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EPIDEMIOLOGY OF CHLAMYDIA TRACHOMATIS INFECTION IN FINLAND DURING 1983–2009
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EPIDEMIOLOGY OF CHLAMYDIA TRACHOMATIS INFECTION IN FINLAND DURING 1983–2009

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Abstract

Chlamydia trachomatis epidemic continues at a slowly, albeit steadily increasing rate in the Western world despite health education, easy/user-friendly diagnostic measures, and effective treatment. In Finland, 8,031 and 13,227 C. trachomatis infections were reported in 1995 and 2012, respectively. Over half of the Chlamydia cases were diagnosed among young women, who suffer from the Chlamydia-related complications such as infertility many years after initial infection. The rates of all but first of the following major Chlamydia-related complications: cervical intraepithelial neoplasia, ectopic pregnancy, hospitalized pelvic inflammatory disease, tubal factor infertility have, however, decreased since the 1990s.

The aim of this study was to clarify the discordance between the apparently increasing incidence of C. trachomatis and decreasing C. trachomatis IgG antibody rates (seroprevalence).

The study material consisted of a random subsample of first trimester serum samples of 7,999 women from the population-based Finnish Maternity Cohort (FMC) registry from 1983 to 2005, and 147,148 women and men with a total of 177,138 C. trachomatis genital infections reported to the Finnish National Infectious Diseases Registry (NIDR) during 1995–2009. Both registries are maintained by the National Institute for Health and Welfare (THL).

Serum IgG antibodies were measured by a C. trachomatis major outer membrane protein-specific peptide enzyme immunoassay (EIA) and the standard micro-immunofluorescence (MIF) method. We found that while C. trachomatis seroprevalences decreased >50% among fertile-aged women the seroconversion rates (seroincidences) were comparable to the NIDR reported rates.

The numbers of annual repeated C. trachomatis infection in the NIDR increased until 2009 by 49% in women and 39% in men. In 2009, about 25% of the females and 20% of the males had had an earlier C. trachomatis infection. During the whole follow-up time, 34% of all the repeat diagnoses occurred within 12 months. Most of the first infections were observed among females and males under 25 years of age, but the numbers of repeated chlamydial infections increased up to the age of 30 years.

The C. trachomatis serotype distribution changed between the 1980s and 1990s, but the leading 1980 serotypes bounced back by 2005. The numbers of women with multiple serotype infections peaked in the 1990s, and serotypes G and J were temporarily replaced by serotypes E and D.

In conclusion, the serological observations fit the polymerase chain reaction (PCR)-based data on C. trachomatis epidemiology. The observed increases in the repeated chlamydial infections among young women and men comply with increasing sexual risk-taking behaviour in Finland. Our observations help to understand the discrepancy between C. trachomatis occurrence and sequelae rates as the overall C. trachomatis infection burden in the population may be decreasing despite the increasing incidence trend.

Keywords: C. trachomatis, epidemiology, repeated infection, serology, serotype


Klamydia IgG vasta-aineet määritettiin entyymi-immunologisesti (EIA) sekä mikroimmunofluoresenssinenketjumuunnoksella (MIF) verinäytteistä. Keskeisimmäin tuloksina havaittiin klamydian serorevalenssin lasku > 50 % hedelmällisessä iässä olevilla naisilla, vaikka infektion ilmennyt vuosina serologiassa aineistossa oli samaan aikaan samankaltainen kuin tartuntatautirekisterissä. Lisäksi havaitsimme, että vuositasolla toistuvien klamydiainfektioiden määrä on tartuntatautirekisteristä perustelkaan lisääntynyt tutkimusaikana naisilla 49 % ja miehillä 39 %. Vuonna 2009 neljännes naisten ja viidenne miesten klamydiainfektioiden määrä oli uusintainfektioiden koko seuran-aikana 34 %, mutta toistuvia infektioiden lukumäärä oli korkeimmilla 1990-luvulla, jolloin serotyyppi G ja J korvasivat serotyyppi E ja D brought to you by the Finnish Maternity Cohort (FMC) for the years 1983-2005, and to the national register of notifications from 1995 to 2009. The cases of chlamydia infection were 147,148 in women and 177,138 in men, respectively. Both registers are maintained by the National Institute for Health and Welfare (THL)

Klamydia IgG antibodies were measured enzymatically (EIA) and microimmunofluorescence flowmetry (MIF) from blood samples. The key findings were that the prevalence of chlamydia seroreversion was increased > 50% in women aged > 50 years, although the seroprevalence in the population was the same as in the national notification register. In addition, we found that the incidence of recurrent infections was increasing from year to year in women 49% and men 39%. In 2009, 4 out of 10 women and 5 out of 10 men had recurrent chlamydia infections, but the highest number of cases occurred in the 1990s, when serotypes G and J replaced serotypes E and D.

In summary, our findings are consistent with the findings of polymerase chain reaction (PCR) tests for chlamydia infection. The incidence of recurrent infections has increased from year to year in women 49% and men 39%. In 2009, 4 out of 10 women and 5 out of 10 men had recurrent chlamydia infections, but the highest number of cases occurred in the 1990s, when serotypes G and J replaced serotypes E and D.

Asiasanat: C. trachomatis, epidemiologia, serologia, serotyyppi, toistuva infektio
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Kempele, April 2013

Erika Wikström
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CIN</td>
<td>Cervical intraepithelial neoplasia</td>
</tr>
<tr>
<td>DFA</td>
<td>Direct fluorescence assay</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EB</td>
<td>Elementary body</td>
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<tr>
<td>EIA</td>
<td>Enzyme immunoassay</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunoassay</td>
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<tr>
<td>EP</td>
<td>Ectopic pregnancy</td>
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<tr>
<td>FMC</td>
<td>Finnish Maternity Cohort</td>
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<tr>
<td>HPV</td>
<td>Human papillomavirus</td>
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<tr>
<td>HSP</td>
<td>Heat shock protein</td>
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<tr>
<td>HSP60</td>
<td>Heat shock protein 60 kDa</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon-gamma</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LCR</td>
<td>Ligase chain reaction</td>
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<tr>
<td>LGV</td>
<td>Lymphogranuloma venereum</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MIF</td>
<td>Microimmunofluorescence</td>
</tr>
<tr>
<td>MSM</td>
<td>Men who have sex with men</td>
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<tr>
<td>MOMP</td>
<td>Major outer membrane protein</td>
</tr>
<tr>
<td>NAAT</td>
<td>Nucleic acid amplification test</td>
</tr>
<tr>
<td>NGU</td>
<td>Non-gonococcal urethritis</td>
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<tr>
<td>NIDR</td>
<td>National Infectious Diseases Register</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PID</td>
<td>Pelvic inflammatory disease</td>
</tr>
<tr>
<td>PROM</td>
<td>Premature rupture of membranes</td>
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<tr>
<td>PTD</td>
<td>Preterm delivery</td>
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<tr>
<td>PTL</td>
<td>Preterm labour</td>
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<tr>
<td>RB</td>
<td>Reticulate body</td>
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<tr>
<td>STI</td>
<td>Sexually transmitted infection</td>
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<tr>
<td>TFI</td>
<td>Tubal factor infertility</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-alpha</td>
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List of original publications

This thesis is based on the following publications, which are referred to by their Roman numerals.


Wikström née Lyytikäinen
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1 Introduction

Wherever humans live, love and reproduce, sexually transmitted infections (STIs), are present. The World Health Organization (WHO) estimates that 448 million new cases of syphilis, gonorrhoea, chlamydial infection and trichomoniasis occur annually and a total of 34 million people are infected with human immunodeficiency virus (HIV) (WHO/UNAIDS 2011). Human papillomavirus (HPV) is the most common STI, aetiological agent of genital warts and cervical intraepithelial neoplasia (CIN) (CDC 2010, WHO 2011). HPV is a necessary cause for cervical cancer, which is the third most common cancer in women worldwide with an estimated 530,000 new cases and 275,000 deaths in 2008 (IARC 2010). Chlamydial genital infection is the most common reported bacterial infection worldwide, with approximately 92 million new cases annually (WHO 1999). The disease burden is highest in sub-Saharan Africa but C. trachomatis is a major cause of preventable blindness worldwide (reviewed by Burton & Mabey 2009).

Asymptomatic C. trachomatis infection is regarded as being very common (Lan et al. 1995, Stamm 1999) and hence the infection often remains undetected and untreated. This has contributed to the spread of a silent C. trachomatis epidemic and underreporting of chlamydial cases furthermore. C. trachomatis morbidity and the economic burden have remained high despite increasing national surveillance programmes and screening (Tuite et al. 2012).

The spectrum of C. trachomatis-related diseases is wide. Women and girls are at a high risk of C. trachomatis complications including endometritis, salpingitis and pelvic inflammatory disease (PID), with associated long-term sequelae such as pelvic pain, ectopic pregnancy, preterm birth and infertility (Paavonen & Eggert-Kruse 1999). Clinical presentation and symptoms associated with untreated Chlamydia infection often appear only years after the initial infection. C. trachomatis infection is also associated with an increased risk of cervical neoplasia (Koskela et al. 2000, Anttila et al. 2001, Lehtinen et al. 2011). Beside non-gonococcal urethritis and accessory gland infection, chlamydial infection is also related to male infertility (Joki-Korpela et al. 2009). Protective immunity to C. trachomatis is considered to be short-lived (Brunham et al. 2005) and repeat genital Chlamydia infection is common (reviewed by Hosenfeld et al. 2009, Batteiger et al. 2010a). Chlamydial incidence is at its highest in the age group of less than 25 years (Hiltunen-Back et al. 2003, Jolly et al. 2005) and gradually decreases with increasing age (Arno et al. 1994, reviewed by Batteiger et al. 2010a).
2010b). Genital infection with *C. trachomatis* has also been shown to be a cofactor in the transmission of HIV in both men and women (reviewed by Fleming & Wasserheit 1999, reviewed by Johnson & Lewis 2008).

The importance of *C. trachomatis* surveillance was documented in Sweden, where Ripa and Nilsson noticed an unexpected 25% decrease in the prevalence of *C. trachomatis* due to a new *C. trachomatis* variant which was not detected by all nucleic acid amplification tests (NAATs) (Ripa & Nilsson 2006). Regardless of increasing chlamydial rates related long-term morbidity is in decline recently (van Valkengoed *et al.* 2004, Low N *et al.* 2006, Paavonen 2012). The reasons for this have remained unclear.

In the present study, our aim was to find an explanation for the conflicting observation of increasing chlamydial case rates and decreasing *C. trachomatis* IgG antibody rates (seroprevalence). Chlamydial serology was studied among healthy Finnish women by measuring serum *C. trachomatis* IgG antibody levels by enzyme immunoassay (EIA). Serological data was compared with reported *C. trachomatis* incidence rate data. The impact of the repeat *C. trachomatis* infections on the increasing chlamydial occurrence was studied in the National Infectious Diseases Register (NIDR).
2 Review of the literature

2.1 General aspects of *Chlamydia trachomatis*

*Chlamydia trachomatis* is a Gram-negative obligate intracellular bacterium that was isolated for the first time in 1959 from the female genital tract (Jones *et al.* 1959). It is surrounded by a rigid cell wall of lipopolysaccharides (LPSs). Owing to its incapacity to synthesize ATP, it is dependent on the host cell. Reinfection with *C. trachomatis* is relatively common, as immunity to the bacterium is limited and serotype-specific (reviewed by Witkin 2002, Brunham 2005).

2.1.1 Growth cycle

The unique chlamydial growth cycle is biphasic and lasts about 48–72 h (Stamm 1999). The extracellular, infectious form (0.3 µm) is called the elementary body (EB) and the intracellular, replicating form (1.0 µm) the reticulate body (RB). *C. trachomatis* infects the epithelial cells of the genitourinary tract, rectum and conjunctiva in both women and men (Paavonen & Eggert-Kruse 1999). Elementary bodies are attached to the susceptible host cell membrane and taken into the cell by phagocytosis. When inside the cell, EBs prevent the fusion of phagosome with lysozyme by remaining in intracellular vacuoles (chlamydial inclusion) (Fig. 1), and transform into RBs, which begin to multiply by binary fission. At the end of the cycle, the RBs transform back to EBs, which are released to the cytoplasm by exocytosis or host cell lysis (Abdelrahman & Belland 2005). In certain circumstances (iron deprivation, nutrient starvation, concomitant DNA-virus infection, administration of antibiotics), the *C. trachomatis* growth cycle may be disturbed, resulting in morphologically altered RBs which may remain in host cells for long periods of time, providing a focus for persistent infection (Beatty *et al.* 1994, reviewed by Wyrick 2010).
Typing of *C. trachomatis* is an important tool for revealing transmission within sexual networks and defining clinical manifestations and pathogenicity (Pedersen *et al.* 2009). *C. trachomatis* strains are historically classified into 15 serotypes based on serologic differences elicited by variable segments of the chlamydial major outer membrane protein (MOMP) (Wang & Grayston 1970, Wang & Greyston 1975, Banks & Schachter 1978). Subsequent isolation of serovariant strains coupled with additional MOMP gene (*ompA*) sequence data has expanded the *ompA*-based classification of chlamydial strains (Millman *et al.* 2004). There are now >20 different *C. trachomatis* serotypes, of which 11 are predominantly isolated from the urogenital tract, including serotypes D, Da, E, F, G, Ga, H, I, Ia, J and K (Morre *et al.* 2000, Millman *et al.* 2004, Mossman 2008). Serotypes A–C cause trachoma and serotypes L1–L3 cause lymphogranuloma venereum (LGV).
infection (Wang et al. 1985, Morre et al. 1998, Dean et al. 2000). Nowadays serotypes are determined by using the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method or by sequencing the gene (ompA) that encodes chlamydial MOMP (Morre 1998).

The most prevalent C. trachomatis serotypes are D, E and F, accounting for approximately 70% of the typed urogenital serotypes (Frost et al. 1993, van der Laar et al. 1996, Morre et al. 2000). This distribution of serovars, with only small variations, has been found in several studies in heterosexual populations all over the world (Borrego et al. 1997, Jurstrand et al. 2001, Jonsdottir et al. 2003, Singh et al. 2003, Suchland et al. 2003, Ngandjio et al. 2004, Yamazaki et al. 2005, Gao et al. 2007, Takahashi et al. 2007). However, in men having sex with men (MSM), the predominant serotypes are G, D and J (Geisler et al. 2002, Lister et al. 2004, Klint et al. 2006). The serotype distribution in Finland was studied in 1987. The highest prevalence was reported for the C. trachomatis BED group, followed by the GF group (Saikku & Wang 1987).

Correspondence of the clinical signs and symptoms related to the Chlamydia serotypes has been controversial. In some studies, serotypes D and F have been associated with asymptomatic infection (Workowsky et al. 1994, Lan et al. 1995), while serotype G has been linked to symptomatic infection (Lan et al. 1995). Van Duynhoven et al. (1998), in a study including 175 men and 135 women attending an STD clinic, reported a correlation between urethral discharge in men and serotypes H and J, and in women lower abdominal pain was predominantly associated with serotypes F and G. More reports on other associations have been published (Batteiger et al. 1989, Dean et al. 1995, van de Laar et al. 1996, Sylvan et al. 2002, Gao et al. 2007). Several larger studies have revealed no association between serotypes and clinical manifestations (Persson & Osser, 1993, Stothard et al. 1998, Geisler et al. 2003, Ngandjio et al. 2004, Lysen et al. 2004, Millman et al. 2006). C. trachomatis serotype G, which is one of the most prevalent serotypes in Finland, has been associated with subsequent development of cervical squamous cell carcinoma (SCC) (Anttila et al. 2001).

A new variant C. trachomatis (nvCT) strain was discovered in Halmstad, Sweden, in 2006 (Ripa & Nilsson 2006). This strain is characterized by a 377-bp deletion in the cryptic plasmid, and because the primers for some of the commercial detection systems contained this deletion area, the strain was undetectable. This strain of serotype E was spread from a single clone (Herrmann et al. 2008, Pedersen et al. 2008).
2.1.3 Natural history of genital C. trachomatis infection

Most C. trachomatis patients have a mild disease and at the time of diagnosis they show no clinical complications. Factors associated with Chlamydia, the duration and resolution of untreated, uncomplicated genital chlamydial infection, are not fully understood (reviewed by Geisler 2010). Both host and microbial factors influence susceptibility, clinical outcome and resolution of Chlamydia infection in an individual and improved understanding of these factors would be important (Geisler et al. 2013).

The transmission probability of C. trachomatis infection is considered to be relatively high. Depending on the study setting, the transmission probability varies from 10% in asymptomatic individuals to as high as 65% in representatives of high-risk population (Lyeke et al. 1980, Quinn et al. 1996, Lin et al. 1998, van Valkengoed et al. 2002, Knauper & Kornik 2004, Rogers et al. 2008). Male-female and female-male transmission frequencies in sexual partnerships were equal (Quinn et al. 1996, Rogers et al. 2008).

Ethical considerations represent a major challenge in studying the natural history of untreated Chlamydia. Most of the studies are prospective or retrospective studies with short follow-up intervals between initial screening and returning for treatment, or they concern another infection with follow-up times of a few weeks or several months (Parks et al. 1997, van Valkengoed et al. 2002, Geisler et al. 2008). Spontaneous resolution rates in these PCR-based studies have been 11–44% in women. Three studies have concerned the natural history of untreated, uncomplicated genital C. trachomatis infection with follow-up times longer than 1 year and about half of the cases cleared the infection in one year (McCormack et al. 1979, Morre et al. 2002b), and clearance increased over time (Molano et al. 2005). Estimates of infection duration in these studies were inaccurate and as the study populations comprised mostly high-risk patients the results are not directly generalizable. In males, rates for spontaneous resolution rates have been both lower and higher compared to females, but studies exist with only short follow-up periods (van Valkengoed et al. 2002, Geisler et al. 2008, Rogers et al. 2008).

The highly immunogenic ompA protein is the most widely studied biological attribute that has been linked to resolution of uncomplicated genital chlamydial infection. Molano et al. (2005) showed that women infected with serogroup B and C ompA types had a longer duration of genital chlamydial infection. Morre et al. (2002a) reported that women with C. trachomatis ompA type E were more likely
to have persistent infection after 1 year of follow-up than those infected with other genotypes. Geisler et al. (2008) demonstrated that individuals infected with genotype J/Ja more often cleared the infection.

Different immunological responses to different serogroups have been found and according to one study, the most prevalent serogroup BED induced the highest antibody response compared with less prevalent groups (Verweij et al. 2009). It is not known why serotypes E and D are the most prevalent in chlamydial genital tract infection (Byrne 2010). Serotype E demonstrates the least genetic and serospecific variability for ompA concerning immune selection (Baehr et al. 1988). One possible explanation might be that serovars E and D are less immunogenic than other serovars and therefore remain undetected (Byrne 2010).

2.1.4 Pathogenesis of chlamydial infections

The outcome of chlamydial infection is influenced by the interaction of pro- and anti-inflammatory cytokines present at the site of infection. Two cytokines, IFN-γ, and interleukin-10 (IL-10), are especially important for immunity to chlamydial infections. In the murine system, a CD4+ T-cell-mediated immune response alone is enough to resolve primary chlamydial infection and to provide immunity against reinfection. Protection is mainly mediated through the action of IFN-γ (Rank et al. 1992, Igietseme et al. 1993, Gondek et al. 2009). Down-regulation of the Th1 (IFN-γ) response at the site of the inflammation during infection may lead to prolonged infection and inflammation (Yang et al. 1996, Öhman et al. 2006).

There is evidence in rodents that prolonged chlamydial infection is mediated by IL-10 (Igietseme et al. 2000). This is due to the ability of IL-10 to down-regulate the expression of Th1-type cytokines. It is assumed that the chronic inflammatory response to Chlamydia is modulated by the immune system with experimental evidence suggesting that the bacterial heat-shock proteins (HSPs), in particular HSP 60-kDa, cross-reacting with human HSP (hHSP) are an important factor in the immunopathogenesis of female genital inflammation (Paavonen & Lehtinen 1996, Stamm 1999, Kinnunen et al. 2001, Tiitinen et al. 2006). The specific balance between pro-inflammatory (TNF-α, IFN-γ) and anti-inflammatory (IL-10) cytokines at the site of inflammation may further influence the pathogenetic mechanisms leading to tissue damage (Öhman et al. 2009). The
aetiopathogenesis of TFI is related to an impaired cell-mediated immune (CMI) response (reviewed by Loomis & Starnbach 2002, Öhman et al. 2011).

There is evidence that inter-individual variation in disease manifestation and the intensity and nature of the immune response is affected by the genetic background of the host, for example HLA class DQ alleles, and IL-10 polymorphism (Kinnunen et al. 2002, Morre et al. 2009, Öhman et al. 2011).

2.2 Epidemiology of C. trachomatis infection

Chlamydial genital infection is the most common reported sexually transmitted bacterial infection (STI). The estimated number of annual new cases worldwide was 92 million in 1999 (WHO 1999). In the USA, about 1.4 million cases of chlamydia were reported in 2011, but an estimated 2.86 million infections occur annually (CDC 2013).

Genital chlamydial infections are common among adolescents and are associated with sexual risk-taking behaviour including multiple or frequently changing partners and failure to use condoms (Hiltunen-Back et al. 2001, Cassell et al. 2006b). Other risk factors include ethnicity, delay in seeking medical care, failure to comply with therapy, failure to inform and treat partners, history of previous STIs, and oral contraceptive use (Gaydos et al. 1998, Hiltunen-Back et al. 2003, Niccolai et al. 2011). In pregnant women, the risk factors appear to be similar to those of non-pregnant women (Shaw et al. 1995, Chokephaibulkit et al. 1997). Women have a 3.5-fold greater risk of being diagnosed with C. trachomatis infection than men in the USA (Miller et al. 2004). In Finland, women have 60% of the C. trachomatis infections reported in the NIDR (Hiltunen-Back et al. 2003) According to one extreme study from USA, by the age of 15, 25% of young urban females had already acquired their first STI, most often C. trachomatis infection, and the time from first sexual intercourse to first acquired STI was less than a year (Tu 2009).

In a systematic review reporting on chlamydial infection among asymptomatic European women, the prevalence ranged from 1.7% to 17% (Wilson et al. 2002). A study conducted by the US Army revealed a prevalence of 9.2% when 13 204 asymptomatic women were screened for C. trachomatis (Gaydos et al. 1998). Recently, large population-based prevalence studies have been reported in several countries. For example, in an HPV vaccination efficacy study, 40 000 15–26-year-old females from 14 countries were enrolled and the baseline chlamydial prevalence was 4% (Paavonen et al. 2008).
Most developed countries base their surveillance of genital *C. trachomatis* infection on the tracking of case reports, often after making *Chlamydia* a legally reportable disease (Rekart & Brunham 2008). Systematic data on the incidence of *C. trachomatis* infection has been available in all Nordic countries since the late 1980s. In the early 1990s there was a decline in *C. trachomatis* incidence but since 1994 an increase has occurred in all the Nordic countries (Moi 2001). In Denmark, Finland, Norway and Sweden, the incidence rates were 262, 207, 296 and 189 cases per 100 000 in 1999 compared with 472, 254, 458 and 396 in 2011 (Denmark rates in 2007) (WHO 2012, data.euro.who.int). Whereas the Nordic countries (Fenton & Lowndes 2004) and Canada (Brunham et al. 2005) have witnessed the downward-upward pattern in chlamydial infection rates, steady increases of chlamydial rates have occurred in the USA, the UK and Australia throughout the last decade (www.cdc.gov/std/Chlamydia2005/CTSurvSupp2005Short.pdf, www.hpa.org.uk, www9.health.gov.au).

Repeat chlamydial infections are relatively common (Veldhuijzen et al. 2005, reviewed by Guy et al. 2012a). The repeat infection rate following treatment in women in US cohorts was 15.5% at 6 months (Hosenfeld et al. 2009) and it was 22% at 12 months in an Australian study (Regan et al. 2009). In men, corresponding repeat *Chlamydia* infection rate was 10.9% at 4 months with follow-up rate of 62.4% in developed countries (a meta-analysis) (Fung et al. 2007). A longitudinal cohort study of young women showed that a new chlamydial infection was more common among women who had prevalent infection at baseline compared with baseline-negative women (78.1% vs. 51.7%, p < 0.001) and about 32% of the participants had one or more repeat infections (Batteiger et al. 2010a).

In Finland, national incidence data on *C. trachomatis* infection has been collected since 1987 by National Institute for Health and Welfare (THL). The numbers of reported cases were 13 796 in 1987 and exceeded 15 000 next year, thereafter a decrease of the reported cases was observed until 1995 (Hiltunen-Back et al. 2003). *C. trachomatis* rates have risen by 60% since 1995 (13 226 cases in 2012) and most of the reported cases were among 15–24-year-old females (www.thl.fi). As in Sweden, the source of the infections has been mainly endemic (Christenson & Sylvan 2011), and only about 10% have been imported (Hiltunen-Back et al. 2001). A sentinel STD network was established in 1995 by THL (Hiltunen-Back et al. 2001). Initially this network consisted of six STD outpatient clinics, three health care centres, two university student health care units and two university hospital departments of gynaecology. Besides testing
STIs, information about partner status, history of previous STIs and use of contraceptives are collected.

A new genetic variant of *C. trachomatis* was discovered in Sweden in 2006 with a 377 base-pair deletion in the plasmid DNA commonly used at nucleic acid amplification tests (NAATs) and it was therefore not detected (Ripa & Nilsson 2006). Investigators found a decreasing number of *Chlamydia* cases in the southern part of Sweden and pointed out that the test did not detect all the cases as a result of the targeting method. So far the new variant has only occasionally been detected outside Sweden and other Nordic countries (de Barbeyrac et al. 2007, Morre et al. 2007, Reischl et al. 2009). In the Gothenburg area, Sweden, 17% of positive specimens contained the new variant in 2009 (Lagergård et al. 2010). The clinical manifestations seem not to differ from those associated with the traditional types (Bjartling et al. 2009). A recent study revealed that despite close proximity to Sweden, the Swedish variant is rare (0.4%) in Finland (Niemi et al. 2011).

### 2.3 Sexually transmitted *C. trachomatis* infections

*C. trachomatis* causes a wide spectrum of genitourinary diseases. In most cases, up to 80%, the infections are asymptomatic (Rahm et al. 1988) but in symptomatic patients the incubation time is usually 7–21 days (reviewed by Manavi 2006).

#### 2.3.1 Genitourinary infections in women

The uterine cervix is the main site of chlamydial infection, but the urethra and rectum may also be involved (Stamm et al. 1980, Paavonen et al. 1982). Urethritis is common, symptoms including increased frequency and dysuria (Stamm et al. 1980, Paavonen & Vesterinen 1982). A culture-negative leucocyturia finding is suggestive of *C. trachomatis* infection (reviewed by Bebear & de Barbeyrac 2009). Since in the cervix columnar cells are infected, the disease presents as endocervitis (Stamm 1999, reviewed by Manavi 2006). Infections are more likely in women with cervical ectopy, since the susceptible cells are more exposed (Faro 1991). In gynaecological examination the cervix may show contact bleeding along with the mucopurulent discharge (Brunham et al. 1984, Paavonen & Eggert-Kruse 1999).
Thirty to fifty per cent of untreated patients with cervicitis also develop endometritis with irregular bleeding (Paavonen et al. 1988, Paukku et al. 1999a, Wiesenfeld et al. 2002). Clinically the patients have abdominal pain, increased menstruation and irregular bleeding. Pelvic inflammatory disease (PID), as one of the key clinical manifestations of chlamydial upper genital tract infections, was described in the 1970s (Mardh et al. 1977). Later studies revealed up to two thirds of PID cases to be associated with C. trachomatis infection (Paavonen et al. 1981, Weström et al. 1982, Stamm 1999). Long-term complications of PID include ectopic pregnancy (EP), tubal factor infertility (TFI) and chronic pelvic pain (Paavonen & Eggert-Kruse 1999). In the literature, the generally assumed risk of developing PID after lower genital tract C. trachomatis infection varies considerably with study setting and is up to 30% (reviewed by Land et al. 2010). In high-risk settings, 2%–5% of untreated women develop PID within the approximately 2-week period between testing positive for C. trachomatis and returning for treatment (reviewed by Haggerty et al. 2010). Rates of PID have markedly declined in many countries since the 1990s (Kamwendo et al. 1996, Moss et al. 2006, Paavonen 2012, Scholes et al. 2012).

Reiter’s syndrome (urethritis, conjunctivitis, arthritis and mucocutaneous lesions) and reactive arthritis (Keat et al. 1978, Kousa1978) have also been associated with genital C. trachomatis infection and are more common in men.

2.3.2 Genitourinary infections in men

C. trachomatis is the most common cause of non-gonococcal urethritis and accessory gland infection in men (Paavonen & Eggert-Kruse 1999). Typical symptoms include discharge and dysuria (Peipert 2003). The urethral orifice may be erythematous. Chlamydial infection may spread through the vas deferens to the epididymis (Peipert 2003). Usually the infection is acute and unilateral. The patient presents with pain, fever and chills. Tenderness starts at the lower pole of the testis but often spreads to the upper pole and the vas deferens may become thickened and indurated. Asymptomacy of genitourinary infections in men is common; up to 50% of men do not experience symptoms (Zelin et al. 1995). Cecil et al. (2001) reported that only 14% of Chlamydia-positive US male military recruits had any symptoms. Prostatitis may also be caused by chlamydia, although results of the different studies are controversial (Cunningham & Beagley 2008). Both women and men can have chlamydial conjunctivitis via auto-inoculation from the genital tract (Bebear & Barbeyrac 2009).
About 15% of cases of proctitis in men who have sex with men (MSM) is caused by *C. trachomatis* (Geisler *et al.* 2002). Clinical symptoms range from minor pain to mucopurulent discharge with associated anal dermatitis and pain on defecation (Black 1997).

### 2.3.3 Lymphogranuloma venereum

Lymphogranuloma venereum (LGV) is a sexually transmitted systemic infection caused by the L1, L2, L2a, L2b and L3 serovars of *C. trachomatis*. L serovars predominantly infect macrophages and monocytes. The LGV serovars of *C. trachomatis* are more invasive than the other genital serovars (Black 1997). LGV is highly prevalent in parts of Africa, Asia and South America. Until 2003 LGV was very rare in developed countries, but since 2003 outbreaks have been reported in the Netherlands, Sweden, Denmark, the United Kingdom, Canada and North America (Nieuwenhuis *et al.* 2004, van de Laar 2005, Ward *et al.* 2007, Stary & Stary 2008, White 2009). Almost all of the European cases, also in Finland, are diagnosed in MSM (Korhonen *et al.* 2012).

The incubation period after sexual intercourse is at least 3–30 days (Mabey & Peeling 2002). The primary lesion is a small papule that evolves into a papulovesicle or papulopustule and then ulcerates. The primary site of lesion in men might be the glans, prepuce, or anterior part of the urethra. In women, the cervix, vagina and labial area are the main primary sites (Mabey & Peeling 2002). Without treatment, painful lymphadenopathia in the inguinal areas and systemic symptoms may occur, including chills, fever, malaise, arthritis and even meningeal signs. Typical symptoms in rectal disease are tenesmus, constipation, anorectal pain, mucopurulent discharge, diarrhoea and abdominal pain (Sethi *et al.* 2009). Proctitis was known for many years in MSM as the “gay bowel syndrome” and LGV as a causative agent was found in 1976 (Kazal *et al.* 1976). LGV might be earlier mistakenly treated for Crohn’s disease (Quinn *et al.* 1981, Forrester *et al.* 2006).

### 2.3.4 *C. trachomatis* infection during pregnancy

Untreated or persistent *C. trachomatis* infection in the upper genital tract (Witkin 1999, Shephard & Jones 1989) is associated with many adverse outcomes during pregnancy, including miscarriage, ectopic pregnancy (EP), preterm delivery (PTD), premature rupture of membranes (PROM) and postpartum endometritis.
In Western countries EP is estimated to occur in about 1–3% of pregnancies (Farguhar 2005, Barnhart 2009). More than 95% of all EPs are located in the Fallopian tube (Corpa 2006, Varma & Gupta 2009). An association between C. trachomatis infections and EP has been supported in several studies with different designs and it is considered to be the most important aetiological agent (Ness et al. 2008b, Haggerty et al. 2010), although Shaw et al. (2010) pointed out recently that the evidence has been mostly descriptive. Past chlamydial PID is associated with increased risk of EP (Chow et al. 1990, Weström et al. 1992), but contradictory results have also been reported from Denmark, where women with at least one positive C. trachomatis result had a lower rate of EP than women with negative results only (Andersen et al. 2005). Furthermore, women with EP are at an increased risk of repeat EP and infertility (Sandvei et al. 1987, Skjeldestad et al. 1998, Ego et al. 2001).

During the 1970s and 1980s, marked increases in the incidence of EP were reported from several countries including Norway (Skjeldestad et al. 1997) and the UK (Rajkhowa et al. 2000). In Australia, EP rates were stable during the 1990s (Chen et al. 2005). Declining trends in EP incidence have been reported from Finland (a 12% decrease from 1988 to 1994) (Mäkinen 2000), Sweden (Kamwendo et al. 2000), France (Coste et al. 2000) and from the 1990s in Norway (Bakken et al. 2006). An observed decline in EP has been attributed to decreased rates of chlamydial infection in Sweden (Egger et al. 1998).

Miscarriage is defined as a pregnancy that ends spontaneously before the foetus has reached a viable gestational age (24 weeks) (Rai & Regan 2006) and it is the most common complication of pregnancy. The proportion of miscarriages that are attributable to infection is approximately 10–15%, but this might be an underestimate. The effect of chlamydial infection in the early stages of pregnancy is unclear (Mardh et al. 2002). Among several studies in which a diagnosis of C. trachomatis infection was settled after positive culture or PCR-results, only one showed a direct association between the presence of C. trachomatis and miscarriage (Rastogi et al. 2000). An increased prevalence of C. trachomatis antibodies in sporadic and recurrent miscarriages has been reported (Quinn et al. 1987, Witkin 1992, Vigil et al. 2002, Kishore et al. 2003) but Chlamydia-seropositive women have been negative for C. trachomatis when using both culture and molecular approaches (Quinn et al. 1987, Witkin et al. 1992). Recently Baud et al. (2008) reported in a prospective study that 14% of women
were *C. trachomatis* IgG positive and that presence of *Chlamydia* specific antibody correlated strongly with recurrent but not with sporadic miscarriages. Other seroepidemiological studies have not revealed any correlation between *C. trachomatis* seropositivity and sporadic (Coste et al. 1991) or recurrent (Paukku et al. 1999b, Sugiura-Ogasawara et al. 2005) miscarriage and the true role of *C. trachomatis* in pregnancy loss remains to be determined.

The role of chlamydial infection in cases of PTD (infant born at less than 37 weeks of gestational age) is unclear. About half of the cases of spontaneous PTD are associated with ascending genital tract infection (Lockwood 2002). Infection sites can be either intrauterine or lower genital tract. Claman et al. (1995) reported a 4-fold risk of a *Chlamydia*-seropositive woman to have a preterm birth compared with a seronegative pregnant woman. Gencay et al. (2000) found that mothers with PTD (22–29 gestational weeks) had a *C. trachomatis* IgM positivity rate of 8.3%. Rours et al. (2011) showed that *C. trachomatis* infection contributes significantly to early premature delivery (PCR-based study). Since 1990, the PTD rates have been reported to increase in the USA (Martin et al. 2010), Europe (Shennan et al. 2006) and Australia (Tracy et al. 2007). A contrasting finding was discovered in Finland recently showing a steady rate of PTD from 1987 to 2005 and the risk of extremely preterm delivery (<28 weeks of gestational age) was decreased in a population-based study concerning 1,137,515 deliveries (Jakobsson et al. 2008). In the USA also, a slight decrease of PTD since 2006 has been reported (Martin et al. 2010).

### 2.3.5 Neonatal infections

Newborns can be infected with *C. trachomatis* by passing through the infected cervix (Bell et al. 1994). There are, however, also case reports of infected newborns delivered by Caesarean section with intact membranes (Ratelle et al. 1997). The two major adverse outcomes are inclusion conjunctivitis in 18–50% and pneumonia in 11–20% of cases (Schachter et al. 1986, Preece et al. 1989, Darville 2005). Symptoms of conjunctivitis usually develop within the first few weeks after delivery and include redness of the conjunctivae, infiltration and pustular discharge (Rours et al. 2008). Pneumonia occurs in four to 17 weeks if the infection remains untreated after delivery (Stamm 1999, Honey & Templeton 2002). The infant may develop crusted rhinitis, coughing and respiratory problems (Stamm 1999).
2.4 Long-term sequelae of *C. trachomatis* infections

Further ascending spread occurs in about 10% of symptomatic patients. Clinically the patients have abdominal pain, tenderness and fever. Chronic inflammation and fibrosis of the tubes can lead to infertility and sterility (Paavonen & Egger-Kruse 1999). The disease can spread further, causing oophoritis, perioophoritis, peritonitis or perihepatitis (Fitz-Hugh–Curtis syndrome) (Wolner-Hanssen et al. 1982).

2.4.1 *C. trachomatis* infection and infertility

Tubal factor infertility (TFI) is a major consequence of PID (Paavonen & Lehtinen 1996, Paavonen 1998). The risk of TFI after chlamydial PID is estimated to be 10–20% according to some cost-effectiveness studies of chlamydial screening (Paavonen et al. 1998, Howell et al. 1998, reviewed by van Valkengoed et al. 2004). Weström et al. (1992) followed over 1200 women with laparoscopically confirmed PID for several years. On average, 11.4% of women with PID became infertile, < 4% after one episode of PID and 40% after three PID episodes. Seroepidemiological studies have indicated that chlamydial infections account for a large proportion of cases of TFI by demonstrating a strong link between the presence of serum antibodies to *C. trachomatis* and the presence of tubal pathology (Punnonen et al. 1979, Cates & Wasserheit 1991).

TFI is strongly associated with recurrent or persistent *C. trachomatis* infection, which results in chronic salpingeal inflammatory responses (Beatty et al. 1994) maintained by mononuclear lymphocytes (Patton et al. 1989). Elevated serum levels of antibodies to chlamydial heat shock protein 60 (CHSP60) correlate with chronic chlamydial infection, PID and TFI (Toye et al. 1993, Peeling et al. 1997, Witkin 2002, Kinnunen et al. 2003, Karinen et al. 2004) and lymphocyte proliferative responses to CHSP60 occur more often in PID patients than in healthy controls (Witkin et al. 1993, Kinnunen et al. 2002). A specific role for CHSP60, in the pathogenesis of salpingitis, has been suggested in a study involving an experimental monkey model of infection (Patton 1994). Heat shock proteins are highly conserved proteins in all organisms ranging from bacteria to man. They are important antigens that induce both humoral and cell-mediated immunity. Chlamydial HSP60 is expressed throughout the chlamydial developmental cycle (Shaw et al. 2000). Because there is high amino acid sequence homology between microbial and human HSPs (Jones et al. 1993), an
induced immune response against microbial HSPs may give rise to an autoimmune inflammatory reaction in the hosts. With chronic chlamydial infections (Betsou et al. 1999) and tubal occlusion (LaVerda et al. 2000) it has also been shown that *C. trachomatis*-specific T-cells are present in the fallopian tubes of women with TFI (Kinnunen et al. 2000) and a considerable proportion are targeted to CHSP60 antigen (Kinnunen et al. 2002). Acute chlamydial PID progresses to TFI in only some patients, suggesting that genetic background, HLA antigens (Ortiz et al. 1996, Kim et al. 1999) and cytokine gene polymorphism may be involved (Conway et al. 1997, Öhman et al. 2011). TFI rates have declined recently and the risk of TFI after one episode of chlamydial lower genital tract infection is 4–5%, which is lower than earlier estimates (Paavonen 2012).

A number of serological studies have investigated the role of *C. trachomatis* in male infertility, sperm quality and IVF outcome, the results being highly variable (Paavonen & Eggert-Kruse 1999, Gonzales et al. 2004). A case-control study of the associations between serum antichlamydial antibodies and male infertility among couples showed that the prevalence of IgG antibodies to *C. trachomatis* was higher among men from infertile couples than control men and they also had lower sperm counts (Joki-Korpela et al. 2009). There is no consensus of opinion as regards including male partner chlamydial antibody testing in infertility work-up (Paavonen 2012). Screening men is important, as they might at least serve as a reservoir of chlamydial infection in couples. Further studies are needed to provide more definitive information on the role of *C. trachomatis* in male infertility.

2.4.2 *C. trachomatis* infection and cancer

It has been demonstrated both in prospective seroepidemiological studies and longitudinal PCR-based (cytological material) studies that past infection with *C. trachomatis* is a cofactor of cervical neoplasia (Lehtinen et al. 1996, Koskela et al. 2000, Anttila et al. 2001, Wallin et al. 2002, Silins et al. 2005, Naucler et al. 2007) especially together with HPV18/43 infections (Luostarinen et al. 2013). An association of *C. trachomatis* with cervical cancer has also been found in HPV DNA (PCR-detected) adjusted analyses of cross-sectional studies (Smith et al. 2004, Madeleine et al. 2007). It has been shown in cohort studies that *C. trachomatis* infection is an independent risk factor of both incident high risk HPV (hrHPV) infection and persistence of hrHPV DNA (Samoff et al. 2005, Silins et
In a recent joint cohort study (17,622 females aged 15–26) it was shown that the presence of *C. trachomatis* resulted in an excessive risk of cervical intraepithelial neoplasia grade 2+ (CIN2+) as a cofactor with hrHPV and it was also an independent risk factor of CIN2+, which takes the results of earlier study evidence a step further (Lehtinen *et al.* 2011). The mode of *C. trachomatis* pathogenetic action in CIN remains unknown, but large-scale longitudinal interaction studies suggest that *C. trachomatis* may interfere with immune surveillance of persistent, carcinogenic infections with hrHPV (Luostarinen *et al.* 2004, Arnheim *et al.* 2005). It has been suggested that *C. trachomatis* prolongs or prevents spontaneous healing of cervical HPV infection (Silins *et al.* 2005).

*C. trachomatis* has also been associated with ovarian cancer in some studies (Risch & Howe 1995), but recent seroepidemiological studies do not support this (Ness *et al.* 2008a, Idahl *et al.* 2010). Riska *et al.* (2006) found no association between past chlamydial infection and fallopian tube carcinoma. *C. trachomatis* infection has not been found to increase the risk of prostate cancer (Anttila *et al.* 2005, Dennis *et al.* 2009, Hrbacek *et al.* 2011).

### 2.5 Diagnosis of *C. trachomatis* infection

#### 2.5.1 Nucleic acid amplification tests

Nucleic acid amplification tests (NAATs) are nowadays the tests of choice for diagnosing *C. trachomatis* infection (Puolakkainen *et al.* 1998). A first void urine (FVU) sample and self-obtained vaginal or vulvar swab specimen can be used, which is a major advantage compared with endocervical and urethral swabs required for culture (Schachter *et al.* 1995). NAATs are much more sensitive (over 90%) than culture or antigen tests and show very few false-positive results, specificities approaching 100% (Peeling 1997). The latest versions of NAATs from major manufacturers are all adequate and capable of detecting all known variants of *C. trachomatis* (Lanjouw *et al.* 2009). The most widely known method of DNA amplification technology is PCR (Black 1997), which gives a positive result at the earliest 5–7 days of *C. trachomatis* transmission (Reunala 2006).

In Finland, as many as 99% of patients with *C. trachomatis* were diagnosed by PCR/LCR tests already in 2002 (Hiltunen-Back *et al.* 2003).
2.5.2 Culture

Before NAATs, culture was considered to be the gold standard for detection of *C. trachomatis* in urogenital specimens. It is almost 100% specific but relatively insensitive, 50–85% compared with NAATs (reviewed by Black 1997). Samples need careful transportation and incubation needs a relatively long time (3–7 days). Sampling techniques are also invasive compared with PCR testing and thus more inconvenient for the patient.

2.5.3 Serology

Serological testing for *C. trachomatis* has been used to detect antichlamydial antibodies among women with *Chlamydia*-related complications such as tubal factor infertility (Arya et al. 2005, den Hartog et al. 2008), ectopic pregnancies (Machado et al. 2007), recurrent miscarriages (Baud et al. 2008) and PID (Ness et al. 2008b). A correlation between antibody titres and the severity of tubal inflammation has been shown (Treharne et al. 1979, Akande et al. 2003). After *C. trachomatis* seroconversion, IgG and IgA antibodies peak within a few weeks (Närvänen et al. 1997). Persistence of *C. trachomatis* titers depend on the individual’s immune system and the sensitivity of the serological test used (Horner et al. 2013). Only ever-infected and never-infected individuals can be differentiated by species-specific serological tests (Clad et al. 2000). IgG antibodies persist for years (Yamamoto et al. 1998, Gijsen et al. 2002) even after antibiotic treatment (Puolakkainen et al. 1986, Henry-Suchet et al. 1994, Clad et al. 2000) and are considered to be markers of past *C. trachomatis* infection (Grayston et al. 1990). Seroconversion and *C. trachomatis* IgG antibodies was found in 73–86% of the PCR-positive women at 6 months follow-up (Muvunyi et al. 2011, Geisler et al. 2012) Chlamydial IgG antibody testing in serum is applied in reproductive medicine in fertility work-up but has no place in early diagnosis of *Chlamydia* infections (reviewed by Land et al. 2010). The persistence of IgA antibodies has been thought to reflect chronicity, because the half-life of IgA antibodies is shorter than that of long-lasting IgG according to *C. pneumoniae* studies (Falck et al. 2002)

The following serological tests have been developed to detect chlamydial antibodies: complement fixation (CF) tests, microimmunofluorescence (MIF) tests and enzyme immunoassay (EIA) tests.
In the CF test, the targets of the antibodies are genus-specific LPSs; thus it is not possible to determine species-specific antibody responses by way of this test (Black 1997).

The MIF test developed by Wang and Greyston was initially used for serotyping chlamydial strains (Wang & Greyston 1970, Wang et al. 1975). The test measures antibodies against chlamydial EB antigen, and is able to differentiate both species- and serotype-specific antibodies (Wang & Greyston 1970, Anttila et al. 1998). This test is considered to be the ‘gold standard’ for the serological diagnosis of *C. trachomatis* infections (Dowell et al. 2001). However, MIF is labour-intensive and reading of the assay is operator-dependent and subjective (Tuuminen et al. 2000, Paldanius et al. 2003).

EIA kits with recombinant peptides of MOMP or CHSP60 of *C. trachomatis* as antigen are commercially available for the detection of chlamydial antibodies (Närvänen et al. 1997, Norby et al. 1987, Jones et al. 1992). Comparisons between MIF and EIA tests have been published and one study showed sensitivities between 71% and 85% and specificities of more than 96% for three different EIA kits (Morre et al. 2002a). Bax et al. (2003) showed that peptide–based EIA test is a good choice instead of MIF for the detection of *C. trachomatis* antibodies, with greater specificity. Since EIA-based serological assays are well standardized, less time-consuming, cheaper and less laborious than MIF, they are good alternatives, especially when processing large numbers of samples (Baud et al. 2010) and thus allow large sero-epidemiological studies to be performed (Persson et al. 2002, Low 2008).

### 2.5.4 Antigen detection

The present antigen detection methods are based on the demonstration of genus-specific chlamydial LPSs and they cannot differentiate between chlamydial species (Black 1997). Antigen detection can be performed by direct fluorescence antibody (DFA) techniques or by EIA (Black 1997).

### 2.6 Treatment of genital *C. trachomatis* infection

In treatment of uncomplicated *C. trachomatis* genital infection, azithromycin (1 g orally as a single dose) is the drug of choice, with good outcome and compliance and with minor side effects (Tobin et al. 2004, Workowsky & Berman 2010). Doxycycline (100 mg twice daily for 7–10 days) is the second-line choice. A
meta-analysis revealed that both regimens are equally effective (Lau & Qureshi 2002). In addition, erythromycin (500 mg qid), ofloxacin (300 mg bid) and levofloxacin (500 mg once daily for 7 days) can be administered (Mylonas et al. 2012).

A single dose of 1 g azithromycin is also a first-choice treatment of chlamydia during pregnancy. Alternative treatment includes a course of amoxicillin, 500 mg qid for 7 days, or erythromycin, 500 mg qid for 7 days.

Treatment of complicated chlamydial infection (e.g. PID) requires a longer duration of treatment and a combination of antibiotics. Guidelines recommend 14-day course of doxycycline and metronidazole for mild to moderate PID.

A guideline for the management of rectal LGV infection recommends a course of doxycycline, 100 mg bid for 21 days (McMillan et al. 2007, de Vries 2010). Neonatal chlamydial infection should be treated with systemic erythromycin (Lanjouw et al. 2009).

Resistance to first- and second-line antibiotic treatment is infrequent, but there are reports of associated treatment failure (Lefevre & Lepargneur 1998, Somani et al. 2000, Wang et al. 2005) and recent evidence strongly suggests that treatment failure may occur in more than 5% of patients (Hansfield et al. 2011, Horner 2012).

A patient should be advised to avoid sexual contact during treatment and for 7 days afterwards to prevent reinfection before the treatment has been effective (Paavonen 2012). Test of cure is a controversial topic and there are major regional variations in practice. Retesting within three weeks can yield false-positive results because of the continued presence of dead organisms (Gaydos 1998). Reinfection is more likely than treatment failure and retesting is recommended in three to 12 months after the first positive testing result (Johnson et al. 2002, Workowski & Berman 2010, reviewed by Guy et al. 2012). Pregnant women should be tested 1–3 months after treatment (Peipert 2003).

### 2.7 Prevention of C. trachomatis infection

Besides antibiotic administration, management and prevention of chlamydial infection includes contact tracing, screening, counselling and health education.
2.7.1 Contact tracing

Partner notification regarding STIs is essential to prevent reinfection, the onset of sequelae in untreated partners and onward transmission. It is mandatory by legislation in Finland. More partners are likely to be treated if a health professional contacts them on behalf of the patient (provider referral) than if patients do this themselves (patient referral) (Mathews et al. 2001). In practice, however, both patients (Apoola et al. 2006) and doctors (Hogben et al. 2004) prefer patient referral, which is cheaper and easier to do in primary care, where increasing numbers of STIs are being diagnosed (Cassell et al. 2006a). Patient referral has been reported to reach 40–60% of named sexual partners (Low et al. 2004). Treating a permanent partner at the same time is important for successful treatment (Workowski & Berman 2010, Kissinger & Hogben 2011). Many studies have shown the efficacy of partner-expedited treatment (where a physician gives medication to an index patient to deliver to the partner) of C. trachomatis infection in preventing further spread and reinfection (Golden et al. 2005, reviewed Marrazzo & Cates 2011). This can result in better success in stopping transmission, but on the other hand the partner does not come to testing and other STIs could remain undetected.

2.7.2 Sexual health education

The basis of primary prevention is sexual health education to reduce the risk of contracting STIs. Spouwen et al. (2011) found that chlamydial testing rates were significantly increased (from 26% to 65%) among Dutch adolescents (median age 19 years) when provided with sexual health education and easy access to STI testing. In Finland, sexual health education was added to school curricula in the 1970s. In the late 1990s sexual health education was downgraded, but nowadays it is a mandatory subject in public schools and is taught to schoolchildren aged 13–15. Sexuality issues are also integrated into other school topics such as biology. Health Education is a subject taught in high schools and vocational schools. The quantity and quality of education and health counselling varies considerably (Wellings & Parker 2006, Kontula & Meriläinen 2007, Kemppainen et al. 2012). Specific health clinics for youth are rare in community level in Finland (Sannisto & Kosunen 2009).

As adolescents are at high risk of having STIs, especially genital chlamydial and HPV infections, they should be targeted as regards sexual health education in
more effective ways than earlier. Material on sexual health on the Internet websites, including social networking sites (SNSs, such as Facebook and MySpace) and text messaging for sexual health purposes is only just beginning to emerge (Selkie et al. 2011, Bull et al. 2012, Guse et al. 2012, Perry et al. 2012).

Sexual health among young people in Finland is promoted also through the Internet and, for example, a private foundation, the Finnish Family Federation has its own Internet site, which provides interactive information about sexual health issues, plus a profile in the SNS Facebook (www.vaestoliitto.fi). In addition to services in sexuality and sexual health issues for adolescents and adults, they have a special net service for men (Men’s Moment), which was founded in 2001, because boys and men lacked a place where they could obtain factual and professional high-quality information about sexual health, about development during puberty, and about dating and sex.

A sexual health education session can be arranged in many different ways, and the effectiveness of brief, single-session intervention (counselling and sexual health information) for the purpose of reducing the prevalence of HIV and other STIs was evaluated in a recent review by Eaton et al. (2012). One mechanism that may play an important role in determining the efficacy of brief, single-session interventions is their delivery, among populations at risk of HIV and other STIs, during a so-called teachable moment, which means that the timing in intervention is very important to gain the patients’ full attention (Lawson & Flocke 2009).

Testing services on the Internet are becoming more common for testing of STIs, especially Chlamydia (Owens et al. 2010). Some sites offer kits for at-home testing, for example in Finland, among a large on-going national HPV-vaccine study, an Internet-based testing, treatment and information (www.rokotiitus.net) of Chlamydia is provided for enrolled adolescents for free (Lehtinen et al. 2007). Internet-based Chlamydia testing/screening and information is also available in Sweden (www.klamydia.se) and as the part of the National Chlamydia Screening Programme in the UK (http://www.chlamydiascreening.nhs.uk). Regulatory control is needed to protect Internet consumers from receiving inaccurate results. With regulation and guidance on how to get treated, Internet services could provide a confidential way of testing for STIs among those who are reluctant to use clinics for STI testing.
2.7.3 Condom use

The most important preventive device as regards STIs including Chlamydia is consistent condom use during sexual intercourse (Niccolai et al. 2005). A condom should be used during the whole period of a sex act, including when having oral or anal sex. According to the School Health Promotion Study (2011), which is conducted every two years in Finland, condom use is inconsistent among adolescents. Condom use errors and problems are common worldwide, occurring across a wide spectrum of populations, and in part lead to inconsistent usage (Crosby et al. 2012, reviewed by Sanders et al. 2012). Breakage and slippage of condoms are the most commonly investigated problems, but also other errors of use such as not using condoms throughout sex, not leaving space at the tip, not squeezing air from the tip, putting the condom on inside out, not using water-based lubricants and incorrect withdrawal were reported to be fairly prevalent. Frequent problems also included leakage, condom-associated erection problems, and difficulties with fit and feel. Erection problems were reported while applying the condom (up to 28%) and during intercourse (up to 20%). There is evidence that even phosphodiesterase type 5 inhibitors do not fully overcome condom-associated erection problems (Sanders et al. 2009). These problems should be noted and guidance provided and manufacturers should also work with material aspects so that condom use would be more popular and provide better protection against STIs. Thus a positive effect on the prevalence of STIs could be achieved.

2.7.4 Screening

The primary aim of screening is to reduce morbidity by early detection and treatment of uncomplicated lower genital tract infection. A secondary aim of screening is to reduce the population prevalence of chlamydial infection by reducing transmission (Regan et al. 2008). Screening programmes must be cost-effective and must be made acceptable to patients by using non-invasive procedures. Screening design can be either systemic, consisting of screening the entire target population (for example register-based screening) or opportunistic, targeting individuals in a healthcare setting (Jones & Boag 2007, Bone et al. 2012).

Systematic reviews of the cost-effectiveness of Chlamydia screening young, asymptomatic women suggest that screening is cost-effective, mainly because of the reduction in chlamydial long-term health costs (Scholes et al. 1996,
Ostergaard et al. 2000, Honey et al. 2002, Oakeshott et al. 2010). Other criteria for screening are also fulfilled, as *C. trachomatis* genital infection is common and easy to diagnose by modern PCR methods (NAATs), using urine or vulvar swab samples that can even be self-sampled at home, which has been shown to raise participation in screening (van Bergen et al. 2005, Graseck et al. 2010). Chlamydial infection is easily treated with antibiotics and this also speaks for screening (Paavonen & Eggert-Kruse 1999).

Modern screening programmes can be arranged, for example, by using register-based approach, and attendees can be advised to order sample kits from a programme website and post samples with prepaid packages to healthcare offices. The test result is made available on the website and if positive, a general practitioner or other healthcare person provides treatment and partner notification (van Bergen et al. 2005, Woodhall et al. 2012). Reminders to target people can be sent by text messaging or e-mail (van den Broek 2012).

There is no national screening programme for *C. trachomatis* in Finland although the cost-effectiveness of such a programme was already pointed out more than ten years ago (Paavonen et al. 1998, Paukku et al. 2003). In 2007 The Ministry of Social Affairs and Health (STM) launched the first National Action Programme for the Promotion of Sexual and Reproductive Health, which includes a recommendation for opportunistic *C. trachomatis* screening in Finland. According to this recommendation, which during the past 5 years has proven to be uneffective; all females under the age of 25 attending healthcare services in regard to contraceptives should be tested for *C. trachomatis* infection. Retesting should be performed during control visits and then annually if the patient has had an earlier *C. trachomatis* infection or has a new partner.

The Screening for Chlamydia in Europe (SCREen) project was established to collect data on current and planned genital chlamydial control activities in Europe (Low et al. 2012). In 2007, a questionnaire concerning different aspects of *Chlamydia* epidemiology and control was sent to public health and clinical experts in 33 European countries. Experts in 29 (88%) countries responded. Thirteen of these 29 countries (45%) had no current chlamydial control activities at the time, 6 countries in this group had plans to introduce chlamydial screening programmes and five countries (17%) had case management guidelines only. Three countries (10%) also recommended case finding amongst partners of diagnosed chlamydial cases or people with another sexually transmitted infection. Six countries (21%) further specified groups of asymptomatic people eligible for
opportunistic chlamydia testing. Only two countries (7%) reported a chlamydial screening programme.

Opportunistic screening results in variable rates of testing within target populations. In England considerable resources have been invested in a National Chlamydia Screening Programme that offers chlamydia tests in a variety of settings to adults of less than 25 years (Department of Health 2009) and an average of 15% of the target population was tested in 2008–2009 (Simms et al. 2009). In the Netherlands, a pilot programme of annual postal invitations for Chlamydia screening is being evaluated as a randomized controlled trial among 16–29-year-olds and 21% of invited women and 10% of invited men were tested during the first year (van Bergen et al. 2005). In the US, the annual screening rate in sexually active 15- to 25-year-olds is approximately 41% (Centers for Disease Control and Prevention) and in Sweden, 71% of women have had at least one chlamydia test in a 10-year period (Low et al. 2006). In contrast, in Australia less than 10% of people in the target screening groups have had a chlamydia test (Guy et al. 2011).

Efficacy in reduction of chlamydial long-term health sequelae has been studied mostly in connection with a reduction of chlamydial PID rates (van den Broek et al. 2012). Evaluating screening efficacy in lowering chlamydia-related complications such as PID, ectopic pregnancy, CIN and infertility is difficult. Firstly, the assessed risk of complications after genital chlamydial infection varies widely according to different study settings as regards PID (Land et al. 2010) and EP, from even a decreased risk (Andersen et al. 2005) to an unchanged (Low et al. 2006) or doubled risk (Bakken et al. 2007). The results of these studies concern screened and presumably treated women, so there is a chance of a bias towards low risk if the results are compared with those among women with undiagnosed and untreated chlamydial infection (Bakken 2008). Secondly, the incidence data of these chlamydia-related complication rates also vary globally (Bender et al. 2011). However, there are reports from many industrialized countries, including Finland, indicating that chlamydial incidence is continuously increasing and chlamydia-related complications are decreasing at the same time (Chen et al. 2005, Bender et al. 2011, Paavonen et al. 2012, Scholes et al. 2012). One report from New Zealand showed that testing rates and chlamydial incidence trebled from 1998 to 2008. At the same time, hospital admissions for PID decreased in 1998–2004, but then increased, infertility diagnoses decreased and ectopic pregnancy rates remained unchanged during the follow-up period (Morgan et al. 2011). In a cross-national survey among 15–39-year-old females,
decreasing PID and EP rates were reported from Sweden and Denmark, but ectopic pregnancy rates increased in 15–19-year-olds in Sweden and the Netherlands (Bender et al. 2011). There are no evaluations about the impact of chlamydial screening efficacy concerning CIN rates. There is lack of randomized, controlled trials evaluating efficacy of population chlamydial screening, whether opportunistic or systemic (van Valkengoed et al. 2000, Low et al. 2007, Miller et al. 2008, Gottlieb et al. 2010). In the USA and Denmark, randomized controlled trial of population chlamydial screening demonstrated about 50% reduction in PID, but both studies have been criticized for many weaknesses (Scholes et al. 1996, Ostergaard et al. 2000). In Denmark, Andersen et al. (2011) reported no reduction of chlamydial long-term reproductive morbidity (PID, ectopic pregnancy, infertility diagnosis) after 9 years of intensified screening (compared with a control group) so the end-point results concerning efficacy also vary depending on setting. Mathematical modelling is an important tool in predicting/evaluating effectiveness of chlamydial screening in various settings and provides accurate information how different infection parameters (for example incidence, prevalence, transmission probability, age, gender) affect in assessment (Kretzschmar et al. 2009, Althaus et al. 2010).

2.7.5 Vaccine

By means of mathematical modelling it has been estimated that fully protective vaccination could eliminate Chlamydia epidemics in 20 years (Gray et al. 2009). A partially effective vaccine would reduce disease in men and women, but cost efficacy would be greater as regards vaccinating women only (Gray et al. 2009). Understanding the natural history of Chlamydia infection, host immune responses and how these impact on subsequent pathology is crucial to rational vaccine design. Developing a vaccine against C. trachomatis is still a challenge, but in the past few years, various protective C. trachomatis antigens as potential vaccine candidates have been found (Brunham & Rey-Ladino 2005, Howie et al. 2011, Igietseme et al. 2011, Karunakaran et al. 2011, Brunham & Rappuoli 2013).
3  Aims of the study

This work was performed to elucidate the observation of increasing *C. trachomatis* incidence and simultaneously decreasing *C. trachomatis* seroprevalence by studying the population-based epidemiology of *C. trachomatis* infections in Finland in 1983–2009. EIA-based serological methodology was used to assess *C. trachomatis* IgG antibodies in the FMC population to calculate *C. trachomatis* seroprevalence and seroincidence compared with reported *C. trachomatis* case rates according to laboratory notification.

The specific aims of the study were:

1. To study *C. trachomatis* seroprevalence (I & II) and seroincidence (II) trends in 1983–2003.
2. To compare the *C. trachomatis* incidence rate based on serology with reported *C. trachomatis* laboratory notifications (NIDR) in 1995–2003 (II).
3. To study the role of repeat genital chlamydial infections and the impact of gender- and age-specific proportions on the increasing incidence rates of the *C. trachomatis* infection in the NIDR from 1995 to 2009 (III).
4. To study the occurrence of *C. trachomatis* serotypes in 1983–2005 in Finland (IV).
4 Materials and methods

4.1 Study population

4.1.1 Finnish Maternity Cohort (FMC)

According to the Infectious Diseases Act, approximately 99% of pregnant Finnish women (altogether 850,000) have participated in serological screening of congenital infections (syphilis, HIV and hepatitis B) during the first trimester (gestational weeks 10 to 12) at municipal maternity care units since 1983. Following informed consent, the screening samples from 850,000 women have been collected and stored in the Finnish Maternity Cohort (FMC) serum bank of the National Institute of Health and Welfare (THL). The FMC serum bank comprised over 1.9 million serum samples at the end of 2011 and an average of 60,000 new samples are added annually. After screening, 1–3 mL of serum is stored at -25°C at THL, Oulu, Finland. After the first pregnancy, about 50% of women become pregnant again within 5 years, donating another serum sample to the serum bank.

For measuring C. trachomatis IgG antibody levels in seroprevalence (I &II) and seroincidence (II) studies, a total of 275,505 females (<29 years of age) with a minimum of two serum samples withdrawn during two consecutive pregnancies (within 5 years) before 2005 were identified and divided into 28 strata according to age (<20, 20–22, 23–25 and 26–28) at the midpoint of the two samplings, and 3-year periods (1983–1985, 1986–1988, 1989–1991, 1992–1994, 1995–1997, 1998–2000, 2001–2003), as earlier described by Lehtinen et al. (2006) and Kaasila et al. (2009) in HPV prevalence studies. In each stratum, a subsample of 200 or 400 women was selected randomly. A total of 401 women were excluded because of missing data, and the final number of women with paired sera tested for C. trachomatis IgG antibodies was 7999 (Table 1).

In the C. trachomatis seroprevalence map study (I), we used the results (IgG seropositivity for C. trachomatis) from the first serum samples (withdrawn at first pregnancy) for each subject, and the data was reorganized according to age and calendar year. The mean age of the subjects at the time of the first serum sample was 22 years (range 14–28 years). For statistical analyses, the two youngest and two oldest age groups were combined in two age groups: 14–22-year-old females and women of 23–28 years of age. The proportions of seropositive individuals...
(seroprevalence rates), with 95% confidence intervals (95% CIs), were estimated for three 6-year time periods: 1983–89, 1990–96 and 1997–2003.

For calculating seroincidence, we identified all the women who were negative for *C. trachomatis* IgG antibodies at the time of the first serum sample (n = 6632) and we also assayed *C. trachomatis* IgG antibodies in the second serum samples to find possible seroconversion between the two samples indicating an incident *C. trachomatis* infection. The age groups used for *C. trachomatis* seroprevalence were the same as used in the first study, but for more detailed information we used 3-year time periods (Study II).

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<td>1110</td>
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For studying *C. trachomatis* serotypes from 1983 to 2005 (IV), a subgroup of 1169 females (median 24.4 years of age, range 15.1–29.9) were randomly selected among those testing positive for *C. trachomatis*-specific immunoglobulin G (IgG) antibodies (from previously selected women (n = 7999), Studies I and II), to represent the 1980s (n = 358), 1990s (n = 480) and the beginning of the 2000s (2000–2005, n = 331). For age-dependent serotype analysis, the study population was divided into three age groups: < 23, 23–28- and > 28-year-old females.

The study protocol was approved by the institutional ethics committee and the FMC steering committee.

### 4.1.2 National Infectious Diseases Register

Surveillance of sexually transmitted *C. trachomatis* infections is based on mandatory notifications according to the Communicable Diseases Act and Decree (1987). The THL maintains the NIDR. Laboratory notification includes personal identification number (PIN), gender, age, place of sampling and test method used.
Data are updated continuously. The same person may be reported more than once if the time interval between two infections is longer than three months. Data after 1995 can be accessed through the Internet database (www.3ktl.fi/stat). Between 1987 and 1997, confirmed *C. trachomatis*-positive cases were reported to the NIDR by physicians, and since 1997 by laboratories only (in 1995–1997 by both). *C. trachomatis* incidence data from earlier years (1987–2000) have been reported by Hiltunen-Back *et al.* (2003). According to NIDR data, in 1995, 63% of patients with *C. trachomatis* were diagnosed by antigen detection tests, 16% by culture tests and 21% by polymerase or ligase chain reaction (PCR/LCR) tests. The proportion of NAAT methods in *C. trachomatis* diagnostics rapidly increased from 62% in 1997 to 95% in 2000 and 99.8% in 2002.

*C. trachomatis* incidence rates in our second study (II) were calculated from NIDR data concerning all reported cases of *C. trachomatis*-positive females between 15 and 29 years of age in 1995–2006. To be comparable with the earlier serological *C. trachomatis* incidence data, the subjects were divided into three age groups (15–19, 20–24 and 25–29 years of age) in four different time periods (1995–1997, 1998–2000, 2001–2003 and 2004–2006).

The third study (III) involved all registered *C. trachomatis* episodes, together with sufficient personal information (gender, age and date of the incident *C. trachomatis* infection) in the NIDR from 1995 to 2009. A case was regarded as a new infection episode if the time interval between two laboratory-confirmed and registered episodes in an individual exceeded three months. To analyse the occurrence of single and repeat *C. trachomatis* infection episodes in different age groups, the female and male study populations were divided into three age categories: < 23, 23–28 and > 28 years of age, being comparable to the age categories in our previous studies (I, II) on *C. trachomatis* seroprevalence. To further analyse trends in infection prevalence and proportion of repeat diagnoses, three comparable birth cohorts of < 23 years of age were formed for both females and males. The numbers of *C. trachomatis* infections were counted separately for each year for the period 1995–2009.

### 4.2 Serology

Serum IgG antibodies to *C. trachomatis* were analysed in a single set of experiments by using a commercial enzyme immunoassay (EIA) technique based on a synthetic peptide containing an immunodominant B cell epitope in the *C. trachomatis* major outer membrane protein (MOMP) (AniLabsystems, Helsinki,
Finland) according to the manufacturer's instructions. A peptide-based EIA test is considered to be species-specific with minimal cross-reactivity with *C. pneumonia* antibodies (Land *et al.* 2003). The reproducibility of the test as reported by the manufacturer is high (SD 0.061, coefficient of variation 4.9%) and correlation of results obtained by the AniLabsystems method and another peptide-based method is greater than 0.9, indicating the high specificity and sensitivity of the test. The results were expressed as the mean absorbance (OD 450 nm) of duplicate samples minus the mean absorbance of the reagent blank, divided by the cut-off value. A value greater than 1.4 was considered positive.

MIF analysis (IV): *C. trachomatis* serotype-specific IgG antibodies were assayed by using a MIF method involving EB antigens of *C. trachomatis* serotypes B, C, D, E, F, G, H, I, J and K obtained from the Washington Research Foundation (Seattle, WA, USA). All serotype-specific antigens were tested with commercial *C. trachomatis*-specific monoclonal antibodies (Bio-Rad, Redmond WA, USA) and diluted to optimal concentrations with uniform antigen densities and quality for all experiments. A pool of positive control sera with known antibody titres identifying all serotypes was included in each test series. All slides were read and interpreted by one experienced researcher (H-M.S.) using a single microscope.

### 4.3 Mapping method

The spatio-temporal variation of *C. trachomatis* seroprevalence rates was visualized as a series of maps by smoothing the community-level input data with a 2 × 2 km raster layer. The rates for the biggest cities (with 58 or more study subjects) were shown as coloured circles (with circle size indicating the population of the city). Seroprevalence rates were visualized using 19 colours varying from blue and green for low rates, to yellow and red for high rates. This method has been used for HPV (Lehtinen *et al.* 2006) and cancer mapping studies (Pukkala *et al.* 1987). Areas with less than one inhabitant per square kilometre were masked with a screen to stress the uncertainty related to these rates.

### 4.4 Statistical analysis

The proportions of seropositive individuals (seroprevalence rates), with 95% confidence intervals (95% CIs), were estimated and the statistical significance of trends over time was tested by using the Chi-square test for a linear trend.

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Municipality-specific seroprevalence rates for the same age and year groups were calculated. Random sampling of the 7,999 subjects resulted in coverage of almost all of the 446 communities (in 2007) in Finland. The relative proportions of study subjects among all pregnant women within a community varied from 0 (less than 30 communities) to 10% per community.

The binomial test was used to test for differences in proportions of seroprevalence rates (II). Incidence rates were estimated by the number of seroconversions divided by person-years of follow-up. The time of each seroconversion was assumed to be at the midpoint of the two samplings. Crude incidence rate ratios with 95% confidence intervals were calculated.

In the third study (III) 95% CIs were calculated by using a Confidence Interval Analysis (CIA) program (Gardner & Altman 1989). Categorical variables were compared by Chi-square tests and Chi-square tests for trend, as appropriate. Direct standardization was used to adjust for the effect of detection method (NAATs vs. other diagnostic methods; culture, antigen detection), applying the distribution of the method in each of the three separate cohorts to determine the relative weights.

The χ² test was used to compare categorical variables between the C. trachomatis serotype study groups (IV). Correlation of the ranked order of MIF-based C. trachomatis serotype-specific prevalences between decades was evaluated by Spearman’s ranked correlation.

Statistical analyses were performed with SPSS versions 13.0, 15.0 and 18.0 for Windows software (SPSS Inc., Chicago, IL, USA) and R 2.1.1 and R 2.6.0 software (R Development Core Team, Vienna, Austria).
5 Results

5.1 C. trachomatis seroprevalence 1983–2003 (I & II)

Seventeen percent (1367 of 7999) of pregnant women had C. trachomatis IgG-class antibodies. Seroprevalence rates decreased from the 1990s to the 2000s in both, women under 23 years of age and among 23–28-year-old women. No significant differences between seroprevalence rates of the two age groups were found, as the 95% confidence intervals overlapped at each calendar time point. The highest C. trachomatis seroprevalence rate, of 23.3% (CI 19.1–27.5), occurred in women under < 23 years of age in 1989–91, whereas among 23–28 year-old women the seroprevalence peaked in 1992–1994 (22.2%, CI 19.3–25.1) (Fig. 2). Thereafter, the seroprevalences declined significantly ($p < 0.001$), being 9.2% (6.3–12.2) in 2001–2003 in the younger age group and 12.4% (10.0–14.8) in 1998–2000 in the older age group. Decreasing seroprevalence rates were also observed in geographical information maps, albeit constant clusters of around larger cities and close to the Russian border crossing areas (Fig. 3).

Fig. 2. Chlamydia trachomatis seroprevalence (%) with the number of prevalent cases in pregnant women aged < 23- and 23–28-year-old during 1983–2003.
Fig. 3. *C. Chlamydia trachomatis* seroprevalence (%) in pregnant Finnish women. *C. trachomatis* IgG antibodies were determined in a random sample of first trimester sera from 14–22 and 23–28-year-old women in three consecutive periods. Areas covered with hatched lines in the north indicate sparsely populated areas (less than one inhabitant/km²).

5.2 *C. trachomatis* incidence (II)

We compared *C. trachomatis* incidence data obtained from the FMC (seroconversion rates) and the NIDR (laboratory notifications).
C. trachomatis incidence based on seroconversions 1983–2003

Altogether 6632 (83%) of 7999 women were seronegative for C. trachomatis IgG antibodies at the first pregnancy and were included in the chlamydial incidence study. A total of 161 (2.4%) women seroconverted by the second pregnancy. The seroconversion rates varied from 3.4% to 5.7% in the younger age group and from 0.8% to 2.3% in the older age group.

In the younger age group the seroconversion derived incidence was highest (264 per 10 000 person-years) at the beginning of the 1980s and lowest (165) in 1992–1994. By 2001–03 the incidence rate increased to 188 (Fig. 4). No statistically significant differences in time trends were, however, observed. In the older age group the incidence was lowest (31 per 10 000 person-years) in 1983–1985 and increased to 97 per 10 000 person-years (95% CI 48–146) by 2001–2003 ($p$ for linear trend = 0.10) (Fig. 4).

Fig. 4. C. trachomatis incidence (per 10 000 person years) with the number of seroconversions in pregnant women during 1983–2003.

C. trachomatis incidence rates based on laboratory notifications to the NIDR 1995–2006

According to NAAT-based laboratory notifications to the NIDR, C. trachomatis incidences in women increased in both age groups (15–24- and 25–29-year-olds)
from 1995–1997 (Table 2). The incidence rates were highest in women below 24-years of age, with a 1.5-fold increase from 1995–1997 (122 per 10 000 person years) to 2004–2006 (187 per 10 000 person years).

Overall, the *C. trachomatis* seroconversion and NAAT-based incidences were in line (Table 2).

Table 2. *Chlamydia trachomatis* incidence rates per 10 000 person-years (with 95% confidence intervals) based on seroconversions (FMC = Finnish Maternity Cohort) and laboratory confirmed cases (NIDR = National Infectious Diseases Register).

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<th>NIDR</th>
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<td>166 (68–264)</td>
<td>222 (110–334)</td>
<td>188 (86–290)</td>
</tr>
<tr>
<td>23–28-year-olds</td>
<td>33 (4–62)</td>
<td>64 (24–103)</td>
<td>97 (48–146)</td>
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<tr>
<td>25–29-year-olds</td>
<td>57 (53–61)</td>
<td>64 (60–68)</td>
<td>70 (66–75)</td>
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</table>

5.3 Repeat *C. trachomatis* infections (III)

We analysed the occurrence of single and repeat *C. trachomatis* infections notified to the NIDR in all chlamydial cases reported from 1995 to 2009. Altogether, 147 148 cases with a total of 177 138 *C. trachomatis* infection episodes were available. Most (65.3%) of the 29 990 repeat infections occurred in females (Fig. 5). Of all the repeat infections, 34.1% (35.1% and 30.3% in women and men, respectively) occurred within 12 months. The incidence rate of *C. trachomatis* infection increased 1.44-fold (95% CI 1.40–1.49) when the number of diagnoses increased from 8 031 cases in 1995 to 11 731 in 2000 (Hiltunen-Back et al. 2003, www3.ktl.fi), and thereafter it increased gradually (1.1-fold) to 2009. The increase in the number of *Chlamydia* diagnoses was more rapid in males than in females, especially among 15- to 19-year-old males (2.5-fold from 304 in 1995 to 767 in 2009). In 2009, 13 264 *C. trachomatis* cases were registered, more often in females (59%) than in males (41%). Between 1996 and 2009, the proportion of annual repeat infections increased from 4.9% to 7.3% (49% increase) in females and from 3.8% to 5.3% (39% increase) in males (Fig. 6). Overall, the proportions of cases who had had an earlier *Chlamydia* infection episode during the study period increased considerably from 2002 to 2009 both in females (18% vs. 24.8%, *p* < 0.001) and in males (14.8% vs. 20.3%, *p* < 0.001).
To further clarify the impact of repeated infections on the infection rates, we evaluated the prevalences of single and repeated diagnoses of *C. trachomatis* infections in three birth cohorts (1979–1981, 1982–1984 and 1985–1987) when the cases were <23 years of age. The proportions of the repeat diagnoses, adjusted for the diagnostic method (NAATs vs. other methods), increased significantly in females (17.4–19.5) and in males (10.1–12.6) during the follow-up period of 1995 to 2009 (both p-values for trend < 0.001) (Table 3). The proportions of repeat infections peaked at the age of 25 (37.0%) in women and at the age of 29 (30.9%) in men, respectively (Fig. 7).

**Fig. 5.** Reported *Chlamydia trachomatis* infections in 1995–2009 among females (white bars) and males (grey bars) in the age groups of < 23, 23–28 and > 28 years. The proportions of repeat *Chlamydia* infections are hatched.
Fig. 6. Proportions of repeat C. trachomatis infection diagnoses within a year in females and males in three age groups: < 23 (dark gray bars), 23–28 (light gray bars) and > 28 years old (white bars) during 1995–2009. The lines represent the reported numbers of primary infection episodes.
Table 3. Number of registered *C. trachomatis* infection diagnoses at the age of < 23 years in females and males in three birth cohorts at < 23 years of age in Finland.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Cohort size (n)</th>
<th>Primary infections (n)</th>
<th>Repeat diagnoses (n)</th>
<th>Prevalence of primary infection (95% CI)</th>
<th>Proportion of repeat diagnoses (95% CI)</th>
<th>Adjusted* proportion of repeat diagnoses (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>1979–1981 95187 9552 1923</td>
<td>10.0 (9.8–10.2)</td>
<td>16.8 (16.1–17.4)</td>
<td>17.4 (16.7–18.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1982–1984 99012 11375 2501</td>
<td>11.5 (11.3–1.7)</td>
<td>18.0 (17.4–18.7)</td>
<td>17.8 (17.2–18.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1985–1987 92394 10860 2668</td>
<td>11.8 (11.5–12.0)</td>
<td>19.7 (19.1–20.4)</td>
<td>19.5 (18.9–20.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1979–1981 99150 4106 455</td>
<td>4.1 (4.0–4.3)</td>
<td>10.0 (9.1–10.8)</td>
<td>10.1 (9.2–10.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1982–1984 103837 4994 631</td>
<td>4.8 (4.7–4.9)</td>
<td>11.2 (10.4–12.0)</td>
<td>11.3 (10.4–12.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1985–1987 96730 4875 716</td>
<td>5.0 (4.9–5.2)</td>
<td>12.8 (11.9–13.7)</td>
<td>12.6 (11.7–13.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted NAAT vs. other methods
Fig. 7. Proportions of cases of repeat diagnoses (%) among all notified *C. trachomatis* infections in females (Panel A) and in males (Panel B) in the 1979 age cohort in 1995–2009. The lines represent notified *C. trachomatis* infections in the different years.
5.4 Occurrence of infections with different C. trachomatis serotypes 1983–2005 (IV)

The distribution of C. trachomatis serotypes over two and a half decades was evaluated by the MIF method in a subcohort of baseline C. trachomatis IgG antibody positive FMC participants. The purpose was to study possible changes in C. trachomatis serotype distribution – whether or not there was replacement of more immunogenic with less immunogenic serotypes over time resulting in decreasing chlamydial seroprevalence in the same population.

Strong MIF reactions were observed among 675 subjects of 1169 (57.7%). Altogether, 118 (10.1%) of the 1169 subjects who were initially seropositive for C. trachomatis-specific IgG antibodies, were seronegative for all C. trachomatis serotypes with the MIF method.

C. trachomatis serotype distribution varied considerably over time (Fig. 8). Serotypes G (41.3, 31.0 and 26.7%) and E (22.6, 32.7 and 24.5%) were the most prevalent serotypes over the time period. However, the ranked order of C. trachomatis serotype prevalences by age and calendar time changed because the prevalence of one the least common serotypes, serotype D, peaked in the 1990s (1980s 5.0% vs. 1990s 30.0%, \( p < 0.005 \)). Serotypes G and J were the first and third most frequent C. trachomatis serotypes in the 1980s and 2000s (Table 4). In the 1990s these serotypes were, however, replaced by serotypes E and D, respectively, and the statistically significant correlation in the ranked order of serotypes as compared to 1980s and 2000s was temporarily lost especially in the 23 to 28 year-old women (Table 4). The overall proportions of women with antibodies to two or more C. trachomatis serotypes were similar in the 1980s and in the 2000s (100/358, 27.9% and 92/331; 27.8%, respectively), but differed significantly from the two in the 1990s (203/480; 42.3%, \( p < 0.001 \)).
Fig. 8. *C. trachomatis* serotype distribution in the 1980s (black bars), 1990s (grey bars) and 2000s (white bars) in 675 Finnish females.

Table 4. Ranked order of *C. trachomatis* serotype distribution among the six most common serotypes in IgG antibody-positive women in the 1980s (N = 358), 1990s (N = 480) and in 2000–2005 (N = 330) overall and in the age group 23–28.

<table>
<thead>
<tr>
<th>Order of serotypes</th>
<th>Overall</th>
<th>23–28 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1980s&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1990s&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.</td>
<td>G</td>
<td>E</td>
</tr>
<tr>
<td>2.</td>
<td>E</td>
<td>G</td>
</tr>
<tr>
<td>3.</td>
<td>J</td>
<td>D</td>
</tr>
<tr>
<td>4.</td>
<td>F</td>
<td>J</td>
</tr>
<tr>
<td>5.</td>
<td>D</td>
<td>B</td>
</tr>
<tr>
<td>6.</td>
<td>B</td>
<td>F</td>
</tr>
</tbody>
</table>

<sup>a</sup>r<sub>s</sub> = 0.657 (ns)
<sup>b</sup>r<sub>s</sub> = 0.943 (p = 0.005)
<sup>d</sup>r<sub>s</sub> = 0.543 (ns)
<sup>e</sup>r<sub>s</sub> = 0.943 (p = 0.005)
6 Discussion

6.1 Main findings

Our aim was to study the occurrence of *C. trachomatis* in Finland between 1983–2009. While *C. trachomatis* seroprevalence decreased among pregnant (fertile-aged) women over time, *C. trachomatis* incidence increased from 1995, based on both seroconversion rates in the FMC and NAAT-based diagnoses in the NIDR. Both single and repeat *C. trachomatis* case rates among women and men increased. Finally, the distributions of *C. trachomatis* serotypes fluctuated over time.

6.1.1 *C. trachomatis* prevalence and incidence trends in Finland

According to national surveillance data the number of reported chlamydial cases has been increasing since 1995 (www.3ktl.fi). In our seroepidemiologic studies in pregnant women, we however found a trend of decreasing *C. trachomatis* seroprevalence rates from 1983 to 2003 for both women under 23 years of age (20.8% to 10.6%) and 23–28-year-old women (19.1% to 12.5%).

Reported *C. trachomatis* prevalence varies widely, depending upon the study setting, country, and context, and the true prevalence of this infection is unknown (reviewed by Kalwij et al. 2010). Only a few population-based serological studies have been reported. In the early 1990s in Brazil and the Philippines, *C. trachomatis* seroprevalences were 20.2% and 23.0%, respectively (Smith et al. 2002). In Sweden, *C. trachomatis* seroprevalence was 24.7% among the sexually active women (Jonsson et al. 1995), i.e. at the same level as in our material in the 1990s. In Japan, EIA-based *C. trachomatis* seroprevalence in 9,652 pregnant women decreased from 47.8, 35.3 and 30.5% to 33.1, 30.8 and 23.6% from 1987 to 1997 in women under 19-, 20–24- and 25–29-year-olds, respectively (Yamamoto et al. 1998). According to a seroepidemiological study conducted among pregnant women in Tallinn, the overall prevalence of *C. trachomatis* IgG antibodies was 20.2% in 1997 (Kibur et al. 2000).

We showed that *C. trachomatis* incidence rates based on seroconversion rates were in line with the incidence rates based on laboratory notifications in the NIDR. The increasing incidence trend contradicts decreasing *C. trachomatis* seroprevalence rates. Steady increases of chlamydial incidence rates have also
occurred in the USA, the UK and Australia throughout the last decade (www.cdc.gov/std/Chlamydia2005/CTSurvSupp2005Short.pdf, www.hpa.org.uk, www9.health.gov.au). The temporal downward trend in the overall STI has been attributed to behavioural modification in response to the early HIV campaigns in the 1980s and the beginning of the 1990s (Fenton & Lowndes 2004). Thereafter, however, the rates of newly diagnosed STIs have gradually increased since the mid 1990s in most Western European countries (Fenton & Lowndes 2004). A recent survey among Finnish school students (School Health Promotion Study 2011) showed that the age of sexual debut is decreasing, sexual risk-taking behaviour is increasing and condom use is inconsistent.

It is challenging to understand the discrepancy between the incident chlamydial cases and the seroprevalence rates. Earlier diagnosis and treatment with simple single-dose medication may have led to reduced immune responses and thus lower transient antibody levels in the population – the so-called arrested immunity hypothesis (Brunham & Rekart 2005). The earlier the infection is detected, the less is the immune response and protective immunity against repeat *C. trachomatis* infection. An extensive use of antibiotic treatment, other than penisilline, for other infections (for example respiratory infections) from the beginning of the 1990s (Rautakorpi et al. 2009) could have an impact in decreasing *C. trachomatis* seroprevalence by eradicating an asymptomatic *C. trachomatis* infection at the same time.

Possible changes in the distribution of *C. trachomatis* serotypes over time could have an impact on decreasing chlamydial seroprevalence. The increasing *C. trachomatis* incidence could be a result of detecting symptomatic infections caused by virulent *C. trachomatis* serotypes linked to stronger symptoms (Lan et al. 1995, Anttila et al. 2001, Geisler et al. 2003, Millman et al. 2006, Kari et al. 2008). In contrast, serotype replacement with less immunogenic strains could result in lower chlamydia antibody levels and seroprevalence. One explanation may involve the current intensive STI testing with improved (PCR) methods, resulting in more cases and partners being detected and reported (Dicker et al. 2000). However, the similar increasing trend in the FMC and NIDR data indicates that the trend is real.

Nevertheless, the decreasing *C. trachomatis* seroprevalence in the female population suggests that the overall infection burden (persistent infection/late complications) might be decreasing despite increasing reported case rates. This fits in with the observation that there has been a declining trend in long-term sequelae of *C. trachomatis* infection, e.g. PID (Scholes et al. 2012), preterm
delivery (Jakobsson et al. 2008) and ectopic pregnancy (Mäkinen et al. 1996, Bakken 2006) since the 1990s. However, hospitalized sequelae (disturbances in early/late pregnancy, PID, pelvic pain and infertility) after detection of C. trachomatis are not rare in Finland (Kortekangas-Savolainen et al. 2012).

Population estimates of chlamydia prevalence in the United States also decreased from 1999 to 2008 by 40% in participants aged 14–39 years despite increasing reported case rates (Datta et al. 2012), even after including statistical adjustment for the proportion of NAATs and controlling also for ethnicity or region (Satterwhite et al. 2011).

Generally, the C. trachomatis infection rate (cumulative incidence) is considered to be a reasonable approximation of prevalence (Dicker et al. 1998) and a reliable follow-up indicator of Chlamydia control programmes. Our C. trachomatis infection prevalence data and the seroprevalence data in the same birth cohort of women (less than 23 years of age in 2001–2003; 10.0% vs. 9.2%) fit, which suggests that the use of seroprevalence data is a feasible indicator of the overall infection burden.

**6.1.2 Repeat C. trachomatis infections**

We found that annual rates of repeat C. trachomatis cases increased from 1995 to 2009 by 48 and 39 percent in women and men, respectively. This observation partly explains the increasing C. trachomatis incidence trend, which contradicts decreasing Chlamydia seroprevalence in an asymptomatic population. Among 177 138 newly diagnosed C. trachomatis infections, the prevalence of infection showed a moderate increase in three consecutive birth cohorts of subjects under 23 years of age (1979–1981, 1982–1984 and 1985–1987). In 2009, repeat C. trachomatis infection diagnoses represented 25 and 20 percent of the registered episodes in women and men. Although women represented almost 60% of all reported Chlamydia cases, the proportional increase of cases was higher in males, especially among 15–19-year-old men from 1995 to 2009 (150%). Rietmeijer et al. (2008) also reported a 46.6% increase in registered Chlamydia incidence rates in the USA males between 1999 and 2004.

These increasing rates may reflect a true increase of infection in males or may be a result of changed diagnostic techniques (NAATs) or simply the fact that men may nowadays participate more in Chlamydia testing. Datta et al. (2012) reported a 53% reduction of male Chlamydia prevalence from 1998 to 2008 in the USA, again contradicting the increasing incidence. Testing rates rose 3.5-fold at the
same time (Scholes et al. 2012). Modern means of communication (text messages/e-mail) may also have improved contact tracing (Menon-Johannsson et al. 2006, Guy et al. 2012b, Lim et al. 2012).

Comparison of repeat infection rates in different studies is not straightforward because of different study populations, varying study settings, and, in particular, varying follow-up times (Fung et al. 2007, LaMontagne et al. 2007, Gaydos et al. 2008). In a review (Hosenfeld et al. 2009), the overall median proportion of females reinfected with Chlamydia was 13.9% (38 studies) and modelled reinfection within 12 months demonstrated peak rates of 19% to 20% at 8 to 10 months. Data in a recent study from the USA revealed even earlier peak of 15% prevalence for repeat infection after the first Chlamydia episode, within 2 to 5 months among 15–24-year old women (Heijne et al. 2013). In a systematic internet-based Chlamydia Screening Implementation Programme in the Netherlands, all Chlamydia-positive participants automatically received a testkit after 6 months to facilitate early detection of repeat infections. Baseline Chlamydia positivity was 4.1% and when retested (uptake 66%), positivity doubled to 8.8% (Götz et al. 2013). In the UK, the incidence of the repeat Chlamydia testing was 18.4 and 26.1 per 100 person years, according to data from the National Chlamydia Screening Programme and from the genitourinary medicine clinics, respectively, among 15 to 24-year-olds in 2010 (Woodhall et al. 2013). These results suggest that the rates of annual multiple infections in our study (7.3% in females and 5.3% in males) were low. It is noteworthy that individuals once tested positive are not retested systematically and this has an impact on evaluation of recurrence rate in our study.

Moreover, the concept of repeat chlamydial infection should be properly defined, as the reasons for recurrence may vary. Batteiger et al. (2010a) classified repeat C. trachomatis infections as reinfections or treatment failures, using an algorithm. They found that 84.2% of repeat chlamydial infections were definite, probable or possible reinfections, 13.7% were probable or possible treatment failures and 2.2% persisted without documented treatment. A level of 92.2% treatment use-effectiveness (percentage of successful treatments among all evaluable infection episodes) was, however, observed.

Strengths of our repeat infection study include the population-based material, a consistent reporting system and personal identifiers, which enabled identification of repeat chlamydial cases. A missing detail was the annual number of Chlamydia tests performed, but, for example, in the Oulu University Hospital area in Northern Finland, the number of chlamydial PCR tests rose from 9158 to
13,392 from 2000 to 2010. During this time period, the number of reported *C. trachomatis* cases remained materially stable in this hospital district (www.3ktl.fi/stat), suggesting that the greater number of tests only partly explains the increasing *Chlamydia* rates. Changing over to more sensitive diagnostic tests from 1995 to 2000 partly explains the increased infection incidence (Hiltunen-Back *et al.* 2003) but not the differences observed between the various age groups or the increased rates of repeated diagnoses.

The increased amount of repeat *Chlamydia* infection diagnoses is in line with increasing risk-taking behaviour among adolescents. Nowadays, sexual activity starts earlier than in the 1980s, the number of sex partners has increased and the use of condoms is irregular in Finland and elsewhere (Navarro *et al.* 2002, Wellings *et al.* 2006, Nikula *et al.* 2007, Satterwhite *et al.* 2007, Falah-Hassani *et al.* 2009, Kuortti *et al.* 2009, School Health Promotion Study 2011). Using birth control pills has become more common since 1980s and leaves responsibility of the protection for girls and women. Hiltunen-Back *et al.* (2001) reported previously from Finland that the median time from exposure to becoming diagnosed with *C. trachomatis* infection was almost five weeks and during that time as many as one third of the patients with *C. trachomatis* infection reported having sex with a second partner. In our study, the proportion of repeat infections continued to increase or remained at a high level up to 30 years of age in both sexes, suggesting that sexual risk-taking behaviour continues. A similar phenomenon has also been demonstrated in acquisition of high-risk human papilloma virus in Finland (Lehtinen *et al.* 2006, Kaasila *et al.* 2009).

In conclusion, repeated chlamydial screening and early retesting are very important to reduce occurrence of repeat *Chlamydia* infection.

### 6.1.3 Occurrence of *C. trachomatis* serotypes over time

Over the time period from the 1980s to 2005, antibodies against *C. trachomatis* serotypes G and E were the most prevalent in all age groups. While the proportion of serotype G decreased from 41.3% in the 1980s to 26.5% in the 2000s, serotypes D and E peaked in the 1990s, keeping the overall occurrence of *C. trachomatis* fairly constant over the follow-up time. This suggests that the decreasing *C. trachomatis* seroprevalence (Lyytikäinen *et al.* 2008) is not explained by a shift from one *C. trachomatis* serotype profile in the 1980s to another in the 2000s.
However, the serotype profile, i.e., ranked order of *C. trachomatis* serotypes changed between 1980s and 1990s but reverted back in the 2000s. Correspondingly the prevalence of women with antibodies against two or more serotypes increased in the 1990s in all age groups and then decreased again. Possibly concomitant emergence of Russian *C. trachomatis* serotypes in the 1990s following opening of the border between Finland and Russia after 1991 (Lyytikäinen *et al.* 2008) might also have contributed for the temporary replacement of serotypes G and J by D and E. While serotypes D and E are the most prevalent worldwide (Quint *et al.* 2007), the high prevalence of serotype G in our study parallels findings also from the Russia (Smelov *et al.* 2009), Italy (Donati *et al.* 2009, Marangoni *et al.* 2012) and Greece (Papadogeorgakis *et al.* 2010). We also compared incidences of serotypes D and E over the decades in different parts of Finland. In South Finland incidence of serotype E increased from 1980 to 1990s and decreased from 1990s to 2000s. Incidence of the serotype E increased statistically significantly in Eastern Finland (close to St. Petersburg, Russia) from 1980s to 1990s and also from 1990s to 2000s. In other parts of Finland no statistically significant changes considering serotype E were observed.

The serotype distributions found in our study are different to the *C. trachomatis* serological methods or PCR-based genotype distributions reported in Finnish clinical materials where the most prevalent serogroups were BED and GF in 1980s by MIF method (Saikku & Wang 1987) and genotypes E (40%), F (28%), and G (13%) in 2008 by *ompA* genotyping (Niemi *et al.* 2011). Our study is, however, based on a population-based serum bank concerning pregnant women (Lehtinen *et al.* 2006, Lyytikäinen *et al.* 2008). In the FMC, no data of past history of *C. trachomatis* infection is available and presumably a part of the serologically identified infections have remained asymptomatic/left untreated.

Serotypes D and E have been suggested to be least immunogenic (Byrne 2010). Serotype E demonstrates the least genetic and serospecific variability as regards *OmpA* (Baehr *et al.* 1988) and immune selection. The high level of resistance of serotype E to the inhibitory effects of IFN-γ (Morrison *et al.* 2000, Öhman *et al.* 2011) is in accordance with it being found twice as frequently in women with persistent infection (Morre *et al.* 2002b). There may, however, be other still undefined virulence attributes linked to the competitive advantages of serotypes D and E.

In conclusion, the temporary *C. trachomatis* serotype replacement and the increase of multiple serotype infections among healthy Finnish females may reflect changes in sexual risk-taking behaviour (population mixing) in the 1990s.
6.2 Methodological considerations and limitations of the study

6.2.1 Study population

The Finnish Maternity Cohort (FMC) is the world’s largest serum bank, with 1.9 million serum samples. It covers virtually the entire (over 99%) fertile-aged female population who have become pregnant in Finland since 1983. Although the FMC serum bank covers almost all pregnant women and is population based, it lacks infertile women and to a large part also adolescent women who have not yet been pregnant. However, due to most often clear-cut antibody response, serum banks are ideal for systematic evaluation of occurrence of chlamydial infections and suitable for epidemiological trend analyses together with the national surveillance data. Although the size of the FMC population was large in this study, the seroconversion numbers were quite small at each age-calendar time points, which can have an impact in comparison of seroincidence and the PCR-based incidence in the NIDR.

The NIDR contains all reported *C. trachomatis* cases in 1995–2009 based on a consistent laboratory reporting system with coverage of nearly 100%. This material is also population-based with personal identifiers, which enabled identification of repeat chlamydial diagnoses. However, no data on tests of cure, treatment failure or information about partners are available in the NIDR. According to the Communicable Diseases Act and Decree of 1987, testing and treatment of *C. trachomatis* is free of charge in community healthcare services, student healthcare centres and STD clinics, and probably most *Chlamydia* cases seek testing and treatment when they suspect an infection, have symptoms or if there is partner notification. However, some patients may be treated according to symptoms without testing, and those cases are not registered in the NIDR. Another missed detail in the NIDR is the amount of annual *C. trachomatis* tests performed in laboratories, but, for example, in one large hospital district (Oulu University Hospital), during 2000–2010, the number of chlamydial tests performed rose from 9158 to 13 392 and at the same time the number of reported *C. trachomatis* cases remain stable.

6.2.2 Detection of *C. trachomatis* infection

The serological tests used in our studies have been extensively evaluated (Puolakkainen *et al.* 1998, Morre *et al.* 2002a, Land *et al.* 2003). After infection
with *C. trachomatis*, IgG antibodies are detectable by MIF or EIA for at least 5 to 10 years (Puolakkainen et al. 1986). Antibodies and metabolites of low molecular weight have been reasonably stable for several years in serum stored at -25°C degrees (Jellum et al. 1995).

The peptide-based EIA we used is considered to be species-specific with minimal cross-reactivity with antibodies against *Chlamydia pneumonia* (Land et al. 2003). Reproducibility of the assay as reported by the manufacturer is high (SD 0.061, coefficient of variation 4.9% and correlation of results with those obtained by means of another peptide-based method is greater than 0.9, indicating the high specificity and sensitivity of the test.

*C. trachomatis* serotypes were identified by using the MIF method, which has for long been considered the gold standard for definition of *C. trachomatis* serovars (Wang & Greyston 1970). Study subjects testing positive for *C. trachomatis* antibodies had been preliminarily analysed by using the EIA method and 10.6% of the material was negative for all serotypes when using the MIF method, probably due to the fact that MIF is less sensitive than the EIA method. MIF is a subjective test, and the results depend not only on the observer, but also on the equipment and reagents used. In the MIF method, the reading conditions must be strictly controlled, cases and controls must be tested simultaneously, and the results should be read blindly by only one observer. In the current work, all slides were prepared by an experienced laboratory assistant, who maintained the same antigen density and quality in all test runs. All slides were read and interpreted by one experienced researcher using a single high-quality microscope; thus the bias resulting from these conditions was diminished.

### 6.3 Future prospects

We showed that serology is a useful tool in *C. trachomatis* epidemiology and can give new insights when evaluating the occurrence of *C. trachomatis* infection in the population. Our results of decreasing *C. trachomatis* seroprevalence and increasing incidence show that the course of chlamydial infection is not straightforward.

In general, chlamydial immunity is considered to be weak and short-lived, and possible arrested immunity partly contributes to diminished protection against repeat chlamydial infection. It would be important to study the impact of earlier diagnoses and effective treatment on immune responses. If the arrested immunity hypothesis (Brunham et al. 2005) is proven to be correct, it would explain the
opposite findings of *Chlamydia* seroprevalence and incidence trends in this study. It would also fit with our result of an increasing number of repeat genital chlamydial infections in the NIDR.

Our finding of decreasing *Chlamydia* seroprevalence is in accordance with recent reports of declining *Chlamydia*-related complications in many Western countries. Most of the studies in which the role of *C. trachomatis* as a causative agent in ectopic pregnancy, preterm delivery and miscarriage has been evaluated have been in clinical settings and have involved small numbers of patients. It would be important to study the role of *C. trachomatis* in these complications at a population level.

As men also have high rates of chlamydial infections, which can remain undiagnosed (with a possibility of onward transmission), further studies are needed to evaluate infection prevalence in male populations, for example by screening men during military/non-military service. At the same time, sexual health counselling could be provided.

The increasing number of both single and repeat *C. trachomatis* infection especially among adolescents is alarming and warrants intervention efforts. Instead of opportunistic screening for women under 25 years of age - as per current recommendations - it might be more effective to systematically screen sexually active young women and men, and the usage of modern means such as internet-based testing and self-sampling could improve the participation rate. *Chlamydia* testing in the case of partner change or multiple sex partners has proven effective in finding new cases. Retesting *Chlamydia* from 3 up to 12 months after positive testing could succeed in lowering the repeat infection rate and onward transmission of the *Chlamydia* infection. According to our results, the fact that repeat infection rates are increasing and peak at the age of 25–30 years postulates that retesting/recscreening should be added to the current national guideline of *Chlamydia* screening, and it might also be reasonable to extend that in population up to 30 years of age.

The present findings of this study help in settling the stage for evaluation of the effectiveness of *C. trachomatis* control programmes. This would help efforts to reduce the *Chlamydia* burden. Regular evaluation of the control programmes is also important.
7 Conclusions

A decline in *C. trachomatis* seroprevalence since the middle of the 1990s has been discovered in Finland in healthy Finnish women. Simultaneously demonstrated rising seroconversion rates in the same population are in line with the reported laboratory notifications in the NIDR, suggesting that the increasing infection rates are not only due to increased testing rates and more sensitive test methods. Based on our results, population-based *C. trachomatis* incidence rates can be demonstrated by seroconversions detected in paired serum samples, and serology can be used in epidemiological studies.

Decreasing *C. trachomatis* seroprevalence coincides with the decrease in *C. trachomatis*-associated complications, such as PID and TFI, since the 1990s. To elucidate the discordant *C. trachomatis* seroprevalence and incidence trends we clarified the role of repeat *C. trachomatis* infections over time in the NIDR and found a gradual increase in repeat diagnoses of *C. trachomatis* from 1995 to 2009. Most of the *C. trachomatis* infections, both single and repeated, were found among young women and men. The proportion of repeat infections remained at a high level up to 30 years of age, suggesting that there are core groups who continue sexual risk-taking behaviour. We also found that the increase in *C. trachomatis* infections was more rapid in males than in females, especially in young men, and they serve as a reservoir of *C. trachomatis*.

One hypothesis for the conflicting *C. trachomatis* seroprevalence and incidence rates was that possible changes in *C. trachomatis* serotype distribution could have a role in *C. trachomatis* immunogenicity, e.g. serotype replacement with fewer immunogenic types, and thus lower antibody levels over time. However, no permanent changes in *C. trachomatis* serotype prevalences occurred during the study period. Nevertheless, the numbers of women seropositive for \( \geq 2 \) serotypes peaked in the 1990s, and serotypes G and J were replaced by serotypes E and D, which may be reflected by changes in sexually active population in Finland.

The increasing *C. trachomatis* incidence and simultaneously decreasing seroprevalence warrants further studies in *C. trachomatis* microbial ecology (for example, studies of immunological responses in relation to early detection and treatment). Knowledge of *C. trachomatis* seroprevalence and incidence trends is extremely important since these trends reflect prevention efforts in the population. Comparison of serological and PCR-based occurrence of *C. trachomatis* reveals
the people most in need of intervention, such as screening and sexual health education.

It is not likely that a *C. trachomatis* vaccine will be available in the near future, and other effective intervention attempts must be implemented to prevent *C. trachomatis* transmission. Modern means of communication such as Internet websites and social media could be suitable for reaching young people who are the main risk population for having *C. trachomatis* infection. Testing for *Chlamydia* should be more easily accessible and information about STIs offered earlier than in the current school curriculum (8th grade). The national HPV vaccination programme starting next year could provide an excellent possibility for a “teachable moment” concerning sexual health education for girls at the vaccination site. Simultaneously, attention should be focused on reaching boys and young men.
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