Introduction
1

General introduction
1. General introduction

1.1 Historical perspective of Chlamydia trachomatis

Chlamydia trachomatis (Ct) comprises a collection of serologically distinguishable Gram-negative intracellular bacteria. Ct can colonize different types of tissue, like the conjunctiva, cervix, urethra, oropharynx, proctum and lymph nodes, and leads to a variety of human diseases depending on the anatomical site of infection (i.e., trachoma, lymphogranuloma venereum, pelvic inflammatory diseases, reactive arthritis). Using current highly sensitive methods, viable Ct is also detected in peripheral blood mononuclear cells in skin lesions and synovial fluid (14-15).

Although trachoma and lymphogranuloma venereum have been known for centuries, the etiology remained mysterious (128). More insight in the disease trachoma was obtained in 1907 when Halberstädter and von Prowazek inoculated the eyes of Orang-utans with conjunctival swabs of trachoma patients (36) (figure 1). In Giemsa-stained epithelial cells from the Orangutan’s conjunctiva they discovered intracytoplasmic vacuoles containing numerous particles. Some small particles, now recognized as Ct elementary bodies, were also detected outside the epithelial cells. The intracytoplasmic vacuoles contained larger particles, known as reticulate bodies. Professor Tang isolated Ct in 1957 for the first time and initially recognized it as a viral infection (126). In the 1960s, the use of tissue culture techniques and electron microscopy revealed evidence for bacterial, ribosomes and cell wall structures, suggesting that Ct is a bacterium. During the sexual revolution in the late 1960s it became clear that Ct can be sexually transmitted (78) and in the late 1970s it was even recognized as the most prevalent sexually transmitted bacterial infection (100, 154). Currently, the total of infections is estimated over 92 million cases each year, which is supposed to be an underestimation (153).

In 1963, Wang and Grayston described for the first time the existence of different Ct serotypes (146). They inoculated egg yolk sacs with Ct and subsequently injected the crude extracts into mice to trigger the mice’s immune response. In some of these mice, a protective mechanism against the Ct infection was observed, while other mice were only partly protected, apparently by cross-protection. This observation suggested the existence of different Ct serotypes. Because this method was time-consuming and complex, more easier tests were needed for larger scale epidemiological studies, leading to the development of polyclonal microimmunofluorescence tests (147) and subsequently to the more specific monoclonal microimmunofluorescence tests, targeting the major outer membrane protein (MOMP) (150). While microimmunofluorescence tests contributed to more knowledge on the etiology...
of the different Ct serovars in the early days of Ct research, the technique was replaced at the end of previous century by more sensitive genotyping methods targeting the gene coding for the MOMP (OmpA gene). Currently, different types of genotyping methods are used in sexual network studies, phylogenetic studies and serovar distribution studies resulting in a better understanding of the epidemiology and etiology of the different Ct serovars.

**Figure 1** Halberstädter and von Prowazek inoculating the eyes of an Orang-utan (1906).

### 1.2 Taxonomy of Chlamydiales

All members of the order Chlamydiales are gram-negative, non-motile bacteria with a biphasic developmental cycle. At the end of the 20th century, a proposal for revision of the order Chlamydiales was developed, due to the discovery of a number of Chlamydia-like organisms such as the Simkania strain and Parachlamydia acanthamoebae (26). The revision is based on 16S rRNA sequence similarity and analysis of full-length 16S and 23S rDNAs. Members of the order Chlamydiales share at least 80% sequence identity for the genes encoding the 16S rRNA and 23S rRNA. The new Chlamydiales taxonomy splits the former family Chlamydiaeae into two genera and add three new non-Chlamydiaaceae families. Thus, according the new taxonomy, the species Chlamydia trachomatis, together with the species Chlamydia suis and Chlamydia muridarum, belong to the genus Chlamydia, the family Chlamydiaceae and the order Chlamydiales while the species C.pneumoniae and C.psittaci belong to the new genus Chlamydophila (figure 2). The new taxonomy is better structured than the previous taxonomy, although not commonly accepted by all chlamydiologist (103).

### 1.3 Chlamydia trachomatis developmental cycle

Ct has a biphasic developmental cycle characterized by alternating between two morphological forms, the electron dense round elementary body (EB) and the reticulate body (RB) (Table 1) (73). EBs are small (300nm), metabolically inactive, extracellular forms, that are responsible for dissemination of Ct and are able to attach to and invade susceptible cells.

The EBs interact with the host cell membrane in a two-stage process. First, initial attachment occurs through reversible electrostatic interactions of the Ct MOMP with heparin sulfate containing glycosaminoglycans present on the surface of epithelial cells (118, 124-125, 127). The second, irreversible binding is possibly associated with protein disulfide isomerase, a component of the estrogen receptor complex (20). This irreversible binding step is immediately followed by a type three secretion system (TTSS) mediated injection of the translocated actin-recruiting phosphoprotein (TARP) into the host cell cytoplasm (figure 3). Inside the host cell, TARP is rapidly phosphorylated.

### Table 1

<table>
<thead>
<tr>
<th>Property</th>
<th>Elementary body</th>
<th>Reticulate body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>0.2-0.4 µm</td>
<td>0.6-1.0 µm</td>
</tr>
<tr>
<td>Rigid cell Wall</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Extracellular stability</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Infective</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Induces phagocytosis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Inhibits phago-lysosomal fusion</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Metabolic active</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Replication</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The EBs interact with the host cell membrane in a two-stage process. First, initial attachment occurs through reversible electrostatic interactions of the Ct MOMP with heparin sulfate containing glycosaminoglycans present on the surface of epithelial cells (118, 124-125, 127). The second, irreversible binding is possibly associated with protein disulfide isomerase, a component of the estrogen receptor complex (20). This irreversible binding step is immediately followed by a type three secretion system (TTSS) mediated injection of the translocated actin-recruiting phosphoprotein (TARP) into the host cell cytoplasm (figure 3). Inside the host cell, TARP is rapidly phosphorylated,
triggering phagocytosis of the EB attached to the host membrane (19). Once entered into the cell by phagocytosis, the EB starts an immediate differentiation to the larger (1 μm) metabolically active non infectious RB. The RB expresses directly early genes, associated with inclusion body modification (i.e., inc-like genes CT228, CT229 and EEA1-like gene CT147). The modification of the inclusion body prevents the inclusion body from entry into the endocytic pathway leading to lysosomal fusion. Six to 14 hours after phagocytosis, the RB starts replicating, resulting in extensive growth of the inclusion body, containing up to 10,000 *Chlamydia* particles. Approximately 30 hours after phagocytosis, the RBs differentiate back to EBs and are released by exocytosis or host cell lysis (Figure 4) (1).

1.4 *Chlamydia trachomatis* endogenous plasmid and genomic genes
The first complete Ct genome (serovar D) was sequenced during the late 1990 as part of the *Chlamydia* genome project (117). This provides valuable information about the genome organization and allowed further research in protein expression and gene regulation. The Ct genome comprises 1,042,519 basepairs with 894 open reading frames encoding for predicted proteins. The endogenous plasmid, consisting of 7,493 basepairs, was also sequenced. Subsequently, the endogenous plasmid, the chromosomal OmpA gene and the
Recently, a new variant of Ct has been discovered in Sweden (designated as the Swedish variant). This Swedish variant carries a plasmid showing a 377 basepairs deletion within coding sequence 1 (93). Although the Swedish Ct variant seems normally transmittable, the infection seems to remain more frequently asymptomatic than regular Ct strains (7). Importantly, most nucleic acid amplification Ct detection tests are targeting the endogenous plasmid, because the plasmid copy number is approximately 10 times higher than chromosomal Ct DNA and therefore permits more sensitive Ct detection. The 377bp deletion found in the Swedish strain overlaps the target region for 2 frequently used nucleic acid amplification tests (Roche Diagnostics and Abbott Laboratories). This results in false-negative Ct tests results by these assays for the Swedish strain (61), causing a major underestimation of the Ct prevalence in Sweden (113). The underestimation has a further impact on the Swedish healthcare system, since infected individuals remain untreated, which leads to persistent Ct infections with possible severe late complications. Infected and untreated individuals have also more time to transmit the bacteria to other persons. Immediately after identification of the Swedish strain, Roche and Abbott developed next generation dual target assays, targeting the endogenous plasmid as well as chromosomal DNA (OmpA gene) (35). The proportion of the Swedish variant has declined significantly, since the introduction of the upgraded detection assays, possibly due to faster treatment (48).

1.4.2 MOMP and the OmpA gene
The Major Outer Membrane Protein (MOMP) is the most studied Ct protein and is encoded by the chromosomal OmpA gene. The protein structure comprises 5 transmembrane constant domains and 4 variable domains outside the membrane (figure 5). The differences in MOMP variable epitopes permits classification of the Ct strains into 3 major serogroups and 19 different serovars, designated serogroup B (comprising serovars B, Ba, D, Da, E, L2 and L2a), serogroup C (comprising serovars A, C, H, I, Ia, J, K, L1 and L3) and the intermediate serogroup (comprising serovars F, G, Ga) (52, 79).

Serovars A, B and C are mainly detected in conjunctival samples, while the serovars D-K are mostly found in the anogenital tract as common STD confined to the mucosal layer. In contrast, the serovars L1, L2 and L3 invade the submucosal layer and lymph nodes causing the disease lymphogranuloma venereum (106). It seems that the disease type is not determined by MOMP but other proteins, although it is possible to cluster the serovars into the 3 disease groups (biovars).

chromosomal pmp genes will be discussed in detail, since those genes are the most frequent DNA targets for diagnostic Ct assays.

1.4.1 The endogenous plasmid
Almost all Ct isolates carry a small plasmid which contains 8 open reading frames. Although the exact function of the endogenous plasmid and its proteins remain unclear, it is supposed that the plasmid genes plays an important role in transcriptional regulation of chromosomal genes and contributes to the virulence of the Ct strain (13). Naturally occurring plasmid-free strains of Ct have been reported, but are exceedingly rare and do not spread into the population, possibly due to low virulence (27, 123). Plasmid-free Ct isolates are also unable to accumulate glycogen in their inclusion body, which is a typical characteristic of Ct. For the viability of Ct, the plasmid seems important but not necessary (87).

Adapted from Gerald J Stine 1992, the biology of sexually transmitted diseases Wm. C. Brown, USA.

Figure 4 Developmental cycle of Chlamydia trachomatis.
complete Ct genome revealed a gene family, consisting of 9 genes that encode for autotransporter proteins (pmp genes). (117). While most pmp genes are highly conserved among the different Ct serovars, one pmp gene (pmpH) can be clustered into three clades that are compatible with the biovars (ie ocular trachoma clade, STD clade and LGV clade; Table 2) (122).

The compatibility of the 3 clades with disease suggests that this protein might play a role in pathogenesis, virulence an tropism. Nevertheless, there is still an incomplete understanding of the virulence and host immune response to these proteins, despite the potential importance of pmp in chlamydial biology.

2. Chlamydia trachomatis epidemiology, serovar distribution and clinical manifestation

2.1 Epidemiology of urogenital Ct infections
Genital Ct infections are the most common bacterial sexually transmittable disease worldwide, with between the 90 and 92 million new infections each year (32). Over the past decade, the detection rate of Ct has increased, due to the application of more sensitive Ct detection assays and the rise of Ct screening programs. Infection may occur at any age, but peaks in the 16-25 year age groups, since these persons show high-risk behavior. Important risk groups are female sex workers, homosexual men, unmarried young adults and swingers (25, 141, 159). Risk factors for a Ct infection are high frequency of...
partner change, and unprotected sexual intercourse (60). In the USA, more than a million cases of chlamydial infections were reported in 2007, while the estimated amount of infected individuals was around 3 million (16, 152). In the past decade, the incidence of Ct infections in Europe has also increased. In 2005, over 200,000 cases were reported in 17 European countries, which is certainly an underestimation, since in the Netherlands alone 60,000 Ct infection occur annually (94, 130). This underestimation might be due to the asymptomatic course in the majority of Ct infections and the lack of extensive diagnosis in other countries.

2.2 Distribution of Ct serovars

Throughout the world many serovar-distribution studies are performed. Most distribution studies focus at Ct infections retrieved from the urogenital tract (serovars D-K). In general, similar serovar distributions are observed in different countries. Although some regional differences are observed, serovar E is the most prevalent serovar worldwide. The serovar E prevalence varies between the 30-60% of the Ct positive samples in the countries USA (Alabama), Australia, China, Costa Rica, France, Iceland, Iran, Korea, Russia, Sweden, Taiwan and the Netherlands (6, 28-29, 41, 44, 46, 53, 89, 95, 108, 116, 155). In contrast, serovar E was far less prevalent in Uganda, Thailand, India and Columbia (3, 67, 91, 107). This variation might be due to the different Ct genotyping methods (Thailand) or a small isolated study population (Uganda, India). Overall, serovars D, E and F contribute for over 70% of all typed urogenital serovars, making it difficult to study an association with symptoms (23, 71, 132). In specific subgroups, like men who have sex with men (MSM), a different serovar distribution is observed. The majority of anogenital Ct infections in MSM are caused by serovars G, D and J (4, 31, 49, 55, 120, 143). The difference in serovar distributions between MSM and the general population can be network related, like observed among heterosexual individuals living in an isolated town in Canada (11). Apart from network-associated factors like sexual prevalence, tissue tropism could explain the difference in serovar distribution. In a recent study, an association between rectal tropism and polymorphisms of open reading frames within genomic DNA of serovar G is observed (43). Genovariants from the serovars G, D and J might be more affected by the polymorphisms associated with rectal tropism than other serovars, although those polymorphisms are not located on the OmpA gene. So for a better understanding of tissue tropism we should focus on other Ct genes, independent of the OmpA gene.

2.3 Clinical manifestation and complications

An urogenital Ct infection can cause urethritis in men and vaginitis, cervicitis and urethritis in women. Since most infections are asymptomatic (women: 70%, men: 50%), the great majority of infections remain unnoticed and thus untreated (131). Symptoms in men are dysuria and a clear urethral discharge, both occurring 7-21 days after primary infection. Complications in men can be epididymitis, prostatitis and reactive arthritis (85). At this moment, the role of Ct in the development of male infertility is not fully understood, since contradicting results are published (17, 21). It is suggested that the infertility is caused by strictures of the Vas deferens, due to previous inflammation. Reactive arthritis (previously called Reiter's syndrome) can be triggered by a urogenital Ct infection and is more frequently observed in men (39). Besides the triad of urethritis, conjunctivitis and arthritis, a reactive arthritis can also give rise to mucocutaneous lesions on the penis, mouth, hands and feet (90). The majority of women have no complaints, but symptoms can be a mucopurulent vaginal discharge or post-coital bleeding. A cervical Ct infection might induce an ascending infection causing endometritis, salpingitis, pelvic inflammatory disease (PID) and perihepatitis (Fitz-Hugh-Curtis syndrome). Although high percentages of progression have been given for these complications, it appears that most of the complications as described are the result of symptomatic Ct infections (72). In Europe, Ct infections are responsible for at least 60% of the PID cases (98). PID can lead to late severe reproductive complications later in life due to tubal adhesions. It is estimated that two-third of women with tubal factor infertility and one-third of the women with ectopic pregnancies have Ct antibodies detectable in the serum, indicating a Ct infection earlier in life (81, 85). The course and duration of an untreated, asymptomatic Ct infection is not totally clear. It would be relatively easy to investigate the course and duration of an untreated Ct infection, but ethical considerations, regarding the prominent health risk for untreated individuals, do not allow those prospective cohort studies and restrict the investigations to retrospective studies. However, the primary time point at which a person had been initially infected is hard to determine in retrospective studies. Also, discrimination between a recurrent or persistent Ct infection is not possible, although Ct genotyping might give an indication. Still, various studies have investigated the natural course of Ct infections in minimal prospective studies and larger retrospective studies (30, 45, 62, 68, 72, 84, 97, 105, 134, 140). In general, approximately 50% of the asymptomatic Ct infections were cleared spontaneously within the first year and no complications occurred. After 5 years, 95% of the women had resolved their Ct infection spontaneously without receiving antibiotic treatment. Those results indicate a currently overrated fear for complications due to a Ct infection.
However, it remains important to realize that most of those associations are observed in single studies that cannot be reproduced. Several other studies do not confirm the associations between serovars and symptoms (57, 65, 76, 86, 121). So more studies will be required to confirm or reject the association between symptoms and serovars.

### 2.5 Lymphogranuloma venereum

Lymphogranuloma venereum (LGV) is a sexually transmitted disease, caused by Ct serovars L1, L2, L2a or L3. Originally, LGV was known as an endemic disease in Africa, Southeast Asia, Central/South America and the Caribbean. Until 2003, LGV was only diagnosed sporadically in the Netherlands as a traveler’s disease imported from foreign countries (142). The disease has an aggressive nature with serious late sequelae. The disease LGV comprises 3 stages. In the first stage, a painless erosion/ulcer in the anogenital region appears 3-30 days after infection. This erosion frequently remains unnoticed and is observed in only 4% of the LGV patients (102). A visible erosion or ulcus is difficult to differentiate from a trauma or other ulcerative diseases, like primary Syphilis and Herpes simplex infections. The second disease stage occurs 1-4 weeks after infection and is characterized by lymphadenopathy in the so-called bubonic form of the inguinal lymph nodes in men or retro-peritoneal lymph nodes in women (Figure 6).

However, other lymph nodes (e.g., femoral) in the lower abdomen can also be infected, depending on the infection site. Over time, the infected lymph nodes become abscesses and may rupture, leading to fistulae. If LGV infections remains untreated a tertiary (final) stage will develop, due to progressive spread and tissue destruction. At this stage, irreversible damage to the lymph nodes will lead to an impaired lymph drainage and possibly elephantiasis. Also signs of more cachexic illness can be observed (135).

Since 2003, LGV proctitis (the anorectal variant) is observed in developed countries among men who have sex with men (MSM), caused by the genovariant L2b (37, 77, 114). The majority of the infected MSM (80%) are co-infected with blood borne diseases like HIV and hepatitis C (133). First it was thought that LGV proctitis in MSM was a new epidemic, but retrospective studies performed in San Francisco revealed that LGV was already present in rectal swabs from MSM in 1981, indicating an old unnoticed epidemic (115, 135).

Differentiation between an LGV and non-LGV Ct infection is important, since an LGV infection need prolonged antibiotic treatment (22). Several in-house PCRs are available for differentiation between a LGV serovar and an anogenital (D-K) serovar (18, 37, 70).
3. *Chlamydia trachomatis* diagnostic methods

In the 1970s Ct culture was the standard reference method for diagnosing Ct infections. However, the restrictive specimen collection, transport and storage conditions limited the availability of the test for clinical purposes. During the 1980s, two antigen detection methods were developed. The first assay was a direct immunofluorescence assay (DFA), utilizing a fluorescein-tagged monoclonal antibody to allow Ct elementary bodies to be microscopically visualized in cellular smears collected from the endocervix and conjunctiva (MicroTrak, Trinity Biotech, Ireland) (129). The second detection method was a solid phase enzyme-linked immunosorbent assay (EIA), using polyclonal anti-chlamydial antibodies to capture Ct antigen onto beads (Chlamydiazyme, Abbott Laboratories, USA) (88). Both assays generate a fare more rapid result (2-3 hours) as compared to Ct culture (3-7 days), but had a slightly lower sensitivity. A limitation of DFA is the requirement of expert microscopists, making it laborious and thus less suitable for high-throughput studies compared to the EIA. Currently, other Ct detection methods are available like point of care (POC) rapid assays, DNA probe assays and highly sensitive nucleic acid amplification tests (NAATs). The three POC assays most commonly used are the Clearview (Unipath, UK), Biostar (Thermo Electron Corp, USA) and the QuickVue (Quidel, USA), all based on direct detection of Ct antigens (136). The POC assays give a result within 30-40 minutes ensuring that patients can immediately receive antibiotic treatment. Like HIV rapid tests, the assays have a very high specificity, but a low to moderate sensitivity indicating that a second more sensitive assay is needed to confirm the obtained test result. The main utility of those POC tests are in applications where return for treatment is unlikely or in population screening programs (59, 99). The two most commonly used DNA probe assays are the PACE2 (Gen-Probe Inc, USA) and the Hybrid Capture 2 (HC2) Ct assay (Qiagen, USA) (66, 137). The PACE2 system uses a labeled DNA probe that binds to Ct rRNA, generating a DNA/RNA hybrid. No amplification is required, due to the high copy number of Ct rRNA. Although this assay has a very good specificity, the test is being replaced by the cheaper and more sensitive HC2 assay. The HC2 targets both the genomic *OmpA* gene and the endogenous plasmid DNA by using a labeled RNA probe. The HC2 does not need DNA amplification, due to the use of signal amplification with multiple labeled antibodies specific for DNA/RNA hybrids. The use of signal amplification instead of DNA amplification prevents the HC2 assay against carry-over contamination. The HC2 system is a good alternative for NAATs, although the sensitivity of DNA probe assays is slightly lower (table 4) (8).

![Figure 6 Example of a bubonic inguinal lymph node in a men.](image)

The picture was kindly provided by Henry de Vries.
NAATs are in general the best diagnostic tools for Ct detection, although they are relatively expensive (Table 4) (8, 40). However, NAATs are nowadays the gold standard for Ct detection. Different types of commercially available NAATs like the rRNA based Aptima Combo 2 assay (Gen-probe), the COBAS Amplicor (Roche Diagnostics), the COBAS TaqMan (Roche Diagnostics) and the Ct-DT DEIA (Labo Biomedical Products BV) are used in routine diagnostics. Most assays have different targets (i.e., endogenous plasmid, chromosomal DNA or rRNA), but have more or less the same sensitivity.

4. **Chlamydia trachomatis typing**

4.1 **Chlamydia serotyping**

During the sixties, the first experiments involving Ct serotyping were performed by Wang and Grayston, who developed the mouse toxicity prevention test (MTPT) (146). Briefly, they inoculated egg yolk sacs with “trachoma-inclusion conjunctivitis organisms”. Extracts were subsequently intravenously injected into mice that subsequently triggered an immune response. A protective mechanism in the mice was described when the mice were challenged for the second time with the same isolates of Chlamydia. When the mice were challenged with other isolates, the protective mechanism was less pronounced indicating cross-protection, but more important, the existence of different serovars. The MTPT method was very time-consuming, since it took 7 years to classify 80 Ct strains into 6 serotypes (150). Therefore, a more rapid culturing of elementary bodies of known serotypes in yolk sacs and the microimmunofluorescence (MIF) test was developed which was based on culturing of elementary bodies of known serotypes in yolk sacs and the production of polyvalent antiserum in mice (147, 149). The combination of the MIF test and the ability of culture of cells with Ct inclusion bodies facilitated the discovery of 15 different Ct strains (A-L3) based on variability in the major outer membrane protein (MOMP) (51, 145, 148). Later, other subserovars like Ba, Da and Ga, were discovered. During the 1980s the relatively aspecific polyclonal antibodies were replaced by more specific monoclonal antibodies (119). The above-described principle was the basis for the development of several immunoassays that were used until the early 1990 when DNA sequencing of the OmpA gene became available (138). A disadvantage of immunotyping was the requirement of Ct culture which is time-consuming, expensive and requires expert staff. Also, the sensitivity of culture is low compared to DNA analysis.

4.2 **Chlamydia genotyping**

During the 1990s serotyping was replaced by molecular genotyping of the OmpA gene, encoding MOMP. The first genotyping method consists of a PCR followed by restriction fragment length polymorphism (RFLP) and pattern analyses on a polyacrylamide gel. With this method it was possible to differentiate between 13 genotypes (96). The patterns observed by RFLP were compatible with the results obtained by serotyping, so the name “serovar” could be maintained. The strength of the PCR-RFLP methods is the ability to detect double serovar infections (156). At this moment, RFLP is still performed to differentiate between an LGV and anogenital serovar, even with other alternatives available (38, 54).

With the availability of fluorophore-marked nucleotides enabling DNA sequence analyses, OmpA sequencing became an alternative for RFLP. Sequencing has a higher resolution than RFLP and serotyping, leading to the discovery of new genovariants (69). A drawback with sequencing is the inability to detect double serovar infections, because a multiple serovar infection will lead to a mixed and therefore non-interpretable sequence result.

Currently, different reverse line blot assays are available that can identify the different serovars (6, 155, 158). Those assays are sensitive simple technology reverse hybridization methods, targeting the variable regions in the OmpA gene. The method is highly suitable for epidemiological studies due to its ability to detect multiple serovar infections, the relatively fast results and the ability to perform the assay manually with the use of simple technology (i.e., PCR). However a drawback of the reverse line blot is the lower discriminating power compared to OmpA sequencing. At this moment, new high throughput microsphere suspension assays are developed, based on reverse hybridization technology for the Luminex 100 platform, which can be used in large-scale serovar distribution studies (42). We can consider those microsphere suspension assays as second generation hybridization methods.

At STD clinics, Real-Time PCRs are used for discrimination between a LGV infection and a non-LGV Ct infection (12, 33). The probe target for those Real Time PCRs is a L-specific gap in the polymorphic protein H (pmpH). Those PCRs are all in-house validated methods, since no commercial pmpH Real-Time PCRs are available. Although this method belongs to the Ct genotyping methods, it cannot identify the different serovars and is thus not used in epidemiological studies.

An upcoming genotyping method is the high-resolution typing of Ct strains by multi locus sequence typing (MLST) that uses several other genes as targets (47). This method has a higher discriminatory power then OmpA sequencing.
HPV types, associated with genital warts and high risk (hr) HPV types, associated with cervical cancer. Besides cervical cancer, HPV is also associated with other carcinomas (e.g., anus, vulva/vagina, penis and oropharynx) (83). Worldwide, approximately 53% of the cervical cancers are caused by hr-HPV type 16 and 17.2% by hr-HPV type 18 (111). Taken together, 94.6% of the cervical cancers are caused by the hr-HPV types 16, 18, 45, 31, 33, 52, 58, 35, 59, 56, 51, 39, 68, 73 and 82 (74). The remaining 5.4% are caused by unknown HPV types.

5.3 Chlamydia trachomatis and cervical cancer

Several co-factors, like smoking, oral anticonceptive usage, and other sexually transmitted infections (e.g., HIV infection, Herpes Simplex 2 infection, Ct infection) have been suggested to facilitate the persistence of an HPV infection or even the development of cancer(9). The first report of an association between Ct infections and cervical cancer was already published during the 1970s (101). Multiple, but not all, studies determined an association between Ct antibodies in the serum and squamous cell carcinoma of the Cervix (SCC) or persistence of carcinogenic HPV types (2, 50, 75, 110, 112). However, no association between Ct antibodies or Ct DNA and adenocarcinomas of the cervix were observed, while the columnar cells of the endocervix are the major Ct infection site (58, 92, 157). So based on the pathogenesis of Ct we would expect an association with adenocarcinomas instead of SCCs.

Most studies observed only an association between Ct antibodies and SCC, but one study also observed Ct DNA in cervical tumors (144). Serovar G antibodies were even associated with a higher risk for SCC (2). Overall, these results suggest a role for Ct as a co-factor in cervical carcinogenesis for SCC either by facilitating a persistent HPV infection via interactions with the immune system or by facilitating HPV induced SCC itself due to the inflammatory status of (parts of) the genital tract (64).

While the association between Ct antibodies and SCC has been interpreted by some groups as a causal link, others think it might be caused by residual confounding of other factors that are related to an HPV-positive status. Ct and HPV are both common sexually transmitted infection that share the same risk factors, such as sex at young age or multiple sexual partners. This strong relationship between HPV and Ct can result in inadequate adjustment for HPV, especially when low sensitive HPV serology is used as a marker for an HPV infection. So further studies, restricted to HPV positive cases and controls only, should be performed to overcome the issue of residual confounding.
The current thesis comprises of three sections. In the first section (Chapter 2-5) comprises a technical part, in which several Ct detection and genotyping methods are evaluated. The second section (Chapter 6-9) comprises several studies regarding the Ct serovar distribution and the relation with clinical signs and symptoms. In the third part (Chapter 10, 11), the association between Ct and the development of cervical cancer is investigated. Briefly, the aim of the specific chapters are mentioned below.

Section 1

Chapter 2 describes the evaluation of a sensitive Ct amplification, Ct detection and Ct genotyping method (Ct-DT assay) that allows detection and identification of the 14 major Ct serovars (A-L3). Also, the serovar distribution was determined in samples obtained from the Netherlands and Uganda.

Chapter 3 evaluates the Ct amplification (PCR) and detection step (DEIA) in clinical samples from Costa Rican women by a comparison with Hybrid Capture 2 Ct detection assay results. Also the Ct serovar distribution among Costa Rican women was determined with the Ct genotyping step.

In Chapter 4 a comparison between the Ct-DT RHA (genotyping) and 2 other Ct genotyping methods (ie., OmpA sequencing and LGV/non-LGV differentiation by a pmpH real time PCR) was made.

Chapter 5 describes the evaluation of a new high-throughput microsphere suspension (MS) assay for simultaneously detection and identification of the 14 serovars on the Luminex platform.

Section 2

Chapter 6 contains a description of the first Russian serovar distribution study (St Petersburg). The obtained Russian distribution results were compared with previous performed distribution studies in the Netherlands.

Chapter 7 describes the incidence of concurrent serovar infections at Ct genotyping level. Also, the incidence of multiple anatomical sites (i.e., vagina/cervix, urethra, rectum and pharynx) on detection and genotyping level were investigated.

In Chapter 8 the D-K serovar distribution was determined among MSM infected with non-LGV serovars. Also the percentage of mixed LGV/non-LGV infections were determined in LGV positive MSM. Besides the serovar distribution, this study serves as an evaluation of the LGV/non-LGV differentiation test used at the STI-outpatient clinic in Amsterdam.

Chapter 9 investigates the correlation between the different serogroups and the level of Ct IgG antibodies in the serum.

Section 3

In Chapter 10 the Ct co-factor role in the development of CIN2+ lesions among Costa Rican women was investigated on Ct antibody level and Ct DNA level.

Chapter 11 investigates whether Ct DNA was detected in cervical adenocarcinomas. Also, HPV genotyping was performed for all adenocarcinomas.

Chapter 12 consists of a general discussion and conclusion.
References:


93. RIVM. 2008. posting date. Urogenitale Chlamydia trachomatis en lymphogranuloma venereum. RIVM. [Online].


141. van Veen, M. G., F. D. Koedijk, and M. A. van der Sande. 2006. Analysis of rectal Chlamydia trachomatis serovar distribution including L2 (lymphogranuloma venereum) at the Erasmus MC STI clinic, Rotterdam. Sex Transm Infect 82:207-211.


