Lymphogranuloma venereum in Australia: anorectal *Chlamydia trachomatis* serovar 2b in men who have sex with men.

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ABSTRACT

Lymphogranuloma venereum (LGV) is a sexually transmitted infection that is causing an ongoing epidemic in men who have sex with men (MSM) in Europe, the United Kingdom and North America. Twenty-nine rectal swabs positive for *Chlamydia trachomatis* were analysed by real-time PCR (RT-PCR) for the presence of LGV serovars. Genotyping revealed an identical L2b serovar from four specimens. All patients were MSM and HIV infected. Three of the four presented with a severe ulcerative proctitis. We report a cluster of rectal LGV L2b serovar in Sydney, Australia.
*Chlamydia trachomatis* is an important human pathogen and a common cause of sexually transmitted disease worldwide (13). The *C. trachomatis* species is divided into 15 prototypic serovars labelled A-K and L1, L2, L3 based on analysis of the major outer membrane protein (MOMP) (2). On the basis of disease manifestation serovars A, B, Ba and C cause trachoma, serovars D-K are associated with urogenital infection, and serovars L1, L2 and L3 are responsible for lymphogranuloma venereum (LGV) (6). The L2 serovar can be further separated into L2, L2’, L2a or L2b according to amino acid differences (17).

Unlike other chlamydial urogenital infections that are generally restricted to epithelial surfaces, L serovars are invasive and cause severe inflammation, often with systemic symptoms, and have a preference for lymphatic tissue (3). The manifestations of LGV infection may vary depending on the site of infection. It can present as an inguinal syndrome with painful inguinal lymphadenopathy, or an anorectal syndrome with acute proctitis, inflammation of the colon and rectum and excessive proliferation of intestinal and perirectal lymphatic tissue which may mimic Crohn’s disease (5,13). Untreated the infection may cause chronic complications including fistulae, strictures and genital elephantiasis (13). The correct diagnosis is essential as treatment for LGV infection requires a prolonged course of antimicrobial therapy. Incorrect treatment may result in progressive invasive disease with tissue destruction.

Until recently LGV has been largely restricted to developing regions, and was only rarely seen in industrialised countries. Early sporadic reports of LGV in MSM are present in the literature (17). However since 2003 there have been outbreaks of LGV in men who have sex with men (MSM) from the Netherlands, Belgium, France,
Germany, Sweden, U.K, and North America (1,7,10,11,16,18,20). To date the vast majority of LGV strains have been identified as serovar L2b (19). Only two cases of LGV in MSM have been reported in Australia. One patient was a bisexual male who developed an inguinal lymphadenopathy, while the other presented with anorectal LGV (4,15). The infections were locally acquired in Melbourne and responded to appropriate therapy. There have been no reports of confirmed anorectal LGV serovar L2b in Australia.

We undertook a prospective review of all patients with a positive rectal swab for Chlamydia trachomatis during a 10 month period, to determine the incidence of LGV in a high risk population of MSM.

All rectal swabs submitted to St. Vincent’s Hospital, Darlinghurst, Australia for C. trachomatis nucleic acid testing over a 10 month period (October 2005 to July 2006) were included in the study. Swabs were extracted using the Qiagen QIAamp® DNA mini kit as per manufacturers instructions and underwent strand displacement amplification (SDA) using the ProbTec® system (Becton Dickinson).

Samples in which C. trachomatis DNA was detected underwent real-time PCR to confirm the presence of LGV serovars. Real-time PCR was performed targeting the pmp gene and utilising a Minor Groove Binding Taqman probe as previously described (14).

Further more all positive samples underwent PCR and sequencing targeting the omp1 gene as described by Justrand et al (9). Sequencing was performed in both directions.
to ensure sufficient sequence overlap and fidelity on an ABI Prism 3730 automated sequencer at the SUPAMAC facility (Royal Prince Alfred Hospital, Sydney). The entire Omp1 gene sequences obtained from the four samples were aligned together along with existing sequence data from LGV strains already deposited in GenBank using the PILEUP program (Genetics Computer Group, Version 8, Madison, WI). The individual sequences were then compared to those available in the GenBank databases using the BLASTN program run on the National Centre for Biotechnology Information Server (http://www.ncbi.nlm.nih.gov?BLAST/).

Of the 29 *C. trachomatis* DNA positive samples by SDA, 4 (14%) were positive for LGV by real-time PCR. All 4 samples gave identical sequences and showed a 100% homology with that of the *C. trachomatis* L2b strain deposited under GenBank accession number DQ217607 and the prototype L2b strain from Amsterdam AMSTLGVL2b (GenBank accession number AY586530).

The clinical presentation of the four patients is summarised in table 1. All patients were HIV positive MSM and were not immunosuppressed (CD4 cell counts ranged from 298 to 571 cells/mm$^3$). Two of the patients were initially misdiagnosed. In addition, all patients had concurrent or previous STI's, and participated in high risk sexual behaviour, with 2 of the 4 patients regularly attending MSM sex on premises venues. One patient had a risk factor for acquisition abroad, having lived in the U.S.A prior to presentation. However, this occurred several years prior to first documented cases. Therefore, all patients acquired their disease in Australia as there was no recent history of international travel. It is of interest that our 4 patients had a CD4 count of
>250 cells/mm$^3$ so none were receiving azithromycin prophylaxis for *Mycobacterium avium* complex infection, which may have prevented LGV infection.

LGV has become endemic in MSM in western developed countries. The first clusters of MSM with anorectal LGV were reported from Rotterdam in February 2003 (16). Since the initial reports of LGV in MSM there have been numerous cases from other European countries including the Netherlands, Belgium, France, Germany, Sweden and Britain (7,11,19,20). More recently LGV has emerged in Canada and several States in the U.S.A. (1,10,18). Most cases were caused by serovar L2b with the majority of patients having concurrent HIV infection.

This is the first reported cluster of anorectal LGV serovar L2b among MSM in Australia. There have been two previous unrelated cases from Melbourne, Australia. One case of locally acquired inguinal lymphadenopathy in a bisexual male and the second case of overseas acquired anorectal LGV in a MSM. (4,15). Both cases were caused by an L2 serovar not an L2b which was found in this study. This raises the possibility of two LGV variants currently circulating in Australia and is in keeping with the majority of previously documented LGV strains being serovar L2b.

Lister at al (12) found no LGV strains from 47 anal swabs collected from MSM in Melbourne in 2004. Therefore, it would appear that LGV has recently arrived in Australia. In addition, it seems that infections were locally acquired. This has public health implications which if not addressed may lead to LGV becoming endemic in the MSM community in Australia.
Clinical characteristics of our patients correspond with previous reports. (16,19). LGV can result in significant disability and may facilitate the spread of HIV and other STI’s and blood-borne infections given the ulcerative nature of the disease. Correct diagnosis is also essential as prolonged treatment (3 weeks) with doxycycline or a macrolide antibiotic is required for patients with LGV infections, in contrast to infection with other serovars where only one week of treatment is required (19).

Current commercially available diagnostic test kits for C. trachomatis cannot distinguish the LGV serovars from the other serovars. Recently real-time PCR (RT-PCR) has been used targeting various membrane protein genes. Morre et al (17) used the polymorphic membrane protein H gene (pmp gene) as a PCR target because it has a unique gap in LGV strains of C. trachomatis, compared to other serovars, which makes it highly specific. While this RT-PCR can detect the LGV serovars it cannot distinguish between them. Halse et al (8) have recently described a multiplex RT-PCR that is specific for C. trachomatis serovar L2. RT-PCR provides a rapid screening method to determine if LGV serovars are present; however it only detects serovar L2. We found direct sequencing to be a rapid and simple method to determine the serovar present.

In conclusion, we report a cluster of anorectal LGV serovar L2b among MSM in Sydney, Australia which correspond to recent trends in MSM patients in other industrialised nations. Diagnosis and appropriate therapy, as well as public health initiatives and education of health care professionals are crucial in preventing the dissemination and establishment of LGV as a common STI in Australia.
ACKNOWLEDGEMENTS

We would like to thank Dr. Milliken for providing detailed clinical details for one of the patients.

FIGURE LEGENDS

Table 1. Summary of patients with LGV

REFERENCES


<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Risk Factors</th>
<th>HIV</th>
<th>CD4 (cells/mm³)</th>
<th>Viral load (copies/ml)</th>
<th>Antiretroviral Therapy</th>
<th>Clinical Presentation</th>
<th>Other STI’s</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>43</td>
<td>MSM, Lived in USA.</td>
<td>+</td>
<td>298</td>
<td>N/A</td>
<td>d4T, 3TC, lopinavir/r</td>
<td>PR bleeding, diarrhoea. colonoscopy – showed multiple ulcerative rectal lesions, probable Crohn’s disease</td>
<td>Kaposi’s sarcoma</td>
<td>azithromycin, doxycycline</td>
</tr>
<tr>
<td>RK</td>
<td>55</td>
<td>MSM, No travel</td>
<td>+</td>
<td>310</td>
<td>&lt;50</td>
<td>abacavir, 3TC, nevirapine</td>
<td>Tenesmus, rectal bleeding for 3 months. high resolution anoscopy – extensive ulcer distal to dentate line</td>
<td>anal warts, syphilis</td>
<td>doxycycline</td>
</tr>
<tr>
<td>PD</td>
<td>54</td>
<td>MSM, No travel, SOP venues</td>
<td>+</td>
<td>571</td>
<td>&lt;50</td>
<td>abacavir, tenofovir, atazanavir/r</td>
<td>PR bleeding, sweats, lethargy for 10 days. colonoscopy – ulcerated tumour? SCC</td>
<td>syphilis</td>
<td>doxycycline</td>
</tr>
<tr>
<td>SH</td>
<td>45</td>
<td>MSM, No travel, SOP venues</td>
<td>+</td>
<td>442</td>
<td>130</td>
<td>abacavir, 3TC, nevirapine</td>
<td>anal ulceration, concurrent HSV2</td>
<td>syphilis x3, gonorrhoea, HSV2, HCV</td>
<td>azithromycin</td>
</tr>
</tbody>
</table>

MSM – men who have sex with men  
SOP – sex on premises  
PR – per-rectal  
N/A – not available  
SCC – squamous cell carcinoma