Lymphogranuloma Venereum Prevalence in Sweden among Men Who Have Sex with Men and Characterization of *Chlamydia trachomatis* ompA Genotypes

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Received 17 March 2006/Returned for modification 22 June 2006/Accepted 28 August 2006

An outbreak of lymphogranuloma venereum (LGV) infections has recently been reported from The Netherlands and other European countries. The Swedish surveillance system has identified three LGV cases since 2004, all with clinically suspected infection in men who have sex with men (MSM). In order to assess the prevalence of LGV in a high-risk group of MSM and include clinically atypical cases, retrospective analysis of 197 *Chlamydia trachomatis*-infected men was performed. Sequencing of the ompA gene showed a different serotype distribution compared to recent Swedish studies in heterosexual populations. The most common types were G (45%), D (27%), and J (26%), whereas the normally predominant type E accounted for only 4% of the chlamydia cases. Furthermore, certain ompA genotype variants of the dominant serotypes were highly prevalent among MSM, and the reason for this is discussed. No additional case of LGV was detected by retrospective analysis of the high-risk MSM population. This indicates that, thus far, LGV in Sweden is only a result of sporadic import from infected MSM clusters abroad.

Lymphogranuloma venereum (LGV) is a disease caused by the LGV biovar (the L1, L2, and L3 serotypes) of *Chlamydia trachomatis*. This biovar is rare in countries with well-developed health care systems but is endemic in parts of Africa, Latin America, and Asia. Although LGV has been observed over at least a century (30), in previous decades the bacteria were found only sporadically in Europe and North America. In 2003, however, there was an outbreak in The Netherlands among MSM (27). Since then LGV has been reported in several countries in Europe (11, 16) and North America (5, 21).

In the recent outbreak, typical symptoms of LGV infection, such as lymphadenopathy, have not been seen in some of the cases presented (35, 42), while proctitis has been noted in most cases. Clinical diagnosis is thus not always sufficient to identify LGV infections. Commercial diagnostic tests commonly used for chlamydia diagnostics are of limited use, since they do not distinguish the serotypes of *C. trachomatis* and do not identify LGV. Alternative nucleotide-based methods such as restriction fragment length polymorphism (25, 41), real-time PCR (26), and nucleotide sequencing have been developed in order to facilitate LGV diagnosis.

Since the outbreak of LGV started among MSM in The Netherlands, there has been concern about the spread of infection to MSM in Sweden. Cases diagnosed with infection with *C. trachomatis*, including LGV, are mandatorily reported to the County Medical Officer for Communicable Disease Control and to the Swedish Institute for Disease Control (SMI). In all, 32,256 cases of chlamydia were reported in Sweden in 2004, of which 338 were in MSM. Contact tracing is mandatory for patients with a sexually transmitted infection (STI) listed in the Swedish Communicable Diseases Act, i.e., gonorrhea, chlamydia, syphilis, or human immunodeficiency virus (HIV) infection.

A homosexual Swedish man was diagnosed with LGV in early 2004 (4). After that it was decided that the prevalence of LGV in a risk population, MSM in Stockholm, should be assessed. Here we present a study of all *C. trachomatis*-positive samples collected at a specialized STI clinic for MSM in Stockholm from patients without typical symptoms of LGV. The aim of the present study was to analyze the distribution of *C. trachomatis* serotypes in MSM in Stockholm during a 13-month period and to detect whether there was any spread of asymptomatic LGV in this risk group. *Chlamydia* specimens were genotyped by sequencing of *ompA*, which codes for the major outer membrane protein (MOMP), and the results were compared to other known *ompA* sequences in GenBank. Three LGV cases among MSM were diagnosed from 2004 to 2005, all with classical symptoms of LGV. Samples from the three cases were also genotyped and used for comparison.

**MATERIALS AND METHODS**

**Setting.** Samples from patients infected with chlamydia and diagnosed at the Venhalsan Gay Clinic, Karolinska University Hospital, Stockholm, Sweden, were included in the study. Venhalsan Gay Clinic has existed since 1982 and is the only clinic in Stockholm specialized in sexual health care for MSM. The clinic offers free counseling, testing, and treatment for STIs, including HIV. Tests for HIV and syphilis are offered routinely. In addition, tests for gonorrhea and chlamydia are performed at the patient’s request and also for HPV in cases where there are symptoms or an epidemiological indication. The number of patient visits to the

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* Published ahead of print on 13 September 2006.
counseling and testing service for a new consultation was approximately 3,000 per year in 2004. Furthermore, some 550 HIV-positive patients in 2004 were registered and monitored clinically, with visits at least every 4 months. Samples for chlamydia testing are obtained routinely from these patients when they were first diagnosed with HIV and also later on if there is a risk of exposure or when symptoms of chlamydia were present.

**Epidemiological and clinical data.** Epidemiological and clinical data relating to the cases were obtained from medical records and contact tracing investigations.

**Clinical samples.** All samples from patients without typical LGV symptoms routinely diagnosed with *C. trachomatis* during the 13-month period February 2004 to March 2005 were retrospectively analyzed further by DNA sequencing. A total of 203 patients provided 227 samples, comprising 120 rectal swabs, 81 urine samples, 16 urethral swabs and 10 throat swabs. In addition, samples from three LGV cases diagnosed in 2004 and 2005 in Swedish MSM were sequenced.

**Isolation of DNA.** DNA was extracted from the swab samples by QIAamp DNA minikit (QIAGEN, Hilden, Germany), whereas DNA from urine samples was isolated by using the MagAttract DNA Mini M48 kit on the BioRobot M48 workstation (QIAGEN).

**PCR.** Amplification of the *ompA* gene was performed according to the method of Lynsén et al. (24).

**Sequencing.** The nested PCR product was approximately 1,000 bp (primers included) and was purified by using the QIAquick PCR purification kit (QIAGEN). The purified product was added to a reaction mixture containing BigDye terminator v3.1 (Applied Biosystems, Foster City, CA) and the primers described in Table 1. Thermal cycling and subsequent purification were performed according to instructions of the manufacturer, except that the annealing temperature was 55°C. For analysis, an ABI3130 instrument (Applied Biosystems) was used.

**Cloning.** Nested PCR products from doubly infected samples were cloned by using the TOPO TA cloning system (Invitrogen, Carlsbad, CA).

**Analysis of sequences.** The sequences obtained from each sample were assembled and edited with the Seqscape software (Applied Biosystems). The resulting consensus for each sample was compared by BLAST to *C. trachomatis* serotype.

**Reverse consensus for each sample was compared by BLAST to* C. trachomatis* serotype.

**TABLE 1. Primers used for sequencing**

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</tr>
<tr>
<td>Ctrl120 F</td>
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</tr>
<tr>
<td>Ctrl280 F</td>
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</tr>
<tr>
<td>Reverse</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Ctrl284 R</td>
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</table>

**RESULTS**

**LGV prevalence.** Prior to August 2006, only three cases of LGV (detected between January 2004 and March 2005) had been diagnosed as a result of clinical symptoms associated with the disease. A total of 227 samples positive for *C. trachomatis* from 203 MSM with no clinical or epidemiological suspicion of LGV were included in the study and retrospectively analyzed. These 203 patients represented 81% (*n* = 252) of MSM reported to SMI with chlamydia infection in Stockholm county and 53% (*n* = 385) of MSM reported in Sweden during the 13-month study period. Samples from 197 patients (97%) were successfully genotyped, but no case of LGV was found.

**Characteristics of the study group.** Of the study group of 203 chlamydia-positive MSM, 8 (4%) reported that they had sex with both men and women. The patients were between 16 and 68 years old, with a mean age of 33 years. Of the patients, 35% (71 of 203) were found to be coinfected with another STI, including HIV, when diagnosed with chlamydia. The most common coinfection was gonorrhea 24% (49 of 203), followed by HIV 10% (20 of 203), condyloma (HPV) 4% (8 of 203), and syphilis 3% (6 of 203). Of the HIV-infected patients, 20% (4 of 20) were diagnosed with chlamydia and HIV at the same time.

The number of sexual contacts during the 3 months prior to chlamydia diagnosis ranged between 1 and 85, with a mean of four partners. Information about how these sexual contacts were established was available for 73% (148 of 203) of the patients. Of these, 33% (49 of 148) had had a steady partner, 30% (44 of 148) had met contacts through the internet, 26% (38 of 148) had found them through friends, 16% (24 of 148) had found partners at blue movies or saunas, 14% (21 of 148) had found partners at a bar or gay club, 7% (10 of 148) had found partners somewhere else, and 1% (2 of 148) stated that they had had no new contacts. A total of 78% (158 of 203) of the patients reported having had sexual contacts in Sweden during the 3-month period, 11% (23 of 203) had sexual contacts abroad, and 15% (31 of 203) did not specify where their contacts had been. Of the patients who specified eventual condom use during the 3 months before chlamydia was diagnosed, 59% (94 of 160) reported to have used unprotected anal intercourse, and 99% (171 of 173) had used unprotected oral-genital sex. The reasons for examination were contact tracing (43% [of whom 14% had symptoms]), symptoms (30%), routine examination (29% [of whom 5% had symptoms]), and unknown reasons (3%).

**ompA genotyping.** In all, the *ompA* sequences of seven serotypes were detected (Table 2). Serotype G was predominant, being detected in 45% of the cases. The D and J serotypes constituted an intermediate group, accounting for 27 and 26% of the cases, respectively. Serotype E was found in only 4% of the cases, whereas serotypes B and F occurred in 1% or less of the study population.

Two samples contained two serotypes, and cloning of the PCR products from these cases successfully separated the four
variants, which were subsequently sequenced. Twenty-two patients provided samples from more than one site (Table 3). Four persons were infected by two different serotypes at different sites. Thus, six patients were infected by two different serotypes at the same time. One patient was infected with serotype D in samples collected 5 weeks apart. His partner was not treated, so this was most likely a reinfection rather than a result of inadequate treatment.

In the ompA gene there are four variable regions (VD1 to VD4), flanked and interspaced by five constant regions (CD1 to CD5). Altogether, 12 different genetic variants were found among the seven serotypes in the present study (Table 4). The three most common genotypes—G1, D1, and J—accounted for 91% of the patients. Of the G variants, type G1 was by far the most common (87 of 89 G specimens), and it was identical to cases previously observed in Sweden (24). The G3 single case of G2 had one additional nonsilent mutation in G/UW57, except for a nonsilent point mutation in VD4. The predominant G variant was G1, D1, and J. Serotypes B and F were only found in urine samples, but since these serotypes were only detected in three patients, this can probably be explained by random distribution. Furthermore, no association was found for specific genotypes when related to patient age, the number of sexual contacts, sexual contacts abroad, meeting place, or the presence of other STIs.

**Clinical symptoms and association with serotypes.** Results from proctoscopy examination were available for 200 patients. No symptoms of proctitis were noted in 87% of the cases examined. Of the remaining cases, 7% had rectal pain, 5% had rectal discharge, 3% had blood in stool, 2% were constipated, 1% had a wound or abscess, and 3% reported other symptoms.

![Table 3](image)

<table>
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![Table 4](image)

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<td>Reference sequence</td>
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<td>M14738</td>
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<sup>a</sup> The positions refer to the reference sequences and cannot be directly compared between different serotypes. * positions with nucleotide changes identical to those in another Swedish population (24).

<sup>b</sup> This sequence is identical in the partial overlap with AMSTLGVL2b (AY586530) that has caused the outbreak in The Netherlands.

<sup>c</sup> GenBank sequence from this study.
Information about genital symptoms was available for 200 patients. No symptoms were noted in 70% of the cases, but 20% of the remaining cases had dysuria, 17% had urethral discharge, 2% had a wound or abscess, 1% had enlarged or sore lymph nodes, and 1% had other symptoms. No association was seen between genotype and the presence or kind of clinical symptoms. This analysis was, however, hampered by the fact that 35% of the patients were infected with other concurrent STIs.

**DISCUSSION**

The three cases of LGV found in Sweden, detected between January 2004 and March 2005, were all diagnosed as a result of suspected clinical symptoms. In the retrospective analysis of the almost 200 chlamydia-infected MSM, no case of LGV was detected. In recent retrospective studies of chlamydia cases in The Netherlands (42), Belgium (44), and Switzerland (12) higher prevalences were observed. In these studies, however, the selection of study material was different, and therefore they cannot easily be compared to the present one. Nevertheless, it is clear that in this retrospective study we have targeted an endemic core group for STIs, since 10% were infected with HIV and 35% were diagnosed with concurrent STIs, including HIV. The prevalence of HIV in this group is low compared to studies performed in San Francisco (20). Considering the infrequent condom use and the high number of partners, combined with the many sexual contacts abroad, it is most likely that we would have detected cases of LGV if there had been any hidden spread in Sweden during the study period.

The sequences from the three LGV cases were identical, and a BLAST search revealed that they were of the L2b type (AY586530). This type has been found in the ongoing outbreak in The Netherlands (35) but has also been detected in a retrospective study of LGV cases in San Francisco going back to the early 1980s (36). Although the three cases shared the same ompA sequence, they did not seem to have any connection with each other. Samples collected from reported sexual contacts of the three Swedish cases all tested LGV negative. Two of the cases reported recent sexual contacts with men from other European countries, such as Switzerland, Italy, France, and the United Kingdom. One patient reported only one contact in Stockholm, who was LGV negative, suggesting that the information provided about previous sexual contacts was incomplete.

An LGV-positive urethral sample was obtained from the first LGV case. Two urethral samples and two skin lesion swabs from inguinal buboes were collected from the second case, but one skin lesion sample was not positive in PCR and was therefore not sequenced. A urethral sample was successfully sequenced from the third LGV case, but a rectal swab and a urine sample were negative in PCR. Thus, although no systematic collection of specimens was undertaken, it is clear that LGV may be detected from different sites.

Three major studies have investigated the serotype distribution of heterosexually acquired *C. trachomatis* infections in Sweden (19, 24, 31). Serotype E dominated (37–60%) and serotype F was the second most common (17–24%). Studies in other countries show similar distributions (13, 33, 43). In our study, however, a totally different distribution of serotypes was seen (Table 2), with serotypes E and F comprising only 4% and less than 1%, respectively. In contrast, serotype G was predominant (45%), whereas in previous Swedish studies it occurred in 3 to 11% of the cases. Other common serotypes were D (27%) and J (26%), which occurred in 9 to 14% and 4 to 7% of cases in the other studies.

The serotype distribution in the present study resembles that found among MSM in Seattle (14). However, the ratios seem to differ over time, since a study in Seattle 10 years earlier (3) showed D to be the most common serotype (41%), followed by G (21%) and J (19%), a distribution that is more similar to a recent study in Melbourne (23). Although the distribution of serotypes can vary over time, it is clear that the most common serotypes among MSM are D, G, and J, whether in Sweden, Australia, or the United States. It is interesting that three of the eight patients who reported having sex with both men and women were carrying strains more commonly found among heterosexuals, namely, B, F, and D2. This further strengthens the observation that certain strains are more prevalent among MSM. Diverging distributions of serotypes among MSM compared to the population at large have been noted in Australian and American studies (2, 22).

In the present study, the predominant serotypes were represented by the genetic variants D1, G1, and J, which have only one point mutation each compared to the reference strains D/IC-Cal8, G/UW-57, and J/UW-36. The mutations were silent in conserved regions (D1 and J), reflecting the importance of these domains in maintaining the structural integrity of the protein, or nonsilent in VD4 (G1), the surface-exposed domain that is most variable. The reference strains were isolated at least 16 to 35 years ago (34, 45, 46), and even though the MOMP is very variable owing to surface exposure and strong immune pressure, the nucleotide substitution rate is very low in ompA. Therefore, it is not surprising that the genotypes obtained in our study have been found in previous work: G1 and J in Sweden (19, 24), Iceland (18) and among MSM in Australia (23) and D1 in Iceland (18) and among MSM in Australia (23). Of the 12 genotypes detected in our study, the E2 (2 of 7 E) and G2 (1 of 89 G) were not found in GenBank and were only found exceptionally, which further emphasizes the stability of the MOMP.

In a previous phylogenetic study of ompA in *C. trachomatis* it was concluded that there was no evolutionary relationship between serotypes and biological or pathological phenotypes (tissue tropism, disease presentation, and epidemiological success) (37). This lack of association between serotype and clinical symptoms has also been noted in other studies (13, 32). It was therefore interesting that certain genetic variants of ompA were predominant in MSM populations and rare in heterosexual populations. The D1 variant comprised 89% in our study and 80% of all D cases among MSM in Melbourne (23), in contrast to only 3% for this genotype in a nonselected population in Iceland (18) and its complete absence in two Swedish studies (19, 24). The only J variant found in the present study was the only J type detected in the Australian MSM study, whereas it accounted for 40% of J samples in one of the Swedish studies (19) and was absent in the other study in Sweden (24). It thus appears not only that strains of certain serotypes dominate among MSM populations but also that some genetic variants are associated with infections in MSM.
Many patients reported recent sexual contacts abroad, suggesting that strains are shared with MSM populations in other countries. The genotype distribution described indicates that the strains concerned are more easily transferred among MSM around the globe than to heterosexual populations in the same country. This is supported by an internationalization of contact patterns and meeting arenas (9). It therefore seems that the reason some strains are more common within the MSM community is behavior rather than cell tropism or other biological explanations.

Of the ompA variants predominating in MSM, nucleotide changes were found to be silent in CDs or to lead to a single amino acid change in VD4. As discussed above, these substitutions are unlikely to confer any biological property that could explain the association of specific ompA variants with MSM. On the other hand, the association with specific ompA variants could be linked to other genome regions harboring functions for the colonization of different tissues. It has recently been demonstrated that the ompA phylogeny differs significantly from the genetic background represented by housekeeping genes, as well as by intergenic noncoding regions and the pmp family (6). Consequently, the strains with the ompA variants commonly observed in MSM may contain other genes that confer tissue tropism or propensity for colonization. Several studies have tried to associate ompA serotypes with clinical symptoms and disease. Serotype G has been reported to be associated with cervical cancer (1) but, since the ompA phylogenetic trees show incongruence with both the type of disease manifestation and tissue tropism (10, 15, 39), it is likely that these traits must be found in other regions of the genome.

In the last two decades, the molecular epidemiology of C. trachomatis has essentially been based on ompA. The variability of this gene can be measured as genetic variants as a proportion of all chlamydia cases. It has been reported to be up to 81% in small high-risk groups (7, 29, 38, 40), but in the present study the variability was only 6%. This is similar to large less-selected or nonselected study populations, where the variability was between 4 and 8% (18, 19, 24). Although ompA phylogenetic trees show incongruence with both the type of disease manifestation and tissue tropism (10, 15, 39), it is likely that these traits must be found in other regions of the genome.

In summary, LGV infections are at present found only exceptionally in Sweden and appear to represent sporadic imported cases from European countries with major outbreaks. It is also noted that some MOMP serotypes and specific genetic ompA variants are more common among MSM than in heterosexual populations, but it is unlikely that these genotypes confer any tissue tropism or increased pathogenesis.

ACKNOWLEDGMENTS

This study was funded by the National Institute of Public Health of Sweden.

We thank Bengt Wretlund for providing chlamydia samples, Anders Karlsson for suggestions when planning the study, and Fredrik Peterson for the collection of clinical data.

REFERENCES


