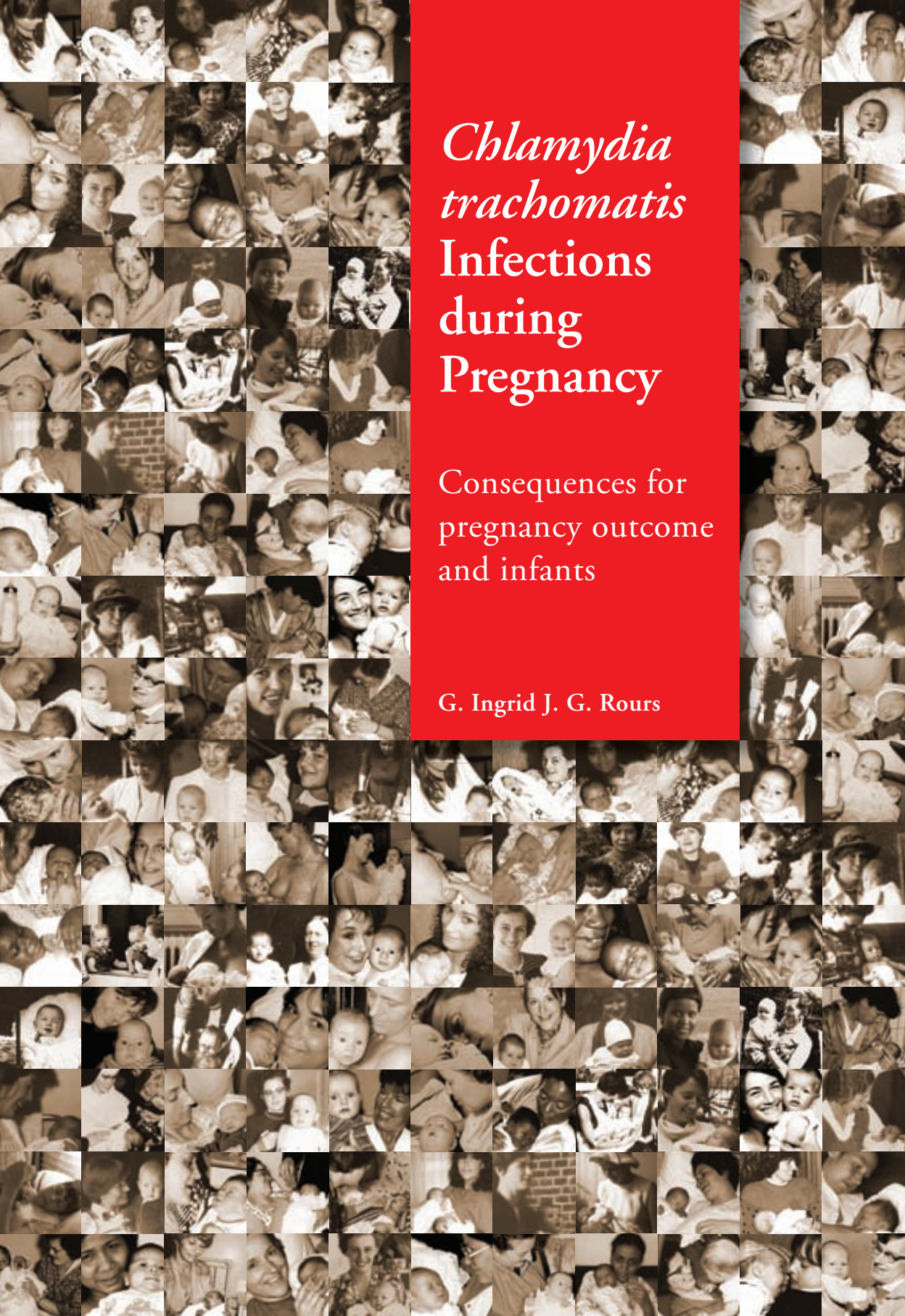

Chlamydia trachomatis Infections during Pregnancy

Consequences for pregnancy outcome and infants

G. Ingrid J. G. Rours

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Chlamydia trachomatis Infections during Pregnancy

Consequences for pregnancy outcome and infants

Chlamydia trachomatis infecties tijdens de zwangerschap

Gevolgen voor zwangerschapsuitkomst en pasgeborenen

Proefschrift

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op gezag van rector magnificus

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I dedicate this thesis to my loving parents,
Anna Christina Lemmen and Friedrich Leonard Rours

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Foreword

The inspiration for this research was born in the 1990's during my rotation as a trainee paediatrician attached to the teaching hospitals affiliated to the University of the Witwatersrand Medical School, Johannesburg, South Africa.
The knowledge and concern then about *C. trachomatis* infections in pregnant women and newborns was superceded by other more prevalent infections like syphilis, tuberculosis and HIV. However, one particular patient drew my attention to *C. trachomatis* infection as an important pathogen requiring equal or increased attention.
Despite full intervention a newborn on ventilatory support in the neonatal intensive care unit did not improve. Re-evaluation of the medical files, revealed that a conjunctivitis had been ignored in the history and initial examination. A subsequent nasopharyngeal swab from the newborn, and urine test from the mother, confirmed the diagnosis of a *C. trachomatis* infection. After appropriate treatment the newborn improved and could be taken off the ventilator.
I discovered that obstetricians did not routinely test women for *C. trachomatis* and neither was it common protocol for paediatricians to test newborns and infants with respiratory tract infection for *C. trachomatis*. So, in a in a time, place and population with a high prevalence of syphilis in pregnant women and newborns and a rapidly increasing prevalence of HIV/AIDS, no attention was paid to another, easily treatable, sexually transmitted infection that could also cause severe disease in women and infants.
Wondering how often this preventable and treatable condition was being missed in our paediatric and obstetric population, I set out to find answers. Unwittingly, that was the beginning of this PhD thesis.

Returning to practise in the Netherlands, it became clear that chlamydial screening for pregnant women and testing infants with conjunctival or respiratory tract infection for *C. trachomatis* was also not part of medical routine. I continued to research and this time to work towards a PhD.

Abbreviations

AIDS	acquired immune deficiency syndrome
ANC	antenatal clinic
ATP	adenosine triphosphate
°C	degree Celsius
CEA	cost-effectiveness analysis
CER	cost-effectiveness ratio
CI	confidence interval
CPP	chronic pelvic pain
CT	*Chlamydia trachomatis*
CTI+	*Chlamydia trachomatis*-positive infants
CTI-	*Chlamydia trachomatis*-negative infants
CTM+	*Chlamydia trachomatis*-positive mothers
CTM-	*Chlamydia trachomatis*-negative mothers
CS&T	costs for screening and treatment
DFA	direct fluorescent antibody test
DNA	deoxyribonucleic acid
EB	elementary body
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
FIR	foetal inflammatory response
FTA-ABS	fluorescent treponemal antibody test
GP	general practitioner
HC	hybrid capture
HELLP	haemolysis, elevated liver enzymes, low platelets
HIV	*Human Immunodeficiency Virus*
hMPV	*human Metapneumovirus*
hRSV	*human Respiratory syncytial virus*
LCR	ligase chain reaction
LGV	lymphogranuloma venereum
LPS	lipopolysaccharide
MIF	micro-immunofluorescence
MIR	maternal inflammatory response
MOMP	major outer membrane protein
NAAT	nucleic acid amplification technique
NASBA	nucleic acid sequence based amplification
NO	number

OR	odds ratio
PAR	population attributable risk
PCR	polymerase chain reaction
PF	population fraction
PI	placental inflammation
PID	pelvic inflammatory disease
PROM	premature rupture of membranes
QALY	quality-adjusted life year
RB	reticulate body
RNA	ribonucleic acid
RPR	rapid plasma reagin
RR	relative risk
SC	savings on complications
SD	standard deviation
SDA	strand displacement amplification
SDS	standard deviation score
SGA	small for gestational age
STD	sexually transmitted disease
STI	sexually transmitted infection
TMA	transcription-mediated amplification
TPHA	*Treponema pallidum* haemagglutination assay
WHO	World Health Organisation

Part I

Introduction

Background

Chlamydia trachomatis is the most common bacterial sexually transmitted infection (STI) worldwide. Chlamydia is responsible for a significant proportion of genitourinary tract infections in adult males and females, but like STIs in general, it is primarily a woman's health care issue since the manifestations and consequences are more damaging to the reproductive health of women than of men [1]. Acute *C. trachomatis* infection is easy to treat, but the majority of chlamydial infections remain asymptomatic and untreated, and may lead to serious complications such as pelvic inflammatory disease (PID), ectopic pregnancy, infertility and chronic pelvic pain in women [2-5], as well as conjunctivitis and respiratory tract infection in infants [6, 7]. The health-economic impact of *C. trachomatis* infections may therefore be enormous. Vaccines are not yet available. Hence, health gain and financial benefits can only be achieved by active case finding and treatment of *C. trachomatis* infection, which can be done on an individual level or nationwide in a screening program.

References

[1] Paavonen J, Eggert-Kruse W. Chlamydia trachomatis: impact on human reproduction. Hum Reprod Update. 1999;5(5):433-47.

[2] Wiesenfeld HC, Hillier SL, Krohn MA, Amortegui AJ, Heine RP, Landers DV, et al. Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. Obstet Gynecol. 2002;100(3):456-63.

[3] Peipert JF. Clinical practice. Genital chlamydial infections. N Engl J Med. 2003;349(25):2424-30.

[4] Manavi K. A review on infection with Chlamydia trachomatis. Best Pract Res Clin Obstet Gynaecol. 2006;20(6):941-51.

[5] Malik A, Jain S, Hakim S, Shukla I, Rizvi M. Chlamydia trachomatis infection & female infertility. Indian J Med Res. 2006;123(6):770-5.

[6] Hammerschlag MR. Chlamydial infections. J Pediatr. 1989;114(5):727-34.

[7] Darville T. Chlamydia trachomatis infections in neonates and young children. Semin Pediatr Infect Dis. 2005;16(4):235-44.

Chapter 1

Aims of this thesis

This thesis concentrates on *C. trachomatis* infections in pregnant women and infants by evaluating the burden of disease, test methods, and the costs of prenatal screening in order to provide information for future discussions about the need for routine screening for *C. trachomatis* in pregnant women in the Netherlands.

The specific aims and research questions were:

The assesment of the prevalence and risk factors during pregnancy

1. What is the prevalence of *C. trachomatis* infection during pregnancy?

2. What are risk factors for *C. trachomatis* infection in pregnant women?

The evaluation of complications of C. trachomatis infection during pregnancy

3. Is *C. trachomatis* infection during pregnancy associated with increased risk for adverse pregnancy outcomes such as stillbirth, premature birth and low birth weight?

4. What is the rate of *C. trachomatis* transmission from women to neonates?

5. Can we find evidence of vertical transmission by detection of *C. trachomatis* in infants with neonatal conjunctivitis and respiratory disease?

The assesment of the feasibility of screening

6. Is pooling of urine specimens a good method to improve cost-effectiveness of screening for *C. trachomatis* during pregnancy?

7. Is routine screening for *C. trachomatis* a cost-effective approach during pregnancy?

The studies that were done in order to address the research questions are shown in figure 1.

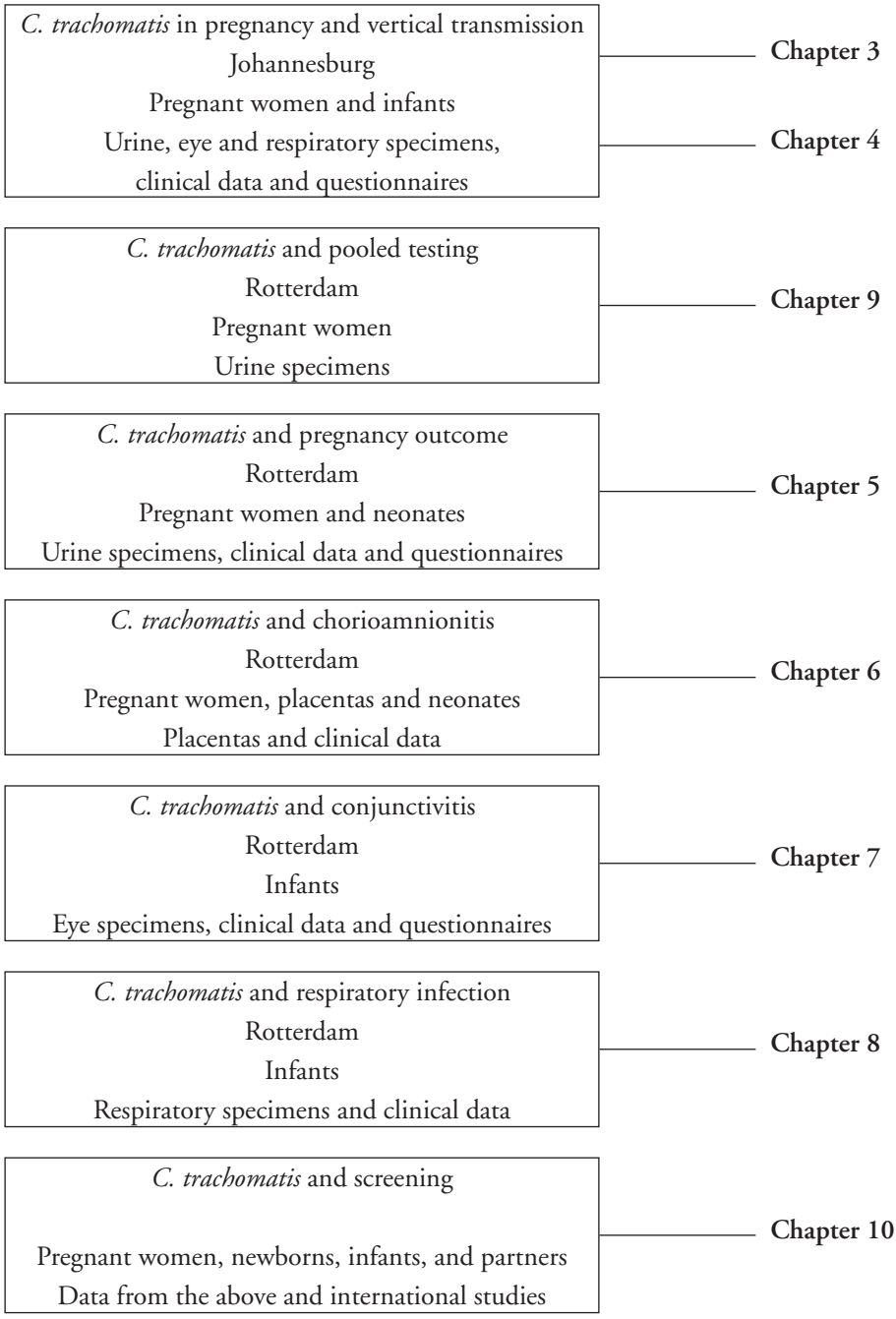


Figure 1 Studies presented in this thesis

Chapter 2

Chlamydia trachomatis infections in pregnant women and infants

Epidemiology

The World Health Organization estimated in 2001 that per year 92 million new cases of *Chlamydia trachomatis* infection occur worldwide [1]. This includes 5.2 million new cases of genital infection yearly in Western Europe of which around 2.9 million are in women, and 15.9 million in Sub-Saharan Africa of which 8.2 million in women (Table I). Overall, there is an increasing trend in the chlamydia positivity rate in recent years, which may partly be due to the development of more sensitive laboratory tests, but also reflects a true increase in infections [2]. Global prevalence rates of *C. trachomatis* infection in asymptomatic nonpregnant women and pregnant women are similar and have been described to vary from 0-37% depending on the study population, setting and test methods used [3, 4]. Unexpectedly, high prevalences of up to 17% have been documented for asymptomatic women in Europe [5].

Table 1 Estimated number of new cases of genital *Chlamydia trachomatis* infection among adults, by gender and United Nation global region

	New cases (million)	
	Males	**Females**
North America	1.77	2.16
Western Europe	2.28	2.94
Australia & New Zealand	0.14	0.17
Latin America & Carribean	4.19	5.12
Sub-Saharan Africa	7.65	8.24
North Africa & Middle East	1.71	1.44
Eastern Europe & Central Asia	2.72	3.25
East Asia & Pacific	2.56	2.74
South & South East Asia	18.93	23.96
Overall	**41.95**	**50.03**

In the Netherlands approximately 60.000 new *C. trachomatis* infections are diagnosed annually with an increase in infections observed in recent years [6-8]. Of these infections 35.000 will be in women [9, 10]. The first nationwide screening study in the Netherlands showed a prevalence rate of 2.5% in women [11]. However, differences have been reported between regions ranging from 0.6% to 4.9% [11-14].

Transmission

Since most *C. trachomatis* infections, up to 80% in women and 50% in men, remain asymptomatic, a pool of individuals with subclinical infection continues to be responsible for the risk of transmission within the community [15]. Transmission among adults mainly occurs via sexual contact (horizontal transmission) and the transfer of infected secretions which primarily affect mucosal membranes such as the cervix, urethra, rectum, conjunctiva and pharynx. Transmission can also occur via genital-ocular auto-inoculation.

The rate of transmission depends on the sexual behaviour within a community, the mean duration of infectiousness and the probability of transmission per sexual contact. Many risk factors have been associated with transmission of *C. trachomatis* infection. Young age and urban residence are the most consistent risk factors, but low socio-economic class, single marital status, ethnicity, and educational level have also been associated with chlamydial infection [16-18]. Behavioural factors that have been associated with increased risk for *C. trachomatis* transmission are first sexual contact at young age, multiple sexual contacts, intercourse with a recent new partner, other STIs, previous history of an STI, a partner with current or previous STIs, the use of oral contraceptives, lack of barrier contraceptives, and late antenatal clinic booking [17-19]. Overall, male-female and female-male transmission rates have been found to be similar being 68% [20]. Importantly, during passage through an infected birth canal *C. trachomatis* can be spread to newborns, who subsequently may become infected (vertical transmission) [21, 22]. The risk of vertical transmission is directly related to the prevalence of *C. trachomatis* infection in pregnant women and has been reported to be as high as 75% [22-24].

Microbiology

Chlamydia genus

Chlamydia trachomatis is a member of the family Chlamydiaceae, or Chlamydiales [25], which contain a single genus: *Chlamydia*. Chlamys, 'mantle', refers to the appearance of the intracytoplasmic inclusions in infected cells, which lie like a mantle around the host cell's nucleus. Because of this appearance, chlamydiae were initially thought to be protozoa and described as 'mantled animals'. Later chlamydiae were classified as viruses because of their small size, and as parasites because of their obligate intracellular existence. At present chlamydiae are known as highly specialized gram-negative bacteria.

Chlamydia species

Regularly, new members of the Chlamydiaceae are discovered. For humans the most important species are *Chlamydia trachomatis* and *Chlamydia pneumoniae* as well as *Chlamydia psittaci*,

although the latter is not a primary human pathogen [25-27]. The common characteristics of chlamydiae are a small genome size (1.1-1.2 million nucleotides), a deficiency in endogenous adenosine triphosphate (ATP) production, a characteristic bacterial double cell wall (inner and outer membrane) similar to other gram-negative bacteria with a lipopolysaccharide but absent peptidoglycan layer, the presence of RNA, DNA, and ribosomes, and the own protein and nucleic acids synthesis. The distinguishing characteristics between the three species *C. trachomatis*, *C. pneumoniae* and *C. psittaci* concern the host range, clinical expression, and antibiotic susceptibility (due to folate biosynthesis), the staining characteristics (due to glycogen inclusions), inclusion morphology, shape of the elementary body, and limited DNA sequence homology.

Table 2 Distinguishing characteristics of *Chlamydia* species

	C. trachomatis	***C. pneumoniae***	***C. psittaci***
host	humans, mice	humans	rarely in humans, birds, mammals,
clinical disease	see page 34	pneumonia, asthma, endocarditis, arthritis	pneumonia
folate biosynthesis	yes	no	no
inclusion staining	idodine+	iodine-	iodine-
inclusion morphology	oval, granular, vacuolar	oval, dense	variable, dense, lucent
elementary body shape	coccoid	pear-shaped	coccoid
DNA homology	10	100	10

Chlamydia trachomatis

C. trachomatis was the first *Chlamydia* species to be discovered and has been divided into subgroups based on antigenic variation in the major outer membrane proteins (MOMP) (serovars) and on clinical expression (biovars) (Table 3). Seventy percent of the non-lymphogranuloma venereum (LGV) STIs are due to serovars D, E and F, which are also responsible for neonatal disease. This thesis deals with urogenital and neonatal chlamydial infections, which are caused by serovars D through K.

Chlamydia trachomatis structure and pathophysiology

C. trachomatis has a distinctive two-phased life cycle (Figure I), in which it has two characteristic forms: an elementary body and a reticulate body [28]. The elementary body (EB) is a small (0.3-0.4 mm), metabolically inactive but infectious form. EBs have a rigid, thick outer membrane

Table 3 *Chlamydia trachomatis* subgroups with presentation and distribution

Serovar	Biovar	Distribution
A, B, Ba, C	trachoma	Asia, Middle East, Africa, Australia
B, D, Da, E, F, G, Ga, H, I, Ia, J, K	urogenital, neonatal, and ocular disease	worldwide
L1, L2, L2a, L3	LGV	worldwide

that is composed primarily of lipopolysaccharide and proteins with extensive disulfide cross-linking bonds among three cysteine proteins (MOMP, OmpB and OmpA), which make EBs resistant to the environmental conditions outside the host cells. The reticulate body (RB) is a larger (0.9 mm), intracellular metabolically active and replicating form, which is not infectious. RBs have a fragile membrane that lacks the cross-linking disulphide bonds.

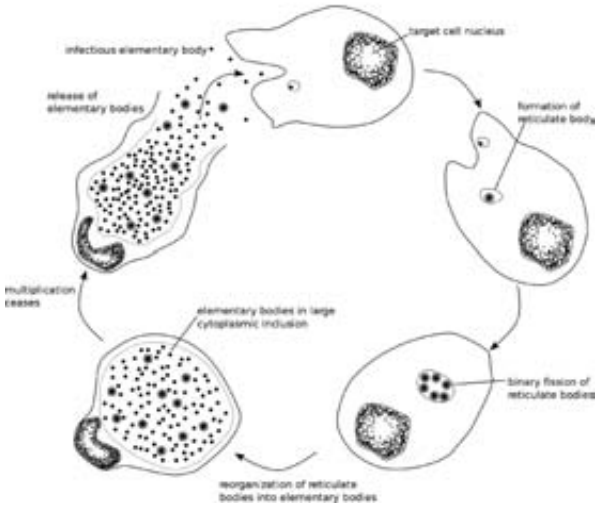


Figure 1 *Chlamydia trachomatis* life cycle

The developmental cycle of *C. trachomatis* begins when infectious EBs attach to receptors on the surface of a suitable host cell (non-ciliated columnar cells and macrophages) and induce their own entry via receptor-mediated endocytosis. The ingested EBs reside within a membrane-limited endosome and escape fusion and destruction by the host cells' lysosomes by an unknown mechanism that may be based on its unique cell wall structure. The EBs enlarge, the DNA becomes less dense, ribosomes are produced that make the cytoplasm more granular, and transformation

follows into metabolically active RBs (within 8 hours after infection). The RBs remain strictly intracellularly, synthesize mRNA and multiply via binary fission in the cytoplasm of the host cells while using the host cell's ATP, sugars and amino acids for continued multiplication. This way the RBs form multiple intracellular microcolonies or inclusion bodies in the endosomes (12-30 hours). During the process chlamydiae protect the cells in an organism against destruction by the organism's immune system. However, there is still a lack of consensus regarding the mechanisms involved in apoptosis inhibition by chlamydiae [29]. After a large number of fissions, the RBs reorganize, condense and transform back into infectious EBs at which time an inclusion body contains up to 1000 infectious EBs (30-40 hours). Eventually, the nutrients of the host cell expend and the host cell releases the EBs by exocytosis, after which infection of other cells may take place (48-72 hours).

The infected cells produce and secrete inflammatory mediators, stimulate infiltration of polymorphonuclear cells and lymphocytes, and secrete growth factors, which leads to the formation of lymphoid follicles, chronic inflammation and even fibrotic changes. After an incubation period of 10 days (varying between 7 and 21 days) patients may present with a variety of clinical manifestations that result from the host inflammatory response and cell destruction. The host is assumed to have some sort of protective immune response to *C. trachomatis* since chlamydial infections have been found to be self-limiting in up to 45% of infected women [30, 31], and asymptomatic infection may last for months or even years [32, 33]. However, the acquisition of protective immunity due to previous infection remains unclear. This is especially so since it has been shown that recurrent infection increases the risk for complications [34, 35]. Due to its latent, insidious and potentially chronic character the severity and chronicity of chlamydial disease varies.

Diagnosis

Nucleic acid amplification techniques (NAATs) are the latest diagnostic methods and have replaced culture as the method of choice to diagnose chlamydial infection [36]. Hower, various laboratory tests, culture and non-culture methods, are available and may still be used to diagnose *C. trachomatis* infection (Table 4) [36-40]. The sensitivity of these tests depends on several factors: the nature of the disease and subsequent site of specimen collection, the quality of the specimen and transport medium, the intrinsic quality of the test and the precision with which the test is carried out.

Specimen collection

Appropriate sites for specimen collection in women are the cervix, vulva or urethra, but in specific clinical disease rectal, nasopharyngeal or throat swabs may be the most suitable; also a

needle aspiration of the fallopian tube or a biopsy sample from the endometrium or inguinal bubo. Appropriate sites for specimen collection in infants are the conjunctiva, posterior nasopharynx, throat or tracheobronchial aspirates, and ear. In children only in case of suspected abuse vaginal, urethral or rectal specimens may be indicated. NAATs are not approved for these sites in children. However, recently NAATs on urines were shown to be adequate forensic tests, being more sensitive than culture and less invasive than swabs, and would reduce further trauma and discomfort for abused children [41].

When taking specimens one should remember that chlamydiae are obligate intracellular pathogens and that specimens should therefore contain epithelial cells from the involved sites rather than exudates. Purulent discharges are inappropriate for most tests and should be removed from the site before a sample is taken. A variety of swabs can be used to obtain specimens. However, toxicity related to material in swabs may be a problem. The tip of a swab should preferably be made of cotton or dacron, because these cause less inhibition than nylon or alginate tips. The shaft is best made of inert material such as plastic or metal instead of wood [38, 39]. A cytobrush appears to collect more cells than swabs and is prefered for endocervical specimens.

Specimens taken for culture require a special transport medium such as a 2SP medium, which contains buffered salt, sucrose and antibiotics (vancomycin, gentamicin, amphotericin B and nystatin) that do not inhibit chlamydiae. Furthermore, specimens should be stored refrigerated at 4°C if inoculated in cell monolayers within 48 hours, or frozen at -70°C if stored for a longer period [38, 39]. Specimens for other tests should be handled according to the companies' instructions for procedures to maintain the sensitivity and specificity of the test.

Diagnostic methods

Different diagnostic methods to detect *C. trachomatis* infection, advantages and disadvantages are shown in table 4 [37-42]:

1. **Cytology**

   Cell scrapings can be stained with iodine and examined for the presence of typical intracytoplasmic inclusion bodies. Since none of the other *Chlamydia* species contains glycogen, which stains with iodine, the finding of iodine-stained inclusion bodies is specific for *C. trachomatis* [43]. This method is not as sensitive as the other methods to diagnose *C. trachomatis* infection, but still exceeds 60% when diagnosing neonatal conjunctivitis.

2. **Culture**

   Culture has been considered the gold standard for many years, and still is the method of choice for medico-legal issues and antibiotic susceptibility testing. Cell culture is the only method to detect viable organisms and therefore highly specific to diagnose *C. trachomatis* infection [37]. *C. trachomatis* is able to grow when specimens are brought to 37°C, inoculated onto the surface of confluent monolayers of susceptible cells (McCoy, Hela or BHK lines), centrifuged (to enhance ingestion of chlamydiae), and overlaid with a growth medium that

contains cyclohexamide to inhibit host cell metabolism and increase the number and size of inclusions [44, 45]. After 48-72 hours incubation of the infected cell monolayers, infected cells can be examined for growth of inclusion bodies, which may be microscopically visualised. Culture has several disadvantages (Table 4) and a low sensitivity for urine [46].

3. **Antigen detection by enzyme immunoassay technique**

   Enzyme immunoassay (EIA) techniques can be used to detect *C. trachomatis* [47]. The specimen is incubated with an antibody preparation to detect chlamydial lipopolysaccharide (LPS) on the membrane of EBs, after which an enzyme substrate is added to produce coloured particles that can be visualized microscopically or measured by spectrophotometry [48]. EIA has several disadvantages (Table 4) and is less sensitive than cultures and NAATs (40% to 75%); especially with samples that contain few organisms (asymptomatic infections). The specificity is 97%.

4. **Direct fluorescent antibody detection**

   For the direct fluorescent antibody test (DFA) the specimen is smeared on a slide, air dried and fixed, and stained with fluorescein-labeled monoclonal antibodies that will bind to the major outer membrane proteins (MOMP) of EBs in specimens, which produces brightly fluorescing and morphologically distinctive particles for direct visualization by fluorescence microscopy [49]. DFA is highly specific (99%) compared to culture, but has several disadvantages (Table 4) and a sensitivity of 75% to 85% [50].

5. **Serology**

   Serological tests are of limited value in diagnosing *C. trachomatis* infection in the individual, because antibodies may not occur in every case of uncomplicated infection and tests do not distinguish current from past infection (Table 4). However, serology may be used to detect chlamydial infection in newborns and in women with tubal factor infertility or LGV infection when aspirates are not obtainable [51-54]. Detection of IgG antibodies in newborns requires repeat testing to observe a rise in antibody titre. Detection of high titer (^{3}32) anti-chlamydial IgM antibodies is indicative of recent infection and infection in newborns [55].

6. **Nucleic Acid Amplification Techniques**

   NAATs are based on the amplification and detection of specific DNA or RNA nucleic acid sequences unique to *C. trachomatis* in specimens. NAATs are using different target, probe or signal amplification technologies such as polymerase chain reaction (PCR), strand displacement amplification (SDA), transcription-mediated amplification (TMA), nucleic acid sequence based amplification (NASBA), ligase chain reaction (LCR; now defunct), or hybrid capture (HC). NAATs are highly specific (99% to 100%) if cross contamination is avoided and have a high sensitivity of 90-95%, which is higher than in all other methods [46, 56-58]. Furthermore, NAATs can be used with non-invasive specimens such as urine or vulvovaginal swabs [42, 59], are (partially) automated and may be used for pooling of specimens, which makes them very useful for screening programs [60-62]. The main disadvantages of NAATs are the costs and the reduced performance if inhibitors (oestrogens, nitrates, crystals, blood)

Table 4 Diagnostic methods for *Chlamydia trachomatis*

Method	Advantages	Disadvantages
Diagnosis		
NAATs: • PCR • SDA • TDA	• high sensitivity • suitable for urines and vulvo-vaginal swabs • validated for extragenital sites, including rectum • automated	• expensive • not licensed for extragenital sites • less performance in presence of inhibitors • false positive results in some settings
Cell culture	• suitable for all specimen types • only method to detect viable organisms	• special transport medium • not automated • labour intensive • requires expertise • time consuming (3-7 days) • subject to contamination • low sensitivity for urine • toxicity of certain swabs during pregnancy • storage at -70°C if processing is delayed
EIA	• inexpensive • quick results • rapid handling of large numbers of specimens • can be accepted for point-of-care tests	• not appropriate for urines and self-collected swabs • low sensitivity; especially in specimens with few organisms (40-70%) • false positives: cross reaction with other chlamydiae and bacteria
DFA	• suitable for all specimen types • rapid turnaround time	• labour intensive • time consuming • not automated • requires expertise • low sensitivity for urine
Serology	• suitable when other specimens are not obtainable	• not standardized • no distinction between past and present infection • need of acute and convalescent samples • antibodies not always present in uncomplicated infections
Specimen collection		
Clinician-obtained	• ability to obtain good quality samples, which may increase sensitivity e.g. endocervical swab	• less acceptable to some patients • more expensive in staff time
Self-collected	• more acceptable to some • less clinical facilities required • self-collected vaginal swabs equivalent to clinician-obtained swabs	• may be less sensitive

NAATs: nucleic acid amplification techniques, PCR: polymerase chain reaction, SDA: strand displacement amplification, TMA: transcription-mediated amplification, EIA: Enzyme immunoassay, DFA: Direct fluorescent antibody test

are present in urine specimens [59, 63]. Furthermore, not all NAATs are equal in performance and some in house NAATs from several laboratories and for example the Cobas Amplicor do not detect strains with no plasmid or the Swedish new variant of *C. trachomatis*, for which

new dual target NAATs using real-time PCR have been developed [64, 65]. The most commonly used NAATs at the moment are the BD Probe Tec, Cobas Amplicor, Cobas TaqMan, and Aptima.

Clinical manifestations

C. trachomatis infections in pregnant women

Pregnant women are a special group at risk for *C. trachomatis* infection. Pregnant women may develop chlamydial clinical disease like non-pregnant women, but are also at increased risk for post-partum PID and subsequent infertility (Table 5). Moreover, *C. trachomatis* infection during pregnancy may jeopardise the pregnancy. The majority, up to 80%, of pregnant and non-pregnant women, have no symptoms. Others have only mild symptoms or non-specific symptoms that easily escape medical attention [66]. Some of these infections may disappear spontaneously, others become overt cervicitis or urethritis or persist silently [67]. Cervicitis or urethritis may lead to non-specific and usually mild complaints like mucopurulent vaginal discharge, vaginal pruritis, intermenstrual or post-coital bleeding, dysuria or frequent micturation.

Persisting 'silent infections' may remain local, but may also ascend to the upper genital tract where they can lead to symptomatic or asymptomatic inflammation of the endometrium, salpinges and abdominal cavity resulting in endometritis/chorioamnionitis, salpingitis and pelvic inflammatory disease (PID) [66, 68, 69]. Women with endometritis may present with low-grade abdominal pain, cramping and intermenstrual bleeding. Salpingitis may lead to tubal scarring and subsequent subfertility or infertility or ectopic pregnancy [70, 71]. Women with PID may suffer from dyspareunia, pelvic pain, fever, chills, nausea and vomiting. However, usually the symptoms of PID are non-specific and may be missed. Further spread of infection in the abdominal cavity will lead to peritoneal inflammation and can result in a perihepatitis with hepatic capsular "violin string" adhesions, the Fitz Hugh Curtis syndrome, causing nausea and vomiting and right upper quadrant pain, as well as in perisplenitis, perinephritis, periappendicitis, perisigmoiditis and peritonitis [72-74]. Furthermore women may develop proctitis, inclusion conjunctivitis, and arthritis. Arthritis may in 1% of women occur isolated, as a so-called sexually acquired reactive arthritis, but may also be part of a Reiter's syndrome, a triad of symptoms consisting of urethritis, conjunctivitis and polyarthritis with or without dermatitis and balanitis (in men) [75]. Lymphogranuloma venereum has been described rarely in pregnant women [76, 77].

The effect of pregnancy on *C. trachomatis* infection

Various changes in pregnancy have been proposed to influence *C. trachomatis* infection [16]. First, cervical ectopy (related to oestrogen levels) has been associated with *C. trachomatis* infection and with pregnancy, and is supposed to increase shedding of *C. trachomatis* and/or increase

the risk of chlamydial infection. Second, pregnancy is physiologically immunosuppressive and alters the immune responses progressively with advancing gestation to a nadir at 32 weeks gestation, which may affect replication and shedding of *C. trachomatis*. Third, maternal antichlamydial antibodies cross the placenta after 5-6 weeks gestation and are found in breastmilk, which may be protective for neonates. However, if such protection occurs, then it is only partial since up to 75% of neonates who are exposed to *C. trachomatis* at the time of delivery become infected [22, 24].

The effect of previous *C. trachomatis* infections on pregnancy

Pelvic Inflammatory Disease

Pelvic Inflammatory Disease (PID) is a consequence of a complex interaction of genetic, immunological and bacterial virulence factors, set off by multimicrobial etiology including enteric organisms, *Mycoplasma hominis* and possibly *Ureaplasma urealyticum*, anaerobic bacteria, and sexually transmitted organisms such as *Neisseria gonorrhoeae* and *C. trachomatis* [78-82]. Comparison of PID studies is difficult because of the difference in clinical case definition in the absence of pathognomonic symptoms and signs, the absence of one simple accurate diagnostic test over time and the various pathogens that cause PID. The role of *C. trachomatis* in PID, however, has been well established but there are different opinions concerning the complication rate following *C. trachomatis* infection in PID. These are explained by differences in classification, incorrect diagnoses, and unjustified attribution of complications to chlamydial infections. The incidence of PID following *C. trachomatis* infection has been described to vary from 0% to 40%, with the lowest rate in asymptomatic women and the highest in symptomatic women or women at higher risk for an ascending STI (e.g. symptomatic partner, co-infection with other STI, visitor STD clinic) [31, 35, 68, 82-92]. Recurrent chlamydial infections have been found to result in an increasing risk of PID after each infection, which may be as high as a four-fold increased risk of PID after two previous chlamydial infections and a six-fold increased risk after three or more infections [35, 68]. PID may result in chronic pelvic pain (18% to 30%), ectopic pregnancy, and tubal factor infertility.

Ectopic pregnancy

Previous *C. trachomatis* infection has been associated with an increased risk for ectopic pregnancy in subsequent pregnancies as evidenced by serological studies, isolation of *C. trachomatis* from fallopian tubes, and detection of chlamydial DNA from cervical, endometrial or salpingectomy tissues [93-97].The risk of a chlamydia-related PID leading to ectopic pregnancy, has been estimated to vary between 0% and 25% [34, 83, 91, 93, 98].The risk for ectopic pregnancy has been found to increase with increasing number of infections: two-fold after two previous chlamydial infections and five-fold after three or more infections respectively [35].

Infertility

C. trachomatis is considered the most important cause of tubal obstruction leading to tubal infertility [99, 100]. IgG antibody testing for *C. trachomatis* has been proposed as a first screening test in combination with medical history taking to identify women with tubal factor subfertility [54, 101-103]. High rates of *C. trachomatis* infection, past and present, were found to be associated with infertility by many investigators [104-108]. Overall, the risk of chlamydia-related PID leading to infertility, has been estimated to vary between 0% and 20% [34, 71, 83, 108, 109].

The effect of *C. trachomatis* infection on pregnancy

In the first trimester

Spontaneous abortion

C. trachomatis has been associated with spontaneous (recurrent) abortions though not consistently [110-116]. In one study 14 of 66 (21%) women from couples with spontaneous abortions consulting a reproductive medicine centre tested positive for *C. trachomatis* by direct immunofluorescence compared to 23 of 59 (9%) women without spontaneous abortions and term pregnancies ($P<0.05$). The infection rate increased to 69% ($P<0.001$) when both partners of the couples were considered. In the same study, oocytes from hamsters were incubated with spermatozoa from the infected partners and, using electron microscopy, the presence of *C. trachomatis* was demonstrated on the surface of and inside the oocytes [110]. Various models for studying the pathogenesis of chlamydia-related spontaneous abortions have been proposed, being either direct zygote infection or an immune response to heat shock proteins expressed by the zygote that is triggered by previous *C. trachomatis* infection, and reactivation of latent chlamydial infection or endometrial damage from past chlamydial infection [110, 114].

Induced abortion

Women undergoing surgical or medical termination of pregnancy have been reported to have *C. trachomatis* infection with prevalences as high as 17% [117-120]. If left untreated, up to 72% of *C. trachomatis* infected women with termination of pregnancy may develop PID, endometritis or salpingitis post-surgery, which risk has been shown to reduce drastically to 8% or even less with treatment [87, 120-124].

In the second and third trimester

Premature rupture of membranes, premature delivery, prematurity

C. trachomatis infection during pregnancy may influence pregnancy outcome and has been associated with chorioamnionitis, premature rupture of the membranes and premature delivery [125-144]. However, the literature regarding these effects of *C. trachomatis* infection on pregnancy outcome is conflicting, which seems primarily due to differences in study design, population and microbiological tests that were used [125-151]. While earlier studies based on serology and

cultures were at variance regarding premature delivery, there seems to be growing evidence with the use of the more recent NAATs that prematurity is associated with *C. trachomatis* infection [140-145].

Low birth weight

C. trachomatis infection during pregnancy has been associated with low birth weight [128, 132, 133, 136, 152-155]. However, again the literature is contradictory and other studies could not confirm such an association [142, 145, 154, 156]. In some studies an association of *C. trachomatis* infection with low birth weight could only be confirmed in subgroups of women with elevated antichlamydial IgM antibodies [130, 157] .

Stillbirth

Between 10% and 25% of stillbirths in developed countries, and even more in developing countries, may be caused by infection [158]. *C. trachomatis* has been indicated to cause in utero infection in the fetus leading to stillbirth [127, 159]. Intrauterine chlamydial infection has been evidenced by infants with chlamydial infections who were born via caesarean section [160-164]. In cord blood of prematurely born neonates IgM antibodies to *C. trachomatis* can be detected, which is suggestive of fetal chlamydial infection [149]. This hypothesis was confirmed by studies in which 12%-16% of neonates born to chlamydia-positive women had anti-chlamydial antibodies in their cord blood [165, 166].

Post-partum effects of *C. trachomatis* infections

C. trachomatis infection during pregnancy may continue after delivery and cause post-partum endometritis, salpingitis, or PID [167-169]. In contrast to early post-partum endometritis, which occurs within 48 hours after delivery and is mainly related to caesarean section, *C. trachomatis* usually causes late post-partum endometritis and develops between two days and six weeks after delivery [167, 170, 171]. Women are usually not seriously ill, but may present with secondary post-partum haemorrhage, with or without fever, lower abdominal pain, and vaginal discharge. *C. trachomatis* infection can spread into the fallopian tubes resulting in salpingitis increasing the risk for infertility or ectopic pregnancy.

The effect of *C. trachomatis* infections on newborns and infants

At the time of delivery, newborns may acquire *C. trachomatis* infections from pregnant women during passage through an infected birth canal. Hence, the occurrence of *C. trachomatis* infection in infants is directly related to the prevalence of maternal urogenital infections [163, 172]. Infants born by caesarean section are considered to be at lower risk of acquiring chlamydial infection [140]. However, several anecdotal reports of *C. trachomatis* infections in newborns after delivery by caesarean section, with and without premature rupture of the membranes,

indicate that intrauterine infection can occur [161-163, 173-175]. The overall risk for infants born to women with untreated chlamydial infections is approximately 50-75%, with infection occurring at one or more anatomic sites [22].

Conjunctivitis

Neonatal conjunctivitis, also called inclusion conjunctivitis of the newborn, is the most common symptomatic disease and has been shown to occur in 20% to 50% of infected infants [22, 24]. *C. trachomatis* has become the most frequent identifiable cause of neonatal conjunctivitis in many countries [176, 177]. The majority of chlamydial conjunctivitis cases resolve spontaneously during the first few months of life. However, untreated persistent infection can lead to acute discomfort and distress for both infant and mother. Infants usually present at the end of the first week until three months of age with tears, redness and swelling of one or both eyes and serosanguinous or mucopurulent discharge [178-182]. Although conjunctivitis may be quite severe, corneal ulceration or follicle formation rarely occur in infants and recovery is without visual impairment. Once neonatal conjunctivitis has been diagnosed, simultaneous silent infection of the respiratory tract should be suspected which in due time may cause acute or chronic respiratory disease [23, 178, 183].

Upper respiratory tract infection

The nasopharynx is the most frequent site for perinatally acquired *C. trachomatis* infection and may be found in up to 70% of infants born to chlamydia-positive women [22]. Nasopharyngeal infection is usually asymptomatic and self-limited, but may cause rhinitis as part of the prodromal phase of chlamydial pneumonia [184-186]. However, isolated cases of chlamydial rhinitis neonatorum have been described in infants between two weeks and three months of age presenting with a running nose, sneezing, nasal obstruction and epistaxis that may be complicated with apnoeic episodes [187-189]. Nasopharyngeal infection may be preceeded by conjunctivitis suggesting that conjunctivas are portals of entry and that the nasopharynx is infected by a 'spillover' effect [23, 190]. However, nasopharyngeal infection has also been described in infants with pneumonia without evidence of conjunctivitis suggesting that infection occurs in its own right in the respiratory tract [184]. Though nasopharyngeal infection is usually self-limited, infection may also persist for periods of up to one year [183].

There are sporadic reports of *C. trachomatis* being cultured from infants with acute otitis media, otitis media with effusion and chronic otitis media, suggesting that chlamydial infection may be a potential cause of otitis media in early infancy [186, 191, 192].

Lower respiratory tract infection

C. trachomatis pneumonia occurs in 5% to 20% of exposed infants [21, 22]. Chlamydial pneumonia has a characteristic presentation with an insidious onset between 3 and 12 weeks of age. Infants are usually afebrile with mild tachypnoea and a distinctive pertussis-like, non-productive staccato paroxysmal cough but without a post-tussic inspiratory whoop. The coughing spells may result in cyanosis and emesis. If left untreated, the cough may take weeks to clear and can run a long and intermittent course. Chest auscultation reveals vesicular breath sounds and crepitations, with no or minimal wheezing. Chest X-rays are not distinctive and show hyper-expansion with bilateral, diffuse interstitial and patchy alveolar infiltrates [22, 24, 186, 193]. Serum immunoglobulins are consistently elevated and mild absolute eosinophilia (> 300 cells/mm^3) may be present. Nasopharyngeal aspirates usually test positive for *C. trachomatis*. Respiratory failure has been described with chlamydial infection, but is relatively uncommon and mainly described in premature infants.

In premature neonates *C. trachomatis* pneumonia has been described to present differently. *C. trachomatis* has been detected in the pharynx, trachea, and lungs as early as within 48 hours after birth in premature neonates [164, 174, 175]. Initially, the clinical presentation has been described as resembling the idiopathic respiratory distress syndrome and may improve. However, newborns may develop apnoeic spells and feeding problems, and may go on to need ventilatory support [164, 173, 194]. In contrast to X-rays in full-term neonates, in premature neonates lung hypoexpansion and hypotransparency have been reported with a fine reticular pattern, bilateral opacifications and cystic interstitial emphysema [173].

An association has been suggested between *C. trachomatis* lower respiratory tract infection in infancy and bronchial hyperresponsiveness and asthma. However, the literature regarding such associations is sparse [195, 196].

Infection at other sites

C. trachomatis has been detected in the rectum and vagina of newborns. Usually, there is a late appearance of infection at these sites, which raises the question whether it is the result of direct infection at birth or rather spread from the respiratory tract [21, 197]. Since untreated perinatally aquired chlamydial infection may persist for years, *C. trachomatis* may still be detected in the urogenital tract of children [198, 199]. Chlamydial detection in children, however, should always raise the suspicion of sexual abuse [200, 201], and require, in view of the medico-legal aspects, a thorough anamnesis, physical examination and appropriate microbiological analysis [41, 202-204].

Table 5 Clinical spectrum of *Chlamydia trachomatis* infection in women and infants

Women	Pregnant women	Newborns
cervicitis	cervicitis	
urethritis	urethritis	
bartholinitis	bartholinitis	
endometritis	chorioamnionitis	
salpingitis	salpingitis	
pyosalpinx	pyosalpinx	
ovarian abscess	ovarian abscess	
perihepatitis	perihepatitis	
perisplenitis*	perisplenitis*	
perinephritis *	perinephritis*	
periappendicitis*	periappendicitis*	
perisigmoiditis*	perisigmoiditis*	
peritonitis	peritonitis	
	abortion	intra-uterine death*
	ectopic pregnancy	
	stillbirth	dysmaturity*
	premature rupture membranes	
	premature labor	
	premature delivery	prematurity*
	postpartum endometritis	
	postpartum salpingitis	
proctitis	proctitis	
conjunctivitis	conjunctivitis	conjunctivitis
reactive arthritis	reactive arthritis	
pharyngitis	pharyngitis	pharyngitis
		rhinitis
		pneumonia
		otitis media
		infection of rectum*
		infection of vagina*

*rare complication of *C. trachomatis* or not proven

Treatment

C. trachomatis spends most of its life inside epithelial cells, as previously described. The metabolically active RB is likely to be the primary target of antibiotics, which act by inhibition of protein synthesis, growth and division of RBs, DNA-gyrase activity, or cell wall biosynthesis.

These processes occur inside the inclusions within the host cells, which is why pharmacotherapy requires the use of antibiotics that are able to gain acces to and exhibit activity in intracellular sites. The 72-hour life cycle and the asynchronous nature of chlamydial infection requires the maintenance of adequate antibiotic concentrations in tissues for longer periods.

Treatment in adults

To prevent recurrent transmission within partnerships, it is important that management of chlamydial infection is based on similtaneous treatment of infected women and their sexual partners [205]. Table 6 shows the current treatment choices for *C. trachomatis* infection as recommended by the CDC [206, 207]. Single-dose treatment with azithromycin has been a significant development in the management of chlamydial infection. Azithromycin concentrates extensively within cells and has a long tissue half-life, which makes it suitable for a single dose regimen. A seven-day course of doxycycline is the recommended alternative with a similar cure rate [208], but other macrolides, quinolones, sulfonamides, rifampicin and clindamycin also have activity against *C. trachomatis*. Antibiotic resistance has only rarely been described for chlamydial infection

Treatment in pregnant women

In most countries there is agreement that both symptomatic and asymptomatic chlamydia-positive pregnant women should be treated considering the possibility of complications. However, for pregnant women therapeutic options are more restricted due to the fetus. The teratogenic and embryopathic effects of tetracyclines on bone growth and dentition, the interference of doxycycline and quinolones with normal skeletal growth, the growth retardation and postnatal hemorrhage in neonates due to rifampicin, and the increased risk that sulfonamides have in bringing about neural tube, cardiovascular and urinary tract defects or adverse pregnancy outcomes has been described in humans or animal studies [209-213]. The current alternatives for treatment of chlamydial infection in pregnant women include multi-day treatment with erythromycin, amoxycillin or clindamycin, or single dose treatment with azithromycin [214, 215].
In recent years, more data and clinical experience have become available to support the efficacy, safety and tolerability of azithromycin in pregnant women [206]. Azithromycin has been shown to be similar or better in treatment success compared to erythromycin and amoxicillin, and to cause similar or less total adverse events and gastrointestinal side effects (nausea, diarrhoea, abdominal pain), which resolve spontaneously [216-221]. Azithromycin may be more costly, but the price is decreasing and its single dose regimen will increase compliance, which makes it increasingly more cost-effective [215].
Erythromycin used to be the first choice to treat chlamydial infection during pregnancy. Erythromycin has been shown to be similar or less efficacious than azithromycin, but the long treatment period, multiple dosing regime, and gastrointestinal side effects decrease compliance significantly

[217, 218]. In addition, the use of erythromycin in pregnant women and infants has been associated with an increased risk for maternal hepatotoxicity and infantile pyloric stenosis [222-224].
Amoxicillin has similar or less efficacy, similar or more reported side effects, and similar or less compliance than azithromycin [216-218, 221]. In addition, amoxicillin also requires a long treatment period and multiple dosing regime, and may precipitate hypersensitivity reactions [225]. Therefore, amoxicilin is less convenient for pregnant women than azithromycin, but is still a recommended alternative during pregnancy and first choice in some countries, including the Netherlands.
Clindamycin may be another alternative but is more expensive than azithromycin, has gastrointestinal side effects similar to erythromycin and may cause pseudomembranous enterocolitis [215, 226].

Treatment in infants

Systemic treatment has been demonstrated to be more effective than topical application of antibiotics to treat chlamydial neonatal conjunctivitis, and infection at other sites [227]. Official guidelines for neonatal chlamydial infection at present recommend systemic treatment with erythromycin (Table 6) [207, 227]. However, erythromycin in neonates and infants has been shown to cause gastrointestinal side effects, increase the risk for hypertrophic pyloric stenosis, and to have drug interactions with theophylline, carbamazepine, warfarin, cyclosporin and digoxin [223, 228]. In addition, a 20% treatment failure rate has been described, which may require multiple courses of therapy [182, 229, 230]. Single-dose azithromycin might be an alternative for the treatment of neonatal chlamydial infection. However, although single-dose azithromycin is being used in paediatric endemic trachoma [231, 232], only one study has been published regarding the use of azithromycin in neonatal inclusion conjunctivitis [233]. Single

Table 6 Treatment in uncomplicated *Chlamydia trachomatis* infections

	First choice	**Alternative**
women	Azithromycin po	Doxycycline po
	1 g in single dose	100 mg bid X 7 days
partners	Azithromycin po	Doxycycline po
	1 g in single dose	100 mg bid X 7 days
pregnant women	Azithromycin po	Amoxicillin po
	1 g in single dose	500 mg tid x 7 days
infants	Erythromycin po	Azithromycin po
	12.5 mg/kg qid x 14 days	20 mg/kg daily x 3 days

PO: orally

dose treatment (20 mg/kg) was compared to a three-dose regimen (20 mg/kg once daily for three days) and the authors concluded that the efficacy of the latter regimen was similar to erythromycin treatment, but that modification of the concentration of azithromycin might improve efficacy even further. More data on the tolerability and efficacy of azithromycin and other new oral macrolides such as clarithromycin or roxithromycin, for infants are still needed [234].

Prevention

C. trachomatis prophylaxis

Previously, ocular prophylaxis with 1% silver nitrate ophthalmic drops, 0.5% erythromycin ophthalmic ointment, 1% tetracycline ointment and 2.5% povidone-iodine (betadine) solution have been used attempting to prevent chlamydial disease in newborns and infants. However, topical prophylaxis may prevent some chlamydial neonatal conjunctivitis, but complete irradication of chlamydial conjunctivitis and prevention of subsequent chlamydial infection at other sites cannot be achieved [235, 236].

C. trachomatis screening

By definition screening is a public health service in which members of a defined population, who do not necessarily perceive they are at risk of or are already affected by a disease or its complications, are asked a question or offered a test to identify those individuals who are more likely to be helped than harmed by further tests or treatment to reduce the risk of a disease or its complications [237].

The above seems applicable to *C. trachomatis* infection in pregnant women suggesting a good potential for *C. trachomatis* screening during pregnancy. However, in order to decide whether screening for *C. trachomatis* infection in pregnant women would be a good policy to serve public health we should consider the 10 screening criteria as described by Wilson and Jungner [238]. These state that (1) there should be knowledge about the disease, which means that the disease should be acknowledged as an important problem, that the natural course of the disease should be adequately understood, and that there should be a recognisable latent or early symptomatic stage. (2) There should be knowledge about the test, which includes that a test should be suitable for examination, acceptable to a population, and case finding should be a continuous process instead of an occasional action. (3) Treatment for the disease should be acceptable for patients with recognized disease, should be readily available and there should be an agreed policy concerning whom to treat. (4) The costs of case finding should be economically balanced in relation to possible expenditures on medicale care as a whole.

Pregnant women as a target population for *C. trachomatis* screening

The natural history of *C. trachomatis* infection is largely known, although infection in pregnant women is not yet completely understood. *C. trachomatis* is infection is sexually transmitted, which means that pregnant women by definition belong to the population at risk for chlamydial disease. Up to 80% of women are asymptomatic. Hence, they will not seek medical care or perceive themselves as being at risk and may easily be missed while they may already be affected by chlamydial infection or its complications. *C. trachomatis* infection in pregnant women may therefore be an important problem for women and infants, but the extent of the health problem can vary between different populations. Chlamydial infection has been shown to occur in between 0.6% and 4.9% of sexually active women in the Netherlands and may be assumed to be similar among pregnant women [11-13]. Some of these women will develop PID, ectopic pregnancies and become subfertile or infertile [9, 10]. By offering pregnant women a test, infected women can be identified and treated to reduce the risk of chlamydial disease and its complications. Moreover, detection and treatment of chlamydia-positive women will reduce the risk of chlamydial infection and complications for their offspring, and also for their partners. An advantage of screening during pregnancy may be that most pregnant women, at least in the developed world and in increasing numbers also in the developing world, spontaneously seek antenatal care. Such visits offer a good opportunity to include a *C. trachomatis* test as part of a routine antenatal care program, as is done by some members of the European Union and other countries [207, 239, 240].

Tests for *C. trachomatis* infection during pregnancy

Various test methods for *C. trachomatis* were described before. The high sensitivity and automation of the more recent NAATs make these tests suitable for screening. In addition, NAATs have a high performance while using non-invasive specimens such as first-void urines or self-obtained vulval or vaginal swabs, which will lower the threshold for women to participate in screening [42, 241, 242]. Urines are well-accepted specimens by the majority of women and are mainly used for screening at present [242, 243]. Bacterial loads are generally lower in urines, which may have an adverse effect on NAATs and produce a lower detection rate in urines than in clinician-obtained swabs [243-245]. NAAT results in urines have also been shown to be inferior when urines are from asymptomatic women [57, 244, 246], and when pooling of urine specimens has been applied [62, 247, 248]. In contrast, others have described similar sensitivity with pooling compared to individual testing [60, 61]. To date limited data are available regarding NAAT performance on urines from pregnant women, especially while pooling urines.

Acceptable treatment against *C. trachomatis* infection for pregnant women

Efficacious antibiotics available to treat *C. trachomatis* infection in pregnancy have been described on page 36. Single dose treatment with azithromycin will increase compliance and is

readily available in most countries and is now recommended by the CDC [206, 207, 240]. However, in some countries the drug of choice is still under debate, this being the case in the Netherlands, where amoxicillin is still advised [249, 250].

Cost-effectiveness of screening

Apart from the target population, availability of non-invasive sampling, high quality testing and effective treatment, the cost-effectiveness of screening is the other important issue to address in order to assess the implementation of a *C. trachomatis* screening program [251-255]. From a health economic point of view, the costs of detecting chlamydia-positive individuals during a screening program should be in balance with the possible expenditures on medical care as a whole. Previous reports regarding *C. trachomatis* screening in family planning clinics showed that screening was cost-effective, but queried the relative merits of total screening versus selective screening and the best diagnostic test method to be used [256, 257]. Screening programs are generally considered to be cost-effective if the prevalence of C. *trachomatis* infection is higher than 3 to 6% [42, 255, 258-260]. This was also concluded from a Dutch study regarding antenatal screening for asymptomatic *C. trachomatis* infection in pregnancy [259]. However, at that time no actual data were available concerning pregnant women and pregnancy outcome in the Netherlands.

Psychosocial consequences of screening

Screening women for *C. trachomatis* infection, and other STIs, with subsequently a positive test result may lead to negative feelings such as guilt, shame, stigmatisation, a negative effect on womens' self-esteem, a sense of responsibility, self-recrimination, awareness of their body and sexuality, anxiety for partner notification and distrust within a relationship, and uncertainty about future reproductive health [261-263]. The literature regarding the psychosocial consequences of chlamydial screening is limited, especially in pregnant women. During pregnancy such emotions may even weigh more heavily, especially because a diagnosis of chlamydial infection would commonly be unexpected and because women may have an additional feeling of responsibility for and uncertainty about the consequences for the health of the fetus. Before implementing a screening program, the impact of diagnostic communication and retesting after treatment of infection as well as possible psychosocial consequences are important aspects to be taken into account [263, 264].

References

[1] World Health Organisation. Global Prevalence and Incidence of Selected Curable Sexually Transmitted infections. Overview and Estimates. Geneva: WHO. 2001.

[2] Fine D, Dicker L, Mosure D, Berman S. Increasing chlamydia positivity in women screened in family planning clinics: do we know why? Sex Transm Dis. 2008;35(1):47-52.

[3] Ross JM, Furr PM, Taylor-Robinson D, Altman DG, Coid CR. The effect of genital mycoplasmas on human fetal growth. Br J Obstet Gynaecol. 1981;88(7):749-55.

[4] Hardy PH, Hardy JB, Nell EE, Graham DA, Spence MR, Rosenbaum RC. Prevalence of six sexually transmitted disease agents among pregnant inner-city adolescents and pregnancy outcome. Lancet. 1984;2(8398):333-7.

[5] Wilson JS, Honey E, Templeton A, Paavonen J, Mardh PA, Stray-Pedersen B. A systematic review of the prevalence of *Chlamydia trachomatis* among European women. Hum Reprod Update. 2002;8(4):385-94.

[6] van Bergen JE. [Increased incidence of gonorrhea and *Chlamydia trachomatis* infections in family practice in southeast Amsterdam, 1996-2000]. Ned Tijdschr Geneeskd. 2001;145(35):1691-3.

[7] Laar MJWvd. SOA en AIDS in Nederland. Bilthoven, The Netherlands: Rijksinstituut voor Volksgezondheid en Milieu; 2000. Report No.: 441500011.

[8] van der Snoek EM, Gotz HM, Mulder PG, Verkooyen RP, van der Meijden WI. Prevalence of STD and HIV infections among attenders of the Erasmus MC STD clinic, Rotterdam, The Netherlands, during the years 1996 to 2000. Int J STD AIDS. 2003;14(2):119-24.

[9] Fact Sheet STD's in the Netherlands. STD AIDS Netherlands (Soa en Soa bestrijding in Nederland). 1996.

[10] Health Council of the Netherlands. Screening for Chlamydia. The Hague: Health Council of the Netherlands, 2004; publication no 2004/07.

[11] van Bergen J, Gotz HM, Richardus JH, Hoebe CJ, Broer J, Coenen AJ. Prevalence of urogenital *Chlamydia trachomatis* increases significantly with level of urbanisation and suggests targeted screening approaches: results from the first national population based study in the Netherlands. Sex Transm Infect. 2005;81(1):17-23.

[12] van den Hoek JA, Mulder-Folkerts DK, Coutinho RA, Dukers NH, Buimer M, van Doornum GJ. [Opportunistic screening for genital infections with *Chlamydia trachomatis* among the sexually active population of Amsterdam. II Over 90% participation and almost 5% prevalence]. Ned Tijdschr Geneeskd. 1999;143(13):668-72.

[13] van Valkengoed IG, Boeke AJ, van den Brule AJ, Morre SA, Dekker JH, Meijer CJ, et al. [Systematic home screening for *Chlamydia trachomatis* infections of asymptomatic men and women in family practice by means of mail-in urine samples]. Ned Tijdschr Geneeskd. 1999;143(13):672-6.

[14] Bax CJ, Oostvogel PM, Mutsaers JA, Brand R, Craandijk M, Trimbos JB, et al. Clinical characteristics of *Chlamydia trachomatis* infections in a general outpatient department of obstetrics and gynaecology in the Netherlands. Sex Transm Infect. 2002;78(6):E6.

[15] Fenton KA, Korovessis C, Johnson AM, McCadden A, McManus S, Wellings K, et al. Sexual behaviour in Britain: reported sexually transmitted infections and prevalent genital *Chlamydia trachomatis* infection. Lancet. 2001;358(9296):1851-4.

[16] Smith JR, Taylor-Robinson D. Infection due to *Chlamydia trachomatis* in pregnancy and the newborn. Baillieres Clin Obstet Gynaecol. 1993;7(1):237-55.

[17] Thomas AG, Brodine SK, Shaffer R, Shafer MA, Boyer CB, Putnam S, et al. Chlamydial infection and unplanned pregnancy in women with ready access to health care. Obstet Gynecol. 2001;98(6):1117-23.

[18] Chen MY, Fairley CK, De Guingand D, Hocking JS, Tabrizi S, Wallace EM, et al. Screening pregnant women

for Chlamydia: what are the predictors of infection? Sex Transm Infect. 2008;85(1):31-5.

[19] Lee VF, Tobin JM, Harindra V. Re-infection of *Chlamydia trachomatis* in patients presenting to the genitourinary medicine clinic in Portsmouth: the chlamydia screening pilot study - three years on. Int J STD AIDS. 2004;15(11):744-6.

[20] Quinn TC, Gaydos C, Shepherd M, Bobo L, Hook EW, 3rd, Viscidi R, et al. Epidemiologic and microbiologic correlates of *Chlamydia trachomatis* infection in sexual partnerships. JAMA. 1996;276(21):1737-42.

[21] Schachter J, Grossman M, Sweet RL, Holt J, Jordan C, Bishop E. Prospective study of perinatal transmission of *Chlamydia trachomatis*. JAMA. 1986;255(24):3374-7.

[22] Hammerschlag MR. Chlamydial infections. J Pediatr. 1989;114(5):727-34.

[23] Heggie AD, Lumicao GG, Stuart LA, Gyves MT. *Chlamydia trachomatis* infection in mothers and infants. A prospective study. Am J Dis Child. 1981;135(6):507-11.

[24] Darville T. *Chlamydia trachomatis* infections in neonates and young children. Semin Pediatr Infect Dis. 2005;16(4):235-44.

[25] Stephens RS, Myers G, Eppinger M, Bavoil PM. Divergence without difference: phylogenetics and taxonomy of Chlamydia resolved. FEMS Immunol Med Microbiol. 2009;55(2):115-9.

[26] Heuer D, Kneip C, Maurer AP, Meyer TF. Tackling the intractable - approaching the genetics of Chlamydiales. Int J Med Microbiol. 2007;297(7-8):569-76.

[27] Baud D, Regan L, Greub G. Emerging role of Chlamydia and Chlamydia-like organisms in adverse pregnancy outcomes. Curr Opin Infect Dis. 2008;21(1):70-6.

[28] Murray A. Medical Microbiology, third edition, Chapter 44.

[29] Sharma M, Rudel T. Apoptosis resistance in Chlamydia-infected cells: a fate worse than death? FEMS Immunol Med Microbiol. 2009;55(2):154-61.

[30] Parks KS, Dixon PB, Richey CM, Hook EW, 3rd. Spontaneous clearance of *Chlamydia trachomatis* infection in untreated patients. Sex Transm Dis. 1997;24(4):229-35.

[31] Morre SA, van den Brule AJ, Rozendaal L, Boeke AJ, Voorhorst FJ, de Blok S, et al. The natural course of asymptomatic *Chlamydia trachomatis* infections: 45% clearance and no development of clinical PID after one-year follow-up. Int J STD AIDS. 2002;13 Suppl 2:12-8.

[32] McCormack WM, Alpert S, McComb DE, Nichols RL, Semine DZ, Zinner SH. Fifteen-month follow-up study of women infected with *Chlamydia trachomatis*. N Engl J Med. 1979;300(3):123-5.

[33] Bell TA, Stamm WE, Wang SP, Kuo CC, Holmes KK, Grayston JT. Chronic *Chlamydia trachomatis* infections in infants. JAMA. 1992;267(3):400-2.

[34] Westrom L, Joesoef R, Reynolds G, Hagdu A, Thompson SE. Pelvic inflammatory disease and fertility. A cohort study of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopic results. Sex Transm Dis. 1992;19(4):185-92.

[35] Hillis SD, Owens LM, Marchbanks PA, Amsterdam LF, Mac Kenzie WR. Recurrent chlamydial infections increase the risks of hospitalization for ectopic pregnancy and pelvic inflammatory disease. Am J Obstet Gynecol. 1997;176(1 Pt 1):103-7.

[36] Schachter J. Which test is best for Chlamydia? Curr Opin Infect Dis. 1999;12(1):41-5.

[37] Black CM. Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. Clin Microbiol Rev.

1997;10(1):160-84.

[38] Morse AS, Ballard RC, Holmes KK, Moreland A.A. Atlas of Sexually Transmitted Diseases and AIDS. 2003:73-97.

[39] Chernesky MA. The laboratory diagnosis of *Chlamydia trachomatis* infections. Can J Infect Dis Med Microbiol. 2005;16(1):39-44.

[40] van de Laar MJ, Fontaine J. ECDC guidance on Chlamydia control in Europe: next steps. Euro Surveill. 2009;14(26).

[41] Black CM, Driebe EM, Howard LA, Fajman NN, Sawyer MK, Girardet RG, et al. Multicenter Study of Nucleic Acid Amplification Tests for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Children Being Evaluated for Sexual Abuse. Pediatr Infect Dis J. 2009;28(7):608-13.

[42] Watson EJ, Templeton A, Russell I, Paavonen J, Mardh PA, Stary A, et al. The accuracy and efficacy of screening tests for *Chlamydia trachomatis*: a systematic review. J Med Microbiol. 2002;51(12):1021-31.

[43] Chiappino ML, Dawson C, Schachter J, Nichols BA. Cytochemical localization of glycogen in *Chlamydia trachomatis* inclusions. J Bacteriol. 1995;177(18):5358-63.

[44] Ripa KT, Mardh PA. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated mccoy cells. J Clin Microbiol. 1977;6(4):328-31.

[45] Ripa KT. Microbiology Diagnosis of *Chlamydia trachomatis* infection. Infection. 1982;10 Suppl 1:S19-24.

[46] Mouton JW, Goessen WHF, MeijdenWI, Verkooijen RP,. Diagnostiek van *Chlamydia trachomatis*: wat is de gouden standaard? Infectieziekten Bulletin. 1998 10:241-5.

[47] Bakir TM, Hossain A, De-Silva S, Siddiqui A, Sengupta BS, el-Sheikh MM, et al. Enzyme immunoassay in the diagnosis of *Chlamydia trachomatis* infections in diverse patient groups. J Hyg Epidemiol Microbiol Immunol. 1989;33(2):189-97.

[48] Chernesky MA, Mahony JB, Castriciano S, Mores M, Stewart IO, Landis SJ, et al. Detection of *Chlamydia trachomatis* antigens by enzyme immunoassay and immunofluorescence in genital specimens from symptomatic and asymptomatic men and women. J Infect Dis. 1986;154(1):141-8.

[49] Cles LD, Bruch K, Stamm WE. Staining characteristics of six commercially available monoclonal immunofluorescence reagents for direct diagnosis of *Chlamydia trachomatis* infections. J Clin Microbiol. 1988;26(9):1735-7.

[50] Svensson LO, Mares I, Olsson SE, Nordstrom ML. Screening for *Chlamydia trachomatis* infection in women and aspects of the laboratory diagnostics. Acta Obstet Gynecol Scand. 1991;70(7-8):587-90.

[51] Rabenau HF, Kohler E, Peters M, Doerr HW, Weber B. Low correlation of serology with detection of *Chlamydia trachomatis* by ligase chain reaction and antigen EIA. Infection. 2000;28(2):97-102.

[52] Dabekausen YA, Evers JL, Land JA, Stals FS. *Chlamydia trachomatis* antibody testing is more accurate than hysterosalpingography in predicting tubal factor infertility. Fertil Steril. 1994;61(5):833-7.

[53] Keltz MD, Gera PS, Moustakis M. Chlamydia serology screening in infertility patients. Fertil Steril. 2006;85(3):752-4.

[54] Mouton JW, Peeters MF, van Rijssort-Vos JH, Verkooyen RP. Tubal factor pathology caused by *Chlamydia trachomatis*: the role of serology. Int J STD AIDS. 2002;13 Suppl 2:26-9.

[55] Schachter J, Grossman M, Azimi PH. Serology of *Chlamydia trachomatis* in infants. J Infect Dis. 1982;146(4):530-5.

[56] Kluytmans JA, Goessens WH, Mouton JW, van Rijsoort-Vos JH, Niesters HG, Quint WG, et al. Evaluation of Clearview and Magic Lite tests, polymerase chain reaction, and cell culture for detection of *Chlamydia trachomatis* in urogenital specimens. J Clin Microbiol. 1993;31(12):3204-10.

[57] Goessens WH, Mouton JW, van der Meijden WI, Deelen S, van Rijsoort-Vos TH, Lemmens-den Toom N, et al. Comparison of three commercially available amplification assays, AMP CT, LCx, and COBAS AMPLICOR, for detection of *Chlamydia trachomatis* in first-void urine. J Clin Microbiol. 1997;35(10):2628-33.

[58] Van Dyck E, Ieven M, Pattyn S, Van Damme L, Laga M. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by enzyme immunoassay, culture, and three nucleic acid amplification tests. J Clin Microbiol. 2001;39(5):1751-6.

[59] Van Der Pol B, Quinn TC, Gaydos CA, Crotchfelt K, Schachter J, Moncada J, et al. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of *Chlamydia trachomatis*. J Clin Microbiol. 2000;38(3):1105-12.

[60] Currie MJ, McNiven M, Yee T, Schiemer U, Bowden FJ. Pooling of clinical specimens prior to testing for *Chlamydia trachomatis* by PCR is accurate and cost saving. J Clin Microbiol. 2004;42(10):4866-7.

[61] Morre SA, Welte R, Postma MJ. Major improvements in cost effectiveness of screening women for *Chlamydia trachomatis* using pooled urine specimens and high performance testing. Sex Transm Infect. 2002;78(1):74-5.

[62] Krepel J, Patel J, Sproston A, Hopkins F, Jang D, Mahony J, et al. The impact on accuracy and cost of ligase chain reaction testing by pooling urine specimens for the diagnosis of *Chlamydia trachomatis* infections. Sex Transm Dis. 1999;26(9):504-7.

[63] Mahony J, Chong S, Jang D, Luinstra K, Faught M, Dalby D, et al. Urine specimens from pregnant and non-pregnant women inhibitory to amplification of *Chlamydia trachomatis* nucleic acid by PCR, ligase chain reaction, and transcription-mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory activity. J Clin Microbiol. 1998;36(11):3122-6.

[64] Unemo M, Rossouw A, James V, Jenkins C. Can the Swedish new variant of *Chlamydia trachomatis* (nvCT) be detected by UK NEQAS participants from seventeen European countries and five additional countries/regions in 2009? Euro Surveill. 2009;14(19).

[65] Reischl U, Straube E, Unemo M. The Swedish new variant of *Chlamydia trachomatis* (nvCT) remains undetected by many European laboratories as revealed in the recent PCR/NAT ring trial organised by INSTAND e.V., Germany. Euro Surveill. 2009;14(32).

[66] Cates W, Jr., Wasserheit JN. Genital chlamydial infections: epidemiology and reproductive sequelae. Am J Obstet Gynecol. 1991;164(6 Pt 2):1771-81.

[67] Sheffield JS, Andrews WW, Klebanoff MA, Macpherson C, Carey JC, Ernest JM, et al. Spontaneous resolution of asymptomatic *Chlamydia trachomatis* in pregnancy. Obstet Gynecol. 2005;105(3):557-62.

[68] Wiesenfeld HC, Hillier SL, Krohn MA, Amortegui AJ, Heine RP, Landers DV, et al. Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. Obstet Gynecol. 2002;100(3):456-63.

[69] Manavi K. A review on infection with *Chlamydia trachomatis*. Best Pract Res Clin Obstet Gynaecol. 2006;20(6):941-51.

[70] Westrom L. Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. Am J Obstet Gynecol. 1980;138(7 Pt 2):880-92.

[71] Svensson L, Mardh PA, Westrom L. Infertility after acute salpingitis with special reference to *Chlamydia trachomatis*. Fertil Steril. 1983;40(3):322-9.

[72] Gatt D, Jantet G. Perisplenitis and perinephritis in the Curtis-Fitz-Hugh syndrome. Br J Surg. 1987;74(2):110-2.

[73] van Dongen PW. Diagnosis of Fitz-Hugh-Curtis syndrome by ultrasound. Eur J Obstet Gynecol Reprod Biol. 1993;50(2):159-62.

[74] Mardh PA, Wolner-Hanssen P. Periappendicitis and chlamydial salpingitis. Surg Gynecol Obstet. 1985;160(4):304-6.

[75] Keat A, Thomas B, Dixey J, Osborn M, Sonnex C, Taylor-Robinson D. *Chlamydia trachomatis* and reactive arthritis: the missing link. Lancet. 1987;1(8524):72-4.

[76] Heaton S, Hammerschlag MR, Roblin PM, Di Pasquale RC. Lymphogranuloma venereum in a pregnant woman. Sex Transm Dis. 1988;15(3):148-9.

[77] Duncan ME, Jamil Y, Tibaux G, Pelzer A, Mehari L, Darougar S. Chlamydial infection in a population of Ethiopian women attending obstetric, gynaecological and mother and child health clinics. Cent Afr J Med. 1996;42(1):1-14.

[78] Westrom L, Wolner-Hanssen P. Pathogenesis of pelvic inflammatory disease. Genitourin Med. 1993;69(1):9-17.

[79] Cohen CR, Brunham RC. Pathogenesis of Chlamydia induced pelvic inflammatory disease. Sex Transm Infect. 1999;75(1):21-4.

[80] Debattista J, Timms P, Allan J. Immunopathogenesis of *Chlamydia trachomatis* infections in women. Fertil Steril. 2003;79(6):1273-87.

[81] den Hartog JE, Morre SA, Land JA. *Chlamydia trachomatis*-associated tubal factor subfertility: Immunogenetic aspects and serological screening. Hum Reprod Update. 2006;12(6):719-30.

[82] Risser WL, Risser JM. The incidence of pelvic inflammatory disease in untreated women infected with *Chlamydia trachomatis*: a structured review. Int J STD AIDS. 2007;18(11):727-31.

[83] van Valkengoed IG, Morre SA, van den Brule AJ, Meijer CJ, Bouter LM, Boeke AJ. Overestimation of complication rates in evaluations of *Chlamydia trachomatis* screening programmes--implications for cost-effectiveness analyses. Int J Epidemiol. 2004;33(2):416-25.

[84] Boeke AJ, van Bergen JE, Morre SA, van Everdingen JJ. [The risk of pelvic inflammatory disease associated with urogenital infection with *Chlamydia trachomatis*; literature review]. Ned Tijdschr Geneeskd. 2005;149(16):878-84.

[85] Low N, Harbord RM, Egger M, Sterne JA, Herrmann B. Screening for Chlamydia. Lancet. 2005;365(9470):1539.

[86] Jones RB, Mammel JB, Shepard MK, Fisher RR. Recovery of *Chlamydia trachomatis* from the endometrium of women at risk for chlamydial infection. Am J Obstet Gynecol. 1986;155(1):35-9.

[87] Osser S, Persson K. Postabortal pelvic infection associated with *Chlamydia trachomatis* and the influence of humoral immunity. Am J Obstet Gynecol. 1984;150(6):699-703.

[88] Paavonen J. *Chlamydia trachomatis* in acute salpingitis. Am J Obstet Gynecol. 1980;138(7 Pt 2):957-9.

[89] Stamm WE, Guinan ME, Johnson C, Starcher T, Holmes KK, McCormack WM. Effect of treatment regimens for *Neisseria gonorrhoeae* on simultaneous infection with *Chlamydia trachomatis*. N Engl J Med. 1984;310(9):545-9.

[90] Ostergaard L, Andersen B, Moller JK, Olesen F. Home sampling versus conventional swab sampling for screening of *Chlamydia trachomatis* in women: a cluster-randomized 1-year follow-up study. Clin Infect Dis. 2000;31(4):951-7.

[91] Scholes D, Stergachis A, Heidrich FE, Andrilla H, Holmes KK, Stamm WE. Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. N Engl J Med. 1996;334(21):1362-6.

[92] Paavonen J, Kiviat N, Brunham RC, Stevens CE, Kuo CC, Stamm WE, et al. Prevalence and manifestations of endometritis among women with cervicitis. Am J Obstet Gynecol. 1985;152(3):280-6.

[93] Bakken IJ, Skjeldestad FE, Lydersen S, Nordbo SA. Births and ectopic pregnancies in a large cohort of women tested for *Chlamydia trachomatis.* Sex Transm Dis. 2007;34(10):739-43.

[94] Lan J, van den Brule AJ, Hemrika DJ, Risse EK, Walboomers JM, Schipper ME, et al. *Chlamydia trachomatis* and ectopic pregnancy: retrospective analysis of salpingectomy specimens, endometrial biopsies, and cervical smears. J Clin Pathol. 1995;48(9):815-9.

[95] Brunham RC, Peeling R, Maclean I, Kosseim ML, Paraskevas M. *Chlamydia trachomatis*-associated ectopic pregnancy: serologic and histologic correlates. J Infect Dis. 1992;165(6):1076-81.

[96] Bakken IJ, Skjeldestad FE, Nordbo SA. *Chlamydia trachomatis* infections increase the risk for ectopic pregnancy: a population-based, nested case-control study. Sex Transm Dis. 2007;34(3):166-9.

[97] Egger M, Low N, Smith GD, Lindblom B, Herrmann B. Screening for chlamydial infections and the risk of ectopic pregnancy in a county in Sweden: ecological analysis. BMJ. 1998;316(7147):1776-80.

[98] Westrom L, Bengtsson LP, Mardh PA. Incidence, trends, and risks of ectopic pregnancy in a population of women. Br Med J (Clin Res Ed). 1981;282(6257):15-8.

[99] Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G, et al. Genital tract infections and infertility. Eur J Obstet Gynecol Reprod Biol. 2008;140(1):3-11.

[100] Mardh PA. Tubal factor infertility, with special regard to chlamydial salpingitis. Curr Opin Infect Dis. 2004;17(1):49-52.

[101] den Hartog JE, Lardenoije CM, Severens JL, Land JA, Evers JL, Kessels AG. Screening strategies for tubal factor subfertility. Hum Reprod. 2008;23(8):1840-8.

[102] Malik A, Jain S, Rizvi M, Shukla I, Hakim S. *Chlamydia trachomatis* infection in women with secondary infertility. Fertil Steril. 2009;91(1):91-5.

[103] Coppus SF, Opmeer BC, Logan S, van der Veen F, Bhattacharya S, Mol BW. The predictive value of medical history taking and Chlamydia IgG ELISA antibody testing (CAT) in the selection of subfertile women for diagnostic laparoscopy: a clinical prediction model approach. Hum Reprod. 2007;22(5):1353-8.

[104] Malik A, Jain S, Hakim S, Shukla I, Rizvi M. *Chlamydia trachomatis* infection & female infertility. Indian J Med Res. 2006;123(6):770-5.

[105] Karinen L, Pouta A, Hartikainen AL, Bloigu A, Paldanius M, Leinonen M, et al. Association between *Chlamydia trachomatis* antibodies and subfertility in the Northern Finland Birth Cohort 1966 (NFBC 1966), at the age of 31 years. Epidemiol Infect. 2004;132(5):977-84.

[106] Barlow RE, Cooke ID, Odukoya O, Heatley MK, Jenkins J, Narayansingh G, et al. The prevalence of *Chlamydia trachomatis* in fresh tissue specimens from patients with ectopic pregnancy or tubal factor infertility as determined by PCR and in-situ hybridisation. J Med Microbiol. 2001;50(10):902-8.

[107] den Hartog JE, Land JA, Stassen FR, Kessels AG, Bruggeman CA. Serological markers of persistent *C. trachomatis* infections in women with tubal factor subfertility. Hum Reprod. 2005;20(4):986-90.

[108] Brunham RC, Maclean IW, Binns B, Peeling RW. *Chlamydia trachomatis*: its role in tubal infertility. J Infect Dis. 1985;152(6):1275-82.

[109] Cates W, Jr., Rolfs RT, Jr., Aral SO. Sexually transmitted diseases, pelvic inflammatory disease, and infertility: an epidemiologic update. Epidemiol Rev. 1990;12:199-220.

[110] Vigil P, Tapia A, Zacharias S, Riquelme R, Salgado AM, Varleta J. First-trimester pregnancy loss and active *Chlamydia trachomatis* infection: correlation and ultrastructural evidence. Andrologia. 2002;34(6):373-8.

[111] Rastogi S, Salhan S, Mittal A. Detection of *Chlamydia trachomatis* antigen in spontaneous abortions. Is this organism a primary or secondary indicator of risk? Br J Biomed Sci. 2000;57(2):126-9.

[112] Avasthi K, Garg T, Gupta S, Grewal RK, Ram S. A study of prevalence of *Chlamydia trachomatis* infection in women with first trimester pregnancy losses. Indian J Pathol Microbiol. 2003;46(1):133-6.

[113] Osser S, Persson K. Chlamydial antibodies in women who suffer miscarriage. Br J Obstet Gynaecol. 1996;103(2):137-41.

[114] Witkin SS, Ledger WJ. Antibodies to *Chlamydia trachomatis* in sera of women with recurrent spontaneous abortions. Am J Obstet Gynecol. 1992;167(1):135-9.

[115] Quinn PA, Petric M, Barkin M, Butany J, Derzko C, Gysler M, et al. Prevalence of antibody to *Chlamydia trachomatis* in spontaneous abortion and infertility. Am J Obstet Gynecol. 1987;156(2):291-6.

[116] Licciardi F, Grifo JA, Rosenwaks Z, Witkin SS. Relation between antibodies to *Chlamydia trachomatis* and spontaneous abortion following in vitro fertilization. J Assist Reprod Genet. 1992;9(3):207-10.

[117] Patel A, Rashid S, Godfrey EM, Panchal H. Prevalence of *Chlamydia trachomatis* and Neisseria gonorrhoeae genital infections in a publicly funded pregnancy termination clinic: empiric vs. indicated treatment? Contraception. 2008;78(4):328-31.

[118] Baczynska A, Hvid M, Lamy P, Birkelund S, Christiansen G, Fedder J. Prevalence of *Mycoplasma genitalium*, *Mycoplasma hominis* and *Chlamydia trachomatis* among Danish patients requesting abortion. Syst Biol Reprod Med. 2008;54(3):127-34.

[119] Renton A, Thomas BM, Gill S, Lowndes C, Taylor-Robinson D, Patterson K. *Chlamydia trachomatis* in cervical and vaginal swabs and urine specimens from women undergoing termination of pregnancy. Int J STD AIDS. 2006;17(7):443-7.

[120] Barbacci MB, Spence MR, Kappus EW, Burkman RC, Rao L, Quinn TC. Postabortal endometritis and isolation of *Chlamydia trachomatis*. Obstet Gynecol. 1986;68(5):686-90.

[121] Blackwell AL, Thomas PD, Wareham K, Emery SJ. Health gains from screening for infection of the lower genital tract in women attending for termination of pregnancy. Lancet. 1993;342(8865):206-10.

[122] Sorensen JL, Thranov I, Hoff G, Dirach J. Early- and late-onset pelvic inflammatory disease among women with cervical *Chlamydia trachomatis* infection at the time of induced abortion--a follow-up study. Infection. 1994;22(4):242-6.

[123] Stray-Pedersen B, Biornstad J, Dahl M, Bergan T, Aanestad G, Kristiansen L, et al. Induced abortion: microbiological screening and medical complications. Infection. 1991;19(5):305-8.

[124] Sorensen JL, Thranov I, Hoff G, Dirach J, Damsgaard MT. A double-blind randomized study of the effect of erythromycin in preventing pelvic inflammatory disease after first trimester abortion. Br J Obstet Gynaecol. 1992;99(5):434-8.

[125] Andrews WW, Klebanoff MA, Thom EA, Hauth JC, Carey JC, Meis PJ, et al. Midpregnancy genitourinary tract infection with *Chlamydia trachomatis*: association with subsequent preterm delivery in women with bacterial vaginosis and *Trichomonas vaginalis*. Am J Obstet Gynecol. 2006;194(2):493-500.

[126] Tamiolakis D, Kotini A, Jivannakis T, Papadopoulos N. Induction of hepatic granulopoiesis due to chorioamnionitis

during the second trimester of development. Eur J Obstet Gynecol Reprod Biol. 2003;110(2):164-8.

[127] Gibbs RS. The origins of stillbirth: infectious diseases. Semin Perinatol. 2002;26(1):75-8.

[128] Donders GG, Moerman P, De Wet GH, Hooft P, Goubau P. The association between Chlamydia cervicitis, chorioamnionitis and neonatal complications. Arch Gynecol Obstet. 1991;249(2):79-85.

[129] Thomas GB, Jones J, Sbarra AJ, Cetrulo C, Reisner D. Isolation of *Chlamydia trachomatis* from amniotic fluid. Obstet Gynecol. 1990;76(3 Pt 2):519-20.

[130] Harrison HR, Alexander ER, Weinstein L, Lewis M, Nash M, Sim DA. Cervical *Chlamydia trachomatis* and mycoplasmal infections in pregnancy. Epidemiology and outcomes. JAMA. 1983;250(13):1721-7.

[131] McGregor JA, French JI, Richter R, Vuchetich M, Bachus V, Seo K, et al. Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment. Am J Obstet Gynecol. 1990;163(5 Pt 1):1580-91.

[132] Cohen I, Veille JC, Calkins BM. Improved pregnancy outcome following successful treatment of chlamydial infection. JAMA. 1990;263(23):3160-3.

[133] Ryan GM, Jr., Abdella TN, McNeeley SG, Baselski VS, Drummond DE. *Chlamydia trachomatis* infection in pregnancy and effect of treatment on outcome. Am J Obstet Gynecol. 1990;162(1):34-9.

[134] Alger LS, Lovchik JC, Hebel JR, Blackmon LR, Crenshaw MC. The association of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and group B streptococci with preterm rupture of the membranes and pregnancy outcome. Am J Obstet Gynecol. 1988;159(2):397-404.

[135] Sweet RL, Landers DV, Walker C, Schachter J. *Chlamydia trachomatis* infection and pregnancy outcome. Am J Obstet Gynecol. 1987;156(4):824-33.

[136] Gravett MG, Nelson HP, DeRouen T, Critchlow C, Eschenbach DA, Holmes KK. Independent associations of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. JAMA. 1986;256(14):1899-903.

[137] Martius J, Krohn MA, Hillier SL, Stamm WE, Holmes KK, Eschenbach DA. Relationships of vaginal Lactobacillus species, cervical *Chlamydia trachomatis*, and bacterial vaginosis to preterm birth. Obstet Gynecol. 1988;71(1):89-95.

[138] Karinen L, Pouta A, Bloigu A, Koskela P, Paldanius M, Leinonen M, et al. Serum C-reactive protein and *Chlamydia trachomatis* antibodies in preterm delivery. Obstet Gynecol. 2005;106(1):73-80.

[139] French JI, McGregor JA, Parker R. Readily treatable reproductive tract infections and preterm birth among black women. Am J Obstet Gynecol. 2006;194(6):1717-26; discussion 26-7.

[140] Yu J, Wu S, Li F, Hu L. Vertical transmission of *Chlamydia trachomatis* in Chongqing China. Curr Microbiol. 2009;58(4):315-20.

[141] Andrews WW, Goldenberg RL, Mercer B, Iams J, Meis P, Moawad A, et al. The Preterm Prediction Study: association of second-trimester genitourinary Chlamydia infection with subsequent spontaneous preterm birth. Am J Obstet Gynecol. 2000;183(3):662-8.

[142] Blas MM, Canchihuaman FA, Alva IE, Hawes SE. Pregnancy outcomes in women infected with *Chlamydia trachomatis*: a population-based cohort study in Washington State. Sex Transm Infect. 2007;83(4):314-8.

[143] Odendaal HJ Schoeman J. The association between *Chlamydia trachomatis* genital infection and spontaneous preterm labour. South African Journal of Obstetrics and Gynaecology. 2006;12(3):146-9.

[144] Rastogi S, Das B, Salhan S, Mittal A. Effect of treatment for *Chlamydia trachomatis* during pregnancy. Int J

Gynaecol Obstet. 2003;80(2):129-37.

[145] Kovacs L, Nagy E, Berbik I, Meszaros G, Deak J, Nyari T. The frequency and the role of *Chlamydia trachomatis* infection in premature labor. Int J Gynaecol Obstet. 1998;62(1):47-54.

[146] Martin DH, Koutsky L, Eschenbach DA, Daling JR, Alexander ER, Benedetti JK, et al. Prematurity and perinatal mortality in pregnancies complicated by maternal *Chlamydia trachomatis* infections. JAMA. 1982;247(11):1585-8.

[147] Association of *Chlamydia trachomatis* and *Mycoplasma hominis* with intrauterine growth retardation and preterm delivery. The John Hopkins Study of Cervicitis and Adverse Pregnancy Outcome. Am J Epidemiol. 1989;129(6):1247-57.

[148] Claman P, Toye B, Peeling RW, Jessamine P, Belcher J. Serologic evidence of *Chlamydia trachomatis* infection and risk of preterm birth. CMAJ. 1995;153(3):259-62.

[149] Gencay M, Koskiniemi M, Ammala P, Fellman V, Narvanen A, Wahlstrom T, et al. *Chlamydia trachomatis* seropositivity is associated both with stillbirth and preterm delivery. APMIS. 2000;108(9):584-8.

[150] Ismail MA, Pridjian G, Hibbard JU, Harth C, Moawad AA. Significance of positive cervical cultures for *Chlamydia trachomatis* in patients with preterm premature rupture of membranes. Am J Perinatol. 1992;9(5-6):368-70.

[151] FitzSimmons J, Callahan C, Shanahan B, Jungkind D. Chlamydial infections in pregnancy. J Reprod Med. 1986;31(1):19-22.

[152] Rastogi S, Kapur S, Salhan S, Mittal A. *Chlamydia trachomatis* infection in pregnancy: risk factor for an adverse outcome. Br J Biomed Sci. 1999;56(2):94-8.

[153] Martin DH, Eschenbach DA, Cotch MF, Nugent RP, Rao AV, Klebanoff MA, et al. Double-Blind Placebo-Controlled Treatment Trial of *Chlamydia trachomatis* Endocervical Infections in Pregnant Women. Infect Dis Obstet Gynecol. 1997;5(1):10-7.

[154] Germain M, Krohn MA, Hillier SL, Eschenbach DA. Genital flora in pregnancy and its association with intrauterine growth retardation. J Clin Microbiol. 1994;32(9):2162-8.

[155] Legris M, Hainaut F, Crimail P, Catalan F. [Results of detection and early treatment of *Chlamydia trachomatis* infections in pregnancy]. J Gynecol Obstet Biol Reprod (Paris). 1989;18(5):581-5.

[156] Donders GG, Desmyter J, De Wet DH, Van Assche FA. The association of gonorrhoea and syphilis with premature birth and low birthweight. Genitourin Med. 1993;69(2):98-101.

[157] Berman SM, Harrison HR, Boyce WT, Haffner WJ, Lewis M, Arthur JB. Low birth weight, prematurity, and postpartum endometritis. Association with prenatal cervical *Mycoplasma hominis* and *Chlamydia trachomatis* infections. JAMA. 1987;257(9):1189-94.

[158] McClure EM. Goldenber RL. Infection and stillbirth. Semin Fetal Neonatal Med. 2009.

[159] Mardh PA. Influence of infection with *Chlamydia trachomatis* on pregnancy outcome, infant health and life-long sequelae in infected offspring. Best Pract Res Clin Obstet Gynaecol. 2002;16(6):847-64.

[160] La Scolea LJ, Jr., Paroski JS, Burzynski L, Faden HS. *Chlamydia trachomatis* infection in infants delivered by cesarean section. Clin Pediatr (Phila). 1984;23(2):118-20.

[161] Bell TA. *Chlamydia trachomatis* infection in dizygotic twins delivered by caesarean section. Genitourin Med. 1988;64(5):347-8.

[162] Shariat H, Young M, Abedin M. An interesting case presentation: a possible new route for perinatal acquisition of Chlamydia. J Perinatol. 1992;12(3):300-2.

[163] Bell TA, Stamm WE, Kuo CC, Wang SP, Holmes KK, Grayston JT. Risk of perinatal transmission of *Chlamydia trachomatis* by mode of delivery. J Infect. 1994;29(2):165-9.

[164] Attenburrow AA, Barker CM. Chlamydial pneumonia in the low birthweight neonate. Arch Dis Child. 1985;60(12):1169-72.

[165] Fejgin MD, Cohen I, Horvat-Kohlmann M, Charles AG, Luzon A, Samra Z. *Chlamydia trachomatis* infection during pregnancy: can it cause an intrauterine infection? Isr J Med Sci. 1997;33(2):98-102.

[166] Koskiniemi M, Ammala P, Narvanen A, Saikku P, Soderlund M, Koskela P, et al. Stillbirths and maternal antibodies to *Chlamydia trachomatis*. A new EIA test for serology. Acta Obstet Gynecol Scand. 1996;75(7):657-61.

[167] Hoyme UB, Kiviat N, Eschenbach DA. Microbiology and treatment of late postpartum endometritis. Obstet Gynecol. 1986;68(2):226-32.

[168] Plummer FA, Laga M, Brunham RC, Piot P, Ronald AR, Bhullar V, et al. Postpartum upper genital tract infections in Nairobi, Kenya: epidemiology, etiology, and risk factors. J Infect Dis. 1987;156(1):92-8.

[169] Temmerman M, Laga M, Ndinya-Achola JO, Paraskevas M, Brunham RC, Plummer FA, et al. Microbial aetiology and diagnostic criteria of postpartum endometritis in Nairobi, Kenya. Genitourin Med. 1988;64(3):172-5.

[170] Wager GP, Martin DH, Koutsky L, Eschenbach DA, Daling JR, Chiang WT, et al. Puerperal infectious morbidity: relationship to route of delivery and to antepartum *Chlamydia trachomatis* infection. Am J Obstet Gynecol. 1980;138(7 Pt 2):1028-33.

[171] Minkoff H. Prematurity: infection as an etiologic factor. Obstet Gynecol. 1983;62(2):137-44.

[172] Schachter J, Sweet RL, Grossman M, Landers D, Robbie M, Bishop E. Experience with the routine use of erythromycin for chlamydial infections in pregnancy. N Engl J Med. 1986;314(5):276-9.

[173] Sollecito D, Midulla M, Bavastrelli M, Panero A, Marzetti G, Rossi D, et al. *Chlamydia trachomatis* in neonatal respiratory distress of very preterm babies: biphasic clinical picture. Acta Paediatr. 1992;81(10):788-91.

[174] Colarizi P, Chiesa C, Pacifico L, Adorisio E, Rossi N, Ranucci A, et al. *Chlamydia trachomatis*-associated respiratory disease in the very early neonatal period. Acta Paediatr. 1996;85(8):991-4.

[175] Mardh PA, Johansson PJ, Svenningsen N. Intrauterine lung infection with *Chlamydia trachomatis* in a premature infant. Acta Paediatr Scand. 1984;73(4):569-72.

[176] Sandstrom I. Etiology and diagnosis of neonatal conjunctivitis. Acta Paediatr Scand. 1987;76(2):221-7.

[177] Sergiwa A, Pratt BC, Eren E, Sunona TC, Hart CA. Ophthalmia neonatorum in Bangkok: the significance of *Chlamydia trachomatis*. Ann Trop Paediatr. 1993;13(3):233-6.

[178] Preece PM, Anderson JM, Thompson RG. *Chlamydia trachomatis* infection in infants: a prospective study. Arch Dis Child. 1989;64(4):525-9.

[179] Alexander ER, Harrison HR. Role of *Chlamydia trachomatis* in perinatal infection. Rev Infect Dis. 1983;5(4):713-9.

[180] Dannevig L, Straume B, Melby K. Ophthalmia neonatorum in northern Norway. II. Microbiology with emphasis on *Chlamydia trachomatis*. Acta Ophthalmol (Copenh). 1992;70(1):19-25.

[181] Hammerschlag MR. Neonatal conjunctivitis. Pediatr Ann. 1993;22(6):346-51.

[182] Hammerschlag MR, Chandler JW, Alexander ER, English M, Koutsky L. Longitudinal studies on chlamydial infections in the first year of life. Pediatr Infect Dis. 1982;1(6):395-401.

[183] Harrison HR, English MG, Lee CK, Alexander ER. *Chlamydia trachomatis* infant pneumonitis: comparison

with matched controls and other infant pneumonitis. N Engl J Med. 1978;298(13):702-8.

[184] Beem MO, Saxon EM. Respiratory-tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. N Engl J Med. 1977;296(6):306-10.

[185] Schachter J. Chlamydial infections N Engl J Med. 1978;298(10):428-34, 90-5, 540-9.

[186] Tipple MA, Beem MO, Saxon EM. Clinical characteristics of the afebrile pneumonia associated with *Chlamydia trachomatis* infection in infants less than 6 months of age. Pediatrics. 1979;63(2):192-7.

[187] Cohen SD, Azimi PH, Schachter J. *Chlamydia trachomatis* associated with severe rhinitis and apneic episodes in a one-month-old infant. Clin Pediatr (Phila). 1982;21(8):498-9.

[188] Shinkwin CA, Gibbin KP. Neonatal upper airway obstruction caused by chlamydial rhinitis. J Laryngol Otol. 1995;109(1):58-60.

[189] Iskandar NM, Naguib MB. *Chlamydia trachomatis*: an underestimated cause for rhinitis in neonates. Int J Pediatr Otorhinolaryngol. 1998;42(3):233-7.

[190] Helin I, Mardh PA. Mother-to-infant transmission of *Chlamydia trachomatis* and its consequences for the baby. Scand J Infect Dis Suppl. 1982;32:135-40.

[191] Ogawa H, Hashiguchi K, Kazuyama Y. Isolation of *Chlamydia trachomatis* from the middle ear aspirates of otitis media. Acta Otolaryngol. 1990;110(1-2):105-9.

[192] Hashiguchi K, Ogawa H, Koga K, Tateno H, Yamazaki Y. Otitis media with effusion associated with *Chlamydia trachomatis* infection in children. Auris Nasus Larynx. 1990;17(3):149-55.

[193] Chen CJ, Wu KG, Tang RB, Yuan HC, Soong WJ, Hwang BT. Characteristics of *Chlamydia trachomatis* infection in hospitalized infants with lower respiratory tract infection. J Microbiol Immunol Infect. 2007;40(3):255-9.

[194] Herieka E, Dhar J. Acute neonatal respiratory failure and *Chlamydia trachomatis*. Sex Transm Infect. 2001;77(2):135-6.

[195] Bjornsson E, Hjelm E, Janson C, Fridell E, Boman G. Serology of Chlamydia in relation to asthma and bronchial hyperresponsiveness. Scand J Infect Dis. 1996;28(1):63-9.

[196] Webley WC, Tilahun Y, Lay K, Patel K, Stuart ES, Andrzejewski C, et al. Occurrence of *Chlamydia trachomatis* and *Chlamydia pneumoniae* in paediatric respiratory infections. Eur Respir J. 2009;33(2):360-7.

[197] Bell TA, Stamm WE, Kuo CC, Wang SP, Holmes KK, Grayston JT. Delayed appearance of *Chlamydia trachomatis* infections acquired at birth. Pediatr Infect Dis J. 1987;6(10):928-31.

[198] de Barbeyrac B, Benali L, Clerc M, Garapon S, Bebear C, Gromb S. *Chlamydia trachomatis* infection in children: do not forget perinatal acquisition: a case report of a 7-year old girl, *C. trachomatis* infected, presumed sexually assaulted. J Forensic Leg Med. 2010;17(2):96-8.

[199] Hammerschlag MR. Sexually transmitted diseases in sexually abused children: medical and legal implications. Sex Transm Infect. 1998;74(3):167-74.

[200] Bechtel K. Sexual abuse and sexually transmitted infections in children and adolescents. Curr Opin Pediatr. 2010;22(1):94-9.

[201] Girardet RG, Lahoti S, Howard LA, Fajman NN, Sawyer MK, Driebe EM, et al. Epidemiology of sexually transmitted infections in suspected child victims of sexual assault. Pediatrics. 2009;124(1):79-86.

[202] Hammerschlag MR. Appropriate use of nonculture tests for the detection of sexually transmitted diseases in children and adolescents. Semin Pediatr Infect Dis. 2003;14(1):54-9.

[203] Hammerschlag MR. Nucleic acid amplification tests (polymerase chain reaction, ligase chain reaction) for the diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in pediatric emergency medicine. Pediatr Emerg Care. 2005;21(10):705.

[204] Simmons KJ, Hicks DJ. Child sexual abuse examination: is there a need for routine screening for *N. gonorrhoeae* and *C. trachomatis*? J Pediatr Adolesc Gynecol. 2005;18(5):343-5.

[205] Geisler WM. Approaches to the management of uncomplicated genital *Chlamydia trachomatis* infections. Expert Rev Anti Infect Ther. 2004;2(5):771-85.

[206] Geisler WM. Management of uncomplicated *Chlamydia trachomatis* infections in adolescents and adults: evidence reviewed for the 2006 Centers for Disease Control and Prevention sexually transmitted diseases treatment guidelines. Clin Infect Dis. 2007;44 Suppl 3:S77-83.

[207] Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep 2006;55(RR-11):1-94. 2006.

[208] Thorpe EM, Jr., Stamm WE, Hook EW, 3rd, Gall SA, Jones RB, Henry K, et al. Chlamydial cervicitis and urethritis: single dose treatment compared with doxycycline for seven days in community based practises. Genitourin Med. 1996;72(2):93-7.

[209] Demers P, Fraser D, Goldbloom RB, Haworth JC, LaRochelle J, MacLean R, et al. Effects of tetracyclines on skeletal growth and dentition. A report by the Nutrition Committee of the Canadian Paediatric Society. Can Med Assoc J. 1968;99(17):849-54.

[210] Siddiqui MA, Janjua MZ. Effect of prenatal doxycycline administration on skeletal differentiation in long bones of Albino rat. J Pak Med Assoc. 2002;52(5):211-4.

[211] Noel GJ, Bradley JS, Kauffman RE, Duffy CM, Gerbino PG, Arguedas A, et al. Comparative safety profile of levofloxacin in 2523 children with a focus on four specific musculoskeletal disorders. Pediatr Infect Dis J. 2007;26(10):879-91.

[212] Chouraqui JP, Bessard G, Favier M, Kolodie L, Rambaud P. [Haemorrhage associated with vitamin K deficiency in pregnant women and newborns. Relationship with rifampicin therapy in two cases]. Therapie. 1982;37(4):447-50.

[213] Czeizel AE, Rockenbauer M, Sorensen HT, Olsen J. The teratogenic risk of trimethoprim-sulfonamides: a population based case-control study. Reprod Toxicol. 2001;15(6):637-46.

[214] Hueston WJ, Lenhart JG. A decision analysis to guide antibiotic selection for Chlamydia infection during pregnancy. Arch Fam Med. 1997;6(6):551-5.

[215] Miller JM, Martin DH. Treatment of *Chlamydia trachomatis* infections in pregnant women. Drugs. 2000;60(3):597-605.

[216] Kacmar J, Cheh E, Montagno A, Peipert JF. A randomized trial of azithromycin versus amoxicillin for the treatment of *Chlamydia trachomatis* in pregnancy. Infect Dis Obstet Gynecol. 2001;9(4):197-202.

[217] Pitsouni E, Iavazzo C, Athanasiou S, Falagas ME. Single-dose azithromycin versus erythromycin or amoxicillin for *Chlamydia trachomatis* infection during pregnancy: a meta-analysis of randomised controlled trials. Int J Antimicrob Agents. 2007;30(3):213-21.

[218] Rahangdale L, Guerry S, Bauer HM, Packel L, Rhew M, Baxter R, et al. An observational cohort study of *Chlamydia trachomatis* treatment in pregnancy. Sex Transm Dis. 2006;33(2):106-10.

[219] Adair CD, Gunter M, Stovall TG, McElroy G, Veille JC, Ernest JM. Chlamydia in pregnancy: a randomized

trial of azithromycin and erythromycin. Obstet Gynecol. 1998;91(2):165-8.

[220] Wehbeh HA, Ruggeirio RM, Shahem S, Lopez G, Ali Y. Single-dose azithromycin for Chlamydia in pregnant women. J Reprod Med. 1998;43(6):509-14.

[221] Jacobson GF, Autry AM, Kirby RS, Liverman EM, Motley RU. A randomized controlled trial comparing amoxicillin and azithromycin for the treatment of *Chlamydia trachomatis* in pregnancy. Am J Obstet Gynecol. 2001;184(7):1352-4; discussion 4-6.

[222] Howe E, Howe E, Benn RA. Hepatotoxicity due to erythromycin ethylsuccinate. Med J Aust. 1993;158(2):142-4.

[223] Mahon BE, Rosenman MB, Kleiman MB. Maternal and infant use of erythromycin and other macrolide antibiotics as risk factors for infantile hypertrophic pyloric stenosis. J Pediatr. 2001;139(3):380-4.

[224] Cooper WO, Griffin MR, Arbogast P, Hickson GB, Gautam S, Ray WA. Very early exposure to erythromycin and infantile hypertrophic pyloric stenosis. Arch Pediatr Adolesc Med. 2002;156(7):647-50.

[225] Keller H. Comparison of the adverse effect profile of different substances such as penicillins, tetracyclines, sulfonamides and quinolones. Infection. 1991;19 Suppl 1:S19-24.

[226] Devenyi AG. Antibiotic-induced colitis. Semin Pediatr Surg. 1995;4(4):215-20.

[227] Patamasucon P, Rettig PJ, Faust KL, Kusmiesz HT, Nelson JD. Oral v topical erythromycin therapies for chlamydial conjunctivitis. Am J Dis Child. 1982;136(9):817-21.

[228] Klein JO. History of macrolide use in pediatrics. Pediatr Infect Dis J. 1997;16(4):427-31.

[229] Heggie AD, Jaffe AC, Stuart LA, Thombre PS, Sorensen RU. Topical sulfacetamide vs oral erythromycin for neonatal chlamydial conjunctivitis. Am J Dis Child. 1985;139(6):564-6.

[230] Stenberg K, Mardh PA. Treatment of chlamydial conjunctivitis in newborns and adults with erythromycin and roxithromycin. J Antimicrob Chemother. 1991;28(2):301-7.

[231] Chidambaram JD, Alemayehu W, Melese M, Lakew T, Yi E, House J, et al. Effect of a single mass antibiotic distribution on the prevalence of infectious trachoma. JAMA. 2006;295(10):1142-6.

[232] Melese M, Alemayehu W, Lakew T, Yi E, House J, Chidambaram JD, et al. Comparison of annual and biannual mass antibiotic administration for elimination of infectious trachoma. JAMA. 2008;299(7):778-84.

[233] Hammerschlag MR, Gelling M, Roblin PM, Kutlin A, Jule JE. Treatment of neonatal chlamydial conjunctivitis with azithromycin. Pediatr Infect Dis J. 1998;17(11):1049-50.

[234] Zar HJ. Neonatal chlamydial infections: prevention and treatment. Paediatr Drugs. 2005;7(2):103-10.

[235] Hammerschlag MR, Cummings C, Roblin PM, Williams TH, Delke I. Efficacy of neonatal ocular prophylaxis for the prevention of chlamydial and gonococcal conjunctivitis. N Engl J Med. 1989;320(12):769-72.

[236] Ali Z, Khadije D, Elahe A, Mohammad M, Fateme Z, Narges Z. Prophylaxis of ophthalmia neonatorum comparison of betadine, erythromycin and no prophylaxis. J Trop Pediatr. 2007;53(6):388-92.

[237] UK National Screening Committee. Definition of screening. First Report of the National Screening Committee. 1998:12.

[238] Wilson JMG Jungner G. Principles and practice of sceening for disease. Geneva: World Health Organization. 1968.

[239] Bernloehr A, Smith P, Vydelingum V. Antenatal care in the European Union: a survey on guidelines in all 25 member states of the Community. Eur J Obstet Gynecol Reprod Biol. 2005;122(1):22-32.

[240] U.S. Preventive Services Task Force. Screening for Chlamydial infection: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2007;147(2):128-34.

[241] Mills N, Daker-White G, Graham A, Campbell R. Population screening for *Chlamydia trachomatis* infection in the UK: a qualitative study of the experiences of those screened. Fam Pract. 2006;23(5):550-7.

[242] Pimenta JM, Catchpole M, Rogers PA, Perkins E, Jackson N, Carlisle C, et al. Opportunistic screening for genital chlamydial infection. I: acceptability of urine testing in primary and secondary healthcare settings. Sex Transm Infect. 2003;79(1):16-21.

[243] Oakeshott P, Hay P, Hay S, Steinke F, Rink E, Thomas B, et al. Detection of *Chlamydia trachomatis* infection in early pregnancy using self-administered vaginal swabs and first pass urines: a cross-sectional community-based survey. Br J Gen Pract. 2002;52(483):830-2.

[244] Logan S, Browne J, McKenzie H, Templeton A, Bhattacharya S. Evaluation of endocervical, first-void urine and self-administered vulval swabs for the detection of *Chlamydia trachomatis* in a miscarriage population. BJOG. 2005;112(1):103-6.

[245] Thomas BJ, Pierpoint T, Taylor-Robinson D, Renton AM. Quantification of *Chlamydia trachomatis* in cervical and urine specimens from women attending a genitourinary medicine clinic: implications for screening strategies. Int J STD AIDS. 1998;9(8):448-51.

[246] van Doornum GJ, Schouls LM, Pijl A, Cairo I, Buimer M, Bruisten S. Comparison between the LCx Probe system and the COBAS AMPLICOR system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in patients attending a clinic for treatment of sexually transmitted diseases in Amsterdam, The Netherlands. J Clin Microbiol. 2001;39(3):829-35.

[247] Kacena KA, Quinn SB, Howell MR, Madico GE, Quinn TC, Gaydos CA. Pooling urine samples for ligase chain reaction screening for genital *Chlamydia trachomatis* infection in asymptomatic women. J Clin Microbiol. 1998;36(2):481-5.

[248] Peeling RW, Toye B, Jessamine P, Gemmill I. Pooling of urine specimens for PCR testing: a cost saving strategy for *Chlamydia trachomatis* control programmes. Sex Transm Infect. 1998;74(1):66-70.

[249] Brocklehurst P, Rooney G. Interventions for treating genital *Chlamydia trachomatis* infection in pregnancy. Cochrane Database Syst Rev. 2000(2):CD000054.

[250] Health Council of the Netherlands. Screening for Chlamydia, The Hague, nr 2004/07. 2004:1-106.

[251] Postma MJ, Welte R, van den Hoek JA, van Doornum GJ, Jager HC, Coutinho RA. Cost-effectiveness of partner pharmacotherapy in screening women for asymptomatic infection with *Chlamydia trachomatis*. Value Health. 2001;4(3):266-75.

[252] Postma MJ, Welte R, van den Hoek JA, Morre SA. Comparing cost effectiveness of screening women for *Chlamydia trachomatis* in systematic and opportunistic approaches. Sex Transm Infect. 2002;78(1):73-4.

[253] Postma MJ, Welte R, Morre SA. Cost-effectiveness of widespread screening for *Chlamydia trachomatis*. Expert Opin Pharmacother. 2002;3(10):1443-50.

[254] Welte R, Kretzschmar M, Leidl R, van den Hoek A, Jager JC, Postma MJ. Cost-effectiveness of screening programs for *Chlamydia trachomatis*: a population-based dynamic approach. Sex Transm Dis. 2000;27(9):518-29.

[255] Honey E, Augood C, Templeton A, Russell I, Paavonen J, Mardh PA, et al. Cost effectiveness of screening for *Chlamydia trachomatis*: a review of published studies. Sex Transm Infect. 2002;78(6):406-12.

[256] Henry-Suchet J, Sluzhinska A, Serfaty D. *Chlamydia trachomatis* screening in family planning centers: a review

of cost/benefit evaluations in different countries. Eur J Contracept Reprod Health Care. 1996;1(4):301-9.

[257] Howell MR, Quinn TC, Brathwaite W, Gaydos CA. Screening women for *Chlamydia trachomatis* in family planning clinics: the cost-effectiveness of DNA amplification assays. Sex Transm Dis. 1998;25(2):108-17.

[258] Paavonen J, Puolakkainen M, Paukku M, Sintonen H. Cost-benefit analysis of first-void urine *Chlamydia trachomatis* screening program. Obstet Gynecol. 1998;92(2):292-8.

[259] Postma MJ, Bakker A, Welte R, van Bergen JE, van den Hoek JA, de Jong-van den Berg LT, et al. Screening for asymptomatic *Chlamydia trachomatis* infection in pregnancy; cost-effectiveness favorable at a minimum prevalence rate of 3% or more. Ned Tijdschr Geneeskd. 2000;144(49):2350-4.

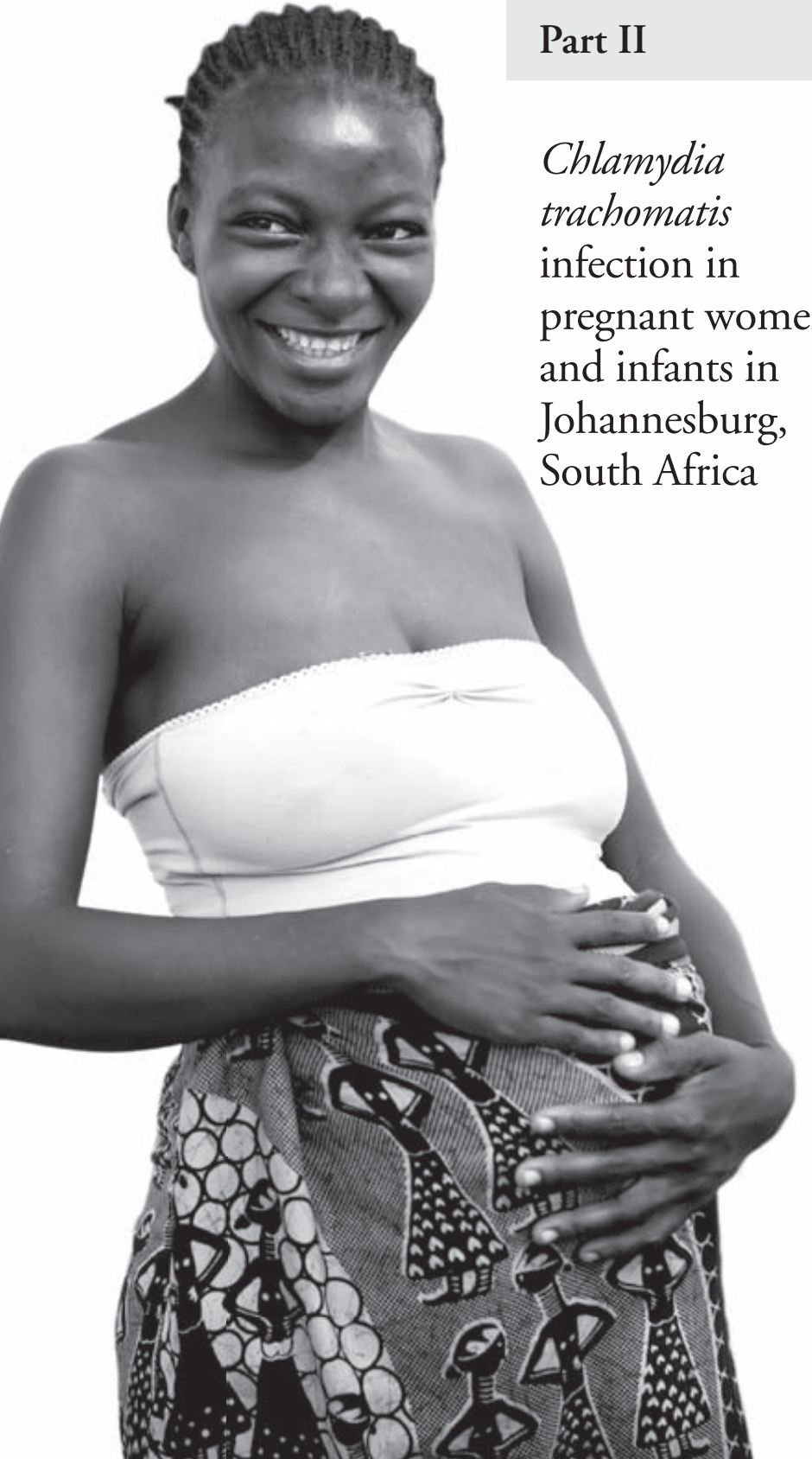
[260] Genc M, Mardh A. A cost-effectiveness analysis of screening and treatment for *Chlamydia trachomatis* infection in asymptomatic women. Ann Intern Med. 1996;124(1 Pt 1):1-7.

[261] Niccolai LM, Livingston KA, Teng FF, Pettigrew MM. Behavioral intentions in sexual partnerships following a diagnosis of *Chlamydia trachomatis*. Prev Med. 2008;46(2):170-6.

[262] Kangas I, Andersen B, Olesen F, Moller JK, Ostergaard L. Psychosocial impact of *Chlamydia trachomatis* testing in general practice. Br J Gen Pract. 2006 Aug;56(529):587-93.

[263] Piercy H. The importance of contextualisation in giving a diagnosis of genital chlamydial infection: findings from a qualitative study. J Fam Plann Reprod Health Care. 2006;32(4):227-30.

[264] Piercy H. “It feels good to be told that I’m all clear”: patients’ accounts of retesting following genital chlamydial infection. Sex Transm Infect. 2006;82(4):330-3.



Part II

Chlamydia trachomatis infection in pregnant women and infants in Johannesburg, South Africa

Chapter 3

Sexually transmitted infections in pregnant urban South African women: socio-economic characteristics and risk factors

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Abstract

Objective The study was conducted to assess the prevalence of sexually transmitted infections in pregnant urban South African women and to determine associations with demographic and socio-economic characteristics, and clinical symptoms.

Methods Pregnant urban South African women presenting for delivery to the Johannesburg Hospital were eligible for the study. Data concerning maternal health, socio-economic circumstances and life style risk factors were obtained through questionnaires. Results of serological testing for HIV and syphilis and delivery characteristics were obtained from obstetric or antenatal clinic records. At inclusion a urine specimen was obtained and blood was taken for serology.

Results Overall, 48% of 766 women carried one or more sexually transmitted infection during their pregnancy. Infection with HIV, *Treponema pallidum, Chlamydia trachomatis* and *Neisseria gonorrhoeae* was detected in 18%, 23%, 12% and 9% of women, respectively. Rates of detection of HIV, active syphilis and chlamydial infection, were found to be age-dependent. Predictive factors for infection included lack of antenatal care, multiple pregnancies, being unmarried, unemployed status and lack of a regular monthly income.

Conclusions The prevalence of sexually transmitted infections was extremely high among these pregnant urban South African women. Not elicited symptoms, but differences in demographic and socio-economic characteristics were associated with infection.

Introduction

Worldwide, sexually transmitted infections (STIs) remain a significant public health problem with a disproportionate burden of the complications of infection affecting women of reproductive age and their infants. STIs do not only cause acute and chronic illness in these women, but may also jeopardise procreation by inducing infertility and pregnancy loss, while significantly contributing to perinatal morbidity and mortality [1, 2]. In addition, conventional STIs have been shown to increase both the risk of acquisition of HIV and increase the rates of shedding of HIV in persons already dually infected. In turn, HIV infection may alter the presentation and clinical course of conventional STIs [3-5]. The World Health Organization has estimated an incidence of 340 million new cases of curable STDs among adults in 1999 [1]. In South Africa, STIs are endemic but have increased in number with the emergence of the HIV/AIDS epidemic and have a rate of approximately 5,000-15,000 cases per 100,000 [6, 7]. In this study, we have determined the prevalence of selected STIs in a group of pregnant urban South African women presenting for delivery. A special focus was made on HIV, syphilis, chlamydial and gonococcal infections since these can be transmitted vertically from mother to child. We have also attempted to identify demographic factors that indicate increased risk of maternal infection.

Methods

Patients

The study was undertaken in the Department of Obstetrics and Gynaecology at the Johannesburg Hospital, South Africa, which is the major academic hospital that provides primary and secondary care for the population of Johannesburg as well as tertiary care for the whole of the Gauteng province. The hospital serves a population covering all socio-economic classes and races, but most obstetric patients are of lower socio-economic status. At the time of the study black women constituted 96% of all deliveries. In order to avoid confounding factors and because the number of women in other groups (white, coloured, Indian) were too small, the study focused on black women. Some pregnant women seek antenatal care while others attend clinics at the hospital, or are referred as a result of complications. Women were not approached for inclusion in the study if they required emergency caesarean section after presentation to the obstetric ward, had an (incomplete) abortion, or were fully dilated upon arrival. Overall, 935 consecutive black pregnant women delivering at the hospital between October 1996 and January 1997 were considered eligible for inclusion in the study. Most women spoke English; otherwise the study was explained in their home language. Women were excluded if complete microbiological and serological STI results were not available either as a result of refusal of testing or failure to trace results, if urine could not be obtained before delivery, or because specimen bottles

were empty on arrival in the laboratory. Eventually, the data generated in 766 cases were analyzed.

Data collection

In each case, a female doctor or nurse administered a questionnaire. Data were collected concerning maternal health and socio-economic circumstances, including maternal age, parity and gravidity, antenatal clinic attendance, underlying disease and substance abuse, as well as marital status (traditional western or African style), number of sexual partners, employment and residential area. Socio-economic information such as medical insurance status and a regular monthly income of the women's household was obtained from a computerized outpatient record system, which was also used to verify employment status and location of residence. Results of serological testing for HIV and syphilis were obtained from obstetric or antenatal clinic records, if available, or as a result of testing on admission to the study. Subsequently, following delivery, information about the mode of delivery, gestational age, birth weight and gender of the newborn were obtained from obstetric and neonatal records.

Laboratory methods

If testing had not been performed prior to entry into the study, women were asked to provide a venous blood sample to test for syphilis and HIV (following pre-test and post-test counselling guidelines). Maternal HIV status was determined using two different HIV ELISA tests: the third generation HIV1/2 test (Abbott Laboratories, North Chicago, USA) and the Access HIV1/2 test (Sanofi Diagnostics Pasteur S.A., Marnes la Coquette, France). Screening for syphilis was undertaken using the rapid plasma reagin (RPR) test (Immutrep, Omega Diagnostics, Alloa, Scotland) and the *Treponema pallidum* haemagglutination assay (TPHA). Positive reactions were confirmed by a fluorescent treponemal antibody test (FTA-ABS). For the purpose of this study, active infections were defined as follows: (1) a positive TPHA with a positive RPR titre greater or equal to 1:4 with or without a positive FTA-ABS or (2) a positive TPHA with a positive RPR titre equal to 1:2 with a positive FTA-ABS IgM. Fresh, first-void urine specimens were tested for chlamydial and gonococcal infection using the Ligase Chain Reaction (LCx, Abbott Laboratories, Abbott Park, IL, USA).

Statistical methods

Percentages and continuous variables were compared between groups using the Mann-Whitney test or Chi-square/Fisher's exact test. Multivariate analyses for putative risk factors for the presence of any STI were performed using logistic regression. After adjusting for age, the association between various STIs was investigated using the Mantel-Haenszel procedure. P=0.05 (two-sided) was considered the limit of significance.

Consent

The study was approved by the Committee for Research on Human Subjects of the Witwatersrand University, Johannesburg, South Africa and written informed consent was obtained from all participants.

Results

A total of 766 pregnant women were screened for all four STIs (HIV, syphilis, chlamydial and gonococcal infection). Overall, 366 (48%) women were found to be infected with at least one STI at the time of sampling; 35% had one STI and 13% had mixed infections (11% with two, 1% with three and a single woman with all four). Patient characteristics for the total group as well as differences in characteristics between women with and without an STI are shown in table 1. Although the median age of the study subjects was 26 years, the youngest was 13 and only a third of women were primigravida. The majority was unmarried (70%) and had received antenatal care (93%) during the current pregnancy. A minority reported underlying diseases such as diabetes, hypertension, or renal disease. Substance abuse was rare. Almost one-third stated they were employed, but several unemployed participants reported a regular monthly income for their household. Only four women had private medical insurance. After inclusion into the study, 20% of women delivered by caesarean section.

Table 1 Characteristics of the total population, and women with and without an STI

Characteristics women	Total n=766	No STI n=400	Any STI n=366	P-value
age	26 (13-44)	26 (15-44)	26 (13-44)	0.60
parity	1.2 (1.3)	1.1 (1.3)	1.3 (1.2)	0.004
pregnancy	2.4 (1.3)	2.3 (1.3)	2.5 (1.3)	0.001
antenatal care	709 (93)	378 (95)	331 (90)	0.04
vaginal delivery	621 (81)	318 (80)	303 (83)	0.30
husband	229 (30)	136 (34)	93 (25)	0.01
employment	251 (33)	143 (36)	108 (30)	0.08
regular income	295 (39)	167 (42)	128 (35)	0.05
underlying disease	81 (11)	42 (11)	39 (11)	1.00
alcohol use	14 (2)	6 (2)	8 (2)	0.60
tobacco use	9 (1)	5 (1)	4 (1)	1.00

STI: sexually transmitted infection, n: number

Age given in median (range); parity, gravidity in means (SD); others in number of patients (%)

Between women with and without an STI no difference was found in age distribution, rates of caesarean section, presence of an underlying disease or substance abuse. However, univariate analyses showed a significant difference between the two groups in respect of parity (P=0.004), gravidity (P=0.001), rates of attendance for antenatal care (P=0.04) as well as marital status (P=0.01) and the availability of a regular monthly income (P=0.05). Differences in employment status also tended towards significance (P=0.08). Women with an STI did not report more genitourinary symptoms during the three-month period prior to delivery than those without an STI. In addition, pregnancy outcome reflected by gestational age (P=0.70) and birth weight (P=0.20) were similar for both groups as were rates of neonatal admission. Neonatal death was recorded twice as often among newborns born to women with an STI: 14 (4%), compared to 8 (2%) of those born to uninfected women. However, this difference was not found to be significant (P=0.33).

Associations between demographic characteristics and the presence of any STI when employing multivariate analyses were: history of two or more pregnancies (with no difference between women with two, three or more pregnancies), being single, and unemployed (Table 2). The lack of antenatal clinic attendance (univariate analysis P=0.04) tended towards significance (P=0.07). Grouping the women according to the number of associated factors present (two or more pregnancies, single marital status, no employment and lack of antenatal care), it was noted that the STI prevalence in women with no factor present was 13%, while the prevalence rate was 75% in those with four factors present (Figure 1).

Table 2 Multivariate analysis of risk factors for an STI in pregnant women

Risk factor	Odds ratio* (95% CI)	P-value
age		
• group 2: 20-24 years	0.8 (0.4-1.5)	0.44
• group 3: 25-29 years	0.8 (0.4-1.5)	0.42
• group 4: ≥ 30 years	0.7 (0.4-1.5)	0.38
no antenatal care	1.7 (1.0-3.0)	0.07
partner ≥ 1 boyfriend	1.8 (1.3-2.5)	<0.001
no employment	1.4 (1.0-1.9)	0.05
pregnancy ≥ 2	2.2 (1.5-3.3)	<0.001

STI: sexually transmitted infection

* Reference categories are age group 1 < 20 years, antenatal care, husband, employment and first pregnancy respectively

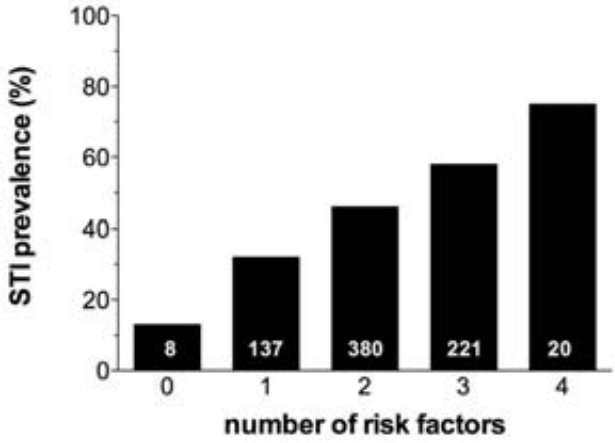

Figure 1 Prevalence of an STI according to number of risk factors (multiple pregnancies, single, lack of employment and lack of antenatal care)
Numbers of patients per category shown in bars

Of the 366 women with an STI, 141 tested positive for HIV (18%), 176 for active syphilis (23%), and 92 and 65 for *Chlamydia trachomatis* (12%) and *Neisseria gonorrhoeae* (9%), respectively. None of the women reported specific treatment for chlamydial infection in the 3 months prior to delivery. However, among the women who tested negative for *C. trachomatis*, 32/674 (5%) reported receiving antibiotics for reasons other than chlamydial infection but which may have affected chlamydial status, compared to 1/92 (1%) of the chlamydia-positive women who received such antibiotics (P=0.17).

On evaluation of the STIs separately, rates of detection of HIV, active syphilis and chlamydial infection, were found to be age-dependent (Figure 2). Rates of HIV seropositivity were significantly higher among women under 30 years of age (21%) than among women 30 years and older (12%) (P=0.04). For syphilis, a significant trend was noted with the lowest prevalence among the youngest women (14%) and a higher rate in older women (30%) (P=0.01). In contrast, an equally significant trend, but in the opposite direction, was observed for chlamydial infection with a highest prevalence (22%) in younger women and a lower rate of infection (5%) being detected among women in the older group (P<0.001). The prevalence for gonococcal infection was found to be highest in women less than 20 years (13%), but was not age-dependent (P=0.61).
Rates of reported genitourinary symptoms during the three-month period prior to delivery were unrelated to the STIs demonstrated. Gestational age and birth weight as well as neonatal admission and neonatal death did not differ between STI groups. However, neonates born to women with

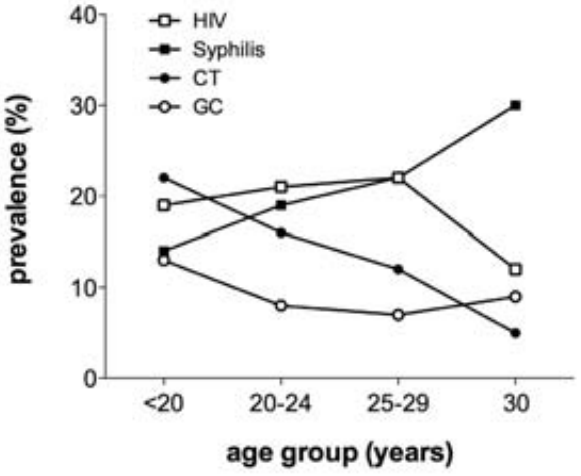

Figure 2 Prevalence of HIV, syphilis, chlamydia and gonorrhoea by age group

serological evidence of active syphilis that were untreated, were born at a significantly lower gestational age (P=0.001) than those born to women treated for the disease. There was also a trend towards lower birth weight (P=0.08). Rates of neonatal admission and death were three times higher in the untreated syphilis group than in the treated group (19% versus 6%), but this difference was not found to be significant owing to few patients in the untreated group. And although 9% of newborns born to women positive for gonorrhoea alone died in this study, no statistical association between maternal gonococcal infection and neonatal death could be demonstrated. Overall, 132 different residential areas were reported, but no significant association of maternal carriage of individual STIs with residential area could be demonstrated.

With increasing age of the women, a significant trend was observed for an increase in antenatal clinic attendance (P=0.04), as was an association with multiple pregnancies (P<0.001), being employed (P<0.001), the availability of a regular monthly income (P<0.001), and a decrease in single marital status (P<0.001). Women who did not receive antenatal care were more likely to be seropositive for HIV (P=0.004) and positive for gonococcal infection than their counterparts who had attended antenatal clinics (P=0.05). In all age groups a significant association could be shown between HIV seropositivity and active syphilis (P<0.001, age-adjusted OR=2.3), as well as between gonococcal and chlamydial infection in women less 20 years of age (P=0.01).

Discussion

The prevalence of sexually transmitted infections (nearly 50% positive for at least one STI) was extremely high among the group of pregnant urban black South African women who presented for delivery in this study. Other South African reports have also shown high STI rates in pregnant women with varying rates recorded for individual STIs [8, 9]. HIV, syphilis, chlamydial and gonococcal rates detected in this study were 18%, 23%, 12% and 9%, respectively. The highest HIV prevalence was shown in young women in their late teens (19%) and early twenties (22%) with a significant decline after age 30. A similar age distribution has been shown elsewhere and can be explained in part by the natural course of HIV infection [10, 11]. The presented high rate of syphilis infections has been observed by others in Africa while previous South African studies demonstrated rates ranging from 7% to 20% among antenatal clinic attendees [8, 12, 13], up to 31% among unbooked pregnant women [14]. The prevalence of active syphilis recorded here may still prove to be an underestimation because complicated deliveries taken directly to the operating theatre were not included in our study as were women with foetal loss early in pregnancy, and the risk of perinatal mortality among neonates born to women with syphilis is known to be at least twice that of those without [15-17]. Chlamydial infection rates also vary widely in South Africa [8, 9, 12, 18, 19]. In our study chlamydial infection was the most prevalent STI in women less than 20 years (22%) and thereafter decreased significantly with age. This is not surprising since increased sexual activity with multiple partners and unprotected sex is known to occur more in younger age groups. Gonorrhoea was the least prevalent STI, but the rate detected was consistent with those previously recorded in South Africa [8, 12, 18, 20]. Gonorrhoea was also most prevalent in women less than 20 years of age (13%), but no significant differences were recorded between age groups.

The WHO recommends a syndromic approach to the management of STIs in developing countries [21]. However, in this study the typical symptoms for an STI appeared to be insufficiently sensitive and specific to be used to estimate the risk for an STI. This is not surprising since 60-70% of chlamydial and gonococcal infections in women are known to be asymptomatic and detection and subsequent treatment of latent syphilis is routinely achieved by comprehensive antenatal screening [22, 23]. Additionally, urogenital symptoms can be so non-specific that they may not be recognized as part of a disease process but regarded as part of a pregnancy and consequently not reported, or they may be due to other infections for which no diagnostic test was performed. Similar studies have also indicated a poor correlation between reported symptoms and STIs during pregnancy [8, 24, 25].

However, univariate and multivariate regression analyses showed significant demographic and socio-economic risk factors (two or more pregnancies, single marital status, unemployed status, lack of regular income and antenatal care) associated with STIs. These data can be used to predict

risk for an STI among women late in pregnancy since we showed retrospectively that the presence of an STI increased from 13% in women with no risk factors to 75% in those with four risk factors. Figure 2 indicates that young women under 25 years of age are mainly at risk for STIs, which is not surprising since single, poor women are more likely to have numerous sexual partners and therefore to be at increased risk for STIs [26].

STIs have generally been accepted as major factors for HIV transmission and this study confirms a significant association between active syphilis in women of all ages with positive HIV status, but not with either chlamydial or gonococcal infection [3, 27]. Co-infection of chlamydia with gonorrhoea was frequently recorded in women less than 20 years of age.

Nearly half the women studied carried an STI, of which 18% tested positive for HIV. Therefore the majority of STIs could be managed appropriately had proper diagnostic testing been available at the antenatal clinic. Antenatal clinic attendance (at least one attendance in our study) was much higher than WHO estimates [27, 28], but women under 20 years (84%) were less likely to seek antenatal care than older women (92%-95%). Although a difference between early and late booking was not recorded, a significant difference in the presence of STIs between women with and without antenatal care could still be observed; especially for HIV infection and gonorrhoea. Since 1994, free health care for pregnant women has been available in South Africa and it has clearly been shown that health benefits can be obtained by educating young women about the importance of antenatal care, the risks of unprotected sex, and by encouraging early booking for delivery [29].

Serological screening for syphilis among antenatal clinic attendees as recommended by the WHO and Centers for Disease Control and Prevention is offered freely to all women in South Africa [30]. Early detection of syphilis using the RPR test is inexpensive, simple to perform and facilitates immediate and appropriate treatment to significantly prevent adverse pregnancy outcome, postnatal morbidity and mortality [31]. Our study confirmed other reports indicating that a positive RPR and TPHA as such are not associated with increased risk for prematurity or low birth weight except if these women are left untreated [19]. Routine serological testing of pregnant women for syphilis at antenatal clinics may act as an appropriate entry point for initiation of on-site rapid HIV testing, which could subsequently lead to increased awareness of serostatus and optimising opportunities for STI/HIV prevention as well as reducing vertical transmission of HIV by providing anti-retrovirals during labour and postpartum as well as choices with regard to infant feeding [27, 32, 33]. At the time of the study, HIV treatment was in most South African clinics not available and no medication was given to prevent transmission of infection to newborns. This situation has changed considerably, but the opportunity of linking serological testing for HIV and routine syphilis screening has not been fully exploited. At

present, HIV testing is readily available throughout South Africa as is anti-retroviral treatment. Positive women should be treated as soon as possible with active follow-up of the women, their newborns and partners.
Asymptomatic chlamydial and gonococcal infections may also result in neonatal infection, complicated pregnancy outcome, post-partum pelvic inflammatory disease and transmission to sexual partners [19, 34, 35] In our study, a syndromic approach, based on elicited symptoms, failed to discriminate between infected and uninfected women, and routine screening by culture or nucleic acid amplified tests would prove too expensive and time consuming while currently available rapid tests lack sensitivity [36]. However, without testing for chlamydia and gonorrhoea, 21% of infections would have remained undiagnosed and therefore would not have been treated.

Overall, this study confirms the high rates of infection with HIV, *T. pallidum, C. trachomatis* and *N. gonorrhoeae* among pregnant women in this urban South African community. Furthermore, we have shown that a risk assessment approach based on socio-demographic factors could prove more effective than provision of mass treatment or dependence on syndromic management principles in women who are largely asymptomatic. This population would therefore benefit from basic approaches to decrease the burden of STIs such as provision of information on STIs including the recognition of subtle symptoms associated with chlamydial and gonococcal infections, the importance of safe sex and antenatal care, and the offer of HIV testing in addition to routine serological screening for syphilis. Since treatment of syphilis and chlamydial and gonococcal infections is one of the most cost-effective health interventions available in developing countries in terms of cost per healthy life-year saved, additional screening for chlamydial and gonococcal infection would be desirable at least for women at highest risk. However, if screening were not feasible, combined treatment with a single dose of azithromycin and ceftriaxone for high-risk women and treatment of their partner may be a safe and cost-effective strategy to reduce the burden of both chlamydial and gonococcal infections.

References

[1] An overview of selected curable sexually transmitted diseases. Geneva: World Health Organization, 1995

[2] Marais NF, Wessels PH, Smith MS, Gericke A and Richter A. *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* infections in women. Prevalence, risks and management at a South African infertility clinic. J Reprod Med 1991;36:161-4

[3] O'Farrell N, Windsor I and Becker P. HIV-1 infection among heterosexual attenders at a sexually transmitted diseases clinic in Durban. S Afr Med J 1991;80:17-20

[4] Wasserheit JN. Epidemiological synergy. Interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases. Sex Transm Dis 1992;19:61-77

[5] Plummer FA. Heterosexual transmission of human immunodeficiency virus type 1 (HIV): interactions of conventional sexually transmitted diseases, hormonal contraception and HIV-1. AIDS Res Hum Retroviruses 1998;14 Suppl 1:S5-10

[6] Puren AJ. The HIV-1 epidemic in South Africa. Oral Dis 2002;8 Suppl 2:27-31

[7] Pham-Kanter GB, Steinberg MH and Ballard RC. Sexually transmitted diseases in South Africa. Genitourin Med 1996;72:160-71

[8] Sturm AW, Wilkinson D, Ndovela N, Bowen S and Connolly C. Pregnant women as a reservoir of undetected sexually transmitted diseases in rural South Africa: implications for disease control. Am J Public Health 1998;88:1243-5

[9] Wilkinson D, Abdool Karim SS, Harrison A, et al. Unrecognized sexually transmitted infections in rural South African women: a hidden epidemic. Bull World Health Organ 1999;77:22-8

[10] Rollins NC, Dedicoat M, Danaviah S, et al. Prevalence, incidence, and mother-to-child transmission of HIV-1 in rural South Africa. Lancet 2002;360:389

[11] Wilkinson D, Abdool Karim SS, Williams B and Gouws E. High HIV incidence and prevalence among young women in rural South Africa: developing a cohort for intervention trials. J Acquir Immune Defic Syndr 2000;23:405-9

[12] Dietrich M, Hoosen AA, Moodley J and Moodley S. Urogenital tract infections in pregnancy at King Edward VIII Hospital, Durban, South Africa. Genitourin Med 1992;68:39-41

[13] Delport SD, Ballard RC, Cameron NA and Rothberg AD. Prevention of congenital syphilis by effective maternal screening at antenatal clinics. S Afr Med J 1993;83:710-1

[14] Mlisana KP, Monokoane S, Hoosen AA, Moodley J, Adhikari M and Taylor L. Syphilis in the 'unbooked' pregnant woman. S Afr Med J 1992;82:18-20

[15] Folgosa E, Osman NB, Gonzalez C, Hagerstrand I, Bergstrom S and Ljungh A. Syphilis seroprevalence among pregnant women and its role as a risk factor for stillbirth in Maputo, Mozambique. Genitourin Med 1996;72:339-42

[16] Bam RH, Cronje HS, Muir A, Griessel DJ and Hoek BB. Syphilis in pregnant patients and their offspring. Int J Gynaecol Obstet 1994;44:113-8

[17] Wilkinson D, Sach M and Connolly C. Epidemiology of syphilis in pregnancy in rural South Africa: opportunities for control. Trop Med Int Health 1997;2:57-62

[18] O'Farrell N, Hoosen AA, Kharsany AB and van den Ende J. Sexually transmitted pathogens in pregnant women in a rural South African community. Genitourin Med 1989;65:276-80

[19] Donders GG, Desmyter J, De Wet DH and Van Assche FA. The association of gonorrhoea and syphilis with premature birth and low birthweight. Genitourin Med 1993;69:98-101

[20] Welgemoed NC, Mahaffey A and Van den Ende J. Prevalence of *Neisseria gonorrhoeae* infection in patients attending an antenatal clinic. S Afr Med J 1986;69:32-4

[21] Management of patients with sexually transmitted diseases. Geneva: World Health Organization, 1991

[22] Schrijver D. Epidemiology of sexually transmitted diseases: the global picture. Geneva: World Health Organization, 1990:639-654

[23] Mabey D. Sexually transmitted diseases in developing countries. Trans R Soc Trop Med Hyg 1996;90:97-9

[24] Vuylsteke B, Laga M, Alary M, et al. Clinical algorithms for the screening of women for gonococcal and chlamydial

infection: evaluation of pregnant women and prostitutes in Zaire. Clin Infect Dis 1993;17:82-8

[25] Wilkinson D, Ndovela N, Harrison A, Lurie M, Connolly C and Sturm AW. Family planning services in developing countries: an opportunity to treat asymptomatic and unrecognised genital tract infections? Genitourin Med 1997;73:558-60

[26] Hunter. The materiality of Everyday Sex: thinking beyond 'prostitution'. African studies 2002;61:100-119

[27] Matambo JA, Moodley D and Moodley J. HIV seroprevalence and rapid testing in unbooked pregnant African women. Int J Gynaecol Obstet 1999;66:289-90

[28] Coverage of maternity care. Maternal and newborn health/safe motherhood. Geneva: World Health Organization, 1997

[29] Opai-Tetteh ET, Hoosen AA and Moodley J. Re-screening for syphilis at the time of delivery in areas of high prevalence. S Afr Med J 1993;83:725-6

[30] 1989 sexually transmitted diseases treatment guidelines: extracted from the Centers for Disease Control guidelines. Pediatr Infect Dis J 1990;9:379-82; discussion 382-4

[31] Hira SK, Bhat GJ, Chikamata DM, et al. Syphilis intervention in pregnancy: Zambian demonstration project. Genitourin Med 1990;66:159-64

[32] Kassler WJ, Dillon BA, Haley C, Jones WK and Goldman A. On-site, rapid HIV testing with same-day results and counseling. AIDS 1997;11:1045-51

[33] Soderlund N, Zwi K, Kinghorn A and Gray G. Prevention of vertical transmission of HIV: analysis of cost effectiveness of options available in South Africa. BMJ 1999;318:1650-6

[34] Donders GG, Moerman P, De Wet GH, Hooft P and Goubau P. The association between Chlamydia cervicitis, chorioamnionitis and neonatal complications. Arch Gynecol Obstet 1991;249:79-85

[35] Ross SM, Windsor IM, Robins-Browne RM, Ballard RC, Adhikari M and Fenn DB. Microbiological studies during the perinatal period. An attempt to correlate selected bacterial and viral infections with intra-uterine deaths and preterm labour. S Afr Med J 1984;66:598-603

[36] Donders GG, van Gerven V, de Wet HG, van Straten AM and de Boer F. Rapid antigen tests for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are not accurate for screening women with disturbed vaginal lactobacillary flora. Scand J Infect Dis 1996;28:559-62

Chapter 4

Carriage of *Chlamydia trachomatis* during pregnancy: consequences for mother and infant

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Abstract

Objective The aim of this study was to determine the rate of *C. trachomatis* transmission from mother to infant in a setting where tetracycline eye prophylaxis is routinely provided, and to assess the postnatal consequences of chlamydial carriage during pregnancy for mother and child.
Methods Pregnant urban South African women presenting for delivery to the Johannesburg Hospital were eligible for the study. At inclusion a urine specimen was obtained to test for *C. trachomatis* and *N. gonorrhoeae*, and blood (-results) were obtained for HIV and syphilis. At follow-up six weeks post-delivery, maternal and neonatal health were assessed via standardized questionnaires, a full physical examination for infants and urogenital examination for women, and a venous blood sample, chest X-ray and conjunctival and nasopharyngeal swabs of infants.
Results A total of 77 chlamydia-positive women and their newborns were followed-up. The chlamydial transmission rate from mother to infant was found to be 30%. *C trachomatis* was detected in the conjunctivae of 39% and in the nasopharynx of 83% of these infants. Postnatal genitourinary symptoms were found in 52% and signs in 78% of chlamydia-positive mothers with 18% developing post-partum pelvic inflammatory disease.
Conclusions *C. trachomatis* infection was transmitted from mothers to infants despite the use of tetracycline eye prophylaxis. Eye prophylaxis appeared to prevent overt ocular, but not nasopharyngeal infection. Postnatal maternal genitourinary symptoms and signs, in combination with symptoms and signs in the infant, should alert clinicians to the possibility of neonatal and maternal complications of chlamydial infection.

Introduction

Chlamydial urogenital infection during pregnancy has been well documented as a cause of acute and chronic maternal illness including extra-uterine pregnancy, infertility and pregnancy loss, as well as perinatal morbidity and mortality [1-4]. Previous studies undertaken in South Africa have indicated maternal chlamydial infection rates varying between 4.7% and 13% among antenatal clinic attendees [5-10], and a vertical transmission rate of approximately 50% has been documented in a study in which no ocular prophylaxis for neonatal conjunctivitis was employed [11]. In this study we endeavoured to determine the rate of chlamydial transmission from mother to infant in a setting where tetracycline eye prophylaxis is routinely provided, using chlamydial culture and nucleic acid amplification techniques. In addition we explored the relationship between maternal chlamydial infection and post-partum maternal symptomatology.

Methods

Patients

Black women presenting for delivery at the Johannesburg Hospital, South Africa, between October 1996 and January 1997 were considered for inclusion in the present study. Methods of testing for genital tract pathogens at the time of delivery and rates of infection in relation to demographic risk factors and subsequent pregnancy outcome have been described elsewhere [12]. Women received cefoxitin as antimicrobial prophylaxis before caesarean sections. Immediately following delivery, newborns received a ribbon of 1% tetracycline eye ointment in each eye as routine prophylaxis against ophthalmia neonatorum. All women (and newborns) were given a follow-up appointment six weeks post-delivery together with verbal and written information about the study and a copy of the consent form.

At follow-up, the initial population was separated into chlamydia-positive (CTM+) and chlamydia-negative women (CTM-) on the basis of the urinary nucleic acid amplification test results at delivery. Matching of mothers and their infants was done according to the following parameters: maternal age (± 2 years), antenatal clinic attendance (ANC), HIV and syphilis serological status, mode of delivery and neonatal gender and birth weight (± 250 grams). Subsequently, the characteristics of infants of CTM+ were compared to infants of CTM-, as were the characteristics of chlamydia-positive infants (CTI+) of CTM+ compared to those of chlamydia-negative infants (CTI-) of CTM+.

CTM+, who did not return for scheduled follow-up visits, were traced by telephone at home, work or another private number, by letter in English, Zulu, and Sotho, or, if all else failed, by a home visit by the principal investigator. The same procedure was followed to trace CTM-matched controls, but this required no more than a telephonic approach since more controls

were suitable for each CTM+. As a result of the uncertainty regarding return for scheduled follow-up, it was necessary to record the data of CTM+ and infants at the time of follow-up, and subsequently search for a match either among CTM- and infant pairs, which had already returned for their appointment, or from those still to return. The latest follow-up to be accepted for inclusion was 12 weeks after delivery.

All CTI+ were treated with erythromycin 50 mg/kg/day orally divided into four doses for 14 days. Mothers were given instructions for the treatment of their infants. In addition, a prescription for erythromycin 500 mg orally four times a day for 7 days was provided for their own treatment, and they and their partners were referred to the sexually transmitted diseases clinic for follow-up.

Data collection

A female doctor or experienced nurse confidentially administered a questionnaire pre-delivery and at the six week follow-up. House staff and the principal investigator cared for all cases and controls and all treatment and diagnostic procedures (including those not related to *C. trachomatis* infection) were conducted at their discretion. Data concerning pre-delivery maternal health and socio-economic circumstances in relation to the presence of any sexually transmitted infection (STI) and isolated *C. trachomatis, N. gonorrhoeae*, HIV and syphilis have been reported elsewhere [12]. The maternal follow-up questionnaire included information on urogenital complaints, unscheduled visits to a doctor or clinic and the intercurrent use of antibiotics. The questionnaire regarding infant characteristics included information about eye, and respiratory problems, unscheduled visits to a doctor or clinic, use of antibiotic treatment and breastfeeding. In all cases, mothers underwent a urogenital examination and infants a full physical examination including weight measurement. From the infants, conjunctival and nasopharyngeal swabs were collected for chlamydial culture and ligase chain reaction (LCR), a venous blood sample to determine the presence of an eosinophilia and detection and quantitation of specific anti-chlamydial antibodies, and a chest X-ray.

Laboratory methods

Eye specimens for isolation and detection of *C. trachomatis* were obtained by stroking the everted lower palpebral conjunctiva with a sterile dacron swab. Specimens for culture were immersed in a 2SP medium, transported on ice and subsequently frozen at -70°C. Attempts to isolate *C. trachomatis* were made in monolayers of cycloheximide-treated McCoy cells [13]. Specimens for LCR testing were placed in a commercial transport medium, transported on ice, frozen at -70°C, and processed according to the manufacturer's instructions as for genital specimens (LCx, Abbott Laboratories, Abbott Park, Il, USA). Nasopharyngeal swabs for isolation and LCR were obtained by stroking the nasopharynx while rotating the swab 360°, placed in a transport medium, stored and processed for isolation of *C. trachomatis* by culture or LCR as

described above. LCR testing has been studied less frequent than PCR, but is used and has been proven to be more sensitive than cell culture in pharyngeal specimens [14-16]. A two ml blood sample was taken to perform a full blood count by standard methods from which absolute eosinophil values were calculated using a cut-off value of 300/mm³ [17], and to measure specific IgG antibodies to *C. trachomatis* using a cut-off value of 16. Type-specific antibody to *C. trachomatis* was detected and quantified by means of the modified micro-immunofluorescence method [18].

Radiological methods

In order to prevent bias, two radiologists who were blinded to maternal and infant chlamydial status evaluated infant chest X-rays [17]. Radiographic characteristics for clinical comparison were scored for hyperinflation, consolidation, broncho-alveolar markings, interstitial markings, lymphnodes, and a final conclusion was recorded as normal or abnormal.

Statistical methods

Percentages and continuous variables were compared between groups using the Mann-Whitney test or Chi-square/Fisher's exact test or McNemar test. P=0.05 (two-sided) was considered the limit of significance.

Ethical considerations

The study was approved by the Committee for Research on Human Subjects of the Witwatersrand University, Johannesburg, and written informed consent was obtained from all participants.

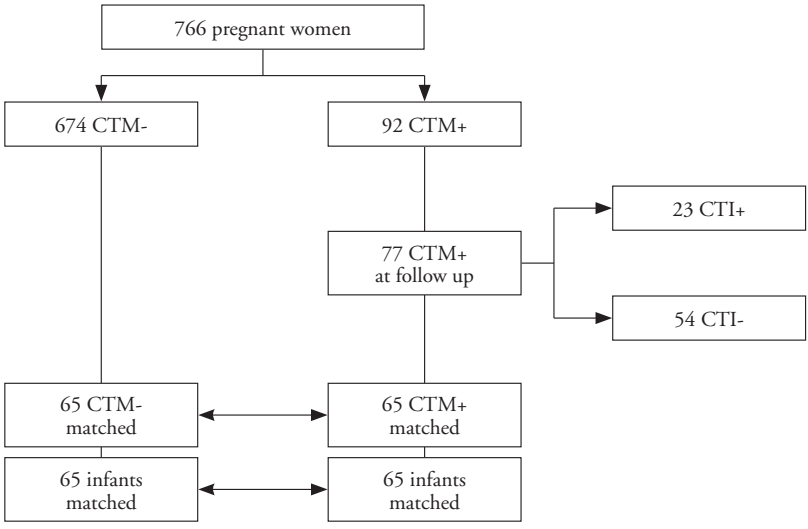

Figure 1 Study population of mothers and infants

CTM+ = chlamydia-positive mothers, CTM- = chlamydia-negative mothers

CTI+ = chlamydia-positive infants, CTI- = chlamydia-negative infant

Results

During the study period 766 pregnant women were screened for HIV, syphilis, *C. trachomatis* and *N. gonorrhoeae* and rates of infection detected were 18%, 23%, 12% and 9%, respectively. Chlamydia-positive mothers (CTM+) (with or without another STI) were found to be significantly younger (24.4 ± 4.8 years) than chlamydia-negative mothers (CTM-) (27.0 ± 5.7 years, P<0.001), had fewer previous pregnancies (2.0±1.0 versus 2.4±1.3, P=0.05), and tested less often positive for syphilis (14% versus 24%, P=0.03). No significant difference between the two groups was detected regarding HIV (17% versus 19%) or gonococcal status (12% versus 8%), ANC attendance (90% versus 93%), rate of caesarean section (12% versus 20%), and gestational age or birth weight of the newborns. No significant differences in rates of urinary, cervical or uterine symptoms were reported at the time of delivery.

Seventy-seven of the initial 92 CTM+ (84%) were available for follow-up (Figure 1). LCR and/or culture testing for *C. trachomatis* were positive in 23 of their infants reflecting an overall rate of concordance of 30% (Table 1). Nineteen infants of these infants (83%) had a positive nasopharyngeal and nine (39%) had a positive conjunctival test. Five infants (22%) were colonised in both sites. All conjunctival cultures were negative. Nasopharyngeal specimens were positive in 13 of 23 infants (57%) by LCR and in 9 of 20 infants (45%) by culture. Three nasopharyngeal cultures were contaminated with other bacteria and were therefore not evaluable. After matching CTM+ with CTM- and their infants, 65 mother-infant pairs were available for comparison owing either to failure of CTM+ or CTM- to return for follow-up or lack of matching

Table 1 Patterns of detection of *C. trachomatis* by culture and LCR in conjunctival and nasopharyngeal specimens obtained from infants at follow-up

Number of specimens	Nasopharyngeal culture	Nasopharyngeal LCR	Conjunctival LCR
1	+	+	+
2	+	+	_
2	+	_	+
2	+	_	_
2	_	+	+
2	_	+	_
2	_	_	+
2	NE*	+	+
2	NE*	+	_
2	NE*	-	+
Total 23	9/20 (45%)	13/23 (57%)	9/23 (39%)

*NE = not evaluable, + = positive, - = negative, eye cultures were negative

controls. When comparing the two groups with regards to matching criteria, the maternal age was 25±5 years, gestational age 37±1week, parity1±1, gravidity 2±1, ANC 94% and 98%, rate of vaginal deliveries 88%, and the HIV and syphilis seropositivity rates were 9% and 8% respectively. Matching for gonococcal status was not performed, but seven CTM+ (11%) and two CTM- (3%) were found to harbour *N. gonorrhoeae*. Urine was collected after rupture of the membranes from 18% and 23% of CTM+ and CTM-, respectively.

A comparison between CTM+ and CTM- for symptoms reported at the time of delivery for the three-month period prior to delivery is shown in table 2. One CTM+ had fever while one mother in each group reported urinary frequency. None received specific treatment for chlamydial infection, but one CTM+ and two CTM- received antibiotics for other diagnoses that could be effective against the organism.

At the follow-up visit (Table 2), CTM+ had significantly more urogenital symptoms (34 versus one; P<0.001) and signs (51 versus 13; P<0.001) than CTM-. Chlamydial infection was found to be strongly associated with symptoms of dysuria, vaginal discharge and lower abdominal pain. Post-coital bleeding was only reported in CTM+ and the association with chlamydial infection tended towards significance. Altogether, 16 CTM+ had one complaint, 12 had two, five had three and one woman had four complaints. One CTM+ reported joint pains. In both groups, eight women reported a previous diagnosis for which treatment had been provided during their pregnancy (urinary tract infection, vaginal discharge, and vaginal candidiasis). Examination

Table 2 Association of *Chlamydia trachomatis* infection with maternal symptoms and signs

	CTM+ N=65 (%)	CTM- N=65 (%)	P-value
Symptoms at delivery			
dysuria	0	8 (12)	0.008
vaginal discharge	12 (18)	13 (20)	1.00
post-coital bleeding	2 (3)	2 (3)	1.00
lower abdominal pain	8 (12)	7 (11)	1.00
Symptoms at follow-up			
dysuria	8 (12)	0	0.008
vaginal discharge	27 (42)	0	<0.001
post-coital bleeding	5 (8)	0	0.06
lower abdominal pain	19 (30)	1 (2)	<0.001
Signs at follow-up			
vaginal discharge	49 (75)	13 (20)	<0.001
bleeding	6 (9)	0	0.03
cervical excitation tenderness	12 (18)	1 (2)	0.003

CTM+ = chlamydia-positive mothers, CTM- = chlamydia-negative mothers

Table 3 Differences between infants born to chlamydia-positive and chlamydia-negative womens

	CTM+ N=65 (%)	CTM- N=65 (%)	P-value
History			
birth weight (grams ± SD)	3155 ± 476	3180 ± 454	0.06
eye problems	19 (29)	7 (11)	0.08
nasal problems	32 (49)	15 (23)	0.005
chest problems	19 (29)	10 (15)	0.08
exclusively breastfed	28 (43)	13 (20)	0.05
Examination			
FU weight (grams ± SD)	5600 ± 1005	6027 ± 1072	0.006
FU age (weeks ± SD)	10.2 ± 3.2	12.2 ± 2.7	0.001
weight gain (grams/week)	246 ± 72	238 ± 73	0.68
conjunctivitis	2 (3)	2 (3)	1.00
nasal obstruction/sneezing	37 (57)	7 (11)	<0.001
respiratory rate (mean ± SD)	56 ± 11	45 ± 5	<0.001
intercostal recession	21 (32)	4 (6)	<0.001
Laboratory results			
eye LCR	8/65 (12)	0	0.008
nasopharyngeal LCR	12/65 (18)	0	<0.001
eye culture	0	0	-
nasopharyngeal culture	7/58 (12)	0	0.02
MIF titre ≥ 16	19/65 (29)	3/65 (5)	0.001
eosinophil count ≥ 300/mm^3	32/65 (49)	30/65 (46)	0.90

CTM+ = chlamydia-positive mothers, CTM- = chlamydia-negative mothers, FU = follow-up

of the women also showed a significant association of chlamydial infection with signs of lower and upper genital tract disease: 36 CTM+ having one sign, 14 having two and one three signs compared to 12 CTM- with one sign and one with two signs.

At follow-up, the weight of infants born to CTM+ was significantly lower than that of infants to CTM- as was the age. Subsequent calculation of weight gain per week showed no significant difference between groups (Table 3). Comparison of infant groups showed no significant difference for intercurrent visits to a clinic or doctor, or intercurrent treatment. Infants of CTM+ had more frequent eye and respiratory problems, presented more frequently with nasal obstruction or sneezing than those born to CTM-, and were significantly more often exclusively breastfed. On examination, bronchial breathing or crepitations were not detected, and wheezing was documented in only one infant of a CTM+. Sneezing or nasal obstruction was documented much more frequently in infants of CTM+, as were higher respiratory rates and intercostal recession.

The latter signs, however, were not associated with significant differences in eosinophil counts or radiographic chest X-ray changes. Chest X-rays showed similar rates of hyperinflation and atelectases, and no hilar lymphadenopathy, consolidation or pleural effusion in both groups.
Although overall 23 infants were found to be chlamydia positive, in the nested case control analysis, 19 CTI+ born to 65 CTM+ (29%) were compared with matched infants of CTM-. Chlamydia was not detected in specimens from infants of CTM-. Among the 19 infected newborns, eight (42%) had a positive conjunctival LCR and 12 (63%) a positive nasopharyngeal LCR while seven of 16 evaluable nasopharyngeal cultures (43%) were positive. Evidence of infection on the basis of elevated specific chlamydial IgG titres was significantly different between infant groups: 19 infants of CTM+ (29%) compared to three infants of CTM- (5%) had elevated anti-chlamydia antibody titres (Table 3). However, only nine of these 19 infants were in the group of 19 CTI+ detected by LCR or culture.
Subsequently, the characteristics of CTM+ of 23 CTI+ were compared to CTM+ of 54 CTI-. No significant differences were found with respect to maternal age, parity, gravidity, ANC attendance, gestational age, rate of vaginal deliveries, marital status, regular monthly income or employment. In addition, rates of maternal symptoms and signs were similar both at delivery and at follow-up. More CTM+ of CTI+ tested positive for HIV (13% versus 9%), syphilis (13% versus 11%) and gonorrhoea (13% versus 11%) compared to CTM+ of CTI-, but these differences were not significant. No mother had received previous treatment for chlamydial infection, but one CTM+ of a CTI- received antibiotics that should treat chlamydial infection. The only significant difference was that urine was more often obtained after rupture of the membranes from seven mothers of 23 CTI+ (30%) compared to six mothers of 54 (11%) CTI- (P=0.05). All except one CTI+ were delivered vaginally. No difference between groups was reported for unscheduled visits to a clinic or for intercurrent treatment. More CTI+ were exclusively breastfed: 13/23 (57%) versus 18/54 (33%); (P=0.08). CTI+ had a lower birthweight and lower weight at follow-up, but these differences were not significant. The weight gain per week was also similar in both groups as were rates of reported symptoms and signs, eosinophil counts and chest X-ray changes. CTI+ had more atelectases, 3 of 22 (14%) versus 1 of 53 (2%) (P=0.07), and hyperinflation, 9 of 22 (41%) versus 16 of 53 (30%) (P=0.43), compared to CTI-. Hilar lymphadenopathy, consolidation or pleural effusion was not seen in either group. However, specific anti-chlamydial antibodies were significantly elevated in 12 of 23 (52%) CTI+ compared to 10 of 54 (19%) CTI- (P=0.005).

Discussion

The high rates of sexually transmitted infection detected during the course of this study are consistent with similar studies conducted in South Africa and elsewhere on the African continent

[5, 19, 20]. The correlation of maternal infection with demographic and other factors has been described elsewhere [12]. The follow-up rate (84%) of chlamydia-positive women (CTM+) and controls (CTM-) was higher than generally known from African follow-up clinics and offered the opportunity to compare groups of women and infants [21, 22]. In this paper we endeavoured to determine the risks and consequences of maternal chlamydial infection during pregnancy both to the mother following delivery and to her newborn. The overall transmission rate in our study (30%) was lower than reported by others [23-25], but similar to studies in which ocular prophylaxis was given at delivery to protect newborns against acquisition of neonatal chlamydial or gonococcal conjunctivitis [26-28].

It is remarkable that despite the use of tetracycline ocular prophylaxis, *C. trachomatis*-specific DNA was still detected in the conjunctivae of 39% of CTI+ at follow-up, but no cases of isolation-positive chlamydial conjunctival infection could be detected. While culture is able to detect only viable organisms, LCR is capable of detecting both viable and non-viable organisms. This implies that, in the absence of culture positive conjunctival samples, a positive LCR result in a newborn may be the result of contamination of the conjunctivae by *C. trachomatis* from the maternal cervico-vaginal fluid during delivery. We would assume, however, that a positive LCR result from a specimen obtained six to twelve weeks after birth indicates detection of infection in the infant rather than maternal contamination.

It is clear that the use of tetracycline eye prophylaxis decreased the bacterial load significantly and that overt chlamydial conjunctival infection can be prevented. However, it is also clear that complete eradication of chlamydial colonisation is not achieved [27, 29]. This underlines once again the need for systemic treatment to prevent late ocular infection or its recurrence.

The nasopharynx was the most frequent site where chlamydial infection was detected in infants. The finding that chlamydial cultures and LCR tests were both positive in a high proportion of infants actually indicates that direct transmission of infection to the nasopharynx occurred rather than contamination of the nasopharynx with chlamydial DNA via the conjunctivae. These findings also indicate that the use of prophylactic tetracycline eye ointment has no apparent influence on nasopharyngeal colonization and the possible development of chlamydial neonatal pneumonia [26]. Ocular prophylaxis may therefore only reduce the index of suspicion of chlamydial infection in the newborn since fewer infants will present with overt conjunctivitis as an indicator that other sites may be infected. Subsequently, without a history of neonatal conjunctivitis, infection at other sites may remain unnoticed and may cause sequelae later in life such as chronic lung disease and hyperreactive airways [3, 30]. Therefore systemic treatment should be used to treat chlamydial infection in infants rather than antibiotic prophylaxis [31, 32].

Minor symptoms in infants such as sneezing and nasal obstruction, and increased respiratory rates and intercostal recession were associated with antenatal chlamydial infection in their mothers. The significant difference in respiratory rates between groups may in part be due to the difference in age at follow-up. However, nasal obstruction and intercostal recession are not age-related and are therefore more likely to reflect evidence of chlamydial nasopharyngeal infection. The increased respiratory rates and recession in infants in this study may be more related to nasal obstruction as a result of relatively early manifestation of upper respiratory chlamydial infection rather than to established chlamydial pulmonary disease since chest X-ray findings did not support a diagnosis of pneumonia. Newborns are obligatory nasal breathers. Oral respiration is usually acquired by two months, but may take up to six months of age to develop fully [33]. Chlamydial infection, or another infection for which no test was performed in this study, may therefore be more likely to cause obstructive nasopharyngeal disease leading to increased respiratory rates or recession [34, 35]. The classical radiological changes described in cases of chlamydial neonatal pneumonia and the detection of an eosinophilia on haematological examination proved either non-specific or relatively insensitive in our hands and may again be due to the presentation of early respiratory disease [17, 36]. Chest X-rays are therefore not indicated as a screening tool and should only be used if there is a clinical indication of pneumonia. Likewise, detection of an eosinophilia appeared unhelpful at this stage. In contrast, significantly more elevated titres of anti-chlamydial antibody were found in infants born to CTM+ compared to infants born to CTM- as well as in CTI+ compared to CTI- both born to CTM+. Only single blood samples were obtained and therefore a significant rise in antibody titres could not be detected. These single elevated anti-chlamydial antibody titres may be associated with infection in the newborn, but may also reflect passive transfer of maternal antibody to the child. Single serological sampling is therefore not useful in establishing a diagnosis. However, sequential sampling for the detection of a rise in anti-chlamydial antibody titres remains useful.

We found that significantly more infants born to CTM+ were exclusively breastfed and that, although not statistically significant, more CTI+ were exclusively breastfed than CTI-. An association of chlamydial infection in the infant with breastfeeding has been recorded in a previous study [23], suggesting the possibility of postpartum transmission as a result of more intimate handling of the infant by the mother.

In this study significant differences between CTM+ and CTM- were detected in respect of post-partum urogenital symptom rates. On follow-up examination, chlamydial infection was associated with post-partum vaginal discharge, bleeding and cervical excitation tenderness [37-39]. Most symptoms and signs are probably due to chlamydial infection, but the findings may in part be confounded by gonococcal infection in some CTM+ and CTM-.

In conclusion, we have shown that chlamydial infection in pregnant women places the newborn at risk and that topical antibiotic prophylaxis at birth is insufficient to prevent chlamydial infection in the infant. Furthermore, a careful examination of both mothers and infants at follow-up and the finding of maternal symptoms or signs of genital tract disease should alert clinicians to the possibility of chlamydial infection in the infant. Likewise, the finding of conjunctivitis and/or respiratory tract infection in the infant should alert physicians to the possibility of asymptomatic maternal post-partum chlamydial infection of the genital tract, which requires prompt treatment with systemic antibiotics. The long-term implications of these inapparent infections for both infant and child health, and maternal fertility in developing societies remain areas in need of further study. Meanwhile consideration should be given to the possible implementation of antenatal screening for chlamydial infection in this high-risk population during the last month of pregnancy.

References

[1] Kramer DG, Brown ST. Sexually transmitted diseases and infertility. Int J Gynaecol Obstet 1984;22:19-27

[2] Gencay M, Koskiniemi M, Ammala P, et al. *Chlamydia trachomatis* seropositivity is associated both with stillbirth and preterm delivery. APMIS 2000;108:584-8

[3] Mardh PA. Influence of infection with *Chlamydia trachomatis* on pregnancy outcome, infant health and life-long sequelae in infected offspring. Best Pract Res Clin Obstet Gynaecol 2002;16:847-64

[4] Marais NF, Wessels PH, Smith MS, Gericke A and Richter A. *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* infections in women. Prevalence, risks and management at a South African infertility clinic. J Reprod Med 1991;36:161-4

[5] Sturm AW, Wilkinson D, Ndovela N, Bowen S and Connolly C. Pregnant women as a reservoir of undetected sexually transmitted diseases in rural South Africa: implications for disease control. Am J Public Health 1998;88:1243-5

[6] van Rensburg HJ, Odendaal HJ. The prevalence of potential pathogenic micro-organisms in the endocervix of pregnant women at Tygerberg Hospital. S Afr Med J 1992;81:156-7

[7] Dietrich M, Hoosen AA, Moodley J and Moodley S. Urogenital tract infections in pregnancy at King Edward VIII Hospital, Durban, South Africa. Genitourin Med 1992;68:39-41

[8] O'Farrell N, Hoosen AA, Kharsany AB and van den Ende J. Sexually transmitted pathogens in pregnant women in a rural South African community. Genitourin Med 1989;65:276-80

[9] Donders GG, Desmyter J, De Wet DH and Van Assche FA. The association of gonorrhoea and syphilis with premature birth and low birthweight. Genitourin Med 1993;69:98-101

[10] Wessels PH, Viljoen GJ, Marais NF, de Beer JA, Smith M and Gericke A. The prevalence, risks, and management of *Chlamydia trachomatis* infections in fertile and infertile patients from the high socioeconomic bracket of the South African population. Fertil Steril 1991;56:485-8

[11] De Souza JJL, Fehler HG, de Vos G, Ballard RC. The implications of fetal exposure to *Chlamydia trachomatis*.

In: 10th Conference on Priorities in Perinatal Care in South Africa. Johannesburg: Dept of Obstetrics and Paediatrics, Coronation Hospital and STD Research Unit, South African Institute for Medical Research and University of the Witwatersrand, 1991

[12] Rours GIJG, Verkooijen RP, Hop WCJ, Ye Htun, Radebe F, Rothberg AD, Cooper PA, de Groot R, Verbrugh HA, Ballard RC. Sexually Transmitted Infections in Pregnant Urban South African Women; Socio-Economic Characteristics and Risk Factors. The Southern African Journal of Epidemiology & Infection 2006;21(1):14-19

[13] Thewessen EA, Freundt I, van Rijsoort-Vos JH, Stolz E, Michel MF and Wagenvoort JH. Comparison of HeLa 229 and McCoy cell cultures for detection of *Chlamydia trachomatis* in clinical specimens. J Clin Microbiol 1989;27:1399-400

[14] Winter AJ, Gilleran G, Eastick K and Ross JD. Comparison of a ligase chain reaction-based assay and cell culture for detection of pharyngeal carriage of *Chlamydia trachomatis*. J Clin Microbiol 2000;38:3502-4

[15] Bird M, Dawson CR, Schachter JS, et al. Does the diagnosis of trachoma adequately identify ocular chlamydial infection in trachoma-endemic areas? J Infect Dis 2003;187:1669-73

[16] Stary A MS, Vinzelj-Horvath E M. Diagnosis of *Chlamydia trachomatis* and *Neisseria Gonorrhoeae* on genital, pharyngeal and rectal samples by the ligase chain reaction. In: Thirteenth Meeting of the International Society for Sexually Transmitted Diseases Research. Denver, Co.: International Society for Sexually Transmitted Diseases Research, 1999

[17] Tipple MA, Beem MO and Saxon EM. Clinical characteristics of the afebrile pneumonia associated with *Chlamydia trachomatis* infection in infants less than 6 months of age. Pediatrics 1979;63:192-7

[18] Treharne JD, Darougar S and Jones BR. Modification of the microimmunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. J Clin Pathol 1977;30:510-7

[19] Pham-Kanter GB, Steinberg MH and Ballard RC. Sexually transmitted diseases in South Africa. Genitourin Med 1996;72:160-71

[20] Wilkinson D, Abdool Karim SS, Harrison A, et al. Unrecognized sexually transmitted infections in rural South African women: a hidden epidemic. Bull World Health Organ 1999;77:22-8

[21] Laga M, Plummer FA, Piot P, et al. Prophylaxis of gonococcal and chlamydial ophthalmia neonatorum. A comparison of silver nitrate and tetracycline. N Engl J Med 1988;318:653-7

[22] Sherman GG, Jones SA, Coovadia AH, Urban MF and Bolton KD. PMTCT from research to reality-results from a routine service. S Afr Med J 2004;94:289-92

[23] Frommell GT, Rothenberg R, Wang S and McIntosh K. Chlamydial infection of mothers and their infants. J Pediatr 1979;95:28-32

[24] Hammerschlag MR, Chandler JW, Alexander ER, et al. Erythromycin ointment for ocular prophylaxis of neonatal chlamydial infection. JAMA 1980;244:2291-3

[25] Schachter J, Grossman M, Sweet RL, Holt J, Jordan C and Bishop E. Prospective study of perinatal transmission of *Chlamydia trachomatis*. JAMA 1986;255:3374-7

[26] Hammerschlag MR, Chandler JW, Alexander ER, English M and Koutsky L. Longitudinal studies on chlamydial infections in the first year of life. Pediatr Infect Dis 1982;1:395-401

[27] Talley AR, Garcia-Ferrer F, Laycock KA, et al. Comparative diagnosis of neonatal chlamydial conjunctivitis by

polymerase chain reaction and McCoy cell culture. Am J Ophthalmol 1994;117:50-7

[28] van Bogaert LJ. Ophthalmia neonatorum revisited. Afr J Reprod Health 1998;2:81-6

[29] Hammerschlag MR, Cummings C, Roblin PM, Williams TH and Delke I. Efficacy of neonatal ocular prophylaxis for the prevention of chlamydial and gonococcal conjunctivitis. N Engl J Med 1989;320:769-72

[30] van Bogaert LJ. Controversies in the approach to ophthalmia neonatorum. S Afr Med J 1997;87:76-7

[31] Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. MMWR 2002:,51(RR-6):1-25

[32] Pediatrics Aao. Red Book: Report of the Committee on Infectious Diseases. 25 ed., 2000

[33] Osguthorpe JD, Shirley R. Neonatal respiratory distress from rhinitis medicamentosa. Laryngoscope 1987;97:829-31

[34] Schachter J. Chlamydial infections (third of three parts). N Engl J Med 1978;298:540-9

[35] Shinkwin CA, Gibbin KP. Neonatal upper airway obstruction caused by chlamydial rhinitis. J Laryngol Otol 1995;109:58-60

[36] Harrison HR, English MG, Lee CK and Alexander ER. *Chlamydia trachomatis* infant pneumonitis: comparison with matched controls and other infant pneumonitis. N Engl J Med 1978;298:702-8

[37] Latif AS. Syndromic management of sexually transmitted diseases. Part 2-The management of genital discharge. Cent Afr J Med 1998;44:236-41

[38] Latif AS. Syndromic management of sexually transmitted diseases. Part 4-The management of lower abdominal pain in women. Cent Afr J Med 1998;44:293-6

[39] Hoffman L, Ma OJ, Gaddis G and Schwab RA. Cervical infections in emergency department patients with vaginal bleeding. Acad Emerg Med 2002;9:781-5

Part III

Chlamydia trachomatis infection in pregnant women and infants in Rotterdam, The Netherlands

Chapter 5

Chlamydia trachomatis infection associated with preterm delivery: a population-based prospective cohort study

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Abstract

Background *C. trachomatis* infection is the most prevalent bacterial sexually transmitted infection and may influence pregnancy outcome.

Objective This study was conducted to assess the effect of chlamydial infection during pregnancy on premature delivery and birthweight.

Methods Pregnant women attending a participating midwifery practice or antenatal clinic between February 2003 and January 2005 were eligible for the study. From 4,055 women self-administered questionnaires and urine samples, tested by PCR, were analysed for *C. trachomatis* infection. Pregnancy outcomes were obtained from midwives and hospital registries. Gestational ages and birthweights were analysed for 3,913 newborns.

Results The *C. trachomatis* prevalence was 3.9%, but varied by age and socio-economic background. Chlamydial infection was, after adjustment for potential confounders, associated with preterm delivery before 32 weeks (OR 4.35 [95% CI 1.3-15.2]) and 35 weeks gestation (OR 2.66 [95% CI 1.1-6.5]), but not with low birthweight. Of all deliveries before 32 weeks and 35 weeks gestation 14.9% [95% CI 4.5-39.5] and 7.4% [95% CI 2.5-20.1] was attributable to *C. trachomatis* infection.

Conclusion *C. trachomatis* infection contributes significantly to early premature delivery and should be considered a public health problem, especially in young women and others at increased risk of *C. trachomatis* infection.

Introduction

Chlamydia trachomatis is an important cause of sexually transmitted infections (STIs) in women, which may lead to pelvic inflammatory disease, tubal infertility, ectopic pregnancy, and chronic abdominal pain [1-4]. *C. trachomatis* infection during pregnancy may in addition influence pregnancy outcomes leading to premature rupture of membranes, prematurity, low birthweight and perinatal mortality, and cause neonatal conjunctival and respiratory infection [5-7]. The literature regarding these detrimental effects of *C. trachomatis* infection on pregnancy outcome, however, yields conflicting results that seem primarily due to differences in study design, population and microbiological tests employed [8-24].

Screening for *C. trachomatis* in pregnant women has revealed prevalence rates varying from 0% to 37% and various associated risk factors including, in particular, age and socio-economic status [25-27].

In the Netherlands, a *C. trachomatis* prevalence of 2.5% was reported in women [28]. However, prenatal screening for *C. trachomatis* is not routine obstetrical practice in the Netherlands and data on pregnant women and pregnancy outcome are lacking.

The objective of this study was to assess the prevalence of *C. trachomatis* infection in pregnant women and to investigate the association of chlamydial infection with the risks of preterm delivery and low birthweight or being small for gestational age.

Methods

Design

This *Chlamydia trachomatis* study was embedded in the Generation R Study, which is a population-based, non-interventional, prospective cohort study designed to identify early environmental and genetic determinants of growth, development and health of children, starting from foetal life until adolescence [29, 30]. Pregnant women, who attended one of the participating midwifery practices or antenatal clinics and who were expected to deliver in Rotterdam, were eligible for the study. Regular health care workers (midwives, obstetricians) informed the women about the study. Most women spoke Dutch, otherwise the study was explained and questionnaires were provided in their native language. Enrolment was scheduled in early pregnancy (gestational age < 18 weeks) at the first routine foetal ultrasound examination, but was allowed until delivery. Women who were scheduled for termination of pregnancy and women with a pregnancy resulting in miscarriage or perinatal death prior to or at the first ultrasound were not included in the study. The Generation R study started in 2002 [29]. Inclusion for the Chlamydia sub-study was between February 2003 and January 2005.

Risk factors

Data were obtained using confidentially administered standardized questionnaires at the time of inclusion. Questions that were not answered were registered as missing data. Maternal age was defined as age at enrolment of the study. Ethnicity was defined according to the classification of Statistics Netherlands [30]. Most women were Dutch (49%), Surinamese (9%), Turkish (9%) or Moroccan (7%). Educational levels of participating women were defined in groups by highest attained education (primary school, secondary school, higher education). Marital status was defined as married if married or living together in partnership. Further information was obtained regarding gravidity, number of sexual partners in the year prior to pregnancy, history of an STI, and the use of cigarettes, alcohol and drugs.

Microbiological diagnosis

Women provided a first-void urine specimen to test for *C. trachomatis* at enrolment. Urines were stored at 4°C, transported the same or following working day, and processed within 24 hours of receipt by the laboratory. DNA was isolated from pooled urine specimens using the MagNA Pure LC Bacterial DNA isolation Kit III (Roche Molecular Systems, Inc, Alameda, USA) and amplified by polymerase chain reaction (PCR) (Cobas Amplicor, Roche Molecular Diagnostics, Branchburg USA) [31]. In brief, pools were made of five individual urines by adding 200 µl of each of the urines into one tube. From each pool the full 1000 µl were taken, and centrifuged for 10 minutes. Subsequently, 900 µl were removed and the pellet was resuspended in 100 µl of the remaining supernatant, mixed with 130 µl lysis buffer and 20 µl proteinase K, incubated for 10 minutes and thereafter denatured for 10 minutes. Finally, DNA was isolated in the automated MagNA Pure LC using a sample volume of 250 µl and an elution volume of 100 µl. Then, 25 µl was used for PCR. Urines from positive pools were individually re-tested and reported as negative or positive.

Pregnancy outcomes

Information about pregnancy outcomes (miscarriage, perinatal death, gestational age, birthweight) was obtained postnatally from midwives and hospital registries. Gestational age was established by foetal ultrasound examination. Preterm birth was defined as delivery at a gestational age of less than 37 weeks with subgroups of gestational ages less than 32 weeks and less than 35 weeks. Low birthweight was defined below 2500 grams. Birthweight measurements were converted into gestational age adjusted standard deviation scores (SDSs) [32]. Small for gestational age (SGA) was defined as birthweight less than 2 SDS below the mean for gestation.

Ethical aspects

The *C. trachomatis* study was embedded within the framework of the Generation R Study, which is a population-based prospective cohort study designed to identify environmental and

genetic causes of normal and abnormal growth, development and health from fetal life until young adulthood [29, 30]. Both studies were approved by the Medical Ethical Committee for Research on Human Subjects of the Erasmus University Medical Centre, Rotterdam. Written informed consent was obtained from all participants. Generation R provided the data anonymously to protect the privacy of participants.

Data analysis

Associations of *C. trachomatis* infection during pregnancy with socio-economic and life style risk factors of women were assessed using multiple logistic regression models. Unequivocal confounders were selected based on previous studies. Associations were studied for each risk factor while adjusting for all other risk factors and for the confounders alcohol, drugs and smoking. We imputated the missing data in other risk factors and confounders with multiple imputations. Preterm birth was analysed using multiple logistic regression models. We adjusted for maternal age, ethnicity, gravidity, education, and smoking).

The Kaplan-Meier procedure was used to illustrate the proportion of women who delivered at given gestational ages. The Breslow test was used to calculate the significance of the difference between gestational ages at delivery of chlamydia-positive and chlamydia-negative women in the Kaplan-Meier procedure.

The proportion of preterm delivery attributable to *C. trachomatis* infection in women in the total population was assessed using the population attributable risk (PAR) [33]. The PAR was calculated with the observed relative risk (RR) and the population fraction with chlamydial infection (PF), using the formula:

$$PAR = [(RR - 1)PF] / [1 + (RR - 1)PF].$$

Birthweight as a continuous variable was analysed using multiple linear regression models. Low birthweight and SGA were analysed using multiple logistic regression models. The latter regressions were adjusted for known determinants of low birthweight (maternal age, ethnicity, gravidity), socio-economic status and life style related variables (education, smoking).

Measures of association are presented with 95% confidence intervals (CI).

Statistical analysis was performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA). Data imputation was done with the *MI* procedure in SAS 9.13.

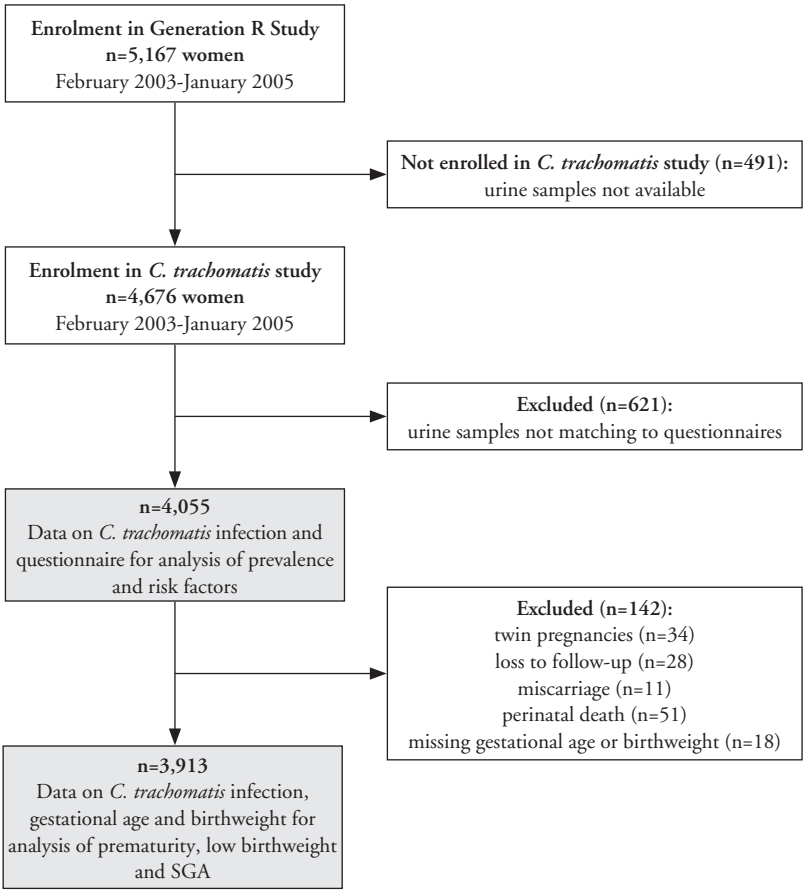

Figure 1 Profile of the Generation R sub-study on *Chlamydia trachomatis*

Results

Population for analysis

During the study period 5,167 pregnant women were enrolled in the overall Generation R study (Figure 1), of whom 4,676 women (90%) provided a urine sample and were enrolled in the Chlamydia sub-study. Of these, 621 urines could not be matched to the respective questionnaires in the database. Urine samples and data from 4,055 women were used to analyse the prevalence and risk factors for *C. trachomatis* infection. Half the women were included within the first 14 weeks of their pregnancy, 95% within 22 weeks.

Prevalence

C. trachomatis infection was detected in 157 of 4,055 (3.9%) women. Age-specific prevalences were 13.5% in women age 20 years or less, 6.7% between 21 and 25 years, 3.3% between 26

Table 1 Socio-economic and lifestyle risk factors of *Chlamydia trachomatis* infection in pregnant women

	Chlamydia trachomatis infection			
Risk factors	Negative n=3,898	Positive n=157 (%)	Crude OR (95% CI)	Adjusted OR (95% CI)#
Age groups (n=4,055)				
< 21 years	217	34 (13.5)	9.4 (5.6-15.8)**	1.8 (1.2-2.6)**
21-25 years	741	53 (6.7)	4.3 (2.7-6.8)**	1.3 (1.0-1.7)
26-30 years	1,194	41 (3.3)	2.1 (1.3-3.4)**	0.9 (0.7, 1.2)
> 30 years	1,746	29 (1.6)	1.00	1.00
Ethnicity (n=3,730)				
Dutch	1,748	33 (1.8)	1.00	1.00
Cape Verdean	149	18 (10.8)	6.4 (3.5-11.6)**	1.5 (0.9-2.5)
Antillean	109	21 (16.2)	10.2 (5.7-18.2)**	2.3 (1.4-3.7)**
Surinamese	310	31 (9.1)	5.3 (3.2-8.8)**	1.4 (1.0-2.1)
Moroccan/Turkish	610	14 (2.2)	1.2 (0.7-2.3)	0.5 (0.3-0.9)*
other (non-) western	668	19 (2.8)	1.5 (0.9-2.7)	0.7 (0.4-1.1)
Education (n=3,656)				
primary school	389	24 (5.8)	3.3 (1.9-5.8)**	1.3 (0.9-1.8)
secondary school	1,573	79 (4.8)	2.7 (1.8-4.2)**	0.9 (0.7-1.2)
higher education	1,562	29 (1.8)	1.00	1.00
Marital status (n=3,648)				
not married	479	65 (11.9)	5.9 (4.1-8.4)**	1.6 (1.3-2.0)**
married	3,034	70 (2.3)	1.00	1.00
Gravidity >1 (n=4,011)				
no	1,719	81 (4.5)	1.00	1.00
yes	2,138	73 (3.3)	0.7 (0.5-1.0)	0.9 (0.7-1.0)
Multiple sexual partners in year prior to pregnancy (n=3,289)				
no	2,918	99 (3.3)	1.00	1.00
yes	251	21 (7.7)	2.5 (1.5-4.0)**	1.2 (0.7-2.2)
History of STI (n=3,313)				
no	2,778	100 (3.5)	1.00	1.00
yes	370	25 (6.3)	1.9 (1.2-3.0)**	1.4 (0.7-2.5)
do not know	38	2 (5.0)	1.5 (0.4-6.2)	0.8 (0.3-2.2)

Values are frequencies and odds ratios (95% CI), *P<0.05, **P<0.01

#Adjusted for all other risk factors, use of drugs, alcohol and smoking with multiple imputation of missing data in the other factors

and 30 years and 1.6% in women over 30 years. The prevalence was highest in Antillean (16.2%), Cape Verdean (10.8%) or Surinamese (9.1%) women, and in women with low education (5.8%), single marital status (11.9%), first pregnancies (4.5%), multiple sexual partners in the past year (7.7%) or a history of an STI (6.3%) (Table 1).
Women with missing data on ethnicity, education and smoking had higher rates of chlamydial infection than women with these data recorded: 6.5% versus 3.6% (P=0.01), 6.3% versus 3.6% (P=0.01) and 4.8% versus 3.4% (P=0.04), respectively. Women with missing data on marital status, gravidity, multiple sexual partners, history of an STI, use of alcohol or drugs did not have significant higher rates of chlamydial infection than women with these data recorded.

Risk factors

In the unadjusted analysis age below 30 years, Antillean, Cape Verdean and Surinamese ethnicity, education, single marital status, multiple sexual partners in the year prior to pregnancy and history

Table 2 Pregnancy outcomes of women and their association with *Chlamydia trachomatis* infection

	Chlamydia trachomatis infection			
Outcomes	**Negative**	**Positive**	**Percentage positive (95% CI)**	**P-value ***
All outcomes (n=4,055)				
all women enrolled	3,898	157	3.9 (3.3-4.5)	
Live pregnancy outcomes (n=4,055)				
live singleton birth	3,780	151	3.8 (3.2-4.4)	1.0 (reference)
live twin birth	30	4	11.8 (0.3-21.9)	0.05
lost to follow up	26	2	7.1 (0.0-17.3)	0.29
Adverse pregnancy outcomes				
Demise (n=4,055)				
miscarriage	11	0	0	1.00
perinatal death	51	0	0	0.27
Gestational age ** (n=3,913)				
term, ≥ 37 weeks	3,583	140	3.8 (3.2-4.4)	1.0 (reference)
prematurity, < 37 weeks	180	10	5.3 (2.1-8.5)	0.33
prematurity, < 35 weeks	57	7	10.9 (3.1-18.8)	0.01
prematurity, < 32 weeks	18	4	18.2 (0.7-36.7)	0.01
Birthweight ** (n=3,913)				
≥ 2500 gram	3,589	140	3.8 (3.1-4.4)	1.0 (reference)
< 2500 gram	174	10	5.4 (2.1-8.7)	0.25

* P-value (Chi-square or Fishers exact test) for comparison of *C. trachomatis* prevalence with respective outcome categories of live singleton birth, gestational age ≥ 37 weeks, and birthweight ≥ 2500 gram

** Including live singleton birth outcomes only

of an STI were significantly associated with *C. trachomatis* infection (Table 1). In the adjusted analysis age below 21 years, Antillean ethnicity and single marital status remained risk factors for *C. trachomatis* infection; other maternal factors were not independently associated. Moroccan or Turkish women had a lower risk for *C. trachomatis* infection.

Pregnancy outcomes

Pregnancy outcomes are shown in table 2. Of the 4,055 pregnancies 3,931 resulted in live singleton births and 34 in live twins. Women who gave birth to twins were more often chlamydia-positive than women who had singletons (P=0.05). Miscarriage (n=11) and perinatal death (33 stillbirth, 18 neonatal death) occurred in 1.5% pregnancies. These adverse events were not associated with *C. trachomatis* infection during pregnancy. Preterm delivery occurred in 190 women (4.9%) of which prematurity before 32 weeks (0.6%) and 35 weeks (1.6%) of gestation was significantly associated with *C. trachomatis* infection (Table 2). No significant association was observed with low birthweight.

After exclusion of women who were lost to follow up, or who had twin pregnancies, a miscarriage or perinatal death, the effect of *C. trachomatis* infection on gestational age and birthweight was further analysed for 3,913 women and neonates.

Gestational age. The distribution of gestational ages according to the chlamydial status of women is shown in a Kaplan-Meier plot (Figure 2). Regarding premature delivery, chlamydia-positive women had a significantly shorter duration of gestation (P Breslow=0.02).

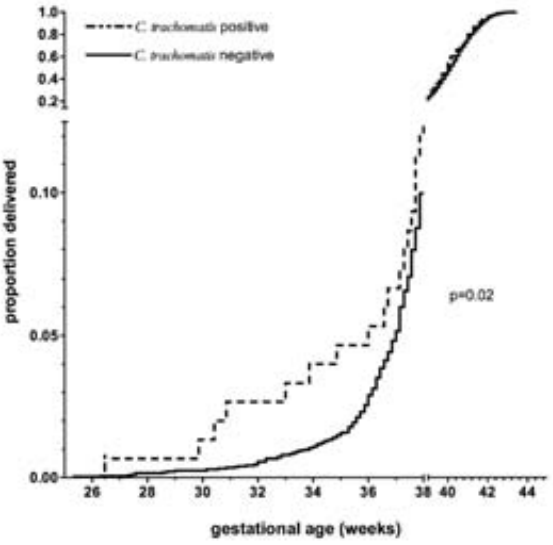

Figure 2 Kaplan-Meier analysis of gestational age at delivery in women with and without *Chlamydia trachomatis* infection

Unadjusted analysis (Table 3) showed that chlamydia-positive women had a significantly higher risk of preterm delivery before 32 weeks compared to delivery at term (OR 5.7 [95% CI 1.9-17.0]). After adjustment for the potential confounders maternal age, ethnicity, education, gravidity, and smoking a significant four-fold increased risk (OR 4.4 [95% CI 1.3-15.2]) of chlamydia-positive women for preterm delivery before 32 weeks remained. Chlamydia-positive women also had a significantly higher risk of preterm delivery before 35 weeks (OR 3.1 [95% CI 1.4-7.0]), which also remained significant after adjustment for the above-stated confounders (OR 2.7

Table 3 Risk for preterm delivery among women with *Chlamydia trachomatis* infection

	Risk for preterm delivery		
	< 32 weeks n=22	< 35 weeks n=64	< 37 weeks n=190
Unadjusted odds ratio			
C. trachomatis infection	5.7 (1.9-17.0)**	3.1 (1.4-7.0)**	1.4 (0.7-2.8)
Adjusted odds ratio			
C. trachomatis infection	4.4 (1.3-15.2) *	2.7 (1.1-6.5) *	1.2 (0.6-2.4)

Analyses are done versus delivery ≥ 37 weeks (n=3,724)

#Adjusted for maternal age, ethnicity, education, gravidity and smoking with multiple imputation

Values are odds ratios (95% confidence interval), *P<0.05, **P<0.01

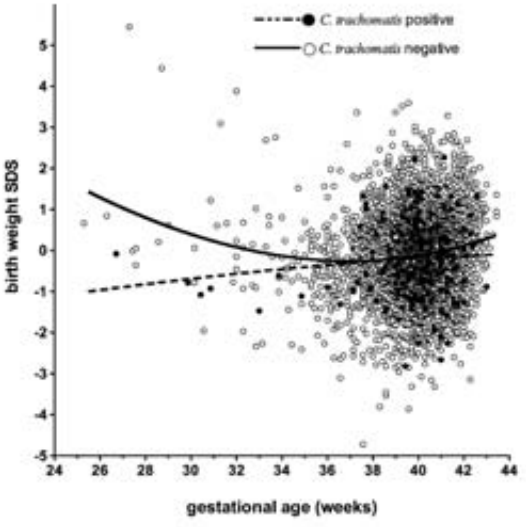

Figure 3 Birthweight standard deviation scores versus gestational ages of neonates born to women with and without *Chlamydia trachomatis* infection

[95% CI 1.1-6.5]). Chlamydia-positive women did not have an increased risk of preterm delivery before 37 weeks.

The fraction of all premature deliveries before 32 weeks gestation attributable to *C. trachomatis* infection in women was 14.9% (95% CI 4.5-39.5). The PAR of *C. trachomatis* infection for preterm delivery before 35 weeks gestation was 7.4% (95% CI 2.5-20.1).

Birthweight. Crude analysis of the difference in birthweight (in grams) between neonates born to chlamydia-positive and chlamydia-negative women showed a significant difference (-114 grams [95% CI -206, -23]), which disappeared after adjustment for the potential confounders gestational age, maternal age, ethnicity, education, gravidity and smoking (-20 grams [95% CI -98, 58]). A comparison of low birthweight (n=184) among neonates born to chlamydia-positive and chlamydia-negative women also showed no significant difference, neither in the unadjusted analysis (OR 1.5 [95% CI 0.8-2.8]) nor in the adjusted analysis (OR 1.0, [0.4-2.2]). Analysis of the correlation between birthweights of neonates, expressed in SDSs, and their gestational ages according to the maternal Chlamydia status is demonstrated in figure 3. This figure suggests that neonates born to chlamydia-positive women had, on average, a lower birthweight SDS, especially when prematurely born. However, this difference in birthweight SDSs between neonates born to chlamydia-positive and chlamydia-negative women did not reach statistical significance, also not after stratification by gestational ages (Table 4). Furthermore, neonates

Table 4 Differences in birthweight between neonates born to women with and without *Chlamydia trachomatis* infection

	Number	Mean birth weight	Difference in birthweight SDS*	
Gestational age	**(%)**	**SDS**	**Unadjusted**	**Adjusted**
< 32 weeks (n=22)				
C. trachomatis –	18 (82)	0.83		
C. trachomatis +	4 (18)	–0.72	–1.54 (–3.6-0.5)	–1.43 (–4.6-1.7)
< 35 weeks (n=64)				
C. trachomatis –	57 (89)	0.13		
C. trachomatis +	7 (11)	–0.87	–0.99 (–2.2-0.2)	–0.79 (–2.2-0.7)
< 37 weeks (n=190)				
C. trachomatis –	180 (95)	–0.26		
C. trachomatis +	10 (5)	–0.85	–0.59 (–1.4-0.2)	–0.38 (–1.2-0.4)
≥ 37 weeks (n=3,723)				
C. trachomatis –	3,583 (96)	–0.08		
C. trachomatis +	140 (4)	–0.19	–0.10 (–0.3-0.1)	–0.12 (–0.1-0.3)

SDS: standard deviation score

Adjusted for maternal age, ethnicity, education, gravidity and smoking

Difference and 95% confidence interval calculated with linear regression analysis

born to chlamydia-positive women were not more often SGA than neonates born to chlamydia-negative women, 3.3% versus 3.6% respectively (P=0.88).

Discussion

This study provides evidence that *C. trachomatis* infection during pregnancy is associated with preterm delivery, but not with low birthweight or being small for gestational age. Young age, Antillean ethnicity and single marital status were independent risk factors for *C. trachomatis* infection in pregnant women.

The strength of this study is its population-based, non-interventional and prospective design with a large number of well-described participants, adjustments for many potential confounders, use of a highly sensitive microbiological test method and near perfect follow-up until delivery (97%). A potential weakness is that the Generation R cohort is slightly skewed towards a relatively affluent and healthy study population [30]. However, we do not expect this to effect our results since the present study was designed to assess the effects of *C. trachomatis* infection on pregnancy outcomes in a non-hospital based, low risk population. Another weakness could be that not all women initially enrolled in the Generation R study could be tested for *C. trachomatis*. We had a non-response of 9.5% (491 women) for the Chlamydia study. This may in part be true non-response, but is more likely due to a change in the Generation R routine with a later start of the inclusion of urine collections for the Chlamydia study. Another 12.0% (621 women) had to be excluded because their urines could not be matched to the respective questionnaires in the database. This was due to logistical problems in the pilot phase of the Chlamydia study. However, all risk factors were similarly distributed among tested and untested women and no differences were found in median gestational age and mean birthweight (39.8 weeks versus 39.8 weeks; p=0.84, 3408 grams versus 3407 grams; p=0.92). Furthermore, our numbers were too small to properly assess an association of *C. trachomatis* infection with miscarriage or perinatal death. Of all enrolled women with *C. trachomatis* results, 62 (1.5%) had a miscarriage or perinatal death. Data on gestational age and birthweight were not available for women with these adverse pregnancy outcomes, but likely is that these women delivered relatively more often prematurely. None of these women tested positive for *C. trachomatis*. Theoretically, our effect estimates for the associations of chlamydial infection with gestational age and birthweight could be biased and exaggerated when these associations would differ between all fetuses and fetal 'survivors'. This would be the case if *C. trachomatis* infection would have a `protective` effect on early fetal death. However, this is most unlikely [8, 20].

Previous studies of associations between maternal chlamydial infection and subsequent spontaneous preterm delivery produced mixed results. Most of these studies were small compared to our study (less than 1,000 women included) and were published 10 to over 20 years ago. Early studies were often based on serology, which does not reliably distinguish current from past infection [9, 11, 14, 15]. One case-control study found an association between the presence of IgM anti-chlamydial antibody, but not IgG antibody, and preterm delivery [11]. Studies using cervical culture for *C. trachomatis* at that time also yielded conflicting results [9, 10, 13, 14]. More recently, sensitive DNA amplification techniques have been used to screen pregnant women for *C. trachomatis* [21-24]. One case-control study, nested in a large USA study among 2,929 pregnant women, used this methodology and reported a two- to three-fold increased risk of preterm delivery before 35 weeks gestation [21]. Interestingly, the same study group reported later that *C. trachomatis* infection in midterm pregnancy was not associated with an increased risk of preterm delivery among 2,470 women enrolled in an antibiotic treatment trial for bacterial vaginosis or *Trichomonas vaginalis* infection [23]. Since all women were infected and bacterial vaginosis itself may induce preterm delivery [10, 34], the findings of the latter study cannot be extrapolated to the population at large. In a prospective South-African study among low-risk pregnant women [22], chlamydia-positive women (cases, n=40) had a relative risk of 2.20 for preterm delivery before 37 weeks gestation (controls, n=303). In this study women were also tested for Syphilis, Gonorrhoea and bacterial vaginosis, which were found not to be associated with preterm delivery. Another population-based retrospective cohort study in the USA found that *C. trachomatis* infected pregnant women (cases, n=851) had a relative risk of only 1.50 for preterm delivery before 37 weeks gestation (controls, n=3,404) [24]. However, the true effect of *C. trachomatis* infection on pregnancy outcomes cannot be ascertained from that study since all women were screened and, when positive, treated for *C. trachomatis* in the first trimester of their pregnancy. In contrast, our study is prospective, non-interventional and population-based (n=4,055), which makes that our findings better estimate the effect of *C. trachomatis* infection on pregnancy and are more predictive for the population as a whole.

Preterm birth represents a major problem for obstetrics and neonatology due to its increasing frequency and accompanying socio-economic impact. We found chlamydial infection to be associated with preterm delivery before 32 weeks and before 35 weeks gestation. The association was much stronger for 32 weeks than for 35 weeks gestation indicating that *C. trachomatis* infection contributes relatively more to early than to late prematurity. For such an important issue, a four-fold increased risk of preterm delivery before 32 weeks gestation implies that a considerable proportion (14.9% in our cohort) of preterm deliveries before 32 weeks gestation is attributable to chlamydial infection in pregnancy. Despite a considerable confidence interval around the estimate, this would classify *C. trachomatis* among the important infective risk factors of early prematurity. The attributable fraction (7.4%) remained significant for preterm delivery before

35 weeks. It should be noted, however, that the number and proportion of premature deliveries attributable to chlamydial infection highly depends upon the *C. trachomatis* prevalence in a given population, and that some confounding as a result of co-infection by other genital pathogens cannot be excluded. Furthermore, we did not correct for a previous history of termination of pregnancy in these women since this potential confounder was only recently established [35]. Finally, we had no information concerning the use of (macrolide) antibiotics during pregnancy, but suggest that such use would mitigate, rather than exaggerate, the detrimental effect of *C. trachomatis* infection on pregnancy outcome. Furthermore, pregnant women are not routinely tested and treated for *Ureaplasma urealyticum* or Mycoplasma genitalium in the Netherlands; neither are women with premature rupture of the membranes routinely treated with antibiotics.

Extrapolation of our findings to the Netherlands, where approximately 3,000 neonates are born before 32 weeks gestation annually [36], showed that *C. trachomatis* infection in pregnancy contributes approximately 450 cases to this burden; for deliveries before 35 weeks gestation these numbers are 7,100 and 525, respectively. Our finding of an association between *C. trachomatis* infection with premature delivery may be useful in a cost-benefit analysis of *C. trachomatis* screening during pregnancy, especially among women with increased risk for infection.
Interestingly, *C. trachomatis* infection was more prevalent among women who had twins than among women who had singletons, a finding not reported before. Since *C. trachomatis* infection is associated with infertility and assisted conception for infertility is associated with twin pregnancies, an explanation for our findings may be that chlamydia-positive women were not treated effectively during their infertility work-up or that they were reinfected. This observation warrants further study into medical histories of women with twin deliveries versus women with singletons.

The major risk factors we found are amongst the many previously described including young age, urban residence, low socio-economic class, specific ethnic groups, single marital status and recent changes in sexual partnerships or sexual promiscuity [9, 25, 37, 38]. Adolescents are at highest risk of infection, which was also observed in this study [27]. Urban residence has been described to be of importance, which effect we could not evaluate since all participating women resided in Rotterdam. The risk factors we describe are similar to those reported in another Dutch community-based study [28, 39]. Importantly, on global scale *C. trachomatis* prevalences vary widely [25], which will directly affect the incidence of complications attributable to this infection, including preterm delivery.

One may question the design of our study since women screened and found positive for *C. trachomatis* infection were not treated. The design of our study would face ethical barriers in those countries that have national guidelines or directives advocating *C. trachomatis* screening

and treatment in routine antenatal care, and would be impossible to perform [40, 41]. However, in most countries of the European Union, including the Netherlands, screening and treatment of chlamydial infection during pregnancy remains controversial and is currently not recommended in routine antenatal care [42, 43]. Our study provides novel data that may well have an impact on the future antenatal screening strategy in the Netherlands and elsewhere. In addition, the main study, the Generation R study, was designed as a non-interventional follow-up study [29, 30]. All following substudies, including ours, had to fit into this overall design. Furthermore, Generation R provided the data anonymously to protect the privacy of participants. We, therefore, had no access to names or addresses of chlamydia-positive women.

In conclusion, *C. trachomatis* urogenital infection in pregnant women increases the risk of preterm delivery, especially early prematurity, such that a significant proportion of preterm deliveries can be attributed to this infection. In order to improve birth outcomes, health systems should consider additional focus on *C. trachomatis* infection, especially in young women and others at increased risk of *C. trachomatis* infection.

References

[1] Mardh PA. Influence of infection with *Chlamydia trachomatis* on pregnancy outcome, infant health and life-long sequelae in infected offspring. Best Pract Res Clin Obstet Gynaecol. 2002;16(6):847-64.

[2] Wiesenfeld HC, Hillier SL, Krohn MA, Amortegui AJ, Heine RP, Landers DV, et al. Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. Obstet Gynecol. 2002;100(3):456-63.

[3] Peipert JF. Clinical practice. Genital chlamydial infections. N Engl J Med. 2003;349(25):2424-30.

[4] Manavi K. A review on infection with *Chlamydia trachomatis*. Best Pract Res Clin Obstet Gynaecol. 2006;20(6):941-51.

[5] Darville T. *Chlamydia trachomatis* infections in neonates and young children. Semin Pediatr Infect Dis. 2005;16(4):235-44.

[6] Rours GIJG, Hammerschlag MR, Ott A, De Faber TJ, Verbrugh HA, de Groot R, et al. *Chlamydia trachomatis* as a cause of neonatal conjunctivitis in Dutch infants. Pediatrics. 2008;121(2):e321-6.

[7] Rours GIJG, Hammerschlag MR, Van Doornum GJ, Hop WC, de Groot R, Willemse HF, et al. *Chlamydia trachomatis* respiratory infection in Dutch infants. Arch Dis Child. 2009;94(9):705-7.

[8] Martin DH, Koutsky L, Eschenbach DA, Daling JR, Alexander ER, Benedetti JK, et al. Prematurity and perinatal mortality in pregnancies complicated by maternal *Chlamydia trachomatis* infections. JAMA. 1982;247(11):1585-8.

[9] Harrison HR, Alexander ER, Weinstein L, Lewis M, Nash M, Sim DA. Cervical *Chlamydia trachomatis* and mycoplasmal infections in pregnancy. Epidemiology and outcomes. JAMA. 1983;250(13):1721-7.

[10] Gravett MG, Nelson HP, DeRouen T, Critchlow C, Eschenbach DA, Holmes KK. Independent associations of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. JAMA. 1986;256(14):1899-903.

[11] Sweet RL, Landers DV, Walker C, Schachter J. *Chlamydia trachomatis* infection and pregnancy outcome. Am J Obstet Gynecol. 1987;156(4):824-33.

[12] Alger LS, Lovchik JC, Hebel JR, Blackmon LR, Crenshaw MC. The association of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and group B streptococci with preterm rupture of the membranes and pregnancy outcome. Am J Obstet Gynecol. 1988;159(2):397-404.

[13] Association of *Chlamydia trachomatis* and *Mycoplasma hominis* with intrauterine growth retardation and preterm delivery. The John Hopkins Study of Cervicitis and Adverse Pregnancy Outcome. Am J Epidemiol. 1989;129(6):1247-57.

[14] Ngassa PC, Egbe JA. Maternal genital *Chlamydia trachomatis* infection and the risk of preterm labor. Int J Gynaecol Obstet. 1994;47(3):241-6.

[15] Claman P, Toye B, Peeling RW, Jessamine P, Belcher J. Serologic evidence of *Chlamydia trachomatis* infection and risk of preterm birth. CMAJ. 1995;153(3):259-62.

[16] Nadisauskiene R, Bergstrom S, Stankeviciene I, Spukaite T. Endocervical pathogens in women with preterm and term labour. Gynecol Obstet Invest. 1995;40(3):179-82.

[17] Martin DH, Eschenbach DA, Cotch MF, Nugent RP, Rao AV, Klebanoff MA, et al. Double-Blind Placebo-Controlled Treatment Trial of *Chlamydia trachomatis* Endocervical Infections in Pregnant Women. Infect Dis Obstet Gynecol. 1997;5(1):10-7.

[18] Kovacs L, Nagy E, Berbik I, Meszaros G, Deak J, Nyari T. The frequency and the role of *Chlamydia trachomatis* infection in premature labor. Int J Gynaecol Obstet. 1998 Jul;62(1):47-54.

[19] Rastogi S, Kapur S, Salhan S, Mittal A. *Chlamydia trachomatis* infection in pregnancy: risk factor for an adverse outcome. Br J Biomed Sci. 1999;56(2):94-8.

[20] Gencay M, Koskiniemi M, Ammala P, Fellman V, Narvanen A, Wahlstrom T, et al. *Chlamydia trachomatis* seropositivity is associated both with stillbirth and preterm delivery. APMIS. 2000;108(9):584-8.

[21] Andrews WW, Goldenberg RL, Mercer B, Iams J, Meis P, Moawad A, et al. The Preterm Prediction Study: association of second-trimester genitourinary chlamydia infection with subsequent spontaneous preterm birth. Am J Obstet Gynecol. 2000;183(3):662-8.

[22] Odendaal HJ Schoeman J. The association between *Chlamydia trachomatis* genital infection and spontaneous preterm labour. South African Journal of Obstetrics and Gynaecology. 2006;12(3):146-9.

[23] Andrews WW, Klebanoff MA, Thom EA, Hauth JC, Carey JC, Meis PJ, et al. Midpregnancy genitourinary tract infection with *Chlamydia trachomatis*: association with subsequent preterm delivery in women with bacterial vaginosis and Trichomonas vaginalis. Am J Obstet Gynecol. 2006;194(2):493-500.

[24] Blas MM, Canchihuaman FA, Alva IE, Hawes SE. Pregnancy outcomes in women infected with *Chlamydia trachomatis*: a population-based cohort study in Washington State. Sex Transm Infect. 2007;83(4):314-8.

[25] Smith JR, Taylor-Robinson D. Infection due to *Chlamydia trachomatis* in pregnancy and the newborn. Baillieres Clin Obstet Gynaecol. 1993;7(1):237-55.

[26] Wilson JS, Honey E, Templeton A, Paavonen J, Mardh PA, Stray-Pedersen B. A systematic review of the prevalence of *Chlamydia trachomatis* among European women. Hum Reprod Update. 2002;8(4):385-94.

[27] Adams EJ, Charlett A, Edmunds WJ, Hughes G. *Chlamydia trachomatis* in the United Kingdom: a systematic review and analysis of prevalence studies. Sex Transm Infect. 2004;80(5):354-62.

[28] van Bergen J, Gotz HM, Richardus JH, Hoebe CJ, Broer J, Coenen AJ. Prevalence of urogenital *Chlamydia trachomatis* increases significantly with level of urbanisation and suggests targeted screening approaches: results from the first national population based study in the Netherlands. Sex Transm Infect. 2005;81(1):17-23.

[29] Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22(12):917-23.

[30] Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update until the age of 4 years. Eur J Epidemiol. 2008;23(12):801-11.

[31] Rours GI, Verkooyen RP, Willemse HF, van der Zwaan EA, van Belkum A, de Groot R, et al. Use of Pooled Urine Samples and Automated DNA Isolation To Achieve Improved Sensitivity and Cost-Effectiveness of Large-Scale Testing for *Chlamydia trachomatis* in Pregnant Women. J Clin Microbiol. 2005;43(9):4684-90.

[32] Niklasson A EA, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). Acta Paediatr Scand. 1991;80(8-9):756-62.

[33] Rothman KJ, Greenland S. Modern epidemiology. second ed: Lippincott-Raven 1998.

[34] Martius J, Krohn MA, Hillier SL, Stamm WE, Holmes KK, Eschenbach DA. Relationships of vaginal Lactobacillus species, cervical *Chlamydia trachomatis*, and bacterial vaginosis to preterm birth. Obstet Gynecol. 1988;71(1):89-95.

[35] Moreau C, Kaminski M, Ancel PY, Bouyer J, Escande B, Thiriez G, et al. Previous induced abortions and the risk of very preterm delivery: results of the EPIPAGE study. BJOG. 2005;112(4):430-7.

[36] The Netherlands Perinatal Registry. Perinatal Care in the Netherlands 2003. Bilthoven: Stichting Perinatale Registratie; 2006.

[37] Thomas AG, Brodine SK, Shaffer R, Shafer MA, Boyer CB, Putnam S, et al. Chlamydial infection and unplanned pregnancy in women with ready access to health care. Obstet Gynecol. 2001;98(6):1117-23.

[38] Chen MY, Fairley CK, De Guingand D, Hocking JS, Tabrizi S, Wallace EM, et al. Screening pregnant women for chlamydia: what are the predictors of infection? Sex Transm Infect. 2008;85(1):31-5.

[39] Gotz HM, van Bergen JE, Veldhuijzen IK, Broer J, Hoebe CJ, Steyerberg EW, et al. A prediction rule for selective screening of *Chlamydia trachomatis* infection. Sex Transm Infect. 2005;81(1):24-30.

[40] Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep 2006;55(RR-11):1-94.

[41] U.S. Preventive Services Task Force. Screening for Chlamydial infection: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2007;147(2):128-34.

[42] Bernloehr A, Smith P, Vydelingum V. Antenatal care in the European Union: a survey on guidelines in all 25 member states of the Community. Eur J Obstet Gynecol Reprod Biol. 2005;122(1):22-32.

[43] Health Council of the Netherlands. Screening for Chlamydia, the Hague, The Netherlands, nr 2004/07:1-106.

Chapter 6

Chlamydia trachomatis and placental inflammation in early preterm delivery

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Abstract

Objective To evaluate the relationship between the presence of *Chlamydia trachomatis* and signs of placental inflammation in women who delivered at 32 weeks gestation or less.

Study Design Placental histology and clinical data were prospectively obtained from 304 women and newborns at the Erasmus MC. *C. trachomatis* testing of placentas was done retrospectively using PCR.

Results *C. trachomatis* was detected in 76 (25%) placentas. Histological evidence of placental inflammation was present in 123 (40%) placentas: in 41/76 (54%) placentas with *C. trachomatis* versus 82/228 (36%) placentas without *C. trachomatis* infection (OR 2.1, 95% CI 1.2-3.5). *C. trachomatis* infection correlated with the progression (P=0.003) and intensity (P=0.002) of placental inflammation on the maternal side, but not on the foetal side.

Conclusion *C. trachomatis* DNA was detected in the placenta of 25% of women with early premature delivery, and was associated with histopathological signs of placental inflammation.

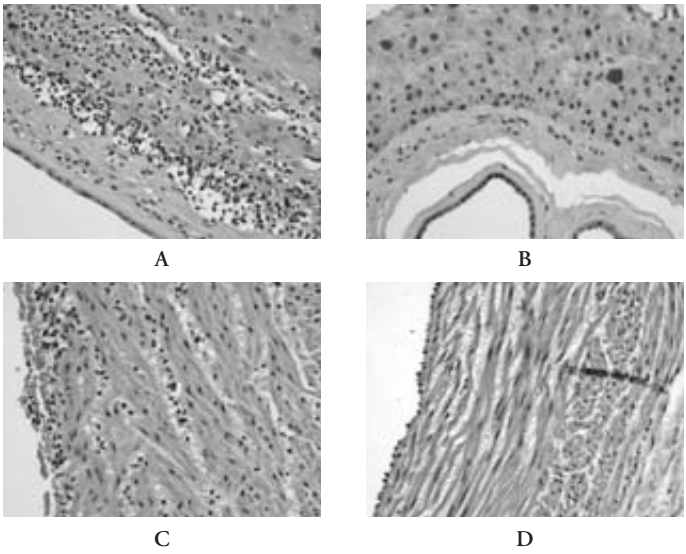

Figure 1 Histological signs of placental inflammation

(a) necrotizing chorioamnionitis (maternal stage 3): degenerating subamnionic neutrophilic granulocytes and thickened amnionic basement membrane in placental membranes, (b) no maternal inflammation, (c) umbilical vasculitis (foetal stage 2): neutrophilic granulocytes infiltrate the smooth muscle of the umbilical arteries, (d) no foetal inflammation

Introduction

Preterm birth is the main cause of perinatal morbidity and mortality worldwide accounting for 60%-80% of deaths of infants born without congenital abnormalities [1]. Preterm labor is associated with placental inflammation, especially with acute chorioamnionitis [2, 3]. Many studies associated clinical and histological chorioamnionitis with acute neonatal morbidity and mortality and, at least to some degree, also with neurological impairments and chronic lung disease [4-8]. *Chlamydia trachomatis* is the most common bacterial sexually transmitted infection worldwide, including in the Netherlands [9]. Starting as cervicitis, chlamydial infection may ascend and infect the placenta or amniotic fluid, which may subsequently lead to premature delivery. The literature regarding the association of maternal *C. trachomatis* infection with premature delivery, however, is conflicting due to differences in study design, population and microbiological test methods [10-18].

The aims of this study were to detect *C. trachomatis* infection in placentas of women with early preterm delivery and to associate *C. trachomatis* infection with histological signs of placental inflammation. In addition, we studied the association of *C. trachomatis* infection with delivery characteristics and neonatal outcome in this selected cohort of women.

Methods

Patients and Design

The chorioamnionitis study, during which the placentas were collected that we used in the current *Chlamydia trachomatis* study, was prospective, observational, and non-interventional. Pregnant women, who delivered live newborns between May 2001 and February 2003 at the Erasmus MC-Sophia, Rotterdam, The Netherlands, at a gestational age of 32 weeks or less, were eligible for the study. Regular health care workers (residents, research-nursing staff, neonatologists) informed the women about the study. Enrolment took place immediately after delivery when the newborns were admitted to the neonatal intensive care unit. Women were excluded from the study if the placenta was not available for histological examination and, at a later stage, if no more placental tissue was available to test for *C. trachomatis*. Antenatal, perinatal and neonatal data were obtained from maternal and neonatal medical records and prospectively stored in a database. Follow-up of neonates was until a postnatal age of 28 days or postmenstrual age of 36 weeks. Neonates, who were transferred to another hospital, were followed in order to complete the data. Placentas were examined for evidence of infection or other pathology. Retrospectively, the placentas were examined for the presence of *C. trachomatis*.

Histopathology

Immediately after delivery placentas and membranes were fixed in formalin for at least 16 hours. Sampling was performed according to a standard protocol with two membrane rolls, two cross sections of the cord and three representative blocks of the placental disk as a minimum. The tissues were thereafter, according to routine standard methodology for pathological specimens, embedded in paraffin until histopathological examination. To prevent inter-examiner variation, the same pathologist who was specialized in perinatal pathology examined all placentas for histological evidence of inflammation. The pathologist was blinded to clinical information. Placental inflammation was categorized according to a maternal inflammatory response (MIR) and foetal inflammatory response (FIR) as suggested by the Amniotic Fluid Infection Nosology Committee [19], and was considered positive if there was any evidence of a MIR and/or FIR. The MIR was divided into three stages (acute subchorionitis or chorionitis, acute chorioamnionitis, necrotizing chorioamnionitis) representing progression of disease, and two grades representing intensity of disease; the FIR was similarly divided into three stages (chorionic vasculitis or umbilical phlebitis, umbilical vasculitis or umbilical panvasculitis, (subacute) necrotizing funisitis or concentric umbilical perivasculitis) and two grades [19].

After histopathological examination, the placentas were stored in paraffin blocks at room temperature. Before testing for *C. trachomatis*, the paraffin blocks were cut on different days using the same microtome with single use disposable blades. Care was taken to clean materials between cases to avoid contamination. The paraffin blocks were cut until the surface was straight after which five samples per paraffin block each of 10-µm thickness were cut for further microbiological analysis.

Microbiology

DNA Isolation

The tissue samples were deparaffinized with xylene at room temperature, followed by washes in ethanol. Bacterial DNA was isolated using the QIA Amp Tissue Kit (QIAgen, Hilden, Germany). Briefly, after a first step of lysis with proteinase K, DNA was bound to a QIAamp spin column. The column was then washed twice and the purified DNA was eluded from the column in an elution buffer AE and preheated at 70°C according to the manufacturer's instructions.

DNA amplification and detection

Real-time PCR was carried out using the Lightcycler 2.0 system combined with the FastStart DNA Master SYBR Green I kit (Roche Diagnostics, Almere, The Netherlands). The primers NLO 5'-ATGAAAAAACTCTIGAAATCG-3' (position 1-21) and NRO 5'-CTAACTG-TAACTGCGTATTT-3' (position 1128-1108) were used to amplify *C. trachomatis* plasmid

DNA [20]. Each PCR reaction contained 0.5 µM of each primer and 3 mM $MgCl_2$. After an initial denaturation of 10 minutes at 95 ºC, the PCR reactions were subjected to 40 cycles of 15 sec at 95 ºC, 10 sec at 60 ºC and 20 sec at 72 ºC. To check the specificity of the amplification products, a melting curve analysis was performed consisting of heating from 65 ºC to 95 ºC at a rate of 0.1 sec per step and holding for 20 sec at each step for data acquisition. The melting temperature for the specific amplicons was 82 ºC. For each sample, the PCR was done using a separate internal validation (β-globine PCR). For each run a negative control (water) and three positive controls (dilutions of purified *C. trachomatis*) were included.

Ethical aspects

The study was approved by the Medical Ethics Committee for Research on Human Subjects of the Erasmus MC, Rotterdam, The Netherlands.

Statistics

Statistical analysis was performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc. Chicago, Illinois, USA). For comparison of categorical variables between groups the Fisher's Exact test or Pearson's Chi-square test was used. For comparison of the continuous variables the two-tailed Student's T-test was used. The Mantel-Haenszel test was used to asses trends. Statistical significance was considered $P<0.05$.

Results

Three hundred and twenty three pregnant women were eligible for the study. Placental tissue and clinical data were available for 304 (94%) women and their newborns.

C. trachomatis in relation to clinical characteristics

C. trachomatis was detected by PCR in 76/304 (25%) women. The demographic and antenatal clinical characteristics of women with and without *C. trachomatis* are compared in table 1. Maternal age, parity, gravidity, and ethnicity were similar in women with and without *C. trachomatis* infection. Clinical diagnoses of pre-eclampsia ($P<0.01$) and HELLP (haemolysis, elevated liver enzymes, low platelets) syndrome ($P<0.01$) were significantly more prevalent among chlamydia-negative women whereas leucocytosis ($P<0.05$) and a clinical diagnosis of chorioamnionitis ($P<0.01$) were significantly more often prevalent among chlamydia-positive women. No significant differences were observed regarding other symptoms and signs of disease at the time of delivery. Eighty-four women received antibiotics for imminent premature delivery. Chlamydia-positive women received more often antibiotics than chlamydia-negative women: 32/76 (42%) versus 52/228 (23%); (OR 2.5, 95% CI 1.4-4.4). Interestingly, erythromycin was prescribed

Table 1 Clinical characteristics of pregnancies according to *Chlamydia trachomatis* status

	***C. trachomatis* negative n=228**	***C. trachomatis* positive n=76**	**OR (95% CI)**
Antenatal characteristics	**Mean ± SD**	**Mean ± SD**	
maternal age	30.9 ± 5.3	30.1 ± 5.0	NA
para	1.8 ± 1.3	1.9 ± 1.1	NA
gravida	2.2 ± 1.7	2.0 ± 1.2	NA
Symptoms and signs	**n (%)**	**n (%)**	
abdominal pain	5 (2)	4 (5)	2.5 (0.6-9.4)
cervical discharge	18 (8)	8 (11)	1.4 (0.6-3.3)
PROM	63 (28)	24 (32)	1.3 (0.7-2.2)
temperature raised	38 (17)	19 (25)	1.7 (0.9-3.1)
CRP raised	50 (22)	20 (26)	1.3 (0.7-2.3)
leucocytosis	16 (7)	11 (15)	2.2 (1.0-5.1)
Clinical diagnosis			
pre-eclampsia	91 (40)	17 (22)	0.4 (0.2-0.8)
HELLP syndrome	58 (25)	7 (9)	0.3 (0.1-0.7)
clinical chorioamnionitis	62 (27)	34 (45)	2.2 (1.3-3.7)
foetal distress	114 (50)	27 (36)	0.6 (0.3-1.0)
Delivery characteristics			
caesarean section	146 (64)	33 (43)	0.4 (0.3-0.8)
gestational age	29.1 ± 1.9	29.1 ± 1.9	NA
small for gestational age	67 (29)	12 (16)	0.5 (0.2 - 0.9)

NA: not applicable, PROM: premature rupture of membranes, CRP: C-reactive protein,
HELLP: haemolysis, elevated liver enzymes, low platelets

significantly more often to chlamydia-positive than to chlamydia-negative women: 14 (18%) versus 20 (9%); (OR 2.4, 95% CI 1.1-5.2). Prescription of amoxicillin-clavulanic acid and amoxicillin did not differ significantly between the two groups (data not shown).

The delivery characteristics are presented in table 1. Chlamydia-positive women delivered significantly less often by caesarean section than chlamydia-negative women (P<0.01), and delivered less often a newborn after foetal distress (P<0.05). Nine (27%) chlamydia-positive women who delivered by caesarean section had PROM and fifteen (35%) women who delivered vaginally. Neonates born to chlamydia-positive women were significantly less often small for their gestational

Table 2 Risk of *Chlamydia trachomatis* infection according to progression and intensity of inflammation in maternal and foetal placental tissues

	Total	***C. trachomatis* positive**	
Placenta tissue	**n**	**n (%)**	**OR (95% CI)***
no placenta inflammation	181	35 (19)	1.0 (reference)
placenta inflammation	123	41 (33)	2.1 (1.2-3.5)
Maternal tissue			
no inflammation	190	38 (20)	1.0 (reference)
inflammation	114	38 (33)	2.0 (1.1-3.5)
progression**			
stage 1	32	9 (28)	1.6 (0.6-3.9)
stage 2	55	17 (31)	1.8 (0.9-3.7)
stage 3	27	12 (44)	4.3 (1.7-11.2)
intensity***			
grade 1	86	25 (29)	1.6 (0.9-3.1)
grade 2	28	13 (46)	4.7 (1.9-11.9)
Foetal tissue			
no inflammation	236	53 (22)	1.0 (reference)
inflammation	68	23 (34)	1.8 (0.9-3.3)
progression			
stage 1	15	3 (20)	0.8 (0.2-3.5)
stage 2	16	8 (50)	3.5 (1.1-10.7)
stage 3	37	12 (32)	1.7 (0.7-3.7)
intensity			
grade 1	30	11 (37)	2.0 (0.8-4.8)
grade 2	38	12 (32)	1.6 (0.7-3.6)

*OR compared to placenta without inflammation, ** P=0.003 for trend, *** P=0.002 for trend

age (P<0.05) (Table 1). Since these differences could also be due to group differences in maternal morbidity, we adjusted for the two major confounders pre-eclampsia and HELLP. After such adjustment the differences disappeared.

Neonatal outcomes such as umbilical cord blood pH and base excess, Apgar scores, respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular haemorrhage, periventricular

leucomalacia, and neonatal mortality rate were not significantly different between neonates born to chlamydia-positive and chlamydia-negative women (data not shown).

C. trachomatis in relation to histological signs of placental inflammation

Histological evidence of placental inflammation (PI) was present in 123/304 (40%) women of whom 64 (52%) had inflammation diagnosed in both maternal and foetal tissue, 50 (41%) in maternal tissue only, and four (3%) in foetal tissue only (Figure 1). Five (4%) placentas showed other specific features including peripheral funisitis, acute villitis, acute intervillositis with intervillous abscesses, or the presence of decidual plasma cells, but had no signs of a maternal or foetal inflammatory response. Chlamydia-positive women had significantly more often histopathological signs of placental inflammation than chlamydia-negative women: 41/76 (54%) versus 82/228 (36%); (OR 2.1, 95% CI 1.2-3.5) (Table 2).

The progression and intensity of inflammation of maternal and foetal tissues and its relation to *C. trachomatis* infection were assessed separately. Chlamydia-positive women had significantly more often signs of maternal progression stage 3 (Figure 1) and maternal intensity grade 2 in their placentas than chlamydia-negative women (Table 2). In addition, a significantly increasing trend towards more frequent detection of *C. trachomatis* was observed with increasingly higher scores for progression (P=0.003) and intensity (P=0.002) of inflammation (Table 2). On the foetal side, chlamydia-positive women had significantly more often foetal progression stage 2 in their placentas than chlamydia-negative women, but no trend was observed between the prevalence of *C. trachomatis* and the progression or intensity of inflammation in placental tissue (Table 2).

Discussion

Chlamydia trachomatis DNA was detected in a high proportion (25%) of placentas from women who had early preterm delivery (≤ 32 weeks), and *C. trachomatis* infection was associated with histopathological signs of placental inflammation. The presence of *C. trachomatis* did not appear to affect either delivery or neonatal outcomes.

The strength of this study is the prospective, observational design during which the placentas and clinical data were obtained, the large number of well-described participants, a high follow-up rate, and the use of nucleic acid amplification technique (NAAT) for the detection of *C. trachomatis*. In order to prevent contamination of samples during the process of cutting, during DNA isolation or during the PCR test itself, precautions were taken. Paraffin blocks were cut in badges on different days by two different people using a similar microtome with single-use disposable blades, and care was taken to clean materials between cases. In addition, the order of cutting was recorded and no clustering was observed in the detection of *C. trachomatis* in the

cuts. In addition, DNA isolation was done on different days by different laboratory technicians in different locations, and pipetting was performed using aerosol-resistant tips. Furthermore, the PCR was done using an internal validation (β-globine PCR) for each sample separately, and for each run using a negative control (water) and three positive controls (dilutions of purified *C. trachomatis*). None of the negative controls became positive. Therefore, contamination seems unlikely to have influenced the results of the study. A potential weakness of the study is that confounding as a result of co-infection by other pathogens was not tested as part of the study. Indeed, the finding of many cases of placental inflammation in the absence of *C. trachomatis* infection would support the role of other inflammation-inducing pathogens in this cohort of women. Another limitation was that *C. trachomatis* testing was done retrospectively and prospectively no specimens were collected from the infants, which made it impossible to study vertical transmission.

The PCR technique has been used previously to detect *C. trachomatis* in placentas [21-23], but reports focusing on the use of PCR to detect *C. trachomatis* in placentas of early preterm deliveries are very limited and from much smaller cohorts. One case report described a stillborn at 36 weeks of gestation in whose placenta *C. trachomatis* was identified [21]. In a Chinese study 59 specimens of chorionic villi were examined that had been collected from women attending an antenatal clinic for artificial abortion within the first trimester of pregnancy, of which three cases (5%) were found to be *C. trachomatis* positive [22]. In a Croatian study of women with a miscarriage between four and 19 weeks gestation, *C. trachomatis* was detected in only one of 108 placental tissues examined [23].

A 25% prevalence of *C. trachomatis* in pregnant women is high. It is much higher than the *C. trachomatis* prevalence in the general population from which these pregnant women originated [24]. However, the current study population was a selected group of women who all delivered at 32 weeks gestation or earlier. The findings, though, suggest an association between *C. trachomatis* infection and preterm delivery. Indeed, the prevalence is similar to that found in one of our previous population-based studies among 4,055 pregnant women in the same region and during the same time as the present study, and for whom the referal centre is the hospital in which women and neonates were enrolled for the present study. In the latter study we found a *C. trachomatis* prevalence of 4% among all pregnant women and 18% among the women who delivered at 32 weeks gestation or less [25]. Others also reported an association between *C. trachomatis* infection and preterm delivery [16-18, 26-28]

We found no difference in maternal age, parity, gravidity, ethnicity, abdominal pain or cervical discharge between chlamydia-positive and chlamydia-negative women. One might have expected to find chlamydia-positive women to be younger than the control group. However, our findings are not surprising since chlamydia-positive women were not compared with a healthy

control group, but with another group of women with disease who had significant other risks for premature delivery such as pre-eclampsia and HELLP syndrome [29]. Individual symptoms and signs indicative of maternal infection were not significantly associated with *C. trachomatis* infection. However, symptoms and signs resulting in a clinical diagnosis of chorioamnionitis and leucocytosis, both of which are known to be related to infection, were significantly associated with the presence of *C. trachomatis*.

The delivery characteristics and neonatal outcome did not differ between the chlamydia-positive and chlamydia-negative group, although *C. trachomatis* infected women seemed less likely to deliver by caesarean section for presumed foetal distress and their newborns were less often small for their gestational age. Again, these differences were due to comparison with another group of women with disease, pre-eclampsia and HELLP syndrome, instead of a healthy control group as shown by loss of these differences after adjustment for the latter confounders. We found no difference in acute or chronic respiratory pathology between neonates born to chlamydia-positive and chlamydia-negative women. However, since no respiratory specimens were obtained from the newborns and follow-up was done for only 28 days, we cannot ascribe any respiratory disease to *C. trachomatis*. Neither were specimens collected to diagnose neonatal conjunctivitis.

Histologically proven chorioamnionitis is considered the gold standard against which other clinical predictors of inflammation should be measured [2, 30, 31]. Histological evidence of placental inflammation was present in 40% of the women of whom virtually all had signs of maternal inflammation whereas only half the time foetal inflammation was present. Only maternal inflammation would be concordant with infection originating from the maternal side. Indeed, *C. trachomatis* was detected more often in placentas with maternal inflammation than in those with foetal inflammation. In addition, we found an association between the prevalence of *C. trachomatis* detection and progression and intensity of tissue inflammation only with maternal inflammation. Our findings, therefore, suggest that ascending *C. trachomatis* infection during pregnancy may extend into the placental tissues. Such invasion of placental tissue is likely to produce an inflammatory response that may trigger preterm labor and delivery [32].

C. trachomatis is transmitted from an infected mother to her newborn during passage through the birth canal. However, there are several, mostly anecdotal, reports of newborns delivered by caesarean section that were infected with *C. trachomatis* [33-37]. Ascending chlamydial infection in women undergoing caesarean section may be due to PROM, but the possibility of a transmembrane or transplacental route has also been suggested in the pathogenesis of neonatal chlamydial infection [34, 36]. In our study 43% of chlamydia-positive women gave birth by caesarean section of which 27% had PROM. Unfortunately, we are unable to confirm the latter hypothesis since no specimens were collected from the newborns. The chorioamnionitis study was not designed as such.

In conclusion *C. trachomatis* DNA was frequently detected in the placenta of women with early preterm delivery. *C. trachomatis* infection was associated with signs of maternal inflammation in the placenta, but not with any adverse outcome. Additional research is needed to study a transmembrane or transplacental route of transmission for chlamydial infection.

References

[1] McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med. 1985;312(2):82-90.

[2] Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N Engl J Med. 1988;319(15):972-8.

[3] Lettieri L, Vintzileos AM, Rodis JF, Albini SM, Salafia CM. Does "idiopathic" preterm labor resulting in preterm birth exist? Am J Obstet Gynecol. 1993;168(5):1480-5.

[4] Salafia CM, Vogel CA, Vintzileos AM, Bantham KF, Pezzullo J, Silberman L. Placental pathologic findings in preterm birth. Am J Obstet Gynecol. 1991;165(4 Pt 1):934-8.

[5] Watterberg KL, Demers LM, Scott SM, Murphy S. Chorioamnionitis and early lung inflammation in infants in whom bronchopulmonary dysplasia develops. Pediatrics. 1996;97(2):210-5.

[6] De Felice C, Toti P, Laurini RN, Stumpo M, Picciolini E, Todros T, et al. Early neonatal brain injury in histologic chorioamnionitis. J Pediatr. 2001;138(1):101-4.

[7] Mehta R, Nanjundaswamy S, Shen-Schwarz S, Petrova A. Neonatal morbidity and placental pathology. Indian J Pediatr. 2006;73(1):25-8.

[8] Hagberg H, Wennerholm UB, Savman K. Sequelae of chorioamnionitis. Curr Opin Infect Dis. 2002;15(3):301-6.

[9] Laar M.J.W.vd, Ossewaarde J.M. Sexually transmitted diseases in the Netherlands; update 1996; report no.: 441500006. 1997.

[10] Harrison HR, Alexander ER, Weinstein L, Lewis M, Nash M, Sim DA. Cervical *Chlamydia trachomatis* and mycoplasmal infections in pregnancy. Epidemiology and outcomes. JAMA. 1983;250(13):1721-7.

[11] Gravett MG, Nelson HP, DeRouen T, Critchlow C, Eschenbach DA, Holmes KK. Independent associations of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. JAMA. 1986;256(14):1899-903.

[12] Sweet RL, Landers DV, Walker C, Schachter J. *Chlamydia trachomatis* infection and pregnancy outcome. Am J Obstet Gynecol. 1987;156(4):824-33.

[13] Association of *Chlamydia trachomatis* and *Mycoplasma hominis* with intrauterine growth retardation and preterm delivery. The John Hopkins Study of Cervicitis and Adverse Pregnancy Outcome. Am J Epidemiol. 1989;129(6):1247-57.

[14] Ngassa PC, Egbe JA. Maternal genital *Chlamydia trachomatis* infection and the risk of preterm labor. Int J Gynaecol Obstet. 1994;47(3):241-6.

[15] Claman P, Toye B, Peeling RW, Jessamine P, Belcher J. Serologic evidence of *Chlamydia trachomatis* infection and risk of preterm birth. CMAJ. 1995;153(3):259-62.

[16] Andrews WW, Goldenberg RL, Mercer B, Iams J, Meis P, Moawad A, et al. The Preterm Prediction Study:

association of second-trimester genitourinary chlamydia infection with subsequent spontaneous preterm birth. Am J Obstet Gynecol. 2000;183(3):662-8.

[17] Odendaal HJ Schoeman J. The association between *Chlamydia trachomatis* genital infection and spontaneous preterm labour. South African Journal of Obstetrics and Gynaecology. 2006;12(3):146-9.

[18] Blas MM, Canchihuaman FA, Alva IE, Hawes SE. Pregnancy outcomes in women infected with *Chlamydia trachomatis*: a population-based cohort study in Washington State. Sex Transm Infect. 2007;83(4):314-8.

[19] Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2003;6(5):435-48.

[20] Lan J, Ossewaarde JM, Walboomers JM, Meijer CJ, van den Brule AJ. Improved PCR sensitivity for direct genotyping of *Chlamydia trachomatis* serovars by using a nested PCR. J Clin Microbiol. 1994;32(2):528-30.

[21] Gencay M, Puolakkainen M, Wahlstrom T, Ammala P, Mannonen L, Vaheri A, et al. *Chlamydia trachomatis* detected in human placenta. J Clin Pathol. 1997;50(10):852-5.

[22] Dong ZW, Li Y, Zhang LY, Liu RM. Detection of *Chlamydia trachomatis* intrauterine infection using polymerase chain reaction on chorionic villi. Int J Gynaecol Obstet. 1998;61(1):29-32.

[23] Matovina M, Husnjak K, Milutin N, Ciglar S, Grce M. Possible role of bacterial and viral infections in miscarriages. Fertil Steril. 2004;81(3):662-9.

[24] van Bergen J, Gotz HM, Richardus JH, Hoebe CJ, Broer J, Coenen AJ. Prevalence of urogenital *Chlamydia trachomatis* increases significantly with level of urbanisation and suggests targeted screening approaches: results from the first national population based study in the Netherlands. Sex Transm Infect. 2005;81(1):17-23.

[25] Rours GIJG, Duijts L, Moll HA, de Groot R, Jaddoe VW, Hofman A, Steegers EAP, Mackenbach JP, Ott A, Willemse HFM, van der Zwaan AE, Verbrugh HA, Verkooijen RP. Pregnancy outcomes in women infected with *Chlamydia trachomatis*: a population-based prospective study cohort. Proceedings Sixth Meeting of the European Society for Chlamydia Research 2008; P32. 2008.

[26] Martin DH, Koutsky L, Eschenbach DA, Daling JR, Alexander ER, Benedetti JK, et al. Prematurity and perinatal mortality in pregnancies complicated by maternal *Chlamydia trachomatis* infections. JAMA. 1982;247(11):1585-8.

[27] Martius J, Krohn MA, Hillier SL, Stamm WE, Holmes KK, Eschenbach DA. Relationships of vaginal Lactobacillus species, cervical *Chlamydia trachomatis*, and bacterial vaginosis to preterm birth. Obstet Gynecol. 1988;71(1):89-95.

[28] Gencay M, Koskiniemi M, Ammala P, Fellman V, Narvanen A, Wahlstrom T, et al. *Chlamydia trachomatis* seropositivity is associated both with stillbirth and preterm delivery. APMIS. 2000;108(9):584-8.

[29] Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84.

[30] Pankuch GA, Appelbaum PC, Lorenz RP, Botti JJ, Schachter J, Naeye RL. Placental microbiology and histology and the pathogenesis of chorioamnionitis. Obstet Gynecol. 1984;64(6):802-6.

[31] Romero R, Salafia CM, Athanassiadis AP, Hanaoka S, Mazor M, Sepulveda W, et al. The relationship between acute inflammatory lesions of the preterm placenta and amniotic fluid microbiology. Am J Obstet Gynecol. 1992;166(5):1382-8.

[32] Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000;342(20):1500-7.

[33] Abiose A, Murdoch I, Babalola O, Cousens S, Liman I, Onyema J, et al. Distribution and aetiology of blindness and visual impairment in mesoendemic onchocercal communities, Kaduna State, Nigeria. Kaduna Collaboration

for Research on Onchocerciasis. Br J Ophthalmol. 1994;78(1):8-13.

[34] Shariat H, Young M, Abedin M. An interesting case presentation: a possible new route for perinatal acquisition of Chlamydia. J Perinatol. 1992;12(3):300-2.

[35] Bell TA. *Chlamydia trachomatis* infection in dizygotic twins delivered by caesarean section. Genitourin Med. 1988;64(5):347-8.

[36] La Scolea LJ, Jr., Paroski JS, Burzynski L, Faden HS. *Chlamydia trachomatis* infection in infants delivered by cesarean section. Clin Pediatr (Phila). 1984;23(2):118-20.

[37] Givner LB, Rennels MB, Woodward CL, Huang SW. *Chlamydia trachomatis* infection in infant delivered by cesarean section. Pediatrics. 1981;68(3):420-1.

Chapter 7

Chlamydia trachomatis as a cause of neonatal conjunctivitis in Dutch infants

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Abstract

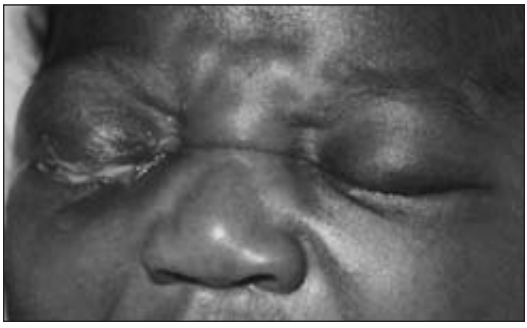
Background *Chlamydia trachomatis* is the most common sexually transmitted pathogen in adults, which at delivery may be transmitted from mother to child and cause conjunctivitis and pneumonia. In the Netherlands, prenatal chlamydial screening and treatment of pregnant women is not routine practice. The contribution of *C. trachomatis* to neonatal ophthalmic disease has not been studied in the Netherlands and remains unclear.

Objective To asses whether *Chlamydia trachomatis* is a cause of neonatal conjunctivitis in a Dutch population and evaluate the clinical presentation and treatment.

Methods At the Erasmus MC-Sophia and Rotterdam Eye Hospital, two cohorts of infants less than three months of age presenting with conjunctivitis were studied, one retrospectively (July 1996 to July 2001), and one prospectively (September 2001 to September 2002). Laboratory diagnosis was based on bacterial culture and PCR for *C. trachomatis.*

Results *C. trachomatis* was detected in 27 (64%) of 42 retrospectively studied infants and 14 (61%) of 23 prospectively studied infants. Mucopurulent discharge was present in 35 (95%) of 37, swelling of the eyes in 27 (73%) of 37, conjunctival erythema in 24 (65%) of 37, respiratory symptoms in 14 (38%) of 37 and feeding problems in five (14%) of 37 infants respectively. Before microbiological diagnosis, general practitioners prescribed anti-chlamydial antibiotics locally to five (12%) and systemically to four (10%) of 41 infants who tested positive for *C. trachomatis*, and ophthalmologists to 21 (51%) and seven (17%) of 41, respectively.

Conclusions *C. trachomatis* was the major cause of bacterial conjunctivitis in this population. Clinically, differentiation from other pathogens was not possible. Many infants who tested positive for *C. trachomatis* did not receive appropriate antibiotic treatment.



Neonatal *Chlamydia trachomatis* conjunctivitis, courtesy of MR Hammerschlag

Introduction

The occurrence of *Chlamydia trachomatis* infection in infants is directly related to the prevalence of maternal urogenital infections and vertical transmission rates [1-4]. The overall risk for infants born to women with untreated chlamydial infection is approximately 50 to 75%, with infection occurring at one or more anatomic sites. Conjunctivitis may occur in 20 to 50% of infected infants, nasopharyngitis in up to 70% and pneumonia in 5 to 20% [5]. *C. trachomatis* has become the most frequent identifiable cause of neonatal conjunctivitis in many countries [6-9]. The majority of chlamydial conjunctivitis cases heal spontaneously during the first few months of life. However, untreated persistent infections can lead to acute discomfort and distress for both infant and mother, as well as to chronic eye disease. Simultaneous silent infection of the respiratory tract may cause acute or chronic respiratory disease [3, 10, 11].

Screening of pregnant women for *C. trachomatis* infection was recommended by the CDC more than a decade ago [12, 13]. In 2004 the Dutch National Health Council advised against routine chlamydial screening of Dutch pregnant women, because local data with respect to chlamydial infection in pregnant women and the contribution of *C. trachomatis* to neonatal disease (pregnancy outcome, conjunctivitis, respiratory tract infection) provided insufficient evidence to support screening [14, 15]. Recently we reported a prevalence of *C. trachomatis* infection in pregnant women of 6.4% [16]. Most of these infections had been missed in routine care, suggesting that *C. trachomatis* transmission to infants may often remain unnoticed.

The main objective of this study was to establish whether *C. trachomatis* was a cause of neonatal conjunctivitis in infants referred to hospital-based care in Rotterdam, the second largest city in the Netherlands. In addition, we evaluated the clinical presentation of and prescribed treatment for chlamydial conjunctivitis compared with other infections.

Materials and Methods

Study population

We conducted our study at the Rotterdam Eye Hospital (REH) and Erasmus MC-Sophia (SCH), Rotterdam, The Netherlands. Rotterdam has a multi-ethnic population. Chlamydial screening is not standard practice in pregnant women in the study area; testing is done on clinical suspicion. Neonatal ocular prophylaxis against chlamydia or gonorrhoea is not routinely provided. Most newborns with conjunctivitis are treated at mother and child health clinics or by general practitioners (GPs). Infants with persistent conjunctivitis are referred to the REH or SCH depending upon the parents' or referring GP's choice. We studied infants retrospectively between July 1996 and July 2001 and prospectively between September 2001 and September 2002. Infants less than three months of age presenting to the REH or SCH with bacterial

(mostly persistent) conjunctivitis to the REH or SCH were eligible for the study. We defined bacterial conjunctivitis as having conjunctival erythema, swelling of the eyelids and/or mucopurulent discharge. In the retrospective study, infants who were diagnosed by ophthalmologists with viral conjunctivitis (conjunctival erythema only) and who were neither microbiologically tested nor treated with antibiotics and improved spontaneously were excluded from the study. Similarly, infants with dacryostenosis (nasolacrimal duct obstruction) were excluded from the study. Infants diagnosed with chlamydial conjunctivitis in the REH were referred to the SCH for further investigation and systemic treatment. Infants diagnosed with *C. trachomatis* conjunctivitis in our prospective study were treated systemically with erythromycin suspension (ethylsuccinate), 50 mg/kg/day in 3 to 4 oral doses for 10-14 days and were invited for a single follow-up visit. Parents were asked to return to the clinic if the infant had respiratory symptoms. Parents themselves were referred to the sexually transmitted disease clinic for investigation and treatment.

Microbiological diagnosis

Eye swabs were taken for routine bacterial culture, including gonococcal culture, and chlamydial and gonococcal polymerase chain reaction (PCR) (Cobas Amplicor, Roche Molecular Diagnostics, Pleasanton, USA). Specimens were obtained by swabbing the conjunctiva of the everted lower eyelid using a sterile Dacron swab. Bacteriologic cultures were processed according to standard procedures for aerobic bacteria in a clinical microbiology laboratory. Chlamydial and gonococcal PCRs were performed according to the manufacturer's instructions.

Treatment

Antibiotics that were prescribed and considered to be effective against *C. trachomatis* were erythromycin, azithromycin, clarithromycin, tetracycline and doxycyline, and chloramphenicol.

Data collection

We collected retrospective data through a systematic review of medical records. We used a standardised questionnaire and laboratory investigations to collect prospective data. Recorded variables included the following: age at presentation, gender, complaints (conjunctival erythema, swelling of the eyelids, mucopurulent discharge, unilateral/bilateral involvement, and respiratory or feeding problems), physical examination, diagnostic tests, diagnosis, and therapy by GPs and ophthalmologists before microbiological diagnosis.

Statistical analysis

We used SPSS 10.0.0 statistical software (SPSS Inc., Chicago, IL) for our analyses. We used uncorrected chi-square tests to compare categorical variables. We calculated risk ratios (with 95% confidence intervals) to examine factors associated with a diagnosis of chlamydia conjunctivitis.

Results

Demographics

Retrospectively, 64 infants with conjunctivitis were identified; 36 (56%) were male. The median age was 2 weeks, with a range of 0 to 13 weeks. Prospectively, 23 infants were enrolled; 12 (52%) were male. The median age was 1 week, with a range of 0 to 7 weeks.

Diagnostic tests

Five of 64 retrospectively studied infants were clinically diagnosed with bacterial conjunctivitis without performing a laboratory test. The remaining 59 infants had a bacterial culture done, including gonococcal culture; 42 infants also had a chlamydial PCR and 17 infants a gonococcal PCR. With inclusion of all tests, 50 (85%) of 59 tested infants had a positive result. All 23 of the prospectively studied infants had bacterial and gonococcal cultures done, as well as a chlamydial and gonococcal PCR. Nineteen infants (83%) had a pathogen detected. Table 1 shows the frequency of isolated species. Detection of *C. trachomatis* was significantly higher than of other pathogens (P<0.001), with similar rates in retrospectively and prospectively studied infants: 27 (64%) of 42 and

Table 1 Identified pathogens among infants less 3 months of age with conjunctivitis

Pathogens detected by test method	Retrospective cohort	Prospective cohort	Total infants	P-value*	RR (95% CI)*
Bacterial culture, n	59	23	82		
S. aureus, n (%)	11 (19)	0 (0)	11 (13)		
H. influenzae, n (%)	5 (8)	2 (9)	7 (9)		
S. pneumoniae, n (%)	4 (7)	0 (0)	4 (5)		
N. gonorrhoeae, n (%)	2 (3)	0 (0)	2 (2)		
other pathogens, n (%)	4 (7)†	3 (13)‡	7 (9)		
cultures with pathogens, n (%) #	24 (41)	5 (22)	29 (35)		
PCR	n = 17	n = 23	n = 40		
N. gonorrhoeae, n (%)	1 (6)	0 (0)	1 (3)		
PCR	n = 42	n = 23	n = 65		
C. trachomatis, n (%)	27 (64)	14 (61)	41 (63)	<0.001	1.8 (1.3-2.5)

RR: risk ratio; CI: confidence interval

* P-value is for the difference in the yield between the non-chlamydia pathogen culture (29 of 82) and *C. trachomatis* PCR (41 of 65); RR reflects the higher likelihood of finding *C. trachomatis* than another pathogen in these infants

Three co-infections included *H. influenzae*, twice with *N. gonorrhoeae* and once with *S. pneumoniae*

† Other pathogens included *Moraxella catarrhalis, Escherichia coli, Stenotrophomonas maltophilia, haemolytic streptococcus*

‡ Other pathogens included two *M. catarrhalis* and one *N. meningitidis*

14 (61%) of 23, respectively. Three infants in the retrospective cohort with *Haemophilus influenzae* had a second pathogen diagnosed.

Clinical presentation

We examined the age of 69 infants with microbiologically confirmed conjunctivitis. Of these infants, 41 had chlamydial conjunctivitis and 28 had another infection (Table 2). Infants with chlamydial conjunctivitis were 2.3 times (95% CI 1.0-5.2 times) more likely than those with other infections to present between one and six weeks of age than within the first week of life. We were able to examine clinical information in 37 infants with chlamydial conjunctivitis (23 diagnosed retrospectively and 14 prospectively) and 22 with another pathogen (17 diagnosed retrospectively and 5 prospectively). Because there was no significant difference between the retrospective and prospective study, and the number of infants in each study was small, the overall results of a total of 37 evaluable infants who tested positive for *C. trachomatis* and 22 infants with another pathogen are shown in table 2. Mucopurulent discharge was the presenting

Table 2 Clinical presentation of neonatal conjunctivitis by causative pathogen

	C. trachomatis	Other pathogens		RR
	n (%)	n (%)	P-value*	(95% CI)
Age at presentation	41	28	0.03	
< 1 week	4 (10)	9 (32)		1.0 (reference)
1-6 weeks	34 (83)	15 (54)		2.3 (1.0-5.2)
> 6 weeks	3 (7)	4 (14)		1.4 (0.4-4.5)
Number of symptoms	37	22	0.26	
One symptom	8 (22)	9 (41)		1.0 (reference)
mp discharge	7 (19)	9 (41)		
redness	1 (3)	0 (0)		
Two symptoms	9 (24)	5 (23)		1.4 (0.7-2.6)
mp discharge + swelling	6 (16)	3 (14)		
mp discharge + redness	2 (5)	2 (9)		
redness + swelling	1 (3)	0 (0)		
Three symptoms	20 (54)	8 (36)		1.5 (0.9-2.7)
mp discharge + swelling + redness				
Extra-ophthalmic symptoms	37	20		
respiratory	14 (38)	6 (30)	0.55	1.3 (0.6-2.8)
feeding	5 (14)	2 (10)	0.70	1.4 (0.3-6.4)

RR: risk ratio; CI: confidence interval, mp: mucopurulent

*P-value for the test of heterogeneity of age or number of symptom categories; P-value of extra-ophthalmic symptoms for the difference between pathogen categories

symptom for 35 (95%) of 37 infants who tested positive for *C. trachomatis*, swelling of the eyelids for 27 (73%) of 37, and conjunctival erythema for 24 (65%) of 37 compared with 22 (100%) of 22, 11 (50%) of 22, and 10 (45%) of 22, respectively, for infants with other pathogens; 27 (73%) of 37 infants who tested positive for *C. trachomatis* had bilateral eye involvement compared with 17 (77%) of 22 of infants with other pathogens. The presence of extraophthalmic symptoms did not differ between infants with chlamydia and those with another pathogen (Table 2). Feeding difficulties corresponded with respiratory complications in infants who tested positive for *C. trachomatis*. In the other group, these were separate cases. Additional symptoms in infants who tested positive for chlamydia included rhinitis (12 of 37), cough (4 of 37), excessive mucous (3 of 37), and wheezing or breathing difficulty (2 of 37). In the group of infants with other pathogens, 4 of 20 had rhinitis; one had cough, and one had wheezing and crepitations.

Therapy

Both GPs and ophthalmologists prescribed topical antibiotics as eye ointment, gel, or drops, including tetracycline, aminoglycosides with or without steroids (gentamicin, soframycin, or tobramycin), fusidic acid, polymyxin/trimethoprim, ofloxacin, and erythromycin. In addition, GPs also prescribed chloramphenicol. Both GPs and ophthalmologists prescribed systemic treatment, including penicillin, clarithromycin and erythromycin. In addition, GPs used co-trimoxazole and ophthalmologists used amoxicilin, augmentin, flucloxacillin, and cefotaxime.

Antibiotic treatment by diagnosis as prescribed by GPs and ophthalmologists is shown in table 3. Comparison of treatment in the retrospective and prospective cohort showed that ophthalmologists gave empiric antibiotics to 17 (63%) of 27 infants who tested positive for *C. trachomatis* in the retrospective versus 14 (100%) of 14 in the prospective study, (P<0.01), anti-chlamydial antibiotics to 14 (52%) of 27 and 7 (50%) of 14 infants (P=0.9), and systemic treatment to 6 (22%) of 27 and one (7%) of 14 infants, respectively (P=0.22).

Table 3 Antibiotic treatment prescribed by GPs and ophthalmologistss

	GPs	Ophthalmologists	
Antibiotic treatment according to diagnosis	**n/N (%)**	**n/N (%)**	**P-value ***
Any confirmed bacterial conjunctivitis			
any antibiotic	30/69 (43)	51/69 (74)	<0.001
***C. trachomatis* conjunctivitis**			
any antibiotic	20/41 (49)	31/41 (76)	0.01
anti-chlamydial antibiotic	5/41 (12)	21/41 (51)	<0.001
systemic anti-chlamydial antibiotic	4/41 (10)	7/41 (17)	0.33

* P-value for the difference in treatment choice between GPs and ophthalmologists

Follow-up

At follow-up, no more conjunctivitis was observed, and no symptoms or signs of respiratory tract infection were present.

Discussion

This study demonstrated that *C. trachomatis* was the major cause of neonatal conjunctivitis in this population of infants referred to paediatric or eye hospitals in Rotterdam. On clinical presentation, no distinction could be made between chlamydial conjunctivitis and conjunctivitis caused by other pathogens. Empiric management to treat chlamydial conjunctivitis was frequently inappropriate.

The main limitation of this study was that we could only include infants with conjunctivitis who were referred to specialist hospitals. Other limitations were the incomplete diagnostic work-up in the retrospective cohort and missing data on clinical symptoms in a few infants. Also, the number of children in analyses was relatively small, too small to draw firm conclusions. Although we cannot show what proportion of all neonatal conjunctivitis is caused by *C. trachomatis*, we have shown that it is an important cause of (persistent) conjunctivitis. Determining the incidence of chlamydial neonatal conjunctivitis and transmission rates would require a prospective study testing all pregnant women and their newborns.

The identification rate of pathogenic bacteria in our study was 84%, which is higher than in most reports [17-20]. In addition, *C. trachomatis* was the organism isolated most often. This is likely to be related to our study design and to the Dutch referral system, in which mainly infants with persistent conjunctivitis are referred to ophthalmologists. However, we may even have underestimated the contribution of chlamydia to persistent neonatal conjunctivitis, because we excluded the infants with a clinical diagnosis of viral conjunctivitis. Underdiagnosis of *C. trachomatis* persistent conjunctivitis may also be suggested by the relatively low observed number of five cases per year in the retrospective study versus 14 in the prospective year. However, the latter increase may also reflect an actual increase of chlamydial infection in Dutch adults [21].

To put our results into context, we related the findings from our prospective study to obstetric data from the SCH in that year. There were 1,648 deliveries in total, of which 731 were in women under 30 years of age. With an antenatal prevalence of 6.4% [16], about 47 women would be expected to have *C. trachomatis* infection. With an estimated transmission rate of 50 to 75% and 20 to 50% of these developing conjunctivitis [5], we would expect 9 to 24 infants with chlamydial conjunctivitis, which corresponds with our findings. This may suggest that all

of the infants with conjunctivitis were detected. However, not all studied infants were born to women delivering at the SCH, but also in other hospitals in Rotterdam or at home.

The clinical presentation of chlamydial conjunctivitis has been extensively described by others and our study shows similar results to previous reports [3, 4, 9, 22-24]. Most infants who tested positive for *C. trachomatis* presented between the ages of one and six weeks (because of the slow reproductivity of the organism), and with all three of the symptoms (erythema, swelling, discharge). Ophthalmologists more often prescribed antibiotics that were effective against chlamydial infection than GPs. Still, only half of the infants with chlamydial conjunctivitis received effective antibiotics before microbiological diagnosis, and less than 20% received systemic treatment. In the prospective study, even fewer infants received systemic treatment. This probably reflects the agreement made with ophthalmologists to refer infants to a paediatrician after diagnosis. We cannot draw any conclusions about chlamydial conjunctivitis in primary health care from our study. Acute conjunctivitis may be correctly treated by GPs with topical antibiotics according to Health Care Insurance Board guidelines. However, *C. trachomatis* is probably not being considered in the differential diagnosis by some GPs, because only 12% of infants with chlamydia had received antibiotics that were active against *C. trachomatis.*

Our results may suggest the need to institute eye prophylaxis for ophthalmia neonatorum, which is not practiced in the Netherlands. However, we do not want to advocate the application of neonatal eye prophylaxis, because prophylaxis does not prevent all chlamydial neonatal conjunctivitis, and the absence of conjunctivitis as an indicator of chlamydial infection may delay the proper diagnosis of (silent) chlamydial infection at other sites. Another motivation against the decision to start routine eye prophylaxis is that this may lead to overtreatment of newborns in a country with easy access to medical care. We would rather like to use our findings to indicate the need for proper (systemic) antibiotic treatment of infants who test positive for *C. trachomatis* and screening of pregnant women.

Chlamydial screening for pregnant women is not standard practice in the study area or in the rest of the Netherlands. Testing is only done when clinically warranted. In the prospective year of the study, only 1% of 1,648 deliveries were tested for *C. trachomatis*, of which 4 tested positive. These figures may also reflect the underestimation of chlamydial infection in pregnant women. The value of screening may warrant further discussion [16]. To prevent one case of chlamydial conjunctivitis, 31 to 78 pregnant women need to be screened. Assuming a specificity of 99.8% or more [16], this could result in 0.06 to 0.16 false-positive mothers and 2 to 5 truly positive mothers (and their partners); furthermore, this could result in the prevention of 1.4 to 2.5 nasopharyngitis cases and 0.2-1.0 pneumonia cases. Previously we described the costs for *C. trachomatis* screening of pregnant women by different DNA-isolation methods in individual

and pooled urine samples [16]. The cost per *C. trachomatis* case detected when using the Cobas Amplicor on individual urine samples was 275 euros and when using a combined method of isolation with the MagNA Pure bacterial DNA-isolation kit (Roche Molecular Diagnostics, Pleasanton, USA), and subsequent amplification and detection by Cobas Amplicor on pooled urine samples, as we did, was 108 euros.

In the absence of a chlamydial screening program, we urge clinicians to have a higher index of suspicion for neonatal and maternal chlamydial infection. Furthermore, we recommend that in areas where prenatal chlamydial screening and treatment of pregnant women is not routine practice, infants with signs of conjunctivitis that persist for more than 72 hours while applying frequent normal saline eye irrigation should have a full microbiological evaluation including *N. gonorrhoeae* and *C. trachomatis*. While awaiting laboratory results, empiric treatment in this population should include erythromycin eye drops or ointment to both eyes. We recommend that confirmed chlamydial conjunctivitis should be treated systemically with erythromycin suspension or alternatively azithromycin [13, 25].

Current national treatment guidelines for neonatal chlamydial conjunctivitis are from the Health Care Insurance Board and recommend tetracycline eye drops (with or without oral erythromycin or tetracycline) [26]. The current CDC recommendation, however, is to treat all neonatal chlamydial conjunctivitis systemically with a 14-day course of erythromycin suspension [13]. Systemic treatment has been demonstrated to be more effective than topical treatment, and infants with conjunctivitis are often infected at other sites as well [27].

In conclusion the high rate of *C. trachomatis* in infants with persistent conjunctivitis confirms the importance of this infection in the Netherlands. GPs and ophthalmologists need to consider *C. trachomatis* in the differential diagnosis of neonatal conjunctivitis and use appropriate antibiotics. Targeted screening for *C. trachomatis* prenatally to prevent infection and related complications in both mother and infants should again be considered in those countries where it is not routine practice.

References

[1] Bell TA, Stamm WE, Kuo CC, Wang SP, Holmes KK, Grayston JT. Risk of perinatal transmission of *Chlamydia trachomatis* by mode of delivery. J Infect. 1994;29(2):165-9.

[2] Schachter J, Sweet RL, Grossman M, Landers D, Robbie M, Bishop E. Experience with the routine use of erythromycin for chlamydial infections in pregnancy. N Engl J Med. 1986;314(5):276-9.

[3] Preece PM, Anderson JM, Thompson RG. *Chlamydia trachomatis* infection in infants: a prospective study. Arch Dis Child. 1989;64(4):525-9.

[4] Alexander ER, Harrison HR. Role of *Chlamydia trachomatis* in perinatal infection. Rev Infect Dis. 1983;5(4):713-9.

[5] Hammerschlag MR. Chlamydial infections. J Pediatr. 1989;114(5):727-34.

[6] Laga M, Plummer FA, Nzanze H, Namaara W, Brunham RC, Ndinya-Achola JO, et al. Epidemiology of ophthalmia neonatorum in Kenya. Lancet. 1986;2(8516):1145-9.

[7] Sergiwa A, Pratt BC, Eren E, Sunona TC, Hart CA. Ophthalmia neonatorum in Bangkok: the significance of *Chlamydia trachomatis*. Ann Trop Paediatr. 1993;13(3):233-6.

[8] Sandstrom I. Etiology and diagnosis of neonatal conjunctivitis. Acta Paediatr Scand. 1987;76(2):221-7.

[9] Dannevig L, Straume B, Melby K. Ophthalmia neonatorum in northern Norway. II. Microbiology with emphasis on *Chlamydia trachomatis*. Acta Ophthalmol (Copenh). 1992;70(1):19-25.

[10] Mordhorst CH, Dawson C. Sequelae of neonatal inclusion conjunctivitis and associated disease in parents. Am J Ophthalmol. 1971;71(4):861-7.

[11] Harrison HR, English MG, Lee CK, Alexander ER. *Chlamydia trachomatis* infant pneumonitis: comparison with matched controls and other infant pneumonitis. N Engl J Med. 1978;298(13):702-8.

[12] Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. MMWR Morb Mortal Wkly Rep. 1989; 38 (suppl 8): 1-43; 1989.

[13] Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep 2006;55(RR-11):1-94.

[14] Health Council of the Netherlands. Screening for Chlamydia. The Hague, The Netherlands: Health, No 2004/7:1-106.

[15] Laar MJWvd. SOA en AIDS in Nederland. Bilthoven, The Netherlands: Rijksinstituut voor Volksgezondheid en Milieu; 2000. Report No.: 441500011.

[16] Rours GI, Verkooyen RP, Willemse HF, van der Zwaan EA, van Belkum A, de Groot R, et al. Use of Pooled Urine Samples and Automated DNA Isolation To Achieve Improved Sensitivity and Cost-Effectiveness of Large-Scale Testing for *Chlamydia trachomatis* in Pregnant Women. J Clin Microbiol. 2005;43(9):4684-90.

[17] Sandstrom KI, Bell TA, Chandler JW, Kuo CC, Wang SP, Grayston JT, et al. Microbial causes of neonatal conjunctivitis. J Pediatr. 1984;105(5):706-11.

[18] Fransen L, Van den Berghe P, Mertens A, Van Brussel K, Clara R, Piot P. Incidence and bacterial aetiology of neonatal conjunctivitis. Eur J Pediatr. 1987;146(2):152-5.

[19] Di Bartolomeo S, Mirta DH, Janer M, Rodriguez Fermepin MR, Sauka D, Magarinos F, et al. Incidence of *Chlamydia trachomatis* and other potential pathogens in neonatal conjunctivitis. Int J Infect Dis. 2001;5(3):139-43.

[20] Persson K, Ronnerstam R, Svanberg L, Pohla MA. Neonatal chlamydial eye infection: an epidemiological and clinical study. Br J Ophthalmol. 1983;67(10):700-4.

[21] Kolader ME, Thiesbrummel HFJ, Peetam E. Year Report STD Clinic 2004. Amsterdam, The Netherlands: Municipal Health Services Amsterdam; 2004.

[22] Hammerschlag MR. Neonatal conjunctivitis. Pediatr Ann. 1993;22(6):346-51.

[23] Stenberg K, Mardh PA. Chlamydial conjunctivitis in neonates and adults. History, clinical findings and follow-up. Acta Ophthalmol (Copenh). 1990;68(6):651-7.

[24] Hammerschlag MR, Chandler JW, Alexander ER, English M, Koutsky L. Longitudinal studies on chlamydial infections in the first year of life. Pediatr Infect Dis. 1982;1(6):395-401.

[25] Hammerschlag MR, Gelling M, Roblin PM, Kutlin A, Jule JE. Treatment of neonatal chlamydial conjunctivitis with azithromycin. Pediatr Infect Dis J. 1998;17(11):1049-50.

[26] Loenen AC. Farmacotherapeutical Kompass. Amstelveen, The Netherlands: Health Care Insurance Board: 2005.

[27] Patamasucon P, Rettig PJ, Faust KL, Kusmiesz HT, Nelson JD. Oral v topical erythromycin therapies for chlamydial conjunctivitis. Am J Dis Child. 1982;136(9):817-21.

Chapter 8

Chlamydia trachomatis respiratory infection in Dutch infants

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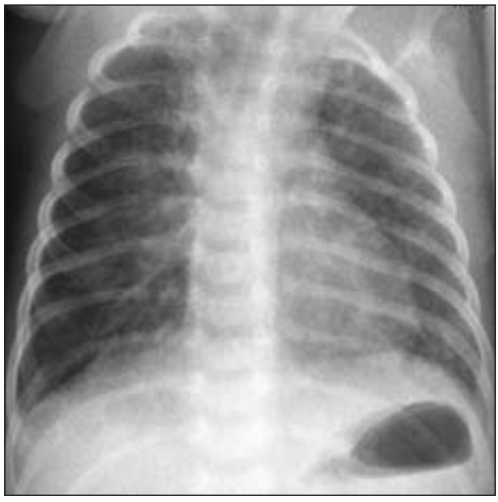
Abstract

Background *Chlamydia trachomatis* is the most common bacterial pathogen causing sexually transmitted infections in Dutch adults. As prenatal screening for *C. trachomatis* and treatment of pregnant women is not routine practice in the Netherlands, perinatal transmission of *C. trachomatis* may therefore occur.

Methods We evaluated the presence of *C. trachomatis* in infants less than six months of age who presented with respiratory complaints to the Erasmus MC-Sophia. Respiratory specimens, primarily nasopharyngeal swabs, were tested for *C. trachomatis,* respiratory viruses and *Mycoplasma pneumoniae* using PCR, viral isolation in cell cultures and direct immunofluorescence.

Results *C. trachomatis* respiratory tract infection was confirmed to be relatively common with detection in 10 (7%) of 148 infants tested. *C. trachomatis* had not been tested for by the attending physicians, but was the second most frequently detected respiratory pathogen after human Respiratory Syncitial Virus, which was found in 41 (28%) infants.

Conclusion Perinatal respiratory infection with *C. trachomatis* was common and underestimated in this population.



Chlamydia trachomatis pneumonia, courtesy of FJ Dijkstra [6]

Introduction

Although *Chlamydia trachomatis* is currently the most prevalent sexually transmitted bacterial infection in the Netherlands, pregnant women are not screened for *C. trachomatis* infection in the Netherlands and neither is it a notifiable disease. The prevalence of *C. trachomatis* infection among pregnant women in Rotterdam, however, is approximately 6% [1]. Recently, we reported that *C. trachomatis* was a major cause of neonatal conjunctivitis in infants less than three months of age in our population in Rotterdam [2].
In the present study we evaluated the presence of *C. trachomatis* respiratory tract infection in infants presenting with respiratory complaints in the same region in Rotterdam, The Netherlands.

Methods

Design

The study was conducted at the Erasmus MC-Sophia, Rotterdam, The Netherlands. *C. trachomatis* screening is not standard practice in pregnant women in the study area; testing is done on clinical suspicion only. Respiratory specimens (nasopharyngeal aspirates or broncho-alveolar lavages) were collected from infants less than 6 months of age who presented with symptoms and signs compatible with respiratory tract infection to the hospital between January 2002 and January 2003. All specimens were prospectively tested for viral pathogens and *Mycoplasma pneumoniae*, and retrospectively tested for *C. trachomatis*. Clinical data, eosinophil count and chest X-ray results were collected through a systematic review of medical records.

Microbiological diagnosis

Initially, the samples were sent to the virology laboratory specifically requesting a test for respiratory viruses. Routine virological testing for respiratory pathogens was performed using direct immunofluorescence on cells in respiratory specimens, virus isolation in cell cultures and immunofluorescence, and included human Respiratory Syncitial Virus (hRSV), influenza A, B and C viruses, human para-influenza virus types 1-4, adenovirus and rhinovirus. Following a diagnosis of picornavirus, nucleic acid amplification techniques were performed with specific primers. Detection of human Metapneumovirus (hMPV), *M. pneumoniae* and *C. trachomatis* was done by PCR.

Satistical analysis

Data were analyzed using SPSS 10.0.0 (SPSS Inc, Chicago, IL). P value <0.05 was considered significant.

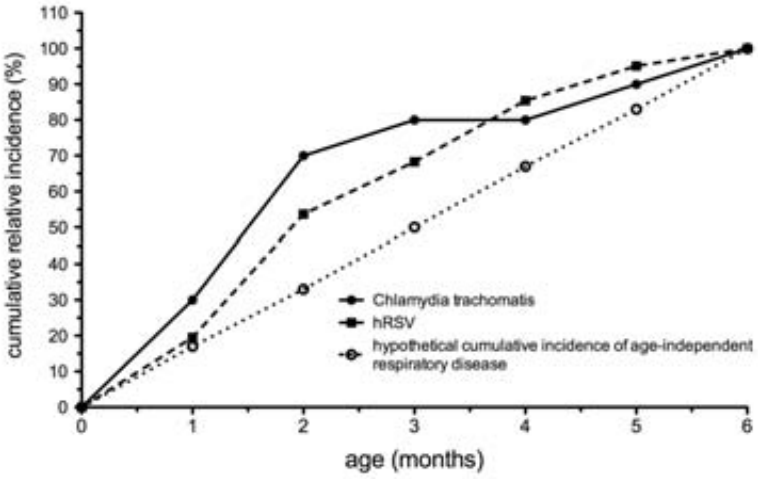


Figure 1 Cumulative incidence of *Chlamydia trachomatis* and *hRSV* infection in infants presenting with respiratory infection

Results

A total of 157 infants were eligible for the study. From nine infants the specimens were consumed while testing for viral pathogens during admission. From 148 (94%) infants, 187 respiratory specimens (184 nasopharyngeal aspirates and three broncho-alveolar lavage fluids) were available for *C. trachomatis testing*. Mean age of the infants was 67 days ± 49 days (range 1 to 172 days), 68 (46%) of 148 were female and 80 (54%) of 148 were male.

Potential respiratory pathogens were identified in 67 (45%) of 148 infants. *C. trachomatis* was detected in 10 (7%) infants. Among the viral pathogens, hRSV was most frequently detected in 41 (28%) infants, followed by rhinovirus (6 of 148 [4%]), influenza A virus (3 of 147 [2%]), adenovirus (2 of 147 [1%]), para-influenza 3 virus (2 of 147 [1%]) and para-influenza 1 virus (1 of 147 [1%]). Influenza B and C virus, para-influenza 2 and 4 virus, hMPV and *M. pneumoniae* were not detected. One (10%) *C. trachomatis* positive infant was also infected with hRSV.

Comparison of *C. trachomatis* and viral pathogens by age group (less than three months versus three to six months) showed that both *C. trachomatis* and hRSV infections were more often observed in the younger group (Figure 1). Eight (80%) of 10 *C. trachomatis* infections occurred before three months of age. hRSV was the most common pathogen detected in 28% of infants in both age groups.

The underlying pathology and clinical characteristics of the 10 *C. trachomatis* positive infants and their mothers are shown in table 1. Most infants had significant underlying disease except for patient 4 and 5. Five infants had a history of conjunctivitis: patient 1 had *Klebsiella pneumoniae* cultured from a conjunctival specimen four days after birth and patient 3 had *Haemophilus*

Table 1 Characteristics of *Chlamydia trachomatis* positive infants and their mothers

Infant number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Underlying pathology	meconium stained liquor, pulmonary hemorrhage eci	hypoplastic left heart syndrome	IUGR, dysmorphism, microcephaly, unilateral choanal atresia	-	-	antenatal asphyxia, generalized hypotonia, epilepsy	hypoplastic left heart syndrome	acute myeloid leukemia	VLCAD,hypertrophic cardiomyopathy	chronic eczema, feeding intolerance
Characteristics Mother										
maternal age	32	31	26	24	28	31	27	23	35	21
gravidity	1	1	2	1	4	2	1	1	5	1
term delivery	+	+	+	+	+	+	+	+	+	+
vaginal delivery	+	+	+	+	+	C/S	+	+	+	+
Infant										
AGA	+	+	SGA	+	+	+	+	+	+	+
age at testing (days)	11	17	26	32	33	45	48	65	147	168
rhinorrhoea/congestion	+	-	-	+	+	+	+	+	+	-
fever	-	-	+	-	-	-	-	+	+	-
cough	-	+	-	-	+	-	+	-	-	-
apnoea	-	-	-	-	+	-	-	-	-	-
cyanosis	+	+	-	-	+	+	+	-	-	-
retractions	-	-	+	-	+	+	-	-	-	+
wheezing	-	+	-	-	+	-	-	-	-	+
crepitations	+	-	-	-	-	-	-	-	-	-
dyspnoea	+	+	+	-	+	+	+	+	-	+
tachypnoea	+	+	-	+	-	+	+	+	-	+
oxygen requirement	+	+	+	-	+	+	+	-	-	-
feeding difficulties	+	+	+	+	+	+	+	+	+	+
X-ray chest										
hyperinflation	-	-	-	-	+	-	+	-	ND	+
atelectasis	+	+	-	-	+	+	-	-	ND	-
interstitial infiltrates	-	-	-	-	-	-	-	-	ND	+
consolidation	+	-	-	-	-	-	-	-	ND	-
Laboratory										
leucocytes (.10^9/l)	12.7	9.0	9.0	14.1	4.6	10.8	9.4	1.3	23.0	26.0
eosinophils (mm^3)	889	720	90	1128	ND	ND	752	0	ND	780
Viral pathogens										
hRSV	-	-	-	-	+	-	-	-	-	-

IUGR: intra-uterine growth retardation, VLCAD: very long-chain acetyl coenzymeA dehydrogenase deficiency, C/S: caesarean section, AGA: appropriate for gestational age, SGA: small for gestational age, ND: not done

influenzae at 22 days of age; eye cultures from the other infants showed no growth. None of the eye samples were tested for *C. trachomatis*. Seven infants had signs compatible with upper respiratory tract infection and most infants had signs compatible with lower respiratory tract infection. However, only one patient (#10) had a characteristic chest X-ray with hyperinflation and interstitial infiltrates compatible with chlamydial pneumonia. Bacterial cultures of the nasopharynx, sputum and/or blood were negative for all infants except patient 3 and 5, who had *H. influenzae* and *M. catarrhalis* with *K. oxytoca* in the sputum.
Two *C. trachomatis* positive nasopharyngeal specimens were obtained from patient 8: at the age of 65 days and 174 days. On both occasions she had rhinorrhea and congestion.

Discussion

In this study *C. trachomatis* was detected in 7% of infants less than 6 months of age presenting to hospital with respiratory tract infection. The role of *C. trachomatis* as a causative pathogen for respiratory disease in young infants is well described in the literature [3, 4]. All respiratory specimens in our study were sent to the laboratory for viral tests, but none were sent for *C. trachomatis*. This suggests that the attending physicians have a low index of suspicion for *C. trachomatis* infection in these infants.

The main limitations of the study are the retrospective design, lack of controls, and the fact that we only included infants who presented to the hospital. Therefore, this study cannot be regarded as a population-based study of the frequency of chlamydial infections. Neither can we be sure that *C. trachomatis* is the causative pathogen of the respiratory complaints in these infants instead of being coincidental finding. It does, however, demonstrate the relative importance of perinatally acquired *C. trachomatis* infection among infants with respiratory complaints in this population.

The nasopharynx is the most frequent site for perinatally acquired *C. trachomatis* infection, but only about 20% of infants with nasopharyngeal infection go on to develop pneumonia. Nasopharyngeal infection is usually asymptomatic and self-limiting but may persist for periods of up to one year. *C. trachomatis* pneumonia has a characteristic presentation. Infants usually present between 3 and 12 weeks of age with tachypnoea, a distinctive (staccato paroxysmal) cough and are usually afebrile. Chest auscultation reveals crepitations, with no or minimal wheezing. Chest X-rays show hyper-expansion with bilateral, diffuse interstitial and patchy alveolar infiltrates. Peripheral eosinophilia and elevated anti-*C. trachomatis* IgM antibodies may be present [3, 4]. In our study, we found 10 infants with *C. trachomatis* nasopharyngeal infection and only one infant had the characteristic presentation of chlamydial pneumonia with infiltrates present on chest X-ray. Interestingly, more than half the infants were dyspnoeic, cyanotic or

required oxygen support. Respiratory failure has been described with chlamydial infection, but is relatively uncommon and mainly described in preterm infants with an early onset respiratory distress syndrome [5]. Therefore, the more severe, atypical course of chlamydial infection in our infants may rather be due to underlying pathology, which included significant cardiopulmonary disease, antenatal hypoxia, acute myeloid leukemia and intrauterine growth retardation with microcephaly and dysmorphic features.

In conclusion, this study demonstrated that perinatal respiratory infection with *C. trachomatis* is common in the Netherlands. We recommend that in countries such as the Netherlands, where screening for *C. trachomatis* is not part of routine antenatal care, testing for *C. trachomatis* should be included in diagnostic and treatment protocols for respiratory disease in infants during the first six months of life.

References

[1] Rours GIJG, Verkooyen RP, Willemse HF, van der Zwaan EA, van Belkum A, de Groot R, et al. Use of Pooled Urine Samples and Automated DNA Isolation To Achieve Improved Sensitivity and Cost-Effectiveness of Large-Scale Testing for *Chlamydia trachomatis* in Pregnant Women. J Clin Microbiol. 2005;43(9):4684-90.

[2] Rours GIJG, Hammerschlag MR, Ott A, De Faber TJ, Verbrugh HA, de Groot R, et al. *Chlamydia trachomatis* as a cause of neonatal conjunctivitis in Dutch infants. Pediatrics. 2008;121(2):e321-6.

[3] Darville T. *Chlamydia trachomatis* infections in neonates and young children. Semin Pediatr Infect Dis. 2005;16(4):235-44.

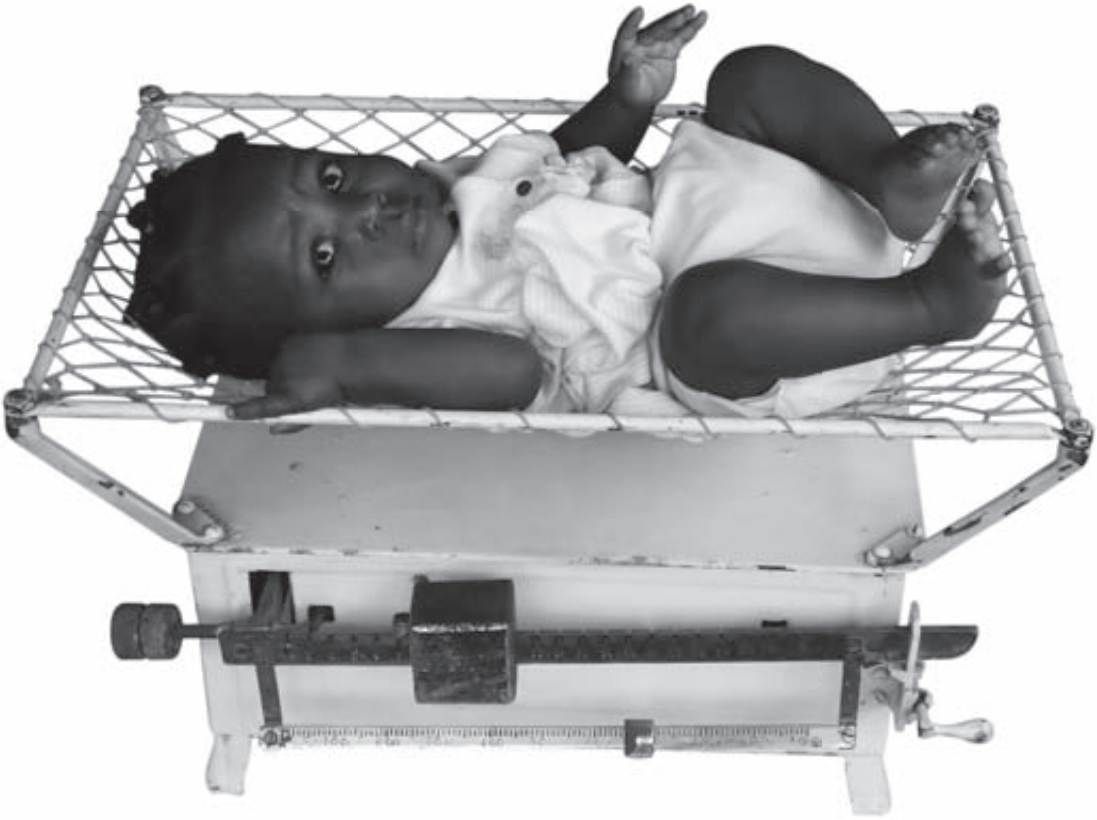
[4] Chen CJ, Wu KG, Tang RB, Yuan HC, Soong WJ, Hwang BT. Characteristics of *Chlamydia trachomatis* infection in hospitalized infants with lower respiratory tract infection. J Microbiol Immunol Infect. 2007;40(3):255-9.

[5] Sollecito D, Midulla M, Bavastrelli M, Panero A, Marzetti G, Rossi D, et al. *Chlamydia trachomatis* in neonatal respiratory distress of very preterm babies: biphasic clinical picture. Acta Paediatr. 1992;81(10):788-91.

[6] Dijkstra FJ, Thjie HT, Breukels MA. An infant with atypical pneumonia. Ned. Tijdschr Geneesk. 2010;78(1):25-26

Part IV

Cost-effectiveness of *Chlamydia trachomatis* screening in pregnant women



Chapter 9

Use of pooled urine samples and automated DNA isolation to achieve improved sensitivity and cost-effectiveness of large-scale testing for *Chlamydia trachomatis* in pregnant women

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J Clin Microbiol. 2005;43(9):4684-90

Abstract

Background The success of large-scale screening for *Chlamydia trachomatis* depends on the availability of non-invasive samples, low costs and high-quality testing.
Objective This study was conducted to evaluate pooled testing for *C. trachomatis* in pregnant women while using urine specimens.
Methods First void urine specimens from 750 consecutive asymptomatic pregnant women from the Rotterdam area, The Netherlands, were collected. Initially, we investigated the performance of three different DNA isolation methods with 350 of these urines and 70 pools of five of the same subset of urine samples. The routinely used Cobas Amplicor test was compared to the Cobas Amplicor with prior DNA isolation by the MagNA Pure large-volume kit and with prior DNA isolation by the MagNA Pure bacterial DNA isolation kit. Next, using all 750 urines, the Cobas Amplicor performance for individual testing was compared to pooled testing with the standard Cobas Amplicor procedure and subsequently to pooled testing with the Cobas Amplicor in combination with the MagNA Pure bacterial DNA isolation kit.
Results The combination of the Cobas Amplicor with prior DNA isolation by the MagNA Pure bacterial DNA isolation kit provided the best DNA test for pooled urines, with a sensitivity twice that of the other methods. The sensitivity of the Cobas Amplicor was 65% on individual and 42% on pooled urines, but improved to 92% on pooled urines with the MagNA Pure bacterial DNA isolation kit, making this combination the best screening method. The *C. trachomatis* prevalence in this population appeared to be 6.4%. Additionally, the cost of the combined MagNA Pure bacterial DNA isolation kit and Cobas Amplicor method on pooled urines was only 56% of the cost of the standard Cobas Amplicor test applied to individual urines. Costs per positive case detected in the combined method were 39% of standard costs.
Conclusion Pooled testing for *C. trachomatis* infection in asymptomatic pregnant women can be developed for large scale testing provided the Cobas Amplicor method is used together with prior chlamydial DNA isolation by the MagNA Pure Bacterial DNA Isolation Kit. This combination significantly improves sensitivity and decreases costs.

Introduction

Chlamydia trachomatis is one of the major sexually transmitted pathogens, and high prevalences of chlamydial infection have been documented for asymptomatic women in many European countries [1]. Asymptomatic carriers are of substantial importance in the transmission of *C. trachomatis* infection within a community. Asymptomatic chlamydial infection in pregnant women imposes an additional risk for acute and chronic consequences for the women themselves and their (unborn) offspring [2-6]. In the Netherlands, *C. trachomatis* causes most sexually transmitted infections with approximately 60,000 new cases estimated for a total population of 16 million in the year 2000. Studies in general practice have shown an increase in the incidence of chlamydial infections [7], but data covering other specific target groups outside of the sexually transmitted disease (STD) outpatient clinics are sparse [8]. Dutch population-based screening for *C. trachomatis* is still under debate, with cost-effectiveness of screening, complexity of sampling, the reliability of test methods, and the nature of the target population as major issues of discussion [9].

In order to investigate the prevalence of chlamydial infection during pregnancy in Rotterdam, and the risk factors and consequences of chlamydial infection during pregnancy for women and newborns, a follow-up study was planned. We explored different methods for *C. trachomatis* testing with respect to sensitivity and cost-effectiveness. The preferred method for the detection of asymptomatic chlamydial infection with a low threshold should involve urine specimens in combination with nucleic acid amplification techniques (NAATs) [10]. However, bacterial loads in urine are generally low, which has an adverse effect on the sensitivity of NAATs [11]. Urines of asymptomatic women generate inferior NAAT results, sometimes 10% lower in sensitivity than attained for male urines [12, 13]. To reduce the costs of chlamydial screening in low-prevalence populations, pooling of urine specimens has been suggested. Although some studies suggested 100% sensitivity of pooled testing compared to individual testing [14, 15], other studies showed a lower sensitivity [16, 17], which decreased most significantly when eight or more urines were pooled [16, 18]. Another important aspect is that large-scale screening programs require automation of test procedures, which should simultaneously improve the quality of testing and should reduce the costs.

To date limited data are available concerning NAATs performed on urines from (asymptomatic) pregnant women as well as for NAATs for pooled urines. We present a study among 750 pregnant women in which the performance and costs of testing with both pooled urines and automated specimen preparation (using the MagNA Pure LC system and DNA amplification with the Cobas Amplicor system) for the detection of asymptomatic *C. trachomatis* infection were evaluated.

Materials and Methods

Patient population

Pregnant women and their offspring were enrolled in the Generation R study, a prospective multi-center, population-based cohort trial that includes 10,000 children and women in Rotterdam, The Netherlands. The study focuses on growth, development, and health of children from intra-uterine fetal life to adolescence [19]. Pregnant women before 24 weeks of gestational age who were Dutch residents and expected to deliver in the Rotterdam area were approached to take part in the study. After informed consent was obtained, women were asked for a fresh first-void urine specimen, preferably at a gestational age of 12 weeks. For the current study, 750 urine specimens were tested anonymously.

DNA amplification

Throughout the study, the automated *C. trachomatis* Cobas Amplicor PCR system (Roche Diagnostics, Almere, The Netherlands) was used according to the manufacturer's instructions to detect chlamydial DNA in specimens processed by any of the methods described below [12]. Positive specimens were subjected to quantitative LightCycler PCR (version 3.5) to assess the bacterial load [20]. For this purpose, DNA was isolated from each specimen according to method IIIB (see below). The PCR protocol was based on the use of the FastStart DNA MasterPLUS SYBR Green I kit (Roche), the primers 5'-GGACAAATCGTATCTCGG-3' and 5'-GAAACCAACTCTACGCTG-3', and 40 amplification cycles. The same dilution range of *C. trachomatis* serovar E (10^0, 10^{-2}, and 10^{-4} [relative *C. trachomatis* concentrations]) was included in each run and used to calculate the concentration of target DNA relative to the initial copy number in the undiluted control. Since this control was not subjected to titration, the absolute number of bacteria could not be determined.

Processing of specimens

The 750 samples were analyzed in two separate batches. Initially, a group of 350 samples was tested according to six different protocols as outlined below and in figure 1 (methods IA to IIIB). Afterwards, all 750 samples were tested individually using the Cobas Amplicor, tested in pools of five according to the Cobas Amplicor procedure, and tested in pools of five with preceding DNA purification by use of the MagNa Pure bacterial DNA isolation kit III.

Method IA: Cobas Amplicor on individual urines

Single urine specimens were processed according to the instructions of the Cobas Amplicor manufacturer (Roche Diagnostics). In short, a 500 µl-urine specimen was diluted with 500 µl of washing buffer and centrifuged at 14,000 rpm. The pellet was resuspended in 250 µl lysis buffer and centrifuged again after addition of 250 µl diluent. The supernatant (50 µl) was used for PCR. The results were reported as negative or positive.

Method IB: Cobas Amplicor on pooled urines

Pools for the Cobas Amplicor were made by adding 100 µl of five different urines into one tube. The 500 µl-urine specimen was further processed as described above, and 50 µl of the supernatant was used for PCR. The urines from negative pools were reported as negative. Urines from positive pools were individually retested and reported as described for method IA.

Method IIA: MagNA Pure large-volume kit on individual urines

The MagNA Pure LC DNA Isolation Kit-Large Volume (Roche Diagnostics) was used to isolate DNA from urines according to the manufacturer's instructions. From individual urines a 1,000-µl specimen was used. DNA was isolated in the automated MagNA Pure LC instrument using an elution volume of 100 µl, of which 25 µl was used for PCR. The results were reported as negative or positive.

Method IIB: MagNA Pure large-volume kit on pooled urines

The MagNA Pure LC DNA Isolation Kit-Large Volume (Roche Diagnostics) was used according to the manufacturer's instructions. Pools were made of five urines by adding 200 µl of each of the five urines into one tube. From these pools the full 1,000-µl specimen was taken and used without further processing. DNA was isolated in the automated MagNA Pure LC instrument using an elution volume of 100 µl, of which 25 µl was used for PCR. The urines from negative pools were reported as negative. Urines from positive pools were individually retested and reported as described for method IIA.

Method IIIA: MagNA Pure bacterial DNA isolation kit on individual urines

The MagNA Pure LC Bacterial DNA Isolation Kit III (Roche Diagnostics) was used to isolate DNA from individual urines. From single urines 500 µl was taken and centrifuged for 10 min at 14,000 rpm. Subsequently 400 µl was removed and the pellet was resuspended in 100 µl of the remaining supernatant, mixed with 130 µl lysis buffer and 20 µl proteinase K, incubated for 10 min at 65 °C, and denatured for 10 min at 95°C. Finally, DNA was isolated in the automated MagNA Pure LC instrument using a sample volume of 250 µl and an elution volume of 100 µl. Again, 25 µl was used for PCR. The results were reported as negative or positive.

Method IIIB: MagNA Pure bacterial DNA isolation kit on pooled urines

The MagNA Pure LC Bacterial DNA Isolation Kit III (Roche Diagnostics) was used to isolate DNA from pooled urines. Pools were made of five urines by adding 200 µl of each of the five urines into one tube. From each pool the full 1,000 µl was taken and centrifuged for 10 min at 14,000 rpm. Subsequently 900 µl was removed, and the pellet was resuspended in 100 µl of the remaining supernatant, mixed with 130 µl lysis buffer and 20 µl proteinase K, incubated for 10 min at 65°C, and thereafter denatured for 10 min at 95°C. Finally, DNA was isolated in the automated MagNA Pure LC instrument using a sample volume of 250 µl and an elution volume of 100 µl. Again, 25 µl was used for PCR. The urines from negative pools were reported as negative. Urines from positive pools were individually retested and reported as described for method IIIA.

Figure 1 summarizes the various volumes used in each test method. In method IIA, IIB, IIIA, and IIIB, the elution buffer did not contain $MgCl_2$ and consequently could not be used directly in the PCR. Therefore, the eluate for amplification was mixed 1:1 with $MgCl_2$- containing diluent from the Cobas Amplicor system. In the PCR 50 µl of this mixture was used.

Discrepancy analysis

A specimen was considered to be truly positive if one or more of the test methods described above gave results that were positive for individual samples. When a pool was positive, all individual samples were retested according to the same procedure as used for the pool in order to identify the positive specimen(s). A positive pool result was considered to be truly positive when one or more individual samples within the pool appeared to be positive by either method. A positive pool result was considered to be false positive when none of the individual samples within the pool turned out positive. A negative pool result was considered true negative in the presence of a positive internal inhibition control as included in the commercial Cobas Amplicor kit. All individual samples and pooled samples were retested when results were discrepant. When the internal control was negative, the sample contained inhibitors. Retesting was performed after diluting the specimen 10-fold and heating the sample for 10 min at 95°C.

Costs

We calculated the costs of materials and reagents for individual and pooled testing by the standard Cobas Amplicor method and by the Cobas Amplicor in combination with the MagNA Pure bacterial DNA isolation kit. We used list prices available at the time of the study. We assumed full runs for each test method, which consist of 20 specimens plus a positive and a negative control per run for the Cobas Amplicor and 32 MagNa Pure specimens. We calculated total costs and costs per positive case detected. We also calculated the costs per positive case using the standard Cobas Amplicor for individual urines versus the combination of the Cobas Amplicor with the MagNA Pure bacterial DNA isolation kit for pooled urines. This was done for hypothetical prevalences in a population ranging between 1% and 10%. Calculations were based on full runs and pools of 5 urines and the sensitivity determined for the Cobas Amplicor method with individual urines and for the combined method with pooled urines.

Statistical analysis

Binomial 95% confidence intervals (CI) were calculated for the prevalences and sensitivities of the different DNA isolation methods. McNemar's test was used to compare the two methods. The nonparametric Kruskal-Wallis H test was used to compare median results.

Results

Comparison of three different DNA isolation methods

Figure 1 summarizes the results of the analysis of the initial 350 urine specimens. Individual urines processed according to methods IA, IIA, or IIIA scored positive in 15, 14 and 27 cases, respectively. This equals sensitivities of 51.7%, 48.3% and 93.1% when calculated on the basis of the number of true positives (n=29). The specificity was 100% for all tests.

Nine pools were positive with the standard Cobas Amplicor test method (method IB). The use of the MagNA Pure large-volume DNA Isolation Kit also yielded nine positive pools and the use of the MagNA Pure bacterial DNA isolation kit resulted in 19 positive pools, which included the 9 pools that were positive by the standard Cobas Amplicor test as well as by the MagNA Pure large-volume DNA Isolation Kit. Including the MagNa Pure bacterial DNA isolation kit clearly provided the most sensitive test method (McNemar's test, $P<0.01$), with equal sensitivities when testing pooled urines compared to individual urines.

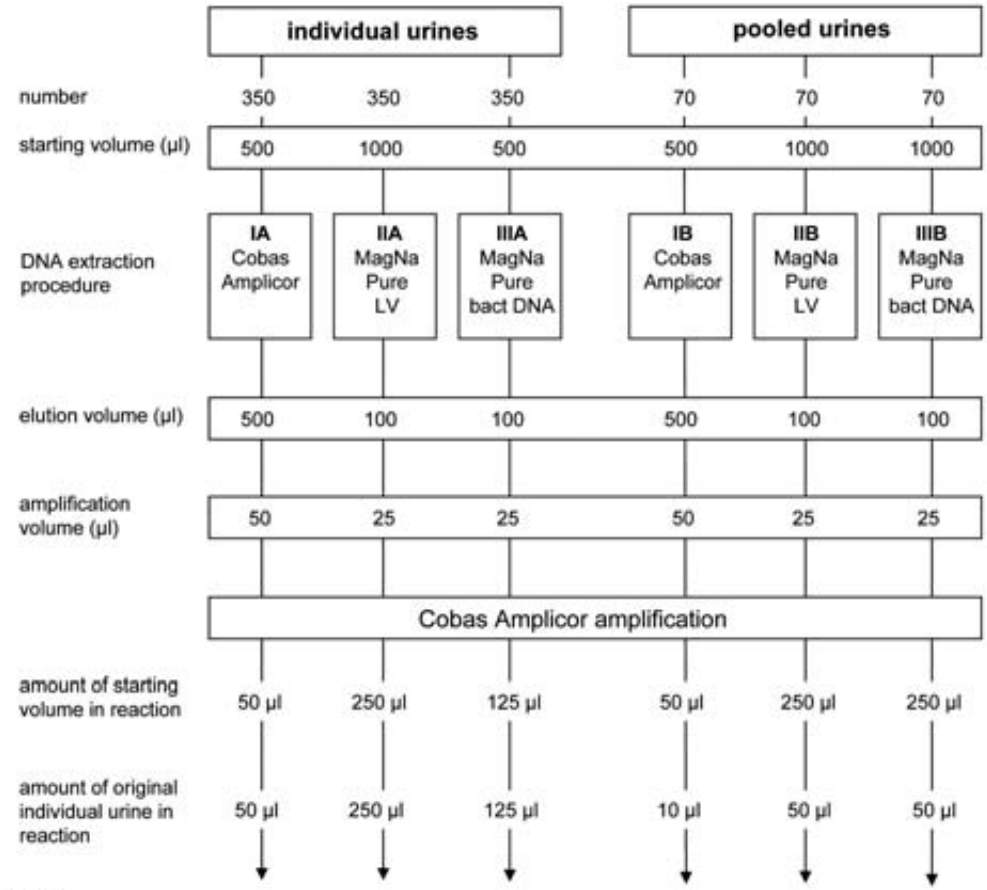
Comparison of the Cobas Amplicor method for individual urines with pooled urines

Pooling of urines was compared to individual testing with the Cobas Amplicor method on all 750 urines; results are summarized in table 1. Testing individual urines by the Cobas Amplicor method yielded 31 positive test results out of 750 specimens, resulting in an estimated prevalence for *C. trachomatis* of 4.1% among these pregnant women. Testing of pooled urines by the Cobas Amplicor method resulted in 15 positive pools out of 150 pools. Subsequent individual testing of the 75 urines from these 15 pools by the Cobas Amplicor yielded 20 positive tests, which with a total of 750 urines resulted in an estimated prevalence of 2.7%. Eleven specimens would have been reported falsely negative when using the Cobas Amplicor test only on pooled urines (11/730 = 1.5%), which proved the sensitivity of standard processing of pooled urines by the Cobas Amplicor method to be 65% compared to individual testing of urines by the Cobas Amplicor method. The number of truly positive samples was 48.

Performance of the MagNA Pure bacterial DNA isolation kit with pooled urines

Pooled urines were tested using the standard Cobas Amplicor method as described above, and results were compared to the performance of the combination of the Cobas Amplicor method with the MagNA Pure bacterial DNA isolation kit (Table 1). All 750 urines were tested in pools of five urines. A total of 34 pools tested positive by the Cobas Amplicor after DNA isolation was done with the MagNA Pure bacterial DNA isolation kit. Subsequent testing of the 170 individual urine specimens yielded 44 positive urines compared to 20 urines by the standard Cobas Amplicor method (McNemar's test, $P<0.001$).

Two pools which were positive after DNA isolation with the MagNA Pure bacterial DNA isolation kit could not be confirmed by individual testing of urines in either isolation method and



Results

method	IA	IIA	IIIA	IB	IIB	IIIB
pools positive	NR	NR	NR	9	9	19
(%)				(12.9)	(12.9)	(27.1)
individual positives	15	14	27	12	11	27
prevalence estimate (%)	(4.3)	(4.0)	(7.7)	(3.4)	(3.1)	(7.7)
Sensitivity %	51.7	48.3	93.1*	41.4	37.9	93.1*
Specificity %	100	100	100	100	100	100

Figure 1 Methods and results of individual and pooled testing by different DNA isolation methods

Sensitivity values marked * indicate that the MagNA Pure bacterial DNA isolation kit provided the best method for DNA processing, P<0.01 (McNemar's test), with equal levels of sensitivity and specificity for pooled urines and individual urines. NR: not relevant

were considered false positive. One other pool was positive after DNA isolation by the MagNA Pure bacterial DNA isolation kit, but the individual urines were negative. However, one urine from this pool was positive in the standard Cobas Amplicor assay for individual urines. Therefore, the pool/urine result was considered to be a true positive.

Altogether, 48 urines were positive for *C. trachomatis* after individual testing by the Cobas Amplicor method with or without the prior use of the MagNA Pure bacterial DNA isolation kit, revealing a prevalence of *C. trachomatis* infection of 6.4% in this population.

When positive individual testing in either method is considered as the gold standard, routine individual testing of urines with the Cobas Amplicor method proved to have a sensitivity of 65%. This sensitivity dropped to 42% when the Cobas Amplicor method on pooled urines was used. However, when using pooled urines with the combination of the Cobas Amplicor method after initial DNA isolation was done with the MagNA Pure bacterial DNA isolation kit, the sensitivity was 92% (see Table 1 for exact figures).

Table 1 Test results and costs of individual and pooled urines by different DNA isolation methods

Procedure	No. of positive tests/no. tested	No. of positive-women/ no.tested	Estimated prevalence % (95% CI)	Estimated sensitivity % (95% CI)	Total costs (euro)	Cost per case detected (euro)
Cobas Amplicor			4.1	65		
individual urines	31/750	31/750	(2.8-5.8)	(49-78)	8,522	275
Cobas Amplicor			2.7	42		
pooled urines	15/150	20/750	(1.6-4.1)	(28-57)	2,562	128
MagNA Pure Bacterial DNA Kit			5.9	92		
pooled urines	34/150	44/750	(4.3-7.8)	(80-98)	4,770	108

Inhibition

The Cobas Amplicor procedure showed inhibition for one (0.7%) of the pools and for 37 (4.9%) of the individually tested urines. After DNA isolation by MagNA Pure LC procedures, no (0%) inhibition was found among pooled urines and only once (0.6%) while testing individual urines.

Bacterial load in pools

Positive urine specimens were subjected to quantitative LightCycler PCR to assess the bacterial load. Figure 2 illustrates the relative *Chlamydia trachomatis* DNA concentrations of pooled urines observed with the use of the LightCycler PCR in relation to the standard Cobas Amplicor

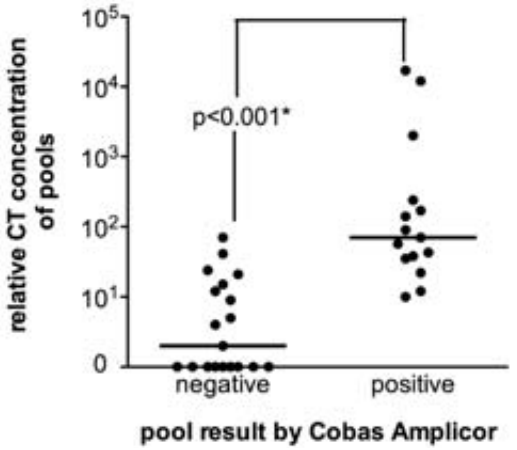


Figure 2 Relative *Chlamydia trachomatis* (CT) concentrations of pooled urines observed via LightCycler in relation to standard Cobas Amplicor test results

Horizontal lines represent the medians of the relative *C. trachomatis* concentration of pooled urines

*P-value (Kruskal-Wallis H test)

test results. True positive pools, which tested negative by the standard Cobas Amplicor method, had significantly lower relative *C. trachomatis* concentrations than positive pools (Kruskal-Wallis H test, P<0.001), confirming that bacterial titres do contribute significantly to the sensitivity of testing. Figure 3 illustrates the relative frequency distributions of the bacterial loads established in the urine samples obtained from these essentially symptom-free females. Note that most of the loads are relatively low but that no correlation with the bacterial load in urine samples from symptomatic patients has been made.

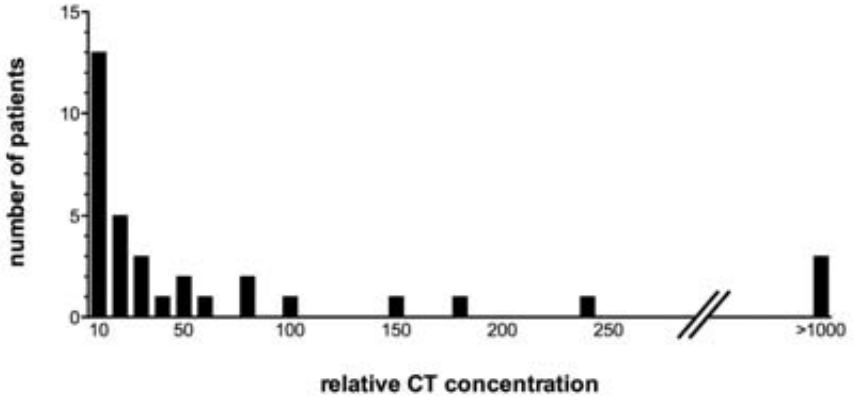


Figure 3 Relative *Chlamydia trachomatis* (CT) concentrations versus number of patients falling in different titer classes

Note that most patients fall within the low-titer classes

Costs

The Dutch costs for a Cobas Amplicor test was 10.33 euros per sample (isolation 0.82 euro, amplification 4.71 euro, detection 4.80 euros) and for the MagNA Pure isolation 4.04 euros per sample with a full run. The cost of the combined method (isolation with the MagNA Pure and subsequent amplification plus detection by the Cobas Amplicor) was 13.55 euros. The total costs for 750 specimens were, therefore, 8,522 euros for the Cobas Amplicor method with individual urines (750 tested with 75 controls) and 2,562 euros when pooled testing by the Cobas Amplicor was followed by individual testing (150 pools with 15 controls plus 15 positive pools times 5 individual tests with 8 controls, making 248 tests). The costs for pooled and individual urines with the prior use of the MagNA Pure bacterial DNA isolation kit were 4,770 euros (150 pools with 15 controls plus 34 positive pools times 5 individual urines with 17 controls, making 352 tests). The calculation of screening costs per positive detected case of *C. trachomatis* infection incorporated the sensitivities found in this study: 65% for the Cobas Amplicor test for individual urines, 42% for the Cobas Amplicor for pooled urines, and 92% for the use of the MagNA Pure bacterial DNA isolation kit in combination with the Cobas Amplicor PCR test for pooled urines. Screening costs per positive detected case were lowest with the use of the latter combination (Table 1).

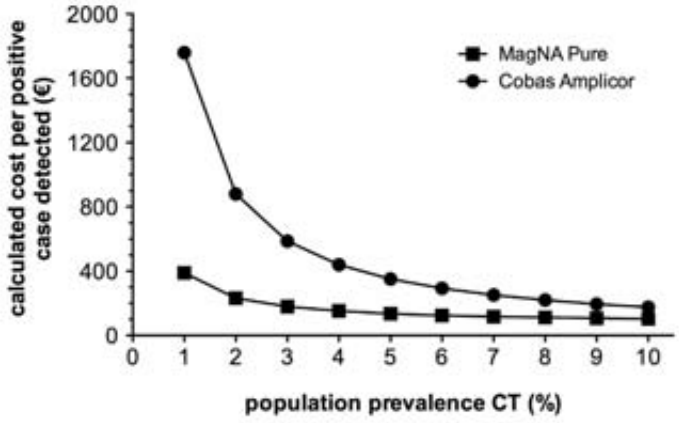


Figure 4 Costs per positive *Chlamydia trachomatis* case detected in relation to population prevalences

● Cobas Amplicor results for individual urines ■ pooled MagNA Pure plus Cobas Amplicor results

Figure 4 illustrates the difference in costs between the standard Cobas Amplicor used for individual urines compared to the use of the MagNA Pure bacterial DNA isolation kit in combination with the Cobas Amplicor PCR test for pooled urines for hypothetical prevalences ranging from 1% to 10%.

Discussion

Technological aspects

We analyzed 750 individual urine samples by several methods. Overall, 31 samples tested positive upon individual testing using the Cobas Amplicor platform. When pooled urines were used without prior DNA purification the sensitivity of the test dropped significantly, only 20 women tested positive. However, upon usage of the MagNa Pure DNA isolation system, an overall number of 48 women tested truly positive (see Table 1 for a summary). So this study shows that pooling of urines combined with prior DNA isolation by use of the MagNA Pure bacterial DNA isolation kit is a reliable and cost-effective way to both increase the sensitivity of testing (by 27%) and decrease the costs per detected case (by 62%) during large scale-testing for asymptomatic *C. trachomatis* infection among pregnant women. Furthermore, we show a 6.4% prevalence of *C. trachomatis* carriership in apparently healthy pregnant women in this Dutch area.

We used the Cobas Amplicor test in our study because it is fully automated and its performance is good [12, 13, 21, 22], being less prone to experimental variation than the Amplicor test [23, 24]. However, the sensitivity of the Cobas Amplicor for female urines is in the range of 80 to 90%, as has been shown in STD outpatient populations [12, 13]. A major problem with the use of urine specimens is inhibition. Urinalysis has shown that various substances are responsible for inhibition [25], and that between 2% and 4% of urine specimens contain inhibitors [21, 26]. The sensitivity, however, could be improved by using a modified specimen-processing procedure [27]. In our study the inhibition was slightly higher when using the Cobas Amplicor method for individual urines (4.9%), but much lower when using the same method for pooled urines (0.7%). However, automated DNA isolation from urines by use of the MagNA Pure bacterial DNA isolation kit prior to the Cobas Amplicor reduced the inhibition significantly in both individual and pooled testing. This significantly improved the sensitivity of *C. trachomatis* detection. In addition, the use of the MagNA Pure bacterial DNA isolation kit prior to the Cobas Amplicor resulted in a higher sensitivity than automated DNA isolation with the MagNA Pure large-volume kit, which may be explained by the additional use of proteinase K prior to DNA isolation in the bacterial DNA kit.

Sample pooling and cost aspects

Pooling of urine specimens is important to reduce the costs of screening. However, some describe a significant reduction of the sensitivity [14, 15], whereas others reported a similar sensitivity with pooling [16-18]. In our study, pooling with the Cobas Amplicor method resulted in a significant reduction of the sensitivity, which is probably due to the dilution of positive specimens and –as shown- not to the introduction of inhibitors from other urines in a pool. However, the use of the MagNA Pure bacterial DNA isolation kit restored and even improved the sensitivity. The combined procedure was the only method producing acceptable

results with pooled urines. Therefore, pooling of urines in large screening programs for the detection of asymptomatic *C. trachomatis* infections should only be used in conjunction with DNA isolation methods that yield highly purified DNA. It should be noted that the sensitivity of our procedure was 92% and not 100%. A low copy number of chlamydial targets in positive urine specimens in our population of asymptomatic women- as shown in figure 2- can explain this. Other variables influencing the sensitivity are the quality of specimens and the timing of sampling [28]. The sensitivity of screening could be improved by testing multiple specimens obtained at various time points, but this would compromise the cost-benefit ratio of screening programs.

C. trachomatis screening among pregnant women

Pregnant women could be a specific target group for *C. trachomatis* screening. Antenatal screening, as recommended by the Centers for Disease Control [29], may be beneficial for decreasing morbidity amongst women themselves, but also to prevent vertical (infant) and horizontal (partner) transmission [2-5, 30]. Screening of pregnant women usually yields prevalences similar to those of non-pregnant women. In Europe, the prevalence of *C. trachomatis* infection among asymptomatic women was recently estimated to range from 1.7% to 17%, depending on setting, context and country [1]. The prevalence of 6.4% in apparently healthy pregnant Dutch women is much higher than previously reported in asymptomatic women in general practices (2,9% and 4,9% in 1996 and 1997) or in a general obstetric and gynecological population (4.5% in 2002) [8, 31, 32], and approaches the chlamydial prevalence of 7.3% that was found in 1998 amongst women consulting the STD outpatient clinic in Rotterdam [33]. However, these figures must be interpreted with caution since test format is clearly important. Testing of individual urines by the Cobas Amplicor method without prior DNA isolation by the MagNA Pure bacterial DNA isolation kit yielded a much lower prevalence of 4.1%.
Screening programs are considered to be cost-effective when the prevalence of *C. trachomatis* infection is higher than 3% to 6% [9, 10, 34, 35]. The introduction of improved technology for screening may reveal higher prevalences, rendering screening programs cost-effective. It was shown that Cobas Amplicor testing was more cost-effective with pooled urines compared to individual urines, but pooling reduced sensitivity. However, usage of the MagNA Pure bacterial DNA isolation kit increased sensitivity and appeared to be more cost-effective: the calculated costs per detected case in the combined method with pooling were a mere 39% of the costs of individual testing with the Cobas Amplicor.

In conclusion, we show that pooled testing for *C. trachomatis* infection in asymptomatic pregnant women can be developed for large-scale testing provided that the Cobas Amplicor method is used together with prior chlamydial DNA isolation by use of the MagNA Pure bacterial DNA isolation kit. This combination significantly improves sensitivity and decreases costs.

References

[1] Wilson JS, Honey E, Templeton A, Paavonen J, Mardh PA and Stray-Pedersen B. A systematic review of the prevalence of *Chlamydia trachomatis* among European women. Hum Reprod Update 2002;8:385-94

[2] Bell TA, Stamm WE, Kuo CC, Wang SP, Holmes KK and Grayston JT. Risk of perinatal transmission of *Chlamydia trachomatis* by mode of delivery. J Infect 1994;29:165-9

[3] Claman P, Toye B, Peeling RW, Jessamine P and Belcher J. Serologic evidence of *Chlamydia trachomatis* infection and risk of preterm birth. CMAJ 1995;153:259-62

[4] Gencay M, Koskiniemi M, Ammala P, et al. *Chlamydia trachomatis* seropositivity is associated both with still-birth and preterm delivery. APMIS 2000;108:584-8

[5] Gencay M, Koskiniemi M, Fellman V, Ammala P, Vaheri A and Puolakkainen M. *Chlamydia trachomatis* infection in mothers with preterm delivery and in their newborn infants. APMIS 2001;109:636-40

[6] Mardh PA. Influence of infection with *Chlamydia trachomatis* on pregnancy outcome, infant health and life-long sequelae in infected offspring. Best Pract Res Clin Obstet Gynaecol 2002;16:847-64

[7] van Bergen JE. Increased incidence of gonorrhea and *Chlamydia trachomatis* infections in family practice in southeast Amsterdam, 1996-2000. Ned Tijdschr Geneeskd 2001;145:1691-3

[8] Bax CJ, Oostvogel PM, Mutsaers JA, et al. Clinical characteristics of *Chlamydia trachomatis* infections in a general outpatient department of obstetrics and gynaecology in the Netherlands. Sex Transm Infect 2002;78:E6

[9] Postma MJ, Bakker A, Welte R, et al. Screening for asymptomatic *Chlamydia trachomatis* infection in pregnancy; cost-effectiveness favorable at a minimum prevalence rate of 3% or more. Ned Tijdschr Geneeskd 2000;144:2350-4

[10] Watson EJ, Templeton A, Russell I, et al. The accuracy and efficacy of screening tests for *Chlamydia trachomatis*: a systematic review. J Med Microbiol 2002;51:1021-31

[11] Thomas BJ, Pierpoint T, Taylor-Robinson D and Renton AM. Quantification of *Chlamydia trachomatis* in cervical and urine specimens from women attending a genitourinary medicine clinic: implications for screening strategies. Int J STD AIDS 1998;9:448-51

[12] Goessens WH, Mouton JW, van der Meijden WI, et al. Comparison of three commercially available amplification assays, AMP CT, LCx, and COBAS AMPLICOR, for detection of *Chlamydia trachomatis* in first-void urine. J Clin Microbiol 1997;35:2628-33

[13] van Doornum GJ, Schouls LM, Pijl A, Cairo I, Buimer M and Bruisten S. Comparison between the LCx Probe system and the COBAS AMPLICOR system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in patients attending a clinic for treatment of sexually transmitted diseases in Amsterdam, The Netherlands. J Clin Microbiol 2001;39:829-35

[14] Currie MJ, McNiven M, Yee T, Schiemer U and Bowden FJ. Pooling of clinical specimens prior to testing for *Chlamydia trachomatis* by PCR is accurate and cost saving. J Clin Microbiol 2004;42:4866-7

[15] Morre SA, Welte R and Postma MJ. Major improvements in cost effectiveness of screening women for *Chlamydia trachomatis* using pooled urine specimens and high performance testing. Sex Transm Infect 2002;78:74-5

[16] Krepel J, Patel J, Sproston A, et al. The impact on accuracy and cost of ligase chain reaction testing by pooling urine specimens for the diagnosis of *Chlamydia trachomatis* infections. Sex Transm Dis 1999;26:504-7

[17] Peeling RW, Toye B, Jessamine P and Gemmill I. Pooling of urine specimens for PCR testing: a cost saving strategy for *Chlamydia trachomatis* control programmes. Sex Transm Infect 1998;74:66-70

[18] Kacena KA, Quinn SB, Howell MR, Madico GE, Quinn TC and Gaydos CA. Pooling urine samples for ligase chain reaction screening for genital *Chlamydia trachomatis* infection in asymptomatic women. J Clin Microbiol 1998;36:481-5

[19] Hofman A, Jaddoe VW, Mackenbach JP, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr Perinat Epidemiol 2004;18:61-72

[20] Wood H RU, Peeling R. Rapid Detection and Quantification of *Chlamydia trachomatis* in Clinical Specimens by LightCycler PCR. In: Reischl U WC, Cockerill F, ed. Rapid Cycle Real-Time PCR, Methods and Applications, Microbiology and Food Analysis: Springer, 2002:115-132

[21] Van der Pol B, T. C. Quinn, C. A. Gaydos, K. Crotchfelt, J. Schachter, J. Moncada, D. Jungkind, D. H. Martin, B. Turner, C. Peyton, and R. B. Jones. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of *Chlamydia trachomatis*. J. Clin.Microbiol. 2000;38:1105-1112

[22] Vincelette J, Schirm J, Bogard M, et al. Multicenter evaluation of the fully automated COBAS AMPLICOR PCR test for detection of *Chlamydia trachomatis* in urogenital specimens. J Clin Microbiol 1999;37:74-80

[23] Ossewaarde JM, van Doornum GJ, Buimer M, Choueiri B and Stary A. Differences in the sensitivity of the Amplicor *Chlamydia trachomatis* PCR assay. Genitourin Med 1997;73:207-11

[24] Peterson EM, Darrow V, Blanding J, Aarnaes S and de la Maza LM. Reproducibility problems with the AMPLICOR PCR *Chlamydia trachomatis* test. J Clin Microbiol 1997;35:957-9

[25] Mahony J, Chong S, Jang D, et al. Urine specimens from pregnant and nonpregnant women inhibitory to amplification of *Chlamydia trachomatis* nucleic acid by PCR, ligase chain reaction, and transcription-mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory activity. J Clin Microbiol 1998;36:3122-6

[26] Chan EL, Brandt K, Olienus K, Antonishyn N and Horsman GB. Performance characteristics of the Becton Dickinson ProbeTec System for direct detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in male and female urine specimens in comparison with the Roche Cobas System. Arch Pathol Lab Med 2000;124:1649-52

[27] Niederhauser C, Kaempf L. Improved sensitivity of the *Chlamydia trachomatis* Cobas Amplicor assay using an optimized procedure for preparation of specimens. Eur J Clin Microbiol Infect Dis 2003;22:118-21

[28] Chernesky M, Jang D, Chong S, Sellors J and Mahony J. Impact of urine collection order on the ability of assays to identify *Chlamydia trachomatis* infections in men. Sex Transm Dis 2003;30:345-7

[29] Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep 1998; 47(RR-1):1-111

[30] Rastogi S, Das B, Salhan S and Mittal A. Effect of treatment for *Chlamydia trachomatis* during pregnancy. Int J Gynaecol Obstet 2003;80:129-37

[31] van den Hoek JA, Mulder-Folkerts DK, Coutinho RA, Dukers NH, Buimer M and van Doornum GJ. [Opportunistic screening for genital infections with *Chlamydia trachomatis* among the sexually active population of Amsterdam. II Over 90% participation and almost 5% prevalence]. Ned Tijdschr Geneeskd 1999;143:668-72

[32] van Valkengoed IG, Boeke AJ, van den Brule AJ, et al. [Systematic home screening for *Chlamydia trachomatis*

infections of asymptomatic men and women in family practice by means of mail-in urine samples]. Ned Tijdschr Geneeskd 1999;143:672-6

[33] van der Snoek EM, Chin ALRA, de Ridder MA, Willems PW, Verkooyen RP and van der Meijden WI. Prevalence of sexually transmitted diseases (STD) and HIV-infection among attendees of STD Outpatient Clinic, Dijkzigt University Hospital in Rotterdam; comparative analysis of years 1993 and 1998. Ned Tijdschr Geneeskd 2000;144:1351-5

[34] Honey E, Augood C, Templeton A, et al. Cost effectiveness of screening for *Chlamydia trachomatis*: a review of published studies. Sex Transm Infect 2002;78:406-12

[35] Paavonen J, Puolakkainen M, Paukku M and Sintonen H. Cost-benefit analysis of first-void urine *Chlamydia trachomatis* screening program. Obstet Gynecol 1998;92:292-8

Chapter 10

Cost-effectiveness of *Chlamydia trachomatis* screening in Dutch pregnant women

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Abstract

Background *Chlamydia trachomatis* infections are largely asymptomatic. In pregnancy chlamydial infections may influence pregnancy outcomes and its prevention is based on screening. The success of large-scale screening for *C. trachomatis* depends on the target population (prevalence, participation), availability of non-invasive sampling, high-quality testing, effective treatment, and the cost-effectiveness of screening.

Objective Cost-effectiveness analysis of *C. trachomatis* screening during pregnancy.

Methods We designed a pharmaco-economic decision analysis model, which included potential health outcomes of *C. trachomatis* infection such as PID, infertility and chronic abdominal pain as well as ectopic pregnancy, premature delivery and neonatal disease. We estimated the cost-effectiveness from a societal perspective using the most recent prevalence data from a population-based prospective cohort study among pregnant women in the Netherlands. We calculated the prevented costs by linking health outcomes with health care costs and productivity losses. Cost-effectiveness was estimated in base-case-, sensitivity- and scenario analysis.

Results In the base-case analysis the costs to detect 1,000 pregnant women with *C. trachomatis* were estimated at €378,300. Cost savings on complications were estimated at €814,400 resulting in net cost savings. Sensitivity analysis showed that net cost savings remained with a test price up to €28, an averted proportion of complications of only 25% and a risk for PID of only 0,4%. Scenario analysis showed even more cost savings with targeted screening for women less than 30 years of age or with first pregnancies.

Conclusions *C. trachomatis* screening of pregnant women in the Netherlands is cost-saving.

Introduction

Chlamydia trachomatis is one of the major sexually transmitted pathogens in industrialized countries [1]. Treacherously, about 80% of infected women remain asymptomatic. Asymptomatic chlamydial infection during pregnancy poses risks for women due to PID and ectopic pregnancy [2-5], and may, due to vertical transmission, lead to neonatal chlamydial conjunctivitis and respiratory tract infection [6-9]. These complications may lead to major health costs, which can only be prevented by active case finding and early treatment of *C. trachomatis* infection.
C. trachomatis screening, however, is not part of routine antenatal care in all countries, because little is known about the cost-effectiveness of such screening. Recently we found *C. trachomatis* infection during pregnancy to increase the risk for early premature delivery in chlamydia-positive women four-fold [10]. This finding implies that screening for *C. trachomatis* may be more cost-effective than previously estimated.
In this study we estimate the cost-effectiveness of *C. trachomatis* screening in pregnant women in the Netherlands based on a static model. Our approach builds on recently reported local data and a decision tree of possible complications.

Materials and Methods

General Model Design

Preferably, we used data from local studies, but if unavailable we used international results. The model we used for the cost-effectiveness analysis (CEA) of screening consists of an epidemiologic part and an economic part. We used the epidemiologic part to estimate the impact of screening on the prevalence of *C. trachomatis* in the population. The economic part we used to evaluate the prevented complications and costs, and the costing of a screening program. We linked the two models by using the output of the epidemiologic part as input for the economic part resulting in a formal cost-effectiveness ratio (CER).

Epidemiologic model

To analyze the cost-effectiveness, we used a recent update of a health-economic model that was previously developed for *C. trachomatis* screening in the Dutch setting [11-15].
For the epidemiologic part of the model we could use a dynamic or a static version. The dynamic model version includes explicitly the transmission dynamics of *C. trachomatis* infection in the population, i.e. "a force of infection that varies" with the changing prevalence of *C. trachomatis* infection in the population [14-17]. A screening program will have a decreasing effect on the chlamydial prevalence in a population with a subsequent decrease in the overall probability to encounter an infected partner and indirectly protection for future sex partners. Dynamic models

are especially warranted to screen populations with frequently changing sexual partners [18]. The static model version, on the contrary, includes "a constant force of infection" or a constant probability to encounter an infected partner. In the static model only the cures are taken into account that are directly related to screening and subsequent treatment, but not to the changing prevalence or risk due to averted chlamydial infections. Static models suffice to assess populations in which frequent change of sexual partners is not common [18]. We have applied this static version of our model to evaluate screening for *C. trachomatis* infection in pregnancy before [11]. However, at that time, actual data concerning pregnant women and pregnancy outcomes were not available in the Netherlands. In the current study we applied the static model version to large-scale observational data on the prevalence and outcome of *C. trachomatis* infection in Dutch pregnant women [10].

Epidemiologic Data

We used the prevalence data from a prospective multi-center, population-based *C. trachomatis* study among 4,055 pregnant women aged 15 to 46 years in Rotterdam, The Netherlands, which were gathered between 2003 and 2005 [10]. This collection represents the start of the Generation R study [19, 20]. Pooled fresh first void urine specimens were tested by a nucleic acid amplification technique [21], and showed that 157 women (3.9%) tested positive for *C. trachomatis*. Age-specific prevalences were 13.5% in women 20 years and younger, 6.7% between 21 and 25 years, 3.3% between 26 and 30 years, and 1.6% in women over 30 years. The women were followed regarding their pregnancy outcomes. Pregnancy-specific prevalences were 4.5% in women with a first pregnancy, 3.1% in women with a second pregnancy and 3.6% for next pregnancies. Adverse pregnancy outcomes such as miscarriage, low birth weight and perinatal death were not found to be associated with *C. trachomatis* infection of the women in this population, but chlamydia-positive women had an increased risk for premature delivery before 35 weeks and 32 weeks gestation [10]. This risk was inserted in our model. (see below)
Figures with respect to maternal complications were used from previous international studies [14]. Data regarding the outcome of premature delivery, e.g. the duration of admission to a neonatal intensive care unit in the Netherlands, were obtained from The Netherlands Perinatal Registry [22].

Risks, Disease Costs and QALYs

Figure 1 shows the medical decision-tree we used for our analysis and reflects the progression of asymptomatic *C. trachomatis* infection in pregnant women in a screening and no-screening scenario, thereby combining the acute disease and long-term complications of chlamydial infection for the women themselves, and complications in their newborns and partners (reduced by 50% due to screening and treatment) [14].
Table 1 shows the risks we used in the CEA-model for screening of *C. trachomatis* infection during pregnancy, which were previously reported [14]. In the current analysis, we added the elevated

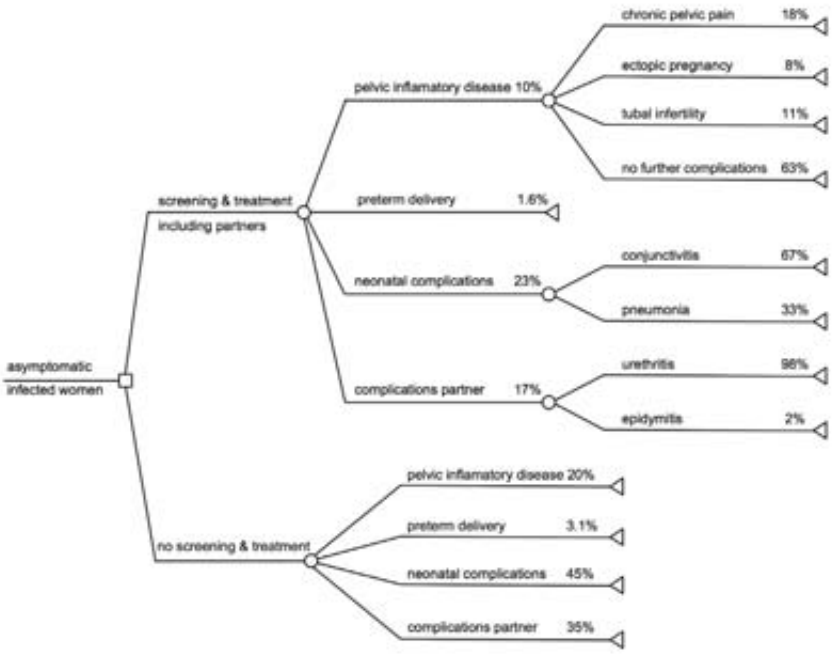

Figure 1 Medical decision tree for *Chlamydia trachomatis* screening during pregnancy based on complications in women, newborns and partners

*Percentages may add up over 100% as complications can be in different individuals: pregnant women, newborns and partners

risk for preterm delivery before 35 weeks gestation, which was directly calculated from the study on pregnancy outcomes [10]. We calculated the risks for preterm delivery for chlamydia-positive women (4.6%) and chlamydia-negative women (1.5%). The 3.1% difference between groups reflects the elevated risk for preterm delivery in women with *C. trachomatis* infection compared to the base-case risk in the absence of infection (Table 1).

The probabilities for neonatal conjunctivitis and pneumonia as well as preterm delivery were based on live-born neonates only and were adjusted taking into account that 1.5% of pregnancies result in miscarriages and perinatal death [10, 23]. The probabilities for ectopic pregnancy and tubal infertility after (post-partum) PID were interpreted as referring to the next pregnancy and were only applied in the model if a next pregnancy wish was expected to occur. The probability for a next pregnancy wish was based on Dutch data for having a second, third or next pregnancy, and was estimated at 78%, 50%, and 45% respectively [22]. We made calculations for these pregnancy rates individually in the sensitivity analysis (below), which analyses were aggregated for reporting the base-case analysis (see below).

Besides maternal and neonatal complications, we included symptomatic and asymptomatic disease of male partners according to our previous approach [12]. We assumed that pregnant

women have a 68% probability of having infected partners [24]. The infected male partners have 50% probability of becoming symptomatic with urethritis and 50% probability of remaining asymptomatic. However, of the latter group another 2% will develop epididymitis [12].
The costs include the direct medical costs of treatment for *C. trachomatis* infection and complications, and the indirect costs of production losses. Previously reported costs were updated using appropriate deflators to achieve 2007 price levels [14, 25](www.cbs.nl). According to the Dutch guidelines for pharmacoeconomic practice we discounted the costs of chronic pelvic pain (CPP), ectopic pregnancy and infertility at 4% per year [26], taking into account that CPP costs occur during 5 subsequent years and that the average duration between two pregnancies is 2.5 years [25]. The costs for prematurity were based on data from the St Radboud UMC, Nijmegen (personal communication L. Collee).
The assumed quality-adjusted life year (QALY) losses that we applied in the model are also listed in table 1 [15, 27, 28]. Given the lack of valid studies regarding the quality of life impact following prematurity, no such QALYs were included for either the newborns or the parents. As for the costs, the QALY gains were similarly discounted, but at a rate of 1.5% per annum [26].

Intervention Costs

The costs of testing were assumed at €12 [14], which included the discounts that would presumably be achieved in a large-scale use of PCR-kits as would be the case in a screening program. If a woman would test positive for *C. trachomatis*, she and her partner would be treated or referred to a General Practitioner (GP) for treatment: costs at €20.4 per GP-visit. We included a prescription for amoxicillin for pregnant women, azithromycin for partners and erythromycin for infants in the model. Internationally, single-dose azithromycin is often the preferred treatment for *C. trachomatis*. However, current Dutch guidelines recommend amoxicillin during pregnancy [29]. The costs were rated at €10.77 for amoxicillin and at €12.02 for azithromycin, which both included the pharmacist's fee [14, 29]. The effectiveness of the antibiotics was inserted in the model at 95% [13]. Reinfection in the absence of partner treatment was not included in the model.

Cost-effectiveness Analysis

The risks, costs and QALY losses were linked within the static cost-effectiveness model as previously described [11]. We calculated the cost-effectiveness ratio (CER) using the formula:
$CER = (C_{S\&T} - S_C)/\text{QALYs gained}$, in which the costs for screening and treatment ($C_{S\&T}$) minus the savings on complications (S_C) are divided by the QALY losses averted (QALYs gained) [30]. These calculations were consistently done for various age groups and screening in first pregnancies, second and subsequent pregnancies.
In the cost-effectiveness framework, we assumed a 100% specificity of testing and a crude 50% of complications that could be averted by screening and subsequent effective treatment [14].

Table 1 Complications of *Chlamydia trachomatis* infection with risks, costs and QALY losses per case

Complication	Risk	Risk base	Costs (€)	QALY
Current pregnancy				
Mothers				
asymptomatic PID	12%	CT-infection*	na	na
symptomatic PID	8%	CT-infection*	1,621	0.35
CPP	18%	any PID	2,072	1.94
Infants**				
neonatal conjunctivitis	30%	CT-infection***	45	0.02
neonatal pneumonia	15%	CT-infection***	1,944	0.06
preterm delivery <35 weeks	3.1%	CT-infection***	32,000	ni
Next pregnancy				
ectopic pregnancy	8%	any PID	3,982	0.04
tubal infertility	11%	any PID	2,107	2.74
Male partners				
symptomatic urethritis	34%	CT-infection	83	0.01
epididymitis	0.68%	CT-infection	940	0.01

*All *C. trachomatis* infected pregnant women

**Risks adjusted for 1.5% of pregnancies resulting in miscarriages and stillbirths

****C. trachomatis* infected women delivering a live-born baby

QALY: quality-adjusted life year, PID: pelvic inflammatory disease, CT: *C. trachomatis,*

na: not applicable, CPP: chronic pelvic pain, ni: not included

The latter reflects the possibility that *C. trachomatis* infection may already have caused irreversible damage.

The calculations were performed in a base-case analysis, sensitivity analyses and in scenario analyses [30]. We did a base-case analysis using the above-mentioned assumptions on risks, costs, QALYs, discount rates and effectiveness of *C. trachomatis* screening for all pregnant women based on the medical decision tree (figure 1). Next, we performed a sensitivity analysis directed towards the test price (ranging from €5 up to the official price of €35), towards the assumed 50% of complications being averted (ranging from 25% to 75%) and towards the risk for PID to develop after *C. trachomatis* infection (ranging from 0,43 to 40%) [31]. PID has been consistently found to be the most sensitive parameter for the cost-effectiveness of screening for *C. trachomatis* in a general population [13, 32], but has also been criticized to be overestimated [31]. Eventually, we performed a scenario analysis on possible target groups for screening based on age (cut-offs at 20, 25 and 30 years) and pregnancy rate (first, second or next pregnancies) as well as on the discount rate (ranging from 0 to 4%) for the costs and the QALYs.

Results

Base-case Analysis

In the base-case analysis, we estimated an investment of €378,300 in order to detect *C. trachomatis* infection for 1,000 pregnant women and subsequently treat them and their respective partners. This estimate consisted of €312,800 for testing and €65,500 for (partner) treatment. The cost savings on complications were estimated at €814,400. A distribution of the savings is shown in figure 2a: the major savings were due to the prevention of preterm delivery (65%), followed by neonatal pneumonia (17%) and PID (8%). Eventually, the resulting net costs were negative indicating overall net cost savings.

Regarding major outcomes, per 1,000 pregnancies identified 73 cases of symptomatic PID, CPP, ectopic pregnancy and infertility would be averted in addition to 225 cases of neonatal conjunctivitis, pneumonia and preterm delivery, and 162 male complications. Correspondingly, we estimated that over the various complications in total 59 QALYs were to be gained per 1,000 pregnancies detected, which distribution is shown in figure 2b: the major QALY gains were due to CPP (61%), infertility (26%) and neonatal pneumonia (8%). In the absence of any data, no QALY gains were included for prematurity.

Sensitivity Analysis

The sensitivity analysis of the test price is shown in figure 3, which clearly demonstrates that the net cost savings remain as long as test costs are below approximately €30. The exact break-even test costs (rendering net costs equaling €0) were estimated at €28.

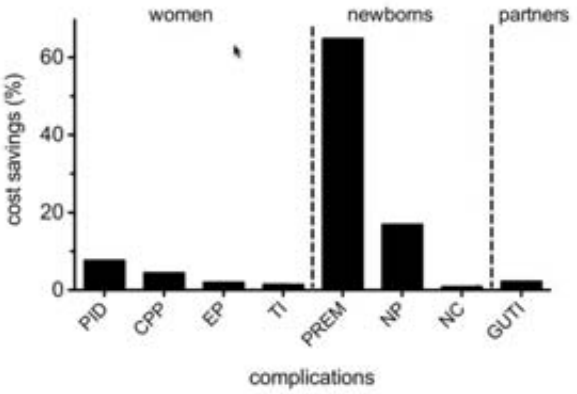

Figure 2a Distribution of cost-savings on complications

PID: pelvic inflammatory disease, CPP: chronic pelvic pain, EP: ectopic pregnancy, TI: tubal infertility, PREM: preterm delivery, NP: neonatal pneumonia, NC: neonatal conjunctivitis, GUTI: genito-urinary tract infection

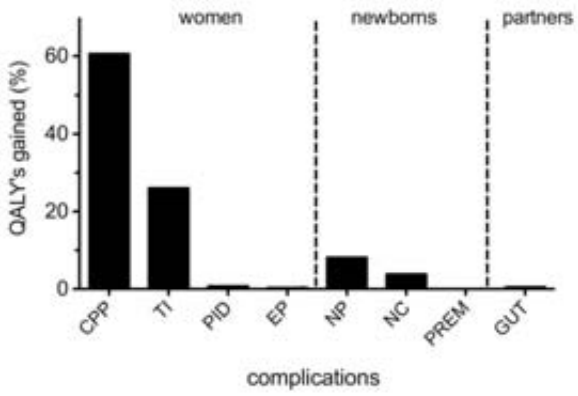

Figure 2b Distribution of QALYs gained on complications

CPP: chronic pelvic pain, TI: tubal infertility, PID: pelvic inflammatory disease, EP: ectopic pregnancy, NP: neonatal pneumonia, NC: neonatal conjunctivitis, PREM: preterm delivery, GUTI: genito-urinary tract infection. No QALYs included for preterm delivery

Varying the proportion of complications that can be averted between 25% and 75% (50% in the base-case), rendered estimated cost savings over the whole range for the base-case test costs at €12. Break-even test costs were estimated at €13 and €44, respectively. For the low proportion of only 25% averted complications, cost-effectiveness remained below €20,000 per QALY until test costs were raised to €35, at which test price the cost-effectiveness was still only €19,400 per QALY.

Varying the risk for PID from 0.4% to 40% (20% in the base-case), again rendered savings over the whole range for the base-case test costs. Break-even test costs were estimated at €26 and €30, respectively. For a PID risk at 0.4%, the cost-effectiveness still remained below €4,000 per QALY when test costs were raised up to €35.

Scenario Analysis

The results of a cost-effectiveness analysis may vary by the prevalence in any given population; by age and by pregnancy rate.

For age-specific screening at base-case test costs, results indicated cost savings for screening pregnant women ≤ 20 years of age (break-even test costs > €35), women aged 21-25 years (break-even test costs > €35) and women aged 26-30 years (break-even test costs €30 and CER €2,400 per QALY for test costs at €35). For women > 30 years of age, the screening program did not exhibit cost savings anymore with a CER of €1,300 per QALY (break-even test costs at €10, €28,800 per QALY at test costs of €35).

For screening by pregnancy rate, the most favorable estimate was derived for screening in first pregnancies with cost savings of €7,800 additional to any QALY gained. The cost savings for second pregnancies were €6,500 and €7,600 for next pregnancies.

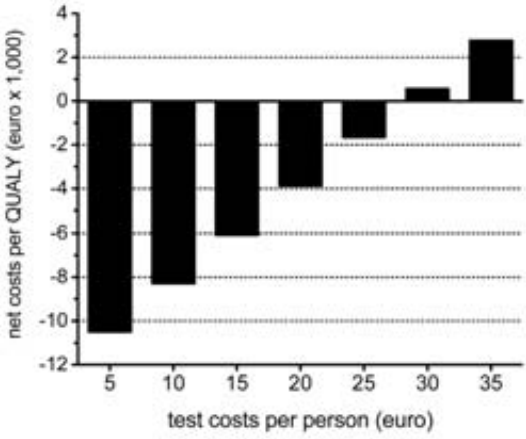

Figure 3 Sensitivity analysis on the test price

Base-case assumption at test price of €12

Figure 4 shows the costs per QALY gained for screening in pregnant populations with a *C. trachomatis* prevalence from 0% onwards, illustrated for second pregnancy rates (curves for first and next pregnancies not shown as these are almost identical and nearly overlapping). At base-case costs (€12), the results indicated cost savings for screening of pregnant women in populations with a *C. trachomatis* prevalence beyond 1.7%. At the extremes, if test costs would be as low as €5 cost savings would already occur beyond a prevalence of 0.7% and with test costs as high as €35 cost savings would occur beyond a prevalence of 5%. However, for the latter high test costs, the cost-effectiveness would still be below €20,000 per QALY gained for prevalences above 2%.
In addition, figure 4 can also be used to estimate the cost-effectiveness for age-specific screening during pregnancy at varying test costs. With prevalences, as in our population, of 13.5% in women 20 years and younger, or 6.7% in women between 21 and 25 years the net costs per QALY gained remained negative even at the extreme of €35 test costs. When screening women between 26 and 30 years with a prevalence of 3.3%, the net costs per QALY gained remained negative at a test price below €20. The net costs per QALY gained would only be cost saving if screening of women over 30 years - with a prevalence of 1.6% - would be done at a test price below €12.
At base-case test costs, the cost savings remained if discount rates were multi-variately ranged from 0 to 4% for both costs and QALYs. At the extreme, the cost-effectiveness worsened only slightly from €2,800 when discounting according to Dutch guidelines (costs at 4% and QALYs at 1.5%) to €2,900 when discounting both the costs and QALYs at 4% and the test costs at €35 [26].

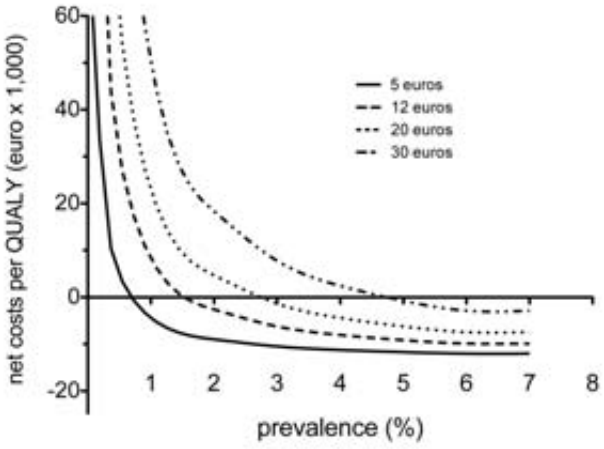

Figure 4 Costs per QALY gained by prevalence when screening for *Chlamydia trachomatis* in pregnant women using different test costs

Discussion

The present health-economic analysis clearly shows that screening for *C. trachomatis* would be cost-saving if all pregnant women would be included in a screening program in the Netherlands at a test price up to €28, despite the conservative approach we used throughout our design. Subsequent sensitivity analysis shows that a screening program remains cost saving, or at least highly cost-effective, if relevant parameters such as test price, complication rate and PID are varied within plausible ranges. Furthermore, the scenario analysis shows that the health economic gain can be further increased if a screening program would take aim at specific groups of pregnant women including women below thirty years of age or women with first pregnancies only.

C. trachomatis infection during pregnancy may lead to serious complications like ectopic pregnancy and premature delivery, vertical transmission with subsequent neonatal conjunctivitis and respiratory tract infection [2, 6-9], and post-partum pelvic inflammatory disease (PID), chronic pelvic pain (CPP) and infertility [33-35]. Pregnant women may, therefore, be a specific target group for *C. trachomatis* screening. Antenatal screening, as recommended by the CDC [36], would be beneficial to decrease morbidity amongst women themselves, but also to prevent vertical (infant) and horizontal (partner) transmission. At present seven of 25 member states of the European Union have included *C. trachomatis* screening in their guidelines for antenatal care in normal pregnancies [37]. In the Netherlands, however, chlamydial testing is not part of routine antenatal care since local data regarding *C. trachomatis* infection in pregnant women and perinatal transmission were lacking and the cost-effectiveness of a national screening program remained largely hypothetical.

In the current cost-effectiveness study of a screening program for *C. trachomatis* we have chosen to use a static model approach since the absence of frequently changing sexual partners is likely to be a reasonable assumption in relationships during pregnancy. We included the use of NAATs on pooled urine specimens to test for *C. trachomatis* in a screening program, because of the non-invasive nature of sampling, a high sensitivity and specificity of testing and a reduction of the total amount and subsequent costs of testing [21]. We worked with the recently reported *C. trachomatis* prevalence of 3.9% that was found in apparently healthy Dutch pregnant women and took into account the increased risk for preterm delivery [10]. The latter finding has recently been reported to occur in *C. trachomatis* infected pregnant women in other countries as well [3-5, 38]. Including preterm delivery in the model increased the public healthcare costs dramatically [39]. Since women were already pregnant in this model, we included the probability for ectopic pregnancy and tubal infertility only for a possible second or third pregnancy. Higher pregnancy rates are not common in the Netherlands and were conservatively not included in the model. Furthermore, we worked with 50% of complications being avertable since treatment may occur at a moment that infection may already have caused irreversible damage. We discounted the costs of CPP, ectopic pregnancy and infertility as well as the QALYs per year according to standard practice in correcting for time preference in health-economic modeling. Reinfection in the absence of partner treatment was not included in the model. We assumed that women who were married or living in registered partnership (85%) [10], and single women with a steady relationship, would take medication at the same time as their partners and have no sexual contact outside the relationship during the time of the current pregnancy. Similarly, for the single women without a steady relationship, we assumed that they would take medication with or without the partner when test findings were positive and that they had no further new sexual contacts during the current pregnancy.

Our results indicate that the benefits of averted direct and indirect costs are much higher than the investment costs for *C. trachomatis* screening of pregnant women despite the conservative approach we used throughout our design. In the current approach for cost-effectiveness analysis we did not include the potentially high costs of long term complications due to premature birth such as lifelong impairments, disability or handicap in hearing, vision, language and speech, neuromotor dysfunction, mental development, musculoskeletal and respiratory tract complications. We also didn't calculate the extra costs and production losses of parents of premature newborns directly after birth and in the years to follow, which may be expected to extend into adolescence or possibly even into adulthood, nor the potential production losses of the prematurely born individuals themselves during their lives [40-46]. Furthermore, we didn't include QALY losses due to premature birth for both the infants and their parents. Despite this conservative approach, both the base-case analysis and sensitivity analysis demonstrated that *C. trachomatis* screening would be highly cost-effective. While varying the proportion of averted complications to as little

as 25% and calculating with a risk for PID as low as 0.4%, the cost-effectiveness remained below €20,000 per QALY. In the Netherlands, a cost-effectiveness below €20,000 per QALY gained is informally taken as a threshold for accepting preventive programs from a health-economic point of view. Our results also demonstrate that screening of pregnant women for *C. trachomatis* in the Netherlands would be cost-saving if screening would be based on either pregnancy rate or age, with the exception of screening women over 30 years of age. However, screening of women over 30 years of age would still be considered cost-effective in the Dutch population.

If we would extrapolate our CEA of *C. trachomatis* screening to all pregnant women in the Netherlands, approximately 200,000 pregnancies per year, the total costs for screening and treatment could be estimated at €2,737,000 annually. Nationally, this would be counterbalanced by savings on costs related to the complications of *C. trachomatis* infection that would mount to €5,893,000, which means net savings of more than €3 million each year. In addition to this economic gain, 426 QALYs would be gained nationwide.

Our conclusion is that screening pregnant women for *C. trachomatis* is highly cost-effective and, in most cases, cost-saving in the Netherlands.

References

[1] Wilson JS, Honey E, Templeton A, Paavonen J, Mardh PA, Stray-Pedersen B. A systematic review of the prevalence of *Chlamydia trachomatis* among European women. Hum Reprod Update. 2002;8(4):385-94.

[2] Mardh PA. Influence of infection with *Chlamydia trachomatis* on pregnancy outcome, infant health and life-long sequelae in infected offspring. Best Pract Res Clin Obstet Gynaecol. 2002;16(6):847-64.

[3] Andrews WW, Goldenberg RL, Mercer B, Iams J, Meis P, Moawad A, et al. The Preterm Prediction Study: association of second-trimester genitourinary chlamydia infection with subsequent spontaneous preterm birth. Am J Obstet Gynecol. 2000;183(3):662-8.

[4] Karinen L, Pouta A, Bloigu A, Koskela P, Paldanius M, Leinonen M, et al. Serum C-reactive protein and *Chlamydia trachomatis* antibodies in preterm delivery. Obstet Gynecol. 2005;106(1):73-80.

[5] Blas MM, Canchihuaman FA, Alva IE, Hawes SE. Pregnancy outcomes in women infected with *Chlamydia trachomatis*: a population-based cohort study in Washington State. Sex Transm Infect. 2007;83(4):314-8.

[6] Hammerschlag MR. Chlamydial infections. J Pediatr. 1989 May;114(5):727-34.

[7] Darville T. *Chlamydia trachomatis* infections in neonates and young children. Semin Pediatr Infect Dis. 2005;16(4):235-44.

[8] Rours GIJG, Hammerschlag MR, Ott A, De Faber TJ, Verbrugh HA, de Groot R, et al. *Chlamydia trachomatis* as a cause of neonatal conjunctivitis in Dutch infants. Pediatrics. 2008;121(2):e321-6.

[9] Rours GIJG, Hammerschlag MR, Van Doornum GJ, Hop WC, de Groot R, Willemse HF, et al. *Chlamydia*

trachomatis respiratory infection in Dutch infants. Arch Dis Child. 2009;94(9):705-7.

[10] Rours GIJG, Duijts L, Moll HA, de Groot R, Jaddoe VW, Hofman A, Steegers EAP, Mackenbach JP, Ott A, Willemse HFM, van der Zwaan AE, Verbrugh HA, Verkooijen RP. Pregnancy outcomes in women infected with *Chlamydia trachomatis*: a population-based prospective study cohort Proceedings Sixth Meeting of the European Society for Chlamydia Research 2008; P32. 2008.

[11] Postma MJ, Bakker A, Welte R, van Bergen JE, van den Hoek JA, de Jong-van den Berg LT, et al. Screening for asymptomatic *Chlamydia trachomatis* infection in pregnancy; cost-effectiveness favorable at a minimum prevalence rate of 3% or more. Ned Tijdschr Geneeskd. 2000;144(49):2350-4.

[12] Postma MJ, Welte R, van den Hoek JA, van Doornum GJ, Jager HC, Coutinho RA. Cost-effectiveness of partner pharmacotherapy in screening women for asymptomatic infection with *Chlamydia trachomatis*. Value Health. 2001;4(3):266-75.

[13] Welte R, Kretzschmar M, Leidl R, van den Hoek A, Jager JC, Postma MJ. Cost-effectiveness of screening programs for *Chlamydia trachomatis*: a population-based dynamic approach. Sex Transm Dis. 2000;27(9):518-29.

[14] de Vries R, van Bergen JE, de Jong-van den Berg LT, Postma MJ. Systematic screening for *Chlamydia trachomatis*: estimating cost-effectiveness using dynamic modeling and Dutch data. Value Health. 2006;9(1):1-11.

[15] de Vries R, van Bergen JE, de Jong-van den Berg LT, Postma MJ. Cost-utility of repeated screening for *Chlamydia trachomatis*. Value Health. 2008;11(2):272-4.

[16] Kretzschmar M, Welte R, van den Hoek A, Postma MJ. Comparative model-based analysis of screening programs for *Chlamydia trachomatis* infections. Am J Epidemiol. 2001;153(1):90-101.

[17] Kretzschmar M. Mathematical epidemiology of *Chlamydia trachomatis* infections. Neth J Med. 2002;60(7 Suppl):35-41; discussion 2-3.

[18] Welte R, Postma M, Leidl R, Kretzschmar M. Costs and effects of chlamydial screening: dynamic versus static modeling. Sex Transm Dis. 2005;32(8):474-83.

[19] Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22(12):917-23.

[20] Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update until the age of 4 years. Eur J Epidemiol. 2008;23(12):801-11.

[21] Rours GI, Verkooyen RP, Willemse HF, van der Zwaan EA, van Belkum A, de Groot R, et al. Use of Pooled Urine Samples and Automated DNA Isolation To Achieve Improved Sensitivity and Cost-Effectiveness of Large-Scale Testing for *Chlamydia trachomatis* in Pregnant Women. J Clin Microbiol. 2005;43(9):4684-90.

[22] The Netherlands Perinatal Registry. Perinatal Care in the Netherlands 2003. Bilthoven: Stichting Perinatale Registratie; 2006.

[23] Jentink J, van de Vrie-Hoekstra NW, de Jong-van den Berg LT, Postma MJ. Economic evaluation of folic acid food fortification in the Netherlands. Eur J Public Health. 2008;18(3):270-4.

[24] Quinn TC, Gaydos C, Shepherd M, Bobo L, Hook EW, 3rd, Viscidi R, et al. Epidemiologic and microbiologic correlates of *Chlamydia trachomatis* infection in sexual partnerships. JAMA. 1996;276(21):1737-42.

[25] Statistics Netherlands (CBS), The Hague, The Netherlands. http://statline.cbs.nl/statweb?/LA=en. 2007.

[26] The Health Care Insurance Board (CVZ). Amstelveen, The Netherlands; Guidelines for Pharmacoeconomic Research (in Dutch: Richtlijnen voor Farmaco-economisch Onderzoek). 1999.

[27] Chlamydia A. In: Stratton KR, Durch JS, Lawrence RS, eds. Vaccines for the 21st Century: A Tool for Decision-making. Washington, DC: National Acad Press. 2000.

[28] Coyne KS, Payne C, Bhattacharyya SK, Revicki DA, Thompson C, Corey R, et al. The impact of urinary urgency and frequency on health-related quality of life in overactive bladder: results from a national community survey. Value Health. 2004;7(4):455-63.

[29] Loenen AC. Farmacotherapeutical Kompass. Amstelveen, The Netherlands. The Health Care Insurance Board (CVZ): 2007.

[30] Gold MR SJ, Russell LB, Weinstein MC. Cost-Effectiveness in Health and Medicine. 1996.

[31] van Valkengoed IG, Morre SA, van den Brule AJ, Meijer CJ, Bouter LM, Boeke AJ. Overestimation of complication rates in evaluations of *Chlamydia trachomatis* screening programmes--implications for cost-effectiveness analyses. Int J Epidemiol. 2004;33(2):416-25.

[32] Postma MJ, Welte R, Morre SA. Cost-effectiveness of widespread screening for *Chlamydia trachomatis*. Expert Opin Pharmacother. 2002;3(10):1443-50.

[33] Wiesenfeld HC, Hillier SL, Krohn MA, Amortegui AJ, Heine RP, Landers DV, et al. Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. Obstet Gynecol. 2002;100(3):456-63.

[34] Peipert JF. Clinical practice. Genital chlamydial infections. N Engl J Med. 2003;349(25):2424-30.

[35] Manavi K. A review on infection with *Chlamydia trachomatis*. Best Pract Res Clin Obstet Gynaecol. 2006;20(6):941-51.

[36] Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep 2006;55(RR-11):1-94.

[37] Bernloehr A, Smith P, Vydelingum V. Antenatal care in the European Union: a survey on guidelines in all 25 member states of the Community. Eur J Obstet Gynecol Reprod Biol. 2005;122(1):22-32.

[38] Odendaal HJ Schoeman J. The association between *Chlamydia trachomatis* genital infection and spontaneous preterm labour. South African Journal of Obstetrics and Gynaecology. 2006;12(3):146-9.

[39] Russell RB, Green NS, Steiner CA, Meikle S, Howse JL, Poschman K, et al. Cost of hospitalization for preterm and low birth weight infants in the United States. Pediatrics. 2007;120(1):1-9.

[40] McCormick MC, Bernbaum JC, Eisenberg JM, Kustra SL, Finnegan E. Costs incurred by parents of very low birth weight infants after the initial neonatal hospitalization. Pediatrics. 1991;88(3):533-41.

[41] Walther FJ, den Ouden AL, Verloove-Vanhorick SP. Looking back in time: outcome of a national cohort of very preterm infants born in the Netherlands in 1983. Early Hum Dev. 2000;59(3):175-91.

[42] Petrou S. The economic consequences of preterm birth during the first 10 years of life. BJOG. 2005;112 Suppl 1:10-5.

[43] Stoelhorst GM, Rijken M, Martens SE, Brand R, den Ouden AL, Wit JM, et al. Changes in neonatology: comparison of two cohorts of very preterm infants (gestational age <32 weeks): the Project On Preterm and Small for Gestational Age Infants 1983 and the Leiden Follow-Up Project on Prematurity 1996-1997. Pediatrics. 2005;115(2):396-405.

[44] Marlow N, Wolke D, Bracewell MA, Samara M. Neurologic and developmental disability at six years of age after

extremely preterm birth. N Engl J Med. 2005;352(1):9-19.

[45] Lindstrom K, Winbladh B, Haglund B, Hjern A. Preterm infants as young adults: a Swedish national cohort study. Pediatrics. 2007;120(1):70-7.

[46] Underwood MA, Danielsen B, Gilbert WM. Cost, causes and rates of rehospitalization of preterm infants. J Perinatol. 2007;27(10):614-9.

Part V

Summary and considerations

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Acknowledgements

Chapter 11

Summary

Considerations

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Samenvatting

Summary

The studies described in this thesis aimed to assess the health-related importance of *Chlamydia trachomatis* infection during pregnancy for women and their newborns in order to provide information for future decision making regarding the need for *C. trachomatis* screening in pregnant women.

In **chapter 1** we presented the research questions, and related studies, that we addressed in this thesis. Main topics included:

1) What is the prevalence of *C. trachomatis* infection during pregnancy?
2) What are risk factors for *C. trachomatis* infection in pregnant women?
3) Is *C. trachomatis* infection during pregnancy associated with increased risk for adverse pregnancy outcomes such as stillbirth, premature birth and low birth weight?
4) What is the rate of *C. trachomatis* transmission from women to neonates?
5) Can we find evidence of vertical transmission by detection of *C. trachomatis* in infants with neonatal conjunctivitis and respiratory disease?
6) Is pooling of urine specimens a good method to improve cost-effectiveness of *C. trachomatis* testing during pregnancy?
7) Is routine screening for *C. trachomatis* a cost-effective approach during pregnancy?

In **chapter 2** we reviewed the literature regarding *C. trachomatis* infection in relation to pregnant women and infants. Chlamydial infection is characterized by invasion of the host cell, intracellular reproduction, and an inflammatory response that may persist and cause chronic inflammation and fibrotic changes. *C. trachomatis* infection, however, is most often asymptomatic. Due to its latent, insidious and potentially chronic character the severity of infection may range from asymptomatic and selflimiting disease to infection with severe complications such as pelvic inflammatory disease, infertility, and chronic pelvic pain. Moreover, *C. trachomatis* infection in pregnant women may lead to adverse pregnancy outcome and neonatal disease. Different test methods can be used to diagnose (latent) *C. trachomatis* infection of which NAATs are the gold standard at present. NAATs are highly sensitive and can be used with most specimens including urine and vaginal swabs. Various antibiotic treatment options for *C. trachomatis* infection are discussed. Single-dose treatment with azithromycin is recommended, because of high compliance, treatment succes, and less adverse events. Pregnant women were proposed as a significant target population for *C. trachomatis* screening. Prenatal chlamydial screening was contemplated according to the Wilson and Jung criteria. Some of these had not yet been explored in the Netherlands.

In **chapter 3** we asessed the prevalence of sexually transmitted infections in 766 pregnant urban South African women at the time they presented for delivery. In addition, associations of sexually transmitted infections with demographic and socio-economic characteristics and with clin-

ical symptoms were determined. Overall, 48% carried one or more sexually transmitted agents during their pregnancy. Infection with HIV, *Treponema pallidum, C. trachomatis* and *Neisseria gonorrhoeae* was detected in 18%, 23%, 12% and 9% of women. Predictive factors for infection included lack of antenatal care (OR 1.7, 95% CI 1.0-3.0), multiple pregnancies (OR 2.2, 95% CI 1.5-3.3), being unmarried (OR 1.8, 95% CI 1.3-2.5), and being unemployed (OR 1.4, 95% CI 1.0-1.9). HIV seropositivity was significantly higher among women under 30 years of age (21%) than among women 30 years and older (12%). For syphilis a significant trend was noted with the lowest prevalence among the youngest women (14%) and a higher rate in the older group (30%). For *C. trachomatis* infection a trend was observed with the highest prevalence in the younger women (22%) and the lowest among the older women (5%). We concluded that nearly half of the pregnant women carried one or more of the common sexually transmitted pathogens, and that chlamydial and gonococcal infections remained undetected and untreated during pregnancy. Furthermore, that infection was not associated with symptoms, but with differences in demographic and socio-economic characteristics.

In **chapter 4** we studied the rate of transmission of *C. trachomatis* infection from 77 pregnant South African women (described in chapter 3) to their newborns in a setting where tetracycline eye prophylaxis is routinely provided to prevent neonatal conjunctivitis. Furthermore, we evaluated the postnatal consequences of chlamydial carriage during pregnancy for women and infants. LCR and/or culture for *C. trachomatis* were positive in 23 of their infants reflecting a transmission rate of 30%. Nineteen (83%) of these 23 infants had a positive nasopharyngeal test and nine (39%) had a positive conjunctival test; five infants (22%) were colonised at both sites. All conjunctival cultures were negative. Nasopharyngeal specimens were positive in 13 (57%) of 23 infants by LCR and in 9 (45%) of 20 infants by culture. Infants of chlamydia-positive women were reported to have significantly more often nasal problems than infants of chlamydia-negative women (32 (49%) of 65 infants versus 15 (23%)) of 65 infants, which were also found on physical examination (37 (57%) versus 7 (11%)), as were higher respiratory rates and intercostal recession.
Postnatal genitourinary symptoms and signs were found in 34 (52%) and 51 (78%) of chlamydia-positive women, with 12 women (18%) developing post-partum pelvic inflammatory disease. We concluded that chlamydial infection in pregnant women places the newborn at risk and that topical tetracycline prophylaxis at birth is insufficient to prevent chlamydial infection in the infant. Furthermore, we suggested that the finding of postnatal maternal genitourinary symptoms and signs, in combination with symptoms and signs in the infant, should alert clinicians to the possibility of neonatal and maternal complications of chlamydial infection.

In **chapter 5** we studied the prevalence of and risk factors for *C. trachomatis* infection during pregnancy in 4,055 Dutch pregnant women who attended one of the midwifery practices or

antenatal clinics that participated in the Generation R study in Rotterdam, The Netherlands. Furthermore, we assessed the effect of chlamydial infection during pregnancy on premature delivery and birthweight. *C. trachomatis* infection was detected in 157 (4%) women. The prevalence was inversely correlated with age with the highest prevalence in women age 20 years or less (14%) and lowest in women over 30 years (2%). The prevalence was highest in Antillean (16%), Cape Verdean (11%) or Surinamese (9%) women, and in women with low-level education (6%), single marital status (12%), first pregnancies (5%), multiple sexual partners in the past year (8%) or a history of an STI (6%). In the unadjusted analysis the latter risk factors, except for pregnancy rate, were significantly associated with *C. trachomatis* infection. In the adjusted analysis age below 21 years (OR 1.8, 95% CI 1.2-2.6), Antillean ethnicity (OR 2.3, 95% CI 1.4-3.7) and single marital status (OR 1.6, 95% CI 1.3-2.0) remained risk factors for *C. trachomatis* infection; other maternal factors were not independently associated. For the second part of the study, pregnancy outcomes (gestational ages and birth weights) were analysed for 3,913 newborns. We found that 18% (95% CI 0.7-36.7) of women who delivered before 32 weeks and and 11% (95% CI 3.1-18.8) of women who delivered before 35 weeks gestation tested positive for *C. trachomatis* infection. Chlamydial infection was, after adjustment for potential confounders, associated with preterm delivery before 32 weeks (OR 4.4, 95% CI 1.3-15.2) and 35 weeks gestation (OR 2.7, 95% CI 1.1-6.5), but not with low birth weight. Of all deliveries before 32 weeks and 35 weeks gestation 15% (95% CI 4.5-39.5) and 7% (95% CI 2.5-20.1) were attributable to *C. trachomatis* infection. We concluded that *C. trachomatis* contributes significantly to early premature delivery which should be considered a public health problem, especially in young women and others at increased risk of *C. trachomatis* infection.

In **chapter 6** we evaluated the relationship between the presence of *C. trachomatis* and signs of placental inflammation in the placentas of 304 women who delivered before 32 weeks of gestation at the Erasmus MC-Sophia, Rotterdam. *C. trachomatis* was detected in 76 (25%) placentas. Histological evidence of placental inflammation was present in 123 (40%) placentas: in 41 (54%) of 76 placentas with *C. trachomatis* versus 82 (36%) of 228 placentas without *C. trachomatis* infection (OR 2.1, 95% CI 1.2-3.5). A significant increase towards more frequent detection of *C. trachomatis* infection was observed with increasing progression (P=0.003) and intensity (P=0.002) of placental inflammation on the maternal side, but not on the fetal side. We concluded that *C. trachomatis* was frequently detected in women with early premature delivery, and that *C. trachomatis* infection was associated with histopathological signs of placental inflammation.

In **chapter 7** we evaluated whether *C. trachomatis* was a cause of neonatal conjunctivitis in infants less than three months of age presented to the Erasmus MC-Sophia and the Rotterdam Eye Hospital, Rotterdam. In addition, we evaluated the clinical presentation of and prescribed treatment for chlamydial conjunctivitis compared with other infections. *C. trachomatis* was

detected in 27 (64%) of 42 retrospectively studied infants and 14 (61%) of 23 prospectively studied infants. Mucopurulent discharge was present in 35 (95%) of 37 infants, swelling of the eyes in 27 (73%) of 37, and conjunctival erythema in 24 (65%) of 37 infants. Respiratory symptoms occurred in 14 (38%) of 37 and feeding problems in five (14%) of 37 infants. Before microbiological diagnosis, general practitioners prescribed anti-chlamydial antibiotics to five (12%) and systemic antibiotics to four (10%) of 41 infants who tested positive for *C. trachomatis*. Ophthalmologists prescribed anti-chlamydial antibiotics to 21 (51%) and systemic antibiotics to seven (17%) of 41 infants, respectively. We concluded that *C. trachomatis* was the major cause of bacterial conjunctivitis in this population and that clinical differentiation from other pathogens was not possible. Many infants who tested positive for *C. trachomatis* did not receive appropriate antibiotic treatment.

In **chapter 8** we evaluated the presence of *C. trachomatis* in infants less than six months of age who presented with respiratory complaints to the Erasmus MC-Sophia during a one-year period. Respiratory specimens, primarily nasopharyngeal swabs, were tested for *C. trachomatis,* respiratory viruses and *Mycoplasma pneumoniae* using PCR, viral cell cultures and direct immunofluorescence. *C. trachomatis* respiratory tract infection was detected in 10 (7%) of 148 infants tested. Eight (80%) of 10 *C. trachomatis* infections occurred before three months of age. *C. trachomatis* had not been tested for by the attending physicians, but was the second most frequently detected respiratory pathogen after hRSV, which was found in 41 (28%) infants. We concluded that perinatal respiratory infection with *C. trachomatis* was relatively common and underdiagnosed in this population of infants less than six months of age.

In **chapter 9** we evaluated *C. trachomatis* testing in pregnant women. First void urine specimens were collected from 750 asymptomatic pregnant women in Rotterdam. Initially, we investigated the performance of three different DNA isolation methods with 350 of these urines and 70 pools of five individual urines of the same subset. The routinely used Cobas Amplicor test was compared to the Cobas Amplicor with prior DNA isolation by the MagNA Pure large-volume kit and the MagNA Pure bacterial DNA isolation kit. We found that 15, 14 and 27 urine specimens tested positive resulting in sensitivities of 52%, 48% and 93%, respectively. Subsequently, using all 750 urines, the Cobas Amplicor performance for individual testing (32 positive tests) was compared to pooled testing with the standard Cobas Amplicor procedure (20 positive tests) and to pooled testing with the Cobas Amplicor in combination with the MagNA Pure bacterial DNA isolation kit (44 positive tests), resulting in a sensitivity of 65%, 42% and 92% respectively ($P<0.001$). The prevalence of *C. trachomatis* in this population appeared to be 6%. In addition, we demonstrated that with pooling of urine specimens the costs of the combined MagNA Pure Bacterial DNA Isolation Kit and Cobas Amplicor method were only 56% of the costs of the standard Cobas Amplicor test applied to individual urines. The costs per

positive case detected in the combined method were only 39% of the standard test costs. We concluded that pooled testing for *C. trachomatis* infection in pregnant women could be developed for large-scale testing when the Cobas Amplicor test was used in combination with the MagNA Pure Bacterial DNA Isolation Kit. This combination significantly improved the sensitivity and decreased the costs.

In **chapter 10** we performed a cost-effectiveness analysis of *C. trachomatis* screening in pregnant women in the Netherlands based on a static model. We designed a pharmaco-economic decision analysis model, which included potential health outcomes of *C. trachomatis* infection in pregnant women such as PID, infertility and chronic abdominal pain as well as ectopic pregnancy, premature delivery and neonatal disease, and disease of the partner. We estimated the cost-effectiveness from a societal perspective using the prevalence data we found in chapter 5. We calculated the prevented costs by linking health outcomes with health care costs and productivity losses. Cost-effectiveness was estimated in base-case-, sensitivity- and scenario analysis. In the base-case analysis the costs to detect 1,000 pregnant women with *C. trachomatis* were estimated at €383,000. Cost savings on complications were estimated at €805,300 resulting in extensive net cost savings. A sensitivity analysis showed that net cost savings remained with a test price up to €28, an averted proportion of complications of only 25% and a risk for PID of only 0,4%. A scenario analysis showed even more cost savings with targeted screening for women less than 30 years of age or with first pregnancies. We concluded that *C. trachomatis* screening of pregnant women is highly cost-effective, and in most cases, cost-saving in the Netherlands.

Methodological considerations

The specific methodological considerations of each study in this thesis have been presented in the separate chapters. In this paragraph selection bias, information bias and confounding as well as ethical issues are discussed in general for the Dutch and South African follow-up study.

Selection bias

Of all eligible children at birth, 61% participated in the Dutch Generation R Study. National and regional registries do not have the subject characteristics for all eligible children and their parents to enable a detailed non-response analysis [1]. However, the percentages of women from different ethnicity, lower socio-economic status and the percentages of women or children with medical complications were lower among the participants than expected from the population figures in Rotterdam [1, 2]. This selection towards a more affluent and healthy study population may have an impact on some determinants and outcome analyses separately, affecting the frequency rates and, as a consequence, the statistical power and generalizibility of the results. The prevalences found in the study should therefore be interpreted against the background of potential selection mechanisms. This selection bias leads only to bias in aetiological studies if the selection mechanisms are both related to the determinant and outcome. Not all women who were initially enrolled in the generation R study could be tested for *C. trachomatis*. We had a non-response of 9.5% (491 women) for the Chlamydia study. This may in part be a true non-response, but is more likely due to a change in the routine of the overall Generation R study when urine specimens had to be collected. Another 12.0% (621 women) had to be excluded because their urines could not be matched to the respective questionnaires in the database due to logistical problems in the pilot phase of the Chlamydia study. However, all risk factors were similarly distributed among tested and untested women and no differences were found in median gestational age and mean birth weight (39.8 weeks versus 39.8 weeks; P=0.84, 3408 grams versus 3407 grams; P=0.92). Also, no differences were found in the proportion of *C. trachomatis* infections between women included and not included in the analyses of gestation age and birth weight. Therefore, selection bias seems unlikely. Finally, our numbers were too small to properly assess the association of *C. trachomatis* infection with low incidence outcomes, including miscarriage and perinatal death. Of all women enrolled in the study and with information about *C. trachomatis* infection, only 62 (1.5%) had a miscarriage or perinatal death. Data on gestational age and birth weight were not available for women with the latter adverse pregnancy outcomes, but it is likely that these women had premature deliveries relatively more often. None of these women had a *C. trachomatis* infection. Theoretically, our effect estimates for the associations of chlamydial infection with gestational age and birth weight could be biased and exaggerated when these associations would differ between all foetuses and foetal 'survivors'. This would be the case if *C. trachomatis* infection would have a 'protective' effect on early foetal death. However, this is unlikely [3, 4].

In the South African study, all consecutive pregnant black women delivering at the Johannesburg Hospital were included in the study. The hospital serves a population covering all socio-economic classes and races, but most obstetric patients are of lower socio-economic status. This may cause a selection towards a less healthy study population, which may influence some determinants, outcome and frequency rates. On the other hand, women who required an emergency caesarean section after presentation to the obstetric ward, and women who had an (incomplete) abortion or who were fully dilated upon arrival in the hospital were not included in the study. Non-inclusion of these women and newborns with medical complications caused a selection towards a more healthy study population, which may influence some determinants and outcomes, frequency rates, and the statistical power and generalizibility of the results. The prevalences found in this study should therefore be interpreted carefully considering the role of potential selection mechanisms.

Information bias

In the Dutch Chlamydia study information about socio-economic and life style risk factors of women was collected using self-administered questionnaires. The use of such self-reported questionnaires may be better than in-person interviews to obtain information. However, younger women may lack self-confidence and subsequently underreport certain risk factors such as number of sexual partners, history of sexually transmitted infections, and the use of alcohol or drugs, which may cause an underestimation of effects.

In the South African study information about socio-economic and life style risk factors of women was obtained through questionnaires administered by a nurse or docter. The choice for this method was made, because many women were illiterate and would not have been able to provide any or reliable information. To minimise underreporting of certain risk factors due to sense of shame, a female doctor or nurse did the questionnaires. However, underreporting of certain risk factors such as number of sexual partners or history of sexually transmitted infections may still have occurred and may cause an underestimation of effects. Most women spoke English. Otherwise the study was explained and the questionnaire obtained in their home language in order to prevent misunderstanding of questions and subsequent information bias due to linguistic problems. Privacy was always striven for. However, the questionnaires sometimes had to be done behind curtains in the presence of one up to three more women in the room. Again, this may have lead to underreporting of certain risk factors, which may subsequently have caused an underestimation of effects.

Confounding

One of the strengths of the Dutch Chlamydia study was that we were able to adjust for many potential confounders. However, regarding the number and proportion of early and late premature deliveries attributable to chlamydial infection some confounding as a result of co-infection by other genital pathogens could not be excluded. Furthermore, we did not correct for a previous history of termination of pregnancy in these women since this potential confounder was only recently established [5]. Finally, we had no information concerning the use of (macrolide) antibiotics during pregnancy. However, pregnant women are not routinely tested and treated for *Ureaplasma urealyticum* or *Mycoplasma genitalium* in the Netherlands; neither are women with premature rupture routinely treated with antibiotics. Regarding the use of (macrolide) antibiotics, it is most likely that such use would mitigate the detrimental effect of *C. trachomatis* infection on pregnancy outcome rather than exaggerate it.

At the time of the study 96% of all deliveries in the South African study was by black women. To avoid confounding by white, coloured, or Indian women we focused on black women. In this study none of the women reported specific treatment for *C. trachomatis* infection in the three months prior to delivery, but chlamydia-negative women reported more often to have received antibiotics for other reasons than *C. trachomatis* infection that may have affected their chlamydial status. However, this difference was not statistically significant. A previous history of termination of pregnancy was also not corrected for in this study. Furthermore, regarding the number and proportion of respiratory tract infection in infants that may have been attributable to chlamydial infection confounding as a result of (co-) infection by other pathogens could not be excluded since we did not test for other respiratory pathogens.

Ethical considerations

One may question the ethics of the Dutch *C. trachomatis* study since we did not treat the women who were screened and found to be positive for *C. trachomatis*. Prospective studies regarding *C. trachomatis* infection in pregnant women and pregnancy outcome or perinatal transmission would face ethical barriers in countries that have national guidelines or directives advocating *C. trachomatis* screening and treatment in routine antenatal care and would be impossible to carry out [6, 7]. However, in most countries of the European Union, including the Netherlands, screening and treatment of chlamydial infection during pregnancy remains controversial and routine antenatal screening for *C. trachomatis* is currently not recommended [8]. One of the reasons for the Dutch health Council to advise against screening for *C. trachomatis* was the lack of local data [9]. A large prospective study like ours would also be very difficult to perform in the Netherlands because of financial recources and logistics. Therefore it was a

unique opportunity to participate in the Generation R study, which is a population-based, non-interventional, prospective cohort study designed to identify early environmental and genetic determinants of growth, development and health of children, starting from foetal life until adolescence [1, 10]. We were allowed to insert our *C. trachomatis* study into the Generation R Study provided that the study would remain non-interventional. Furthermore, the data would be provided anonymously in order to protect the privacy of the participants. Both the Generation R Study and the *C. trachomatis* study were approved by the Medical Ethical Committee for Research on Human Subjects of the Erasmus University Medical Centre, Rotterdam. Written informed consent was obtained from all participants. Since we had to conform to the overall study design of the Generation R study and had no access to names or addresses we were not able to treat chlamydia-positive women or refer them to regular health care providers. However, the study did not interfere with current Dutch standard medical practice. Standard medical practice does not include screening for *C. trachomatis* antenatally. Pregnant women are only tested and treated for *C. trachomatis* during their pregnancy if they are identified as being at risk of infection due to clinical signs or via contact tracing from a chlamydia-positive partner.

In the chorioamnionitis study and respiratory study, samples were tested retrospectively with a few years in between specimen collection and testing, after which period we did not trace the women or infants.

In the retrospective part of the conjunctivitis study chlamydia-positive infants had been treated according to standard practice of the ophthalmologists at that time. In the prospective part of the conjunctivis study chlamydia-positive infants were treated with erythromycin and parents were referred to their general practitioner, the municipal public health service or a sexually transmitted disease clinic.

In the South-African study, great effort was made to invite participants to return for follow-up. At inclusion, the women were given a copy of the consent form, an information letter about the study, and a follow-up appointment. Chlamydia-positive women and infants who did not return for their follow-up appointment were traced by phone calls, by letters in English, Zulu, Sotho and Xhosa, and by home visits. This way, a much higher follow-up rate was achieved than is generally known from African follow-up clinics [11]. At their follow-up visit, all infants born to chlamydia-positive women were treated with erythromycin. All chlamydia-positive women and their partners were also given a prescription for treatment with erythromycin. In addition they were counseled and referred to the sexually transmitted disease clinic for further follow-up of *C. trachomatis* or other infections for which they had tested positive.

References

[1] Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, et al. The Generation R Study: Design and cohort profile. Eur J Epidemiol. 2006;21(6):475-84.

[2] Center for Research and Statistics (COS). http://www.cos.rotterdam.nl. Rotterdam. 2005.

[3] Martin DH, Koutsky L, Eschenbach DA, Daling JR, Alexander ER, Benedetti JK, et al. Prematurity and perinatal mortality in pregnancies complicated by maternal *Chlamydia trachomatis* infections. JAMA. 1982;247(11):1585-8.

[4] Gencay M, Koskiniemi M, Ammala P, Fellman V, Narvanen A, Wahlstrom T, et al. *Chlamydia trachomatis* seropositivity is associated both with stillbirth and preterm delivery. APMIS. 2000;108(9):584-8.

[5] Moreau C, Kaminski M, Ancel PY, Bouyer J, Escande B, Thiriez G, et al. Previous induced abortions and the risk of very preterm delivery: results of the EPIPAGE study. BJOG. 2005;112(4):430-7.

[6] Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep 2006;55:1-94. 2006.

[7] U.S. Preventive Services Task Force. Screening for Chlamydial infection: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2007;147(2):128-34.

[8] Bernloehr A, Smith P, Vydelingum V. Antenatal care in the European Union: a survey on guidelines in all 25 member states of the Community. Eur J Obstet Gynecol Reprod Biol. 2005;122(1):22-32.

[9] Health Council of the Netherlands. Screening for Chlamydia. The Hague: Health Council of the Netherlands, nr 2004/07. 2004:1-106.

[10] Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update until the age of 4 years. Eur J Epidemiol. 2008;23(12):801-11.

[11] Sherman GG, Jones SA, Coovadia AH, Urban MF, Bolton KD. PMTCT from research to reality-results from a routine service. S Afr Med J. 2004;94(4):289-92.

Conclusion

In this thesis we endeavoured to provide information for future studies, discussions and decision making about the need for routine *C. trachomatis* screening in pregnant women in the Netherlands. The presented studies were performed with the aim to answer specific research questions.

1: *The assessment of the prevalence and risk factors during pregnancy*

The prevalence of *C. trachomatis* infection was found to be 12.0% in South African pregnant women and 3.9% in Dutch pregnant women with the highest prevalence in young women: 22.0% and 13.5%, respectively. In the Dutch study, age below 30 years, Antillean, Cape Verdean and Surinamese ethnicity, lower education, single marital status, multiple sexual partners in the year prior to the pregnancy and history of an STI were associated with *C. trachomatis* infection. Young age, single marital status and Antillean ethnic status appeared independent risk factors for infection. Determination of the typical symptoms for chlamydial urogenital infection was insufficiently sensitive and specific to estimate the risk. The willingness to participate in screening was good in both studies. We also had a high follow-up rate in both studies, although it must be stated that the follow-up rate in the South African study would have been much lower without the continuous effort to trace the women.

2: *The evaluation of complications of C. trachomatis infection during pregnancy*

The extent of morbidity associated with *C. trachomatis* infection during pregnancy was demonstrated for chorioamnionitis, adverse pregnancy outcome in terms of preterm delivery, and vertical transmission as evidenced by neonatal conjunctivitis and respiratory tract infection, and postpartum PID. A population attributable risk of *C. trachomatis* infection for preterm delivery before 32 weeks and 35 weeks of gestation was found to be 15% and 7% in our population. A vertical transmission rate of 30% was found even in the presence of tetracycline eye prophylaxis.

3: *The assessment of the feasibility of screening*

Screening pregnant women for *C. trachomatis* infection by means of nucleic acid amplification techniques on pooled urine specimens was shown to have a good sensitivity and to decrease the costs for a screening program. An estimation of the cost-effectiveness of a screening program in pregnant women, while using the data of our study and international data in a pharmaco-economic decision analysis model, showed that such a screening program would be highly cost-effective in the Netherlands.

Previously we stated that the success of a large-scale screening program depends on proper selection of the target population, non-invasive sampling, high-quality testing, the availability of effective treatment, and the cost-effectiveness of screening. We addressed these items and effective treatment is readily available in the Netherlands. Based on this thesis we can conclude that *C. trachomatis* infections in pregnant women and infants are highly underdiagnosed and, hence, not treated, and that that there is an association between *C. trachomatis* infection during pregnancy and early premature delivery. But, since we did not adjust for potential confounding as a result of co-infection by other genital pathogens and a previous history of termination of pregnancy, we did not prove a causal relation beween *C. trachomatis* infection during pregnancy and premature delivery and that treatment of pregnant women for *C. trachomatis* infection will prevent premature delivery. However, considering the high rate of *C. trachomatis* detection in women with premature deliveries less than 32 and 35 weeks of gestational age in the outcome study as well as the high rate of *C. trachomatis* detection in the placentas of women with preterm delivery before 32 weeks in the placenta study in association with histopathological signs of inflammation, the fact that it is biologically plausible that *C. trachomatis* is involved in preterm delivery, and other studies have shown a decrease in preterm deliveries with antibiotic treatment that is effective against *C. trachomatis*, we think it is very likely that the relation between *C. trachomatis* infection and premature delivery is causal. We think that all conditions are met for pregnant women to be a good target population for *C. trachomatis* screening in the Netherlands, but while implementing a screening program the latter considerations should still be explored.

Recommendations

- *C. trachomatis* infection in pregnant women must be considered an important public health problem in the Netherlands.

- We strongly recommend further study into the implementation of an antenatal *C. trachomatis* screening program for pregnant women in the Netherlands with emphasis on the effects of treatment of chlamydia-positive women (and their partners) on pregnancy outcomes.

- In the absence of a screening program we urge clinicians -general practitioners, midwives, obstetricians, gynaecologists, neonatologists, paediatricians and ophthalmologists- to have a higher index of suspicion for maternal and neonatal *C. trachomatis* infection.

- Women with (imminent) premature delivery should be tested for *C. trachomatis*.

- Infants less than three months of age with signs of conjunctivitis that persist for more than 72 hours should have a microbiological evaluation that includes *C. trachomatis* diagnostics.

- Likewise, infants less than six months of age with signs of respiratory tract infection that require microbiological evaluation should be tested for *C. trachomatis.*

- Chlamydia-positive infants should be treated appropriately, which implies the systemic application of appropriate antibiotics.

- Last but not least, we emphasize the importance of primary prevention through continuous education about antenatal care, sexual health, and relationship education as well as awareness campaigns and promotion of condom use both in the Netherlands and in South Africa.

Future research and perspectives

We did a literature search for evidence of an evaluation of the cost-effectiveness of an already implemented screening program for *C. trachomatis* among pregnant women, but found no reports. We advocate an implementation study in the Netherlands with respect to the cost-effectiveness of screening for C. *trachomatis* infection during pregnancy. We recommend a stepwise implementation of such antenatal screening program, suggesting a start in the major cities and, if appearing cost-effective, to be extended to the rest of the Netherlands with careful evaluation at regular intervals.

Chlamydia trachomatis screening for pregnant women in the context of routine antenatal care in the Netherlands

Most pregnant women in the Netherlands seek antenatal care in early pregnancy spontaneously. This affords a propitious opportunity for relatively uniform and efficient *C. trachomatis* screening since inclusion would not require much extra organisational effort in the already existing structure of routine antenatal care that is being delivered by midwives and general practitioners (75%-80%), and by gynaecologists (20%) [1]. Women are routinely screened for infections such as HIV, syphilis, Hepatitis B and rubella [2]. Conveniently, screening for *C. trachomatis* could be included at this time for which the logistics already exist. Likewise, the notification of test results and provision of treatment could be included in the existing logistics. Retesting of chlamydia-positive women after they received treatment would require some extra effort, but could be included at a regular follow-up visit. Annually, around 200.000 women are pregnant in the Netherlands. Extrapolation of our figures would mean that on a yearly basis 7.800 pregnant women may test positive for *C. trachomatis*, who are at increased risk for developing PID, chronic pelvic pain, tubal infertility, and ectopic pregnancies as well as, assumed, for preterm delivery before 35 weeks of gestation and all subsequent complications for their newborns such as respiratory distress syndrome, intraventricular haemorrhage, periventricular leucomalacia, persistent ductus arteriosus, necrotizing enterocolitis, sepsis, and others. Transmission will put the newborns at risk of conjunctival or respiratory chlamydial infection and the partners for symptomatic urethritis and epididymitis. These complications together with the resulting societal costs and loss of quality of life may be prevented by incorporation of *C. trachomatis* screening in the existing routine antenatal care.

Chlamydia trachomatis screening for pregnant women in the context of routine antenatal care in South Africa

We had insufficient data to evaluate the cost-effectiveness of an antenatal screening program for *C. trachomatis* in South Africa. Theoretically, such screening program would be even more rewarding in South Africa and in other developing countries. However, logistically a screening

program would be much more difficult to realise in South Africa because of the lack of financial resources, the fact that not all pregnant women seek antenatal care – especially the younger ones-, late antenatal clinic booking, the use of different antenatal clinics during a single pregnancy, and difficulties in follow-up. At present, routine screening for HIV and syphilis is offered at most South African antenatal clinics, and it may be suggested to include rapid onsite testing for *C. trachomatis* at the same time. However, recent studies in developing countries have reported rather poor sensitivities of rapid *C. trachomatis* tests and many positive women would still be missed despite a great effort and costs [3, 4]. Another approach might be to offer on site single dose treatment with azithromycin to all pregnant women. However, many women would be treated without being infected, costs would be unnecessarily high, and emergence of antimicrobial resistance may aggrevate rather than help eradicate *C. trachomatis* infections in this susceptible group of women. We showed that a syndromic approach for *C. trachomatis* infection during pregnancy was not useful, but that demographic and socio-economic characteristics could be helpful to treat sexually transmitted infections in this population. Therefore, we propose on site single dose treatment with azithromycin for pregnant women at increased risk for infection in South Africa, with additional provision of single dose treatment for partners.

References

[1] The Netherlands Perinatal Registry. Perinatal Care in the Netherlands 2007. Bilthoven: Stichting Perinatale Registratie; 2009:1-152. 2009.

[2] Heringa M. Computer-aided screening in antenatal care. 1998.

[3] Saison F, Mahilum-Tapay L, Michel CE, Buttress ND, Nadala EC, Jr., Magbanua JP, et al. Prevalence of *Chlamydia trachomatis* infection among low- and high-risk Filipino women and performance of Chlamydia rapid tests in resource-limited settings. J Clin Microbiol. 2007;45(12):4011-7.

[4] Yin YP, Peeling RW, Chen XS, Gong KL, Zhou H, Gu WM, et al. Clinic-based evaluation of Clearview Chlamydia MF for detection of *Chlamydia trachomatis* in vaginal and cervical specimens from women at high risk in China. Sex Transm Infect. 2006;82 Suppl 5:v33-7.

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Samenvatting

De doelstelling van de in dit proefschrift beschreven studies was om het gezondheidsgerelateerde belang van *Chlamydia trachomatis* infecties tijdens de zwangerschap voor vrouwen en pasgeborenen te evalueren om zodoende een bijdrage te leveren aan een toekomstige besluitvorming over *C. trachomatis* screening tijdens de zwangerschap.

In **hoofdstuk 1** presenteren we de onderzoeksvragen en de studies die in dit proefschrift beschreven zijn. De belangrijkste vragen waren:

1) Wat is de prevalentie van *C. trachomatis* infectie tijdens de zwangerschap?
2) Wat zijn risicofactoren voor *C. trachomatis* infectie tijdens de zwangerschap?
3) Is *C. trachomatis* infectie tijdens de zwangerschap geassocieerd met een verhoogd risico voor ongunstige zwangerschapsuitkomsten zoals doodgeboorte, vroeggeboorte en laag geboortegewicht?
4) Wat is het risico van *C. trachomatis* transmissie van vrouwen naar pasgeborenen?
5) Kunnen we bewijs vinden van verticale transmissie door het aantonen van *C. trachomatis* bij zuigelingen met conjunctivitis en luchtweginfecties?
6) Is samenvoegen van urine monsters een goede methode om de kosten-effektiviteit van testen voor *C. trachomatis* tijdens de zwangerschap te verbeteren?
7) Is routinematige screening voor *C. trachomatis* een kosten-effectieve benadering tijdens de zwangerschap?

In **hoofdstuk 2** hebben we een literatuur overzicht gegeven over de rol en het belang van *C. trachomatis* infectie bij zwangere vrouwen en zuigelingen. Chlamydia infectie wordt gekarakteriseerd door invasie van gastheercellen, intracellulaire reproductie en ontstekingsprocessen, welke kunnen persisteren en chronisch inflammatoire en fibrotische veranderingen kunnen veroorzaken. *C. trachomatis* infectie verloopt echter meestal asymptomatisch. Door het latente, sluimerende en mogelijk chronische karakter van de infectie kan het beloop asymptomatisch en zelfherstellend zijn, maar kan het ook leiden tot ernstige complicaties zoals 'pelvic inflammatory disease' (PID), infertiliteit en chronische buikpijn. Tevens kan *C. trachomatis* infectie tijdens de zwangerschap leiden tot een ongunstige zwangerschapsuitkomst en ziekte bij de pasgeborene. Verschillende test methoden kunnen worden gebruikt om (latente) *C. trachomatis* infecties aan te tonen, waarvan de NAATs momenteel de gouden standaard zijn. NAATs hebben een hoge sensitiviteit en kunnen gebruikt worden met de meeste materialen waaronder ook urine en vaginale uitstrijkjes. De mogelijkheden voor antibiotische behandeling van infecties door *C. trachomatis* worden besproken. Eenmalige behandeling met azithromycine wordt aanbevolen in verband met de goede compliantie, het succes van de behandeling en de geringe bijwerkingen. Zwangere vrouwen worden voorgesteld als een belangrijke doelgroep screening op *C. trachomatis.* Prenatale *C. trachomatis* screening wordt daarbij beschouwd aan de hand van de Wilson en Jung criteria, waarvan sommige nog niet uitgezocht zijn in Nederland.

In **hoofstuk 3** rapporteren we de prevalentie van sexueel overdraagbare infecties in 766 zwangere vrouwen in stedelijk Zuid-Afrika op het moment dat zij kwamen bevallen, en de associaties van sexueel overdraagbare infecties met demografische en socio-economische karakteristieken en klinische symptomen. In totaal hadden 48% van de vrouwen een of meer sexueel overdraagbare infecties tijdens hun zwangerschap, waarvan 18% geinfecteerd was met HIV, 23% met *Treponema pallidum,* 12% met *C. trachomatis* en 9% *Neisseria gonorrhoeae*. Voorspellende factoren voor infectie waren het ontbreken van prenatale zorg (OR 1.7, 95% CI 1.0-3.0), multigraviditeit (OR 2.2, 95% CI 1.5-3.3), en alleenstaand (OR 1.8, 95% CI 1.3-2.5) en niet werkzaam zijn (OR 1.4, 95% CI 1.0-1.9). HIV seropositiviteit was significant hoger onder vrouwen jonger dan 30 jaar (21%) dan onder vrouwen van 30 jaar en ouder (12%). Voor Syphilis was een significante trend waarneembaar met de laagste prevalentie onder de jongste vrouwen (14%) en de hoogste in de oudere groep (30%). Voor *C. trachomatis* infectie was een trend waarneembaar met de hoogste prevalentie in de jonge (22%) en de laagste in de oudere vrouwen (5%). We concluderen dat bijna de helft van de zwangere vrouwen één of meerdere sexueel overdraagbare pathogenen droeg, en dat chlamydia en gonorrhoea niet gediagnosticeerd en behandeld werden tijdens de zwangerschap. Tevens dat infectie niet geassocieerd was met symptomen, maar met verschillen in demografische en socio-economische karakteristieken.

In **hoofdstuk 4** tonen we de resultaten van een studie over de overdracht van *C. trachomatis* infectie van 77 zwangere Zuid-Afrikaanse vrouwen (beschreven in hoofdstuk3) naar hun pasgeborenen in een setting waar tetracycline oog profylaxe routinematig wordt gegeven ter preventie van neonatale conjunctivitis. Tevens evalueren we de postnatale gevolgen van *C. trachomatis* dragerschap tijdens de zwangerschap voor moeder en kind. LCR en/of kweek voor *C. trachomatis* was positief in 23 kinderen resulterend in een transmissiepercentage van 30%. Hiervan hadden 19 (83%) kinderen een positieve nasopharynx test en 9 (39%) een positieve conjunctiva test; vijf (22%) kinderen testten positief voor beiden. Alle conjunctiva kweken waren negatief. Nasopharynx materiaal was positief in 13/23 (57%) zuigelingen met LCR en in 9/20 (45%) zuigelingen met kweek. Zuigelingen van chlamydia-positieve vrouwen hadden significant vaker nasale klachten dan die van chlamydia-negatieve vrouwen (32/65 (49%) versus 15/65 (23%)), hetgeen ook bij lichamelijk onderzoek werd gevonden (37 (57%) versus 7 (11%)), als ook hogere ademhalingsfrekwenties en intercostaal intrekken. Postnatale urogenitale klachten en symptomen werden gevonden bij 34 (52%) en 51 (78%) van de chlamydia-positieve vrouwen, waarvan 12 vrouwen (18%) PID ontwikkelden. We concluderen dat *C. trachomatis* infectie tijdens de zwangerschap een risico vormt voor de pasgeborene en dat locale tetracycline profylaxe onvoldoende is om infectie bij de zuigeling te voorkomen. Tevens stellen we dat het vinden van postnatale maternale urogenitale klachten en symptomen in combinatie met klachten en symptomen van de zuigeling, de arts bedachtzaam moeten maken op de mogelijkheid van neonatale en maternale complicaties van *C. trachomatis* infectie.

In **hoofdstuk 5** rapporteren we de prevalentie en risicofactoren van *C. trachomatis* infectie tijdens de zwangerschap in 4,055 zwangere Nederlandse vrouwen die naar een van de verloskundige praktijken of prenatale klinieken gingen die meededen aan de Generation R studie in Rotterdam, Nederland, en het gevolg van *C. trachomatis* infectie tijdens de zwangerschap voor premature partus en geboortegewicht. *C. trachomatis* werd gevonden bij 157 (4%) van de vrouwen. De prevalentie was omgekeerd evenredig met leeftijd met een prevalentie van 14% in vrouwen van 20 jaar en jonger en 2% in vrouwen ouder dan 30 jaar. De prevalentie was het hoogst onder Antilliaanse (16%), Kaap Verdiaanse (11%) of Surinaamse (9%) vrouwen, alleenstaande vrouwen (12%), en vrouwen met een laag opleidingsniveau (6%), primigraviditeit (5%), meerdere sexuele partners in het afgelopen jaar (8%) of een sexueel overdraagbare infectie in de voorgeschiedenis (6%). In de ongeadjusteerde analyse waren deze risicofactoren, met uitzondering van graviditeit, significant geassocieerd met *C. trachomatis* infectie. In de geadjusteerde analyse bleven leeftijd jonger dan 21 jaar (OR 1.8, 95% CI 1.2-2.6), Antilliaanse etniciteit (OR 2.3, 95% CI 1.4-3.7) en alleenstaand zijn (OR 1.6, 95% CI 1.3-2.0) risicofactoren voor infectie met *C. trachomatis*; andere maternale factoren waren niet onafhankelijk geassocieerd. In het tweede deel van het onderzoek werden de zwangerschapsuitkomsten (zwangerschapsduur en geboortegewicht) van 3,913 pasgeborenen geanalyseerd. We vonden dat 18% (95% CI 0.7-36.7) van de vrouwen die voor een zwangerschapsduur van 32 weken en 11% (95% CI 3.1-18.8) van de vrouwen die voor 35 weken bevielen positief waren voor *C. trachomatis*. Na adjusteren voor mogelijke confounders was *C. trachomatis* infectie geassocieerd met vroeggeboorte vóór 32 weken (OR 4.4, 95% CI 1.3-15.2) en 35 weken (OR 2.7, 95% CI 1.1-6.5) zwangerschapsduur, maar niet met laag geboortegewicht. Van alle partussen voor een zwangerschapsduur van 32 weken en 35 weken waren 15% (95% CI 4.5-39.5) en 7% (95% CI 2.5-20.1) toe te schrijven aan *C. trachomatis* infectie. We concluderen dat *C. trachomatis* significant bijdraagt aan het optreden van vroege prematuriteit, hetgeen als een algemeen gezondheidsprobleem beschouwd zou moeten worden, vooral in jonge vrouwen en andere vrouwen met verhoogd risico op *C. trachomatis* infectie.

In **hoofdstuk 6** bestuderen we de relatie tussen de aanwezigheid van *C. trachomatis* en tekenen van placentaire ontsteking in de placentas van 304 vrouwen die vóór een zwangerschapsduur van 32 weken bevielen in het Erasmus MC-Sophia, Rotterdam. *C. trachomatis* werd in 76 (25%) placentas aangetoond. Histologische tekenen van placentaire ontsteking werden in 123 (40%) placentas gevonden: in 41/76 (54%) placentas met *C. trachomatis* versus 82/228 (36%) placentas zonder *C. trachomatis* infectie (OR 2.1, 95% CI 1.2-3.5). *C. trachomatis* werd significant vaker waargenomen met toenemende progressie (P=0.003) en intensiteit (P=0.002) van placentaire ontsteking aan maternale zijde, maar niet aan foetale zijde. We concluderen dat *C. trachomatis* vaak gevonden werd in de placentas van vrouwen met vroege prematuriteit, en dat *C. trachomatis* infectie geassocieerd was met histopathologische tekenen van placentaire ontsteking.

In **hoofdstuk** 7 hebben we onderzocht of *C. trachomatis* een oorzaak was van neonatale conjunctivitis in zuigelingen onder de drie maanden die gepresenteerd werden in het Erasmus MC-Sophia en Rotterdam Oogziekenhuis, Rotterdam. Tevens evalueren we de klinische presentatie en voorgeschreven behandeling voor *C. trachomatis* conjunctivitis vergeleken met conjunctivitis ten gevolge van andere infecties. *C. trachomatis* werd vastgesteld in 27/42 (64%) retrospectief bestudeerde zuigelingen en 14/23 (61%) prospectief bestudeerde zuigelingen. Mucopurulente afscheiding werd gezien in 35/37 (95%) zuigelingen, zwelling van de ogen in 27/37 (73%), en erytheem van de conjunctivae in 24/37 (65%) zuigelingen. Respiratoire klachten waren aanwezig in 14/37 (38%) en voedingsproblemen in vijf (14%) van de 37 zuigelingen. Vóór het stellen van de microbiologische diagnose, schreven huisartsen anti-chlamydia antibiotica voor aan vijf (12%) en systemische antibiotica aan vier (10%) van de 41 zuigelingen die positief waren voor *C. trachomatis*. Oogartsen schreven anti-chlamydia antibiotica voor aan 21 (51%) en systemische antibiotica aan zeven (17%) van de 41 zuigelingen. We concluderen dat *C. trachomatis* de belangrijkste verwekker was van bacteriele conjunctivitis in deze populatie en dat klinisch onderscheid van andere pathogenen niet mogelijk was. Veel zuigelingen die positief waren voor *C. trachomatis* kregen niet de juiste behandeling met antibiotica.

In **hoofdstuk 8** bestuderen we de aanwezigheid van *C. trachomatis* in zuigelingen jonger dan zes maanden die gedurende een jaar met respiratoire klachten gepresenteerd werden aan het Erasmus MC-Sophia. Respiratoire materialen, vooral nasopharynx watten, werden getest voor *C. trachomatis,* respiratoire virussen en *Mycoplasma pneumoniae* met PCR, virale celkweken en directe immunofluorescentie. *C. trachomatis* respiratoire infectie werd gevonden in 10/148 (7%) geteste zuigelingen. Acht (80%) van de 10 *C. trachomatis* infecties werden gezien bij zuigelingen onder de leeftijd van drie maanden. Er werden door de betrokken artsen geen testen voor *C. trachomatis* aangevraagd, maar het was het tweede meest gevonden respiratoire pathogeen na hRSV dat gevonden werd in 41 (28%) zuigelingen. We concluderen dat perinatale respiratoire infecties met *C. trachomatis* relatief vaak voorkomen en ondergediagnosticeerd worden in deze groep zuigelingen jonger dan zes maanden.

In **hoofdstuk 9** hebben we diverse test methoden voor *C. trachomatis* onderzocht in verband met de kosten-effektiviteit van screening tijdens de zwangerschap. Van 750 asymptomatische zwangeren vrouwen in Rotterdam werden urines verzameld. Aanvankelijk onderzochten we de kwaliteit van drie verschillende DNA isolatie methoden met 350 van deze urines en 70 pools van vijf urines van dezelfde subset. De gewone Cobas Amplicor test werd vergeleken met de Cobas Amplicor in combinatie met voorafgaande DNA isolatie met de MagNA Pure large-volume kit en de MagNA Pure bacterial DNA isolation kit. We vonden dat 15, 14 en 27 urines positief testten, hetgeen resulteerde in een sensitiviteit van 52%, 48% en 93%, respectievelijk. Vervolgens, met gebruik van alle 750 urines, werd de kwaliteit van de Cobas Amplicor voor

individuele testen (32 positieve testen) vergeleken met gepoolde testen met de standaard Cobas Amplicor procedure (20 positieve testen) en met gepoolde testen met de Cobas Amplicor in combinatie met de MagNA Pure bacterial DNA isolation kit (44 positive testen), resulterend in een sensitiviteit van 65%, 42% en 92% respectievelijk (P<0.001). De prevalentie van *C. trachomatis* was 6% in deze populatie. Tevens toonden we aan dat met pooling van urines de kosten van de gecombineerde MagNA Pure Bacterial DNA Isolation Kit en Cobas Amplicor methode slechts 56% waren van de kosten van de standaard Cobas Amplicor test voor individuele urines. De kosten per positief vastgestelde casus waren in de gecombineerde methode slechts 39% van de standaard kosten. We concluderen dat gepooled testen voor *C. trachomatis* infectie tijdens de zwangerschap bruikbaar is voor grootschalig onderzoek door gebruik te maken van de Cobas Amplicor test in combinatie met de MagNA Pure Bacterial DNA Isolation Kit. Deze combinatie verbeterde de sensitiviteit significant en verlaagde de kosten.

In **hoofdstuk 10** hebben we een kosten-effektiviteits analyse gemaakt van *C. trachomatis* screenen onder zwangere vrouwen in Nederland op basis van een statisch model. We ontwikkelden een pharmaco-economisch beslismodel, waarin mogelijke gezondheidsuitkomsten van *C. trachomatis* infectie tijdens de zwangerschap opgenomen werden zoals PID, infertiliteit and chronische buikpijn als ook ectopische zwangerschap, premature partus en neonatale ziekte, en ziekte van de partner. We begrootten de Kosten-effectiviteit vanuit een maatschappelijk perspectief waarbij we de gevonden prevalentie uit hoofdstuk 5 gebruikten. We berekenden de kosten die voorkomen zouden kunnen worden door de gezondheidsuitkomsten te koppelen aan gezondheidskosten en productiviteitsverlies. Kosten-efektiviteit werd geschat in base-case-, sensitiviteits- en scenario analyses. In de base-case analyse werden de kosten om 1,000 zwangere vrouwen met *C. trachomatis* te diagnosticeren op €378,300 geschat. Kosten-besparingen betreffende complicaties werden geschat op €814,400, hetgeen resulteerde in omvangrijke netto kostenbesparingen. Een sensitiviteits analyse liet zien dat netto kostenbesparingen bleven bestaan bij een test prijs tot €28, met een preventie van complicaties van slechts 25% en een risico voor PID van slechts 0,4%. Een scenario analyse toonde zelfs meer kostenbesparingen met gerichte screening voor vrouwen onder 30 jaar en primigravida. We concluderen dat *C. trachomatis* screening van zwangere vrouwen in Nederland zeer kosten-effectief is en in de meeste gevallen zelfs kostenbesparend is.

Chapter 12

List of publications

Affiliation co-authors

PhD Portfolio

Curriculum Vitae

List of publications

Manuscripts based on this thesis

Rours GIJG, Hammerschlag MR, Van Doornum GJ, Hop WC, de Groot R, Willemse HF, Verbrugh HA, Verkooyen RP. *Chlamydia trachomatis* respiratory infection in Dutch infants. Arch Dis Child. 2009;94(9):705-7.

Rours GIJG, Hammerschlag MR, Ott A, De Faber TJ, Verbrugh HA, de Groot R, Verkooyen RP. *Chlamydia trachomatis* as a cause of neonatal conjunctivitis in Dutch infants. Pediatrics. 2008;121(2):e321-6.

Rours GIJG, Duijts L, Moll HA, de Groot R, Jaddoe VW, Hofman A, Steegers EAP, Mackenbach JP, Ott A, Willemse HFM, van der Zwaam EAE, Verbrugh HA, Verkooyen RP. Pregnancy outcomes in women infected with *Chlamydia trachomatis*: a population-based prospective study cohort. ISBN 978 87 984259 3 9. Proceedings Sixth Meeting of the European Society for Chlamydia Research 2008.

Rours GIJG, Verkooyen RP, Hop WCJ, Ye Htun, Radebe F, Rothberg AD, Cooper PA, de Groot R, Verbrugh HA, Ballard RC. Sexually transmitted infections in pregnant urban South African women; socio-economic characteristics and risk factors. The Southern Journal of Epidemiology and Infection 2006; 21 (1):14-19.

Rours GIJG, Hop WCJ, Ye Htun, Radebe F, Rothberg AD, Cooper PA, de Groot R, Verbrugh HA, Verkooyen RP, Ballard RC. Carriage of *Chlamydia trachomatis* during pregnancy: consequences for mother and infant. The Southern Journal of Epidemiology and Infection 2006; 21 (1): 20-25.

Rours GIJG, Verkooijen RP, Willemse HF, van der Zwaan EA, van Belkum A, de Groot R, Verbrugh HA, Ossewaarde JM. Use of pooled urine samples and automated DNA isolation to achieve improved sensitivity and cost-effectiveness of large scale testing for *Chlamydia trachomatis* in pregnant women. J Clin Microbiol. 2005; 43(9):4684-90.

Rours GIJG, Verkooijen RP, Willemse HF, van der Zwaan EA, van Belkum A, de Groot R, Verbrugh HA, Ossewaarde JM. Effect of DNA isolation and Pooling of Urines on the Sensitivity of PCR Detection of *Chlamydia trachomatis* in Asymptomatic Pregnant Women. ISBN 963 482 666 0. Proceedings Fifth Meeting of the European Society for Chlamydia Research 2004.

Submitted manuscripts based on this thesis

Rours GIJG, *Chlamydia trachomatis* infection in pregnant women and infants; review

Rours GIJG*, Duijts L*, Moll HA, Arends LR, de Groot R, Jaddoe VW, Hofman A, Steegers EAP, Mackenbach JP, Ott A, Willemse HFM, van der Zwaan EAE, Verkooyen RP, Verbrugh HA. *shared first authorship *Chlamydia trachomatis* infection during pregnancy associated with preterm delivery: a population-based prospective cohort study.

Rours GIJG, de Krijger RR, Ott A, Willemse HFM, de Groot R, Zimmermann LJI, Kornelisse RF, Verbrugh HA, Verkooijen RP. *Chlamydia trachomatis* and placental inflammation in early preterm delivery.

Rours GIJG, Verkooyen RP, de Groot R, Verbrugh HA, Postma MJ. Cost-effectiveness of *Chlamydia trachomatis* screening in Dutch pregnant women.

Other publications

Been JV*, **Rours GIJG***, Kornelisse RF, Jonkers F, de Krijger R, Zimmermann LJI.*shared first authorship. Chorioamnionitis alters the response to surfactant in preterm infants. Journal of Pediatr. 2010;156(1):10-15.

Been JV*, **Rours GIJG***, Kornelisse RF, Lima Passos V; Kramer BW, Schneider TJ, De Krijger R, Zimmermann LJI.*shared first authorship. Histologic chorioamnionitis, fetal inflammation and antenatal steroids: effects on neonatal outcome in preterm infants. Am J Obstet Gynecol. 2009;201(6):587.e1-8.

Been JV, Kornelisse RF, **Rours GIJG**, Lima Passos V, De Krijger R, Zimmermann LJI. Early postnatal blood pressure in preterm infants: effects of chorioamnionitis and timing of antenatal steroids. Pediatric Res. 2009;66(5):571-6.

Chlamydophilia pneumoniae, RIVM-Clb, Landelijke Coordinatie Infectieziektebestrijding, LCI-Richtlijnen. ISBN 978-90-6960-187-8, Infectieziektebestrijding, Deel I, Editie 2008.

Chlamydophilia pneumoniae, RIVM-Clb, Landelijke Coordinatie Infectieziektebestrijding, LCI-Richtlijnen. ISBN 978-90-6960-187-8 Infectieziektebestrijding, Deel II, Editie 2008.

Rours GIJG. Onderzoekservaringen in een ontwikkelingsland: Zuid-Afrika. Nederlands Tijdschrift voor Researchverpleegkundigen. 2004; 1:8-10.

Gaytant MA, **Rours GIJG**, Steegers EA, Galama JM, Semmekrot BA. Congenital cytomegalovirus infection after recurrent infection: case reports and review of the literature. Eur J Pediatr. 2003; 162(4):248-53.

Hjálmarsson B, **Rours GIJG**, de Groot R. Een puber met multipele vergrote lymfeklieren. Probleemgeoriënteerd denken in de kinder-geneeskunde. ISBN 90 5898 024 3. De Tijdstroom 2002.

Other manuscripts submitted

Ketharanathan N, Lincke CR, **Rours GIJG.** Lemierre's syndrome and orthodontic brackets.

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PhD Portfolio

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General academic skills	Year	Work load ECT
Systematic literature search, Erasmus MC, Rotterdam	2009	0.6
Endnote X, Erasmus MC, Rotterdam	2009	0.2
MSc Clinical epidemiology, NIHES, Rotterdam		
- Biomedical English writing and Communication	2007	4.0
- Integrity in research	2008	0.6
- SPSS for Windows	2010	0.4

Research Skills		
MSc Clinical epidemiology, NIHES, Rotterdam,		
- Introduction to Clinical Research	2008	0.7
- Principles of research in medicine and epidemiology	2009	0.7
- Decision-making in medicine	2009	0.7
- Methods of clinical research	2009	0.7
- Clinical decision analysis	2009	0.7
- Clinical trials	2009	0.7
- Pharmaco-epidemiology	2009	0.7

Presentations at Conferences	
18th ISSTDR/BASSH, London, United Kingdom, 2009. Cost-effectiveness of *Chlamydia trachomatis* screening in Dutch pregnant women. Poster	1.4
6th Meeting of the European Society for Chlamydia Research, Aarhus, Denmark, 2008. Pregnancy outcomes in women infected with *Chlamydia trachomatis*. Poster	1.4
5th Annual Amsterdam Chlamydia Meeting, Amsterdam, The Netherlands, 2008. *Chlamydia trachomatis* infection during delivery and preterm delivery. Oral	1.4
Dutch Society for Medical Microbiologists, Papendal, The Netherlands, 2008. Consequences of *C. trachomatis* infection during pregnancy for newborns. Poster	1.4

22nd IUSTI-EUROPE Conference on STI, Versailles, France, 2006. Clinical presentation and consequences of *Chlamydia trachomatis* (placental) infection in preterm delivery. Oral 1.4

22nd IUSTI-EUROPE Conference on STI, Versailles, 2006. *Chlamydia trachomatis* as a cause of placental infection in early preterm delivery. Poster 1.4

16th Biennial meeting of the ISSTDR, Amsterdam, The Netherlands, 2005. Improved Sensitivity and Cost-effectiveness of Large Scale *Chlamydia trachomatis* testing in Pregnant Women using Pooled Urines and Automated DNA Isolation. Poster 1.4

16th Biennial meeting of the ISSTDR, 2005, Amsterdam, The Netherlands, 2005. *Chlamydia trachomatis* and respiratory disease in infants in a dutch inner city. Poster 1.4

5th Meeting of European Society for Chlamydia Research, Budapest, Hungary, 2004. Neonatal conjunctivitis and the significance of *Chlamydia trachomatis*. Oral 1.4

1th Annual Amsterdam Chlamydia Meeting, Amsterdam, The Netherlands, 2004. Risk factors and Consequences of *Chlamydia trachomatis* infection during pregnancy for women and infants. Oral 1.4

Congress High Tech & Poor Health, Amsterdam, The Netherlands, 2003. Sexually transmitted infections in pregnant urban South African women: Strategies for health gain? Oral 1.4

21st Annual meeting ESPID, Sicily, Italy, 2003. *Chlamydia trachomatis* and neonatal conjunctivitis in an innercity in the Netherlands. Poster 1.4

IUSTI/STD/HIV 6th World congress & 38th IUSTI General Assembly, Sun City, South Africa, 1999. Consequences of maternal chlamydial and gonococcal infection for mothers and neonates in an urban South African population. Oral 1.4

Presentations at Universities and Public Schools	
Consequences of *C. trachomatis* infection during pregnancy for infants. Department of Obstetrics & Gynaecology, Erasmus MC, Rotterdam, The Netherlands, 2009.	1.0
Manifestation of infections and skin lesions in children with HIV/AIDS. Department of Tropical Medicine, Public School of Health, Utrecht, The Netherlands, 2001.	1.0
Consequences of chlamydial carriership in pregnant women for mother and child. Research day, Department Medical Microbiology & Infectious Diseases, Rijswijk, The Netherlands, 2001.	1.0
Paediatrics and work experience in South Africa. Department of Tropical Medicine, Public School of Health, Utrecht, The Netherlands, 2001.	1.0
Reviewer	
Sexually Transmitted Infections	0.2
European Journal of Clinical Microbiology & Infectious Diseases	0.2
Teaching activities	
Post-academic Infectious Disease Course, Vlieland, 2003	1.4
Post-academic Infectious Disease Course, Vlieland, 2003	1.4
PAOG, Erasmus MC, Rotterdam, 2002	1.4
Supervision Master's theses	5.0
PAOK, Erasmus MC, Rotterdam, 2002	1.4
Seminars and Workshops	
Infectious Disease Prevention, Department Medical Microbiology & Infectious Diseases, Erasmus MC, Rotterdam, The Netherlands, 2008	0.3
PhD day, Erasmus MC, Rotterdam, The Netherlands, 2007, 2008, 2009	0.9
Total	45.1