

## Outbreak report

# EXPERIENCES WITH THE NEW GENETIC VARIANT OF *CHLAMYDIA TRACHOMATIS* IN ÖREBRO COUNTY, SWEDEN – PROPORTION, CHARACTERISTICS AND EFFECTIVE DIAGNOSTIC SOLUTION IN AN EMERGENT SITUATION

M Unemo (magnus.unemo@orebro.ll.se)<sup>1</sup>, P Olcén<sup>1</sup>, I Agné-Statling<sup>1</sup>, A Felldt<sup>1</sup>, M Jurstrand<sup>1,2</sup>, B Herrmann<sup>3</sup>, K Persson<sup>4</sup>, P Nilsson<sup>5</sup>, T Ripa<sup>5</sup>, H Fredlund<sup>1</sup>

1. Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden
2. Clinical Research Centre, Örebro University Hospital, Örebro, Sweden
3. Department of Clinical Microbiology, Uppsala University Hospital, Uppsala, Sweden
4. Department of Clinical Microbiology, Malmö University Hospital, Malmö, Sweden
5. Department of Clinical Microbiology and Infection Control Halland, County Hospital, Halmstad, Sweden

A *Chlamydia trachomatis* variant that contains a 377 bp deletion in the cryptic plasmid was recently reported in Sweden. This deletion includes the targets for Cobas Amplicor, Cobas TaqMan48, and Abbott m2000. We examined the proportion and characteristics of this variant in Örebro county, Sweden and developed an effective diagnostic solution. In total, 2,401 consecutive *C. trachomatis* culture samples and 536 PCR samples from symptomatic and asymptomatic patients and screened females were included. Culture, Cobas Amplicor, and LightMix 480HT were used for diagnosis. A mutant-specific PCR, plasmid sequencing, *omp1* sequencing and multilocus sequence typing (MLST) were used to identify and characterise mutants. In total, 162 (6.7%) of the cultured samples were positive for *C. trachomatis*. However, 61 (38%) of those were negative when using Cobas Amplicor, and 60 of these were subsequently confirmed as the new variant. 13 of these mutant isolates were further characterised genetically, and all were of identical genotype E and the unique MLST sequence type: 21, 19, 1, 2, 1. Of all culture-positive samples, 161 of 162 were positive in the LightMix 480HT assay. The single negative sample was only weakly positive in culture, and negative in all PCRs. Of the 536 PCR samples, 37 were positive in both Cobas Amplicor and LightMix 480HT, 13 were only positive in LightMix 480HT (mutants), and two were only positive in Cobas Amplicor. Mutated *C. trachomatis* were prevalent in Örebro county in the period from October 2006 to February 2007, and it appeared to be a single clone. LightMix 480HT seemed sensitive, specific, and enabled high throughput diagnostics. However, rare low positive samples may be false-negative. Frequent surveillance and evaluations of diagnostic methods worldwide are crucial.

### Introduction

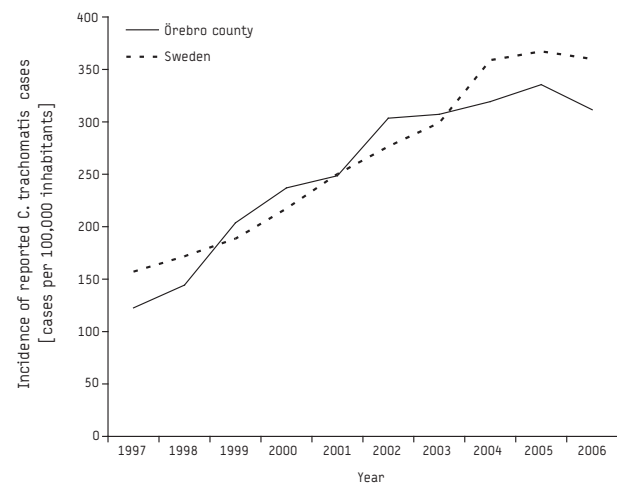
In Halland, Sweden, a new variant of *Chlamydia trachomatis* (nvCT) was recently reported, which contained a 377 bp deletion in the cryptic plasmid (GenBank accession no. EF121757) [1]. This deletion includes the targets for diagnostic systems widely used in Sweden and in other countries, i.e. Cobas Amplicor (Roche Diagnostics), Cobas TaqMan48 (Roche Diagnostics), and Abbott m2000 (Abbott Laboratories) [1,2]. BD ProbeTec ET (Becton Dickinson), which targets a plasmid sequence outside the deleted region, is used in some of the Swedish counties. Aptima Combo

2 (Gen-Probe), which detects *C. trachomatis* specific 16S rRNA sequences, is not used in Sweden.

Currently, nvCT have been identified in several counties across Sweden, with reported proportions from 10% to 66% of total *C. trachomatis* true positive samples [unpublished data]. In Örebro County (275,000 inhabitants), all *C. trachomatis* samples are analysed in the Department of Clinical Microbiology, Örebro University Hospital, using nucleic acid amplification test (NAAT), i.e. Cobas Amplicor, or culture (mainly cervical samples). The incidence of clinically reported *C. trachomatis* cases in Örebro county is similar to the national incidence and increased between 1997 and 2005. However, the incidence decreased from 336 cases per 100,000 inhabitants in 2005 to 311 cases per 100,000

FIGURE 1

The incidence of clinically reported *C. trachomatis* infection, irrespective of diagnostic method, in Örebro county and Sweden from 1997 to 2006



inhabitants in 2006 (Figure 1). Immediately after the first report of nvCT in Sweden, information regarding the diagnostic problem was widely distributed and it was recommended that symptomatic patients and patients with suspected chlamydial infection due to other reasons (contact tracing, etc) should be diagnosed using PCR and/or culture.

The objectives of this study were to examine the proportion and characteristics of mutated *C. trachomatis* in Örebro county, Sweden, and to develop an effective diagnostic solution in an emergent situation.

### Materials and Methods

All consecutive *C. trachomatis* samples received for culturing at the laboratory between 5 October 2006 and 15 January 2007 (n=2,401, mainly cervical and urethral specimens) were included in this study. In addition, 536 consecutive PCR samples (urine (n=447), cervical (n=74), urethral (n=7), rectal (n=2), conjunctival (n=1), and unspecified (n=5) specimens) received between 5 February and 28 February 2007 were included. All these samples came from symptomatic or asymptomatic patients with suspected *C. trachomatis* infection, or were screening samples from women.

For diagnosis of *C. trachomatis*, Cobas AmpliCor PCR (Roche Diagnostics) and/or McCoy cell culture with subsequent identification using fluorescein-labelled monoclonal antibodies (Phadebact Chlamydia IF Test, Bactus AB) were used. In addition, we evaluated the diagnostic value of a robotised system for automatic DNA isolation with magnetic silica particles (MagNa Pure LC System) combined with the new quantitative real-time PCR assay LightMix 480HT (TIB MOLBIOL) that targets a 136 bp fragment of the *omp1* gene (together with LightCycler FastStart DNA Master Hybridization Probes, additional MgCl<sub>2</sub>, and LC Uracil-DNA Glycosylase), performed in 96-well microtiter plates on a LightCycler 480 (Roche Diagnostics). This is the first paper evaluating LightMix 480 HT, with a sensitivity to detect, at least, >=10 copies of *C. trachomatis* DNA (TIB MOLBIOL).

For the genetic characterisation of all suspected mutants, we used a mutant-specific real-time PCR with LightCycler probes flanking the plasmid deletion, and, for selected mutants, we performed plasmid sequencing, *omp1* sequencing and a new assay for multilocus sequence typing (MLST) [3].

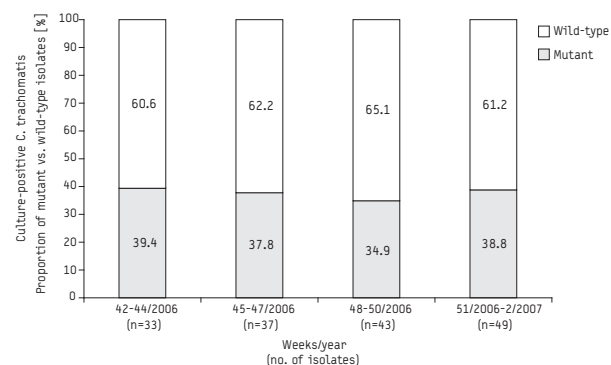
### Results

Of the 2,401 cultured samples, 162 (6.7%) were *C. trachomatis* culture-positive. 101 of these 162 culture-positive samples were also positive in Cobas AmpliCor PCR. The remaining 61 (38%), however, were negative in the Cobas AmpliCor PCR, which only detects "wild-type" *C. trachomatis* and not the nvCT. 60 of those 61 were also positive for nvCT in the mutant-specific PCR, and, in addition, two of those 60 were further analysed by plasmid sequencing and shown to be identical to the mutant previously discovered in Halland [1]. Thus, in Örebro county, based on cultured samples, the proportion of the mutated variant had a mean of 38% (range: 34.9% to 39.4%) during the studied period of 12 weeks (Figure 2).

The mutated isolates were derived from 20 male (mean age: 22 years; range: 17 to 30 years) and 40 female patients (mean age: 21 years; range: 16 to 36 years). There were no obvious differences between the mutated isolates and wild-type isolates, neither with regards to clinical infection nor to growth characteristics in cell culture. 13 of the mutant isolates were genetically characterised

FIGURE 2

Culture-positive *C. trachomatis* and proportion [%] of mutated isolates in Örebro county, week 42, 2006 - week 2, 2007



further and found indistinguishable, i.e. they were all of genotype E, identical to the prototype strain E/Bour [4], and of the unique MLST sequence type: 21 (target region CT046, *hctB*), 19 (CT058), 1 (CT144), 2 (CT172), and 1 (*pbpB*).

For the initial evaluation of the LightMix 480HT diagnostic assay, we used the primary samples of the 162 culture-positive cases described above. 161 (99.4%) of them were positive using LightMix 480HT. Furthermore, we analysed 536 consecutive PCR samples independent of the culture samples. 37 of them were positive in both Cobas AmpliCor and LightMix 480HT, 13 were positive in LightMix 480HT only (mutants), but two urine samples were only positive in Cobas AmpliCor. Consequently, the proportion of the mutated variant among the PCR samples was 25% (13/52). Overall, according to the quantification available for LightMix 480HT, the load of *C. trachomatis* cells/DNA was similar for the mutated isolates and the wild-type strains (not shown). Furthermore, none of the samples included in this study were inhibited either in Cobas AmpliCor or LightMix 480HT according to the internal positive controls.

### Discussion and Conclusions

In Örebro county, Sweden, the new genetic variant of *C. trachomatis* was present and the proportion was high and rather constant during the study period (Figure 2). *Omp1* gene sequencing and MLST, which has a significantly higher discriminatory ability than *omp1* sequencing [3], strongly indicate that the nvCT is of one single clone that has not been prevalent in the community for an extended time period, at least not in high numbers.

LightMix 480HT seemed to be a sensitive, specific, and fast method for high throughput (96 samples analysed on LightCycler 480 in 1.5 hours) identification of *C. trachomatis*. One culture-positive and two Cobas AmpliCor-positive samples were negative using LightMix 480HT. However, the culture-positive sample was reported as only weakly positive in culture, repeatedly negative in all the NAATs (even when using an increased volume of DNA template), and contamination in the culture cannot be excluded. Furthermore, one of the two Cobas AmpliCor-positive samples was derived from a woman that already received treatment. However, rare samples with a low *C. trachomatis* load may be false-negative with LightMix 480HT due to its lower sensitivity for wild-type strains compared to Cobas AmpliCor. This can presumably be explained by the fact that

the target in LightMix 480HT, *omp1*, is a single copy gene, while the target in Cobas Amplicor is the plasmid, which is present in up to 10 copies per bacterial cell. Moreover, optimised and quality assured culture of *C. trachomatis* remains fairly effective and valuable for the diagnosis of the present or potential other mutants, for NAAT inhibited or extra-genital samples, for antibiotic resistance testing if needed in the future, and for research purposes.

The origin of this nvCT is unknown. Extraordinarily, it has so far only been detected in Sweden and very recently in the neighbouring countries Norway (two cases) [5] and Finland [6]. Studies performed in Denmark [JS Jensen, personal communication], the Netherlands [7], and Ireland [8] did not detect the nvCT. Recently, a study was initiated that is aimed at identifying the diagnostic assays currently used, the presence of the mutant, recommendations for laboratories, and actions undertaken in different areas of Europe [9]. The importance of regular local, national, and international surveillance for possible undetected strains needs to be highly emphasised. In particular, because strains like the nvCT, may already be in a stage of early transmission in many countries. These strains have a selective advantage over wild-type strains and can be transmitted more rapidly if they are not being detected and/or eradicated by treatment. This may explain why the Swedish counties that are mainly using the Roche or Abbott systems are the ones with the highest proportion of the nvCT, as these tests may have resulted in an accumulation of undetected and untreated cases that have escaped the mandatory contact tracing. However, in Sweden and many other countries, symptomatic patients are generally treated independently of the test results. It is also important that any unusual decrease in the *C. trachomatis* incidence in different populations and geographic areas is investigated. In addition, regular and more comprehensive evaluation of different diagnostic methods is crucial for maintaining diagnostic quality. The samples included in such an evaluation should reflect not only currently transmitted strains, but also temporally, geographically

and genetically diverse strains. Furthermore, diagnostic assays with a high sensitivity and specificity need to be established and provided, which target at least two different genetic sequences, e.g. a plasmid sequence and a chromosomal sequence such as the *omp1* gene, or at least two divergent assays based on different targets or principles. This is evident for *C. trachomatis*, and could also be considered for other infectious agents.

#### References

1. Ripa T, Nilsson P. A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid: implications for use of PCR diagnostic tests. *Euro Surveill* 2006; 11(11). Available from: <http://www.eurosurveillance.org/ew/2006/061109.asp#2>
2. Söderblom T, Blaxhult A, Fredlund H, Hermann B. Impact of a genetic variant of *Chlamydia trachomatis* on national detection rates in Sweden. *Euro Surveill* 2006; 11(12). Available from: <http://www.eurosurveillance.org/ew/2006/061207.asp#1>
3. Klint M, FuxeLius HH, Goldkuhl RR, Skarin H, RuteMark C, Andersson SG, Persson K, Herrmann B. High-resolution genotyping of *Chlamydia trachomatis* strains by multilocus sequence analysis. *J Clin Microbiol* 2007 Feb 28; [Epub ahead of print].
4. Jurstrand M, Falk L, Fredlund H, Lindberg M, Olcén P, Andersson S, Persson K, Albert J, Bäckman A. Characterization of *Chlamydia trachomatis omp1* genotypes among sexually transmitted disease patients in Sweden. *J Clin Microbiol* 2001; 39:3915-9.
5. Moghaddam A, Reinton N. Identification of the Swedish *Chlamydia trachomatis* variant among patients attending a STI clinic in Oslo, Norway. *Euro Surveill* 2007; 12(3). Available from: <http://www.eurosurveillance.org/ew/2007/070301.asp#3>
6. European Surveillance of Sexually Transmitted Infections (ESSTI). Update on the ESSTI/ECDC survey "New variant of *Chlamydia trachomatis* restricted to Sweden?". ESSTI Newsletter No. 4. Available from: <http://www.essti.org/>
7. de Vries HJC, Catsburg A, van der Helm JJ, BeukeLaar EC, Morré SA, Fennema JSA, Thiesbrummel H. No indication of Swedish *Chlamydia trachomatis* variant among STI clinic visitors in Amsterdam. *Euro Surveill* 2007; 12(2). Available from: <http://www.eurosurveillance.org/ew/2007/070208.asp#3>
8. Lynagh Y, Walsh A, Crowley B. Investigation to determine if newly-discovered variant of *Chlamydia trachomatis* is present in Ireland. *Euro Surveill* 2007; 12(2). Available from: <http://www.eurosurveillance.org/ew/2007/070201.asp#2>
9. van de Laar M, Ison C. Europe-wide investigation to assess the presence of new variant of *Chlamydia trachomatis* in Europe. *Euro Surveill* 2007; 12(2). Available from: <http://www.eurosurveillance.org/ew/2007/070208.asp#4>

Citation style for this article: Unemo M, Olcén P, Agné-Statling I, Feldt A, Jurstrand M, Herrmann B, and al. Experiences with the new genetic variant of *Chlamydia trachomatis* in Örebro county, Sweden – proportion, characteristics and effective diagnostic solution in an emergent situation. *Euro Surveill* 2007;12(4) [Epub ahead of print]. Available online: <http://www.eurosurveillance.org/em/v12n04/1204-223.asp>