SHORT REPORT

Observation of a cytopathogenic effect on cell lines used for routine viral cultures led to the diagnosis of lymphogranuloma venereum

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ABSTRACT

Objectives This article reports the fortuitous recovery of nine *Chlamydia trachomatis* serovar L strains in cell cultures (Vero and LLC-MK_2 cell line) designed for viral culture.

Methods Nine ano-genital swabs were inoculated on confluent Vero, MRC5 and LLC-MK_2 cell cultures. They were collected from HIV-positive patients who were primarily men who have sex with men (MSM) presenting ulcerations that mimicked herpes simplex infections.

Results A cytopathogenic effect was observed on Vero and LLC-MK_2 cells on day 14. The presence of *C trachomatis* serovar L in the cell lines was confirmed by Real Time–PCR.

Conclusions *C trachomatis* serovar L can grow on Vero and LLC-MK_2 cell lines designed for viral cultures. Lymphogranuloma venereum must be considered as a differential diagnosis for herpes-like lesions, particularly in MSM with high-risk behaviours.

INTRODUCTION

Chlamydia trachomatis was identified in the mid-sixties; it causes several sexually transmitted diseases and trachoma. The D to K serovars of *C trachomatis* primarily cause urethritis, cervicitis, salpingitis and mild proctitis. Lymphogranuloma venereum (LGV) is caused by the L serovar. LGV is an inguinal syndrome, but in Europe, it typically presents as an anorectal syndrome among men who have sex with men (MSM). Unlike the other urogenital *Chlamydia* serovars, the L serovar is more invasive and affects the submucosal connective tissue layers.[1]

This bacterium was formerly thought to be a virus due to its intracellular replication and ability to grow on cell lines.[2] Currently, the McCoy (human synovial fluid), HeLa 229 (human cervical epithelial adenocarcinoma) and BGMK (Buffalo Green Monkey Kidney) cell lines are used for its isolation and identification from clinical specimens.[1 3]

Herein, we report the observation of an unexpected cytopathogenic effect (CPE) caused by *C trachomatis* serovar L on cell lines used for routine viral culture, which led to the identification of nine cases of LGV.

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MATERIALS AND METHODS

From April 2010 through September 2011, eight anorectal swabs and one penile swab using FLOQSwabs (Copan, Brescia, Italy) led to an unexpected and atypical CPE on cell lines used for viral cultures. Those swabs were stored in virus transport medium consisting of veal infusion broth and bovine albumin, supplemented with antibiotics (vancomycin, gentamycin and amphotericin B). As a part of routine viral culture procedure, portions of the eluted specimens were inoculated on confluent Vero (African Green Monkey Kidney), MRC5 (Human lung cells) and LLC-MK_2 (Rhesus Monkey Kidney) cell cultures (Vircell, Santa-Fé, Spain) in 24-well or 6-well tissue culture plates (Greiner-Bio One, Frickenhausen, Germany) and incubated at 36°C in a 5% CO_2 atmosphere for 2 weeks for Vero and LLC-MK_2 cells and 3 weeks for MRC5 cells. The media were replaced every week. The cultures were examined every 2–3 days using an inverted microscope. CPE was observed on the Vero and LLC-MK_2 cell lines on day 14, which is the final day of observation before discarding negative cell cultures. The CPE consisted of cell swelling and syncytia formation. No CPE was observed on MRC5 cells during the 3 weeks of observation.

Aliquots of the infected cell lines were sent to the Institute of Tropical Medicine, Antwerp (Belgium), which confirmed the *C trachomatis* L serovar in all nine samples using Real Time–PCR based on the publication by Chen *et al*.[4]

CASE DESCRIPTIONS

The clinical data, symptoms and laboratory findings of the nine patients are summarised in table 1.

It is noteworthy that patient D presented a penile lesion of approximately 1 cm diameter, erythematosus, lightly ulcerated and with recurrences over at least 5 years. The lesion was diagnosed as a herpes simplex infection and treated without success with acyclovir. The swab of this ulceration was positive for *C trachomatis* in cell culture and using the molecular technique.

DISCUSSION

LGV used to occur primarily in Asia, Africa, South America and the Middle East. Prior to 2003, it was unusual in Western Europe, with most cases being imported. According to the Belgian Institute of Public Health, a total of 43 cases of LGV were reported in Belgium between 2004 and 2008 and were exclusively due to *C trachomatis* serovar L2. Of those 43 cases, 99.7% were MSM, including 1 who was bisexual, 95.3% were HIV positive, 14% had a co-infection with syphilis and 11.6% were

Table 1 The clinical data and laboratory findings for the nine patients

Patient	Age	Gender	Sexual orientation	Localisation of the lesion	Symptoms	HIV serology	HIV viral load (copies/ml)	Chlamydia serology	Co-infection
A	21	M	MSM	Anorectal	Haematochezia Proctitis	Positive	77 300	Strongly positive for IgA and IgG	*Neisseria gonorrhoea*
B	48	M	Unknown	Anorectal	Haematochezia Proctitis Diarrhoea	Positive	Unknown	Not performed	CMV colitis
C	38	M	Unknown	Anorectal	Proctitis	Positive	Unknown	Not performed	
D	36	M	MSM	Penis	Recurrent penile ulceration	Positive	<20	Not performed	Syphilis
E	50	M	MSM	Anorectal	Haematochezia Proctitis	Positive	<20	Not performed	Past syphilis
F	52	M	MSM	Anorectal	Haematochezia Proctitis	Positive	<20	Strongly positive for IgA and IgG	HPV (rectum) Cured/healed hepatitis C
G	35	M	MSM	Anorectal	Haematochezia Proctitis	Positive	14 200	Strongly positive for IgA and IgG	Past syphilis
H	50	M	MSM	Anorectal	Proctitis	Positive	<20	Strongly positive for IgA and IgG	Past syphilis Cured/healed hepatitis C Anal HSV2
I	49	M	MSM	Anorectal	Proctitis Anal stricture Anal discharge	Positive	<20	Strongly positive for IgA and IgG	

CMV, cytomegalovirus; HPV, human papilloma virus; HSV2, herpes simplex virus 2; M, male; MSM, men who have sex with men.

co-infected with gonorrhoea. The mean age was 38 years (range 20–58 years).[5]

The emergence of LGV in Europe appears to be restricted to some networks of MSM with high-risk behaviours. The 'new' L2b serovar was found in a cluster of MSM in Rotterdam, The Netherlands, in 2003 and led to the awareness of the problem in Europe. It was retrospectively retrieved in samples collected between 1979 and 1985 from patients living in San Francisco who had been diagnosed with LGV.[6] The hypothesis of a latent and endemic disease or a slowly evolving epidemic rather than a new outbreak was evoked, particularly because no systematic surveillance for LGV was available in Europe before 2003.[6–8]

Consistent with those data, seven of the nine patients diagnosed with LGV in our hospital were MSM; the sexual orientation of the other two was not documented. All patients had a positive HIV serology and some were co-infected with syphilis (1/9), cured or healed hepatitis C (positive serology with undetectable viral load) (2/9), gonococcal urethritis (1/9), anal human papillomavirus (1/9) and anal herpes simplex virus 2 (1/9).

The fortuitous detection of *C trachomatis* serovar L in cases in which the diagnosis of LGV was not suspected highlights the lack of awareness of this disease. Moreover, some presentations may mimic other pathologies, particularly in penile ulcerations or inguinal nodes. A previous case report mentions the detection of *C trachomatis* serovar L in the cell culture of an inguinal lymph node from a patient with a suspicion of cat scratch disease.[9]

CONCLUSION

LGV has been an emerging disease in Europe since 2003, particularly in some networks of MSM with high-risk behaviours. The methods of diagnosing and declaring LGV could be improved, as highlighted in the 2010 surveillance report of the European Centre for Disease Prevention and Control.[10] Virology laboratories using cell cultures should be aware of the possible growth of *C trachomatis* serovar L in cell cultures used for viral detection and should therefore be more vigilant when confronted with an unusual CPE for an ano-genital sample. This observation also demonstrates that cell cultures are still useful in university hospitals in addition to molecular techniques. Indeed, with molecular techniques, you only find what you seek, whereas with cell cultures you can sometimes find what you did not expect.

Key messages

- *Chlamydia trachomatis* serovar L can be grown in the Vero and LLC-MK_2 cell lines, which are typically used for viral cultures.
- Lymphogranuloma venereum can have atypical presentations (recurrent penile ulceration).
- The diagnosis and declaration systems for lymphogranuloma venereum could be improved.

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