# LYMPHOGRANULOMA VENEREUM

Among Men who have Sex with Men

NYNKE DE VRIEZE

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**Lymphogranuloma venereum among men who have sex with men**
Thesis, University of Amsterdam, the Netherlands


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| Layout and cover design: | Design Your Thesis | www.designyourthesis.com |
| Printing: | Ridderprint B.V. | www.ridderprint.nl |
| ISBN: | 978-94-6375-051-6 | |

The printing of this thesis was financially supported by the Public Health Service of Amsterdam (GGD Amsterdam) and the Huidstichting Chanfleury van IJsselsteijn. Their financial contributions were greatly appreciated.



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# LYMPHOGRANULOMA VENEREUM
## Among Men who have Sex with Men

ACADEMISCH PROEFSCHRIFT
ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. ir. K.I.J. Maex

ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op dinsdag 30 oktober 2018, te 10.00 uur
door Nynke Hesselina Neeltje de Vrieze
geboren te Zürich, Zwitserland

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# 01

# GENERAL INTRODUCTION AND AIMS OF THIS THESIS

# CHLAMYDIA TRACHOMATIS SEROVARS

Lymphogranuloma venereum (LGV) is a sexually transmitted disease (STD) caused by the bacterium *Chlamydia trachomatis (Ct)*. *Ct* has evolved into several variants, which can be divided into so-called 'serovars' A to L.* Subsequently the serovars A to L can be grouped into three different biological variants (named biovars) with distinctive clinical infections:

1) Trachoma biovar (serovars A to C);
2) Urogenital biovar (serovars D to K);
3) LGV biovar (serovars L1, L2, and L3).

The trachoma biovar is known to cause the conjunctival infection called trachoma, but can also cause urogenital chlamydia infections. The urogenital biovar is the most prevalent one and causes a condition known as urogenital chlamydia, although infections of the oropharynx and anorectal tract also occur. Bacteria belonging to the LGV biovar differ from the bacteria of other biovars in the sense that they can invade connective as well as lymphatic tissue. In contrast, trachoma and urogenital biovar bacteria are restricted to mucous membranes without further invasion of underlying tissue layers. As a result, LGV biovar infections require longer treatment regimes than non-LGV biovar infections.[1,2]

# CLINICAL PRESENTATION OF LGV

The disease course can be divided into three stages with distinct clinical and pathological features. Stage I: A primary lesion appears varying from 1 to 5mm at the site of inoculation in epithelial linings; it is painless and it usually heals in a few days. This stage may go unnoticed. Stage II: About 2-6 weeks after the appearance of the primary lesion the disease spreads via the underlying connective tissue layers and along the lymphatics. Classically, LGV manifests itself in inguinal or femoral lymph nodes with the formation of severe lymphadenopathy known as 'buboes' (inguinal syndrome). Usually the inflammatory swelling of the lymph nodes in the groin is unilateral. An abscess can arise from the centre of the node; this can perforate towards the exterior and form a fistula. In the present LGV epidemic among men who have sex with men (MSM), LGV proctitis often presents in the secondary stage, with symptoms like anal cramps, tenesmus, discharge, and pain. However, LGV proctitis

**. Classification of Ct was conventionally based on serological antibody typing of the major outer membrane protein (MOMP). Based on this, different strains of Ct were called 'serovars'. Nowadays classification is based on determination of single nucleotide polymorphisms (SNP) within the Omp1 gene, coding for MOMP, known as 'genovars'. In literature, the term 'serovars' is often used for both 'serovars' and 'genovars'.*

can be asymptomatic as well. Stage III: If left untreated, chronic lymphadenitis can lead to elephantiasis 1-20 years after infection. This final stage is also characterized by fibrosis and stenosis, and can lead to irreversible strictures of the genital and anorectal tract and a chronic condition called 'the frozen pelvis' syndrome.[3,4] Stage III is barely seen in the current Western epidemic among MSM.

# HISTORY OF LGV

## Clinical picture

During the pre-scientific era in 1786, John Hunter was the first to describe cases that fit the current LGV clinical pattern.[5] Hunter stated: "Commonly one gland is affected by the absorption of venereal matter, which may distinguish venereal buboes and other diseases of the bodies." Hunter also observed that "buboes are slow in their cure and do not seem to be affected by mercury when early applied." At that time mercury was a standard treatment for syphilis, and with the latter statement he implied a different entity was responsible for this disease. After Hunter's first observations, more similar cases appeared in the medical literature. Nélaton wrote about venereal patients suffering from "single subacute inguinal adenitis with intraganglionic purulent foci" Lejars mentioned "bubo of the groin" and Marion and Gandy described patients with "subacute adenitis of the groin"; they hypothesized that tuberculosis was the cause of these typical complaints.[6-8]

In 1913 Durand, Nicolas, and Facre first described LGV as a distinct clinical and pathological infection.[9] They adopted a new clinical entity called *lymphogranulomatosis*. Like Hunter, Durand *et al*. suspected a venereal cause and rejected the tuberculosis hypothesis of Marion and Gandy. In 1922, Phylactos established that there was one infectious agent capable of causing the different clinical manifestations of lymphogranulomatosis: genital ulceration, proctitis, and the formation of buboes that may be followed by late complications such as genital lymphoedema and rectal strictures.[10] Phylactos distinguished this disease from tuberculosis, Hodgkin's disease, bubonic plague, syphilis, and cancroid, thus establishing the basic entity of the current clinical diagnosis of LGV.

## First attempts to elucidate the cause of LGV

Thus, LGV was already recognized as a clinical entity in the early 20$^{th}$ century. Subsequently, it took decades to elucidate the aetiology of LGV and develop reliable diagnostic tests. Early attempts to isolate or cultivate the causal agent of LGV from the pus of the involved inguinal glands were unsuccessful. A major breakthrough in the diagnosis of LGV was the development of an intra-dermal test in 1925 by Wilhelm Siegmund Frei, a

German dermatologist.[11] Antigen made from aspirated pus from an unruptured LGV bubo produced a delayed type hypersensitivity reaction in other patients with LGV when injected intradermally. It was named 'the Frei test'.

## Psittacosis-Lymphogranuloma-Trachoma (PLT) group

Halberstaedter and von Prowazek had found cytoplasmic inclusion bodies in the conjunctival epithelial cells in patients with ocular trachoma in 1907, and named them 'chlamydozoa' (from Greek: chlamys = cloak and zoa = animals).[12] It took years (until around 1930) before they were observed again by a new generation of scientists. At that time (1934) Bedson *et al*. described a unique developmental cycle of the psittacosis agent (a bird disease) and thought the pathogen was a virus. They reported that the elementary bodies of psittacosis passed through a cycle in the cytoplasm of host cells, now known as 'initial bodies', dividing successively to finally form the relatively small elementary bodies.[13] In the 10 years that followed, similarities were found between the pathogen of trachoma and inclusion conjunctivitis, and the agent of lymphogranuloma and psittacosis. In these two groups of conditions the agents looked alike, stained alike, and exhibited the same sequence of development form when multiplied.[14,15] Around 1935, cultivation of the etiological agent of LGV in embryonated eggs was performed. This made the production of large amounts of antigen possible for the Frei test. Inclusion bodies in infected tissue culture cells of lymphogranuloma were described in 1939.[16] Around 1940 it was discovered that the agent of trachoma and inclusion conjunctivitis belonged to the same group of agents as those of psittacosis and lymphogranuloma. A causal relationship was found between the elementary and initial bodies, which were characteristically present in lesions of these disorders. They also discovered they all went through the same unique developmental cycle.[17,18] Furthermore, McKee demonstrated that a powerful complement-fixing antigen could be prepared by the propagation of the agent of lymphogranuloma in the yolk sac of the embryonated chick egg.[19] It was shown that this antigen gave marked cross fixation with sera from individuals infected with other members of this group of agents: psittacosis, trachoma, and inclusion conjunctivitis.[20] It was concluded that agents of both trachoma and inclusion conjunctivitis showed a definite antigenic relationship to lymphogranuloma and psittacosis and were therefore called the *Psittacosis-Lymphogranuloma-Trachoma (PLT) group*.[21] In 1955 T'ang *et al*. succeeded in isolating 3 strains of an elementary body agent, from then on recognised as the agent of trachoma.[22] Serological differentiation of the PLT group on the basis of their specific cell wall antigens was initiated in 1965 by Schachter *et al*.[23] The similarities of the organisms of the PLT group were found to be so fundamental that this outweighed the characteristics separating the various species. On

taxonomical and nomenclatural grounds, it was deemed correct to include them all under the genus chlamydia. Also, the term 'virus' was rejected and chlamydiae were recognized as bacteria.[24,25]

### Diagnosis of Chlamydia trachomatis and LGV

The Frei test was an indirect method to show an immunological reaction in a patient and since its antigen is common to all chlamydia, the specificity to diagnose LGV was limited.[26] The commercial manufacture of the Frei test was thus discontinued in 1974. Complement-fixation serologic tests and micro immunofluorescence antibody tests replaced the original Frei test and were more sensitive and specific.[27-30]

The development of monoclonal antibodies in 1980 enabled specific and direct detection of *Ct* in cultivated clinical specimens. To culture *Ct*, fertilised eggs were used and later replaced by cultivation in HeLa cell lines.[31,32] The two antigens used to detect *Ct* were species-specific major outer membrane protein (MOMP) and genus-specific lipopolysaccharide (LPS). The latest developments in the diagnosis include the introduction of genovar-specific nucleic acid amplification tests (NAATs). NAAT assays have a high sensitivity and specificity and are able to detect *Ct*-associated DNA or RNA from genital, rectal, and throat swabs, urine, bubo pus, lymph node aspirates, and biopsy specimens.[33,34] To diagnose LGV, it is recommended to first detect *Ct* with a genovar non-specific commercially available NAAT. If the result is positive the diagnosis of LGV should be confirmed by the detection of genovar-specific *Ct* DNA or RNA.[35] These tests are not yet commercially available, but several 'in-house' tests have been developed.[34,36] Several commercial manufacturers have shown interest in incorporating LGV specific assays in multiplex NAAT platforms. Until these reach the market, LGV genovar-specific in-house tests are available in dedicated laboratories and public health settings.

## EPIDEMIOLOGY OF LGV

In the Western world, LGV largely disappeared when antibiotics became available after the Second World War. Since then, LGV has mainly been seen in tropical and subtropical regions. When diagnosed in Western countries, it concerned imported infections among travellers to LGV-endemic regions. This changed in 2003 when the first endemically acquired LGV cases were reported in Rotterdam, the Netherlands, predominantly among HIV-positive MSM.[37] Subsequently, other Western European countries started to report LGV cases; this has led to a sharp increase of reported diagnoses in developed countries. Soon after the initial Dutch report, national and international health authorities launched warnings.

Multiple LGV cases were identified in several large European cities, but also in Australia, Canada, and the USA.[38-48] In the current epidemic, LGV is predominantly detected among MSM in European metropolitan areas, and is rarely seen among women.[49-53] LGV among MSM is associated with high-risk behaviour, which is reflected in the large proportion of men with sexually transmitted co-infections, like HIV.[54,55] The age of LGV patients is higher (median 35 years) than that of cases of non-LGV chlamydia infections, which predominantly affects the population under 25 years old. LGV may also increase the risk for acquisition and transmission of other sexually transmitted infections (STIs), including HIV, and blood-borne infections like hepatitis C virus (HCV).[56,57] In the Western world, the number of reported LGV diagnoses has increased since 2003, primarily among HIV-positive MSM.[58-68] Over 10 years (2003-2013), a total of 4761 cases were reported in Europe.[68] In 2014 alone, 1416 cases of LGV were reported (Figure 1).[69] Three countries (France, the Netherlands, and the United Kingdom) accounted for 87% of notified cases.

Several factors may account for the increase of diagnoses of LGV, including changes in sexual behaviour, but also more frequent testing with increased awareness of LGV and improved detection systems. However, a number of medical professionals/settings still do not perform diagnostic tests on LGV consistently or do not report on LGV cases, suggesting considerable under-diagnosis and under-reporting of LGV.

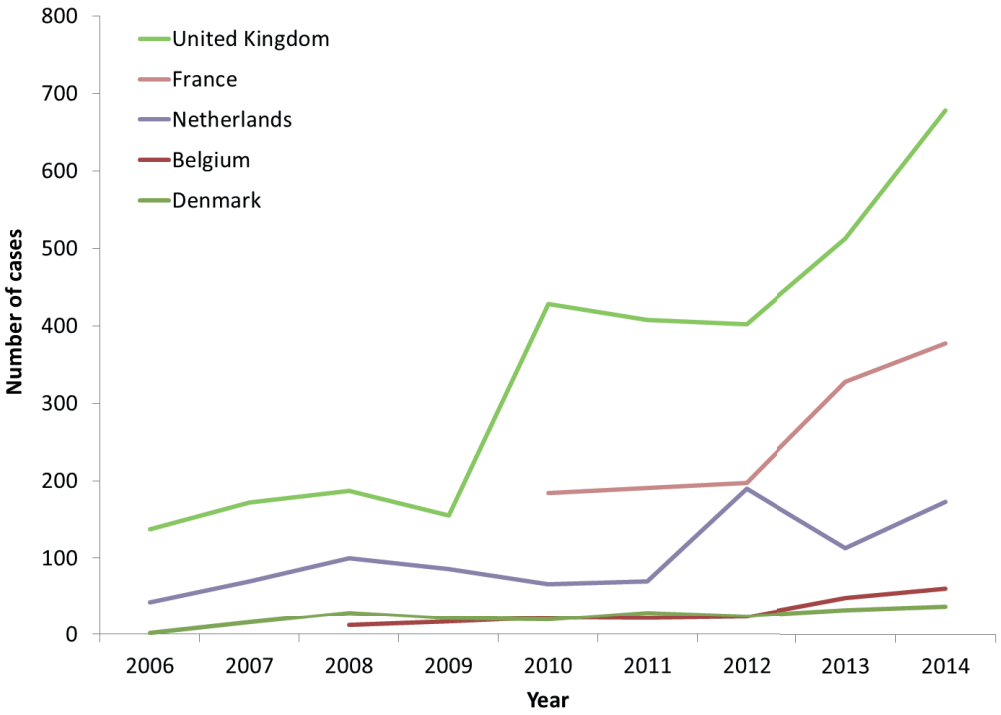


**FIGURE 1.** Number of reported confirmed LGV cases for selected EU/EEA Member States, 2005-2014. European Centre for Disease Prevention and Control, Annual Epidemiological Report 2015. Lymphogranuloma venereum, Stockholm: ECDC;2016.

Figure 2 shows the latest numbers of LGV diagnoses at the STI clinic of Amsterdam. In addition to the absolute numbers, positivity rates are presented. The number of tests is calculated for MSM tested for chlamydia. Until May 2014, MSM engaging in receptive anal sex were tested for anorectal chlamydia and LGV. Since then, all MSM irrespective of having anal sex have been tested. In the past 10 years there has been a steady increase, both in the number and positivity rate of LGV. The ratio of LGV among HIV-negative MSM has increased in the past 2 years. This might indicate risk compensation due to the availability of biomedical interventions against HIV transmission.[70]

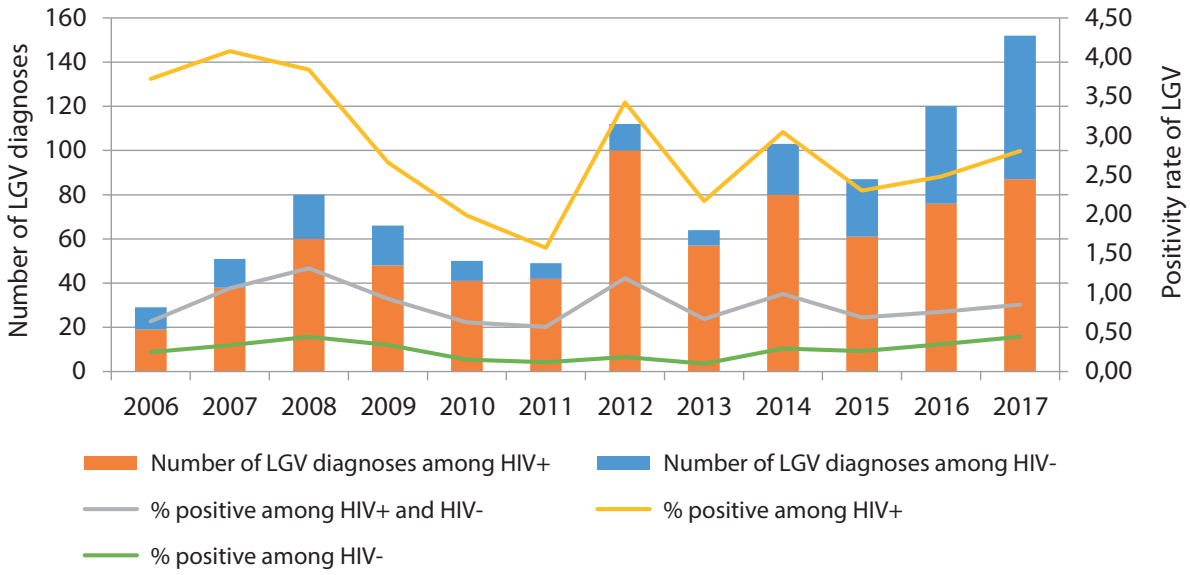


**FIGURE 2.** Number and positivity rate (%) of lymphogranuloma venereum (LGV) among men who have sex with men visiting the STI outpatient clinic, Amsterdam, the Netherlands, by year, from January 2006 to January 2018.

# TRANSMISSION ROUTE OF LGV

The mode of transmission in the current LGV epidemic among MSM in Western countries is unclear. Although it seems logical to assume that anorectal LGV is caused by unprotected genital-anal contact, it is highly remarkable that very few cases of urogenital LGV are reported.[62-66,71] Early on, it was suggested that rectum-to-rectum transmission could play a role based on the frequent use and sharing of sex toys, group sex, and fisting reported by LGV patients.[73] Yet, a significant association with these practices could not be confirmed in more detailed studies.[74]

# CURRENT MANAGEMENT IN CASE OF A SUSPECTED LGV

## Diagnosis

The diagnosis of LGV is not always straightforward. The symptoms and signs of LGV have a significant overlap with other STDs, other infections, drug reactions, and malignancies. A suspicion of LGV is often based on the patient's history and the clinical picture, and is supported by laboratory testing. As mentioned earlier, the classical manifestation of LGV is characterised by inguinal lymphadenopathy. The primary manifestation of infection during the current epidemic is proctitis, but the clinical manifestation is often variable and non-specific.[75-77] Reports on the number and proportion of symptomatic LGV cases have varied. UK cohorts from 2007 showed almost all LGV cases to be symptomatic,[66] in contrast to Dutch studies where a significant proportion of diagnosed cases were asymptomatic.[55,63] A recent study from the UK confirmed the Dutch reported proportion of asymptomatic LGV cases.[78] Asymptomatic infections often cause delayed or missed LGV diagnosis. The variation in clinical presentation and the absence of widely applicable (commercial) tests, which can discriminate between LGV and non-LGV in health care settings, hampers systematic surveillance and suggest that the number of LGV cases is currently under-reported.

## Guidelines

The British and Canadian guidelines only recommend screening for anorectal LGV upon clinical or epidemiological suspicion, after exclusion of other causes of proctitis, or for patients with direct sexual contact with a partner with LGV.[79-82] Since treatment of LGV requires longer treatment regimens than non-LGV chlamydia, it is of importance to differentiate between the two.[83] However, to date, none of the guidelines mentioned above recommend routine screening for asymptomatic MSM, and diagnoses are likely missed. These patients are not receiving the proper treatment required for their infection. The European IUSTI LGV guideline is the only international guideline that recommends screening all MSM reporting receptive anal sexual practices in the previous 6 months, irrespective of symptomatology, for anorectal *Ct* infection using a commercial (non-genovar-specific) platform.[84] Subsequently, screening for LGV proctitis according to local guidelines is recommended for MSM with a *Ct*-positive result.

Very few urethral LGV cases are reported and routine screening for LGV of urine or urethral swabs that are *Ct*-positive is not recommended in any international guideline.

# HIV POST-EXPOSURE PROPHYLAXIS

Apart from LGV, one chapter of this thesis discusses HIV post-exposure prophylaxis (PEP). PEP is a 4-week course of antiretroviral treatment recommended to persons exposed to human body fluids possibly infected with HIV, in order to prevent an established HIV infection.[85] Individuals presenting for PEP after sexual exposure are at risk for concurrent STI. Therefore, when MSM visit the STI clinic with a PEP request, it is an ideal moment for STI screening and safe sex promotion. Ideally PEP is started as soon as possible, but no later than 48 hours after sexual exposure. It is unclear what the optimal moment for STI screening is for patients with a PEP indication. Testing during a consultation within 48 hours after exposure might be too soon to detect an incubating STI. On the other hand, delaying STI screening increases the risk of loss to follow-up and onward transmission.

# AIMS AND OUTLINE OF THIS THESIS

This thesis provides insight into the LGV epidemic in Amsterdam among MSM, with special emphasis on the role of urogenital (urethral and inguinal) LGV. Our aim is 1) to investigate a potentially missed reservoir of urethral LGV that might explain the transmission route of LGV and 2) to formulate recommendations on the diagnosis and treatment of urethral and inguinal LGV. Additionally, our aim is 3) to determine whether additional STI testing among MSM with an indication of HIV PEP leads to more STI diagnoses to prevent ongoing transmission of STI. All clinical data were collected at the STI outpatient clinic of Amsterdam, the Netherlands.

In **chapter 2** an epidemiological and clinical overview of LGV is given. **Chapter 3** reports on the positivity trends of LGV, the symptomatology of anorectal LGV, and the patient characteristics of those with anorectal and inguinal LGV. **Chapter 4** reports on the prevalence of urethral LGV among MSM with an anorectal LGV and their sexual partners, to determine if there is a reservoir of missed urethral LGV that might contribute to the transmission of LGV. **Chapter 5** illustrates pitfalls in diagnoses and management of inguinal LGV with examples from practice. **Chapter 6** reports on the positivity rate of urethral LGV among MSM visiting the STI outpatient clinic in Amsterdam, in order to assess its potential contribution to the current LGV epidemic. **Chapter 7** is the only chapter that does not deal directly with LGV; this chapter reports on a study on the yield of STI screening of MSM with an indication for HIV PEP. Finally, **chapter 8** provides the general discussion. The results of the studies in this thesis are discussed in the context of the recent literature. Moreover, alternative modes of transmission are discussed that could explain the current discrepancy

in the ratio of anorectal to urogenital LGV observed in the current epidemic among MSM. Recommendations for future guidelines on testing, treatment, and clinical management and recommendations for further research are provided.

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# 02

# LYMPHOGRANULOMA VENEREUM AMONG MEN WHO HAVE SEX WITH MEN. AN EPIDEMIOLOGICAL AND CLINICAL REVIEW

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*Expert Rev Anti infect Ther. 2014;12(6):697-704*

# ABSTRACT

Lymphogranuloma venereum (LGV) is a sexually transmitted infection, previously only seen in tropical regions. This changed in 2003 when the first endemically acquired LGV cases were reported in Rotterdam, the Netherlands, among predominantly HIV positive men who have sex with men (MSM). Early diagnosis is important to prevent irreversible complications and to stop further transmission in the community. In contrast to earlier reports, approximately 25% of LGV infections are asymptomatic and form an easily missed undetected reservoir. The majority of reported infections in MSM are found in the anorectal canal and not urogenital, which leaves the mode of transmission within the MSM network unclear. Given the increasing trend, the LGV endemic is clearly not under control. Therefore directed screening must be intensified.

# INTRODUCTION

Lymphogranuloma venereum (LGV) is an invasive ulcerative sexually transmitted infection (STI) caused by the biovars L1, L2 or L3 of *Chlamydia trachomatis*.[1] *C. trachomatis* urogenital infections with biovars D to K are mostly associated with mild to asymptomatic inflammation. In contrast, the LGV biovars usually cause invasive infections and subsequent severe inflammatory responses.[2] LGV among men who have sex with men (MSM) in Western countries has been ongoing for more than 10 years and first came to light in 2003.[3] Thirteen MSM (11 HIV-positive) with severe anorectal LGV infections were described in Rotterdam.[4] Soon after, similar LGV proctitis cases were reported from major cities throughout Europe, the USA and Australia.[5,6] LGV among MSM is associated with high-risk behaviors, like the use of enemas, having sex on sex parties, the use of recreational drugs like γ-hydroxybutyrate, having unprotected receptive anal intercourse, having sex with HIV-positive partners and with anonymous partners.[2,7] High-risk behavior is also reflected in the large number of sexually transmitted coinfections such as HIV (~80.0%), hepatitis C virus (HCV) and previous syphilis infections.[2,8] Acute anorectal LGV can be characterized by anorectal cramps, pain, bloody discharge and/or constipation. An infection can also lead to complications such as lymphedema, strictures and the development of fistula.[9,10] LGV is considered an ulcerative STI, but fewer than one-third of those infected notice any ulcers. If ulceration occurs, it can be found 4–30 days after transmission. The ulcerative nature of LGV likely facilitates the spread of other STI, including bloodborne infections like HCV.[11,12] In 2012, the LGV prevalence among MSM visiting the STI outpatient clinic in Amsterdam doubled compared with the previous year (Figure 1).[8,13] LGV can be treated with antibiotics, preventing sequelae in case of early intervention. Yet, timely diagnosis is often missed because 5–27% of the patients have no complaints and/or correct diagnostic tools are not available.[8,14] This review article will elaborate on the epidemiology, diagnosis, treatment and current knowledge status of LGV.

## Epidemiology

LGV is endemic in many parts of Africa, Southeast Asia, Latin America and the Caribbean.[14] Until 2003, sporadic cases were reported in Europe and North America, among travelers who probably were infected during a visit to LGV-endemic regions. In the 1980s, a small cluster of LGV infections was described among MSM caused by biovar L1 that remained confined to Seattle and did not spread further.[15] Therefore, LGV was considered an imported disease in the Western world. However, in 2003, several cases of LGV proctitis were reported among MSM, first in Rotterdam, followed by reports coming from other countries in Western Europe, North America and Australia.[5,6] Based on molecular genetic research, the LGV infections among MSM turned out to be caused by a specific new variant, biovar L2b (also

known as the 'Amsterdam variant'), which had not been described previously.[16] This strain turned out not to have emerged recently. A retrospective study showed that the LGV L2b already existed in 1981 among MSM in San Francisco.[17] This indicated that the LGV epidemic among MSM in the Western world was not a sudden outbreak, but an endemic infection among MSM that had gone unnoticed for more than 20 years. Until now, the LGV endemic caused by L2b seems to be confined to the MSM community. The majority (~80.0%) is HIV-positive, and many are coinfected with other STIs.[2] A strong association between HCV (until then considered a strictly bloodborne infection) and LGV was found.[11,12] Sporadic female cases with LGV have been described, but it is not expected that the current focused MSM endemic will spread to the heterosexual population on a wide scale soon.[18,19] Recently, *C. trachomatis* strains among MSM and heterosexuals in Amsterdam were examined with molecular genetic techniques.[20] It was shown that the transmission networks did not overlap and were mostly separated, which is likely due to distinct transmission networks of *Chlamydia* separating MSM from heterosexuals. A similar disparity in circulating strains among MSM as opposed to heterosexuals was found in a larger international study also involving participants from Sweden and the USA.[21] Therefore, the transmission of LGV from the MSM community to the heterosexual population seems unlikely at this time.

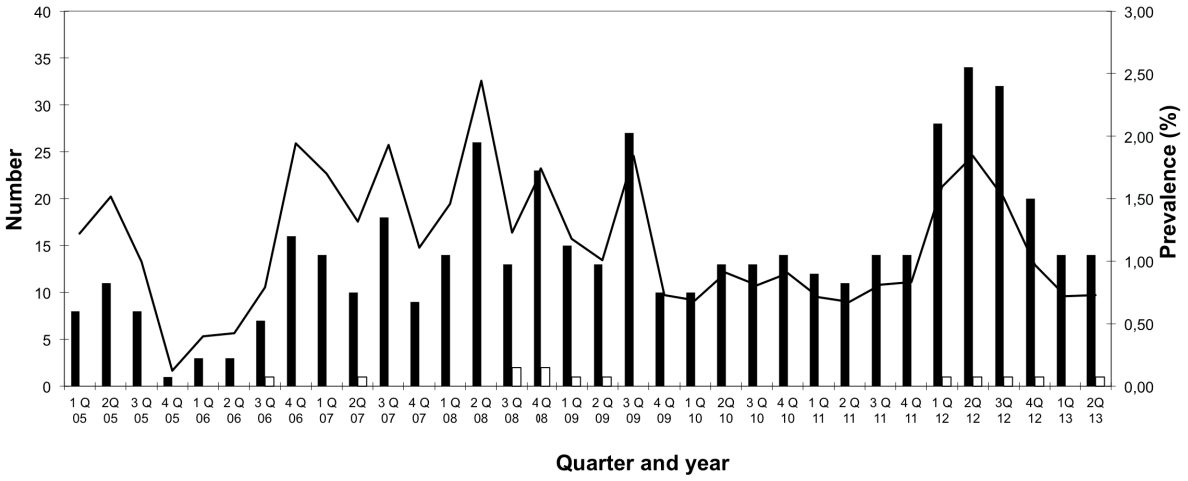


**FIGURE 1.** Number and prevalence of lymphogranuloma venereum cases among men who have sex with men visiting the Amsterdam sexually transmitted infection outpatient clinic between January 2005 and June 2013.

Black bars: Number of anorectal infections, White bars: Number of inguinal infections, Line: Overall prevalence (%).

In the first papers reporting on the LGV epidemic, it was proposed that the transmission of anorectal LGV infection was facilitated via fisting practices and the sharing of toys during sex parties.[4] Nonetheless, no evidence was found to support this route of transmission in later studies.[2,22]

Macdonald *et al*. published recently their findings on the modes and risks of transmission for rectal LGV in gay men.[7] The striking imbalance between rectal and genital infections was confirmed as reported earlier.[8] The authors found that unprotected receptive anal intercourse was the key risk factor. Sex with anonymous contacts, sex under the influence of γ-hydroxybutyrate and fisting were risk factors when comparing cases with asymptomatic controls. Compared with symptomatic controls, rectal douching and unprotected insertive anal intercourse were independent behavioral risk factors.

In a recent UK-based study, there were 66/1281 (5.2%) individuals with an LGV reinfection episode during 2004 until 2010.[23] 'LGV repeaters' reported higher levels of unprotected sex, were more often HIV- and HCV-positive, had more often a concurrent gonorrhea infection and more often visited a London STI clinic than the 'non-repeaters'.

## Clinical presentation

LGV can cause a variety of clinical syndromes, of which the classical inguinal syndrome and the anorectal syndrome are the most common.[10,24]

In the first stage, all syndromes are characterized by ulcerative disease, developing after an incubation period of 4–30 days. Ulcers are usually small, inconspicuous and of short duration (Figure 2).

**FIGURE 2.** Primary stage penile lymphogranuloma venereum ulcer.

Depending on the inoculation site (urethra, cervix or rectum), mucopurulent discharge may be present. Typical anorectal complaints caused by L2b biovar in the current endemic among MSM are anorectal spasms (tenesmus), pain, bloody discharge, peri-anal sores and constipation. The latter is caused by edema of the mucous membrane and underlying tissues. Anoscopic examination may reveal a distal granular or hemorrhagic proctitis with purulent exudate, mucosal ulceration and tumorous masses (Figure 3).

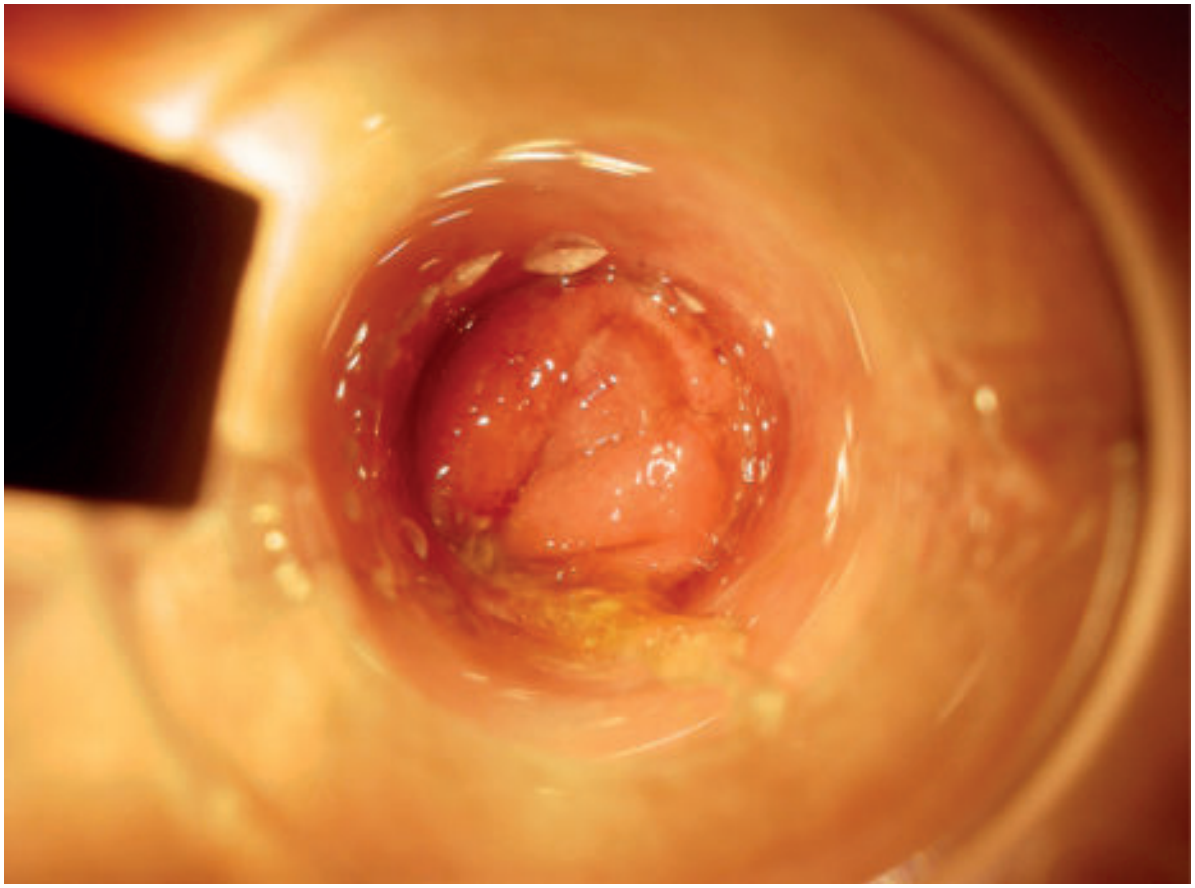

**FIGURE 3.** Anorectal lymphogranuloma venereum with discharge, mucosal inflammation (point bleedings) and edema.

Usually, the anorectal syndrome is not associated with externally palpable lymphadenopathy, because the locoregional draining glands are located internally, in the abdominal cavity. With radiological imaging of the abdomen and pelvic regions, lymphadenopathy can be objectified. If left untreated, the anorectal syndrome can lead to permanent anal strictures, which can lead to soiling, pain, constipation and the development of a megacolon.[10] In the second stage (1–6 weeks after inoculation), the infection spreads beyond the mucous membranes into the underlying connective tissue. In case of the inguinal syndrome, bacterial spread via the locoregional lymphatic system cause subsequent inguinal

lymphadenopathy with the formation of buboes (fluctuating abscessing lymph nodes; Figure 4). If the infection persists, the surrounding connective structures get involved leading to tissue destruction and fistula can arise (Figure 5). Another complication of neglected LGV infection is irreversible damage to the lymphatic drainage system that can result in permanent genital lymphedema (elephantiasis).

LGV proctitis can mimic inflammatory bowel diseases, such as Crohn's disease, both clinically and in histological findings.[25] As a result, LGV can be missed by gastroenterologists in patients with symptomatic proctitis. 'A delay in the correct diagnosis and an initial clinical response to immunosuppressive therapy may lead to insidious progression of LGV infection with the risk of irreversible late complications'. Constitutional symptoms, such as low-grade fever, chills, malaise, myalgia and arthralgia may present during the second stage of disease. From earlier literature, it is known that systemic spread of *C. trachomatis* and reactive inflammatory responses occasionally result in arthritis, pneumonitis or (peri)-hepatitis.[26]

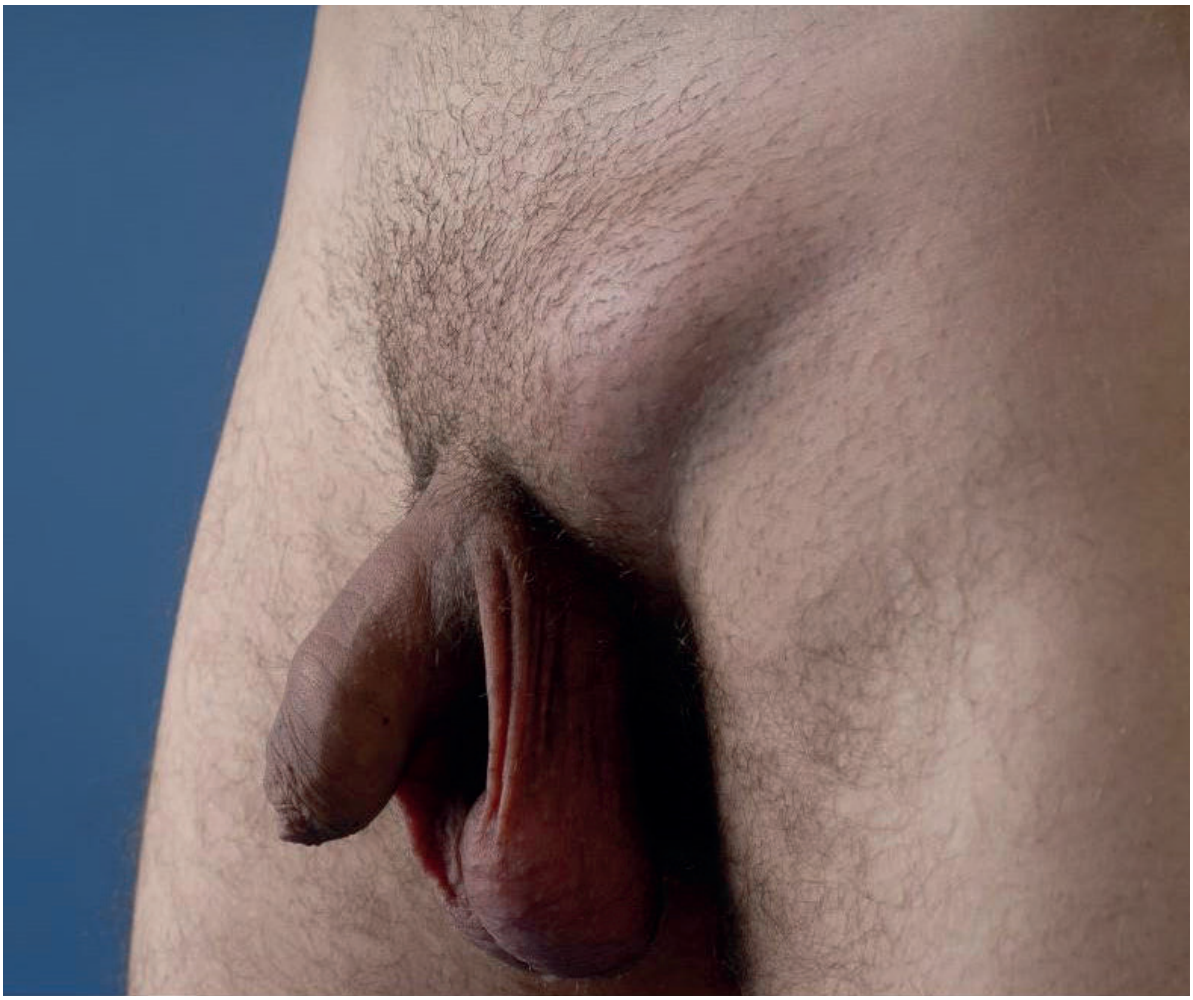

**FIGURE 4.** Second-stage inguinal lymphogranuloma venereum with a bubo in the inguinal region.

**FIGURE 5.** Late-stage inguinal lymphogranuloma venereum with fistulae and sinus formation in the inguinal region. Courtesy of Ricardo Hu, Dermatologic Service, Ministry of Health of the Republic of Suriname, Paramaribo, Suriname.

## Missed extra-anal LGV infections

Since the first reports of LGV in MSM in 2003, the endemic is still ongoing. There have been reports of increasing numbers of cases over recent years in the UK[27], Amsterdam[8], Barcelona[28] and Madrid.[29] It seems most plausible to assume that anorectal LGV infections are caused by unprotected genital-anal contact, but only very few genital, urogenital or pharyngeal LGV infections are reported.[27–31] Underreporting might be the cause, since none of the current guidelines on the management of LGV advise to exclude urogenital or pharyngeal LGV infections. We recently reported a 2.1% urethral LGV prevalence among patients with anorectal LGV and 6.8% urethral LGV prevalence among contacts of patients with anorectal LGV, indicating that urogenital LGV infections are much more common than previously believed.[32] Moreover, only 1 of the 11 urethral LGV cases had the classic inguinal buboes (lymphadenopathy). These findings indicate there is an undiagnosed reservoir of urethral infections, of which the majority remains asymptomatic.

The small number of urogenital LGV infections may reflect the lack of diagnostic testing.[8,32] The reason why urethral LGV infections were considered rare is because LGV is in general considered a symptomatic disease. In a study of unselected MSM attending clinics in the UK, urethral LGV prevalence was estimated at 0.04% compared with 0.9% for anorectal LGV.[31] Yet, this estimation was based on symptomatic patients only. In a recent German study involving 1833 MSM, Chlamydia-positive specimens from the rectum, pharynx and urethra or urine were genotyped, and LGV biovars were found in 17 of 103 anorectal specimens.[33] Unfortunately, the number of extra-anal specimens was far too small to draw sound conclusions on 2 of 13 pharyngeal specimens, and none of eight urethral/urine specimens proved an LGV biovar as causative pathogen. In Madrid, 10/338 (2.6%) Chlamydia-positive urethral samples from symptomatic men were identified to be LGV biovars.[29] Overall, 10/2420 were positive of urethral LGV (0.4%). Although the data on urethral LGV infections are sparse, even less is known on the prevalence of pharyngeal LGV infections. In Finland, a pharyngeal LGV biovar-positive sample was found in an MSM who also had anorectal LGV.[34] LGV was detected in four MSM from the pharynx in the UK.[35] At this moment, no clear screening guidelines for extra-anal LGV infections exist, but should be formulated in the future.

Apart from missed extra-anal LGV infections, tissue tropism could be a second explanation for the discrepant anatomical distribution of LGV infection.[36,37] There are indications that *C. trachomatis* biovars preferentially infect specific tissues, which in part might explain the overrepresentation of anorectal L2b infections among MSM. At the same time, the L2b biovar might infect urethral epithelium only temporarily, long enough to contribute to ongoing transmission, but too short to become symptomatic and clinically detected.

## Missed asymptomatic anorectal LGV infections

Recently, more studies have reported on a significant proportion of asymptomatic anorectal LGV infections. In Amsterdam, 27.2% of anorectal LGV cases were found to be asymptomatic whereas in Madrid, approximately 11% (9/82) of the anorectal LGV cases were reported as asymptomatic.[8,29] In the UK, 22% of LGV infections detected in a case-finding study were asymptomatic at first presentation.[38] On the other hand, Ward *et al*. only found 5% asymptomatic LGV cases in a multicenter case finding study.[31] Because of the retrospective data collection, recall bias could have caused overreporting of complaints experienced at the time of the clinic visit. Moreover, anal coinfections were not excluded in this study, which also might have influenced the reported number of symptomatic cases.

The proportion of asymptomatic LGV infections in the current LGV endemic in MSM could be explained by the relative immunosuppressive state resulting from the concurrent HIV infection in most patients.[8,16,22] It is recommended to screen all MSM for anorectal *C. trachomatis* infection who report anoreceptive sexual contact within the last 6 months. Those who have an anorectal *C. trachomatis* infection should be subsequently screened for LGV proctitis, according to local guidelines (irrespective of symptomatology and/or HIV status).[39]

## Diagnosis

Different approaches based on cultivation, immunofluorescence and ELISA have previously been described to diagnose LGV infections.[40] The current gold standard diagnostic tests are 'inhouse' developed nucleic acid amplification tests (NAAT) designed to identify LGV biovar-specific *C. trachomatis* strains.[39] Compared with the older techniques, NAAT-based assays are known to have much higher sensitivity and specificity.[40]

The clinic-diagnostic procedure to identify LGV is a two-step process. First, the presence of *C. trachomatis* is objectified with a biovar non-specific commercial test. These tests are very sensitive and routinely available in most microbiology laboratory settings. Second, the diagnosis of LGV is confirmed with an LGV biovar specific test. For this purpose, two 'in-house' NAAT have been developed. First, a real-time PCR-based test that specifically detects all *C. trachomatis* LGV biovar strains as described by Morré *et al*.[40] The second test is a real-time quadriplex PCR assay that incorporates an LGV-specific target, a non-LGV-specific target sequence, a *C. trachomatis* plasmid target and the human RNase Pgene as an internal control as described by Chen *et al*.[41] The test characteristics of the two methods are comparable.[42]

Depending on the body location that needs to be scrutinized, specimens can be collected via swabs of ulcers, anorectal, vaginal, urethral or pharyngeal mucosa, bubonic aspirates or first-catch urine.

Although LGV NAAT are considered standard, these tests are also expensive, require specialized laboratory conditions and as a result in many cases are not available, like in resource-poor settings.

If LGV-specific NAAT are not at hand, a Chlamydia IgA specific antibody test can be an alternative choice to make a presumptive LGV diagnosis.[43,44] LGV is likely in case of an elevated antibody titer (far outside the normal ranges) in combination with a confirmed anorectal *C. trachomatis* infection. In the current situation, it is desirable that *C. trachomatis*

positive specimens from MSM can be tested more easily at more laboratories and with increased affordability. Commercialization of LGV NAAT is needed and should be developed in the future.

The 2013 British Association for Sexual Health and HIV recommends LGV-specific NAAT diagnostics only on specimens from patients with clinical syndromes suggestive of LGV.[26] As a result, asymptomatic LGV cases are likely missed when only men with symptoms are tested. As mentioned above, the proportion of asymptomatic LGV cases differs between 5 and 27%, depending on the study. Testing symptomatic men only contributes to ongoing transmission and the likelihood of late complications in affected individuals. For this reason, the 2013 European guideline on the management of LGV recommends to screen all MSM who report receptive anal sexual practices in the previous 6 months for anorectal *C. trachomatis* infection, irrespective of signs and symptoms.[39] Subsequently, in all MSM with proven anorectal *C. trachomatis* infections, screening for LGV proctitis according to local guidelines is advised. In addition, screening for coinfections including *Neisseria gonorrhoeae*, HIV, HCV, hepatitis B and syphilis should be performed. Counseling and hepatitis B vaccination should be offered too before starting therapy. Apart from asymptomatic LGV cases, those who are already symptomatic but not yet treated also contribute to the ongoing transmission.

## Treatment

There is a shortage of robust evidence regarding the efficacy of therapy for rectal chlamydial infections (LGV or non-LGV).[45,47,48] Nonetheless, the first recommended (expert based) choice of treatment for an LGV infection is doxycycline 100 mg twice daily for 21 days.[39] This is considerably longer than the 7 days doxycycline regimen recommended for anorectal non-LGV *C. trachomatis* infections. We have shown that in anorectal LGV infections, *C. trachomatis* RNA could be demonstrated up to 16 days during continuous doxycycline therapy, which justifies the 3-week antibiotic course.[49] The need for a prolonged doxycycline course stresses the importance of additional biovar-specific testing in case of *C. trachomatis* infections in risk groups for LGV (i.e., MSM).

The alternative treatment option for LGV is erythromycin 500 mg four-times daily for 21 days. Disadvantages are gastrointestinal side effects, which occur more often than during doxycycline therapy. Yet erythromycin is the required option in pregnant women and those with a contraindication for doxycycline (e.g., because of allergic reactions). There is a need for antibiotic regimens with a shorter duration to improve patient compliance. Azithromycin has been proposed for shorter duration treatment regimes, but to date there

is no evidence on the efficacy of azithromycin in any dosage for the treatment of anorectal *Chlamydia* infections. Further studies on the efficacy of azithromycin for LGV are necessary. A test of cure is recommended if alternative treatment options are used.

To prevent spontaneous rupture and chronic fistulae, fluctuant buboes in LGV patients should be aspirated promptly through healthy adjacent skin. Surgical incision of buboes is not recommended because this can induce sinus formation. Reconstructive genital surgery should be considered in patients with residual fibrotic lesions, strictures or fistulae, since these permanent sequelae do not benefit from further courses of antibiotics. As LGV is sexually transmitted, it is essential to initiate partner notification when the diagnosis is made.[39] Sexual contacts within the last 4 weeks should be offered STI testing and effective antibiotic treatment for LGV. Screening for syphilis, *C. trachomatis*, *N. gonorrhoeae*, HIV, hepatitis B and HCV should be offered during a follow-up visit 3 months after an LGV diagnosis to exclude reinfections.[50]

# CONCLUSION

The LGV endemic among MSM in the Western world is still prevalent. In Amsterdam, the number of cases doubled in 2012 (116 cases) as compared with the previous year (51 cases), and the same trend is seen in other European countries. Very little is known about the prevalence of LGV among MSM in non-Western society. One small study looked into STI among 43 HIV-negative MSM in Kilifi, Kenia.[51] Among the three anorectal *C. trachomatis* infections found, no LGV biovar infection was detected.

Despite a decade of observation, many clinical and epidemiological questions remain unanswered and the exact modes and risks of transmission are still not elucidated. Unprotected receptive anal intercourse as a key risk factor supports the hypothesis that rectal infection is due to direct inoculation[7], this can occur either directly from a man with urethral infection or indirectly from rectal-to-rectal transmission via fomite spread at sex parties or during group sex.

Based on the current epidemiological data, it seems that anorectal LGV is yet limited to MSM with high-risk behavior, although LGV infections also occur in MSM with low-risk behavior. Since we do not routinely screen women for rectal *C. trachomatis*, let alone LGV, we cannot say the disease is limited to MSM. It is unlikely, but since LGV can be asymptomatic, infections in other populations may be missed.

Because in approximately a 5–27% of the patients with anorectal LGV, no complaints are reported or signs of inflammation are present upon the first consultation, it seems important to consider LGV in all MSM at risk for an anorectal infection, that is, having had receptive anal intercourse in the past 6 months. In case of a proven anorectal *C. trachomatis* infection, further diagnostics to rule out LGV, preferably using a biovar-specific NAAT, is advisable.

Physicians dealing with urogenital and rectal pathology (e.g., STI clinicians, GPs, emergency medicine and gastroenterology specialists) should be alert for LGV in MSM presenting with inguinal lymphadenopathy, genital ulcers or symptoms of proctitis. If a chronic inflammatory bowel disease such as Crohn's disease is considered in MSM, LGV proctitis must always be excluded. In view of the increasing trend, it is clear that the LGV endemic is not under control, and that screening needs to be further intensified. This is important in order to prevent complications in the individual patient, and to avoid further expansion in the community.

## Expert commentary & five-year view

Although it remains unknown how the ongoing LGV endemic will develop in the future, current data underline the importance of more intensified and active testing. Clearly, the reservoir of disease has not been accurately defined. As a result, it seems that a large and important proportion of the LGV endemic is overlooked. The overrepresentation of anorectal infections in the current epidemiological picture remains a puzzle to be solved. Undetected urethral, and possibly pharyngeal infections may be candidates that can explain the dominant number of anorectal infections found.[32]

Since LGV emerged among MSM in 2003, the infection has kept on affecting the same high-risk core population it was found in originally, without an indication to spill over into a larger network.[8] It remains to be seen if the LGV endemic will remain compartmentalized within the high-risk MSM network or will spread to larger communities.

In the coming years, LGV research will need to focus more on the biology of the disease. It is still unclear what pathogenic and/or host mechanisms cause the invasive and aggressive nature of the LGV biovars as opposed to the non-LGV biovars that remain confined to the mucosal layers and in many cases cause little inflammatory responses. Although the genome of the LGV biovar shows no additional genes that could account for the differences in disease outcome, functional gene loss and regions of heightened sequence variation have shown to be important sites for interstrain recombination.[52] Lastly, gene transfer between *C. trachomatis* biovars has been proven to occur.[53,54] This could have considerable and unpredictable effects on the clinical characteristics of urogenital *C. trachomatis* disease and hamper diagnostics and therapeutic interventions.

Efforts to control the transmission of LGV include prevention, early diagnosis and an appropriate treatment of the patient and sexual partners. Future LGV management guidelines will need to address extra-anal and asymptomatic LGV infections. Commercialization of the LGV-specific tests is needed and cost-effectivity studies are required to weigh the benefits against the costs for additional testing.

# KEY ISSUES

- The lymphogranuloma venereum (LGV) endemic among men who have sex with men (MSM) in Western society is caused by a specific biovar L2b, also known as the Amsterdam variant.
- Until now, the LGV endemic seems confined to a core group of MSM with high-risk behavior, with no spill over into the wider MSM community. L2b infections in the heterosexual population are exceedingly rare.
- The LGV endemic in Western society is ongoing and the prevalence is rising in Europe.
- The majority of LGV infections among MSM are found in the anorectal canal. Although urethral LGV infections seem more common than thought before, the transmission mode of LGV among MSM remains unclear.
- LGV is associated with a high prevalence of sexually transmitted infections, including HIV in 80% and hepatitis C in 20% of the cases.
- LGV is asymptomatic in approximately a quarter of the cases upon the first consultation. It is therefore recommended to screen all MSM who practiced receptive anal sex in the previous 6 months for anorectal *Chlamydia trachomatis* infection, irrespective of signs and symptoms, and if found positive, subsequent tests to exclude LGV proctitis should be performed.
- The preferred method to screen for LGV is to first identify *C. trachomatis* by a commercial biovar non-specific nucleic acid amplification tests. If the test result is positive, the same sample can then be used to identify LGV with an 'in-house' developed LGV biovar-specific nucleic acid amplification test assay.
- The first choice of treatment for anorectal LGV is doxycycline 100 mg twice daily for 21 days, as opposed to a 7-day doxycycline regimen sufficient for *C. trachomatis* non-LGV biovar anorectal infections.
- Apart from counseling of index patients, sexually transmitted infection screening and presumptive treatment of all sexual partners in the previous 4 weeks is required.

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# 03

# ANORECTAL AND INGUINAL LYMPHOGRANULOMA VENEREUM AMONG MEN WHO HAVE SEX WITH MEN IN AMSTERDAM, THE NETHERLANDS: TRENDS OVER TIME, SYMPTOMATOLOGY AND CONCURRENT INFECTIONS

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*Sex Transm Infect. 2013;89(7):548-52*

# ABSTRACT

**Objectives.** To examine lymphogranuloma venereum (LGV) trends over time among men who have sex with men (MSM) visiting the Amsterdam sexually transmitted infection (STI) clinic; to investigate anal LGV symptomatology; and to examine the positivity and characteristics of anorectal and inguinal LGV.

**Methods.** We included MSM consultations from whom a swab (from anorectum, bubo or an genital ulcer) was taken for *Chlamydia trachomatis* (*Ct*) screening. Anorectal swabs were taken from all MSM who reported receptive anorectal intercourse in the preceding 6 months. *Ct* positive samples were further tested with a *pmpH* PCR to identify L-genovars. Patient symptoms, clinical and anoscopic inflammatory signs, and STI co-infections were noted; Gram-stained anorectal mucosal smears were examined.

**Results.** Between January 2005 and June 2012, 48,570 consultations among MSM were conducted. In 3628/35,650 visits, anorectal *Ct* infections were diagnosed, including 411 anal LGV (1.2%). Moreover, 65/1649 genital ulcer swabs were *Ct* positive; 10 were inguinal LGV (0.6%). Since January 2011 a significant increase in the positivity of LGV occurred ($p<0.0001$). 89 (27.2%) anorectal LGV cases were asymptomatic. HIV prevalence among anorectal LGV cases was significantly higher ($p=0.008$) than among inguinal LGV cases. STI co-morbidity in anorectal LGV cases remained invariably high during the study period.

**Conclusions.** Since January 2011, LGV positivity in MSM consultations in Amsterdam has risen significantly. The great majority comprise anal LGV; inguinal LGV is rare. Anal LGV is asymptomatic in a quarter of cases. In all MSM with anal *Ct* infections LGV should be excluded, irrespective of symptoms or inflammatory signs.

# INTRODUCTION

The epidemic of lymphogranuloma venereum (LGV) among men who have sex with men (MSM) in industrialised countries first became apparent in 2003.[1] In a report from Rotterdam, the Netherlands, 13 MSM (most of them HIV infected) with severe LGV anorectal infections were presented.[2] Subsequently, similar LGV proctitis cases were reported from major cities throughout Europe.[3] LGV among MSM is associated with high-risk behaviour, which is reflected in the large proportion of sexually transmitted co-infections, like HIV (in up to 82.2% of the cases),[4] hepatitis C and previous syphilis infections.[5] LGV is an invasive ulcerative sexually transmitted infection (STI) caused by *Chlamydia trachomatis* (*Ct*) biovar L.[6] Acute anorectal LGV is characterised by anorectal cramps, pain, bloody discharge and constipation. If left untreated, chronic disease can lead to irreversible anorectal strictures causing soiling, pain, constipation, and megacolon.[7,8] LGV may also increase the risk for acquisition and transmission of other STIs, including HIV and blood-borne infections like hepatitis C virus.[9,10]

The LGV epidemic is ongoing and new cases are being reported every year, mainly from large urban centres throughout Europe, the USA and Australia.[3,11,12] The vast majority of diagnosed LGV infections in this epidemic were anorectal; only few inguinal LGV infections have been reported. This clinical–epidemiological discrepancy leaves the mode of transmission between men unexplained.[3,13]

There is some debate whether anorectal LGV may have an asymptomatic course. We earlier reported that in a considerable proportion of consultations (up to 40.0% of the cases), patients reported few symptoms or had no physical abnormalities.[5,14] In contrast to our findings, studies from the UK showed that only a small proportion of LGV cases were asymptomatic (6.0–17.2%).[15,16]

In this article we report on the positivity trends of LGV, the symptomatology of anorectal LGV, the relative frequencies of anorectal and inguinal LGV, and the differences in demographics and concurrent STIs between anorectal and inguinal LGV cases, among MSM visiting the STI clinic in Amsterdam, the Netherlands, between January 2005 and June 2012.

# METHODS

## Study population and setting

The STI outpatient clinic of the Public Health Service in Amsterdam, the Netherlands, offers free-of-charge examination and treatment for sexually transmitted diseases (STDs). In the clinic an MSM client is defined as a man who reports sexual intercourse in the preceding 6 months with one or more men. In 2011 more than 40% of all MSM visiting STI clinics throughout the Netherlands were visitors of the STI clinic in Amsterdam.[17,18] In Amsterdam, MSM are offered screening for gonorrhoea, syphilis, hepatitis B, chlamydia and HIV as described previously.[19] Each visitor is seen by a trained nurse, who records possible STI related symptoms, inspects the anogenital region, and collects samples for routine diagnostics. If the visitor reports receptive anal sex in the previous 6 months, anoscopy is performed. Anorectal symptoms (anorectal pain, discharge, soiling, blood loss, urge or constipation) and signs of anorectal mucosal inflammation (oedema, purulent discharge, mucosal fragility, ie, bleeding on manipulation, and ulceration) are recorded in the electronic patient file.

## Clinic procedures

Swabs collected from the urethra, from the anorectal mucosa on anoscopy and from ano-genital ulcers, as well as bubo aspirates, were screened for *Ct* by nucleic acid amplification testing (Aptima Combo 2 system; GEN-PROBE, San Diego, California, USA). Since 2005, all anorectal mucosal, ulcer or bubo samples positive for *Ct* were tested further with a *pmpH* based in-house real-time PCR to discriminate between LGV and non-LGV genotypes, as described previously.[20,21] If the *pmpH* test was inconclusive (mainly due to insufficient DNA), the result was considered negative for LGV. If an anorectal mucosa swab or an anorectal ulcer swab was positive for an LGV genotype, anorectal LGV was diagnosed. If a penile ulcer swab or a bubo aspirate was positive for an LGV genotype, inguinal LGV was diagnosed. Samples of the urethra positive for *Ct* were not tested further for LGV.

If the visitor reported anorectal symptoms or if signs of ano-rectal mucosal inflammation were observed on anoscopy, Gram-stained anorectal mucosal smears were made and examined by a trained laboratory technician to exclude gonorrhoea and to count the number of polymorphonuclear leucocytes per high power microscope field (PMNL/hpf). If more than 10 PMNL/hpf were recorded, a diagnosis of presumptive non-specific proctitis was made and doxycycline therapy commenced before the definite results became available.

HIV antibody rapid testing (Determine 1/2; Abbott Laboratories, Abbott Park, Illinois, USA) was offered to all clients, except to visitors already diagnosed with HIV. Reactive samples were confirmed by line immunoassay (Inno-Lia HIV I-II Score; Innogenetics, Ghent, Belgium). When a client declined to be tested, the HIV status was reported as 'unknown'. Syphilis serologic screening to detect both recent and past infections was performed with a *Treponema pallidum* particle agglutination assay (TPPA, Fujirebio, Chuo-ku, Tokyo, Japan), followed by the rapid plasma reagin (RPR, bioMérieux, Marcy l'Etoile, France) in case of a TPPA titre of ≥1:80. In case of genital ulceration, dark-field microscopy to detect spirochetes and PCR on ulcer swabs for T. pallidum and herpes simplex virus (HSV type 1 and 2) were performed.[22] All clinical findings, diagnoses and subsequent treatment were recorded in an electronic patient database along with patient characteristics and information on sexual behaviour.

## Statistical analyses

Data from the electronic patient file were extracted for all MSM who visited the STI clinic in Amsterdam for screening between January 2005 and June 2012. Visitors could be diagnosed with LGV on more than one occasion. For the analysis of the symptomatology of anorectal LGV, men with anorectal gonorrhoea were excluded, since both infections may cause symptoms.

Fisher's exact test and the Mann–Whitney U test were used to compare groups. We analysed whether LGV positivity in all MSM consultations increased over the last six quarters, from January 2011 to June 2012, with the $\chi^2$ test for trend. Trends in the annual proportions of LGV cases with STI co-morbidity (HIV, TPPA and gonorrhoea) were analysed, for the whole study period, with the $\chi^2$ test for trend. All tests were two-sided and were considered significant if the p value was less than 0.05. All statistical analyses were performed using SPSS V.19. Since we used anonymised routine data, no ethical clearance for this study was needed.

# RESULTS

## Consultations of MSM

Between January 2005 and June 2012, 48,570 consultations involving an MSM visitor were done (Figure 1). At 35,650 visits an anorectal swab was obtained. We diagnosed 3628 (10.2%) anorectal *Ct* infections, including 411 anorectal LGV infections (positivity 1.2%). In 1649 consultations genital ulcers were sampled. In total 65 were *Ct* positive; of these, 10 were L-biovar Ct positive. Therefore, the inguinal LGV positivity among MSM was 0.6%.

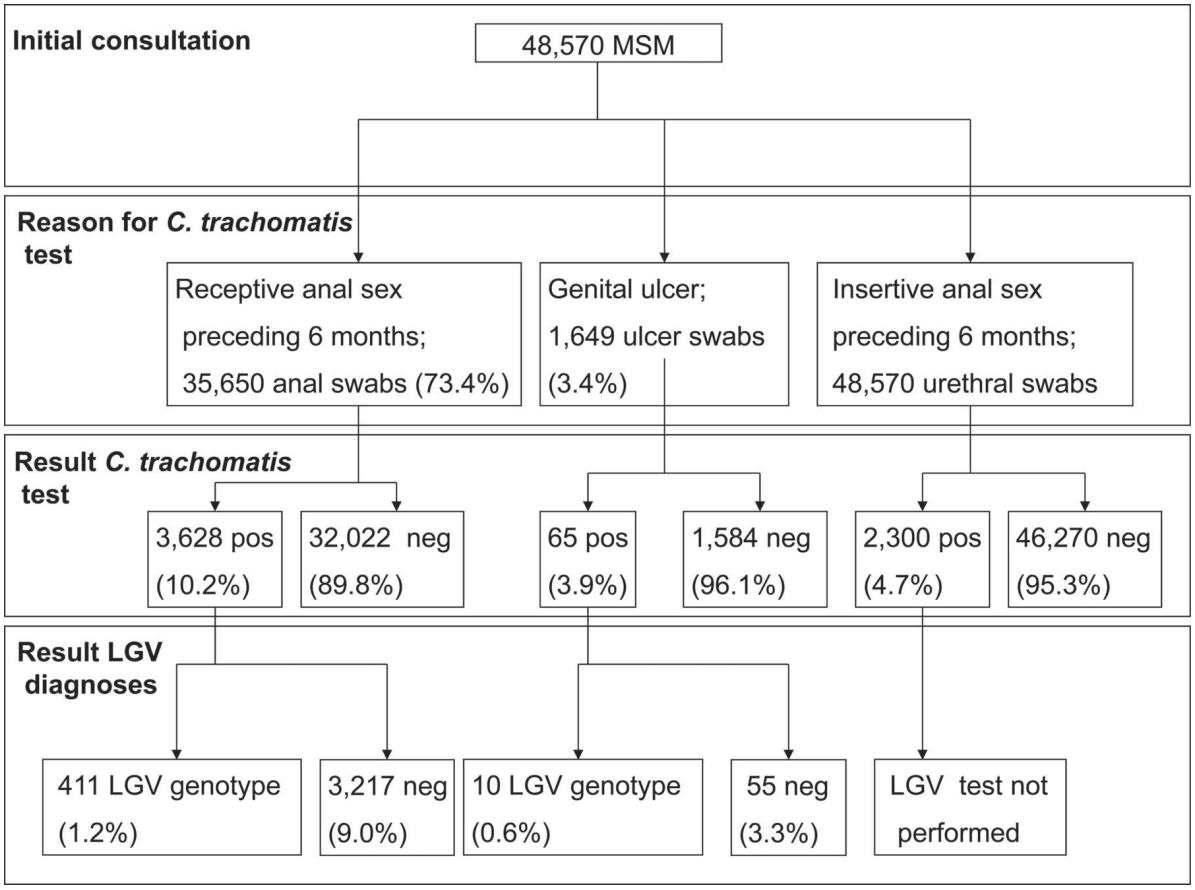


**FIGURE 1.** Flowchart for the diagnosis of lymphogranuloma venereum (LGV) in men who have sex with men (MSM) visiting the sexually transmitted infection outpatient clinic, Amsterdam, the Netherlands, January 2005–June 2012.

## Consultations with LGV infections

In the study period 411 anorectal LGV infections were diagnosed in 365 MSM and 10 inguinal LGV infections in 10 individual MSM. Of the men with an anorectal LGV infection, 46 MSM had a repeat infection; 34 were infected twice, 7 three times, and 5 more than three times. No individual had a dual anorectal and inguinal LGV infection.

Characteristics of anorectal and inguinal LGV consultations are compared in the online supplementary table S1. Overall, the consultations with anorectal and inguinal LGV were predominately of Dutch nationality (respectively, 69.6% and 50.0%; p=0.3) and the median age was, respectively, 41 (IQR 30.3– 48.8) and 43 (IQR 35.0–46.0) years (p=0.9). The prevalence of HIV infection was significantly higher among MSM with anorectal (82.7%) compared to those with inguinal LGV (50.0%, p=0.008). TPPA serology was positive in, respectively, 68.1% and 60.0% (p=0.7). Concurrent gonorrhoea, urogenital and/or anorectal, was detected in 24.6% of anorectal LGV cases. No concurrent gonorrhoea was diagnosed among consultations with inguinal LGV (p=0.1).

## LGV infections 2005–2012

The LGV positivity per quarter fluctuated between 0.1% and 2.5% in the period from 2005 to 2009 (Figure 2, see online supplementary table S2). From 2010 onwards the LGV positivity was stable at around 1% per quarter, but more recently a sharp increase was observed ($\chi^2$ test for trend over six quarters 2011–2012; p<0.0001). In absolute numbers, more LGV cases (n=64) have been found in the first half of 2012 compared to all of 2011 (n=51).

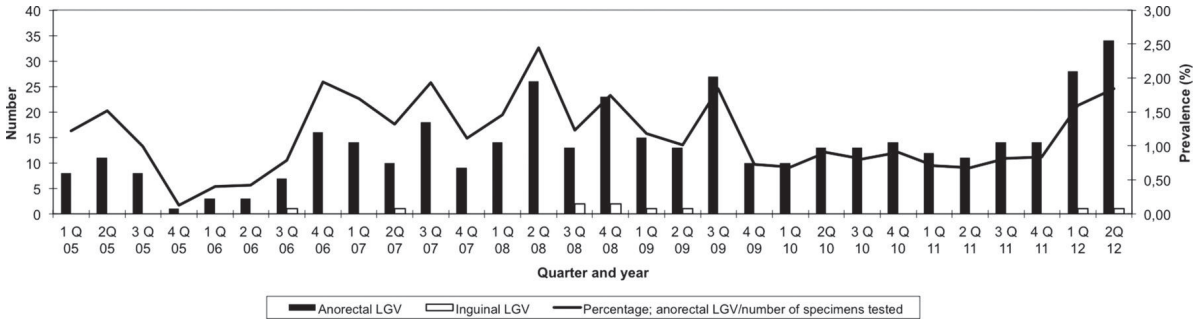


**FIGURE 2.** Number and positivity (%) of lymphogranuloma venereum (LGV) among men who have sex with men visiting the sexually transmitted infection outpatient clinic, Amsterdam, the Netherlands, by quarter from January 2005 to June 2012.

## Clinical presentation of anorectal LGV infections

For the analysis of clinical signs and symptoms of anorectal LGV, we excluded men diagnosed with concurrent anorectal gonorrhoea (n=84) since both infections may cause anorectal symptoms or inflammatory signs. In the remaining 327 anorectal LGV cases, anorectal symptoms were reported in 195 consultations (59.6%) (Table 1). In 167 cases (51.1%), anorectal inflammatory signs were identified on proctoscopy. In 124 (37.9%) consultations, both inflammatory signs and anal symptoms were present; and in 89 (27.2%) consultations with an anal LGV infection, neither anorectal symptoms nor inflammatory signs were recorded. Overall there were 3628 consultations with a positive *Ct* diagnosis. When consultations with a concurrent anorectal gonorrhoea (n=640) were excluded, in 2193/2988 (73.4%) consultations with an anorectal *Ct* infection, neither anal symptoms nor inflammatory signs were recorded.

In 203/238 (85.3%) anorectal LGV cases with anorectal symptoms or inflammatory signs, light microscopic examination of Gram-stained anorectal smears revealed ≥10 PMNL/hpf (Table 1).

**TABLE 1.** Clinical presentation of 327 consultations with anorectal lymphogranuloma venereum, sexually transmitted infections outpatient clinic, Amsterdam, the Netherlands, January 2005-June2012*

| Clinical characteristic | n (%) |
|---|---|
| One or more anorectal symptoms† | 195 (59.6%) |
| One or more anorectal inflammatory signs‡ | 167 (51.1%) |
| One or more anorectal symptoms† AND one or more inflammatory signs‡ | 124 (37.9%) |
| Neither anorectal symptoms nor signs | 89 (27.2%) |
| Anorectal mucous Gram stained smear with >10 PMNL/hpf¥ | 203 (85.3%) |

*Consultations with concurrent anorectal gonorrhoea were excluded.
† Anorectal symptoms: anorectal pain, discharge, soiling, blood loss, urge and/or constipation.
‡ Anorectal inflammatory signs (on anoscopy): oedema, purulent discharge, mucosal fragility (bleeding on manipulation) and/or ulceration. Anoscopic data from one case are missing.
¥ PMNL/hpf: polymorphonuclear leucocytes per light microscopic high power field. Cases without symptoms or signs were excluded. Gram stained smears were performed in 238 consultations.

## Concurrent STI among LGV cases

Of 421 LGV cases, 345 (81.9%) were known to be HIV positive or tested HIV positive at the time of consultation; of 17 (4.0%) cases, the HIV status was unknown. Of all LGV cases, 286 (67.9%) were TPPA seropositive, indicating a previous syphilis or current syphilis infection at the time of consultation, and 101 (24.6%) had concurrent urogenital or anorectal gonorrhoea. During the study period, the proportion of men with LGV who also had HIV, TPPA positivity or gonorrhoea, remained consistently high, and no significant downward trends over time of co-infections among LGV cases were detected ($\chi^2$ test for trend for TPPA: p=0.8; for gonorrhoea: p=0.3); the proportion of cases with HIV co-infection increased over time ($\chi^2$ test for trend; p=0.04; Figure 3 and see online supplementary table S3).

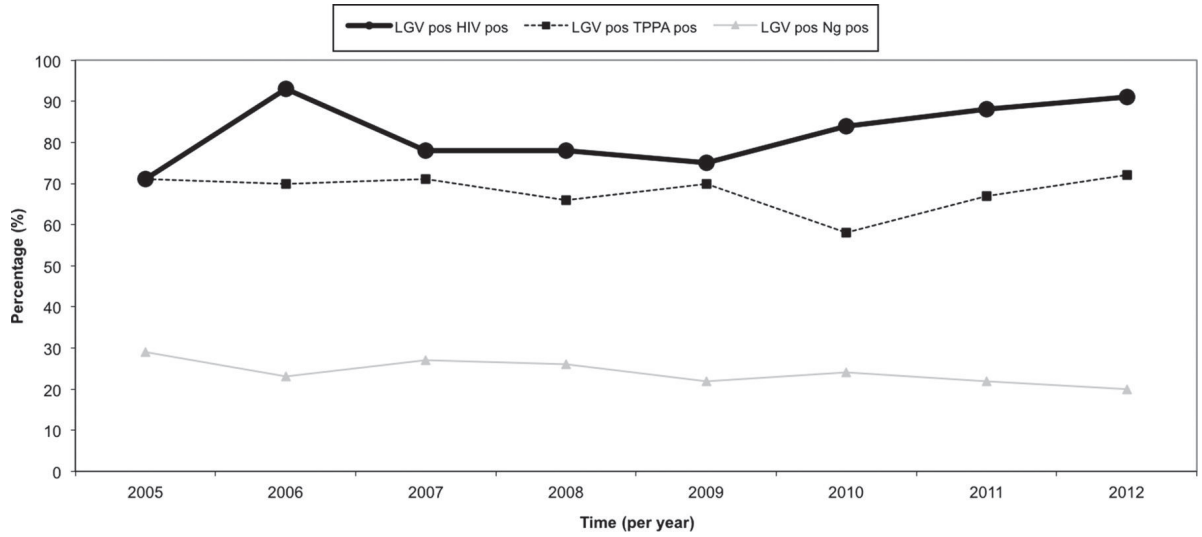


**FIGURE 3.** HIV, syphilis *Treponema pallidum* particle agglutination assay (TPPA) sero-status and concurrent *Neisseria gonorrhoeae* (Ng) infection (%) among men who have sex with men consultations with lymphogranuloma venereum (LGV), by year, sexually transmitted infection outpatient clinic, Amsterdam, the Netherlands, January 2005–June 2012.

# DISCUSSION

Our data show a significant increase of LGV positivity in MSM consultations at the Amsterdam STI clinic over the six quarters since January 2011. In absolute numbers we found more LGV in the first half of 2012 compared to all of 2011. The rise of LGV positivity cannot be attributed to changes in testing policy, since no changes were made during the study period. A similar increase in the number of LGV cases has been reported in the UK.[23] Between October 2004 and March 2011, 14 250 specimens were received for confirmatory testing, of which 1665 were found to be LGV positive[24]; a third of these cases had been diagnosed since January 2010. The LGV endemic among MSM in Western society has been going on for almost 10 years and the recent increasing trends in Amsterdam and the UK indicate that the epidemic is not controlled and screening should be further intensified.

We found a high prevalence of concurrent STI (gonorrhoea, HIV, syphilis) among LGV cases. During the study period the proportion of concurrent STI among LGV cases remained high, with a small but significant increase in the proportion of concur- rent HIV. This negates the idea that the LGV epidemic spreads from the core group of high-risk MSM identified at the start of the epidemic into a broader population. Two recent publications from France[25] and the Netherlands[26] described LGV in women caused by the L2b genovar characteristic for the epidemic in MSM. In conclusion, with only two female cases reported in the 9 years since

the discovery of the epidemic, it seems that the L2b LGV epidemic is still largely confined to a population of high-risk MSM. Nonetheless, a spread of LGV into the broader population is still a possible threat.

With only 10 inguinal LGV cases over 7.5 years, the overt predominance of anorectal LGV infections (n=411) in Amsterdam remains unexplained. The preponderance of anorectal LGV cases has also been reported in other European studies.[16] It has been suggested that anorectal LGV could be transmitted through sharing of toys and fisting during group sex activities,[2] but this could not be confirmed in large systematic studies afterwards.[5,27] Few cases of urethral LGV infection have been reported.[15,28] Missed urethral LGV infections, and possibly pharyngeal LGV[29] could form contributing sources responsible for anorectal LGV infections. As MSM with urethral *Ct* infections are not routinely tested for LGV, perhaps many urethral LGV diagnoses are missed. This could explain the discrepancy between the numbers of reported inguinal and anorectal LGV cases reported.

Compared to anorectal cases, significantly fewer cases of inguinal LGV were HIV infected and they less often had concurrent STIs. This could indicate that inguinal LGV cases show less high-risk behaviour compared to cases with anorectal LGV. Larger numbers of patients, recruited in multicentre studies, are required to analyse in greater depth these differences in risk factors between men with LGV at different anatomical locations.

The IUSTI/WHO guideline for LGV recommends screening for anorectal infections in MSM irrespective of anorectal symptoms.[6] Yet, in many settings LGV testing is performed only in case of inflammatory anorectal signs and/or symptoms. Based on the low proportion of asymptomatic anorectal LGV cases found in the UK,[15,16] the British Association for Sexual Health and HIV recommends LGV testing in case of an anorectal syndrome; that is, the diagnosis is based on clinical suspicion after the exclusion of other aetiologies of proctitis.[30] Here, we found that in 27.2% of the anorectal LGV cases neither symptoms were reported nor were inflammatory signs noticed on routine anoscopy. This is in agreement with earlier reports by our group indicating that a sizeable proportion of anorectal LGV patients are asymptomatic.[5,14] Asymptomatic anorectal LGV could be missed if screening is performed exclusively in men with anorectal symptoms and/or anoscopic proctitis. In settings where anoscopy is not performed routinely, the chance to miss LGV is even greater. In 40.4% of the anorectal LGV cases described here, no symptoms were reported on consultation. Light microscopic examination of anorectal smears is an inexpensive, easy and relatively sensitive test for the syndromic management of LGV infections in MSM.[5] In 85.3% of the studied anorectal LGV cases with symptoms or signs, we detected more than 10 PMNL/hpf. Anorectal smears as screening tool could be of value, especially in settings where expensive

serovar-L specific nucleic acid amplification testing is unavailable or too costly. Likewise, an HIV positive sero-status is a strong predictor for LGV in MSM with a proven anorectal *Ct* infection and can be considered in a syndromic algorithm.[5]

A limitation of our study is the lack of LGV testing of urethral *Ct* positive samples. Currently we are investigating the prevalence of urethral LGV infections in greater detail. Due to the retrospective nature of the study, consistent data on risk parameters like condom use and the number of partners were unfortunately not available for the whole study period.

In conclusion, the LGV epidemic among MSM in Amsterdam is ongoing, and seems to have accelerated in the past year and a half, with a higher number of cases detected in the first half of 2012 compared to the whole of 2011. To date, anorectal LGV is still an STI confined to MSM engaging in high-risk behaviour. Anorectal LGV forms the vast majority of cases; inguinal LGV is rare. The mode of transmission of LGV within the MSM community remains enigmatic; the discrepancy in numbers of anorectal and inguinal infections suggests that many LGV infections are missed. Control methods might have failed due to missed LGV cases like asymptomatic infections and infections at sites which are not routinely tested for LGV, such as the urethra.

Compared to anorectal LGV cases, cases with inguinal LGV are significantly less often co-infected with HIV and they tend to have fewer co-infections like gonorrhoea. Lower co-morbidity in inguinal LGV cases could be a reflection of lower-risk behaviour compared to anorectal LGV cases and requires further study. LGV is asymptomatic in a quarter of the consultations, therefore LGV should be excluded in all MSM with anorectal *Ct* infections, irrespective of anorectal symptoms or inflammatory signs.

# KEY MESSAGES

- Since 2005, lymphogranuloma venereum (LGV) has been prevalent among men who have sex with men (MSM) in Amsterdam, and the incidence has risen significantly since 2011.
- LGV is associated with a high prevalence of sexually transmitted infections, including HIV, in 80% of the cases, suggesting that LGV remains confined to a high-risk population.
- The great majority of LGV cases are anal LGV; inguinal LGV is rare, but perhaps many diagnoses are missed.
- Since LGV is asymptomatic in 27.2%, it should be excluded in all MSM with anal *Chlamydia trachomatis* infections, irrespective of anal symptoms or inflammatory signs.

# SUPPLEMENTARY TABLES

**SUPPLEMENTARY TABLE 1.** Characteristics of 421 visitors diagnosed with lymphogranuloma venereum (LGV), STI outpatient clinic Amsterdam, the Netherlands, January 2005 – June 2012

| Characteristic | Anorectal LGV n=411 (97.6%) | Inguinal LGV n=10 (2.4%) | P value |
|---|---|---|---|
| Age in years | | | 0.9[#] |
| Median age (IQR) | 41(30.3 – 48.8) | 43 (35.0- 46.0) | |
| Nationality | | | 0.3[##] |
| Dutch | 286 (69.6%) | 5 (50%) | |
| Non – Dutch | 125 (30.4%) | 5 (50%) | |
| HIV status* | | | 0.008[##] |
| HIV positive | 340 (82.7%) | 5 (50.0%) | |
| HIV negative or tested negative at a later visit | 54 (13.1%) | 5 (50.0%) | |
| Unknown | 17 (4.1%) | 0 (0.0%) | |
| Syphilis status | | | 0.7[##] |
| TPPA positive | 280 (68.1%) | 6 (60.0%) | |
| TPPA negative | 131 (31.9%) | 4 (40.0%) | |
| Concurrent *N. gonorrhoeae* | 101 (24.6%) | 0 (0.0%) | 0.1 |

[#] Mann-Whitney *U* test
[##] Fisher's exact test
[*] For analyses HIV unknown is excluded
IQR=interquartile range
TPPA=*Treponema pallidum* particle agglutination

**SUPPLEMENTARY TABLE 2.** Number and positivity (%) of lymphogranuloma venereum (LGV) among men who have sex with men visiting the outpatient clinic Amsterdam, the Netherlands, by quarter from January 2005 to June 2012

| Time (per quarter) | LGV anal | LGV inguinal | % positive | Anal tested |
|---|---|---|---|---|
| 1 Q 05 | 8 | 0 | 1,22 | 656 |
| 2 Q 05 | 11 | 0 | 1,52 | 726 |
| 3 Q 05 | 8 | 0 | 1,00 | 804 |
| 4 Q 05 | 1 | 0 | 0,12 | 806 |
| 1 Q 06 | 3 | 0 | 0,40 | 750 |
| 2 Q 06 | 3 | 0 | 0,42 | 706 |
| 3 Q 06 | 7 | 1 | 0,79 | 886 |
| 4 Q 06 | 16 | 0 | 1,94 | 823 |
| 1 Q 07 | 14 | 0 | 1,70 | 824 |
| 2 Q 07 | 9 | 1 | 1,19 | 759 |
| 3 Q 07 | 18 | 0 | 1,93 | 932 |
| 4 Q 07 | 9 | 0 | 1,11 | 811 |
| 1 Q 08 | 14 | 0 | 1,46 | 959 |
| 2 Q 08 | 26 | 0 | 2,44 | 1065 |
| 3 Q 08 | 13 | 2 | 1,23 | 1058 |
| 4 Q 08 | 23 | 2 | 1,74 | 1321 |
| 1 Q 09 | 15 | 1 | 1,18 | 1271 |
| 2 Q 09 | 13 | 1 | 1,01 | 1287 |
| 3 Q 09 | 27 | 0 | 1,84 | 1464 |
| 4 Q 09 | 10 | 0 | 0,73 | 1372 |
| 1 Q 10 | 10 | 0 | 0,69 | 1456 |
| 2 Q 10 | 13 | 0 | 0,91 | 1429 |
| 3 Q 10 | 13 | 0 | 0,81 | 1611 |
| 4 Q 10 | 14 | 0 | 0,90 | 1552 |
| 1 Q 11 | 12 | 0 | 0,71 | 1690 |
| 2 Q 11 | 11 | 0 | 0,68 | 1627 |
| 3 Q 11 | 14 | 0 | 0,81 | 1722 |
| 4 Q 11 | 14 | 0 | 0,83 | 1682 |
| 1 Q 12 | 28 | 1 | 1,60 | 1755 |
| 2 Q 12 | 34 | 1 | 1,84 | 18846 |
| **total** | **411** | **10** | **1,15** | **35650** |

**SUPPLEMENTARY TABLE 3.** HIV, syphilis (TPPA) serostatus and concurrent *N. gonnorrhoeae* (*Ng*) infection (%) among MSM consultations with lymphogranuloma venereum (LGV), by year, STI outpatient clinic Amsterdam, the Netherlands, January 2005 – June 2012

| | **LGV pos HIV pos** | **LGV pos TPPA pos** | **LGV pos *Ng* pos** |
|---|---|---|---|
| 2005 | 71 | 71 | 29 |
| 2006 | 93 | 70 | 23 |
| 2007 | 78 | 71 | 27 |
| 2008 | 78 | 66 | 26 |
| 2009 | 75 | 70 | 22 |
| 2010 | 84 | 58 | 24 |
| 2011 | 88 | 67 | 22 |
| 2012 | 91 | 72 | 20 |

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# 04

# URETHRAL LYMPHOGRANULOMA VENEREUM INFECTIONS IN MEN WITH ANORECTAL LYMPHOGRANULOMA VENEREUM AND THEIR PARTNERS: THE MISSING LINK IN THE CURRENT EPIDEMIC?

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*Sex Transm Dis. 2013;40(8):607-8*

# ABSTRACT

Urethral lymphogranuloma venereum (LGV) is not screened routinely. We found that in 341 men having sex with men with anorectal LGV, 7 (2.1%) had concurrent urethral LGV. Among 59 partners, 4 (6.8%) had urethral LGV infections. Urethral LGV is common, probably key in transmission, and missed in current routine LGV screening algorithms.

# INTRODUCTION

Lymphogranuloma venereum (LGV) has reestablished itself in Western society as an invasive sexually transmitted infection (STI) among men who have sex with men (MSM).[1] Lymphogranuloma venereum is caused by *Chlamydia trachomatis* (*Ct*) biovar L. Prompt antibiotic treatment is effective and curative, although it is more extensive than anogenital biovar non-L *Ct* infections. Broader awareness among clinicians of LGV is needed to enable appropriate investigation and management, prevent irreversible late sequelae, and accelerate the interruption of onward spread. Recently, we see a significant increase in the prevalence of LGV at the STI outpatient clinic in Amsterdam, which indicates that more extensive control measures are warranted.[2]

Most reported LGV cases among MSM involve anorectal infections.[2] Very few urogenital infections, also known as *inguinal* LGV, are described. Even fewer infections of the pharynx have been reported.[3] The overrepresentation of anorectal infections in the LGV epidemic is poorly understood but stresses that the mode of transmission of LGV needs additional clarification. It has been suggested that the transmission of rectal LGV may be related to sexual activities such as 'fisting',[4] but this could not be confirmed later.[5] We postulated that anorectal LGV is transmitted via receptive anal intercourse and that there is a reservoir of missed genital LGV infections in MSM. The primary aim of this study was to determine whether there is a reservoir of missed urethral LGV that might contribute to the ongoing LGV epidemic. We performed LGV typing on all *Ct*-positive urethral samples in index patients with anorectal LGV infections and in visiting partners of indexes with anorectal LGV.

Since 2005, all MSM patients reporting receptive anal intercourse in the past 6 months were tested on anorectal *Ct* infections by Aptima CT system (GEN-PROBE, San Diego, CA)[6,7] and tested further for LGV, as described before. In case the *pmpH* test was inconclusive (mainly due to low *Ct* load), biovar L could not be confirmed and the diagnosis was considered negative for LGV.

Patients who were diagnosed positive (index patients) could notify their partners who were registered at the STI clinic on a voluntary basis. The electronic patient record of the index was linked to the record of the partner. This enabled immediate partner notification in case an infection was diagnosed in an index patient. Moreover, we distributed STI notification slips to infected index patients to distribute to their partners. The notification slip states the date plus the infection found, without personal identification. If the partner returned to the clinic, we could thus identify which infection he was exposed to. Lymphogranuloma venereum partner notification was implemented in case the partner had contact with the index within the past 60 days. For the study, we identified LGV contacts either on the

electronic partner link or via the notification slip. Lymphogranuloma venereum contacts were screened routinely for other STIs and treated with a biovar non-L *Ct* infection regimen (azithromycin 1000 mg orally single dose or doxycycline 100 mg orally twice a day for 7 days) in accordance with the Centers of Disease Control and Prevention 2010 sexually transmitted disease treatment guidelines.[8] The test and treat policy of the STI outpatient clinic in Amsterdam has been described in more detail in reference 5.

We tested urethral biovar L infections retrospectively in urethral *Ct*-positive consultations with an anorectal LGV infection and in urethral *Ct*-positive sexual partners of consultations with an anorectal LGV infection on record between January 2008 and August 2012. All available *Ct*-positive urine samples stored at -80°C, were retrieved, thawed, and tested with the biovar L specific polymerase chain reaction.[6,7] Patient data from LGV index patients and partners were retrieved from the electronic patient database. Because we used anonymous routine data, no ethical clearance for this study was needed.

During the study period, 27,504 MSM consultations reporting receptive anal intercourse in the past 6 months were screened at the STI clinic in Amsterdam. The prevalence of anorectal LGV in these men was 1.2% (n = 341). Within this group, 33 (9.7%) urine samples were *Ct*-positive and 7 of these urine samples were biovar L positive (prevalence 2.1%). Nine (2.6%) urine samples were biovar non-L, 15 (4.4%) samples were inconclusive, and 2 (0.6%) samples were missing.

We located 59 sexual partners of MSM diagnosed as having an anorectal LGV on record. All partners were male, and none were diagnosed as having anorectal LGV. Among these partners, 10 (16.9%) urine samples were *Ct* positive and 4 of these samples were biovar L positive (prevalence 6.8%). One (1.7%) urine sample was biovar non-L, 3 (5.1%) were inconclusive, and 2 (3.4%) were missing.

All 11 patients with anorectal LGV and concurrent urethral LGV (based on a biovar L positive urine sample) had sexual intercourse with exclusively men, and 9 were HIV positive. Five of the 11 patients with urethral LGV did not report symptoms of urethritis, and only 1 of 11 had lymphadenopathy. Concurrent STIs including gonorrhea (4/11), herpes simplex virus (1/11), and syphilis (1/11) were found among index patients with anorectal LGV. No coinfections besides urethral LGV were found in the partner group. In both groups, condom use was inconsistent, and multiple partners were reported in the preceding 6 months (between 2 and 10).

Various guidelines (British Association for Sexual Health and HIV [BASHH], World Health Organization/International Union against Sexually Transmitted Infections [IUSTI] and Centers of Disease Control and Prevention) recommend routine diagnostic methods for ano-rectal LGV in MSM, but none recommend routine screening of urethral LGV infections.[8-10] Here we show previously undiagnosed urethral LGV infections both in MSM with anorectal LGV and among partners of anorectal LGV index patients. Probably, urethral LGV is key in the transmission of LGV in MSM, but remains undetected to date. Many clinicians expect to find unilateral lymphadenopathy in an individual with genital LGV. Because only 1 of the 11 patients with urethral LGV had lymphadenopathy, the absence of this finding does not exclude an infection, and molecular testing is warranted.

Moreover, anorectal LGV infections are often missed because of unawareness of the disease,[11] lack of appropriate diagnostic tools,[12] or the asymptomatic nature of the infection in a considerable proportion of patients.[2] These missed anorectal infections might also contribute to the ongoing LGV epidemic among MSM.

Two above-mentioned guidelines recommend partner treatment of LGV index patients with azithromycin 1000 mg once or doxycycline 100 mg twice a day for 7 days, a regimen considered sufficient to eliminate biovar non-L *Ct* infections. The new BASHH guideline, still under review, suggests extending the duration of partner treatment with doxycycline to 14 days.[9] We showed earlier that anorectal LGV can persist under doxycycline treatment for 16 days, which stresses the importance of prolonged treatment for at least 21 days.[13]

Here we showed that partners of index patients with anorectal LGV (1) harbor asymptomatic urethral LGV infections, (2) had no other bacterial STI requiring antibiotic treatment, and (3) engaged in high-risk behavior. Because under the current guidelines, they were treated insufficiently, it is feasible that their LGV infection was not eliminated, and further transmission was possible. We detected urethral LGV infections in 6.8% of the partners of patients with anorectal LGV. This is based on a small and incomplete number of partners necessitating further studies on the prevalence of urethral LGV. Until that time, it would be advisable to treat partners of patients with LGV with a 3-week course of doxycycline, considered effective to eliminate biovar L *Ct* infections.

A limitation in our study is that we only included partners on record with a legitimate notification. Therefore, our results probably show an underestimation of urethral LGV among partners of LGV index cases. Of the *Ct*-positive urethral samples, 4.4% and 5.1% of patients with anorectal LGV, respectively, and their partners were inconclusive for LGV determination. This is caused by a difference in sensitivity of the biovar-nonspecific

commercial *Ct* test and the LGV-specific in-house developed additional test. As a consequence, an underrepresentation of the true urethral LGV prevalence cannot be excluded.

Lymphogranuloma venereum infection is a serious concern for the MSM community in Western Europe and other industrialized countries. Awareness of, screening for, and prompt treatment of LGV are crucial for the individual patient and to prevent ongoing transmission. We see a significant increase in the prevalence of LGV in Amsterdam in the last year.[2] Urethral LGV might form a potential undetected reservoir. To further clarify this, we plan to estimate the prevalence of urethral LGV infections among the total MSM population visiting the outpatient clinic. Future findings might indicate the need for adjustment of LGV protocols, especially partner treatment and possibly routine screening for urethral LGV.

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# 05

# PITFALLS IN THE DIAGNOSIS AND MANAGEMENT OF INGUINAL LYMPHOGRANULOMA VENEREUM: IMPORTANT LESSONS FROM A CASE SERIES

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*Sex Transm Infect. 2014;90(4):279–82*

# ABSTRACT

Current lymphogranuloma venereum (LGV) guidelines mainly focus on anorectal infections. Inguinal LGV infections have been rare in the current epidemic among men who have sex with men (MSM), but might require a different approach not yet recommended in current guidelines for the treatment and diagnosis of LGV. We describe 4 inguinal LGV cases. Three MSM developed inguinal LGV infection several weeks after a previous consultation, of which two had received azithromycin after being notified for LGV. Three failed the recommended 21 days doxycycline treatment. These inguinal LGV cases highlight 3 pitfalls in the current standard management of LGV: 1) Urethral chlamydia infections in MSM can be caused by LGV biovars that in contrast to non-LGV biovars require prolonged antibiotic therapy. 2) The recommended one gram azithromycin contact treatment seems insufficient to prevent established infections. 3) Inguinal LGV may require prolonged courses of doxycycline, exceeding the currently advised 21 days regimen.

# INTRODUCTION

Lymphogranuloma venereum (LGV) in men who have sex with men (MSM) became apparent in 2003, first in the Netherlands and soon after in other European countries, North America and Australia.[1] Most men were HIV seropositive[2] and engaged in high-risk behaviour.[3] LGV is an invasive ulcerative sexually transmitted disease caused by biovar L-type *Chlamydia trachomatis* (*Ct*).

A short-lived ulcer on the site of entry characterises the first stage of an LGV infection and if inoculation occurs at an internal location such as the anal canal or the urethra, is easily missed.[4] After transmission, the pathogen leaves the mucosal lining at the apical site, invades the underlying connective tissue layers and disseminates via the lymphatics towards loco-regional lymph nodes. A typical sign of secondary stage LGV is the occurrence of 'buboes', fluctuating painful swellings of infected lymph nodes that can suppurate spontaneously and cause chronic suppurating fistulae. The inguinal lymph nodes become involved in cases where the external genitalia are the site of inoculation, in what is often referred to as 'the inguinal syndrome'.

In the current epidemic among MSM, the majority of LGV infections are anorectal and only a few urethral infections have been reported.[5] Most current guidelines recommend partner treatment of LGV index patients with azithromycin 1000 mg once or doxycycline 100 mg twice a day for 7 days, a regimen considered sufficient to eliminate biovar non-L *Ct* infections.[6,7] The new BASHH guideline suggests extending the duration of partner treatment with doxycycline to 14 days.[8] We showed previously that anorectal LGV can persist under doxycycline treatment for 16 days, which highlights the importance of prolonged treatment for at least 21 days.[9]

Here we describe a case series of four MSM who developed inguinal LGV to highlight: (1) pitfalls in the diagnosis of LGV; (2) the importance of correct partner treatment; and (3) the protracted course of some infections.

# METHODS

All data were obtained for routine purposes for which ethics clearance was not necessary. All patients consented to publication. All MSM who reported receptive anal contact in the previous 6 months were screened for anorectal chlamydia with the Aptima single *Ct* test (Gen-Probe, California, USA). If *C. trachomatis* was detected, the sample was further tested using an in-house developed genovar L specific assay to detect LGV.[10] Gram stained smears

of urethral and anorectal samples were used to exclude non-specific urethritis, non-specific proctitis (defined as the presence of >10 polymorphonuclear leucocytes (PMNL) per light microscopic high power field) and gonorrhoea infections (presence of Gram negative diplococci in PMNL). Presumptive therapy for non-specific proctitis and urethritis was doxycycline 100 mg twice daily for 7 days and azithromycin 1000 mg once, respectively. Syphilis, urogenital chlamydia and gonorrhoea, and undiagnosed HIV infections were excluded. Known HIV seropositive patients were screened for hepatitis C. Patients notified by a partner diagnosed with LGV were screened and treated presumptively with 1000 mg azithromycin if the index patient could be identified in the electronic patient file or if the notified partner could show a notification slip. More details on the notification system have been provided elsewhere.[11]

# RESULTS

All four patients reported high-risk behaviour such as unprotected receptive anal and oral intercourse with multiple partners, and all had been diagnosed with multiple bacterial sexually transmitted infections (STI) in the past.

Patient A was a 45-year-old HIV positive, hepatitis C negative MSM who was receiving antiretroviral therapy (ART). He visited the outpatient clinic upon partner notification for LGV. He reported no complaints or signs, and the Gram stained smears were negative. He received the LGV contact treatment of 1000 mg azithromycin once. A week later all screening results came back negative. Seven weeks after the consultation, he visited his general practitioner (GP) with a painful fluctuating swelling in the groin region (Figure 1). Aspirate of this inguinal bubo proved LGV positive. The GP prescribed doxycycline 100 mg twice a day for 21 days, after which the inguinal bubo disappeared and no further abnormalities were observed.

Patient B was a 26-year-old HIV and hepatitis C positive MSM; ART had not yet been commenced. Like patient A, an LGV positive partner had notified him. At his visit he had no signs or symptoms and the Gram stained smears were negative. He was treated with 1000 mg azithromycin once, and 1 week later all his screening results came back negative. He returned to the clinic for a routine screening (no complaints were reported) 16 weeks after this episode. Urogenital chlamydia was diagnosed and treated with 1000 mg of azithromycin once. One week later (17 weeks after his first presentation), he returned to the clinic with a painful swollen penis, and swollen bilateral inguinal lymph nodes. Therapy with 100 mg doxycycline twice a day was started. One week later, patient B returned with five inguinal buboes, of which three had erupted spontaneously and two had increased in size.

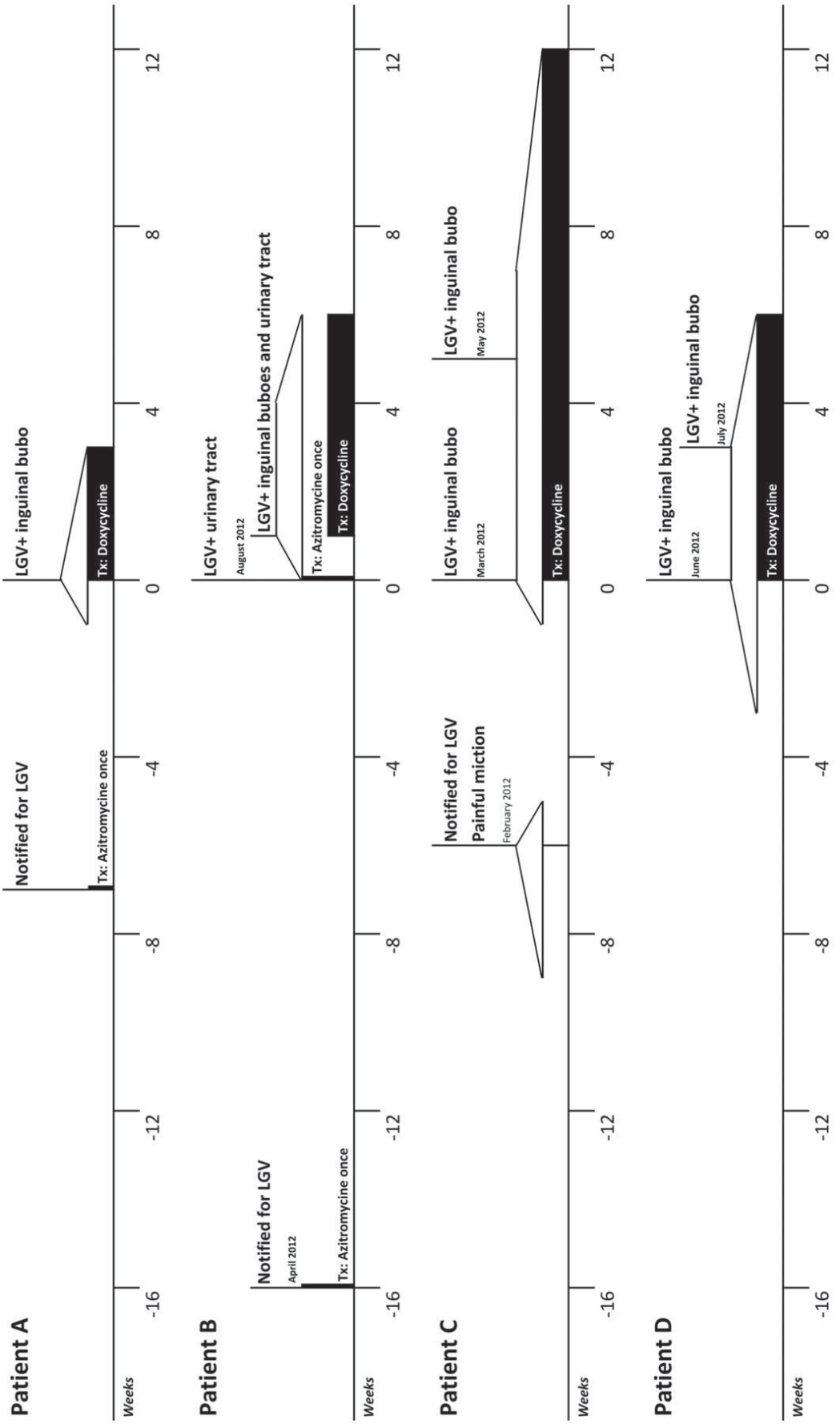


**FIGURE 1.** Timeline of the cases of four men who have sex with men (MSM) who developed bubonic lymphogranuloma venereum (LGV). Tx, treatment.

Urine samples collected then and the week previously, plus bubo pus, all proved LGV positive. The buboes subsided after 3weeks of continuous therapy. Nonetheless, doxycycline was continued for another 2 weeks.

Patient C was a 39-year-old HIV positive, hepatitis C negative MSM who was receiving ART. A partner diagnosed with LGV had notified him, but this could not be confirmed, so he was not presumptively treated with azithromycin. He complained of painful urination which had lasted several weeks, had no STI related signs upon physical examination, and the Gram stain smears were negative. A week later all STI screening results proved negative. Six weeks after his STI clinic consultation, he visited his GP with an inguinal bubo. An aspirate proved LGV positive and *Ct* RNA positive, suggesting live replicating infections.[9] Antibiotic therapy with doxycycline 100 mg twice a day was started. Despite the doxycycline, the inguinal bubo did not subside and the antibiotic regimen was extended. Five weeks after the first LGV positive aspirate, a second aspirate still proved LGV positive. The doxycycline regimen was prolonged for 12weeks by which time the inguinal bubo had finally disappeared.

Patient D was a 33-year-old HIV and hepatitis C negative MSM. He visited the STI outpatient clinic for a solitary painful fluctuating inguinal lymph node lasting 3weeks. All Gram stained smears were negative. Doxycycline 100 mg twice a day was started under the suspicion of inguinal LGV. A week later the aspirate proved LGV DNA positive. After 3 weeks the bubo had not decreased in size and a second aspirate was again LGV positive. Doxycycline twice daily was continued for 6 weeks by which time the bubo had subsided.

# DISCUSSION

Here we describe two men (A and B) who received a single dose of azithromycin after exposure to *Ct* biovar L. Patient C was also exposed to biovar L, but did not receive presumptive antibiotic treatment. In all three patients, urethral and anorectal LGV was ruled out by routine screening. Subsequently, in all three patients, inguinal LGV was diagnosed respectively 7, 16 and 6 weeks after the initial presentation.

Patients C and D did not respond to the advised course for inguinal LGV of doxycycline 100 mg twice a day for 21 days. Both had persisting LGV DNA positive bubo aspirates after receiving 5 and 3 weeks of doxycycline, respectively. Subsequently, doxycycline courses were prolonged for another 12 and 6 weeks, respectively, by which time the buboes had subsided.

## Pitfalls in the diagnosis of LGV

Patients A, B and C were all partners of LGV patients, but none had a urethral *Ct* infection when they visited the clinic after being notified. Presumably the infection had spread into the lymphatic system and had left the epithelial layers. Moreover, two of the three were treated with azithromycin once, but still developed inguinal LGV a few weeks later. This finding supports the suggestion that LGV contacts should be treated with an extended course of doxycycline instead of the now widely recommended single dose of azithromycin.

## Azithromycin for partner treatment

There is insufficient evidence concerning the effectiveness of azithromycin for LGV.[11] Azithromycin is currently recommended in many guidelines as presumptive therapy for the partners of LGV patients.[6,7] Here we describe two partners of LGV patients (A and B) who were treated with azithromycin 1000 mg once as presumptive therapy but who were diagnosed with LGV infections after 7 and 16 weeks, respectively. The new BASHH guideline suggests partner treatment with doxycycline for 14 days. In 2009 we reported that anorectal LGV can persist for up to 16 days during treatment with doxycycline.[9] The treatment of notified LGV partners with doxycycline, possibly for 21 days, seems warranted.

## Protracted course of inguinal LGV infections

Treatment with 21 days of doxycycline is recommended for LGV infections. The duration of therapy is mainly based on clinical experience, and not on evidence.[4,12] Moreover, chlamydia biovar L-type organisms are able to survive under doxycycline therapy for a longer period than chlamydia non-LGV biovars, so a pro- longed course of doxycycline is warranted to successfully eradicate LGV.[9] Patients C and D had persisting *Ct* RNA positive and LGV DNA positive bubo aspirates following the recommended 21 days of doxycycline. Both patients confirmed they used the antibiotic therapy as prescribed. Symptoms only subsided upon extension of the doxycycline course to 12 and 6 weeks, respectively. The therapeutic course of these two patients suggests that 21 days of anti- biotic therapy is insufficient to completely eliminate inguinal LGV infections.[13] Furthermore, it is important to aspirate persisting buboes in cases of inguinal LGV, since the penetration of antibiotics in abscess cavities is very poor. Clinical trials on the optimal treatment of LGV infections are warranted.

## Strengths and weaknesses of this study

The strength of this case series is that it highlights shortcomings in the current clinical guidelines for the management of LGV, in particular the treatment of notified partners and patients with inguinal LGV. A weakness is the uncontrolled nature of any case descriptive study. Although both cases A and B denied having had sexual contact with the LGV indexes

who had notified them after they had received partner treatment, it cannot be excluded that they were re-exposed to other LGV infected partners since they both reported considerable risk behaviour.

We conclude first that the diagnosis of inguinal LGV can be missed in the first stages of the infection when symptoms can be non-specific in the absence of buboes, mild or absent, and routine tests for CT can be negative. Clinicians dealing with STI patients should be aware that clinical manifestations of LGV have not been confined to proctitis[14] and instruct patients notified for LGV to return to the STI clinic once anogenital symptoms occur, even if all STI test came back negative. Second, a 21-day regimen of doxycycline is considered sufficient to treat anorectal LGV infections. In cases of inguinal LGV with bubo formation, live replicating bacteria can be detected after 3 weeks of continuous doxycycline treatment. This could be caused by the sub-optimal penetration of the antimicrobial agent in the bubo cavity. It is therefore important to follow-up LGV patients with buboes and continue doxycycline treatment until symptoms have resolved. In cases of persisting buboes, their contents should be aspirated. Third, the effectiveness of the current recommended presumptive treatment of contacts notified for LGV with 1 g of azithromycin lacks evidence. In an analogy with the current treatment of LGV infections, prolonged courses of doxycycline seem better able to prevent established infections in contacts exposed to LGV.

# KEY MESSAGES

- Inguinal lymphogranuloma venereum (LGV) infections have been rare in the current epidemic among men who have sex with men (MSM) that may require a different approach not yet recommended in current guidelines for the treatment and diagnosis of LGV.
- Urethral chlamydia infections in MSM can be caused by LGV biovars that in contrast to non-LGV biovar infections require prolonged courses of antibiotic therapy.
- The recommended presumptive treatment of contacts notified for LGV with one gram of azithromycin seems insufficient to prevent established infections.
- Inguinal LGV with bubo formation may require prolonged courses of doxycycline, exceeding the currently advised 21 days regimen.

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# 06

# LOW PREVALENCE OF URETHRAL LYMPHOGRANULOMA VENEREUM INFECTIONS AMONG MEN WHO HAVE SEX WITH MEN: A PROSPECTIVE OBSERVATIONAL STUDY, SEXUALLY TRANSMITTED INFECTION CLINIC IN AMSTERDAM, THE NETHERLANDS

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*Sex Trans Dis. 2017:44(9):547-50*

# ABSTRACT

In contrast to anorectal lymphogranuloma venereum (LGV), few urogenital LGV cases are reported in men who have sex with men. Lymphogranuloma venereum was diagnosed in 0.06% (7/12,174) urine samples, and 0.9% (109/12,174) anorectal samples. Genital-anal transmission seems unlikely the only mode of transmission. Other modes like oral-anal transmission should be considered.

# INTRODUCTION

Lymphogranuloma venereum (LGV) is an invasive ulcerative sexually transmitted infection (STI) caused by the *Chlamydia trachomatis* (*Ct*) LGV biovar (*ompA* genovars L1, L2 and L3).[1] Since 2003 an LGV epidemic among men who have sex with men (MSM) is ongoing in Europe, North America, and Australia.[2] LGV is associated with high-risk behavior, reflected in high rates of STI co-infections like human immunodeficiency virus (HIV) (in up to 82.2% of the cases) and hepatitis C.[3-6] It is still unknown whether the increased frequency of STI coinfections found in HIV-infected MSM is due to increased susceptibility associated with immunodeficiency and/or immune restoration, or a network factor associated with risk behavior.[4]

Recent reports suggest an increase of LGV diagnoses in Europe in recent years.[7-9] Mainly anorectal infections have been diagnosed, whereas the frequency of urogenital and pharyngeal LGV diagnoses seems rare.[10-13] Assuming that most anorectal infections are caused by receptive genital-anal contact, the discrepancy between the frequency of anorectal and urethral infections remains unexplained.

In a previous study, alternative transmission modes for the transmission of anorectal infections were suggested, such as fisting and/or sharing of sex toys.[2] Yet, in a more recent systematic study, we found no evidence in support of this hypothesis.[4] Tissue tropism of L2b (the most frequently found strain among MSM) with a predilection to infect anorectal mucosa as opposed to urogenital mucosa was thought to be another explanation for the discrepancy in the frequency of urethral as opposed to anorectal infections, yet we could not prove this.[14]

Currently, most guidelines do not recommend routine testing for urethral LGV,[15,16] except for the European International Union against STI LGV guideline.[17] Earlier, we reported 2.1% LGV positivity rate in MSM with a concurrent anorectal LGV infection and a 6.8% urethral LGV positivity in their sexual partners.[18] This suggested that urethral LGV infections are a possible link in the ongoing transmission of LGV in MSM. To indicate its contribution to the current LGV epidemic, we aimed to determine the positivity rate of urethral LGV among MSM.

# METHODS

## Study Design and Setting

In the STI outpatient clinic of Amsterdam, the Netherlands, MSM are offered free screening for *Ct* (urethral and pharyngeal), *Neisseria gonorrhoeae* (*Ng*) (urethral and pharyngeal), syphilis, hepatitis B, and using an opt-out strategy for HIV.[19,20] If the client reports receptive anal sex in the previous 6 months, additionally, anorectal *Ct* and *Ng* infections are tested. All clinical findings, diagnoses, and subsequent treatment are recorded in an electronic patient database along with patient characteristics and information on sexual behavior.

Prospectively, urine samples were collected from all MSM visiting the STI outpatient clinic between March 2014 and July 2015 and were screened for *Ct* by amplification with a very sensitive molecular screening assay (Aptima Combo test, Hologic, USA). Positive samples were genotyped using an in-house *pmpH* quantitative polymerase chain reaction to differentiate between LGV and non-LGV type infections.[21,22] If the *pmpH* test was non-typable (mainly due to insufficient DNA), or tested positive for a non-LGV infection, the result was considered negative for an LGV infection. The same strategy was used for Ct-positive anorectal samples. MSM without an anorectal test were excluded

## Statistical Analysis and Data Collection

Statistical analyses were performed using SPSS V.19 (SPSS, Chicago, Ill). We analyzed whether determinants of MSM with an anorectal LGV infection differed from MSM with a urethral LGV infection, using Fisher test. Tests were 2-sided and considered significant at P less than 0.05. Sexual preference and number of sexual partners referred to the period 6 months before consultation. HIV status was based on self-reported history of HIV. Urethral symptoms were defined as discharge, dysuria, and/or pruritus. Anorectal symptoms were defined as discharge and/or a burning sensation. A concurrent STI diagnosis was defined as *Ct* (non-LGV) or LGV at another anatomical location (eye/pharyngeal/urogenital/anorectal), *Ng* and/or infectious syphilis diagnosed at the same consultation. Men with a concurrent anorectal LGV and urogenital LGV infection were categorized in the urethral LGV infection group.

# RESULTS

During the inclusion period, 12,564 screening tests were performed for urethral *Ct* infections (Figure 1). In 383 visits, no anorectal test was performed, and in 7 *Ct*-positive tests, no *pmpH* quantitative polymerase chain reaction was executed; these were all excluded from the

analyses. In 12,174 tests, 404 (3.3%) tested positive for urethral *Ct*, of which 319 (78.9%) were negative for an LGV type infection, 78 (19.3%) were nontypable and 7 (1.7%) tested LGV-positive. In total, 1010 (8.0%) samples tested positive for anorectal *Ct*, of which 674 (66.7%) were negative for an LGV type infection, 227 (22.5%) were nontypable and 109 (10.8%) tested LGV-positive. Overall, we found a urethral LGV positivity rate of 0.06% (7/12,174; 95% confidence interval [CI], 0.02–0.12) and an anorectal positivity rate of 0.9% (109/12,174; 95% CI, 0.74–1.08). Of those 7 with urethral LGV, 4 had urethral symptoms, 1 had a concurrent anorectal LGV infection, 3 were HIV co-infected, and 1 was notified for LGV. Of the 108 with anorectal LGV, 39 (36.1%) had anorectal symptoms, 91 (84.3%) were HIV coinfected, and 9 (8.3%) were notified for LGV (Table 1). Compared with MSM with urethral LGV, those with anorectal LGV were significantly more often HIV co-infected (*P*=0,02).

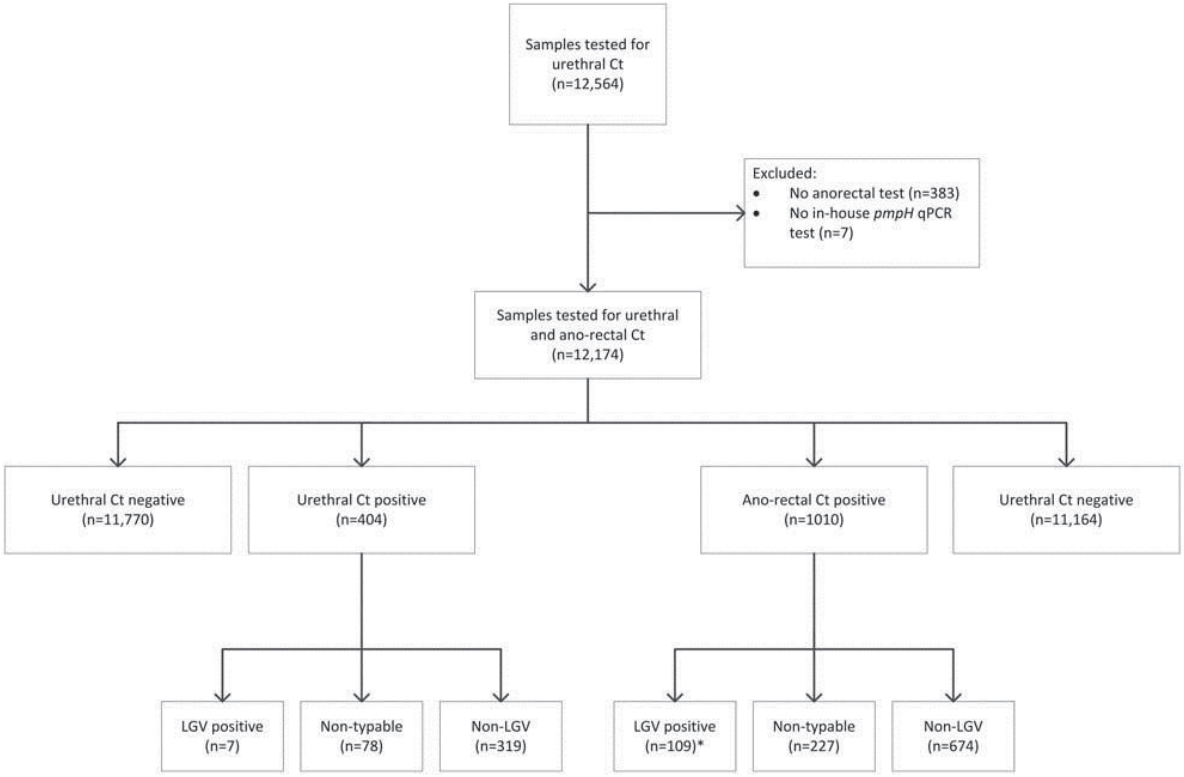


**FIGURE 1.** Study flowchart of 12,564 visits during which *C. trachomatis* (*Ct*) tests were performed in men who have sex with men at the STI outpatient clinic in Amsterdam, March 2014 to July 2015.
*One patient with anorectal LGV had a urethral LGV co-infection; therefore, he was included in the urethral LGV group.

**TABLE 1.** Baseline characteristics of 115 MSM with an LGV infection visiting the STI outpatient clinic in Amsterdam March 2014 to July 2015, by anatomical site.

| | Total (n=115) | | Urethra (n=7) | | Rectum (n=108)* | | |
|---|---|---|---|---|---|---|---|
| **Characteristics** | **No.** | **%** | **No.** | **%** | **No.** | **%** | ***P*†** |
| Age, y | | | | | | | 0.23 |
| <30 | 15 | 13.0 | 2 | 28.6 | 13 | 12.0 | |
| ≥30 | 100 | 87.0 | 5 | 71.4 | 95 | 88.0 | |
| Median (IQR) | 44(16) | | 44(26) | | 44(15.8) | | |
| Ethnicity | | | | | | | 1.00 |
| Dutch | 68 | 59.1 | 4 | 57.1 | 64 | 59.3 | |
| Non-Dutch | 47 | 40.9 | 3 | 42.9 | 44 | 40.7 | |
| No. sex partners preceding 6 months | | | | | | | 1.00 |
| ≤6 | 44 | 38.3 | 3 | 42.9 | 41 | 38.0 | |
| >6 | 71 | 61.7 | 4 | 57.1 | 67 | 62.0 | |
| Median (IQR) | 10(15) | | 7(9) | | 10(15) | | |
| HIV status | | | | | | | 0.02 |
| Positive | 94 | 81.7 | 3 | 42.9 | 91 | 84.3 | |
| Negative | 21 | 18.3 | 4 | 57.1 | 17 | 15.7 | |
| Notified of LGV by a sexual partner | | | | | | | 0.48 |
| No | 105 | 91.3 | 6 | 85.7 | 99 | 91.7 | |
| Yes | 10 | 8.7 | 1 | 14.3 | 9 | 8.3 | |
| Urethral symptoms‡ | | | | | | | 0.03 |
| No | 102 | 88.7 | 4 | 57.1 | 98 | 90.7 | |
| Yes | 13 | 11.3 | 3 | 42.9 | 10 | 9.3 | |
| Anorectal symptoms¥ | | | | | | | 0.09 |
| No | 76 | 66.1 | 7 | 100.0 | 69 | 63.9 | |
| Yes | 39 | 33.9 | 0 | 0.0 | 39 | 36.1 | |
| Concurrent bacterial STI** | | | | | | | 0.08 |
| No | 84 | 73.0 | 4 | 57.1 | 81 | 75.0 | |
| Yes | 31 | 27.0 | 3†† | 42.9 | 27 | 25.0 | |

* One anorectal LGV is included in the "urethral LGV" column due to a concurrent urogenital LGV.
† Based on Fisher exact test; P <0.05 is considered significant.
‡ Symptoms were defined as discharge, dysuria, and/or pruritus.
¥ Symptoms were defined as discharge and/or burning sensation.
** Non LGV *Chlamydia trachomatis*, LGV at another anatomical location, *Neisseria gonorrhoeae* or infectious syphilis.
†† One urogenital LGV positive MSM also had anorectal LGV.
IQR, interquartile range.

# DISCUSSION

The observed positivity rate of urethral LGV infections (0.06%) is 15 times lower compared with the positivity rate of anorectal LGV infections of 0.9% found among MSM at the STI clinic. Therefore, it seems likely that other modes of transmission are needed to explain the current LGV epidemic in MSM. As thought earlier, transmission via toys is not supported based on epidemiological data.[2] Nor does tissue tropism seem a likely explanation for the discrepancy found in the rate of anorectal and urethral LGV infections.[14]

A strength of this study is the high number and unbiased population of MSM that was prospectively tested for both urethral and anorectal LGV because all clients were included irrespective of symptoms, notification, or prioritization based on sexual risk assessment.

The number of *pmpH* non-typable samples from the urethral and anorectal location was high: respectively, 19.3% (78/404) and 22.5% (227/1010). We considered non-typable samples LGV negative because it is known that these samples have a low bacterial load and LGV types are successful growers.[23] Nevertheless, a chance remains that we have missed LGV infections. However, in an earlier study using a genotyping Reverse Hybridization Assay, we showed that at least one third of the *pmpH* non-typable samples were non-LGV types.[23] Moreover, because there was no significant difference in the ratio of non-typable samples from urethral or anorectal locations, the discrepancy between anatomical locations remains unexplained.

In a 2014 convenience sample study from Madrid, Spain, 13,585 samples, including 2420 urethral samples, were tested in 8407 clients of whom 3282 MSM. In total, 10 (2.6%) of 338 urethral *Ct*-positive samples were LGV positive.[11] This is slightly higher than the 1.7% found here. In a 2013 German convenience sample study, 1883 MSM rectal and pharyngeal specimens were tested for LGV and 522 urethral samples were obtained; 8 were *Ct*-positive of which none were LGV-positive.[12] In a 2009 prospective multicentre study from the United Kingdom, 4825 urethral and 6778 rectal samples from consecutive MSM attending for sexual health screening were screened for LGV.[13] The LGV positivity in rectal samples was 0.90% (95% CI, 0.69%–1.16%) and in urethral samples 0.04% (95% CI, 0.01%–0.16%), very comparable to our results. None of the 3 studies tested such a number of MSM both for anorectal and urethral LGV infections as done here. Moreover, we compared characteristics of MSM with LGV at different anatomical locations. As found earlier,[9] men with urethral LGV were significantly less often HIV coinfected as opposed to men with anorectal LGV, respectively, 42.9% and 84.3% ($P = 0.02$), indicating that the latter is a population with higher risk taking behavior. Unfortunately, the number of urethral LGV cases is too small to look into risk behavior in closer detail.

The low prevalence of cases found here does not justify routine screening for urethral LGV. Yet, clinicians should be aware of possible treatment failures as we described earlier in a case series of MSM with advanced inguinal LGV with bubo formation that were likely caused by missed and/or undertreated urethral LGV infections.[24] Apart from direct consequences for individual patients, missed urethral LGV infections likely also contribute to ongoing transmission.

As we described earlier,[9] a considerable part of LGV diagnosis found in this study were asymptomatic: approximately 36% of the anorectal infections, and 4 of the 7 urethral LGV infections. Because LGV requires prolonged treatment and follow-up compared with non-LGV chlamydia infections, this finding stresses the clinical importance to exclude LGV in high-risk groups, irrespective of complaints.

With the skewed anorectal/urethral LGV ratio of 15:1, it seems unlikely that urethral LGV infections are responsible for all anorectal LGV transmissions. Recently, we suggested that oral infections may have a role in LGV transmission via ano-oral sex.[25] Schachter et al. [26] demonstrated in early work that neonates with an initial chlamydia conjunctivitis or pneumonia, subsequently shedded *Ct* from the vagina and rectum. They suggested that the vagina and conjunctivae are exposed to chlamydia at birth and that pneumonia and gastrointestinal infection occur later via oropharyngeal transmission. This paradigm could possibly also account as an explanation for the unanswered findings in the current LGV epidemic in MSM. Apart from genital-anal transmission, oropharyngeal infection may occur via ano-oral sex (also known as rimming).[25] Subsequently, LGV organisms pass through the gastrointestinal tract to cause anorectal LGV proctitis. Whether this paradigm proves right remains to be seen, and its contributing factor to the LGV epidemic in MSM needs to be addressed in future research.

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# 07

# ADDITIONAL GONORRHEA AND CHLAMYDIA INFECTIONS FOUND WITH RAPID FOLLOW-UP SCREENING IN MEN WHO HAVE SEX WITH MEN WITH AN INDICATION FOR HIV POSTEXPOSURE PROPHYLAXIS

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*Sex Trans Dis. 2014;41(8):515-7*

# ABSTRACT

Sexually transmitted infection was found in 16.5% of the men who have sex with men with a postexposure prophylaxis indication. Chlamydia and gonorrhea screening was repeated after 14 days. Among those who were initially sexually transmitted infection negative, 4.1% had chlamydia or gonorrhea. In postexposure prophylaxis-indicated men who have sex with men, repeat chlamydia and gonorrhea screening is advised to diagnose infections not apparent at baseline screening.

# INTRODUCTION

HIV postexposure prophylaxis (PEP) is a 4-week course of antiretroviral treatment recommended to persons exposed to human body fluids possibly infected with HIV to prevent HIV acquisition.[1] It was first introduced to reduce the transmission risk after needle-stick accidents and other occupational exposures to HIV.[2] In 1997, it was introduced for use after sexual exposure.[3] Since 2010, the sexually transmitted infection (STI) outpatient clinic of the Public Health Service in Amsterdam, the Netherlands, offers PEP to HIV-negative patients who have had a considerable risk of recent HIV exposure through unsafe sex.

Individuals presenting for PEP after sexual exposure are at risk for concurrent STI. Therefore, a PEP request is an ideal opportunity for STI screening and safe sex promotion. Early incubating *Chlamydia trachomatis* (*Ct*) and *Neisseria gonorrhoeae* (*Ng*) infections acquired during the sexual exposure for which PEP is sought are possibly missed if STI screening is only performed at the consultation during which an indication for PEP initiation is established. Therefore, we aimed to determine if chlamydia and gonorrhea screening should be repeated in men who have sex with men (MSM) 2 weeks after a PEP indication.

If the client requests PEP or the history suggests that there has been considerable risk for HIV transmission in the previous 72 hours, the option to start PEP is discussed. A medical doctor decides whether PEP is indicated based on the criteria of the Dutch guidelines for sexual exposure,[4] which are in agreement with European guidelines[1] and the Centers for Disease Control and Prevention recommendations.[5]

At the STI clinic, testing for gonorrhea, chlamydia, syphilis, hepatitis B, and HIV is offered to all MSM patients who visit the clinic, as described before.[6-9] Urine and rectal swabs are collected and screened for *Ct* (Aptima Ct single system; GEN-PROBE, San Diego, CA). Pharyngeal swabs are tested for *Ct* and *Ng* (Aptima Combo 2 system; GEN-PROBE). At the initial visit, a trained laboratory technician examines urethral and rectal Gram-stained smears for a presumptive gonorrhea diagnosis (i.e., gram-negative diplococci in polymorphonuclear leukocytes) or a nongonococcal infection (i.e., 910 polymorpho- nuclear leukocytes per light microscopic high-power field, no gram-negative diplococci). Urethral and rectal cultures are used for the definitive *Ng* diagnosis. HIV antibody rapid testing (Determine 1-2; Abbott Laboratories, Abbott Park, IL) is offered to all patients. Serum samples are also further tested by line immunoassay (Inno-Lia HIV I-II Score; Innogenetics, Ghent, Belgium); this result will follow after a few working days. Condylomata acuminata are diagnosed clinically.

Postexposure prophylaxis is not started if the HIV rapid test result is positive. In case the HIV antibody rapid test result is negative or inconclusive, but the result of the line immunoassay result, when it becomes available a few days later, is positive, continuation of PEP medication as treatment of HIV is reviewed with an infectious disease specialist. If the source person of the risk event is tested at the STI clinic and found to be HIV negative, PEP is considered not indicated, and if PEP was already started, it is therefore discontinued. Patients are free to decline PEP. If PEP is accepted, a visit is planned 2 weeks later to repeat screening for urethral, pharyngeal, and anal *Ng* and *Ct* infections.

We included all MSM visiting the STI outpatient clinic in Amsterdam with a PEP request after sexual exposure from April 2010 until December 2012. Men who have sex with men with multiple PEP requests during the inclusion period were included in the analysis multiple times, and thus, we report about "presentations" instead of individuals. An STI presentation could involve multiple sexually transmissible pathogens, or the same pathogen at more than 1 anatomical location. All statistical analyses were performed in SPSS version 19 (SPSS Inc, Chicago, IL). Ethical clearance was not sought because this was an analysis on routinely collected, anonymized data.

From April 2010 until December 2012, a total of 26,733 consultations were performed in MSM. We analyzed 473 PEP requests from 438 unique individual MSM. In 78 (16.5%) of those 473 presentations, at least 1 STI was found (Figure 1). In 334 presentations, PEP was indicated (70.6%). The STI positivity among the 139 presentations in which PEP was not indicated was 16.5% (23/139): 11 attendees (7.9%) were HIV positive, and in 12 presentations (8.6%), 16 STIs other than HIV were diagnosed. In the presentations in which PEP was indicated, 68 STIs were found in 55 (16.5%) of 334. Two attendees had a coinfection, 9 had the same STI at multiple anatomical places, and 1 had a triple infection (both *Ng* and *Ct* and *Ng* at multiple anatomical places). The rectum was the most common anatomical place for an STI in 27 (49.1%) of 55 presentations. Only 6 (10.9%) of 55 presentations with a PEP indication and an STI were symptomatic at screening.

In 14 presentations, PEP was discontinued: in 2, the confirmation of the line immunosorbent assay of the index proved positive; in 9, the partner involved in the sex accident proved HIV negative; in 3, the patient discontinued PEP at own initiative. The remaining 320 completed PEP. A follow-up visit after 2 weeks (range, 10-17 days) was performed in 218 (68.1%) of 320 presentations. In 9 (4.1%) of 218 presentations, at least 1 previously undiagnosed infection was found: 3 rectal *Ct*, 3 rectal *Ng*, 1 pharyngeal *Ct*, and 2 rectal *Ct/Ng* coinfections. No urethral infections were detected at the 2-week visit. All of 9 presentations with a previously undiagnosed infection denied sexual contact since the last consultation.

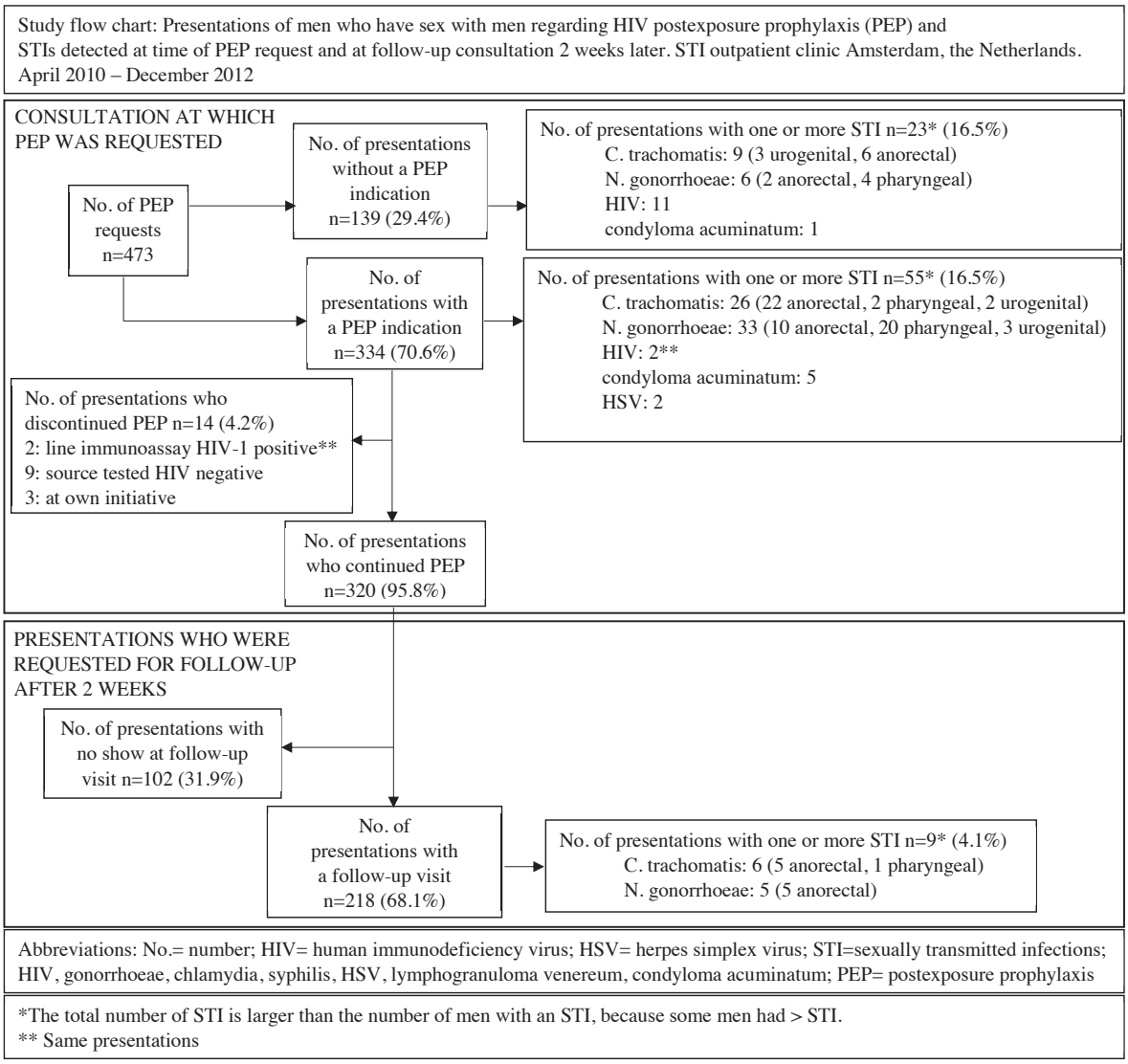


**FIGURE 1.** Study flowchart. Presentations of MSM regarding HIV PEP and STIs detected at the time of PEP request and at follow-up consultation 2 weeks later. STI outpatient clinic; Amsterdam, the Netherlands; April 2010 to December 2012.

This study demonstrates that screening for STI in MSM with a request for PEP after sexual HIV exposure is worthwhile. Of all 473 presentations requesting PEP, 16.5% had at least 1 STI. Of great importance are the 13 (2.7%) of 473 previously undiagnosed HIV infections. Moreover, reconfirming negative/ inconclusive HIV rapid test results with HIV immunoassay in MSM is of importance because 2 additional HIV infections were thus diagnosed. We consider these by rapid test missed HIV primary infections. Because primary HIV infections are known to have very high viral loads, these are of great consequences for ongoing transmission.[10,11]

Repeated chlamydia and gonorrhea screening 2 weeks after the PEP indication revealed 4.1% additional, possibly early incubating, *Ct* and/or *Ng* infections. Our observed prevalence of STI (16.5%) is higher compared with other reported studies in MSM receiving PEP.[12,13] Similar to earlier studies, in 31.9% consultations, the attendees in which PEP was indicated did not show for the follow-up visit.[12,14] Most MSM with an STI were asymptomatic; the same was reported recently by Jamani *et al*.,[14] who found that more than 90% of cases with chlamydia and gonorrhea diagnoses were asymptomatic. This underlines the importance of providing STI screening to all individuals after high-risk exposures regardless of symptoms, unlike the recommendations in the UK PEP guidelines.[15] Offering PEP and prompt STI testing creates an opportunity to shorten the duration of infectiousness of an STI and facilitates discussion regarding risk reduction and safer sex.[16]

Strengths of this study are that our test policy did not change over time, and all PEP requesters were screened routinely, both at the initial visit and 14 days afterward.

Limitations of our study are the no-show ratio for a repeat screening of approximately 32% after 2 weeks and that we cannot exclude that attendees with a new *Ct* or *Ng* infection after 2 weeks did not have sexual intercourse within that period, although they all denied having had sex between the 2 visits. Our outcome differs from a similar report in which 28.1% of the presentations reported sexual activity between the baseline and the 2-week visit.[12] Sampling errors during the baseline visit could have produced false-negative results that were considered "new" infections found during the return visit. Because trained nurses supervised all sample collections and highly sensitive nucleic acid amplification tests were used, we consider the occurrence of sampling error highly unlikely.

Based on these data, we consider that STI and HIV screening should be offered to all MSM with a PEP request after a sexual HIV exposure regardless of symptoms. Immediate screening yields a high rate of especially gonorrhea and chlamydia infections. Moreover, it seems advisable to repeat screening for gonorrhea and chlamydia a few weeks later, to detect infections not apparent at the baseline screening. Whether these later diagnoses were early incubating or newly acquired infections remains unclear. The considerable proportion of patients that will not return for repeat screening a few weeks after the PEP request makes immediate screening indispensable.

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# 08

# GENERAL DISCUSSION

The aim of this thesis is to provide insight into the lymphogranuloma venereum (LGV) epidemic in Amsterdam, with special emphasis on the role of urogenital (urethral and inguinal) LGV. In addition, we assessed the importance of adequate screening for sexually transmitted infections (STIs) among men who have sex with men (MSM) with an indication for HIV post-exposure prophylaxis (PEP). The discussion below will focus on LGV and more specifically on (1) a potentially missed reservoir; (2) considerations of transmission routes; (3) the importance of diagnosing urogenital LGV; and (4) recommendations for future guidelines for urogenital LGV and MSM with a request for PEP. Additionally, proposals on future research will be discussed.

# A POTENTIALLY MISSED RESERVOIR OF LGV

In the current epidemic, the majority of reported LGV among MSM concerns LGV in the anorectal canal and rarely urogenital LGV (**chapter 3, 4, and 6**). Since routine screening for urethral LGV is not recommended in any of the European, British, or Canadian guidelines[1-5] we wanted to investigate whether there is an undiagnosed reservoir of urethral LGV responsible for anorectal LGV.

First, as described in **chapter 3,** the discrepancy between the number of anorectal and inguinal LGV was confirmed: 10 inguinal LGV cases found in 7.5 years compared to 411 anorectal LGV cases (ratio 41:1). Subsequently, in a study reported in **chapter 4** we retrospectively selected urine or urethral samples that were *C. trachomatis* (*Ct*) positive from patients with anorectal LGV, and from partners of index patients with anorectal LGV. These samples were tested with an LGV genotype L specific assay. We found a prevalence of 2.1% (7/341) of urethral LGV in anorectal LGV patients, whereas 6.8% (4/59) of the partners of these patients had asymptomatic urethral *Ct* genotype L infections. These results suggest that a missed reservoir of urethral LGV might play a key role in the transmission of *Ct* genotype L. Since evidence based on retrospective data is potentially biased, we then performed a prospective study to determine the prevalence of urethral *Ct* genotype L infections among MSM.

**Chapter 6** reported on this prospective study. Urine samples were collected from all MSM visiting the STI clinic in Amsterdam and *Ct*-positive samples were tested further to diagnose *Ct* genotype L infections. Our study was unique because unlike others, we tested MSM both for anorectal and urethral LGV according to a standard protocol instead of driven by presented symptoms or at clinicians' discretion. In 12,174 tests, 404 (3.3%) were positive for urethral *Ct* of which 7 (1.7%) were *Ct* genotype L-positive. In contrast, 8.3% (1010/12,174) were positive for anorectal *Ct*, of which 109 (10.8%) were *Ct* genotype L-positive. Thus, we

observed a positivity rate of 0.06% (7/12,174) of urethral LGV among MSM, which is much lower than the positivity rate of anorectal LGV among MSM, which was 0.9% (109/12,174). This is in agreement with previous retrospective European studies that also found low numbers of urethral LGV.[6-9] With an observed anorectal/urethral ratio of 15:1, it seems unlikely that urethral LGV is responsible for all anorectal LGV, and the existence of an unnoticed reservoir of urethral infections is less plausible.

However, we did diagnose some urethral LGV cases. Possibly, a very small group of MSM with a urethral *Ct* genotype L infection function as 'superspreaders'. Their role could be explained in cases where the duration of the urethral infection is much longer than the duration of the anorectal infection, i.e., prevalence = duration x incidence.

# HYPOTHETICAL TRANSMISSION ROUTES

Since the discovery of the outbreak of LGV in the Western world in 2003, its dynamics and potential transmission routes have been investigated to explain the asymmetric distribution of diagnosed anorectal and urogenital LGV. Whether this asymmetric distribution was a true representation of the prevalence in the community, or represents an under-diagnosis of the number of urogenital infections, was unknown at that time. In the early days of the epidemic, high-risk behaviour (fisting, sharing toys, serosorting, and the use of illicit drugs) was considered an important factor in LGV transmission, but was dismissed when investigated in multivariable models based on surveillance data.[10,11] However, these studies did demonstrate that enema use was associated with LGV. Moreover, the very strong association between LGV and HIV co-infection suggested that a breakdown of the mucosal barrier and/or immunosuppression played a role in susceptibility for LGV, although network-related factors could not be ruled out as an underlying explanation for these associations.[12] Tissue tropism also has been proposed as a reason for the imbalance between anorectal versus urethral infections found. It was hypothesised that LGV serovars could have a higher affinity for rectal mucosa than for urethral epithelium. So far, tissue tropism of urogenital *Ct* strains has not been confirmed.[13] Macdonald *et al*. identified unprotected receptive anal intercourse as a key risk factor for rectal LGV, supporting the hypothesis that rectal infection is due to direct inoculation.[14] Yet, this transmission option does not explain the observed asymmetric distribution of anorectal versus urethral LGV diagnoses. It was speculated that the central position of 'LGV repeaters' in the sexual network might be an important factor in the ongoing epidemic. Rönn et al. compared baseline characteristics of repeaters and non-repeaters among MSM from 2004 to 2010. Repeaters were more likely to be HIV-positive

at the first LGV episode and were more likely to have STDs like gonorrhoeae and HCV.[15] The sexual network position was likely to influence the risk of reinfection. Nonetheless, the 'biological' mode of transmission of LGV proctitis among MSM remains enigmatic.

### Alternative transmission route

Under-diagnosis of urethral LGV is, therefore, not a probable explanation of the asymmetric distribution of LGV cases. Alternative transmission modes that could explain the imbalance between anorectal/urogenital LGV diagnoses still need to be clarified. De Vries recently proposed an alternative 'out of the box' explanation: LGV transmission via the oro-anal route.[16] In virtually every animal, chlamydiae strains persist in the gastro-intestinal tract for long periods of time in the absence of apparent inflammation and pathology.[17] Igietseme *et al*. demonstrated in 2001 that mice infected orally with the mouse chlamydia, *C. muridarum*, became infected in the lower intestinal tract and were unable to clear the infection.[18] Oral infection may occur via ano-oral sex or mechanical transmission. Oral infection may result in clinical or subclinical pharyngitis.[19] Although numbers of diagnoses of pharyngeal LGV are low, the pathogen may pass through the gastro-intestinal tract to the large intestine and rectum.[20-22] Here, it could either induce symptomatic LGV proctitis or induce an asymptomatic infection, in both cases contributing to the ongoing transmission.

## THE IMPORTANCE OF DIAGNOSING UROGENITAL LGV

The LGV epidemic appears to persist within the MSM community in Western society.[8,14,23-32] This could have a number of reasons:

1) a proportion of anorectal and urethral LGV is asymptomatic (**chapter 3 and 6**), so many infected men do not seek diagnosis or treatment;
2) in the current epidemic MSM have sexual contacts in an international network. Contact tracing is difficult because of venues for anonymous sex like saunas, darkrooms, and parking areas, and more recently the vast opportunities to meet partners online via social media and dating apps;
3) cases stay undetected because only in specialized and well-equipped settings are MSM tested for *Ct* genotype L, and current guidelines coming from of Europe, the United Kingdom, and Canada[1-5] do not recommend screening for urethral LGV. If an infection is not suspicious for *Ct* genotype L and subtyping is not subsequently performed, LGV patients will receive the standard course of treatment for non-LGV *Ct* (azithromycin 1000mg for urethral *Ct* and doxycycline 100mg twice a day for 7 days for anorectal *Ct*) instead of the recommended treatment for LGV (doxycycline 100 mg twice a day

orally for 21 days). Azithromycin in single- or multiple-dose regimes or a short course of doxycycline (100mg twice a day orally for 7-14 days) has been proposed for treatment of LGV, but consistent evidence is lacking to recommend these drug regimes at present.[33-39]

With a high prevalence of concurrent STDs (gonorrhoeae, HIV, syphilis) (**chapter 3**), LGV is associated with high-risk behaviour. LGV may also facilitate the transmission of other STIs, like blood-borne pathogens such as HIV and HCV.[40,41] Even though there seems to be no large unnoticed reservoir of urethral LGV (**chapter 6**), this is why we consider it important to adequately diagnose and treat LGV in these high-risk communities.

# RECOMMENDATIONS FOR FUTURE GUIDELINES

## Urethral and inguinal LGV

Ideally, all MSM would be tested for urethral and anorectal *Ct* genotype L, regardless of symptoms and available diagnostic capacity. However, the *Ct* genotype L specific nucleic acid amplification test (NAAT) as the gold standard is expensive, requires specialized collection techniques (e.g., anoscopy), and requires specialized laboratory conditions. Affordable commercial tests that are easy to use and inexpensive are desired.[11] These multiplex testing platforms will probably become available in the coming years. Chlamydia-specific serology tests are available and inexpensive, unfortunately serological tests for LGV are not very sensitive or specific.[42] Recently Turingan *et al.* published data about rapid detection and strain typing of *Ct* using a highly multiplexed microfluidic PCR assay. This assay was able to detect *Ct* and to identify LGV and non-LGV strain types. In the future this assay might have significant potential as a rapid point of care diagnostic for *Ct* and *Ct* genotype L.[43] Until that time, based on this thesis, we would recommend the following for urogenital LGV:

***Testing:***

Since routine screening on urethral LGV does not seem cost-effective under the current conditions, we recommend screening for urethral LGV for:

- sexual contacts within the last 4 weeks of patients with anorectal LGV (**chapter 4**);
- MSM with a urethral *Ct* infection and ongoing urethral complaints, despite treatment with azithromycin 1000mg.

We recommend testing of bubo aspirates for suspected inguinal LGV for:

- MSM with inguinal complaints or with inguinal swelling, despite treatment with azithromycin 1000mg (**chapter 5**).

***Treatment***

The recommended regime for urethral LGV and routine treatment of urethral *Ct* infections of doxycycline 100mg twice a day for 3 weeks for all MSM seems undesirable for reasons of overtreatment, unwanted side effects, antimicrobial stewardship, and compliance. We therefore advise:

- treating patients with a proven urethral LGV, with 3 weeks of doxycycline 100mg twice a day;
- treating sexual partners within the last 4 weeks of patients with anorectal LGV, with 3 weeks of doxycycline 100mg twice a day (**chapter 4 and 5**);
- following up with patients with inguinal LGV. Especially in case of buboes, prolonged treatment regimes should be continued upon clinical resolution, since the penetration of antibiotics in abscess cavities is very poor (**chapter 5**).

***Clinical management:***

We advise physicians dealing with urogenital and rectal pathology:

- to be alert for (asymptomatic) LGV among high-risk groups and partners of patients with anorectal LGV (**chapter 3, 4, and 6**);
- to target MSM at risk of LGV for frequent STI screening and interventions to reduce risk behaviour (**chapter 3**);
- to remember the need for sexual health counseling and to inform the MSM population about LGV via narrow casting, e.g., online services, social media, and social/health services.

## MSM with a request for PEP

From April 2010 until December 2012, 473 PEP requests were analyzed. In 78/473 (16.5%) consultations, at least one STI was diagnosed. Moreover, in 13/473 (2.7%) consultations concerning PEP, new HIV infections were detected. This is higher compared to the percentage of new HIV diagnoses among MSM attending the STI clinic in 2016 (0.7%). Of the 13 newly diagnosed HIV infections, 2 proved to be primary HIV infections; these are known to be accompanied by very high viral loads and therefore much more contagious than chronic HIV infection. Based on this thesis we recommend screening for STI among MSM with a request for PEP at an STI clinic: this is worthwhile regardless of whether there is an indication for PEP (**chapter 7**).

# RECOMMENDATIONS FOR FUTURE RESEARCH

The discrepancy in anorectal versus urethral LGV cases remains unexplained to date. More research is needed to confirm the evidence that (urethral) LGV requires 3 weeks of therapy. In addition, it will be necessary to investigate whether different antibiotic regimes are needed to treat symptomatic LGV as opposed to asymptomatic LGV. To improve infection management and sexual health counseling, research should focus on the pathophysiology of the disease to evaluate the mode of transmission. It is important to find clear answers on who should be tested for rectal and extra-rectal LGV and to identify the high-risk group that should be tested for urethral LGV routinely. Finally, it is important to incorporate *Ct* genotype L specific options in commercial diagnostic platforms, to investigate cheaper and more practical screening methods, and to evaluate the cost-benefit ratio of additional testing on urethral LGV.

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# Appendix

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- PORTFOLIO
- CURRICULUM VITAE
- DANKWOORD

# SUMMARY

Lymphogranuloma venereum (LGV) is an invasive ulcerative sexually transmitted disease (STD) caused by serovars L1, L2, or L3 of the bacterium *Chlamydia trachomatis* (*Ct*). LGV was first described in 1786, and in the past 15 years LGV has re-emerged in the Western world among men who have sex with men (MSM). Although unsafe genital-anal sexual contact seems the most logical way of transmission, in the current epidemic, predominantly anorectal LGV have been described and only few cases of urogenital LGV are reported. This makes the mode of transmission unclear. What is also important is that routine screening for urogenital LGV is not recommended in international guidelines, as a result of which there may be an undiscovered reservoir of urogenital LGV.

With a high prevalence of concurrent sexually transmitted infections (STIs), LGV is associated with high-risk behaviour. LGV could also facilitate the transfer of other STIs, and LGV needs longer treatment than other *Ct* infections. An adequate diagnosis and treatment for urogenital LGV are of importance, both for the individual patient, as well as for the community at large that he is part of.

Apart from LGV, one chapter of this thesis discusses STI screening in MSM visiting the STI clinic in Amsterdam for HIV post-exposure prophylaxis (PEP). PEP is a 4-week course of antiretroviral treatment recommended to persons exposed to human body fluids possibly infected with HIV, in order to prevent an established HIV infection.

With this thesis we want to provide insight into the current LGV epidemic in Amsterdam among MSM, with special emphasis on the role of urogenital (urethral and inguinal) LGV. The aims are 1) to investigate a potentially missed reservoir of urethral LGV that might explain the transmission route of LGV and 2) to formulate recommendations on diagnosis and treatment for urethral and inguinal LGV. Additionally, our aim is 3) to determine whether additional STI testing among MSM with an indication of HIV PEP leads to more STI diagnoses to prevent ongoing transmission of STI.

**Chapter 1** is an introductory chapter to the studies described in this thesis. **Chapter 1 and 2** also give a brief overview on the history, epidemiology, clinical observations, and management of LGV.

**Chapter 3** reports on positivity trends of LGV, the symptomatology of anorectal LGV, and the frequencies of anorectal and inguinal LGV. The differences in demographics and concurrent STIs between anorectal and inguinal LGV among MSM visiting the STI outpatient clinic in Amsterdam from January 2005 until June 2012 are also reported. A

significant increase of LGV diagnoses among MSM in the first 6 months of 2012 and a high prevalence of concurrent STI were found. Unlike previously described in the literature, more than 25% of the anorectal LGV cases neither had symptoms nor had inflammatory signs noticed on anoscopy. There was a predominance of anorectal LGV compared to inguinal LGV diagnoses. As MSM with urethral *Ct* infections were not routinely tested for LGV, it is likely that urethral LGV diagnoses were missed.

In **chapter 4** we wanted to determine whether there is a reservoir of missed urethral LGV, which might be of importance in its transmission. From January 2008 until August 2012 we retrospectively performed LGV typing on all *Ct*-positive urethral samples in index patients with anorectal LGV and in partners of patients with anorectal LGV; 27,504 MSM reporting receptive anal intercourse in the past 6 months were screened. The prevalence of anorectal LGV was 1.2% (n=341). Within this group 7/33 *Ct*-positive urine samples were biovar L-positive. Out of 59 sexual partners, none were diagnosed with anorectal LGV, but 4/10 *Ct*-positive urine samples from these partners were biovar L-positive. With this study we found undiagnosed urethral infections in a selected population exposed to LGV. Therefore, urethral LGV might be a key in the transmission of LGV among MSM.

**Chapter 5** describes a case series of four MSM who developed inguinal LGV to highlight: 1) pitfalls in the diagnosis of LGV; 2) the importance of correct partner treatment; and 3) the protracted course of some LGV infections. Patients notified by a partner diagnosed with LGV were screened. We describe two men who received a single dose of azithromycin after exposure to LGV. Urethral and anorectal *Ct* was ruled out but inguinal LGV was diagnosed weeks later. Based on these findings, it was recommended to treat contacts of LGV patients with doxycycline for 21 days. The other two men had persistent inguinal LGV after the advised course of 100mg of doxycycline twice a day for 21 days. Inguinal LGV with bubo formation may require a prolonged course of doxycycline, exceeding the currently advised 21 days.

Keeping the findings on urethral LGV of **chapter 3 and 4** in mind, it is a logical step to **chapter 6:** what is the contribution of urethral LGV to the ongoing transmission in the current LGV epidemic. We wanted to determine the positivity rate of urethral LGV among MSM. Prospectively, urine samples were collected from all MSM visiting the STI outpatient clinic between March 2014 and July 2015 and were screened for *Ct*. Positive samples were genotyped to differentiate between LGV and non-LGV. In 12,174 tests, 404 were positive for urethral *Ct*. Of these 404 urethral *Ct* infections, 7 tested LGV-positive. Overall, we found a urethral LGV positivity rate of 0.06%, which is 15 times lower compared with the positivity rate of anorectal LGV of 0.9%. Therefore, it seems unlikely that urethral LGV is responsible

for all anorectal LGV, and the existence of an unnoticed reservoir of urethral infections is less plausible. The low prevalence of cases found here does not seem to justify routine screening for urethral LGV.

**Chapter 7** is the only chapter that does not deal directly with LGV. Individuals presenting at the STI clinic for a PEP request are at risk for concurrent STI. In **chapter 7** the importance of adequate STI screening of MSM with an indication for HIV PEP is studied. Data from the period April 2010 until December 2012, including 473 patients with a PEP request, were analysed. Of these patients 16.5% had at least one STI, including 13 previously undiagnosed HIV infections. Repeated screening 2 weeks later revealed that 4.1% of patients had additional *Ct* and/or *Neisseria gonorrhoeae* (*Ng)* infections that were not diagnosed at the initial screening. We advise STI and HIV screening for all MSM with a PEP request regardless of symptoms, and that repeat screening should be considered after a few weeks to diagnose incubating infections.

**Chapter 8** discusses the findings of this thesis in a broader context. The majority of reported LGV in MSM in the current epidemic is found in the anorectal canal and not in association with the urogenital tract. Although several transmission routes have been investigated, the 'biological' mode of transmission of LGV proctitis among MSM is still an enigma. We found an anorectal/urethral ratio of 15:1. With this ratio it seems unlikely that urethral LGV is responsible for all anorectal LGV, and the existence of an unnoticed reservoir of urethral infections is less plausible. However, we did diagnose some urethral LGV cases. Possibly, a very small group of MSM with a urethral *Ct* genotype L infection function as 'superspreaders'. An 'out of the box' transmission route is also discussed: the oro-anal route.

To conclude, all recommendations formulated in this thesis are summarised, which we believe will help in the formulation of future guidelines. Finally, perspectives on future LGV research are given.

# SAMENVATTING

Lymphogranuloma venereum (LGV) is een invasieve ulceratieve seksueel overdraagbare aandoening (SOA) veroorzaakt door serovars L1, L2 of L3 van *Chlamydia trachomatis* (*Ct*). LGV werd voor het eerst beschreven in 1786, maar verdween grotendeels in de westerse wereld toen antibiotica na de Tweede Wereldoorlog beschikbaar kwam. Sindsdien werd LGV vooral gezien in tropische en subtropische gebieden. Echter in de afgelopen 15 jaar is LGV opnieuw opgedoken in de westerse wereld onder mannen die seks hebben met mannen (MSM). Alhoewel onveilig genitaal-anaal seksueel contact de meest logische manier van besmetting lijkt, wordt in de huidige epidemie overwegend anorectale LGV beschreven en worden er slechts enkele gevallen van urogenitale LGV gerapporteerd. Dit maakt de manier van besmetting onduidelijk. Wat ook meespeelt, is dat routinematige screening voor urogenitale LGV niet wordt aanbevolen in internationale richtlijnen, waardoor er mogelijk een onontdekt reservoir van urethrale LGV bestaat.

Er bestaat een hoge prevalentie van andere SOA's bij MSM met LGV, hierdoor wordt LGV geassocieerd met risicovol seksueel gedrag. LGV zou ook de besmetting van andere SOA's kunnen vergemakkelijken, en LGV heeft een langere behandeling nodig dan andere *Ct*-infecties. Een adequate diagnose en behandeling van urethrale en anorectale LGV zijn dan ook van belang, zowel voor de individuele patiënt als voor de public health.

Afgezien van LGV, bespreekt één hoofdstuk van dit proefschrift SOA-screening bij MSM die de SOA-polikliniek in Amsterdam bezoeken met een verzoek voor HIV *post-exposure profylaxe* (PEP). PEP is een 4-weekse kuur met een antiretrovirale behandeling die wordt aanbevolen aan personen die risico hebben gelopen op een HIV-besmetting om een HIV-infectie te voorkomen.

Met dit proefschrift willen we inzicht geven in de huidige LGV-epidemie in Amsterdam onder MSM, met speciale nadruk op urogenitale (urethrale en inguinale) LGV. De doelstellingen zijn 1) om een mogelijk gemist reservoir van urethrale LGV te onderzoeken, hetgeen de route van besmetting van LGV zou kunnen verklaren en 2) om aanbevelingen te formuleren voor de diagnose en behandeling van urethrale en inguinale LGV. Daarnaast is het ons doel om 3) te bepalen of aanvullende SOA-testen onder MSM met een indicatie van HIV PEP leidt tot meer SOA-diagnoses om verdere besmetting van SOA te voorkomen.

**Hoofdstuk 1** is een inleidend hoofdstuk van de onderzoeken die in dit proefschrift worden beschreven. **Hoofdstuk 1 en 2** geven ook een kort overzicht van de geschiedenis, epidemiologie, kliniek en management van LGV.

**Hoofdstuk 3** rapporteert de positiviteitstrends van LGV, de symptomatologie van anorectale LGV en de frequenties van anorectale en inguinale LGV. De demografische verschillen en de verschillen in seksueel overdraagbare co-infecties tussen anorectale en inguinale LGV onder MSM die van januari 2005 tot juni 2012 een bezoek brachten aan de SOA-polikliniek in Amsterdam, worden ook gerapporteerd. Een significante toename van LGV-diagnoses bij MSM in de eerste 6 maanden van 2012 en een hoge prevalentie van gelijktijdige SOA's werden gevonden. Anders dan eerder in de literatuur was beschreven hadden meer dan 25% van de anorectale LGV gevallen geen klachten, noch waren er bij anoscopie ontstekingsverschijnselen. Er werd beduidend meer anorectale LGV gevonden in vergelijking met inguinale LGV-diagnoses. Omdat MSM met urethrale *Ct*-infecties niet routinematig werden getest op LGV, is het waarschijnlijk dat urogenitale LGV-diagnosen werden gemist.

In **hoofdstuk 4** wilden we bepalen of er een reservoir van gemiste urethrale LGV is dat van belang kan zijn bij de besmetting. Van januari 2008 tot augustus 2012 hebben we met terugwerkende kracht LGV-typering uitgevoerd op alle *Ct*-positieve urethrale monsters bij indexpatiënten met anorectale LGV en bij partners van patiënten met anorectale LGV; 27,504 MSM die rapporteerden receptieve anale geslachtsgemeenschap te hebben gehad in de afgelopen 6 maanden werden gescreend. De prevalentie van anorectale LGV was 1.2% (n = 341). Binnen deze groep waren 7/33 *Ct*-positieve urinemonsters biovar L-positief. Van de 59 seksuele partners werden er geen gediagnosticeerd met anorectale LGV, maar 4/10 *Ct*-positieve urinemonsters van deze partners waren biovar L-positief. Met deze studie toonden we niet-gediagnosticeerde urethrale infecties aan in een geselecteerde populatie blootgesteld aan LGV. Daarom zou urethrale LGV een sleutel kunnen zijn bij de besmetting van LGV in MSM.

**Hoofdstuk 5** beschrijft een case-serie van vier MSM die inguinale LGV hebben ontwikkeld. Hiermee willen we benadrukken; 1) valkuilen bij de diagnose van LGV; 2) het belang van een correcte partnerbehandeling; en 3) het langdurige beloop van sommige LGV-infecties. Patiënten gewaarschuwd door een partner met LGV werden gescreend. We beschrijven twee mannen die een enkele dosis van 1000mg azitromycine kregen na blootstelling aan LGV. Urethrale en anorectale *Ct* werden uitgesloten, maar weken later werd inguinale LGV gediagnostiseerd. Op basis van deze bevindingen wordt het aanbevolen om de contacten van LGV-patiënten te behandelen met doxycycline 100mg tweemaal daags gedurende 21 dagen. De andere twee mannen hadden een aanhoudende inguinale LGV-infectie na de geadviseerde kuur van doxycycline 100mg tweemaal daags gedurende 21 dagen. Inguinale LGV met bubo vorming kan een verlengde kuur met doxycycline vereisen, die de momenteel aanbevolen 21 dagen overschrijdt.

Met de bevindingen van de urethrale LGV van **hoofdstuk 3 en 4** in onze gedachte, is het een logische stap naar **hoofdstuk 6**: wat is de bijdrage van urethrale LGV aan de voortdurende transmissie in de huidige LGV-epidemie. We wilden de daadwerkelijke aantallen van urethrale LGV onder MSM bepalen. Prospectief werden urinemonsters verzameld van alle MSM die de SOA-polikliniek in Amsterdam bezochten tussen maart 2014 en juli 2015, deze werden gescreend op *Ct*. Positieve monsters werden gegenotypeerd om onderscheid te maken tussen LGV en niet-LGV. In 12,174 testen waren 404 testen positief voor urethrale *Ct*. Van deze 404 urethrale *Ct*-infecties waren er 7 LGV-positief getest. Over het algemeen vonden we een urethrale LGV-prevalentie van 0.06%, wat 15 keer lager is in vergelijking met de prevalentie van anorectale LGV van 0.9%. Daarom lijkt het onwaarschijnlijk dat urethrale LGV verantwoordelijk is voor alle anorectale LGV. Het bestaan van een onopgemerkt reservoir van urethrale infecties is hiermee ook minder aannemelijk. De lage prevalentie van urethrale LGV lijkt het niet te rechtvaardigen om routinematig te screenen op urethrale LGV.

**Hoofdstuk 7** is het enige hoofdstuk dat niet rechtstreeks met LGV te maken heeft. Personen die zich presenteren op de SOA-poli met een verzoek voor HIV PEP lopen het risico op een gelijktijdige andere SOA. In **hoofdstuk 7** wordt het belang van adequate SOA-screening van MSM met een verzoek voor HIV PEP bestudeerd. Gegevens uit de periode april 2010 tot december 2012, waaronder 473 patiënten met een PEP-verzoek, werden geanalyseerd. Van deze patiënten had 16.5% ten minste één SOA, waaronder 13 voorheen niet-gediagnosticeerde HIV-infecties. Herhaalde screening twee weken later toonde aan dat 4.1% van de patiënten bijkomende *Ct*- en/ of *Neisseria gonorrhoeae (Ng*) infecties hadden, die bij de eerste screening niet werden gediagnosticeerd. We adviseren SOA en HIV-screening voor alle MSM met een PEP-verzoek, ongeacht de symptomen. Om incuberende infecties te diagnosticeren moet worden overwogen om na enkele weken SOA screening te herhalen.

**Hoofdstuk 8** bespreekt de bevindingen van dit proefschrift in een bredere context. Het merendeel van de gemelde LGV bij MSM in de huidige epidemie wordt gevonden in het anorectale kanaal en niet in het urogenitale kanaal. Hoewel verschillende transmissieroutes zijn onderzocht, behoeft de "biologische" wijze van overdracht van LGV-proctitis bij MSM nog steeds verder onderzoek. We vonden een anorectale/urethrale ratio van 15:1. Met deze ratio is het onwaarschijnlijk dat urethrale LGV verantwoordelijk is voor alle anorectale LGV en het bestaan van een onopgemerkt reservoir van urethrale infecties is niet aannemelijk. We hebben echter wel enkele urethrale LGV-gevallen gediagnosticeerd. Mogelijk dat een zeer kleine groep van MSM met een urethrale LGV als 'superspreaders' fungeren. Een 'out-of-the-box' transmissieroute wordt ook besproken: de oro-anale route.

Tot slot zijn alle aanbevelingen geformuleerd in dit proefschrift samengevat. We geloven dat het zal helpen bij het formuleren van toekomstige richtlijnen. Perspectieven op toekomstig LGV-onderzoek wordt ook gegeven.

# LIST OF PUBLICATIONS

## Part of this thesis:

- **De Vrieze NHN**, Versteeg B, Bruisten SM, van Rooijen MS, van der Helm JJ, de Vries HJC. Low prevalence of urethral lymphogranuloma venereum infections among men who have sex with men: a prospective observational study, sexually transmitted infection clinic in Amsterdam, the Netherlands. Sex Transm Dis. 2017;44(9):547-50.
  *Author contributions: NdV, BV, SB and HdV were responsible for the design of the study. MvR and NdV collected and interpreted the data. NdV wrote the manuscript. JvdH and HdV supervised the overall study. All authors critically reviewed and approved the final article.*

- Oud EV, **de Vrieze NHN**, de Meij A, de Vries HJC. Pitfalls in the diagnosis and management of inguinal lymphogranuloma venereum: important lessons from a case series. Sex Transm Infect. 2014;90(4):279-82.
  *Author contributions: EO and NdV gathered the data from the electronic patient file. AdM provided information from his own general practice. HdV designed and supervised the study. The first draft was written by EO and critically revised by NdV, AdM and HdV. All authors approved of the final version submitted.*

- **De Vrieze NHN**, de Vries HJC. Lymphogranuloma venereum among men who have sex with men. An epidemiological and clinical review. Expert Rev Anti Infect Ther. 2014;12(6):697-704.
  *Author contributions: NdV and HdV collected the data. NdV drafted the paper. HdV approved the final article.*

- **De Vrieze NHN**, van Rooijen MS, Schim van der Loeff MF, de Vries HJC. Additional gonorrhea and chlamydia infections found with rapid follow-up screening in men who have sex with men with an indication for HIV postexposure prophylaxis. Sex Transm Dis. 2014;41(8):515-7.
  *Author contributions: NdV, MvR and HdV were responsible for the design of the study. MvR and NdV collected and interpreted the data. NdV drafted the paper. MSvdL and HdV supervised the overall study. All authors reviewed and approved the final article.*

- **De Vrieze NHN**, van Rooijen MS, Schim van der Loeff MF, de Vries HJC. Anorectal and inguinal lymphogranuloma venereum among men who have sex with men in Amsterdam, the Netherlands: trends over time, symptomatology and concurrent infections. Sex Transm Infect. 2013;89(7):548-52.

*Author contributions: NdV analysed the data and wrote the manuscript; MvR gathered the data from the electronic patient file and wrote the manuscript; MSvdL supervised the epidemiological analysis and wrote and approved the final manuscript. HdV supervised the overall study and approved the final manuscript.*

- **De Vrieze NHN**, van Rooijen MS, Speksnijder AGCL, de Vries HJC. Urethral lymphogranuloma venereum infections in men with anorectal lymphogranuloma venereum and their partners: the missing link in the current epidemic? Sex Transm Dis. 2013;40(8):607-8.
  *Author contributions: NdV, MvR and HdV were responsible for the design of the study. NdV and MvR collected and interpreted the data. NdV drafted the paper. HdV supervised the overall study. All authors reviewed and approved the final article*

## Other publications

- Van Rooijen MS, Heijman T, **de Vrieze NHN**, Urbanus A, van Leeuwen P, de Vries HJC, Prins M. Earlier detection of Hepatitis C Virus infection through routine Hepatitis C Virus antibody screening of Human Immunodeficiency Virus-positive men who have sex with men attending a sexually transmitted infection outpatient clinic: A longitudinal study. Sex Transm Dis. 2016;43(9):560-5.

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- Cnossen JS, Vollebregt KC, **de Vrieze NHN**, ter Riet G, Mol BW, Franx A, Khan KS, van der Post JA. Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meat analysis. BMJ. 2008;336(7653):1117-20.

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- **De Vrieze NHN**, Laheij-de Boer AM, Toonstra J. De rol van de 308-nm excimerlaser in de behandeling van mycosis fungoides. NTDV. 2014;24(5):353-5.

- **De Vrieze NHN**, Koek MBG, Versteeg L, Sigurdsson V. Home UVB phototherapy for psoriasis: is the prescribing behaviour and the opinions among dermatologists still the same?: Manuscript in preparation.

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# PORTFOLIO

## Oral presentations

| | Year |
|---|---|
| Lymphogranuloma venereum en anorectale chlamydia, Refereeravond, UMCG Groningen, the Netherlands | 2017 |
| Prevalence of urethral lymphogranuloma venereum infections among MSM. 11th Annual Amsterdam Chlamydia meeting, Amsterdam, the Netherlands | 2016 |
| Epidemiology of urethral LGV infections. 10th Annual Amsterdam Chlamydia meeting, Amsterdam, the Netherlands | 2015 |
| Urethral lymphogranuloma venereum infections in men with anorectal lymphogranuloma venereum and their partners; The missing link in the current epidemic? 14th annual scientific meeting of the NVED, Lunteren, the Netherlands | 2013 |
| Urethral lymphogranuloma venereum infections in men with anorectal lymphogranuloma venereum and their partners; The missing link in the current epidemic? Expertmeeting SOAHIV, RIVM Bilthoven, the Netherlands | 2012 |
| Resultaten van 5 jaar Epididymale Sperma Aspiratie-Intra Cytoplasmatische Sperma Injectie (PESA/ICSI) in het VUmc. Voorjaarsvergadering Nederlandse Vereniging voor Urologie, Amsterdam, the Netherlands | 2011 |

## Poster presentations

| | Year |
|---|---|
| Home UVB phototherapy for psoriasis: did the prescribing behaviour and the opinions change over the past 9 years? 24th EADV congress, Copenhagen, Denmark | 2015 |
| Low prevalence of urethral lymphogranuloma venereum infections among homosexual men visiting the STI clinic in Amsterdam, the Netherlands. IUSTI, Barcelona, Spain | 2015 |
| Additional gonorrhea and chlamydia infections found with rapid follow-up screening in men who have sex with men with an indication for HIV post-exposure prophylaxis (PEP). 15th annual scientific meeting of the NVED, Lunteren, the Netherlands | 2014 |

| | |
|---|---|
| Early incubating gonorrhoea and chlamydia infections in MSM with an indication for HIV post-exposure prophylaxis (PEP). STI & AIDS World Congress, Vienna, Austria | 2013 |

## General courses

| | Year |
|---|---|
| Scientific Writing in English for Publication, AMC, Amsterdam, the Netherlands | 2011 |
| Reference manager, better use of pubmed and other medical databases. AMC Amsterdam, the Netherlands | 2009 |

## Conferences attended

| | Year |
|---|---|
| Vulvapathologie cursus, Breukelen, the Netherlands | 2018 |
| 17th Annual Meeting of the European Society for Pediatric Dermatology, Mallorca, Spain | 2017 |
| BSDS Annual Surgery Workshop; Newcastle, United Kingdom | 2017 |
| Jaarsymposium Dermatologie, Utrecht, the Netherlands | 2015-2016 |
| Annual Amsterdam Chlamydia meeting, Amsterdam, the Netherlands | 2014-2016 |
| Scientific meeting of the Dutch Society of Experimental Dermatology (NVED), Lunteren, the Netherlands | 2013-2015 |
| Proctology congress, Proctos Kliniek, Bilthoven, the Netherlands | 2015 |
| European Academy of Dermatology and Venereology (EADV) Congress Amsterdam, the Netherlands | 2014 |
| Dermatoloog in opleiding (DIO) dagen | 2013-2017 |
| Hugh Greenway's Superficial Anatomy and cutaneous surgery. San Diego, United States | 2012 |

## Teaching

| | Year |
|---|---|
| Supervising | |
| E. Oud, scientific internship, AMC/UvA, Amsterdam, the Netherlands | 2013 |

# CURRICULUM VITAE

Nynke de Vrieze was born in Zürich, Switzerland, on July 14, 1984. In high school (Laar & Berg, Laren, the Netherlands) she followed an International Baccalaureate Programme, and after graduating she started medical school at the University of Amsterdam (UvA). During her internship she spent 4 months in Paramaribo, Surinam, where she followed a surgery internship and worked at a medical post in the inlands of Surinam. After she graduated in 2010 she worked for over a year as a urology resident at the VUmc in Amsterdam. Subsequently, she started working as a physician at the STI clinic of the GGD in Amsterdam in combination with scientific research, which led to this thesis. In September 2013 she started her dermatologist training at the UMCU, in Utrecht, under supervision of dr. Vigfús Sigurdsson. Nine months of her training took place in the Tergooi Hospital, Hilversum, under supervision of dr. Bram Preesman. In 2019 she expects to complete her education. In addition to her dissertation and medical training she is also active in the organization of 'Dermatologen in Opleiding (DIO) dagen' (dermatologists in training days), she is still working as a supervisor in the evening at the STI clinic, and is she contributing to the new guideline for lymphogranuloma venereum. Nynke is married to Bob Meijer, they live in Amsterdam and together they have a daughter Fleur (2) and a son Lex (0).

Would you like to see Nynke giving information on post-exposure prophylaxis (PEP)? Download a QR-code reader and scan above.

# CURRICULUM VITAE

Nynke de Vrieze werd geboren in Zürich, Zwitserland, op 14 juli 1984. Op de middelbare school (Laar & Berg, Laren, Nederland) volgde ze een Internationaal Baccalaureaat Programma en na haar eindexamen ging ze studeren aan de medische faculteit van de Universiteit van Amsterdam (UvA). Tijdens haar coschappen verbleef zij 4 maanden in Paramaribo, Suriname, waar zij een keuze coschap chirurgie volgde en werkte op een medische post in de binnenlanden van Suriname. Nadat ze in 2010 haar artsexamen behaalde heeft ze ruim een jaar gewerkt als ANIOS urologie in het VUmc te Amsterdam. Vervolgens is zij gaan werken als SOA arts op de SOA-poli van de GGD in Amsterdam in combinatie met wetenschappelijk onderzoek, wat heeft geleid tot dit proefschrift. In september 2013 startte ze haar opleiding tot dermatoloog in het UMCU, te Utrecht, onder begeleiding van dr. Vigfús Sigurdsson. 9 maanden van haar opleiding vond plaats in het Tergooi Ziekenhuis, te Hilversum, onder begeleiding van dr. Bram Preesman. In 2019 verwacht ze haar opleiding af te ronden. Naast haar proefschrift en opleiding is ze o.a. actief in de organisatie van de Dermatologen in Opleiding (DIO) dagen, is ze nog steeds werkzaam als supervisor op de SOA-avondpoli en schrijft ze mee aan de nieuwe richtlijn voor lymphogranuloma venereum. Nynke is getrouwd met Bob Meijer, ze wonen in Amsterdam en samen hebben zij een dochter Fleur (2) en een zoon Lex (0).

Wil je Nynke voorlichting zien geven over post-exposure profylaxe (PEP)?
Download dan een QR-code reader en scan bovenstaande.

# DANKWOORD

Ik ben begonnen aan het schrijven van dit proefschrift toen ik een aantal jaar geleden werkte als arts/onderzoeker bij de SOA-poli van de GGD. En er is nogal wat gebeurd sinds die tijd; het verdrietige overlijden van mijn schoonvader, aangenomen worden voor de opleiding Dermatologie in het UMCU, vele reizen waaronder zeven weken met de trein door Rusland-Mongolië-China, getrouwd met de liefde van mijn leven, samen een huis gekocht in Amsterdam, twee fantastische kindjes gekregen en vele onuitwisbare momenten met familie, vrienden & collega's. Tussen al deze gebeurtenissen door liep het proefschrift altijd op mijn schouders een beetje mee….soms wat intenser en fanatieker dan de andere keer, maar ergens was het altijd aanwezig.

Ik kan me het moment nog goed herinneren. Het moment dat ik werd aangenomen voor de opleiding Dermatologie (en ik dus de SOA-poli zou gaan verlaten) waarna Henry (mijn promotor) aan mij vroeg of ik mijn onderzoek bij de GGD dan nog wel wilde voortzetten. Hierop heb ik direct positief geantwoord. Maar wanneer ik wederom in het weekend of 's avonds laat aan het werk was heeft toch ook door mijn hoofd gespookt, waarom zei ik geen "nee"??? Maar nu ik het boekje bijna in mijn handen heb, ben ik toch heel erg blij dat de mensen om mij heen en ik erin zijn blijven geloven en hebben doorgezet. En dit is dan ook het perfecte moment om een aantal mensen te bedanken die deze reis met mij zijn begaan.

**Henry** (Prof. dr. H.J.C. de Vries, mijn promotor), waar moet ik beginnen. Je bent er altijd in blijven geloven en je hebt me onvoorwaardelijk gesteund in het hele proces. Je hebt me nooit gepusht maar zei altijd net de juiste dingen, dat als ik het even niet zag zitten ik weer helemaal enthousiast werd. Je bent enorm gedreven voor de wetenschap en infectieziekten en je weet dat goed over te brengen. Als het een week duurde voordat je de volgende nieuwe versie had nagekeken was het lang. Dat maakt het leven van een promovendus een stuk makkelijker! Veel dank dat je me hebt aangenomen bij de GGD destijds, het heeft me verrijkt.

**Maarten** (dr. M.F. Schim van der Loeff, mijn co-promotor), ik ken niemand met zo'n oog voor detail. Veel dank voor je inzet, punctualiteit en epidemiologische en methodologische inzichten. Je was altijd betrokken en ook even benieuwd hoe het met mij ging, naast het onderzoek. Je bent onmisbaar geweest voor het realiseren van dit proefschrift.

**Vigfús** (dr. V. Sigurdsson, mijn opleider), mijn eerste ontmoeting met jou was op een SOA-expert meeting, niet lang daarna werd ik aangenomen voor de opleiding Dermatologie in het UMCU met jou als opleider. Wat was ik ontzettend blij! Je bent een enorm voorbeeld

voor mij als dermatoloog. Pragmatisch, altijd kritisch en met oog voor het hele plaatje. De dingen zijn niet altijd zoals in de boeken! En jouw befaamde uitspraak 'your guess is as good as mine' zal ik nooit vergeten. Dat je als SOA-expert en opleider in mijn commissie zit vind ik een eer!

De promotiecommissie bestaande uit **dr. V. Sigurdsson**, **Prof. dr. P.I. Spuls**, **Prof. dr. J.E.A.M. van Bergen**, **Prof. dr. C.J.P.A. Hoebe**, **Prof. dr. C. Schultsz** en **dr. H.M. Götz**. Ik wil u allen bedanken voor uw bereidheid om deel uit te maken van mijn promotiecommissie en de tijd die u hebt vrijgemaakt voor het beoordelen van mijn proefschrift.

Alle **co-auteurs**, veel dank voor jullie inhoudelijke en tekstuele bijdrage aan de verschillende artikelen.

**Collega's van het UMCU**. Alle oud en huidige **collega arts-assistenten** véél dank voor de leuke en leerzame opleidingsjaren en fantastische AIOS-uitjes! De ontlading na het cabaret, Après ski in Berlijn met RONY (Hester!) en verkleed in Kopenhagen met BRAMA, onvergetelijk! En alle **verpleegkundigen**, **dokters-assistenten**, **baliemedewerkers** en **stafleden**, bedankt voor de fijne samenwerking!

**Collega's van de GGD**, wat een geweldige tijd heb ik met jullie gehad! De laatste jaren werkte ik alleen nog op de avondpoli maar desondanks dat ik daar na een dag werken enigszins gestrest binnen kwam, ging ik altijd de deur uit met een glimlach! Ik kan jullie helaas nooit allemaal persoonlijk bedanken. Extra dank voor **Martijn**; held van de SPSS! Dank voor al jouw uitleg & data & gezelligheid! Grüsse! **Adriaan**; als er op de deur geklopt werd wist ik dat jij het was (hallooooooo), je bent een echt mensen mens! En **Menne**; jouw filmpje voor mijn afscheid was fenomenaal, en naast alle gezelligheid dank voor jouw hulp op de weg naar dit proefschrift! Overige '**chlamydia lovers**', laten we snel weer een date plannen!!

**Friends & Family**. Ik kan ook hier niet iedereen persoonlijk bedanken maar wat ben ik toch gezegend met trouwe en waardevolle vriendschappen en een ontzettend leuke schoonfamilie! Héél veel dank voor jullie steun, gezellige momenten, relativerende vermogen, enthousiasme en schouderklopjes. Ieder op z'n eigen manier.

En nog in het bijzonder wil ik bedanken voor hun onvermoeide steun: **Zoé & Agnes**; mijn paranimfen! Wat hebben we níet met elkaar meegemaakt! Samen uit samen thuis was altijd al ons motto, dat we ook nog alle 3 geneeskunde gingen studeren en samen op de Marnixstraat in Amsterdam gingen wonen sloot daar naadloos op aan :) Al lijkt die tijd alweer ver weg en Agnes woon jij zelfs op Texel (ons wel bekend;), over onze vriendschap bestaat geen twijfel. Zuiver op de graad, 3 musketiers voor altijd!!!! **Edje** (Lara); van de

woensdagavond-borrelavond tot pasodoble, de liefde voor oranje & schaatsen en nu zelfs in hetzelfde jaar een mini gekregen, wat een geluk dat wij maatjes zijn!! En m'n lieve **zus Welmoed**; we gunnen elkaar alles en één blik is vaak genoeg. Ik geniet ervan als we even helemaal kunnen bijpraten als we in de auto zitten te bellen en jeetje wat een feest dat onze kindjes zo leuk spelen. Zo fijn dat jij mijn zus bent!!

**Papa & Mama**. Dank voor jullie onvoorwaardelijke liefde en vertrouwen, altijd! Papa zette wat druk, mams haalde er wat druk af. Beiden waren belangrijk in de weg naar dit proefschrift. Maar pap, ik heb doorgezet! Deze is eigenlijk voor jou! En mams, op een gegeven moment zeiden we "*it giet oan*" en hebben we een plan gemaakt. Dank voor alle goede zorgen, voor mij en voor Fleur & Lex.

**Schatje (Bob)**, wat ben ik mega trots op ons en ons gezin!! Elke dag naast jou wakker worden is een feestje, en wat kunnen we genieten samen. Het was soms best een beetje zwaar voor je: al die keren dat ik ongezellig aan het werk was, dat je mijn inleiding en discussie weer moest lezen en dat je de 'gore' plaatjes onder ogen kreeg (tijdens het eten)…maar onthoud: LGV is okay! Love you so much, **BONYFLEX-4ev**!