Prevalence trends in Sweden for the new variant of *Chlamydia trachomatis*

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Abstract

In 2006, a new variant of *Chlamydia trachomatis* (nvCT) was discovered in Sweden that was not detectable with Abbott m2000 (Abbott) and Amplicor/COBAS Amplicor/TaqMan48 (Roche). The proportion of nvCT was 20–64% of the detected *Chlamydia* cases in counties using Abbott/Roche test systems. Although the ProbeTec system from Becton Dickinson (BD) could detect nvCT, the proportion of nvCT in counties using BD was 7–19%. The objective of the current study was to follow the nvCT proportions from 2007 to 2009 in two counties that used Roche and had introduced test systems able to detect nvCT in late 2006. The nvCT was also followed in two counties that used BD, and in all four counties the effect of nvCT on the serotype distribution of *C. trachomatis* wild-type strains was analysed. A total of 2576 specimens positive for *C. trachomatis* were collected in the four counties at three time points, and analysed for nvCT and serotype E. The proportion of nvCT declined significantly in the two counties using Roche, from 65% and 48% in 2007 to 24% for both counties in 2009 ($p < 0.001$). The nvCT proportion increased in Norrbotten county, which used BD, from 9% in 2007 to 19% in 2009 (p 0.03). In Uppsala county, which also used BD but was surrounded by counties using detection systems from Roche, the proportion of nvCT declined from 24% in 2007 to 18% in 2009 ($p < 0.03$). No major difference in the level of serotype E was seen. The proportion of nvCT seems to rapidly converge in the Swedish counties after the selective diagnostic advantage for nvCT has been lost in the Abbott/Roche counties.

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Introduction

A new variant of *Chlamydia trachomatis* (nvCT) was discovered in Sweden in 2006 that had a 377-bp deletion in the cryptic plasmid [1]. The deleted area included the target sequence used by nucleic acid amplification tests (NAATs) for *C. trachomatis* manufactured by two commercial companies, Abbott m2000 (Abbott) and Amplicor/COBAS Amplicor/TaqMan48 (Roche). This deletion resulted in several thousand false-negative results, and the proportion of nvCT recorded was between 20% and 64% in counties using test systems that were unable to detect nvCT in 2006 and early 2007 [2]. The third NAAT used in Sweden, apart from Abbott/Roche, was the ProbeTec system from Becton Dickinson (BD). This system has always been able to detect nvCT, because another target region on the cryptic plasmid is used. The proportion of nvCT in counties using BD was between 7% and 19% [2] during the same time period.

The major aim of this study was to investigate the prevalence of nvCT in two counties that were unable to detect this variant in 2006 (Örebro and Dalarna, using Roche) and follow the proportions of nvCT after the introduction of test systems able to detect nvCT in 2007. The proportion of nvCT in two counties with effective detection systems even

before 2006 (Uppsala and Norrbotten, using BD) was also determined.

C. trachomatis strains are divided into 19 serovars on the basis of the major outer membrane protein. The nvCT is serotype E, which is the dominant serotype in Sweden among wild-type *C. trachomatis* (wtCT) [3–5]. A second aim of the present study was to investigate whether the reduction of wtCT seen in some counties using Abbott/Roche in 2006 [6] had an effect on the serotype distribution among wtCT. The proportion of serotype E among wtCT was determined and compared between counties with high or low proportions of nvCT.

Materials and Methods

Specimens

Between 115 and 278 consecutive *C. trachomatis*-positive specimens (one per patient) were collected for each county and time-point. In total, 2576 specimens were analysed, and 78 of these were untypeable (Dalarna, $n = 16$; Norrbotten, $n = 24$; Uppsala, $n = 15$; Örebro, $n = 23$) and excluded from analysis. The time periods for specimen collection are specified in Table 1. Only urine samples were collected from Norrbotten at the first time-point in 2007. Additional data on gender, age and clinical setting of diagnoses were collected from medical records.

DNA extraction

In Dalarna and Örebro, the MagNa Pure (Roche) extraction robot was used for specimens analysed in 2007 and 2008. In 2009, the Bullet Bugs'n Beads kit on the robot NorDiag Bullet (NorDiag ASA) was used. DNA from urine specimens collected in Norrbotten and Uppsala was extracted with the M48 biorobot (Qiagen). Lysate from swab samples analysed with BD was diluted ten times before PCR, and if negative in PCR, extracted with the M48 biorobot (Qiagen).

PCR

A duplex PCR that simultaneously detected the deletion on the cryptic plasmid and the *ompA* gene of serotype E was developed. A primer pair from Ripa *et al.* [7] amplified the region of the deletion in the cryptic plasmid. Amplification of nvCT resulted in a 161-bp fragment, and specimens without the deletion had a PCR product of 538 bp.

The serotype E specimens were identified by the primers CtrEF (5′-TCAAAGCACGGTCAAAACGAATT-3′) and CtrER2 (5′-CATCAGTTCCTGCTATGAGTG-3′), targeting *ompA* and amplifying a PCR product of 278 bp. The reaction mixture contained 0.4 μM each primer, 0.2 mM dNTP, 2 mM $MgCl_2$ and 0.75 U of HotStarTaq DNA Polymerase (Qiagen) in a 25-μL reaction mixture. The temperature profile was as follows: initial denaturation for 15 min at 94°C, followed by 40 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 55°C and elongation for 90 s at 72°C. The amplification was terminated with elongation for 7 min at 72°C. The PCR products were separated on a 1% agarose gel stained with ethidium bromide. Samples with two bands of 161 bp and 278 bp were interpreted as nvCT. Samples with only a 538-bp band were interpreted as non-serotype E wtCT, and samples with the 538-bp and an additional 278-bp band were interpreted as wtCT serotype E. Specimens were extracted again if the bands were absent, weak or aberrant. If they displayed absent, weak or aberrant bands after a second analysis, the specimens were classified as untypeable and excluded from study.

A subset of 13 specimens, eight from Uppsala and five from Örebro, was analysed with multilocus sequence typing (MLST) [8].

Reported cases of *C. trachomatis*

Information about the reported number of *C. trachomatis* cases and the number of tests performed was obtained from the Swedish Institute for Infectious Disease Control (SMI, http://www.smittskyddsinstitutet.se, July 2009). The incidence was defined as number of cases from February to June per

TABLE 1. Time-points for specimen collection in the four counties

County (diagnostic system)	Time-points		
	2007	2008	2009
Dalarna (Roche)	25 Jan. to 23 Feb. ($n = 204$)	22 Jan. to 24 Mar. ($n = 204$)	2 Feb. to 2 Apr. ($n = 172$)
Norrbotten (BD)	28 Nov. (2006) to 9 Feb. ($n = 115$)	1 Feb. to 21 May ($n = 242$)	2 Feb. to 2 Apr. ($n = 190$)
Uppsala (BD)	14 Nov. (2006) to 14 Feb. ($n = 260$)	21 Jan. to 27 Mar. ($n = 234$)	2 Feb. to 9 Apr. ($n = 217$)
Örebro (Roche)	1 Feb. to 29 Apr. ($n = 227$)	1 Feb. to 30 Apr. ($n = 278$)	1 Feb. to 30 Apr. ($n = 233$)

BD, Becton Dickinson.