infections with serovar L2. In univariable and multivariable analyses, L2 serovar positive patients were significantly more often HIV positive ($p=0.002$; OR: 6.5; 95% CI: 2.0 to 21.1), and had had sex in the past 6 months with more partners compared to other serovars. Furthermore, patients with L2 proctitis presented far more often with self reported rectal symptoms ($p<0.005$; OR: 19.4; 95% CI: 4.9 to 77.0) and clinical manifestations ($p<0.005$; OR: 15.4; 95% CI: 4.5 to 52.5).

Conclusions: Chlamydial infections with serovar L2 show a different clinical and epidemiological pattern compared to serovar D-K. LGV proctitis is significantly associated with HIV positivity and a high number of sexual partners and causes more rectal symptoms and clinical manifestations. Neither young age nor ethnicity were identified as risk factors for any of the serovars investigated in this study.

It is estimated that 60 000 cases of chlamydial infections annually occur in the Netherlands, making *Chlamydia trachomatis* one of the most common sexually transmitted infections (STIs).¹ International and national epidemiological studies on chlamydial infection demonstrated that young age, ethnicity, degree of urbanisation, and number of sex partners are important risk factors.^{1–3} Other studies evaluated the relation between the infecting *C trachomatis* serovar and clinical manifestations or demographic factors.^{4–6}

The species *C trachomatis* comprises 15 serovars, serovars A–L, and additional variants. The results of different studies on the relation between serovars and clinical manifestations or demographic factors are inconclusive and comparison of the different populations involved is difficult. Recently, important differences between serovar distribution in Amsterdam and Rotterdam were found. Differences in ethnic composition between these two large Dutch cities was considered a possible explanation.⁷

Compared to urogenital infections, little is known of serovar distribution in rectal chlamydial infection.^{2 4 8 9} Serovars D–K have not consistently been investigated together with *C trachomatis* serovar L2. Since the outbreak of lymphogranuloma venereum (LGV) proctitis among Dutch men who have sex with men (MSM) in February 2003, genotyping of all rectal chlamydial infections has been routinely carried out prospectively at the STI clinic of the Rotterdam Erasmus MC.¹⁰ Furthermore, polymerase chain reaction (PCR) positive rectal chlamydial specimens from the period July 2001 to January 2003 were genotyped retrospectively. The aim of this study was to explore the possible relations between demographics, sexual behaviour, clinical manifestations, rectal symptoms, and chlamydial serovars including L2 (lymphogranuloma venereum).

MATERIALS AND METHODS

Study population and study design

The study was conducted at the STI clinic of the Department of Dermatology and Venereology, Erasmus MC Rotterdam, Netherlands. From July 2001 to August 2005, a total of 219 rectal chlamydial infections were detected. Genotyping was done prospectively in all rectal chlamydial infections detected at our STI clinic since the outbreak of proctitis caused by lymphogranuloma venereum in February 2003.¹⁰ Thirty three (15.1%) samples of rectal *C trachomatis* infections from the period July 2001 to January 2003 were genotyped retrospectively. All patients attended the STI clinic on their own initiative because of symptoms related to STI or sexual risk behaviour. Patients underwent a standardised venereological examination as described previously.¹¹ In brief, blood samples were drawn to test for HIV antibodies (microparticle enzyme immunoassay AxSym HIV-1/HIV-2 reagents; Abbott, Santa Clara, CA, USA), syphilis (*Treponema pallidum* particle agglutination (TPPA) test; Serodia-TPPA, Fujirebio Inc, Tokyo, Japan) and hepatitis B (anti-HBc and HBsAg, microparticle enzyme immunoassay IMX; Abbott, Santa Clara, IL, USA). The examination also included testing for urethral gonorrhoea (GC-Lect agarplates; Becton & Dickson Europe, Meylan, France) and urethral or cervical *C trachomatis* infection (Cobas Amplicor PCR system, Roche Diagnostics, Almere, Netherlands). When receptive anal sexual contact had taken place in the last 6 months or when rectal

Abbreviations: LGV, lymphogranuloma venereum; MOMP, major outer membrane protein; MSM, men who have sex with men; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; STIs, sexually transmitted infections; TPPA, *Treponema pallidum* particle agglutination

Table 1 Serovar distribution in patients with rectal *Chlamydia trachomatis* infection attending the Erasmus MC STI clinic

Serovar	Males*		Females		Total (%) (n = 218)
	Homosexual (%) (n = 136)	Bisexual (%) (n = 19)	Heterosexual (%) (n = 58)	Bisexual (%) (n = 5)	
D	32 (23.5)	6 (31.6)	4 (6.9)	–	42 (19.3)
E	3 (2.2)	–	6 (10.3)	–	9 (4.1)
F	3 (2.2)	–	12 (20.7)	–	15 (6.9)
G	25 (18.4)	3 (15.8)	4 (6.9)	–	32 (14.7)
H	17 (12.5)	2 (10.5)	4 (6.9)	1 (20.0)	24 (11.0)
I	1 (0.7)	–	1 (1.7)	1 (20.0)	3 (1.7)
K	–	–	3 (5.2)	–	3 (1.7)
L2	19 (14.0)	2 (10.5)	–	–	21 (9.6)
Untyped	36 (26.5)	6 (31.6)	24 (41.4)	3 (60.0)	69 (31.7)

*One self identified heterosexual male with serovar "untyped" was not included in this table.

symptoms possibly related with STIs were reported, rectal testing for gonococcal and chlamydial infection was done as well. Rectal tests for gonococcal and chlamydial infection were performed as a routine in all MSM.

Data collection

Demographic and sexual behavioural data were collected. These included sex, age, ethnic background, residence, sexual orientation, number of sexual partners during the last 6 months, practice of anal intercourse, use of condoms in anal sex, and earlier diagnoses of STI including HIV infection. All reported rectal symptoms (itch, pain, discharge, discomfort, tenesmus, loss of blood) and clinical manifestations (perianal erythema, discharge, loss of blood) possibly related to the chlamydial infection were registered. When proctitis caused by lymphogranuloma venereum was suspected, proctoscopy was performed.

Genotyping

To detect *C trachomatis* DNA in clinical specimens, the automated *C trachomatis* Cobas Amplicor PCR system was used throughout the study, in accordance with the manufacturer's instructions.

Rectal swabs were collected in 2SP medium and subsequently used for PCR testing. Genotyping of the gene encoding the major outer membrane protein (MOMP) was performed by nested PCR and restriction fragment length polymorphism (RFLP) analysis.^{10–12, 13} Results were reported as one of the 15 "classic" *C trachomatis* serovars. When specimens could not be genotyped by nested PCR and RFLP analysis, the serovars were addressed as "untyped."

Serovar distribution analysis

In the serovar distributions comparison, we classified non-LGV *C trachomatis* serovars in three serogroups: the B group (serovars D and E), the intermediate group (serovars F and G), and the C group (serovars H, I, and K).¹⁴ Serovar L2 was considered as a separate serogroup in the serovar distributions comparison.¹⁵

Statistical methods

Univariable and multivariable logistic regression analyses were used to investigate the association between demographics, sexual behaviour, clinical manifestations, rectal symptoms and serogroup. People with serogroup B were the reference group, and men with serovar L2 were analysed separately.⁴ A test was considered significant if the p value was less than 0.05. All statistical analyses mentioned in this study were done using SPSS for Windows, version 12.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

From July 2001 to August 2005, a total of 219 rectal chlamydial infections were genotyped. In all, 156 (71.2%) patients were males and 63 (28.8%) were females. The median age of the patients was 31 years (interquartile range 26–41); 70.9% were of native Dutch descent, and 59.3% lived in the city of Rotterdam. A total of 136 (87.2%) males identified themselves as homosexual, and 12.2% described their sexual orientation as bisexual. One man stated to be exclusively heterosexual. Of the 63 female patients, 58 (92.1%) were heterosexual and five (7.9%) were bisexually orientated. The median number of sexual partners during the last 6 months was three (interquartile range 2–6) in all patients, five (interquartile range 2–10) in homosexual males, and two (interquartile range 1–3) in heterosexual women.

With respect to HIV serostatus, 29 males were known to be HIV positive, of whom 26 (89.7%) were homosexual and three (10.3%) bisexual. No female patients were known to be HIV positive. In 13 males, a new HIV infection was detected; nine (69.2%) of these men were homosexual and four (30.8%) were bisexually orientated.

An earlier diagnosis of STI was reported by 110 (70.5%) men and 23 (36.5%) women. The STI most often reported in the past was infection with *C trachomatis* in both men and women. Of all patients who reported STI in the past, 50.9% of the men and 52.2% of the women had had a chlamydial infection. The second most often reported STI in the past was infection with *Neisseria gonorrhoeae*, both in men (46.4%) and women (26.1%). Syphilis was the third most often reported past STI in 36.4% of the men and 17.4% of the women.

The practice of anal sex in the previous 6 months was reported by 89.5% of males and 95.1% of females. Of all male patients who reported anal sex in the last 6 months, 12 (8.8%) stated to have had only insertive anal sex in the last 6 months; 15.3% of males who had had anal sexual intercourse "never" or "seldom" use condoms compared to 65.0% of women. Of the male patients, 58.7% said they "always" or "mostly" used condoms during anal sex compared to 25.0% of the female patients.

Of all patients, three men and four women worked as prostitutes in the last 6 months. No males had sex with a prostitute in the last 6 months.

Table 1 summarises the serovar distribution in the patients investigated in the prospective study. The serovars most often detected in rectal chlamydial infections in men were serovar D, G, and L2. In women, serovars most often detected in rectal chlamydial infections were serovar F, E, and H. Serovars A, B, Ba, C, J, L1, and L3 were not detected. All patients with serovar L2 were men, 19 (90.5%) of whom were homosexual and two (9.5%) bisexual.

Table 2 Demographic, sexual behaviour characteristics, and serogroups in patients with rectal *Chlamydia trachomatis* infection attending the Erasmus MC STI clinic

	B group (n = 51)	Intermediate group (n = 47)	C group (n = 30)	L2 (n = 21)	Untyped (n = 70)
	%	%	%	%	%
Age (median, IQR; in years)	32 (27–43)	30 (24–39)	30 (25–41)	39 (37–44)	30 (25–38)
Ethnicity (Dutch)	72.0	68.1	70.0	81.0	69.2
Sexual orientation†					
Male homosexual	66.7	56.6	60.0	90.5*	52.9
Male bisexual	9.8	6.4	6.7	9.5	10.0
Female heterosexual	21.6	34.0	26.7	0	32.9
Female bisexual	2.0	0	6.7	0	2.9
Number of sexual partners‡					
0–1	22.9	21.3	22.2	9.5	28.4
2–4	45.8	38.3	33.3	19.0*	46.3
5–10	18.9	23.4	25.9	42.9*	13.4
More than 10	12.5	17.0	18.5	28.6	11.9
Practice of anal sex‡	89.8	93.3	96.7	95.2	87.0
Insertive anal sex only‡	6.7	2.3	10.0*	0	8.3
Receptive or both receptive and insertive anal sex‡	93.3	97.7	90.0	100	91.7
Condom use in anal sex ((almost) always)‡	40.0	51.1	58.6	70.0*	43.9
Positive history of STI	68.6	55.3	53.3	90.5	52.9
Concomitant rectal gonorrhoea	11.8	17.0	16.7	19.0	11.4
Newly detected HIV infection	7.8	2.1	3.3	14.3	5.7
Known to be HIV positive	11.8	8.5	6.7	47.6**	10.0

* $p \leq 0.05$ compared to individuals with B group serovars. ** $p \leq 0.01$ compared to individuals with B group serovars.

†One self identified heterosexual male with serovar “untyped” was not included in this row of the table.

‡As reported during the last 6 months.

Of all 29 males known to be HIV positive, 10 (34.5%) had serovar L2. Table 2 summarises demographic, sexual behaviour characteristics, concomitant rectal gonorrhoea, HIV infection, and serogroups in patients with rectal *C trachomatis* infection.

Symptoms were reported by 52 (23.7%) of all patients. Table 3 summarises reported symptoms in relation to the serogroups. Infection with *C trachomatis* serovar L2, caused symptoms in 86% of patients. The intermediate group and C group caused symptoms in 7% and 5% of the patients, respectively. B group and untyped rectal chlamydial infections brought about symptoms in 20–25% of patients infected. The symptom most often reported was rectal discharge (61.9% of serovar L2 infections and 4.3% to 15.7% in other groups). Rectal bleeding was reported by more than half (52.4%) of the LGV patients and by 11.8% of the B group individuals.

Clinical manifestations were observed in 33 (15.1%) of all patients. Serovar L2 caused clinical manifestations in 71.4%

of patients. Most frequent manifestations seen in L2 proctitis were discharge and perianal erythema in 61.9% and 57.1% of the patients, respectively. More than half the patients (52.4%) infected with L2 had visible loss of blood.

Group C, intermediate, and untyped serovars caused clinical manifestations in less than 9% of patients infected. Local erythema at the anus was seen most often. In group B, perianal erythema was observed in 11.8% of individuals.

All non-LGV *C trachomatis* infections were treated with azithromycin 1 g orally in a single dose and (suspected) LGV infections with doxycycline 100 mg orally twice daily for 3 weeks. Post-treatment swabs were only taken in those with LGV. All infections with LGV cleared after treatment with doxycycline.

Univariable and multivariable analyses

The possible association between demographics, sexual behavioural data, rectal symptoms, clinical manifestations

Table 3 Self reported symptoms, clinical manifestations and serogroups in patients with rectal *Chlamydia trachomatis* infection attending the Erasmus MC STI clinic

	B group (n = 51)	Intermediate group (n = 47)	C group (n = 30)	L2 (n = 21)	Untyped (n = 70)
	%	%	%	%	%
Self reported symptoms					
Itch	3.9	2.1	6.7	0	7.1
Pain	5.9	2.1	0	28.6*	10.0
Discharge	15.7	6.4	10.0	61.9**	4.3*
Discomfort	0	0	0	23.8	1.4
Tenesmus	2.0	0	3.3	19.0*	1.4
Loss of blood	11.8	2.1	0	52.4**	4.3
None	76.5	91.5	90.0	14.3**	78.6
Clinical manifestations					
Perianal erythema	11.8	6.4	0	57.1**	8.6
Discharge	5.9	0	6.7	61.9**	2.9
Loss of blood	2.0	0	3.3	52.4**	1.4
None	86.3	91.5	93.3	28.6**	91.4

* $p \leq 0.05$ compared to individuals with B group serovars. ** $p \leq 0.01$ compared to individuals with B group serovars.

and serogroup including L2 and “untyped” serovars was explored.

Nearly all L2 serovar positive patients were homosexual males (87.2%; $p = 0.052$; OR: 4.8; 95% CI: 1.0 to 22.8) who were more often HIV positive ($p < 0.0005$; OR: 11.3; 95% CI: 3.5 to 36.7), and had had sex in the last 6 months with more partners ($p = 0.009$; OR: 2.0; 95% CI: 1.2 to 3.5). However, these serovar L2 positive patients said they had used condoms in anal sexual contacts more often ($p = 0.027$; OR: 3.5; 95% CI: 1.2 to 10.6).

In multivariable analyses, both “known to be HIV positive” ($p = 0.002$; OR: 6.5; 95% CI: 2.0 to 21.1) and number of sexual partners in the last 6 months ($p = 0.054$; OR: 2.0; 95% CI: 1.0 to 3.9) remained independently associated with L2 proctitis.

Patients with L2 proctitis presented far more often with self reported rectal symptoms ($p < 0.005$; OR: 19.4; 95% CI: 4.9 to 77.0) and clinical manifestations ($p < 0.005$; OR: 15.4; 95% CI: 4.5 to 52.5). Symptoms such as pain, discharge, tenesmus, and rectal bleeding were more often reported in these patients. At physical examination, all symptoms (perianal erythema, discharge, and loss of rectal blood) were detected more often. Intermediate serogroup patients presented less often with rectal symptoms ($p < 0.012$; OR: 0.19; 95% CI: 0.1 to 0.7). Rectal discharge was reported less often in patients with untyped serovars ($p = 0.043$; OR: 0.23; 95% CI: 0.1 to 1.0).

There was no association between age, ethnic background, practice of anal sex, history of STI, and any serogroup.

DISCUSSION

The aim of this study was to explore the possible association between demographics, sexual behaviour, clinical manifestations, rectal symptoms, and infecting chlamydial serovar including L2 (lymphogranuloma venereum). The vast majority (70.8%) of patients attending the Rotterdam STI clinic with rectal chlamydial infections consisted of MSM.

The serovars most often detected in rectal chlamydial infections in this study were serovars D (19.3%) and G (14.7%). These data are in accordance with chlamydial serovar distributions in MSM attending the STI clinic in Seattle during the period 1994 to 1999. However, in Seattle, rectal chlamydial infections were more often caused by serovar G (47.9%) compared to D (29.6%). Of all MSM in Seattle, 13% were infected with *C trachomatis*, serovar J.⁹ This serovar was not detected in Rotterdam MSM.

In a longitudinal study of more than 7000 female patients and more than 4000 male patients in Seattle, serovar E (31.8%) was the most prevalent, followed by F (18.2%) and D (13.3%) in urethral and cervical *C trachomatis* infections. In 36 urine samples of asymptotically infected men during a screening programme in Amsterdam, serovar E (36.1%) was seen twice as often as serovars D and F. In urine samples of the 40 asymptotically infected women in this study, serovar E (50.0%) was seen three to five times as often as serovars D and F.¹³

Serovar G is relatively uncommon among men and women with urogenital chlamydial infection. It has been postulated that this serovar has biological properties not yet described that allow it to more efficiently infect and persist in the anorectal environment than other serovars.⁹

Chlamydial infections with serovar L2 were seen almost exclusively in HIV positive MSM and MSM with more sexual partners in the last 6 months. These males presented significantly more often with rectal symptoms and clinical manifestations. Rectal discharge and bleeding especially were significantly associated with L2 proctitis. All the clinical manifestations (perianal erythema, discharge, and loss of blood) were significantly more often seen in patients with

Key messages

- Lymphogranuloma venereum (LGV) proctitis causes more rectal symptoms (pain, tenesmus) and clinical manifestations (rectal discharge, bleeding) than rectal *Chlamydia trachomatis* infections with serovar D-K
- In this study conducted at the Rotterdam STI clinic, LGV proctitis was significantly associated with HIV positivity and a high number of sexual partners in the past 6 months, but not with (young) age or ethnicity
- Serovars most often detected in rectal chlamydial infections in this study were serovar D and G

rectal lymphogranuloma venereum. The association between chlamydial infections with serovar L2 in HIV positive MSM and moderate to severe symptomatic proctitis was described previously after the outbreak of LGV proctitis among MSM in the Netherlands.¹⁰ It was suggested that genital ulcer disease may appear atypically and more extensive in HIV positive individuals and may enhance transmission and acquisition of STIs including HIV infection. On the other hand, the introduction of LGV proctitis in restricted sexual networks of MSM based on HIV serosorting (searching sexual contacts within HIV positive groups) may explain the high number of HIV positive individuals with L2 serovar.¹⁶ In the Rotterdam group of MSM with L2 proctitis, high rates of sexual partners, high risk sexual behaviour, and serosorting have been described.^{10 17}

It is important to be aware that our study population largely consisted of MSM from Rotterdam and therefore most likely is not representative of the MSM population in the Netherlands. Extrapolation of our findings should be done with caution. However, the findings in L2 proctitis in Amsterdam are similar to our findings.¹⁸

Furthermore, it is possible that since the outbreak of LGV proctitis among MSM in Rotterdam, more than usual attention has been paid to collection of data concerning presented rectal symptoms and clinical manifestations. After the first detected case of LGV proctitis, proctoscopy was performed routinely in case of suspicion of rectal L2 chlamydial infection. This selective procedure may have introduced information bias.

Despite these limitations, the results of this study clearly showed that chlamydial infections with serovar L2 show a different clinical and epidemiological pattern than infections with serovar D-K. Neither young age nor ethnicity were identified as risk factors for any of the serogroups investigated in this study.

CONTRIBUTORS

RW collected the data from the patient files for this study; EVDS wrote the first draft, finalised the report, and was responsible for the conception and design of the study; WVDM and JO have contributed to the conception and design of the study, critically reviewed the manuscript, and were all involved in the final report; statistical analysis and interpretation of the data was performed by EVDS and PM.

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ECHO

More endoscopists improve outcome for upper GI cancer



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More endoscopists may be the answer to better outcomes for upper gastrointestinal (GI) cancer, as recent improvement seems to owe more to the introduction of nurse endoscopists than the UK government's two week wait scheme for a specialist consultation, according to doctors in one cancer unit.

True enough, the odds of curative resection increased significantly (odds ratio 1.48) in their unit in the two years after the scheme was introduced compared with the two years before, and curative resections for early (stage 1 and 2) cancers rose from 47 to 58. But only two patients (5%) of 38 diagnosed with the cancer out of 623 referred under the scheme had early stage disease compared with 56 (27%) outside it. Furthermore, just over a third of patients with early stage cancer had symptoms consistent with the referral criteria in the scheme, but only two of them were referred under it.

When the scheme was implemented at Norfolk and Norwich University Hospital, in September 2000, it coincided with appointment of two full time nurse endoscopists, which reduced routine waiting times for endoscopy—and probably accounted for the improvement.

Under the scheme guidelines for urgent referrals for upper GI cancer were issued to general practitioners to ensure timely specialist evaluation. Detecting the cancer early is key to curative treatment, but symptoms can be unreliable. This may be why reducing times for routine endoscopy may be the best option.

The UK government has been under pressure to improve its poor record on upper GI cancer outcome in western Europe.

▲ Spahos T, *et al.* *Postgraduate Medical Journal* 2005;**81**:728–730.