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Lymphogranuloma Venereum in Australia: Anorectal *Chlamydia trachomatis* Serovar L2b in Men Who Have Sex with Men▽

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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 analyzed by real-time PCR for the presence of LGV serovars. Genotyping revealed an identical L2b serovar from four specimens. All patients were MSM and human immunodeficiency virus infected. Three of the four presented with severe ulcerative proctitis. We report a cluster of rectal LGV serovar L2b infections in Sydney, Australia.

Chlamydia trachomatis is an important human pathogen and a common cause of sexually transmitted disease worldwide (12). The *C. trachomatis* species is divided into 15 prototypic serovars labeled A to K, L1, L2, and L3 on the basis of analysis of the major outer membrane protein (2). On the basis of disease manifestation, serovars A, B, Ba, and C cause trachoma, serovars D to 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 (16).

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, 12). Untreated, the infection may cause chronic complications including fistulae, strictures, and genital elephantiasis (12). 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 industrialized countries. Early sporadic reports of LGV in men who have sex with men (MSM) are present in the literature (16). However, since 2003 there have been outbreaks of LGV in MSM from The Netherlands, Belgium, France, Germany, Sweden, the United Kingdom, and North America (1, 7, 10, 15, 17, 19, 20). To date, most LGV strains have been identified as serovar L2b (18). 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, 14). The infections were locally acquired in Melbourne and responded to appropriate therapy. There have been no reports of confirmed anorectal LVG serovar L2b in Australia.

We undertook a prospective review of all patients with a rectal swab positive for *C. 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 this study. Swabs were extracted with the QIAGEN QIAamp DNA mini kit in accordance with the manufacturer's instructions and underwent strand displacement amplification with the ProbTec system (Becton Dickinson).

Samples in which *C. trachomatis* DNA was detected underwent real-time PCR (RT-PCR) to confirm the presence of LGV serovars. RT-PCR was performed by targeting the *pmp* gene and using a Minor Groove Binding TaqMan probe as previously described (13).

Furthermore, all positive samples underwent PCR and sequencing targeting the *omp1* gene as described by Jurstrand 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, Australia). The entire *omp1* gene sequences obtained from the four samples were aligned, along with existing sequence data from LGV strains already deposited in GenBank, with the PILEUP program (version 8; Genetics Computer Group, Madison, WI). The individual sequences were then compared to those available in the GenBank databases with the BLASTN program run on the National Center for Biotechnology Information Server (htpp://www.ncbi.nlm.nih.gov?BLAST/).

Of the 29 samples *C. trachomatis* DNA positive by strand displacement amplification, 4 (14%) were positive for LGV by RT-PCR. All four samples gave identical sequences and showed 100% homology with that of the *C. trachomatis* L2b

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TABLE 1. Summary of the clinical characteristics of patients with LGV reported in this study

Patient age (yr)	Risk factors	HIV	No. of CD4 cells/mm^3	Viral load (copies/ml)	Antiretroviral therapy[e]	Clinical presentation	Other STI(s)	Treatment
43	MSM, lived in United States	+	298	NA[c]	d4T, 3TC, lopinavir-ritonavir	PR[b] bleeding, diarrhea; colonoscopy showed multiple ulcerative rectal lesions, probable Crohn's disease	Kaposi's sarcoma	Azithromycin, doxycycline
55	MSM, no travel	+	310	<50	Abacavir, 3TC, nevirapine	Tenesmus, rectal bleeding for 3 mo; high-resolution anoscopy showed extensive ulcer distal to dentate line	Anal warts, syphilis	Doxycycline
54	MSM, no travel, SOP[a] venues	+	571	<50	Abacavir, tenofovir, atazanavir-ritonavir	PR bleeding, sweats, lethargy for 10 days; colonoscopy showed ulcerated tumor, SCC[d]?	Syphilis	Doxycycline
45	MSM, no travel, SOP venues	+	442	130	Abacavir, 3TC, nevirapine	Anal ulceration, concurrent HSV2[f] infection	Tertiary-stage syphilis, gonorrhea, HSV2, HCV[g]	Azithromycin

[a] SOP, sex on premises.
[b] PR, per-rectal.
[c] NA, not available.
[d] SCC, squamous cell carcinoma.
[e] d4T, stavudine; 3TC, lamivudine.
[f] HSV2, herpes simplex virus type 2.
[g] HCV, hepatitis C virus.

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 summarized in Table 1. All patients were human immunodeficiency virus (HIV)-positive MSM and were not immunosuppressed (CD4 cell counts ranged from 298 to 571/mm^3). Two of the patients were initially misdiagnosed. In addition, all patients had concurrent or previous sexually transmitted infections (STIs) and participated in high-risk sexual behavior, with two of the four patients regularly attending MSM sex-on-premises venues. One patient had a risk factor for acquisition abroad, having lived in the United States prior to presentation. However, this occurred several years prior to the first documented cases. Therefore, all patients acquired the disease in Australia as there was no recent history of international travel. It is of interest that our four patients had CD4 cell counts of >250/mm^3 so none were receiving azithromycin prophylaxis for *Mycobacterium avium* complex infection, which might have prevented LGV infection.

LGV has become endemic in MSM in western developed countries. The first clusters of MSM with anorectal LGV were reported in Rotterdam in February 2003 (15). Since the initial reports of LGV in MSM, there have been numerous cases in other European countries, including The Netherlands, Belgium, France, Germany, Sweden, and Britain (7, 18, 19, 20). More recently, LGV has emerged in Canada and several of the United States (1, 10, 17). 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 infections among MSM in Australia. There have been two previous unrelated cases in Melbourne, Australia.

One case of locally acquired inguinal lymphadenopathy occurred in a bisexual male, and the second case of anorectal LGV acquired overseas occurred in an MSM (4, 14). Both cases were caused by an L2 serovar and not an L2b serovar as 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. (11) found no LGV strains in 47 anal swabs collected from MSM in Melbourne in 2004. Therefore, it appears that LGV has recently arrived in Australia. In addition, it seems that the 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.

The clinical characteristics of our patients correspond to those in previous reports (15, 18). LGV can result in significant disability and may facilitate the spread of HIV and other STIs 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 1 week of treatment is required (18).

Current commercially available diagnostic test kits for *C. trachomatis* cannot distinguish the LGV serovars from the other serovars. Recently, RT-PCR targeting various membrane protein genes has been used. Morre et al. (16) 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 infections among MSM in Sydney, Australia, which correspond to recent trends in MSM patients in other industrialized nations. Diagnosis and appropriate therapy, as well as public health initiatives and education of health care profes-

sionals, are crucial in preventing the dissemination and establishment of LGV as a common STI in Australia.

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