Minna Honkila

CHLAMYDIA TRACHOMATIS
INFECTIONS IN NEONATES AND INFANTS
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**Abstract**

Around 3% of pregnant women in Finland have genital *Chlamydia trachomatis* infection, which can be transmitted from mother to newborn at birth. The risk of transmission has been reported to be 10–70% in vaginal deliveries resulting in conjunctivitis in 10–30% of cases and lower respiratory tract infection in 0–20% of cases. Although usually benign, *Chlamydia trachomatis* infections in infancy may result in long-term consequences, including conjunctival and corneal scarring, chronic cough and abnormal lung function.

Based on the transmission rates published in prior studies, chlamydial conjunctivitis should occur in approximately 200 infants and chlamydial lower respiratory tract infection in 100 infants each year in our country, but in clinical practice we rarely encounter or diagnose infants with *Chlamydia trachomatis* infections. To investigate the reason for this discrepancy and to improve the recognition of *Chlamydia trachomatis*-infected infants, we set out to study the risk of vertical transmission of *Chlamydia trachomatis* in a population-based setting, to describe the typical features of *Chlamydia trachomatis* infections in infants and to evaluate the occurrence of *Chlamydia trachomatis* in both neonatal conjunctivitis and lower respiratory tract infections in infants.

When studying the probability of vertical transmission of *Chlamydia trachomatis* a search through two national health registers for 1996–2011 yielded 206 children aged less than four years with a possible *Chlamydia trachomatis* infection. In a cohort of 933 823 births this represented an occurrence of 0.22 per 1000 live births (95% confidence interval 0.19–0.25). The risk of vertical transmission of *Chlamydia trachomatis* leading to a symptomatic infection in infancy was 0.8–1.8%.

A review of patient charts to evaluate the typical features of *Chlamydia trachomatis* infections in infants (124/206) revealed that one-third of the infants with chlamydial conjunctivitis (33/124) had spontaneous bloody discharge from the infected eyes. Almost half of the infants with chlamydial lower respiratory tract infection (15/32) had wheezing, but the characteristic staccato cough was not recorded in any of them. The median diagnostic delay from the onset of symptoms was 13 (range 4–374) days for conjunctivitis and 25 (range 10–149) days for lower respiratory tract infection. One neglected child developed bilateral corneal scars due to untreated chlamydial conjunctivitis.

To investigate the occurrence of *Chlamydia trachomatis* in neonatal conjunctivitis, 173 neonates with clinical conjunctivitis at child health clinics were examined prospectively during 2010–2015 and none of the 163 cases tested had chlamydial or gonococcal conjunctivitis (0%; 95% confidence interval 0%–2.2%). Viral conjunctivitis was diagnosed in 8/167 cases (4.8%; 95% confidence interval 2.1%–9.2%) and non-chlamydial bacterial conjunctivitis in 58/160 (36%; 95% confidence interval 29%–44%).

To investigate the occurrence of *Chlamydia trachomatis* in lower respiratory tract infections, 228 infants aged less than six months with lower respiratory tract infection presenting at the paediatric emergency department of Oulu University Hospital were examined prospectively over a period of a complete epidemiological year. One infant (0.4%; 95% confidence interval 0.01%–2.4%) had lower respiratory tract infection caused by *Chlamydia trachomatis* and another was diagnosed with whooping cough (0.4%; 95% confidence interval 0.01%–2.4%). The majority of the infants with lower respiratory tract infection (203/228) had a respiratory viral infection.

It may be concluded that the risk of mother-to-child transmission of *Chlamydia trachomatis* leading to a clinical illness in the infant in this era of nucleic acid-based diagnostics was less than 2%, which is significantly lower than in earlier studies. The population-based prevalence of neonatal chlamydial conjunctivitis in primary care was less than 2% and that of chlamydial lower respiratory tract infection in a hospital setting less than 2.5%. The long-term prognosis for *Chlamydia trachomatis* infections in infancy was good. Common respiratory viruses were detected in 5% of the neonatal conjunctivitis cases.

**Keywords:** bacterial conjunctivitis, child, *Chlamydia trachomatis*, chlamydial pneumonia, inclusion conjunctivitis, infant, neonatal conjunctivitis, neonate, occurrence, ophthalmia neonatorum, prognosis, respiratory tract infection, serology, vertical transmission, viral conjunctivitis
Noin 3 %:lla suomalaisista raskaana olevista naisista on klamydian (Chlamydia trachomatis) aiheuttama sukupuoletauti, joka voi tarttua äidistä lapsen synnytyksessä. Tartuntariskin on raportoitu olevan alatisyksynynysessä noin 10–70 %. Noin 10–30 % tartunnan saaneista lapsista sairastuu silmätulehdusen ja 0–20 % keuhkokuumeen. Vaikka imeväisten klamydianinfektioit ovat useimmiten lieviä tautuja, imeväissä sairastettua klamydianinfektiot voi aiheuttaa silmän. silmätulehdusta ja seuravaloen arpeutumista, pitkittynyttä yskää ja keuhkfunktion alennemaa.

Aiempien tutkimusten perusteella arvioimme, että Suomessa sairastuu vuosittain noin 200 imeväistä klamydian aiheuttaman silmätulehdukseen ja noin 100 imeväistä klamydiakeuhkokuumeeen. Klininen kokemus on kuitenkin, että kohtaan amme klamydiaa sairastavia imenväisiä varsin harvoin. Tämän ongelman ratkaisemiseksi ja klamydian sairastajien imeväisten parannuksen sektoriin suunnittelimme tutkimuksen, joka tarkoituksena on selvittää väestöyksikön riski klamydian tarttumiselle äidistä vastasyntyneeseen, kuvata imeväisten klamydianinfektioiden tyypilliset piirteet sekä selvittää klamydian osoi imeväisten silmätulehduksesssa ja alle kuuden kuukauden ikäisten imeväisten alahengitystieinfektioissa.


Vastasyntyneen silmätulehdustutkimukseen rekrytoitiin 173 alle 30 päivän ikäistä lasta Oulun kaukon lastenneuvoloissa vuosina 2010–2015. Klamydian tai tippurin aiheuttamaa silmätulehdusta ei todettu yhdekkään 163:stä tutkitusta vauvasta (0 %; 95 % luottamusväli 0 %–2,2 %). Viruksen aiheutuma silmätulehdus todettiin kahdeksalla vauvalle (4,8 %; 95 % luottamusväli 2,1 %–9,2 %) ja jonkin muun bakteerin kuin klamydian aiheuttama silmätulehdus 58:lla vauvalle (36 %; 95 % luottamus- väli 29 %–44 %).

Imenväisten alahengitystieinfektionilmaantuvuuden selittämiseksi tarkastimme 228 alle kuuden kuukauden ikäistä lasta Oulan yliopiston yläharjoituksen alahengitystieinfektioissa vuoden 2010–2015. Klamydian tai tippurin aiheuttama silmätulehdus ei todettu yhdekkään 228:stä alahengitystieinfektionilmaantuvuudesta (0 %; 95 % luottamusväli 0 %–2,4 %) ja hinkuyskä niin ikään yhdekkään (0,4 %; 95 % luottamusväli 0,01 %–2,4 %) ja hinkuyskä niin ikään yhdekkään (0,4 %; 95 % luottamusväli 0,01 %–2,4 %). Valtaosalla (203/228) alahengitystieinfektio- oireista imeväistä viruksen aiheuttaa infektio.

Yhteenvetona voimme todeta, että klamydia aiheuttaa äidistä lapsen alle 2 %:ssä synnytyksissä, mikä on huomattavasti harvinaistempaa kuin aiemmin on johtu. Klamydian aiheuttamien silmätuleh- dusten esiintyvyys oli alle 2 % ja alahengitystieinfektioiden alle 2,5 % alle 2,5 % alueemme lapsiväestössä. Kla- mydian aiheuttamatt pitkäaikaishaitat olivat harvinaisia. Tavallisten hengitystieinfektion osoi vasta- synnytyneiden silmätulehdusissa oli 5 %.

Asiasanat: bakteerikonjunktivi, Chlamydia trachomatis, ennuste, hengitystieinfektio, ilmanantuus, imeväinen, inklusiokonjunktivi, keuhkokuume, klamydia, lapsi, serologia, tarttuminen, viruskonjunktivi
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Oulu, August 2018  Minna Honkila
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BP</td>
<td><em>Bordetella pertussis</em></td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CT</td>
<td><em>Chlamydia trachomatis</em></td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EB</td>
<td>Elementary body</td>
</tr>
<tr>
<td>FMC</td>
<td>Finnish Maternity Cohort</td>
</tr>
<tr>
<td>GC</td>
<td><em>Neisseria gonorrhoeae</em></td>
</tr>
<tr>
<td>i.e.</td>
<td>id est</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IHPS</td>
<td>Infantile hypertrophic pyloric stenosis</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>LRTI</td>
<td>Lower respiratory tract infection</td>
</tr>
<tr>
<td>MOMP</td>
<td>Major outer membrane protein</td>
</tr>
<tr>
<td>NAAT</td>
<td>Nucleic acid amplification test</td>
</tr>
<tr>
<td>NIDR</td>
<td>National Infectious Diseases Register</td>
</tr>
<tr>
<td>NP</td>
<td>Nasopharyngeal</td>
</tr>
<tr>
<td>ON</td>
<td>Ophthalmia neonatorum</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PYR</td>
<td>Person-years at risk</td>
</tr>
<tr>
<td>RB</td>
<td>Reticulate body</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RSV</td>
<td>Respiratory syncytial virus</td>
</tr>
<tr>
<td>Tdap</td>
<td>Tetanus toxoid, reduced diphtheria toxoid and acellular pertussis</td>
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</tbody>
</table>
List of original publications

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


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1 Introduction

Around 3–6% of pregnant women in the western world harbour *Chlamydia trachomatis* (CT) (Kurkinen, Sarkkinen, Kärpänoja, & Ranta, 2006; O’Higgins *et al*., 2017; G. I. Rours *et al*., 2005; G. I. Rours *et al*., 2011), which can be transmitted to the newborn baby during delivery. Such transmission has been reported to occur in 10–70% of vaginal deliveries (Chandler *et al*., 1977; Dannevig, Schive, Straume, & Melby, 1991; M. R. Hammerschlag, Anderka, Semine, McComb, & McCormack, 1979; M. R. Hammerschlag, Chandler, Alexander, English, & Koutsky, 1982; Skjeldstad, Johansen, & Dalen, 1987; Yu, Wu, Li, & Hu, 2009). Chlamydia-infected infants may develop conjunctivitis or lower respiratory tract infection (LRTI) as a clinical disease, the risk of conjunctivitis being approximately 10–30% and that of LRTI up to 20% if the mother has an untreated genital CT infection (Chandler *et al*., 1977; Dannevig *et al*., 1991; M. R. Hammerschlag *et al*., 1979; M. R. Hammerschlag *et al*., 1982; Skjeldstad *et al*., 1987). CT infections in infants are usually mild, but untreated conjunctivitis has been thought to lead to conjunctival and corneal scars and LRTI to chronic cough and abnormal lung function (Forster, Dawson, & Schachter, 1970; Harrison, Taussig, & Fulginiti, 1982; Mordhorst & Dawson, 1971; Weiss, Newcomb, & Beem, 1986).

The prevalence of genital CT infection among pregnant women in Finland is estimated to be 2.7% based on the nucleic acid positivity in the first-catch urine (Kurkinen *et al*., 2006). This figure means that around 1600 infants annually are exposed to CT at delivery. Based on the point estimate of the transmission and disease rates published in previous studies (Rosenman, Mahon, Downs, & Kleiman, 2003), conjunctivitis due to CT should occur in approximately 200 infants and LRTI in 100 infants each year in our country, but despite these figures we rarely encounter infants with CT infections in clinical practice. This may be due to failure to recognise CT-infected infants, leading to underdiagnosis, or alternatively, the transmission rates reported previously, originating from the 1970s and 1980s, overestimate the risk of transmission. We therefore set out to study the risk of vertical transmission of CT in a population-based setting, to describe the typical features of CT infections in infants and to evaluate the occurrence of CT in both neonatal conjunctivitis and LRTIs in infants. If CT infections in infants are underdiagnosed in our country, we should change our clinical practice, i.e. screen pregnant women for genital chlamydia and be more active in testing neonates and infants with either conjunctivitis or LRTI for CT.
2 Review of the literature

2.1 Microbiology of Chlamydia trachomatis

2.1.1 The pathogen

CT is a gram-negative obligate intracellular bacterial pathogen belonging to the genus Chlamydia (Everett, Bush, & Andersen, 1999). It is among the smallest living organisms, with a genome size of 1000 kB (Stephens et al., 1998). CT infects the columnar epithelial cells in humans, its only natural hosts, in whom it causes three types of infection: ocular, genital tract and pulmonary (Witkin, Minis, Athanasiou, Leizer, & Linhares, 2017). CT has an outer cell membrane consisting of proteins and lipopolysaccharides. Earlier studies suggested that CT lacks any peptidoglycan structure for maintaining cell shape in the cell wall, but more recent studies have demonstrated the existence of functional peptidoglycan in replicating CT and other chlamydial species (Liechti et al., 2014). One of the major proteins at the cell surface of CT is the major outer membrane protein (MOMP), which comprises 60% of the total outer membrane proteins (Caldwell, Kromhout, & Schachter, 1981). CT consists of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and ribosomes, but it uses energy phosphate compounds from the host cell during its growth and replication (Darville, 2005).

2.1.2 Genotypes

CT can be divided into more than 20 genotypes, previously known as immunotypes (Millman et al., 2004). The classification was originally based on serological responses to the MOMP epitopes (Stephens et al., 1998; Wang, Kuo, Barnes, Stephens, & Grayston, 1985), but nowadays it involves both serotyping of the MOMP and genotyping of its gene, ompA (Millman et al., 2004). Genotypes A–C are the primary agents of ocular trachoma (Table 1). Multiple infections with these genotypes cause roughening and scarring of the upper eyelid, which leads to inward deviation of the eyelashes (Grayston, Wang, Yeh, & Kuo, 1985). The deviant eyelashes can then further rub and scar the cornea, causing visual impairment. Globally, trachoma is the leading cause of infectious blindness (Flaxman et al., 2017). Genotypes D–K produce infections of the genital tract and are the most common sexually transmitted genotypes (Bebear & de Barbeyrac, 2009; Millman
et al., 2004). Genotypes L1–L3 are associated with infections of the lymphatic system known as lymphogranuloma venereum, which cause more severe and more invasive diseases than the other sexually transmitted genotypes. Vertical transmission of the L1–L3 genotypes is rare, however.

Table 1. *Chlamydia trachomatis* strains and their associations with clinical diseases.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Clinical manifestation</th>
<th>Spreading</th>
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<tbody>
<tr>
<td>A–C</td>
<td>Ocular trachoma</td>
<td>Hand-to-eye, fomites, flies</td>
</tr>
<tr>
<td>D–K</td>
<td>Cervicitis, urethritis, proctitis, conjunctivitis, infant pneumonia</td>
<td>Sexual, perinatal</td>
</tr>
<tr>
<td>L1–L3</td>
<td>Lymphogranuloma venereum</td>
<td>Sexual</td>
</tr>
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### 2.1.3 Developmental cycle

CT has a unique developmental cycle, alternating between two developmental forms, the elementary body (EB) and the reticulate body (RB) (Hackstadt, Fischer, Scidmore, Rockey, & Heinzen, 1997). The EB is a small extracellular form of CT (300–400 nm in diameter) that is infectious but metabolically inactive. It does not form spores, but it has a spore-like structure, making it stable outside the cell. The biphasic developmental cycle starts when the EB attaches to and enters the epithelial cell (Figure 1). The exact mechanism of entry is unclear, although one suggested mechanism is receptor-mediated endocytosis (Wyrick et al., 1989). Inside the host cell, EB is surrounded by a protective endosomal membrane. Surface antigens on the EB prevent fusion of the endosome with the host cell lysosome, thus protecting the CT from enzymatic destruction. Within six to eight hours of entry, the EB differentiates into RB, a larger, replicative form of CT. The RB then starts to divide and multiply by binary fission inside the endosome, now called an intracytoplasmic inclusion. After multiple divisions, the rapidly increased number of RBs redifferentiate back into a new generation of EBs. Within 48–72 hours post infection, the EBs are released from the host cell by cytolysis, exocytosis or extrusion of the whole inclusion and are able to infect new, as yet uninfected host cells. Despite reorganising back into EBs, the RBs can enter a non-replicative but viable state causing persistent chlamydial infection (Witkin et al., 2017). Penicillin-class antibiotics, iron deficiency and interferon-gamma (IFN-γ) are exogenous factors that may induce persistent chlamydial infection (Beatty, Morrison, & Byrne, 1994).
2.1.4 Host responses

CT infects the columnar epithelial cells of the cervix, urethra and conjunctiva. After infection with CT, the epithelial cells start to secrete several pro-inflammatory mediators, such as interleukin-1 (IL-1), IL-6, IL-8 and tumor necrosis factor-alpha (Brunham & Rey-Ladino, 2005). These cytokines trigger inflammation and result in an influx of immune cells such as lymphocytes, macrophages and dendritic cells into the epithelium. There is an inflammatory reaction at the infection site that is mediated by both CD4+ and CD8+ T lymphocytes with a Th1 phenotype, which play an important role in the protective immunity against CT through the production of IFN-\(\gamma\) (Loomis & Starnbach, 2002). IFN-\(\gamma\) in particular plays an essential role in the outcome of the infection, as it inhibits reproduction of the pathogen (Perry, Feilzer, & Caldwell, 1997). In most cases, the primary reaction is
transient and does not result in tissue damage, but reinfections with CT increase the risk of damage to the reproductive system (Hillis, Owens, Marchbanks, Amsterdam, & Mac Kenzie, 1997). It is unclear how much of the tissue destruction is primarily immunologically mediated and how much is caused by the direct cytotoxic effect of the pathogen (Hvid et al., 2007; J. L. Shaw et al., 2011).

CT infection usually lasts several months, but spontaneous recovery has been reported in 20% of polymerase chain reaction (PCR)-positive patients and in 30% of culture-positive adults (Joyner, Douglas, Foster, & Judson, 2002; Parks, Dixon, Richey, & Hook, 1997). The likelihood of being culture-negative in the follow-up increases with age and the duration of the follow-up (Parks et al., 1997). The reason for a relatively long infection before spontaneous clearance is unknown, but it has been suggested that the recruitment of T cells to the genital tract at a sufficient threshold may take several months (Igi etseme & Rank, 1991). It has also been hypothesised that shortening the duration of CT infection in the host with antimicrobials may dull the development of immunity against CT and therefore increase susceptibility to CT in the population (Brunham & Rey-Ladino, 2005).

### 2.2 Epidemiology of Chlamydia trachomatis

The cornerstone of communicable disease surveillance is the detection and reporting of infections, but surveillance systems for CT vary considerably across the world. In Europe, for instance, 21 countries, including Finland, have comprehensive surveillance systems and in 20 countries CT infections are monitored through compulsory notifications, whereas seven European countries have sentinel systems, which only obtain CT diagnoses from selected health-care providers (European Centre for Disease Prevention and Control, 2017).

CT is the world’s most common sexually transmitted infection, with more than 100 million new cases occurring each year (World Health Organization, 2012). The annual incidence rates of CT infection vary globally and range from 9.2 to 72.6 per 1000 in women and from 6.2 to 54.2 per 1000 in men depending on the population studied and the surveillance system maintained (World Health Organization, 2012). In Europe, a total of 394,163 CT infections were reported in the 27 EU/EEA member states in 2015, which corresponds to an incidence rate of 173 per 100,000 of population for the 21 countries with a comprehensive surveillance system (European Centre for Disease Prevention and Control, 2017). The corresponding figures in the USA were 1,598,354 cases for an annual incidence rate of 497.3 per 100,000 (Centers for Disease Control and Prevention, 2017). In Finland the number
of confirmed CT cases was 13,572 in 2015, which corresponds to an annual incidence rate of 248 per 100,000 (Table 2) (European Centre for Disease Prevention and Control, 2017).

The overall trend in reported CT cases in Europe has remained relatively stable during the past few years, although notification rates vary considerably from one country to another, mainly reflecting differences in chlamydia testing rather than actual differences in incidence rates (European Centre for Disease Prevention and Control, 2017). This is attributable to the fact that the United Kingdom was responsible for more than 58% of all the CT notifications in Europe (Table 2), since that country implemented a large screening programme targeted at 15–24-year-olds in 2008.

The incidence of CT infection remains highest in the young and in women. In Europe, the annual notification rate in 2015 was 197 per 100,000 in women and 147 per 100,000 in men, while the overall male-to-female ratio was 0.7:1 (European Centre for Disease Prevention and Control, 2017). The figures are similar in the USA with respect to gender as the annual incidence rate was 657.3 per 100,000 in women and 330.5 per 100,000 in men (Centers for Disease Control and Prevention, 2017). The incidence of CT peaks in young people aged 15–24 years. Young adults in this age range accounted for 61% of all notified CT cases with known age in Europe in 2015, while in the USA the annual incidence rates were 2,643.8 per 100,000 among 20–24-year-olds and 1,929.2 per 100,000 among those aged 15–19 years (Centers for Disease Control and Prevention, 2017). In Finland 80% of the CT infections were reported in the age group 15–29 years, and women accounted for approximately 60% of the reported infections in 2016 (Liitsola & Hiltunen-Back, 2016). The other factors predictive of a positive test for CT include Afro-American race, low socioeconomic status and living in a rural area (Centers for Disease Control and Prevention, 2017). For example, the prevalence of CT infection is approximately six times higher in black inhabitants of the USA than in whites. Behavioural risk factors include having multiple sex partners, a new sex partner, a sex partner with concurrent partners, or a sex partner who has a sexually transmitted infection (K. A. Workowski, Bolan, & Centers for Disease Control and Prevention, 2015).
Table 2. Confirmed Chlamydia trachomatis genital infections in selected countries in Europe in 2015 (European Centre for Disease Prevention and Control, 2017).

<table>
<thead>
<tr>
<th>Country</th>
<th>Surveillance system</th>
<th>Confirmed cases</th>
<th>Rate per 100 000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td>Comprehensive</td>
<td>31 782</td>
<td>561.5</td>
</tr>
<tr>
<td>Finland</td>
<td>Comprehensive</td>
<td>13 572</td>
<td>248.0</td>
</tr>
<tr>
<td>Iceland</td>
<td>Comprehensive</td>
<td>1989</td>
<td>604.4</td>
</tr>
<tr>
<td>Norway</td>
<td>Comprehensive</td>
<td>25 207</td>
<td>487.9</td>
</tr>
<tr>
<td>Sweden</td>
<td>Comprehensive</td>
<td>36 955</td>
<td>379.1</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Comprehensive</td>
<td>226 809</td>
<td>349.6</td>
</tr>
</tbody>
</table>

2.3 Chlamydia trachomatis infections in adults

2.3.1 Clinical manifestations

CT is a serious threat to reproductive health in women, in whom it causes cervicitis, urethritis and Bartholinitis (Bebear & de Barbeyrac, 2009; Paavonen, 2012; Peipert, 2003). In untreated women, CT can spread to the upper genital tract and cause endometritis, salpingitis and pelvic inflammatory disease. Salpingitis may lead to severe complications such as ectopic pregnancy and infertility, through scarring the Fallopian tubes. It may also cause perihepatitis and conjunctivitis. Around 75–90% of CT-infected women are asymptomatic, however (Peipert, 2003; Stamm, 1999).

CT is the leading cause of non-gonococcal urethritis in men (Bebear & de Barbeyrac, 2009; Paavonen, 2012; Peipert, 2003). Epididymitis is a possible complication of chlamydial urethritis, especially in young men, but reproductive consequences are rare. CT may also cause acute proctitis, proctocolitis, reactive arthritis and Reiter’s syndrome (urethritis, conjunctivitis, arthritis and mucocutaneous lesions) in men, and also conjunctivitis. Approximately 50–85% of genital CT infections in men are asymptomatic (Cecil et al., 2001; Zelin, Robinson, Ridgway, Allason-Jones, & Williams, 1995).

2.3.2 Diagnostics

Urogenital CT infection can be diagnosed by testing first-void urine or collecting vaginal or endocervical swabs from women and by testing first-void urine or collecting urethral swabs from men (Centers for Disease Control and Prevention, 2014). In adults, the nucleic acid amplification test (NAAT) is the only non-culture test method that is recommended for routine use, because its performance with
respect to sensitivity (>90%) and specificity (>99%) is better than that of any of
other test available (Gaydos, Theodore, Dalesio, Wood, & Quinn, 2004; Jaschek,
Gaydos, Welsh, & Quinn, 1993; Lee et al., 1995). Culture has long been a gold
standard for its diagnosis, but the sensitivity of this method is only 65% in women
(Lee et al., 1995) and 50% in men (Quinn, Welsh et al., 1996), although it is
possible to obtain higher level of sensitivity by an experienced analyst.

2.3.3 Treatment and prevention

Treatment

Antimicrobial treatment must eradicate both the extracellular and intracellular
forms of CT, thus requiring either a regimen with a long half-time in tissues or
multiple doses of the regimen. The treatment options are a single dose of oral
azithromycin or seven days of oral doxycycline (Geisler et al., 2015; Martin et al.,
1992). Azithromycin and doxycycline have been found to be equally efficacious in
a randomised controlled trial that reported treatment failure in 4% (5/141) of those
receiving azithromycin and 2% (3/125) receiving doxycycline (difference 2%; 95%
confidence interval (CI) 0%–6%) (Martin et al., 1992).

Prevention

Strategies for controlling and preventing CT infections in adolescents and adults
include health education to change risk behaviour, use of a condom and detection
of infected individuals through testing or screening followed by antimicrobial
treatment. Behavioural interventions have managed to reduce the burden of the
disease (Shain et al., 2002), but the most important device for preventing its
transmission is the condom. In a study based on a review of the medical records of
1455 patients with known exposure to CT, condom use was found to be effective
in its prevention, as 13% of the condom users were diagnosed with CT infection as
compared with 34% of the inconsistent condom users (adjusted odds ratio 0.10; 95%
CI 0.01–0.83) (Niccolai, Rowhani-Rahbar, Jenkins, Green, & Dunne, 2005).

The majority of CT-infected individuals are asymptomatic or minimally
symptomatic and do not seek medical care. Programmes designed to control and
prevent CT infections include the detection of infected individuals through
organised and opportunistic screening followed by treatment with antimicrobials.
The efficacy of organised screening programmes has mainly been evaluated through the reduction in reproductive complications associated with CT, but evidence from randomised controlled trials remains controversial. Scholes et al. found in their study, conducted among ~2600 women who were randomly assigned to screening and usual care, that the women allocated to the screening group had a 56% lower incidence of pelvic inflammatory disease than those in the standard care group (relative risk 0.44; 95% CI 0.20–0.90) (Scholes et al., 1996), whilst another randomised controlled trial reported an incidence of pelvic inflammatory disease being 1.3% (15/1191) in screened women compared with 1.9% (23/1186) in controls (relative risk 0.65; 95% confidence interval 0.34–1.2) (Oakeshott et al., 2010).

Vaccinating adolescents before the initiation of their sex life, or alternatively women at reproductive age, would be a more effective and cheaper approach for controlling and preventing CT infections than screening programmes (Sahu et al., 2018; Schautteet, De Clercq, & Vanrompay, 2011), but although there have been many attempts to develop an effective and safe vaccine that induces protective immune responses, there is currently no vaccine available against CT (Brunham & Rey-Ladino, 2005). However, the safety of the biodegradable polymeric nanoparticles-based experimental vaccines, and the available CT vaccine candidates suggest that clinical trials in humans may be initiated in the near future (Sahu et al., 2018).

2.3.4 Transmission

Evaluation of the risk of CT transmission from an infected adult to an uninfected one is a challenging undertaking, but it was found in one previous study that among 101 couples in which at least one member was CT-positive in terms of PCR, half of the partners were also infected (Quinn, Gaydos et al., 1996).

2.4 Chlamydia trachomatis infections in pregnant women

2.4.1 Epidemiology

The prevalences of genital CT infection in pregnant women based on NAAT from either first void urine or a vaginal or an endocervical swab range from 3% to 6% in western countries (Kurkinen et al., 2006; O'Higgins et al., 2017; G. I. Rours et al.,
2005; G. I. Rours et al., 2011). Somewhat higher figures, from 2% to 18%, tended to be quoted during the era of culture-based diagnostics, i.e. between the 1970s and 1990s (Chandler et al., 1977; Dannevig et al., 1991; FitzSimmons, Callahan, Shanahan, & Jungkind, 1986; Frommell, Rothenberg, Wang, & McIntosh, 1979; M. R. Hammerschlag et al., 1979; Heggie, Lumicao, Stuart, & Gyves, 1981; Mårdh, Helin, Bobeck, Lurin, & Nilsson, 1980; Schachter, Grossman, Holt, Sweet, & Spector, 1979; Schachter, Sweet et al., 1986; Skjeldstad et al., 1987; Wu, Shen, & Liu, 1999). Developing countries bear the highest burden of the disease, with reported figures of up to 29% for CT positivity among pregnant women (Laga et al., 1986). The predictive factors for CT infection are principally the same as in non-pregnant women, as young age, Afro-American race and being unmarried contribute significantly to the higher prevalence of CT infection in pregnant women (Chandler et al., 1977; Frommell et al., 1979; Heggie et al., 1981; Mårdh et al., 1980; Preece, Ades, Thompson, & Brooks, 1989; Ratelle, Keno, Hardwood, & Etkind, 1997; E. Shaw, Roberts, & Connor, 1995; Skjeldstad et al., 1987).

**Epidemiology in Finland**

Kurkinen et al., investigating the prevalence of CT infection in the maternity healthcare population of the Päijät-Häme region of Finland in 2003–2004, found that 1.3% of the pregnant women (10/793) were NAAT-positive for CT when screened during the first trimester, and the overall prevalence when taking into account data from the National Infectious Diseases Register (NIDR) concerning pregnant women diagnosed with a CT infection during the same period was 2.7% (95% CI 1.8%–4.2%) (Kurkinen et al., 2006). Furthermore, Lyytikäinen et al., who investigated the annual incidence rates of CT infection among Finnish women by serological methods using Finnish Maternity Cohort (FMC) serum bank samples obtained during two consecutive pregnancies within five years, analysed a total of 7999 serum samples from randomly selected women for CT-specific immunoglobulin (Ig) G antibodies and found that 161 seroconversions had occurred in 6632 women who were seronegative at the baseline. The average seroconversion rate for the total population was 12.7/1000 person-years at risk (PYR) (Lyytikäinen et al., 2008). One earlier study from the 1980s reported a CT prevalence of 1.1% among 92 pregnant women, but this was conducted before the introduction of NAAT-based test methods (Honkonen, Punnonen, & Terho, 1983). In addition, Niinimäki et al. reported that 3.7% (496/13 547) of women ≥18 years
who underwent medical abortion had a positive CT test result (Niinimäki et al., 2011).

### 2.4.2 Perinatal complications

CT infection during pregnancy is associated with an increased risk of perinatal complications and adverse pregnancy outcomes. The risk of preterm labour has been documented in several prospective studies (Claman, Toye, Peeling, Jessamine, & Belcher, 1995; Gencay et al., 1995; Martin et al., 1982; G. I. Rours, Duijts et al., 2011). In a large population-based cohort study of 4055 pregnant women, NAAT-positive mothers were significantly more likely to have a preterm delivery before 32 weeks of gestation, with an odds ratio of 4.4 (95% CI 1.3–15), and before 35 weeks of gestation with an odds ratio of 2.7 (95% CI 1.1–6.5) relative to CT-negative mothers (G. I. Rours et al., 2011). Similar findings have been observed among women with CT IgM or IgG seropositivity compared with a seronegative group (Claman et al., 1995; Gencay et al., 1995). In addition, one case-control study performed among preterm and term babies reported a figure of 29% for CT positivity among the preterm babies but none among the term babies (Bekler et al., 2012). The risk of stillbirth and neonatal death has been found to be 10 times greater among women with genital CT infection than among uninfected controls in a prospective observational study (Martin et al., 1982), and the frequencies of maternal infection, chorioamnionitis and meconium-stained amniotic fluid have also been found to be higher among seropositive women (Gencay et al., 1995). To determine the association between CT and adverse pregnancy outcomes, a few researchers have managed to detect CT DNA and its antigen from human placental tissues, supporting a role for CT in adverse pregnancy outcomes (Baud et al., 2011; Gencay et al., 1997).

By contrast, there are various reports of similar pregnancy outcomes among mothers infected with CT and uninfected women (FitzSimmons et al., 1986; Heggie et al., 1981; Preece et al., 1989), and one additional study found that the frequencies of prematurity and low Apgar scores did not differ significantly between CT-infected and uninfected mothers after antimicrobial treatment for CT (Much & Yeh, 1991).
2.4.3 Treatment

A single dose of oral azithromycin, seven days of oral erythromycin and seven days of oral amoxicillin have all been found in randomised controlled trials to be safe and equally efficacious for treating CT infections during pregnancy (Alary et al., 1994; Jacobson, Autry, Kirby, Liverman, & Motley, 2001), although amoxicillin should be considered an alternative regimen, because exposure to penicillin-class antibiotics may induce persistence of the pathogen (Wyrick, 2010). Erythromycin, on the other hand, has been reported to cause significant gastrointestinal side-effects in up to 50% of pregnant women resulting in a treatment failure, thus limiting compliance with the treatment (Alary et al., 1994; Schachter et al., 1986).

2.5 Vertical transmission of Chlamydia trachomatis

CT is primarily transmitted to infants via exposure to an infected mother’s genital flora during passage through the birth canal (Bell et al., 1994; Yu et al., 2009). Infection after Caesarean section is rare and is associated mainly with early rupture of the amniotic membrane (Givner, Rennels, Woodward, & Huang, 1981; La Scola, Paroski, Burzynski, & Faden, 1984), but there are case reports based on CT antigen detection showing that the infection may also occur after Caesarean delivery with intact amniotic membranes, suggesting a transmembrane or transplacental route of transmission (Shariat, Young, & Abedin, 1992). Serological studies have documented the same genotypes in both mothers and their offspring, confirming the vertical route of transmission (Bell et al., 1992). No evidence exists to support postnatal transmission of CT from the mother or other family members (Darville, 2005; M. R. Hammerschlag, 1994).

Infants born to CT-infected women may become infected at one or more anatomical sites, including the conjunctiva, nasopharynx, rectum and vagina (Schachter et al., 1979; Schachter et al., 1986). The transmission rates among culture-positive mothers can reach as high as 67%, but the variation is wide-ranging, from 9% to 67% for asymptomatic infection (Table 3) (Chandler et al., 1977; Dannevig et al., 1991; Datta et al., 1988; Frommell et al., 1979; M. R. Hammerschlag et al., 1979; M. R. Hammerschlag et al., 1982; Heggie et al., 1981; Mårdh et al., 1980; Preece, Anderson, & Thompson, 1989; Preece et al., 1989; Schachter et al., 1979; Schachter et al., 1986; Skjeldestad et al., 1987; Wu et al., 1999; Yu et al., 2009). Less data exist on the risk of transmission from NAAT-positive mothers. One previous study conducted among 33 selected NAAT-positive
mothers in China demonstrated a transmission rate of 24% for asymptomatic nasopharyngeal (NP) infection in infants (Yu et al., 2009). The nasopharynx is the most common site of asymptomatic infection, with up to 80% of infected infants having positive cultures at that site (M. R. Hammerschlag et al., 1982), followed by asymptomatic rectal and vaginal infections in up to 10% (Schachter et al., 1986).

Symptomatic CT infections in infants usually present with conjunctivitis and/or a LRTI. Conjunctivitis has been found to occur in 9–44% of infants exposed to CT at birth and LRTI in 0–20%, depending on the population studied and the test method used (Table 3) (Chandler et al., 1977; Dannevig et al., 1991; Datta et al., 1988; Frommell et al., 1979; M. R. Hammerschlag et al., 1982; Heggie et al., 1981; Mårdh et al., 1980; Preece et al., 1989; Preece et al., 1989; Schachter et al., 1979; Schachter, Grossman et al., 1986; Skjeldstad et al., 1987; Wu et al., 1999). Pooling data from studies published on the vertical transmission rate since the 1970s, the point estimate for the incidence of conjunctivitis has been calculated to be 15% and that for LRTI 7% among infants exposed to CT at delivery (Rosenman et al., 2003).

There are no known factors that may contribute to the risk of developing a symptomatic illness. One previous study suggested that breast-feeding may increase the risk of conjunctivitis, as 67% of a series of breast-fed infants developed conjunctivitis compared with 22% of bottle-fed infants (p>0.05) (Frommell et al., 1979). A later prospective study of CT infections in infants failed to confirm this association, however (M. R. Hammerschlag et al., 1982).
Table 3. Previous studies of *Chlamydia trachomatis* infections in mothers during pregnancy or post-partum and the outcome for their infants.

<table>
<thead>
<tr>
<th>Study</th>
<th>Mothers</th>
<th>Infants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT-positive detection method</td>
<td>Asymptomatic infection&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>n (%)</td>
</tr>
<tr>
<td>Chandler et al. (Chandler et al., 1977)</td>
<td>18 Culture</td>
<td>12 (67)</td>
</tr>
<tr>
<td>Frommell et al. (Frommell et al., 1979)</td>
<td>18 Culture</td>
<td>11 (61)</td>
</tr>
<tr>
<td>Hammerschlag et al. (M. R. Hammerschlag et al., 1979)</td>
<td>6 Culture</td>
<td>4 (67)</td>
</tr>
<tr>
<td>Schachter et al. (Schachter et al., 1979)</td>
<td>20 Culture</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Mårdh et al. (Mårdh et al., 1980)</td>
<td>23 Culture</td>
<td>5 (22)</td>
</tr>
<tr>
<td>Heggie et al. (Heggie et al., 1981)</td>
<td>95 Culture</td>
<td>27 (28)</td>
</tr>
<tr>
<td>Hammerschlag et al. (M. R. Hammerschlag et al., 1982)</td>
<td>60 Culture</td>
<td>22 (37)</td>
</tr>
<tr>
<td>Schachter et al. (Schachter et al., 1986)</td>
<td>131 Culture</td>
<td>47 (36)</td>
</tr>
<tr>
<td>Skjeldestad et al. (Skjeldestad et al., 1987)</td>
<td>35 Culture</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Datta et al. (Datta et al., 1988)</td>
<td>49 Culture</td>
<td>18 (37)</td>
</tr>
<tr>
<td>Preece et al. (Preece et al., 1989)</td>
<td>174 Antigen</td>
<td>43 (25)</td>
</tr>
<tr>
<td>Dannevig et al. (Dannevig et al., 1991)</td>
<td>9 Culture or antigen</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Wu et al. (Wu et al., 1999)</td>
<td>20 Culture</td>
<td>11 (55)</td>
</tr>
<tr>
<td>Yu et al. (Yu et al., 2009)</td>
<td>33 NAAT</td>
<td>8 (24)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Number of infants with CT infection born to mothers who had CT infection during pregnancy or post-partum; CT, *Chlamydia trachomatis*; NAAT, nucleic acid amplification test; NR, not reported.

### 2.5.1 Prevention of vertical transmission

Screening and treating chlamydia-infected women during pregnancy is currently the only available strategy for preventing transmission of the pathogen to infants.
However, only a few countries have routine screening programmes for CT during pregnancy. In Europe, Estonia has compulsory screening during pregnancy whereas opportunistic testing is available to both symptomatic and asymptomatic individuals in Denmark, Finland, Italy, Romania, Sweden and the United Kingdom (Low et al., 2018). Several studies have shown that screening and treating chlamydia-infected women with antimicrobials is effective in preventing CT infections in infants (FitzSimmons et al., 1986; McMillan et al., 1985; Ottesen, Sahl, Herbstman, Friis, & Philipsen, 1996; Schachter et al., 1986). Two prospective studies compared the clinical outcomes for infants born to culture-positive mothers treated with erythromycin during pregnancy with those for untreated subjects and showed that treatment with erythromycin significantly reduced the risk of CT infection in infants, as 0–7% of the those born to treated mothers developed CT infection as compared with 24–50% of the infants of untreated mothers (p<0.001 and p<0.04) (McMillan et al., 1985; Schachter et al., 1986). Similarly, in two patient series none of the infants born to treated mothers (n=10 and n=16) showed any evidence of CT infection four to eight weeks post-partum (FitzSimmons et al., 1986; Ottesen et al., 1996).

Studies using decision analysis models based on health economics to estimate the costs of screening pregnant women for genital CT suggest that screening either all pregnant women or those aged less than 25 years is cost-effective (Ong et al., 2016; Ottesen et al., 1996; G. I. Rours et al., 2016). In the Netherlands, where the prevalence of CT infection among pregnant women is 3.9%, screening all pregnant women for genital CT has been estimated to be a cost-saving intervention and the savings increase further when screening is targeted at women aged below 30 years (G. I. Rours et al., 2011; G. I. Rours et al., 2016). In another study from Australia, where a CT prevalence of 3% was assumed, the screening of pregnant women aged ≤25 has been estimated to improve the quality of life in adjusted units and provide cost savings (Ong et al., 2016). If the prevalence had been greater than 11% the screening of all pregnant women would have resulted in cost savings. Screening for CT during pregnancy has been found to be an acceptable and non-stigmatizing procedure (Pereboom et al., 2014).
2.6 *Chlamydia trachomatis* infections in infants

2.6.1 History

CT was first discovered in 1907 by Halberstädt and von Prowazek, who described the typical CT inclusion bodies, Halberstädt-Prowazek bodies, in conjunctival scrapings from monkeys (Halberstadt & von Prowazek, 1907). T’ang and colleagues were the first to culture CT from ocular samples of trachoma in 1957 (Tang, Chang, Huang, & Wang, 1957) and two years later, in 1959, CT was also isolated from the neonatal conjunctivitis inclusions (Jones, 1961), whereupon the mothers of the infected infants were found to have inclusions in the epithelial cells of the cervix and the fathers in the epithelial cells of the urethra. These findings revealed the epidemiology of sexually transmitted disease. In the 1960s CT was confirmed as being a bacterium, having been defined since 1935 as a virus, referred to as the trachoma or inclusion conjunctivitis (TRIC) agent.

LRTI caused by CT was recognised in 1975, when Schachter and colleagues published a case report of an infant born to a CT-positive mother (Schachter, Lum, Gooding, & Ostler, 1975). The infant first developed chlamydial inclusion conjunctivitis and later pneumonitis associated with vertically transmitted CT. It was previously thought that CT spreads to the lacrimal ducts and lower respiratory tract from the infected conjunctiva, but since many of the infants with LRTI lack a history of chlamydial conjunctivitis, the infected eye is no longer regarded as the likely portal of entry (C. J. Chen et al., 2007; Chiang et al., 2005; Schachter et al., 1979).

2.6.2 Conjunctivitis

CT has been shown to be a major causative agent for neonatal conjunctivitis in many hospitals (Kakar et al., 2010; Rapoza, Quinn, Kiessling, & Taylor, 1986; I. G. Rours et al., 2008), but its proportion varies considerably between geographical locations, ranging from 0% to 64% (Table 4) (Dannevig, Straume, & Melby, 1992; Di Bartolomeo et al., 2001; Kakar et al., 2010; Krohn, Hillier, Bell, Kronmal, & Grayston, 1993; Mohile, Deorari, Satpathy, Sharma, & Singh, 2002; Pak, Kim, & Lee, 2017; Persson, Ronnerstam, Svanberg, & Polberger, 1986; Prentice, Hutchinson, & Taylor-Robinsin, 1977; Rapoza et al., 1986; I. G. Rours et al., 2008; I. Sandström, 1987a; I. Sandström, Kallings, & Melen, 1988; K. I. Sandström et al., 1984; T. P. Yip et al., 2007). The incubation period for chlamydial conjunctivitis
after delivery is typically five to 14 days (Di Bartolomeo et al., 2001; M. R. Hammerschlag et al., 1982; Heggie et al., 1981; Preece et al., 1989; Quirke & Cullinane, 2008; Rapoza et al., 1986; Rees, Tait, Hobson, Byng, & Johnson, 1977). Presentation before five days of age is rare, but there are a few reports of symptoms being present as early as three days of age (Rees et al., 1977; Schachter et al., 1986). The male-to-female ratio is 1:1 (Quirke & Cullinane, 2008; Rapoza et al., 1986).

Chlamydial conjunctivitis can be either unilateral or bilateral. The first reports from the late 1960s mentioned unilateral presentation in 75% of cases (6/8) (Watson & Gairdner, 1968), whilst one recent study found 73% of chlamydial conjunctivitis cases (27/37) to be bilateral (I. G. Rours et al., 2008). Mucopurulent discharge, redness and swelling of the eyelids have been found to be distinctive clinical symptoms of chlamydial conjunctivitis (Table 5) (American Academy of Pediatrics, 2015a; M. Hammerschlag, 2011; M. Hammerschlag & Kohlhoff, 2014; Rees et al., 1977; I. G. Rours et al., 2008; Royal College of Paediatrics and Child Health, 2016; Schachter et al., 1979; K. A. Workowski et al., 2015), although the manifestations vary extensively and range from mild injection to severe conjunctivitis (Persson et al., 1986; Watson & Gairdner, 1968). Severe chlamydial conjunctivitis is characterised by chemosis and pseudomembrane formation (Chang, Cheng, & Kwong, 2006; Persson et al., 1986; Preece et al., 1989). In addition, the conjunctivae are often friable and may bleed after swabbing (M. Hammerschlag, 2011; M. Hammerschlag & Kohlhoff, 2014). The hospitalisation rate for infants with chlamydial conjunctivitis has been reported to be up to 10% (Jain, 1999).
Table 4. Proportions of *Chlamydia trachomatis* involvement in neonatal conjunctivitis in previous studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Detection method</th>
<th>Conjunctivitis n</th>
<th>CT n</th>
<th>CT n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rees et al. (Rees et al., 1977)</td>
<td>UK</td>
<td>Culture</td>
<td>103</td>
<td>33</td>
<td>33 (32)</td>
</tr>
<tr>
<td>Prentice et al. (Prentice et al., UK 1977)</td>
<td>UK</td>
<td>Culture</td>
<td>149 (eyes)</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Persson et al. (Persson, Ronnerstam, Svanberg, &amp; Pohla, 1983)</td>
<td>Sweden</td>
<td>Culture</td>
<td>281</td>
<td>39</td>
<td>39 (14)</td>
</tr>
<tr>
<td>Sandström et al. (K. I., Sandström et al., 1984)</td>
<td>USA</td>
<td>Culture</td>
<td>19</td>
<td>5</td>
<td>5 (26)</td>
</tr>
<tr>
<td>Sandström et al. (I. Sandström et al., 1987a)</td>
<td>Sweden</td>
<td>Culture</td>
<td>107</td>
<td>13</td>
<td>13 (12)</td>
</tr>
<tr>
<td>Sandström et al. (I. Sandström et al., 1988)</td>
<td>Sweden</td>
<td>Culture</td>
<td>160</td>
<td>33</td>
<td>33 (21)</td>
</tr>
<tr>
<td>Dannevig et al. (Dannevig et al., 1992)</td>
<td>Norway</td>
<td>Culture</td>
<td>269</td>
<td>16</td>
<td>16 (6)</td>
</tr>
<tr>
<td>Krohn et al. (Krohn et al., 1993)</td>
<td>USA</td>
<td>Antigen, culture</td>
<td>97</td>
<td>2</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Di Bartolomeo et al. (Di Bartolomeo et al., 2001)</td>
<td>Argentina</td>
<td>Antigen, NAAT</td>
<td>332</td>
<td>26</td>
<td>26 (8)</td>
</tr>
<tr>
<td>Mohile et al. (Mohile et al., 2002)</td>
<td>India</td>
<td>Antigen</td>
<td>70</td>
<td>17</td>
<td>17 (24)</td>
</tr>
<tr>
<td>Yip et al. (T. P. Yip et al., 2007)</td>
<td>Hong Kong</td>
<td>Antigen, culture, NAAT</td>
<td>192</td>
<td>24</td>
<td>24 (13)</td>
</tr>
<tr>
<td>Rours et al. (I. G. Rours et al., 2008)</td>
<td>Netherlands</td>
<td>NAAT</td>
<td>42</td>
<td>27</td>
<td>27 (64)</td>
</tr>
<tr>
<td>Kakar et al. (Kakar et al., 2010)</td>
<td>India</td>
<td>Antigen</td>
<td>58</td>
<td>18</td>
<td>18 (31)</td>
</tr>
<tr>
<td>Pak et al. (Pak et al., 2017)</td>
<td>Korea</td>
<td>Culture</td>
<td>82</td>
<td>0</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

1 Previously treated and 36 untreated; 2 42 studied retrospectively and 23 prospectively; 3 Neonatal bacterial conjunctivitis; CT, *Chlamydia trachomatis*; NAAT, nucleic acid amplification test.
Table 5. Clinical characteristics of *Chlamydia trachomatis* infections in infants as reported in paediatric textbooks and handbooks (American Academy of Pediatrics, 2015a; M. Hammerschlag, 2011; M. Hammerschlag & Kohlhoff, 2014; Royal College of Paediatrics and Child Health, 2016; K. Workowski & Bolan, 2015).

<table>
<thead>
<tr>
<th>Symptom</th>
<th>The Blue Book</th>
<th>CDC</th>
<th>Feigin and Cherry’s</th>
<th>Nelson</th>
<th>Red Book</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctivitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purulent discharge</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Erythema</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Swollen eyelids</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Blood-stained discharge</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
<td>(+)</td>
<td>–</td>
</tr>
<tr>
<td>Onset</td>
<td>5–14 days</td>
<td>5–12 days</td>
<td>5–14 days</td>
<td>5–14 days</td>
<td>Few days to several weeks</td>
</tr>
</tbody>
</table>

LRTI

<table>
<thead>
<tr>
<th>Symptom</th>
<th>The Blue Book</th>
<th>CDC</th>
<th>Feigin and Cherry’s</th>
<th>Nelson</th>
<th>Red Book</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afebrile &lt;38°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cough</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rales</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>–</td>
<td>–</td>
<td>+/-</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Wheezing</td>
<td>+/-</td>
<td>–</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Tachypnoea</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Staccato cough</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Onset</td>
<td>1–3 months</td>
<td>1–3 months</td>
<td>4–12 weeks</td>
<td>1–3 months</td>
<td>2–19 weeks</td>
</tr>
</tbody>
</table>

*, yes; –, not mentioned; +/- uncommon; (+), after swabbing; CDC, Centers for Disease Control and Prevention; LRTI, lower respiratory tract infection.

2.6.3 Lower respiratory tract infection

The proportion of CT in the aetiology of LRTIs in infants has been reported to be 7–36% in a hospital setting (C. J. Chen *et al*., 2007; Dereli *et al*., 1996; Li *et al*., 2015; Numazaki, Chiba, Yamanaka, Umetsu, & Nakao, 1984; G. I. Rours *et al*., 2009; Souza *et al*., 2012) and 6–19% in outpatient clinic settings (Table 6) (Berman, Shanks, Feiten, Horgan, & Rumack, 1990; Nascimento-Carvalho *et al*., 2017; Zar, Van Dyk, Yeats, & Hanslo, 1999). The majority of infants with chlamydial LRTI become symptomatic between four and eight weeks of age (Beem & Saxon, 1977; Datta *et al*., 1988; Heggie *et al*., 1981; Preece *et al*., 1989; Schachter *et al*., 1979; Schachter *et al*., 1986). Some may already have symptoms related to CT at the age
of two weeks, but cases extending beyond six months are rare (Takase, Khono, Kinoshita, & Niki, 1990). The onset of the disease is often insidious, with gradually worsening respiratory symptoms (Tipple, Beem, & Saxon, 1979).

The association between CT and a distinctive pneumonia syndrome was first confirmed by Beem and Saxon, who described characteristic features of chlamydial LRTI among 20 Afro-American infants aged less than six months with respiratory tract shedding of CT, although they failed to isolate CT from open lung biopsies obtained from two affected infants (Beem & Saxon, 1977). They found that the infants with chlamydial LRTI presented with afebrile, chronic-course tachypnoea and a repetitive staccato cough. Other observational studies have later confirmed these features as being typical of chlamydial LRTI and they have been widely accepted as such in paediatric textbooks (Table 5) (American Academy of Pediatrics, 2015a; C. J. Chen et al., 2007; Chiang et al., 2005; Frommell et al., 1979; M. Hammerschlag, 2011; M. Hammerschlag & Kohlhoff, 2014; M. R. Hammerschlag et al., 1982; Heggie et al., 1981; Li et al., 2015; Preece et al., 1989; Royal College of Paediatrics and Child Health, 2016; Schachter et al., 1979; K. A. Workowski et al., 2015).

A microbiological confirmation of the relation between CT and characteristic respiratory symptoms was obtained by Frommell et al., who isolated CT from lung biopsy material from an affected infant in the 1970s (Frommell, Bruhn, & Schwartzman, 1977). Obtaining lung biopsies is not a routine practise for confirming chlamydial LRTI, since the course of the disease is rarely fatal, but when a biopsy is obtained, the material may show non-specific pleural congestion, mononuclear cell infiltrates with eosinophils and aggregation of neutrophils (Arth, Von Schmidt, Grossman, & Schachter, 1978; Frommell et al., 1977).

Tachypnoea and inspiratory rales are typical findings in physical examinations of chlamydial LRTI cases (Beem & Saxon, 1977; M. R. Hammerschlag et al., 1982; Tipple et al., 1979), but wheezing is regarded as an uncommon finding (M. R. Hammerschlag, 1994; Tipple et al., 1979). Severe respiratory illnesses have been reported in 10% to 20% of CT-infected infants, although around 40% of the severely ill cases have had a viral respiratory co-infection (Li et al., 2015; Numazaki et al., 1984; Numazaki, Asanuma, & Niida, 2003). A few authors have also found that infants with chlamydial LRTI require oxygen therapy more often and over a longer time than those with LRTI of other aetiological origins (Li et al., 2015; Souza et al., 2012). The hospitalisation rate for infants with chlamydial LRTI has been shown to be approximately 10% (Jain, 1999).
Attempts have been made in case-control studies of infants with LRTI to identify features that could distinguish CT-infected individuals from those infected with other aetiological agents, and the following features have been found to be significantly more common in CT-infected cases: onset of symptoms before two months of age, gradually worsening symptoms, prolonged cough, seeking treatment at four to 11 weeks of age, hyperinflation in chest radiographs, eosinophilia and elevated serum immunoglobulins (Harrison, English, Lee, & Alexander, 1978; Li et al., 2015; Tipple et al., 1979).

Radiography

When evaluating 125 CT-infected infants with LRTI alongside over 2000 infants with LRTI of other aetiologies, Radkowski et al. did not report any radiographic findings that were specific for chlamydial LRTI, but several other groups have reported bilateral hyperexpansion and diffuse infiltrates in chest films of infants with chlamydial LRTI, and these in combination with typical clinical symptoms may be sufficient to suggest a diagnosis of CT (Beem & Saxon, 1977; Chiang et al., 2005; Frommell et al., 1979; M. R. Hammerschlag et al., 1982; Harrison et al., 1978; Heggie et al., 1981; Radkowski, Kranzler, Beem, & Tipple, 1981). Infiltrates on chest radiograms may be of many kinds, i.e. interstitial, reticular nodular, atelectasis, coalescence and bronchopneumonia, but pleural effusion and lobar consolidates are usually absent (Radkowski et al., 1981). The clinical symptoms are in any case often milder than the chest radiograph findings would suggest.
Table 6. Proportions of *Chlamydia trachomatis* involvement in lower respiratory tract infections in infants in previous studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Study design</th>
<th>Setting</th>
<th>Detection method</th>
<th>Age</th>
<th>LRTI n</th>
<th>CT n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numazaki et al. (Numazaki et al., 1984)</td>
<td>Japan</td>
<td>Prospective</td>
<td>Hospital</td>
<td>Antibody (IgM)</td>
<td>≤2 years</td>
<td>109</td>
<td>32(^1) (29)</td>
</tr>
<tr>
<td>Dereli et al. (Dereli et al., 1996)</td>
<td>Turkey</td>
<td>Retrospective</td>
<td>Hospital</td>
<td>Antibody (IgM)</td>
<td>1 month to 1 year</td>
<td>42</td>
<td>15 (36)</td>
</tr>
<tr>
<td>Berman et al. (Berman et al., 1990)</td>
<td>USA</td>
<td>Prospective</td>
<td>Outpatient clinic</td>
<td>Culture</td>
<td>2 to 12 weeks</td>
<td>84</td>
<td>16 (19)</td>
</tr>
<tr>
<td>Zar et al. (Zar et al., 1999)</td>
<td>South Africa</td>
<td>Prospective</td>
<td>Outpatient clinic</td>
<td>Antigen</td>
<td>&lt;6 months</td>
<td>100</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Chen et al. (C. J. Chen et al., 2007)</td>
<td>Taiwan</td>
<td>Prospective</td>
<td>Hospital</td>
<td>Antigen</td>
<td>&lt;6 months</td>
<td>60</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Rours et al. (G. I. Rours et al., 2009)</td>
<td>Netherlands</td>
<td>Retrospective</td>
<td>Hospital</td>
<td>NAAT</td>
<td>&lt;6 months</td>
<td>148</td>
<td>10 (7)</td>
</tr>
<tr>
<td>Souza et al. (Souza et al., 2012)</td>
<td>Brazil</td>
<td>Cross-sectional</td>
<td>Hospital</td>
<td>Antibody (IgM)</td>
<td>≤6 months</td>
<td>151</td>
<td>15 (10)</td>
</tr>
<tr>
<td>Li et al. (Li et al., 2015)</td>
<td>China</td>
<td>Prospective</td>
<td>Hospital</td>
<td>NAAT</td>
<td>&lt;6 months</td>
<td>1408</td>
<td>101 (7)</td>
</tr>
<tr>
<td>Nascimento-Carvalho et al. (Nascimento-Carvalho et al., 2017)</td>
<td>Brazil</td>
<td>Prospective</td>
<td>Outpatient clinic</td>
<td>Antibody (IgM)</td>
<td>&lt;6 months</td>
<td>28</td>
<td>3 (11)</td>
</tr>
</tbody>
</table>

\(^1\) 21 culture-positive cases; CT, *Chlamydia trachomatis*; Ig, immunoglobulin; LRTI, lower respiratory tract infection; NAAT, nucleic acid amplification test.
2.6.4 Infections at other sites

Rhinorrhoea and rhinitis have been suggested on three occasions as possible symptoms of chlamydial infection in infants (Dannevig et al., 1992; Iskandar & Naguib, 1998; Kent & Matthews, 1987). In one group of nine selected neonates who were positive for CT in an antigen test, 22% had rhinitis as the only symptom (Iskandar & Naguib, 1998). In addition, rhinitis was reported in 60% of 16 chlamydia-infected neonates (Dannevig et al., 1992), while rhinorrhoea was reported to be the prodromal symptom in 67% of a series of 18 infants with chlamydial LRTI (C. J. Chen et al., 2007). The risk of otitis media is controversial, as one group found that their CT-exposed infants had twice the rate of recurrent otitis media during the first six months of life that the unexposed infants had (Schaefer, Harrison, Boyce, & Lewis, 1985), but an earlier report did not reach such a conclusion (Schachter et al., 1979).

The role of rectal and vaginal infections in infants is unclear, as it has proved impossible to associate these findings with any clinical diseases (Datta et al., 1988; Schachter et al., 1986). It has nevertheless been suggested that CT isolated from a rectal site does indicate a gastrointestinal tract infection and is not caused by the respiratory tract shedding, as rectal shedding starts late and continues long after other sites become negative, so that on some occasions the rectum is the only culture-positive site (Schachter et al., 1986). In addition, infants with a culture-positive rectal infection have been found to have high titres of CT-specific IgM antibodies.

2.6.5 Diagnostics

The laboratory tests available for diagnosing CT infections include both direct and indirect methods (Centers for Disease Control and Prevention, 2014). CT can be detected directly either by culture or by various non-culture tests, while the indirect methods include serological tests. Specimens for CT detection should be obtained from both conjunctival and NP sites in order to increase the likelihood of a positive result, even though the symptoms are only present at one site (Johnson et al., 2002), as it has been found that up to 60% of infants with chlamydial conjunctivitis have a simultaneous asymptomatic NP infection (Rees et al., 1977; I. Sandström et al., 1988; T. P. Yip et al., 2007).
Direct detection of *Chlamydia trachomatis*

Despite the current recommendations for the use of cell culture for conjunctival and NP specimens from infants (Centers for Disease Control and Prevention, 2014), non-culture tests are widely used in clinical practice. The benefits are that they can be performed on various clinical specimens and they do not require any specific storage or transport conditions. In any case, the sensitivity of cell culture, which serves as the gold standard, is only 70–80% (Darville, 2005), whereas NAATs, which amplify the nucleic acid sequences of CT, have a sensitivity of 92–100% and a specificity of 98–100% when used with conjunctival samples (M. R. Hammerschlag *et al*., 1997; Rafiei Tabatabaei *et al*., 2012; P. P. Yip *et al*., 2008) and a sensitivity of 100% and a specificity of 97% with NP specimens when compared with that standard (M. R. Hammerschlag *et al*., 1997). NAATs cannot be used for assessing the efficacy of antimicrobial treatment, however, since many specimens are NAAT-positive at seven to 35 days post-treatment (M. R. Hammerschlag *et al*., 1997). This may be due to non-viable CT that can be detected by NAAT, but not by cell culture.

Antigen tests based on the detection of either chlamydial lipopolysaccharides (enzyme immunoassay) or MOMP (direct fluorescent antibody) have also been found to perform relatively well at conjunctival sites, with a sensitivity of 88–98% and a specificity of 88–100% in comparison to culture (M. R. Hammerschlag *et al*., 1987; M. R. Hammerschlag, Roblin, Gelling, & Worku, 1990; Rapoza *et al*., 1986; Roblin, Hammerschlag, Cummings, Williams, & Worku, 1989), but their performance with NP specimens is less satisfactory, as the sensitivity is around 33–87% and the specificity 92–96% in comparison to culture (M. R. Hammerschlag *et al*., 1987; M. R. Hammerschlag *et al*., 1990; Roblin *et al*., 1989).

**Serology**

Chlamydial IgG antibodies detected from infants during the first months of life reflect maternally transferred antibodies and correlate with the levels of maternal serum antibodies (M. R. Hammerschlag *et al*., 1979; Persson *et al*., 1986). In addition, the presence of local antibodies in tears and NP secretions are related to maternally transferred serum antibodies (M. R. Hammerschlag *et al*., 1979). In a study of 41 infants with proven chlamydial conjunctivitis, 98% had CT-specific IgG antibodies in the cord sera and neither the presence of these antibodies nor the titre differed from the results given by infants without conjunctivitis born to CT-
positive mothers, suggesting that maternal IgG antibodies do not protect infants from developing CT infection (Persson et al., 1986). Infants born to antibody-positive mothers usually lose their maternally transferred IgG-antibodies by nine months of age (Schachter et al., 1986).

In infants with chlamydial LRTI a microimmunofluorescence serum titre of $\geq 1:32$ for CT-specific IgM is considered diagnostic for CT (Darville, 2005). IgM antibodies have been observed to develop as early as five days after infection and to persist for three months (Mahony, Chernesky, Bromberg, & Schachter, 1986), and it has been suggested that IgM antibodies, measured by either enzyme immunoassay or the immunofluorescence test, might be helpful for diagnosing LRTIs caused by CT (Mahony et al., 1986; Numazaki et al., 1984; Puolakkainen et al., 1984; Schachter, Grossman, & Azimi, 1982). However, IgM antibodies have also been shown to develop after chlamydial conjunctivitis without LRTI (Persson et al., 1986), although infants with LRTI typically have higher titres of antibodies than those with conjunctivitis or an asymptomatic NP infection (Beem & Saxon, 1977). Furthermore, it has been suggested that there might be some non-specific production of chlamydial IgM antibodies in cases of infectious mononucleosis, since 19% of a series of children infected with Epstein-Barr virus (14/72) had detectable IgM antibodies against CT (Persson & Broms, 1986). This observation may explain the presence of antibodies against CT in infants with LRTI in whom CT cannot be isolated. The sensitivity of IgM antibodies is approximately 75–80%, i.e. a lack of antibody development during chlamydial LRTI has been demonstrated in 19–25% of antigen-positive cases (Dereli et al., 1996; Numazaki et al., 1984).

**Laboratory studies**

Observational studies have shown eosinophilia (from $>300$ to $\geq 400$ eosinophils/mm$^3$) in the peripheral blood of 50% of both term and preterm babies with a CT infection (Beem & Saxon, 1977; Chiang et al., 2005; Harrison et al., 1978; Heggie et al., 1981). In addition, two case-control studies showed significantly higher levels of eosinophils in the blood of infants with chlamydial LRTI than in that of infants with LRTI of other aetiologies (C. J. Chen et al., 2007; Tipple et al., 1979).
Distribution of genotypes

The most common genotype associated with chlamydial conjunctivitis and LRTI is type E, detected in about a half of all cases (Balla et al., 2017; Di Bartolomeo et al., 2001; Li et al., 2015), followed by type G and type J in conjunctivitis (Balla et al., 2017) and type F and type J in LRTI (Li et al., 2015). The distribution of genotypes detected in infants reflects the distribution of genotypes among women.

2.6.6 Prophylaxis and treatment

Prophylaxis

Several studies have shown that topical ocular prophylaxis with antimicrobial or silver nitrate ointments does not prevent the development of neonatal chlamydial conjunctivitis or infections at other sites (Beem & Saxon, 1977; Bell et al., 1987; J. Y. Chen, 1992; M. R. Hammerschlag et al., 1987; Laga et al., 1988; Rettig, Patamasucon, & Siegel, 1981). Chen et al., who conducted a large clinical trial of different prophylaxis options among 4544 neonates, found that the incidence rates of chlamydial conjunctivitis were similar: 1.3 for tetracycline, 1.5 for erythromycin, 1.7 for silver nitrate, 1.6 for no prophylaxis and 1.4 for erythromycin twice (J. Y. Chen, 1992). No research results are currently available regarding prophylactic treatment with oral antimicrobial therapy for infants born to CT-infected mothers.

Treatment

The recommendation is to treat all CT infections in infants with systemic macrolide therapy, because the infections are often multifocal (Schachter et al., 1979). Observational case series comprising 11 to 46 chlamydia-infected infants have shown that the use of oral erythromycin for 10 to 21 days resulted in clinical cure rates of 80% to 100% in the treatment of both chlamydial conjunctivitis and LRTI (Beem, Saxon, & Tipple, 1979; Rapoza et al., 1986; Rees, Tait, Hobson, Karayiannis, & Lee, 1981; I. Sandström et al., 1988; T. P. Yip et al., 2007). Although clinically cured, around 5–32% of the infants treated with oral erythromycin have had positive cultures for CT at follow-up examinations one to 35 months after treatment (Beem et al., 1979; Rapoza et al., 1986; Rees et al., 1981; I. Sandström et al., 1988; Stenberg & Mårgh, 1991; T. P. Yip et al., 2007). The efficacy of other macrolides such as azithromycin and clarithromycin has not been
documented in controlled trials, but the results in two patient series have suggested that azithromycin may be effective (Y. M. Chen, Hu, & Hou, 2010; M. R. Hammerschlag, Gelling, Roblin, Kutlin, & Jule, 1998). Chen et al. reported that 19 out of 27 (70%) retrospectively studied infants treated with two weekly doses of azithromycin were clinically and microbiologically cured when examined two weeks post treatment (Y. M. Chen et al., 2010).

Topical and oral erythromycin have been found to be equally efficacious for treating chlamydial conjunctivitis in a randomised controlled trial, with failure rates of 21% in the topical treatment group and 14% in the oral treatment group (p<0.69) (Patamasucon, Rettig, Faust, Kusmiesz, & Nelson, 1982). Topical treatment alone is not recommended, however, because it does not eradicate concomitant NP infection (Patamasucon et al., 1982; Rees et al., 1981). Oral erythromycin is excreted in tears and is thus effective in the treatment of CT conjunctivitis (Rettig, 1986).

Risk of infantile hypertrophic pyloric stenosis

Infants receiving oral erythromycin and azithromycin have an increased risk of developing infantile hypertrophic pyloric stenosis (IHPS), as evaluated in retrospective cohort studies (Cooper et al., 2002; Eberly, Eide, Thompson, & Nylund, 2015; Mahon, Rosenman, & Kleiman, 2001). The risk of IHPS is highest during the first two weeks of life. Mahon et al. reported a relative risk of 11 (95% CI 4.5–25) in infants exposed to systemic erythromycin during the first two weeks of life (Mahon et al., 2001), and similarly Cooper et al. found a nearly 8-fold increased risk after exposure before 14 days of age (adjusted incident rate ratio 7.9; 95% CI 2.0–32) (Cooper et al., 2002). Eberly et al., investigating the risk of IHPS after exposure to azithromycin, found that the risk is increased with an adjusted odds ratio of 8.3 (95% CI 2.6–26) during the first 14 days of life and an adjusted odds ratio of 3.0 (95% CI 1.2–7.2) between 15 and 42 days of life (Eberly et al., 2015). There is no evidence to support an increased risk of IHPS among those exposed to topical erythromycin (Mahon et al., 2001) or those born to mothers exposed to erythromycin during pregnancy (Louik, Werler, & Mitchell, 2002).

Treatment of parents

CT infection in an infant is an indicator that the mother is infected, so that the mother and her sexual partner should be treated appropriately and should also be
examined for other sexually transmitted diseases. In addition, contact tracing of sexual partners of CT-infected patients is the responsibility of the diagnosing physician.

### 2.6.7 Duration of infection

CT infection in infants can resolve spontaneously without treatment (San Joaquin, Herrin, & Hautala, 1980; Schachter et al., 1986). Schachter et al. conducted a prospective follow-up of asymptomatic infants with culture-proven CT infection at an NP, vaginal or rectal site, and found that they all became culture-negative by one year of age without treatment, since treatment was prescribed only for symptomatic infants (Schachter et al., 1986). The number of untreated subjects was small, however, as many of the 47 culture-positive infants were symptomatic and received antimicrobial treatment. One case report of an infant with serologically diagnosed chlamydial pneumonia noted that a cure took place without any efficacious treatment for CT (San Joaquin et al., 1980). On the other hand, a CT infection may persist if left untreated, as shown by Bell et al., who showed that 35% of infants with perinatally acquired CT were still infected at the age of 12 months and the infection may persist up to 28.5 months (Bell et al., 1992). In addition, two follow-up studies of children known to have had chlamydial conjunctivitis, reported that chlamydial inclusion bodies were found in conjunctival specimens from two children at the age of three and seven years (Forster et al., 1970; Mordhorst & Dawson, 1971), although no sequelae related to persistent conjunctivitis were detected.

### 2.6.8 Prognosis

Chlamydial infections in infants are generally considered relatively mild diseases, for although they are often chronic, they have a benign course. This is supported by various prospective studies reporting no long-term consequences in either treated and untreated infants during a follow-up of approximately 12 months (Datta et al., 1988; M. R. Hammerschlag et al., 1982; Preece et al., 1989; Schachter et al., 1979; Schachter et al., 1986).
**Conjunctivitis**

Chlamydial conjunctivitis caused by sexually transmitted genotypes was originally considered an entirely benign disease (Mordhorst & Dawson, 1971), until a study performed in the late 1960s involving eight infants with mild chlamydial conjunctivitis reported permanent conjunctival scars in two out of three cases who were followed up to the age of six to 12 months (Watson & Gairdner, 1968). A few years later, Forster et al. reported that 6/9 children (67%) with a history of CT conjunctivitis had micropannus (a vascularised sheet of fibrous tissue overlying the cornea), conjunctival scars, or both at ages ranging from five to 11.5 years (Forster et al., 1970). These findings were further confirmed by Mordhorst and colleagues, who found corneal pannus in seven children, focal corneal vascularisation in two and scarring of the conjunctiva in one out of 16 untreated children with known chlamydial conjunctivitis during the neonatal period (Mordhorst & Dawson, 1971). Other authors have also observed mild superior corneal micropannus in up to 27% of infants with a history of chlamydial conjunctivitis upon follow-up at one year of age (Chandler et al., 1977; Frommell et al., 1979). The risk of micropannus formation is thought to be higher among those treated with topical antimicrobials alone (Chandler et al., 1977; Forster et al., 1970).

**Lower respiratory tract infection**

Chlamydial LRTIs during infancy have been associated with possible adverse pulmonary outcomes in two follow-up studies (Harrison et al., 1982; Weiss et al., 1986). Harrison et al. compared 10 children under six months of age with only serological evidence of chlamydial LRTI with others having a history of LRTI of other aetiologies and found that the children with suspected chlamydial LRTI had significantly more reported chronic cough and abnormal lung function during a follow-up of up to five years than did the controls (Harrison et al., 1982). Pulmonary functions in 18 children hospitalised for chlamydial pneumonia during infancy were studied at the age of seven to eight years by Weiss et al., who found that the children with a history of chlamydial LRTI had significantly impaired pulmonary function as defined by an expiratory airflow of 25–75% and signs of air trapping as compared with the control group (Weiss et al., 1986). These children also had more physician-diagnosed asthma than the controls.
2.7 Differential diagnostics

2.7.1 Neonatal conjunctivitis

Neonatal conjunctivitis (ophthalmia neonatorum, ON), defined as conjunctivitis occurring within the first 30 days of life (American Academy of Pediatrics, 2015b), is a relatively common condition with an occurrence of 15 to 190 per 1000 live births (Dannevig et al., 1992; Di Bartolomeo et al., 2001; Fransen et al., 1987; Pierce, Ward, & Seal, 1982; Prentice et al., 1977). ON can be either infectious or non-infectious in origin and the differential diagnostics include various bacteria, viruses, nasolacrimal duct obstruction and chemical irritation.

Gonococcal conjunctivitis

*Neisseria gonorrhoeae* (GC) is the most crucial bacterial pathogen to be identified in infectious neonatal conjunctivitis. Like CT, GC is also acquired during passage through an infected birth canal. ON caused by GC usually occurs earlier than a chlamydial infection, two to five days after birth, but in some cases it may have a relatively long incubation period (up to eight days after birth) (Rees et al., 1977). Without treatment, ON gonococcal conjunctivitis can rapidly progress to severe infection and permanent visual loss (Moore, MacDonald, & Canadian Paediatric Society, Infectious Diseases and Immunization Committee, 2015).

Non-gonococcal, non-chlamydial bacterial conjunctivitis

The conjunctivae in neonates are vulnerable to infection, because newborn babies lack tears and the amount of antibacterial tear proteins is low at birth (Pierce et al., 1982). Bacterial growth is detected in about 50% of conjunctival cultures from neonates with conjunctivitis (Di Bartolomeo et al., 2001; Fransen et al., 1987; Mohile et al., 2002) and the most commonly isolated bacteria include *Staphylococcus* species, *Streptococcus* species and *Haemophilus* species (Di Bartolomeo et al., 2001; Gigliotti et al., 1981; Prentice et al., 1977; Rapoza et al., 1986; I. Sandström, 1987a; K. I. Sandström et al., 1984).

Skin, respiratory, vaginal and gastrointestinal tract bacterial pathogens are traditionally classified as pathogens when isolated from conjunctivae (American Academy of Pediatrics, 2015b). These pathogens include CT, *Corynebacterium* species, *Escherichia coli*, group A and B streptococci, *Haemophilus influenzae*
(non-typeable), Klebsiella species, Moraxella catarrhalis, GC, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pneumoniae. Moreover, Streptococcus mitis has been associated with an increased risk of conjunctivitis in one case-control study (Krohn et al., 1993). The role of Staphylococcus species in the pathogenesis of conjunctivitis is controversial, as they are frequently isolated from asymptomatic neonates (Pierce et al., 1982). Coagulase-negative staphylococci, alpha-haemolytic streptococci and Acinetobacter species are traditionally regarded as commensals when isolated from the conjunctiva. A recent study of the ocular microbiome conducted among healthy adult volunteers using both culture and DNA sequencing techniques found that Corynebacterium species, Propionibacterium species and coagulase-negative staphylococci were the most commonly identified organisms on the healthy ocular surface (Doan et al., 2016). Of the viruses, herpes simplex virus and adenovirus are associated with clinical conjunctivitis (American Academy of Pediatrics, 2015b; Gigliotti et al., 1981), but the role of the common respiratory viruses is largely unknown.

Bacteria that cause non-gonococcal, non-chlamydial conjunctivitis are probably transmitted to the eyes horizontally rather than from the birth canal (Krohn et al., 1993). This is attributable to fact that Haemophilus influenzae and Streptococcus mitis, for example, are often isolated from the conjunctivae of newborns but rarely from the vagina of the mother. In addition, the mode of delivery is not associated with an increased risk of conjunctivitis (Krohn et al., 1993), by contrast with CT or GC. The distribution of bacteria is related to the onset of the disease, as Staphylococcus aureus is the main pathogen isolated from the samples obtained in maternity wards, whereas coagulase-negative staphylococci, viridans streptococci and CT are more commonly observed after discharge (Dannevig et al., 1992). The clinical characteristics of neonatal non-gonococcal, non-chlamydial bacterial conjunctivitis are similar regardless of the bacteria involved (Prentice et al., 1977).

Nasolacrimal duct obstruction

Congenital nasolacrimal duct obstruction is the most common cause of non-infectious conjunctivitis (Paul & Shepherd, 1994) and the most common cause of failure in the treatment of non-chlamydial conjunctivitis (I. Sandström, 1987b). The incidence of nasolacrimal duct obstruction in infants ranges from 5% to 20% and the condition often resolves without surgery (Petris & Liu, 2017).
Chemical conjunctivitis

Chemical conjunctivitis is non-infectious in origin and is generally caused by the silver nitrate solution which was originally used to treat ON. This treatment was ineffective, however, as 20% of the treated newborn babies developed corneal damage and 3% suffered permanent visual impairment (Oriel, 1991; Schaller & Klauss, 2001). In the late 1800s GC was discovered to be the causative agent in two-thirds of neonatal conjunctivitis cases, and it was also found that administering 2% silver nitrate solution at birth was effective in preventing gonococcal ON (Oriel, 1991). Prophylaxis with silver nitrate eye drops was therefore made mandatory by law in many countries. Silver ions cause considerable chemical irritation of the eyes during the first 24 hours of life in up to 90% of the prophylactically treated neonates (Nishida & Risemberg, 1975) and therefore other ointments such as tetracycline and erythromycin have been adopted instead (Moore et al., 2015). As gonococcal genital infections are rare in many developed countries, prophylaxis against gonococcal ON is no longer provided in Canada, the United Kingdom and the Scandinavian countries, for example. In Finland, GC positivity in the general population is low, 0.06% (Jokiranta et al., 2017). Ocular prophylaxis against GC is not routinely provided for neonates in Finland.

2.7.2 Lower respiratory tract infection

Viral respiratory tract infections

Acute LRTI is the most frequent cause of non-elective hospital admissions of infants in high-income countries, and respiratory viruses account for the majority of all LRTIs in infants, especially among those aged less than six months (Boyce, Mellen, Mitchel, Wright, & Griffin, 2000; Shay et al., 1999; Stockman, Curns, Anderson, & Fischer-Langley, 2012). The introduction of molecular methods has changed viral diagnostics and the research of respiratory tract infections in infants. The causative viral agent can now be found in up to 90% of infants presenting with respiratory tract infections (Miller et al., 2013; Ruohola et al., 2009). Respiratory syncytial virus (RSV) is the most important causative agent in 70–80% of cases during the first year of life (Cangiano et al., 2016; Piedimonte & Perez, 2014; Skjerven et al., 2016), and virtually all children become infected with RSV at least once by two years of age (Glezen, Taber, Frank, & Kasel, 1986). The proportion of rhinovirus is 13% to 34% among infants with LRTI (Cangiano et al., 2016; Miller
et al., 2013; Skjerven et al., 2016). Moreover, 20–40% of infants have been shown to have multiple virus infections (Calvo et al., 2008; Cangiano et al., 2016; Nascimento et al., 2010).

**Whooping cough**

*Bordetella pertussis* (BP) and *Bordetella parapertussis* cause outbreaks of whooping cough every three to four years (Cantey, Sanchez, Tran, Chung, & Siegel, 2014; Tanaka et al., 2003; Vitek, Pascual, Baughman, & Murphy, 2003). Some previous studies have reported 7% to 23% prevalence of BP in infants aged less than six months hospitalised for acute LRTI or other respiratory complaints (Crowcroft et al., 2003; Korppi & Hiltunen, 2007; Nuolivirta et al., 2010), but the prevalence during a non-epidemic season has been found to be less than 2% (Korppi et al., 2016; Siberry, Paquette, Ross, Perl, & Valsamakis, 2006; Walsh et al., 2011). Whooping cough causes significant morbidity and mortality among young infants, especially among those too young to be protected against BP by immunisation. If the disease progresses, it can prove to be fatal (Berger et al., 2013; Cantey et al., 2014; Crowcroft et al., 2003).

Infants usually acquire BP from close contacts, most commonly from their infected mother. To protect unvaccinated infants from whooping cough, Centers for Disease Control and Prevention (CDC) recommends that all pregnant women should receive tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine in the third trimester (between the 27th and 36th weeks) of each pregnancy (Centers for Disease Control and Prevention (CDC), 2013). The vaccination of pregnant women with Tdap is not currently a standard practice in Finland.

**Atypical bacterial pathogens**

Several studies have indicated that the atypical pathogens *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* cause community-acquired LRTIs in children aged ≥12 months (Baer, Engelcke, Abele-Horn, Schaad, & Heininger, 2003; Prapphal et al., 2006; Principi & Esposito, 2001). Chen et al. recently reported that up to 10% of LRTIs in Chinese infants were caused by *Mycoplasma pneumoniae* and that 5% were caused by *Chlamydia pneumonia* (Z. Chen et al., 2013). However, this finding was explained by the high population density in China that predisposes infants to these airborne diseases. Based on the published literature,
the role of atypical respiratory pathogens as causative agents of LRTIs in infants aged less than six months remains largely unknown.
3 Aims of the research

The origin of this work was our clinical observation that we rarely diagnose CT infections in infants. It was hypothesised that either we fail to recognise these infants, or alternatively previous studies have overestimated the risk of vertical transmission.

The specific aims of this work were:

1. to calculate a population-based estimate for the probability of vertical transmission of CT in the era of NAAT-based diagnosis of maternal CT infections (I),
2. to improve the clinical recognition of CT infections in infants by evaluating the typical clinical characteristics and the present delay in diagnosing CT in symptomatic infants (II),
3. to evaluate the risk of long-term consequences of CT infections in infants, given current clinical practises (II),
4. to investigate the aetiology of neonatal conjunctivitis and to examine whether CT is an underdiagnosed causative agent in unselected neonatal conjunctivitis patients in primary care (III), and
5. to investigate the aetiology of LRTIs and to examine whether CT is an underdiagnosed causative agent in LRTIs in infants in a hospital setting (IV).
4 Subjects and methods

This work was carried out at the Department of Children and Adolescents, Oulu University Hospital, and involved 10 child health clinics in the city of Oulu.

4.1 Populations and study design (I–IV)

This thesis includes two retrospective register-based studies, papers I and II, and two prospective observational cohort studies, papers III and IV (Table 7).

<table>
<thead>
<tr>
<th>Paper</th>
<th>Design</th>
<th>Data source</th>
<th>Time period</th>
<th>Number of subjects</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Nationwide register-based study</td>
<td>National Infectious Diseases Register, Care Register for Health Care</td>
<td>1/1996 to 12/2011</td>
<td>206 CT-infected</td>
<td>0–4 years</td>
</tr>
<tr>
<td>II</td>
<td>Nationwide register-based study</td>
<td>Medical records</td>
<td>1/1996 to 12/2011</td>
<td>124 CT-infected (symptomatic)</td>
<td>0–4 years</td>
</tr>
<tr>
<td>III</td>
<td>Prospective observational cohort study</td>
<td>Neonates with conjunctivitis in child health clinics</td>
<td>10/2010 to 9/2015</td>
<td>173 screened</td>
<td>&lt;30 days</td>
</tr>
<tr>
<td>IV</td>
<td>Prospective observational cohort study</td>
<td>Infants with LRTI in the paediatric emergency department</td>
<td>12/2016 to 12/2017</td>
<td>228 screened</td>
<td>&lt;6 months</td>
</tr>
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CT; Chlamydia trachomatis; LRTI, lower respiratory tract infection.

4.2 Probability of vertical transmission of Chlamydia trachomatis (I)

In the population-based nationwide study of the vertical transmission of CT children aged less than four years with a possible CT infection during the period from 1 January 1996 to 31 December 2011 were searched for in two national health registers, the NIDR and the Care Register for Health Care (HILMO). This
particular age group was chosen as it was the youngest age group available in the NIDR for data search purposes. After the possible CT cases had been identified, we ordered copies of the children’s medical records, including laboratory data, from the hospitals, health centres and child health clinics where they had been treated and identified their mothers from the Medical Birth Register, after which we obtained the mothers’ serum samples drawn during the first trimester of the index pregnancy from the FMC serum bank. We then measured IgG antibodies against CT in these maternal serum samples. We also obtained data on the mothers’ possible CT infections from the NIDR.

*National Infectious Diseases Register*

CT infections are monitored in Finland through mandatory notifications made in accordance with the Communicable Diseases Act and Decree of 1987. Between 1995 and 1997 physicians reported all confirmed cases of CT to the NIDR, and from 1998 onwards the laboratories looked after the notification procedure. Each laboratory notification includes the patient’s personal identity number, age and gender, the sampling site and the test method used. Treatment data are not reported to the NIDR.

*Care Register for Health Care*

The Care Register for Health Care (formerly the Hospital Discharge Register), maintained by the National Institute for Health and Welfare, consists of data on patients discharged from in-patient care and specialised outpatient care. The data include the patient’s personal identity number, the reason for seeking care, diagnoses and the date of discharge. We used the International Classification of Diseases (version 10) codes A74* (Other diseases caused by chlamydiae), H13* (Disorders of conjunctiva in diseases classified elsewhere), J16.0 (Chlamydial pneumonia) and P23.1 (Congenital pneumonia due to Chlamydia) to retrieve data from the register.

*Finnish Maternity Cohort serum bank and serology*

The FMC serum bank is a nationwide repository for mothers’ serum samples obtained during the first trimester of pregnancy. The serum bank was established by the National Institute for Health and Welfare in 1983 and since then more than
850,000 pregnant women have given permission for their serum samples to be used for research purposes. In our study, maternal serum samples obtained during the index pregnancies in 1996–2011 were available for 99 (80%) mothers with a CT-infected child. These serum samples were retrieved from storage at –25°C and examined using the commercial enzyme immunoassay (medac Diagnostics, Wedel, Germany) to detect IgG antibodies to CT for estimating the timing of the acquisition of the maternal CT infection. CT-specific IgG antibodies in the mothers’ sera were measured in the laboratory of the National Institute of Health and Welfare in 2015.

**Estimation of the risk of vertical transmission**

We calculated the risk of vertical transmission of CT based on our data and two earlier studies of pregnant women in Finland (Kurkinen et al., 2006; Lyytikäinen et al., 2008). There were 933,823 babies born from 922,263 deliveries in Finland over the period of 16 years (Suomen virallinen tilasto (SVT), 2015), and we estimated the total number of CT-positive mothers during the period concerned using data published by Kurkinen et al. (Kurkinen et al., 2006) and Lyytikäinen et al. (Lyytikäinen et al., 2008). In order to calculate the risk of vertical transmission based on NAAT positivity, we used the number of infected infants from our data as the numerator and the estimated proportion of the deliveries in 1996–2011 in which the mother was assumed to be CT-positive (n=24,901) as the denominator (Kurkinen et al., 2006). In order to calculate the risk of vertical transmission based on the annual seroconversion rate between two pregnancies, we used the number of infected infants in our data as the numerator and the estimated proportion of the deliveries in 1996–2011 in which the mother was assumed to be CT-positive (n=11,712) as the denominator (Lyytikäinen et al., 2008).

4.3 **Clinical features of vertically transmitted Chlamydia trachomatis infections in infants (II)**

We evaluated the signs, symptoms and long-term prognosis of vertically transmitted CT infections in the same register-based population (I). We reviewed copies of all the original medical records, including laboratory data, for children with a possible CT infection up to the age of 16 years as obtained from the hospitals, health centres and child health clinics where they had been treated and used a structured form for data input from the medical records. We also reviewed five
paediatric textbooks and handbooks for the clinical features of CT infection in infants (Table 5) and compared them with the symptoms reported in the medical records of our cases (American Academy of Pediatrics, 2015a; M. Hammerschlag, 2011; M. Hammerschlag & Kohlhoff, 2014; Royal College of Paediatrics and Child Health, 2016; K. A. Workowski et al., 2015).

4.4 Aetiology of neonatal conjunctivitis (III)

We investigated prospectively the aetiology of neonatal conjunctivitis and the proportions of CT, GC, other bacteria and 16 respiratory viruses in 10 child health clinics in the city of Oulu from 12 October 2010 to 25 September 2015. The material for this included all the consecutive neonates who had symptoms of conjunctivitis, defined as the presence of conjunctival discharge, erythema or swelling of the eyelids before the age of 30 days. Parents or legal guardians were asked to complete a standardised questionnaire concerning background characteristics, symptoms and signs of the infection and the duration of the current illness. We also reviewed the hospital records of all the neonates enrolled at Oulu University Hospital, including the Department of Ophthalmology, at least until the age of 18 months, to find out whether there were any long-term consequences of neonatal conjunctivitis. Furthermore, to estimate the coverage of our material, we searched for all the microbiologically confirmed CT diagnoses in neonates aged less than 30 days in the hospital’s catchment area.

4.5 Aetiology of lower respiratory tract infections in infants aged less than six months (IV)

We investigated prospectively the aetiology of LRTIs in infants aged less than six months in paediatric emergency department at Oulu University Hospital, during a complete epidemiological year from 7 December 2016 to 6 December 2017 and assessed the proportions of CT, BP and 16 respiratory viruses. We included in this material all consecutive infants who presented with symptoms of LRTI, defined as cough, wheezing, tachypnoea, dyspnoea, or apnoea with or without fever (temperature higher than 38.0°C). Parents or legal guardians were asked to complete a standardised questionnaire concerning background characteristics, symptoms and signs of the infection and the duration of the current illness. We also reviewed the hospital records of all the infants enrolled at Oulu University Hospital to collect clinical data.
4.6 Microbiological samples (III, IV)

4.6.1 Conjunctival specimens (III)

We trained public health nurses working in the maternity and child health clinics in the city of Oulu to collect conjunctival specimens from the neonates. Before the commencement of this research we prepared a detailed instruction sheet and video concerning the sampling and distributed these to the public health nurses. All the specimen collection materials were packed in ready-for-use sets: Transystem M40 transport cotton tipped swabs (Copan Diagnostics, Inc., California, USA) for bacterial culture, Abbott multi-Collect Specimen Collection swabs (Abbott Molecular Inc., Illinois, USA) for CT and GC PCR, and FLOQSwabs Copan flocked swabs (Copan Diagnostics, Inc., California, USA) for respiratory virus multiplex real-time PCR. Conjunctival specimens were collected from the symptomatic eye, and both eyes were sampled in cases of bilateral conjunctivitis. Before sampling, the area around the infected eye was gently wiped to remove any additional discharge. The bacterial culture was obtained by rolling the cotton tipped swab on the mucosal area of the lower eyelid, after which the inner surface of the lower eyelid was swabbed with a flocked swab two to three times to collect epithelial cells for PCR testing.

Each bacterial culture swab was inserted into a Transystem M40 transport medium tube (Copan Diagnostics, Inc., California, USA), the swab for CT testing into an Abbott multi-Collect Specimen Collection transport medium tube (Abbott Molecular Inc., Illinois, USA) and the swab for multiplex real-time PCR into a 3ml transport medium tube (UTM™, Universal Transport Media; Copan Diagnostics, Inc., California, USA). The swabs were transported to the clinical microbiological laboratory at Oulu University Hospital (NordLab, Oulu) at room temperature within the same working day.

4.6.2 Respiratory specimens (IV)

We trained nurses working in the paediatric emergency department at Oulu University Hospital to collect NP specimens from the infants, again preparing a detailed instruction sheet concerning the sampling procedure and distributing it to the nurses. All the specimen collection materials were packed in ready-for-use sets: Abbott multi-Collect Specimen Collection swabs (Abbott Molecular Inc., Illinois, USA) for CT PCR and FLOQSwabs Copan flocked swabs (Copan Diagnostics,
Inc., California, USA) for BP PCR and respiratory virus multiplex real-time PCR. NP swabs were obtained from the nostril by passing the swab into the nostril towards the nasopharynx and rotating it two to three times to collect epithelial cells, whereas NP aspirates were obtained by inserting a plastic catheter into the nostril and sucking with an electronic device while withdrawing the catheter from the nostril. After suction, a Copan flocked swab (Copan Diagnostics, Inc., California, USA) was dipped into the aspirate.

The swab for CT testing was inserted into an Abbot multi-Collect Specimen Collection transport medium tube (Abbott Molecular Inc., Illinois, USA), the swab for BP testing into a dry transport tube and the swab for multiplex real-time PCR into a 3ml transport medium tube (UTM™, Universal Transport Media; Copan Diagnostics, Inc., California, USA). The swabs for CT and respiratory virus testing were transported to the clinical microbiological laboratory at Oulu University Hospital (NordLab, Oulu) at room temperature within the same working day. The swabs for BP testing were transported to the clinical microbiological laboratory at Helsinki University Hospital (HUSLAB, Helsinki) at room temperature within the same working day.

4.6.3 Detection methods

A 5% sheep blood agar and a chocolate agar were used to culture the bacteria from the conjunctival samples. A PCR (Abbott Molecular Inc., Illinois, USA) was used to detect CT and GC. Nucleic acid was isolated using the Abbott mSample Preparation Systems DNA Reagent and the Abbott m2000s instrument, and detected and amplified using an Abbott RealTime CT/NG Amplification Reagent Kit together with the Abbott m2000rt instrument. A multiplex real-time PCR kit (Seegene Inc., Seoul, Korea) was used to detect respiratory viruses, including adenovirus, bocavirus, enterovirus, influenza viruses A and B, human coronaviruses 229E, NL63, and OC43, human metapneumovirus, parainfluenza viruses 1, 2, 3 and 4, RSV A and B, and rhinovirus. Nucleic acids for the respiratory viruses were isolated using the QIAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Hilden, Germany) and a QIAsymphony SP instrument (Qiagen, Germantown, USA) and amplified and detected using an Anyplex™ II RV16 Detection System (Seegene Inc., Seoul, Korea) and the CFX96™ Real-Time PCR System (Bio-Rad Laboratories Inc., California, USA). An in-house assay based on detection of the BP insertion sequence was used to detect BP at HUSLAB in Helsinki.
The laboratory personnel reported all positive CT, GC and BP findings to the project's physicians. Other microbiological findings from the conjunctivae were available to the primary care physicians as needed.

4.7 Classification of organisms (III, IV)

The following micro-organisms were classified as pathogens: *Bacillus cereus*, group A and B streptococci, CT, *Corynebacterium* species, *Enterococcus faecalis*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella oxytoca*, *Moraxella catarrhalis*, GC, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mitis*, *Streptococcus pneumoniae* and respiratory viruses. The following micro-organisms were classified as non-pathogens: coagulase-negative staphylococci, alpha-haemolytic streptococci and *Acinetobacter* species.

4.8 Sample size calculations (III, IV)

We considered the situation clinically significant, i.e. testing for CT would be routinely needed, if 5% (to an accuracy of ±2.5%) of neonatal conjunctivitis cases were to be caused by CT (III). With a two-sided alpha error of 0.05, 226 neonates with conjunctivitis would have been needed for this. However, after five years of recruitment, none of the recruited neonates had had chlamydial conjunctivitis (0/163). We then calculated the 95% CI for the proportion of CT conjunctivitis among all the neonatal conjunctivitis cases, which was 0% to 2.2%, and declared the research completed, because we had achieved the desired accuracy. Correspondingly, we considered the situation clinically significant, i.e. testing for CT or BP would be routinely needed, if 5% (to an accuracy of ±2.5%) of LRTI cases were to be caused by CT or BP (IV). With a two-sided alpha error of 0.05, 169 infants with LRTI would have been needed. However, we decided to collect samples for at least 12 months to represent a complete epidemiological year.

4.9 Statistical methods

The statistical analyses were performed using SPSS version 24 software (SPSS Inc., Chicago, Illinois, USA) and StatsDirect (StatsDirect Ltd, 2013). We assumed the occurrence of CT infections to follow a Poisson distribution and calculated 95% CIs accordingly (I). We calculated the sample sizes and Clopper-Pearson continuity corrected 95% CIs for the occurrence of the pathogens using StatsDirect statistical
software (III, IV). If the occurrence was zero, 97.5% one-sided CI was calculated. The Exact \( \chi^2 \) test was used to compare the differences between symptoms and bacterial culture findings, and a \( p \) value of <0.05 was considered statistically significant (III).

4.10 Ethical considerations

The Review Board of the National Institute for Health and Welfare, Helsinki, Finland accepted the study protocols I–II and the Regional Ethics Committee of the Northern Ostrobothnia Hospital District, Oulu, Finland approved the research plans III–IV. Parents or legal guardians of the participants were informed about the research (III, IV) and gave their written informed consent.
5 Results

5.1 Chlamydia trachomatis-infected infants (I, II)

Altogether 213 children with a possible CT infection were identified in the national health registers, 151 were found in the NIDR, 38 in the Care Register for Health Care and 24 in both. Seven children originally identified in the Care Register for Health Care were excluded as the review of the medical records did not confirm that they had had a CT infection. Of the remaining 206 possible CT cases, the infection could not be confirmed in 78 cases as 50 of them had an incomplete personal identity number and 28 incomplete medical records. Thus, there were 128 children with a microbiologically confirmed CT infection based on the medical records, 124 of whom had complete medical records available for the review and were thus used to characterize the clinical features of the CT infections. Altogether 123 infants were younger than three months at the time of the onset of CT infection and were used in the vertical transmission calculations. Pregnant women were not routinely screened for CT during the period concerned.

5.2 Occurrence and risk of vertical transmission of Chlamydia trachomatis (I)

The occurrence rate was 0.22 per 1000 live births (95% CI 0.19–0.25) among the 206 children who had a possible CT infection during the 16-year period and 0.13 per 1000 live births (95% CI 0.11–0.16) among the 123 microbiologically confirmed CT cases whose symptoms started before the age of three months. The risk of mother-to-child transmission of CT was 0.8% among the estimated 24 900 NAAT-positive mothers and 1.8% among the estimated 11 712 mothers with a recently acquired infection based on the annual seroconversion rate of CT-specific antibodies (Table 8).
Table 8. The risk of vertical transmission of *Chlamydia trachomatis*.

<table>
<thead>
<tr>
<th>CT in pregnant women</th>
<th>Deliveries (n)</th>
<th>CT-positive mothers (n)</th>
<th>CT-positive infants</th>
<th>Risk of vertical transmission, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAAT-positivity 1.3%</td>
<td>922 263</td>
<td>11 989</td>
<td>206</td>
<td>1.7 (1.5–2.0)</td>
</tr>
<tr>
<td>(screened)</td>
<td></td>
<td></td>
<td>123^3</td>
<td>1.0 (0.9–1.2)</td>
</tr>
<tr>
<td>NAAT-positivity 2.7%</td>
<td>922 263</td>
<td>24 901</td>
<td>206</td>
<td>0.8 (0.7–1.0)</td>
</tr>
<tr>
<td>(all cases)</td>
<td></td>
<td></td>
<td>123^3</td>
<td>0.5 (0.4–0.6)</td>
</tr>
<tr>
<td>Seroconversion rate</td>
<td>922 263</td>
<td>11 712</td>
<td>206</td>
<td>1.8 (1.5–2.0)</td>
</tr>
<tr>
<td>12.7/1000 PYR^2</td>
<td></td>
<td></td>
<td>123^3</td>
<td>1.0 (0.9–1.3)</td>
</tr>
</tbody>
</table>

^1 1.3% of the pregnant women were NAAT-positive for CT when screened during the first trimester, whereas the overall prevalence among all pregnant women was 2.7% (95% CI 1.8–4.2) (Kurkinen *et al*., 2006); ^2 The seroconversion rate was 188 (95% CI 86–290) per 10 000 PYR for those <23 years of age and 97 (95% CI 48–146) per 10 000 PYR among 23–28-year-olds, the average for the total population being 127 per 10 000 PYR (Lyytikäinen *et al*., 2008). ^3 Microbiologically confirmed CT infection onset before three months; CI, confidence interval; CT, *Chlamydia trachomatis*, NAAT, nucleic acid amplification test; PYR, person-years at risk.

5.3 Characteristics of the mothers and timing of the maternal *Chlamydia trachomatis* infection (I)

The mean age of the mothers of the CT-infected infants was 24 years (SD 5.3), and 74% of them were primiparae. All but one mother delivered vaginally. Two mothers were hepatitis C-positive, but none had HIV, hepatitis B or syphilis. The NIDR data showed that 51 (41%) mothers had tested positive for CT at some point in their lives, but only five (4.1%) tested positive during the index pregnancy (Table 9). Just above one third (35/99) of the maternal serum samples were negative for CT-specific IgG antibodies, indicating that the maternal CT infection had probably been acquired after the first trimester of the pregnancy.
Table 9. *Chlamydia trachomatis* infections in mothers whose infants had a chlamydia infection during the first three months of life.

<table>
<thead>
<tr>
<th>Maternal CT infection (n=123)</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data from the NIDR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive CT register data</td>
<td>51</td>
<td>41</td>
</tr>
<tr>
<td>During index pregnancy</td>
<td>5</td>
<td>4.1</td>
</tr>
<tr>
<td>Serum sample available for the index pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive CT serology</td>
<td>64</td>
<td>65</td>
</tr>
<tr>
<td>Negative CT serology</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>

CT, *Chlamydia trachomatis*; NIDR, National Infectious Diseases Register.

5.4 Symptoms and signs of *Chlamydia trachomatis* infections in infants (II)

Conjunctivitis was diagnosed in 110/124 (89%) infants and LRTI in 32 (26%), 18 (15%) having both conjunctivitis and LRTI (Table 10). The most common symptoms of chlamydial conjunctivitis were mucopurulent discharge in 104/110 (95%) infants, erythema in 68 (62%) and swelling of the eyelids in 61 (55%). Bloody discharge from the infected eyes was reported in 33 (30%) infants. Mild conjunctivitis with mucopurulent discharge as the only symptom was present in 24 (22%) cases. The typical signs and symptoms of chlamydia LRTI were cough in 25/32 (78%) infants, rales in 24 (75%) and tachypnoea in 14 (44%). In addition, 19 (59%) infants with LRTI had dyspnoea and 15 (47%) wheezing. Staccato cough was not recorded in any of the infants with chlamydial LRTI.

The symptoms of conjunctivitis developed before 20 days of age in 90% of the cases, but respiratory tract infections appeared more slowly (Figure 2). The infants were on average nine (SD 10) days old at the onset of conjunctival symptoms and 41 (SD 22) days old at the onset of respiratory symptoms. The median delay from the onset of symptoms to CT diagnosis was 13 (range 4–374) days for conjunctivitis and 25 (range 10–149) days for LRTI.
Table 10. Clinical characteristics of *Chlamydia trachomatis* infections in infants as reported in the present study.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctivitis (n=110)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purulent discharge</td>
<td>104</td>
<td>(95)</td>
</tr>
<tr>
<td>Erythema</td>
<td>68</td>
<td>(62)</td>
</tr>
<tr>
<td>Swollen eyelids</td>
<td>61</td>
<td>(55)</td>
</tr>
<tr>
<td>Blood-stained discharge</td>
<td>33</td>
<td>(39)</td>
</tr>
<tr>
<td>Onset, mean (SD), days</td>
<td>9</td>
<td>(10)</td>
</tr>
<tr>
<td>Lower respiratory tract infection (n=32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afebrile &lt;38°C</td>
<td>30</td>
<td>(94)</td>
</tr>
<tr>
<td>Cough</td>
<td>25</td>
<td>(75)</td>
</tr>
<tr>
<td>Rales</td>
<td>24</td>
<td>(75)</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>19</td>
<td>(59)</td>
</tr>
<tr>
<td>Wheezing</td>
<td>15</td>
<td>(47)</td>
</tr>
<tr>
<td>Tachypnoea</td>
<td>14</td>
<td>(44)</td>
</tr>
<tr>
<td>Staccato cough</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td>Onset, mean (SD), days</td>
<td>41</td>
<td>(22)</td>
</tr>
</tbody>
</table>

1 Two outliers (420 days and 850 days) were excluded from the analyses.
Fig. 2. Cumulative appearance of symptoms caused by *Chlamydia trachomatis* in 124 infants. The symptoms of chlamydial conjunctivitis in two children were reported to have started at ages of 420 and 850 days.

5.5 Diagnostics (I, II)

Of the 123 infants who were younger than three months at the time of the onset of infection, the majority (66%) were diagnosed for CT by PCR. Antigen detection was used in 25% of cases, culture in 12% and serology in 4.1%.

5.6 Treatment and hospitalisation (I, II)

The majority of the infants (116/120, 97%) were treated with a systemic macrolide antibiotic after the diagnosis of CT. Treatment data on four infants were missing. Erythromycin was provided for 55 infants (46%), azithromycin for 46 (38%) and clarithromycin for 10 (8.6%), while five (4.3%) cases received both erythromycin
and azithromycin due to a change in the route of administration. Treatment was unsuccessful in eight (6.7%) cases, six of whom were clinical failures, one microbiological and one both clinical and microbiological failure. Out of the treatment failure cases, one infant received initially erythromycin and seven infants were treated with azithromycin. All eight treatment failure cases were provided with a repeat macrolide course. IHPS was not diagnosed in any of the 116 infants treated with a macrolide antibiotic. A total of 49/124 infants (40%) were hospitalised due to the CT infection and two of them (1.6%) were treated in the intensive care unit. Altogether 10 hospitalised infants (8.1%) received oxygen therapy.

5.7 Prognosis (I, II)

A follow-up appointment was arranged for 46/124 (37%) infants, and the majority of them (32; 70%) were found to have been cured. A total of 14 cases (11%) sought further medical care on account of either ocular or pulmonary problems, among whom three (1.6%) had chalazion and another three (1.6%) watery eyes. Prolonged cough or recurrent wheezing was present in seven children (5.6%), but all of them had normal results in the pulmonary function tests. One child with untreated conjunctivitis developed bilateral corneal scars (1:933 823 births), the diagnosis of CT infection having been made at the age of 3.4 years. The CT genotype of this particular case was one of the sexually transmitted ones and not the trachoma genotype. The child had not attended regular health examinations at the child health clinic due to neglect on the part of the legal guardians. None of the infants died of the CT infection, but one infant died of congenital heart disease and one whose CT conjunctivitis was treated before the age of three months died at the age of eight years, the exact cause of death remaining unknown.

5.8 Aetiology of neonatal conjunctivitis (III)

5.8.1 Population

We recruited 173 neonates with conjunctivitis, representing 1.8% of the total of 9600 babies born in the catchment area during the five-year period. The mothers were on average 29 years (SD 5.3) old, 20% being younger than 25 years, and 48% of them primiparae.
5.8.2 Clinical characteristics

The neonates were 7.3 (SD 7.0) days old on average when the symptoms of conjunctivitis appeared. Bilateral conjunctivitis was present in 74/173 cases (43%). The most common symptoms were mucopurulent discharge, in 164/173 (95%) neonates, swelling of the eyelids in 52 (30%) and redness in 42 (24%). Blood-stained discharge was not reported in any of the cases. Legal guardians reported respiratory tract symptoms in 33 (19%) neonates (Table 11). Rhinorrhoea was present in 23 cases (13%), cough in six (3.5%), nasal congestion in three (1.7%) and sneezing in one (0.6%).

Table 11. Symptoms of neonatal conjunctivitis by conjunctival bacterial culture and virus polymerase chain reaction findings.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Bacterial pathogen(^1) n=58</th>
<th>Virus n=8</th>
<th>Non-pathogen n=25</th>
<th>Commensal flora n=67</th>
<th>Negative n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Mucopurulent discharge</td>
<td>54 (93)</td>
<td>8 (100)</td>
<td>23 (92)</td>
<td>64 (96)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Redness</td>
<td>16 (28)</td>
<td>3 (38)</td>
<td>7 (28)</td>
<td>16 (24)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Swelling of the eyelids</td>
<td>20 (34)</td>
<td>3 (38)</td>
<td>5 (20)</td>
<td>22 (33)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Rhinorrhoea</td>
<td>12 (21)</td>
<td>2 (25)</td>
<td>4 (16)</td>
<td>5 (7.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cough</td>
<td>2 (3.4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (6.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4.0)</td>
<td>2 (3.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sneezing</td>
<td>1 (1.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

\(^1\) Symptoms did not differ between the bacterial culture findings (p>0.05).

5.8.3 Microbiological findings

The mean age of the neonates when the conjunctival samples were collected was 20 (SD 10) days. PCR results for CT and GC detection was obtained for 163/173 (94%) neonates, multiplex real-time PCR for respiratory viruses for 167 (97%) and bacterial cultures for 160 (92%) (Table 12). CT or GC was not found in any of the 163 neonates tested (95% CI 0%–2.2%), and no cases of chlamydial or gonococcal neonatal conjunctivitis were diagnosed in the catchment area of the hospital outside the present study either. Respiratory viruses were detected at an ocular site in 8/167 neonates (4.8%; 95% CI 2.1%–9.2%), while rhinovirus was found in four specimens, adenovirus in three and bocavirus in one. Half of the neonates with viral conjunctivitis had bacterial growth in culture. *Moraxella catarrhalis* was isolated
together with rhinovirus in one sample and with bocavirus in one sample, while *Staphylococcus epidermidis* was found together with rhinovirus in one sample and with adenovirus in one sample. Legal guardians reported respiratory tract symptoms in one fourth of the neonates with viral conjunctivitis (2/8). Bacterial pathogens other than CT or GC were present in 58/160 (36%) samples (95% CI 29%–44%), the most commonly identified pathogens being *Staphylococcus aureus* (16%), *Moraxella catarrhalis* (9.4%), *Corynebacterium* species (3.8%), *Streptococcus pneumoniae* (3.1%) and *Haemophilus influenzae* (2.5%). Altogether 25 neonates (16%; 95% CI 10%–22%) had non-pathogenic bacterial growth in bacterial culture, the most commonly identified non-pathogens being coagulase-negative staphylococci and alpha-haemolytic streptococci. A commensal flora of the conjunctiva was present in 67 (42%) cases (95% CI 34%–50%) and eight cultures (5.0%) were negative (95% CI 2.2–9.6). There were no statistically significant associations between the symptoms and the bacterial culture findings (Table 11).
Table 12. Microbiological findings in 173 cases of neonatal conjunctivitis.

<table>
<thead>
<tr>
<th>Microbiological finding</th>
<th>Number of neonates</th>
<th>Proportion (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiratory virus detection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any respiratory virus</td>
<td>8</td>
<td>4.8 (2.1–9.2)</td>
</tr>
<tr>
<td>Rhinovirus¹</td>
<td>4</td>
<td>2.4 (0.7–6.0)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>3</td>
<td>1.8 (0.4–5.2)</td>
</tr>
<tr>
<td>Bocavirus¹</td>
<td>1</td>
<td>0.6 (0.02–3.2)</td>
</tr>
<tr>
<td>Human coronaviruses</td>
<td>0</td>
<td>0 (0–2.2)</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>0</td>
<td>0 (0–2.2)</td>
</tr>
<tr>
<td>Influenza viruses</td>
<td>0</td>
<td>0 (0–2.2)</td>
</tr>
<tr>
<td>Parainfluenza viruses</td>
<td>0</td>
<td>0 (0–2.2)</td>
</tr>
<tr>
<td>Respiratory syncytial viruses</td>
<td>0</td>
<td>0 (0–2.2)</td>
</tr>
<tr>
<td><strong>CT and GC detection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>0</td>
<td>0 (0–2.2)</td>
</tr>
<tr>
<td>GC</td>
<td>0</td>
<td>0 (0–2.2)</td>
</tr>
<tr>
<td><strong>Bacterial culture</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any pathogens</td>
<td>58</td>
<td>36 (29–44)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25</td>
<td>16 (10–22)</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>15</td>
<td>9.4 (5.3–15)</td>
</tr>
<tr>
<td>Corynebacterium species</td>
<td>6</td>
<td>3.8 (1.4–8.0)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>5</td>
<td>3.1 (1.0–7.1)</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>4</td>
<td>2.5 (0.7–6.3)</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>1</td>
<td>0.6 (0.02–3.4)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>0.6 (0.02–3.4)</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>1</td>
<td>0.6 (0.02–3.4)</td>
</tr>
<tr>
<td>Group B streptococcus</td>
<td>1</td>
<td>0.6 (0.02–3.4)</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>1</td>
<td>0.6 (0.02–3.4)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>0.6 (0.02–3.4)</td>
</tr>
<tr>
<td>Streptococcus mitis</td>
<td>1</td>
<td>0.6 (0.02–3.4)</td>
</tr>
<tr>
<td>Non-pathogenic bacterial growth³</td>
<td>25</td>
<td>16 (10–22)</td>
</tr>
<tr>
<td>Commensal flora</td>
<td>67</td>
<td>42 (34–50)</td>
</tr>
<tr>
<td>Negative culture</td>
<td>8</td>
<td>5.0 (2.2–9.6)</td>
</tr>
</tbody>
</table>

¹ 2/8 infants had Moraxella catarrhalis in addition to viruses in the eye samples; ² 4/58 infants had two bacterial pathogens in their eye specimens; ³ The most common non-pathogens were coagulase-negative staphylococcus (n=28) and alpha-haemolytic streptococcus (n=10); CI, confidence interval; CT, Chlamydia trachomatis; GC, Neisseria gonorrhoeae.
5.8.4 Follow-up data

Nasolacrimal duct obstruction was diagnosed in 4/173 infants and toddlers (2.3%) and was treated with either probing or surgery at a median age of 17 months (range 9–36). Two of these cases had *Moraxella catarrhalis* in the conjunctival specimens taken in infancy, one had *Staphylococcus aureus* and one had a negative culture. There were no other long-term ocular sequelae recorded in the children’s hospital records after neonatal conjunctivitis.

5.9 Aetiology of lower respiratory tract infections in infants aged less than six months (IV)

5.9.1 Population

We enrolled 228 infants with LRTI during the one-year period. The median age of the infants at presentation was 70 days (range 6–174) and 50% were boys. The mean birth weight was 3385 g (SD 774) and height 49 cm (SD 3.5). Of the 228 mothers, 25% were primiparae. The median number of siblings was three (range 1–14). One mother had received a Tdap vaccine during the pregnancy. CT genital infection was diagnosed in one mother after delivery, and pertussis was circulating in two families.

5.9.2 Microbiological findings

PCR for CT and multiplex real-time PCR for respiratory virus detection were obtained for all the 228 infants, whereas BP PCR was obtained from 226 (99%) infants. CT PCR was positive in one infant (0.4%; 95% CI 0.01%–2.4%) and BP PCR in one infant (0.4%; 95% CI 0.01%–2.4%) (Table 13), the total proportion of CT and BP being 0.9% (95% CI 1.1%–3.1%). The BP-infected infant also had rhinovirus and bocavirus in the respiratory tract specimens. The NP sample from the baby born to a chlamydia-positive mother was CT-negative, and similarly the babies with pertussis-infected family members were BP-negative. The majority of the infants (203/228, 89%) had a viral respiratory tract infection, the most commonly detected viruses being RSV in 78 infants (34%), rhinovirus in 67 (29%) and parainfluenza viruses in 25 (11%) (Table 13). Multiple respiratory viruses were detected in 30 infants (13%) and one infant had three viruses in the NP specimens. A total of 24 infants (11%) had negative respiratory samples.
Table 13. Findings in the respiratory specimens of 228 infants with a lower respiratory tract infection.

<table>
<thead>
<tr>
<th>Microbiological finding</th>
<th>Number of infants</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>n=228</td>
<td>% (95% CI)</td>
</tr>
<tr>
<td>CT</td>
<td>1</td>
<td>0.4 (0.01–2.4)</td>
</tr>
<tr>
<td>BP¹</td>
<td>1</td>
<td>0.4 (0.01–2.4)</td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>4</td>
<td>1.8 (0.5–4.4)</td>
</tr>
<tr>
<td>Bocavirus</td>
<td>13</td>
<td>5.7 (3.1–9.6)</td>
</tr>
<tr>
<td>Human coronavirus 229E</td>
<td>1</td>
<td>0.4 (0.01–2.4)</td>
</tr>
<tr>
<td>Human coronavirus NL63</td>
<td>3</td>
<td>1.3 (0.3–3.8)</td>
</tr>
<tr>
<td>Human coronavirus OC43</td>
<td>8</td>
<td>3.5 (1.5–6.8)</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>4</td>
<td>1.8 (0.5–4.4)</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>21</td>
<td>9.2 (5.6–14)</td>
</tr>
<tr>
<td>Influenza virus A</td>
<td>9</td>
<td>3.9 (1.8–7.4)</td>
</tr>
<tr>
<td>Influenza virus B</td>
<td>1</td>
<td>0.4 (0.01–2.4)</td>
</tr>
<tr>
<td>Parainfluenza virus 1</td>
<td>4</td>
<td>1.8 (0.5–4.4)</td>
</tr>
<tr>
<td>Parainfluenza virus 2</td>
<td>0</td>
<td>0 (0–1.6)²</td>
</tr>
<tr>
<td>Parainfluenza virus 3</td>
<td>19</td>
<td>8.3 (5.1–13)</td>
</tr>
<tr>
<td>Parainfluenza virus 4</td>
<td>2</td>
<td>0.9 (0.1–3.1)</td>
</tr>
<tr>
<td>Respiratory syncytial virus A</td>
<td>51</td>
<td>22 (17–28)</td>
</tr>
<tr>
<td>Respiratory syncytial virus B</td>
<td>27</td>
<td>12 (8.0–17)</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>67</td>
<td>29 (24–36)</td>
</tr>
<tr>
<td>Coinfections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two viruses</td>
<td>30</td>
<td>13 (9.1–18)</td>
</tr>
<tr>
<td>Three viruses</td>
<td>1</td>
<td>0.4 (0.01–2.4)</td>
</tr>
</tbody>
</table>

¹ n=226 for BP; ² 97.5% one-sided CI; BP, Bordetella pertussis; CI, confidence interval; CT, Chlamydia trachomatis.

5.9.3 Clinical characteristics

The symptoms of LRTI had appeared an average of 6.7 days (SD 11) before presentation (median four days, range 1–100). There were no clinically significant associations between the symptoms and signs and the PCR findings of the respiratory specimens (Table 14). Conjunctivitis was diagnosed in five infants (2.2%), none of whom had CT infection. One infant had blood-stained discharge from the infected eyes. That particular infant did not have LRTI caused by CT, but the conjunctiva was not sampled for chlamydia testing. In the infant with BP, typical whooping was not present at diagnosis, nor was the characteristic staccato cough in the infant with the CT infection. In the viral respiratory infections,
whooping was present in 13–21% and staccato coughs in 3.3–11% of the infants. The duration of symptoms before presentation was similar across the pathogens except for the BP case whose parents reported a duration of 100 days for the cough.

5.9.4 Treatment and hospitalisation

Altogether 140/228 infants (61%) were hospitalised. The infant with the pertussis was hospitalised and received oral azithromycin therapy. The infant with the CT infection was discharged from the emergency department without antibiotic therapy before the diagnosis and received oral azithromycin treatment at home after the positive PCR result for CT. Both the infant with CT infection and the infant with whooping cough were clinically cured. Altogether nine infants (3.9%) were admitted to the intensive care unit, but none of them was intubated. None of the infants included in this series died.
Table 14. Symptoms and signs in 228 infants with a lower respiratory tract infection by polymerase chain reaction findings of the respiratory specimens.

<table>
<thead>
<tr>
<th>Microbiological finding</th>
<th>Rhinorrhoea</th>
<th>Any cough</th>
<th>Paroxysmal cough</th>
<th>Staccato cough</th>
<th>Whooping</th>
<th>Wheezing</th>
<th>Dyspnoea</th>
<th>Apnoea</th>
<th>Age¹</th>
<th>Duration of symptoms²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus, n=3</td>
<td>67%</td>
<td>33%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>33%</td>
<td>33%</td>
<td>77</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Bocavirus², n=2</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>66</td>
<td>2.0</td>
</tr>
<tr>
<td>Human coronavirus, n=9</td>
<td>89%</td>
<td>78%</td>
<td>22%</td>
<td>11%</td>
<td>22%</td>
<td>11%</td>
<td>33%</td>
<td>22%</td>
<td>70</td>
<td>3.1</td>
</tr>
<tr>
<td>Enterovirus³, n=2</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>64</td>
<td>2.5</td>
</tr>
<tr>
<td>hMPV, n=14</td>
<td>71%</td>
<td>86%</td>
<td>50%</td>
<td>14%</td>
<td>14%</td>
<td>14%</td>
<td>57%</td>
<td>0%</td>
<td>85</td>
<td>6.0</td>
</tr>
<tr>
<td>Influenza virus, n=8</td>
<td>88%</td>
<td>88%</td>
<td>50%</td>
<td>0%</td>
<td>13%</td>
<td>13%</td>
<td>38%</td>
<td>0%</td>
<td>98</td>
<td>7.6</td>
</tr>
<tr>
<td>Parainfluenza virus, n=16</td>
<td>81%</td>
<td>75%</td>
<td>81%</td>
<td>13%</td>
<td>13%</td>
<td>19%</td>
<td>25%</td>
<td>0%</td>
<td>76</td>
<td>8.4</td>
</tr>
<tr>
<td>RSV, n=67</td>
<td>81%</td>
<td>93%</td>
<td>63%</td>
<td>7.5%</td>
<td>16%</td>
<td>22%</td>
<td>48%</td>
<td>15%</td>
<td>66</td>
<td>4.8</td>
</tr>
<tr>
<td>Rhinovirus, n=52</td>
<td>90%</td>
<td>81%</td>
<td>33%</td>
<td>9.6%</td>
<td>12%</td>
<td>5.8%</td>
<td>42%</td>
<td>21%</td>
<td>80</td>
<td>6.4</td>
</tr>
<tr>
<td>Multiple viruses, n=29</td>
<td>76%</td>
<td>90%</td>
<td>55%</td>
<td>3.3%</td>
<td>21%</td>
<td>10%</td>
<td>52%</td>
<td>14%</td>
<td>84</td>
<td>8.9</td>
</tr>
<tr>
<td>CT³, n=1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>46</td>
<td>4.0</td>
</tr>
<tr>
<td>BP³4, n=1</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>166</td>
<td>100</td>
</tr>
<tr>
<td>Negative, n=24</td>
<td>50%</td>
<td>50%</td>
<td>46%</td>
<td>13%</td>
<td>29%</td>
<td>13%</td>
<td>38%</td>
<td>33%</td>
<td>55</td>
<td>8.8</td>
</tr>
</tbody>
</table>

¹ The mean age (days); ² The mean duration of symptoms before presentation (days); ³ Results are presented as yes or no due to a small number of cases; ⁴ Infant with a BP had also rhinovirus and bocavirus in the respiratory specimens; BP, Bordetella pertussis; CT, Chlamydia trachomatis; hMPV, human metapneumovirus; RSV, respiratory syncytial virus.
6 Discussion

6.1 A new estimate for the risk of vertical transmission of Chlamydia trachomatis

This research was designed to clarify a major discordance between our clinical experience and the published literature, i.e. to explain why we do not encounter and diagnose infants with CT infections even though genital CT infections are common in expecting mothers. We know from previous investigations that around 3% of pregnant women in Finland have an untreated genital CT infection (Kurkinen et al., 2006; Niinimäki et al., 2011), which may be transmitted to the newborn baby in 10% to 70% of vaginal deliveries (Chandler et al., 1977; Dannevig et al., 1991; M. R. Hammerschlag et al., 1979; M. R. Hammerschlag et al., 1982; Skjeldstad et al., 1987; Yu et al., 2009). For infants exposed to CT at birth the occurrence of CT varies from 10% to 30% for conjunctivitis and from 0% to 20% for LRTI. We consequently calculated that around 1600 babies are exposed to CT at delivery every year in our country, of whom 160–480 should develop conjunctivitis and up to 270 LRTI. The corresponding figures in our university hospital district are 120 every year for exposure, 12–36 for conjunctivitis and 0–20 for LRTI.

In the present study we found only 206 children with a possible CT infection from the comprehensive national health registers over a period of 16 years, representing an occurrence of 0.22 per 1000 live births. Likewise the risk of vertical transmission, based on the incidence and prevalence estimates from previous studies, was less than 1% among NAAT-positive mothers and less than 2% among the mothers with a recently acquired infection based on CT-specific IgG antibodies. The lower than expected occurrence of CT infections in Finland can be explained by two alternative scenarios. Either the vertical transmission rates reported previously overestimate the risk of transmission, or alternatively, we markedly underdiagnose CT infections in infants in our clinical practise.

In order to evaluate whether we fail to recognise, and therefore underdiagnose, neonates and infants with CT infections, we tested all consecutive neonates with conjunctivitis for CT for five years in child health clinics and infants aged less than six months with LRTI in a paediatric emergency department for one year. Our finding was that CT was not diagnosed in any of the neonates enrolled with conjunctivitis and in only one infant with LRTI. Thus, the prevalence of CT
conjunctivitis, based on the upper limit of the 95% CI, was less than 2% and that of LRTI less than 2.5% in our population. To summarise the implications of these new observations, it is plausible that the vertical transmission rates reported in selected populations in the era of the culture-based diagnosis of maternal CT infections (Chandler et al., 1977; Dannevig et al., 1991; M. R. Hammerschlag et al., 1979; M. R. Hammerschlag et al., 1982; Skjeldestad et al., 1987) overestimated the likelihood of vertical transmission of CT compared with our clinical setting primarily based on NAAT diagnostics. The bacterial load in culture-positive mothers is likely to be significantly higher than that of NAAT-positive mothers. This is consequential to the fact that NAAT-based test methods are not dependent on viable pathogens and in theory, are able to detect a single copy of bacterial DNA or RNA. There has been only one previous investigation into the vertical transmission of CT, in a selected series of 33 NAAT-positive mothers (Yu et al., 2009), where it was found that 24% of the neonates born to these mothers had an NP swab positive for CT, indicating an asymptomatic infection, but unfortunately the risk of symptomatic infections was not studied. In our material the population-based risk of vertical transmission leading to a symptomatic infection, i.e. either conjunctivitis or LRTI, was less than 2%.

6.2 Timing of the maternal *Chlamydia trachomatis* infection

Untreated CT infections in pregnant women can cause significant maternal and neonatal morbidity (Gencay et al., 1995; Paavonen, 2012) and to date the optimal method for preventing adverse pregnancy outcomes and infant morbidity is the screening and treatment of expecting mothers during pregnancy. Screening is usually organised at the first prenatal visit and rescreening in the third trimester only if the expecting mother has an increased risk of acquiring an infection (K. A. Workowski et al., 2015). It has been shown here, however, that one third of all maternal CT infections based on CT-specific IgG antibodies measured in the mother’s serum are contracted after the first trimester. The sensitivity of IgG antibodies for a newly acquired infection has been reported to be 67–95%, i.e. 5% to 33% of the infected women do not develop these antibodies (Csango, Sarov, Schiotz, & Sarov, 1988; Dannevig et al., 1991). Considering these data, we conclude that at least one fourth of the present mothers acquired their genital CT infection later during the pregnancy and that these infections would be likely to remain undiagnosed if screening was organised only at the first prenatal visit. This is in agreement with the observation by Jain that 29% of the mothers who gave
birth to a CT-positive infant tested negative for CT at some time during the pregnancy but were not retested at birth (Jain, 1999).

6.3 Recognition of *Chlamydia trachomatis* infections in infants

6.3.1 Chlamydial conjunctivitis

 Conjunctival bleeding after swabbing is stated as a possible finding in CT conjunctivitis in two paediatric textbooks (M. Hammerschlag, 2011; M. Hammerschlag & Kohlhoff, 2014), but neither of the books mentions spontaneous blood-stained discharge as a characteristic finding. We found here, however, that 30% of those with CT conjunctivitis had spontaneous blood-stained discharge from the infected eyes, and consequently we carefully reviewed the past literature and found two earlier patient series in which blood-stained purulent discharge was reported in 2 out of 33 infants (6%) (Rees et al., 1977) and 6 out of 19 infants (32%) (Chang et al., 2006) suffering from perinatally acquired chlamydial conjunctivitis, respectively. Chang *et al.* concluded that haemorrhagic eye discharge had a specificity of 100% and a positive predictive value of 100% for CT conjunctivitis (Chang *et al.*, 2006). We thus suggest that blood-stained discharge, spontaneous or iatrogenic, is a very characteristic feature of chlamydial conjunctivitis and should raise a high suspicion of CT as the causative agent. The exact mechanism of the blood-stained discharge observed in neonatal chlamydial conjunctivitis remains unknown. Any cause of infection and inflammation in the conjunctiva can lead to dilatation of the blood vessels, chemosis and an increase in secretion, but it is unclear why other pathogenic bacteria or viruses do not produce any bloody discharge. One possible explanation suggested in the literature is that CT particularly infects bulbar and palpebral sites on the conjunctiva, sites which contain more blood vessels than the other parts of the eye (Chang *et al.*, 2006).

6.3.2 Chlamydial lower respiratory tract infection

 Beem and Saxon, who first described the typical clinical features of infant pneumonia caused by CT in the late 1970s, found that infants with a CT respiratory tract infection presented with a distinctive cough that was severe and pertussis-like (Beem & Saxon, 1977). The cough was described as a series of closely spaced staccato coughs separated by short inspirations, but without post-tussic whoops.
Since then, a staccato cough has been regarded as a typical sign of chlamydial LRTI (American Academy of Pediatrics, 2015a; Beem & Saxon, 1977; M. Hammerschlag & Kohlhoff, 2014; Royal College of Paediatrics and Child Health, 2016; Tipple et al., 1979; K. A. Workowski et al., 2015). Quite recently, Chen et al. reported that only 22% of their patient series of prospectively examined infants with chlamydial LRTI had a staccato cough (C. J. Chen et al., 2007). Furthermore, such a cough was not recorded or recognised in any of the infants with LRTI in the present series. We consequently suggest that clinicians may not correctly identify this staccato cough and that it cannot be used to differentiate chlamydial LRTIs from those arising from other aetiological agents.

Wheezing has been mentioned in paediatric textbooks as an uncommon finding in chlamydial LRTI (American Academy of Pediatrics, 2015a; M. Hammerschlag & Kohlhoff, 2014; Royal College of Paediatrics and Child Health, 2016), even though several studies have reported wheezing in up to 50% of CT-infected infants with LRTI (Numazaki et al., 1984; Numazaki et al., 2003; Preece et al., 1989; Robinson, Meier, Lee, & Larke, 2014; G. I. Rours et al., 2009; San Joaquin et al., 1980). Our findings strengthen the association between CT and wheezing, as wheezing was detected on clinical examination in almost 50% of the infants with LRTI. Since the infants were not systematically tested for respiratory viruses, it is possible that some of them had a viral respiratory co-infection.

The symptoms of LRTI caused by CT are usually considered relatively mild, with only a few patients requiring ventilator or oxygen support (M. Hammerschlag & Kohlhoff, 2014). Somewhat contrary data were presented by Rours et al., however, as they found in their retrospective analysis that 80% of the infants with chlamydial LRTI (n=10) had dyspnoea and 60% required oxygen therapy (G. I. Rours et al., 2009). Souza et al. also reported a higher need for oxygen therapy among CT-positive infants with LRTI than in CT-negative ones (Souza et al., 2012). In addition, apnoeas have been documented in up to 10% of infants with chlamydial LRTI (Chiang et al., 2005). Our present findings are in line with these reports of severe respiratory complaints associated with CT, as more than half of the infants with chlamydial LRTI in our series had dyspnoea, 8% received oxygen therapy and 2% were admitted to the intensive care unit.

### 6.4 Diagnostic delay

The majority of previous studies of CT infections in infancy have been conducted among selected series of infants born to mothers with a diagnosed CT infection,
implying that the diagnosis of CT infection in the offspring was made soon after
the appearance of symptoms of the infection (Chandler et al., 1977; Dannevig et al., 1991; Frommell et al., 1979; M. R. Hammerschlag et al., 1979; M. R. Hammerschlag et al., 1982; Schachter et al., 1986; Skjeldestad et al., 1987). The
present data show that the diagnostic delay from the onset of the symptoms may be
long, especially among infants with chlamydial LRTI, if the mother has an
undiagnosed genital CT infection. Similar findings were reported by Tipple et al.
who found that 76% of their infants with LRTI caused by CT had been symptomatic
for longer than a week and 49% for three or more weeks by the time of presentation
(Tipple et al., 1979).

6.5 Prognosis for Chlamydia trachomatis infections

Chlamydial conjunctivitis of infancy has been reported to cause conjunctival and
corneal scars (Forster et al., 1970; Mordhorst & Dawson, 1971; Watson & Gairdner,
1968), and bilateral corneal scars were detected in one child in the present series,
corresponding to an occurrence of one per million births. The particular child had
not attended regular healthy-child visits and her chlamydial conjunctivitis was first
diagnosed and treated at the age of three years. All the children treated during
infancy showed no long-term consequences related to CT infection. Even though
6% of the present children had episodes of recurrent wheezing, all of them had
normal lung findings in pulmonary function tests, and we could not confirm chronic
lung problems as potential long-term sequelae of treated chlamydial LRTI
(Harrison et al., 1982; Weiss et al., 1986).

The prevalence of persistent nasolacrimal duct obstruction after the first year
of life is estimated to be 4% (MacEwen & Young, 1991). The occurrence found in
our data was 2%, which is slightly less, but within the range of the figures reported
in the literature. Our purpose was to investigate whether there is any association
between CT and persistent nasolacrimal duct obstruction, since we hypothesised
that CT may be able to cause scarring of the nasolacrimal ducts in infants just as it
does scarring of the Fallopian tubes in women. As chlamydial conjunctivitis was
non-existent in our population, we could not evaluate this hypothesis. None of the
pathogens that we detected had any long-term consequences associated with them.
6.6 Differential diagnostics

6.6.1 Neonatal viral conjunctivitis

There have only been two previous investigations into the viral aetiology of neonatal conjunctivitis (Rapoza et al., 1986; K. I. Sandström et al., 1984), each reporting one case of viral conjunctivitis (1/100 and 1/55), the causative agents being Coxsackie virus A9 and herpes simplex virus type 1. In the present series, however, respiratory viruses were found in 5% of the neonates with clinical conjunctivitis when using multiplex real-time PCR detection methods. This finding provides support for the notion that viruses are also clinically relevant to the aetiology of this disease during the neonatal period. Altogether two neonates with viral conjunctivitis had a concomitant bacterial pathogen in their conjunctival samples, but even after excluding these cases, the proportion of viral conjunctivitis remained at 4%.

6.6.2 Whooping cough and viral respiratory tract infections

The typical clinical features of a whooping cough infection include a paroxysmal cough followed by high-pitched whooping sounds when the person breathes in (Frumkin, 2013). Whereas the symptoms of a chlamydial LRTI develop gradually and the course of the disease is often chronic, BP is an acute and potentially life-threatening respiratory illness (Berger et al., 2013; Cantey et al., 2014; Crowcroft et al., 2003). The present data show that the symptoms of infants with either CT or BP infections were similar to those of infants with viral LRTIs. The typical clinical symptoms for CT or BP, such as staccato cough, whooping or conjunctivitis, were reported in many infants who had a sole viral respiratory infection. This is in accord with previous studies showing that the clinical manifestations of LRTIs in infants are often non-specific and that there is a significant overlap in symptoms and signs between the different aetiologies (Cosnes-Lambe et al., 2008; Korppi & Hiltunen, 2007). In addition, the diagnosis of a viral respiratory infection does not exclude the possibility of an infection with CT or BP, as co-infections with viruses and these bacteria have been reported in up to 16% of infants with acute LRTIs (Cosnes-Lambe et al., 2008; Korppi & Hiltunen, 2007; Nuolivirta et al., 2010; G. I. Rours et al., 2009).
6.7 Strengths and limitations of the research

The strengths of our work lie in the use of nationwide data drawn from comprehensive national health registers. It is therefore one of the first to evaluate the probability of vertical transmission of CT in an unselected population in the era of NAAT-based diagnosis of maternal CT infections. Our findings are further strengthened by the opportunity to obtain data on the mothers from the NIDR and the ability to analyse CT-specific antibodies from serum samples drawn from the mothers during their pregnancies. We also reviewed the medical records of all the children up to the age of 16 years to evaluate the long-term prognosis of CT infections and to validate the national register data.

One additional strength of our study is that we investigated both the viral and the bacterial aetiology of neonatal conjunctivitis with modern diagnostic tools in a community setting. We were also able to evaluate the possible long-term consequences of neonatal conjunctivitis, since there is only one centre in the area concerned that provides ophthalmological surgery for infants and children. In addition, our study is one of the first to investigate prospectively the proportions of both CT and BP among infants aged less than six months presenting with LRTI.

The work has some limitations, however. The data for papers I and II were collected retrospectively from registers, implying that we only had data on diagnosed and probably the most symptomatic CT cases. For papers III and IV, however, we prospectively screened consecutive cases with either conjunctivitis or LRTI and showed that failure to recognise CT infections is not a likely explanation for the low incidence of CT observed in the register-based assessments (papers I and II). The primary limitation of the study is that the vertical transmission rates presented here are based on the figures reported in two earlier Finnish studies that evaluated the incidence and prevalence of CT positivity among pregnant women (Kurkinen et al., 2006; Lyytikäinen et al., 2008). Kurkinen et al. used NAAT from first-void urine in diagnostics (Kurkinen et al., 2006), while Lyytikäinen et al. estimated the incidence of CT based on serological methods (Lyytikäinen et al., 2008). The different methodologies used by previous studies to detect CT infections and the fact that the present study was a register-based work provides a possible bias in the vertical transmission rate calculations. In addition, Kurkinen et al. examined only a small number of pregnant women in one region in Southern Finland over a relatively short period of time (Kurkinen et al., 2006), and therefore the prevalence of CT positivity presented in their study may not represent the overall prevalence of CT positivity among pregnant women throughout the country.
Furthermore, the timing of the maternal CT infection remained somewhat uncertain in Lyytikäinen et al.’s study because the incidence rate was estimated from the seroconversion between two pregnancies within five years (Lyytikäinen et al., 2008). Also, the risk of vertical transmission of CT was two-fold higher when calculated from the seroconversion rate rather than from the overall rate of NAAT positivity, however, both rates are significantly lower than indicated by earlier studies (Chandler et al., 1977; Dannevig et al., 1991; M. R. Hammerschlag et al., 1979; M. R. Hammerschlag et al., 1982; Skjeldstad et al., 1987; Yu et al., 2009).

One limitation is that the neonatal conjunctivitis cases were recruited from child health clinics and not from health centres directly. Child health clinics assess the physical, mental and social growth and development of children under school age and provide vaccinations, but the scope of child health clinics does not cover the diagnosis of diseases or the treatment of acutely ill children. It is therefore possible that some neonatal conjunctivitis cases in the study population were missed. However, a search of all microbiologically confirmed CT diagnoses in neonates in that hospital’s catchment area did not reveal any chlamydial neonatal conjunctivitis cases diagnosed outside this study.

This work was carried out in a country with excellent standards of maternity care and easy access to medical care, which impairs the generalisability of the results to countries with poor maternity care. In addition, the research was not designed to evaluate the need for universal screening programmes for CT targeted at pregnant women, because we were not investigating maternal chlamydial infections or the sequelae related to them. Not all pregnant women are routinely screened for genital CT infection in Finland. However, local practices for CT testing may differ, and there may be some regions that already test either all pregnant women or those known to be at increased risk for genital CT.

Previous authors have detected respiratory viruses, particularly rhinoviruses, in nasal swab samples from asymptomatic subjects during the neonatal period (Sarna et al., 2016). Even though we suggest that respiratory viruses are probable pathogens when found at the ocular site, we cannot confirm that the viruses found here caused the conjunctival symptoms in all the subjects with a positive virus finding. Moreover, we did not evaluate the sensitivity of multiplex real-time PCR for conjunctival specimens, and therefore the true occurrence of respiratory viruses may be even higher than that reported here.
6.8 Clinical implications

The introduction of modern molecular methods has rapidly changed the diagnostics of respiratory tract infections in infants. Clinicians have widely adopted the use of multiplex real-time PCR assays that commonly detect and identify 15–17 respiratory viruses. Recently, panels that also include respiratory bacteria have been implemented in clinical practise. Multiplex panels such as FilmArray® (BioFire Diagnostics, Utah, USA) and Allplex™ Respiratory Panel Assays (Seegene Inc., Seoul, Korea) simultaneously detect BP, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in addition to the *Bordetella parapertussis*, *Legionella pneumophila*, *Haemophilus influenzae* and *Streptococcus pneumoniae* included in the Allplex™ panel.

While the role of the atypical bacteria *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in the pathogenesis of LRTIs in young infants is currently unclear, both CT and BP are clinically important pathogens in young infants, and treatment with antibiotics is recommended for both infections. However, CT is often difficult to recognise from other causative agents of LRTIs in infants, and many clinicians are not completely aware of this particular respiratory pathogen as it is relatively rare. Therefore, CT infection may take a long time to diagnose, as was demonstrated in the present study. CT is not currently included in most respiratory bacterial panels. For clinicians, a multiplex real-time PCR panel that includes CT in addition to BP and common respiratory viruses would be an excellent tool to rapidly and accurately establish the aetiology of LRTIs in young infants.

Although very useful in LRTIs in infants, there are no such panels available for conjunctival specimens. Therefore, if the neonate suffers from conjunctivitis, and especially if the conjunctivitis is characterised by bloody discharge or prolonged symptoms, clinicians should investigate the possibility that CT is a causative agent by sampling and testing for CT in addition to conventional bacterial culture.
7 Conclusions

1. The risk of vertical transmission of CT, less than 2% as deduced from national register data, appeared to be markedly lower than had been reported earlier.

2. Spontaneous blood-stained discharge was a novel sign for the recognition of CT infection in infants, but the distinctive staccato cough was not recognised in any of the present cases.

3. The long-term prognosis for diagnosed and treated cases was excellent. Only one child with a significantly delayed diagnosis of chlamydial conjunctivitis, 1:1 million live births, developed corneal scars.

4. CT was a rare pathogen, causing less than 2% of all the neonatal conjunctivitis cases observed in primary care. Thus, the low incidence of CT observed in clinical practice does not result from underdiagnosis of CT infections in neonates with conjunctivitis in primary care. Respiratory viruses were detected in 5% of the present neonates with clinical conjunctivitis, which is a significantly higher rate than found in previous studies.

5. CT was a causative agent in less than 2.5% of LRTIs observed in infants in a hospital setting, implying that failure to recognise CT infections is not a likely explanation for the low incidence of CT observed in clinical practice. The occurrence of whooping cough was low, less than 2.5%, but the study was performed during a non-epidemic season.
References


109


**List of original publications**


Reprinted with permission from BMJ Publishing Group Ltd. (I), Wolters Kluwer (II) and John Wiley and Sons (III).

Original publications are not included in the electronic version of the dissertation.
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