Recent Developments of Chlamydia trachomatis and Mycoplasma Infections in Women

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RECENT DEVELOPMENTS OF CHLAMYDIA TRACHOMATIS AND MYCOPLASMA GENITALIUM INFECTIONS IN WOMEN

CARINA BJARTLING

Academic Dissertation

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Abstract

The aim of this thesis was to elucidate developments in epidemiology, clinical manifestations and complications in Chlamydia trachomatis (C.trachomatis) and Mycoplasma genitalium (M.genitalium) infection. In study I the frequencies of non-gonococcal salpingitis, gonococcal salpingitis and ectopic pregnancy (EP) were observed over a period of 28 years and correlated to the prevalence of Neisseria gonorrhoeae (N.gonorrhoeae) and C.trachomatis. The frequency of acute salpingitis reflected the prevalence of N.gonorrhoeae and C.trachomatis in our population. The frequency of acute salpingitis and ectopic pregnancy might be used to estimate the occurrence of C.trachomatis during the 1970s and early 1980s before diagnostic facilities became available.. In study II we explored the possible presence of C.trachomatis DNA at the time of EP using freshly frozen tubal tissue and analyzing for C.trachomatis with PCR and a highly sensitive real time PCR test. We also investigated the correlation between c-hsp60 antibodies and h-hsp60. C.trachomatis DNA could not be detected in any of the tubal tissue specimens from our patients with EP although highly sensitive diagnostic methods were used. Prior EP /PID was associated with ct-hsp60 antibodies but not with human hsp60. Comparison of the antibody levels of chlamydial hsp60 and human hsp60 in our patients with EP showed no correlation. In study III we compared clinical manifestations of infections with nvCT and wild type C.trachomatis (wtCT) in both men and women and estimated the frequency of ascending infections (PID) in women. Men and women with nvCT or wtCT infection were similar with regard to sexual lifestyle parameters and they had the same frequency of previous chlamydial infection. Asymptomatic infection seemed more common in women with nvCT infection than in women with wtCT infection. No case of PID associated with nvCT was found. Our findings suggest a difference in virulence between the nvCT and the wtCT. Study IV was performed to investigate the prevalence, clinical findings and complications of M.genitalium in women. In 7,598 women tested for M.genitalium and C.trachomatis the prevalence was 2.1 % and 2.6 % respectively. M.genitalium was associated with cervicitis and the observed association was independent of age and C.trachomatis infection. The frequency of symptoms and clinical signs were higher in patients with C.trachomatis infection suggesting that M.genitalium is a less aggressive pathogen in terms of symptoms and clinical signs. M.genitalium was clearly associated with PID in patients requesting TOP.

Key words: Chlamydia trachomatis, Mycoplasma genitalium, pelvic inflammatory disease, ectopic pregnancy, cervicit, uro-genital infection

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Front page: Chlamydia trachomatis inclusions in McCoy cell culture, stained by immunofluorescence
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To my beloved family

Staffan, Axel, Anna, and Ulf

Kärleken är så förunderligt stark

kuvas av intet i världen

Rosor slår ut ur den hårdaste mark

som sol över mörka gärden

(I folkviseton, Nils Ferlin 1898-1961)

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ORIGINAL PAPERS

This thesis is based on the following papers, which will be referred to in the text according to their Roman numerals.

I The frequency of salpingitis and ectopic pregnancy as epidemiologic markers of *Chlamydia trachomatis*.

Bjartling C, Osser S, Persson K. Acta Obstet Gynecol Scand. 2000 Feb;79(2):123-8. PMID: 10696960

II Deoxyribonucleic acid of *Chlamydia trachomatis* in fresh tissue from the Fallopian tubes of patients with ectopic pregnancy

Bjartling C, Osser S, Persson K. Eur J Obstet Gynecol Reprod Biol. 2007 Sep;134(1):95-100. Epub 2007 Feb 5. PMID: 17280761

III Clinical manifestations and epidemiology of the new genetic variant of *Chlamydia trachomatis*

Bjartling C, Osser S, Johnsson A, Persson K. Accepted for publication in Sexually Transmitted Diseases

IV *Mycoplasma genitalium* is an independent risk factor for cervicitis and is associated with pelvic inflammatory disease after termination of pregnancy

Bjartling C, Osser S, Persson K. Submitted

ABBREVIATIONS

ATP	adenosine triphosphate
Bp	base pair
CSH	Centre of Sexual Health
C.trachomatis	*Chlamydia trachomatis*
DFA	direct fluorescence assay
EB	elementary body
ECDC	European Centre for Disease Prevention and Control
EIA	enzyme immunosorbent assay
ELISA	enzyme linked immunosorbent assay
EP	ectopic pregnancy
FCU	first catch urine
FVU	first void urine
HIV	human immunodeficiency virus
HPF	high power field
hsp	heat shock protein
IUD	intrauterine device
LGV	lymphogranuloma venereum
LNG-IUS	Levonorgestrel intrauterine system
M.genitalium	*Mycoplasma genitalium*
M.pneumoniae	*Mycoplasma pneumoniae*
MIF	microimmunofluorescence
MOMP	major outer membrane protein
NAAT	nucleic acid amplification test
N.gonorrhoeae	*Neisseria gonorrhoeae*
NGU	non-gonococcal urethritis
nvCT	new variant *Chlamydia trachomatis*
omp	outer membrane protein
ORF	open reading frame
PCR	polymerase chain reaction
PID	pelvic inflammatory disease

PMNL	polymorph nuclear leucocytes
RB	reticulate body
STD	sexually transmitted disease
STI	sexually transmitted infection
TFI	tubal factor infertility
TOP	termination of pregnancy
WHO	World Health Organization
wtCT	wild type *Chlamydia trachomatis*

INTRODUCTION

Infections of the genital tract of women do not simply present a short term problem but may also be a future threat to reproductive capacity later in life. Most of these infections are sexually transmitted which also implicates men.

There are more than 30 different sexually transmissible agents. The most common bacteria are *Chlamydia trachomatis* (*C.trachomatis*) and *Neisseria gonorrhoeae* (*N.gonorrhoeae*). While gonorrhoea has decreased in many parts of the developed world, chlamydial genital tract infections still remain a refractory problem world wide. Infections caused by *C.trachomatis* are particularly difficult to confine as a high proportion of these infections are asymptomatic thus making part of the population (those not tested) a reservoir for further transmission. Transmission in the population is depending on large scale screening programs and sexual health units offering individuals possibility for testing and treatment. The nature of and the proportion of complications following a *C.trachomatis* infection is of crucial importance when estimating cost-effectiveness of screening programs.

Mycoplasma genitalium (*M.genitalium*) which was discovered in the early 1980s has recently been proven to be a significant pathogen similar to *C.trachomatis* in several respects, such as preference to the genital tract and mode of transmission.

The highest prevalence of *C.trachomatis* infection is seen in young sexually active men and women below 30 years of age. Complications such as ectopic pregnancy and tubal infertility are not discovered until several years later. It has yet to be seen if *M.genitalium* infections will follow the same pattern.

The main purpose of this thesis was to explore and elucidate the developments in epidemiology, clinical manifestations and complications of *C.trachomatis* and *M.genitalium* infections, mainly with reference to women. The results of the work in this thesis may provide some suggestions and some answers needed in the process of managing these infections in future.

BACKGROUND

Historical overview

C.trachomatis and *M.genitalium* are both bacteria known to cause sexually transmitted infections (STI). While diseases caused by *C.trachomatis* have been recognised for many years the discovery of diseases associated with *M.genitalium* are very recent findings.

Since ancient times 'trachoma' has been known to humans and medical texts from Egypt, China, Rome, Greece, and Arabia all make reference to trachoma (Wright *et al.*, 2008). However, it was much later that the first suggestion of *C.trachomatis* infection in the female and male genital tract was made. In 1907 Halberstaedter, von Prowazec and Körper had first described the classical cytoplasmic inclusions, that bear their names (HPK bodies), discovered in the conjunctival scrapings of orangutans inoculated with material from scrapings of patients with trachoma (Halberstaedter L and von Powazek, 1907).

In 1910 Lindner described inclusions in the urethral epithelium in three of ten men with non-gonococcal urethritis (NGU) (Lindner, 1911). In the same year Heymann reported to have seen the cytoplasmic inclusions (previously described by Halbersteadter, von Prowazec and Körper), in cervical cells from mothers of infants with non-gonococcal ophthalmia (Heymann, 1910). This discovery was confirmed in 1911 by Lindner who detected similar cytoplasmic inclusion bodies in ophthalmia of newborns as well as in cervical and urethral cells from their parents.

Thus, within a few years the aetiology of trachoma, non-gonococcal ophthalmia, NGU and cervical infection in women had been established.

In 1957 the first isolation of *C.trachomatis* was made from patients with trachoma by using embryonated hens' eggs (Tang *et al.*, 1957a; Tang *et al.*, 1957b).

Isolation of *C.trachomatis* from the cervix of a mother (of an infant with conjunctivitis neonatorum) was the first isolation from genital material and was made in 1959 by Jones *et al*. This was a breakthrough in chlamydia research. It was now possible to identify the organism and inoculate it into animals to prove the causal connection in a variety of diseases. This group, based at the Institute of Ophthalmology in London, did a number of groundbreaking studies confirming the aetiology of NGU, associated cervicitis and inclusion conjunctivitis. However, the isolation procedure was difficult and took several weeks to complete.

In 1965 a tissue culture method for more feasible isolation of *C.trachomatis* was introduced by Gordon and Quan. They used an irradiated cell culture of McCoy cells (originally thought to be human cells from synovial fluids but now recognised as epithelial cells from mice) making the procedure more sensitive.

When Ripa and Mård in 1977 introduced cyclohexamide, a pre-treatment of McCoy cells that removed the need for irradiation, they made cell culture considerably more convenient and more sensitive. This method for cell culture is used routinely for the isolation of *C.trachomatis* today.

Immunofluorescence using polyclonal antisera has been used since the 1960s for the detection of chlamydial antigens; both in cell culture and in clinical material but such antisera (used for such studies) were generally the property of a limited number of chlamydial research laboratories and tended to be individually prepared and were of inconsistent quality.

The development of microimmunofluorescence (MIF) tests in the 1970s brought more knowledge about chlamydial infections as this technique made it possible to differentiate *C.trachomatis* into serovars (sometimes also called serotypes) and to measure chlamydial antibodies in a sensitive and specific way (Wang, 1971). During this time *C.trachomatis* became known as a sexually transmitted disease (STD) causing urogenital infections in both men and women.

In the early 1980s the monoclonal antibodies were developed to *C.trachomatis* (Stephens *et al.*, 1982; Wang *et al.*, 1985). These new antibodies gave better specificity and sensitivity in direct immunofluorescence microscopy allowing better detection of *C.trachomatis* in clinical samples and cell culture using direct fluorescence assay (DFA). Serovar-specific monoclonal antibodies were also developed and used as a powerful epidemiological research tool. This was a quantum leap in the use of immunofluorescence and lead to the elucidation of the relationship between the newly discovered major outer membrane protein (MOMP) and the serovars of *C.trachomatis* (Yuan *et al.*, 1989; Wang *et al.*, 1985; Wang and Grayston, 1991).

Direct immunofluorescence with monoclonal antibodies marked the beginning of the trend towards use of non-viability dependent methods for diagnosing *C.trachomatis*. With the advent of enzyme immunoassay (EIA) kits, a test could be completed in a few hours. These tests were also suited to large scale testing and automation. The possibility to test more individuals at a lower cost was now at hand. However, the enzyme immunoassay turned out to have both low sensitivity and low specificity (Jones *et al.*, 1984; Howard *et al.*, 1986; Taylor-Robinson *et al.*, 1987).

In 1980 *M.genitalium* was discovered by Taylor-Robinson. He found the bacterium in two of 13 urethral specimens from men with non-gonococcal urethritis (NGU). It took one month of culture before the bacterium was detectable (Tully *et al.*, 1981).

Although it's been more than thirty years since this discovery, research into *M.genitalium* has been limited as the cultivation of this organism proved to be extremely difficult and only a few urogenital isolates have been obtained (Jensen, 2004).

With the development of the polymerase chain reaction in 1983 by Mullis (Saiki *et al.*, 1985) the situation has changed. The PCR method provided both a highly sensitive and a highly specific way to detect *C.trachomatis* and *M.genitalium*. The invention and development of PCR (which was rewarded

with the Nobel Prize in Medicine in 1993) brought a new era into research in many different fields including the detection of infectious pathogens.

The introduction of mainly PCR based nucleic acid amplification tests (NAATs) for *C.trachomatis* during the 1990s improved sensitivity and specificity. New non-invasive sampling also became feasible. When NAATs were developed for *M.genitalium* it became clear that this agent was associated with non-gonococcal urethritis in males (Jensen *et al.*, 1991). Other clinical conditions associated with this agent have later been observed both in men and women.

Microbiology

Chlamydia trachomatis and new variant Chlamydia trachomatis

C.trachomatis belongs to the genus Chlamydia. Since 1999 when the taxonomic description of *C.trachomatis* was changed there are now three species within this genus, *C.trachomatis*, *Chlamydia suis* (affects only swine) and *Chlamydia muridarum* (affects only mice and hamsters), (Everett *et al.*, 1999).

The chlamydia genome is small with 1,000 to 1,200 kbps compared to other free-living bacteria such as *E.coli* which has a genome of 4,980 kbps. The *C.trachomatis* genome is larger than that of *M. genitalium* (580 kbps) and *M. pneumoniae* (816 kbps), which have the smallest bacterial genomes known so far. The *C. trachomatis* genome codes for approximately 875 proteins with some 75 unique ones not found in *C.pneumoniae*.

The chlamydiae were originally classified as protozoans and then as viruses until the 16S ribosomal RNA analysis placed them as gram-negative bacteria (Stephens, 1999).

Although classified as bacteria, the chlamydiae are small obligate intracellular parasites unable to multiply outside the host cell. Originally thought to be 'energy parasites' it is now known that they are able to make their own adenosine triphosphate (ATP) but nevertheless they also rely on the host cells for this and other nutrients.

The chlamydiae have evolved a unique biphasic developmental cycle in which they can alternate between two functionally and morphologically distinct forms, the elementary body (EB) adapted for extra cellular survival and the reticulate body (RB), adapted for intra cellular environment. The

developmental cycle takes between 48 to 72 hours, *in vitro*, during optimal conditions.

The EB is the infectious form of the bacteria and is initially metabolically inactive and stable (like a spore). These properties allow its extra cellular survival for sufficient time until it encounters a susceptible host cell.

When the EBs attaches to the surface of a host cell they mediate their own internalization. Internalization is thought to occur through invagination of clathrin-coated pits forming an intracellular membrane-bound vacuole known as an inclusion. The EBs will immediately differentiate into RBs and start to multiply forming a chlamydial inclusion.

The RB is metabolically active but non-infectious. As the RBs replicate, the inclusion grows to accommodate the increasing number of organisms. Then, through unknown mechanisms, the RBs begin differentiation back to infectious EBs, which are released from the host cell when the inclusion burst open (on lysis), ready for a new round of infection.

The structure of the cell wall and membranes of the chlamydiae are unique. The outer membrane complex is comprised primarily of three proteins of which the major outer membrane protein (MOMP) is the most important. It was discovered in 1981 by three independent laboratories (Hatch *et al.*, 1981; Salari and Ward, 1981; Caldwell *et al.*, 1981).

The cloning, sequencing and expression of the outer membrane protein (omp A gene) encoding MOMP was a major breakthrough achieved of Stephens *et al*. in 1985 and for *C.trachomatis* serovar L2 in 1986, followed by Pickett in 1987 (Pickett, 1987) for serovar L1.

The serotyping of *C.trachomatis* is based on the serological differentiation of antigenic epitopes on MOMP into 19 human *C.trachomatis* serovars (A to K, Ba, Da, Ia, Ja, L1 to L3 and L2a).

C.trachomatis is divided into two human biovars: 'trachoma' and 'lymphogranuloma venereum' (LGV). The trachoma biovar has currently 15 serovars who primarily infects epithelial cells of mucous membranes and the LGV biovar with four serovars which can invade lymphatic tissue.

Different serovars are associated with different disease pathologies. Serovars A, B, Ba and C are generally associated with blinding trachoma and serovars D-K cause sexually transmitted infections such as urethritis, cervicitis, pelvic inflammatory disease (PID), proctitis, prostatitis, epididymitis, reactive arthritis, conjunctivitis and transmitted from mother to child, neonatal conjunctivitis and neonatal pneumonia.

Serovars L1-L3 cause a rare invasive and systemic sexually transmitted infection normally found in the tropics, known as 'lymphogranuloma venereum'or LGV. An epidemic of LGV proctitis has recently been reported in Europe including Sweden among men-who-have-sex-with-men (MSM).

Strains of *C.trachomatis* normally have a highly conserved small extra-chromosomal element or plasmid which is 7.5 kbp and present in 7–10 copies per EB. Only a few plasmid-free isolates have been described (Peterson *et al.*, 1990; Farencena *et al.*, 1997; Stothard *et al.*, 1998). The only viable clinical isolates that are plasmid free belong to serotypes L2, D and E.

The function of the plasmid is largely unknown but evolutionary preservation (<1 % difference in DNA sequence between strains) suggests an important biological role (Comanducci *et al.*, 1990; Thomas *et al.*, 1997). Recent studies suggest that the cryptic plasmid plays a role in the replication and control of copy number as well as in virulence (Pickett *et al.*, 2005; O'Connell and Nicks, 2006; Carlson *et al.*, 2008; Li *et al.*, 2008).

In October, 2006, Ripa and Nilsson, reported a new genetic variant of *C.trachomatis* in Sweden. This new variant had a deletion of 377 bps in the plasmid which included the site used for PCR detection by two commercial

PCR-based tests. The tests failed to detect the new variant of *C.trachomatis* (nvCT) leading to an uncontrolled spread in the population.

Recently Seth- Smith and co-workers (in collaboration with our laboratory) sequenced the genomes of six *C.trachomatis* isolate including the new variant strain from our laboratory in Malmö (Seth- Smith submitted). The plasmid of the nvCT Sweden2 (pSW2) was found to have further changes. Sweden 3, the matched parental or wtCT was suggested to be the progenitor of Sweden 2 as they had identical sequences of the chromosomal *ompA* gene. The difference in size between pSW2 and pSW3 is accounted for by a deletion of 377bp and a duplication of 44bp at a different locus. (Figure 1)

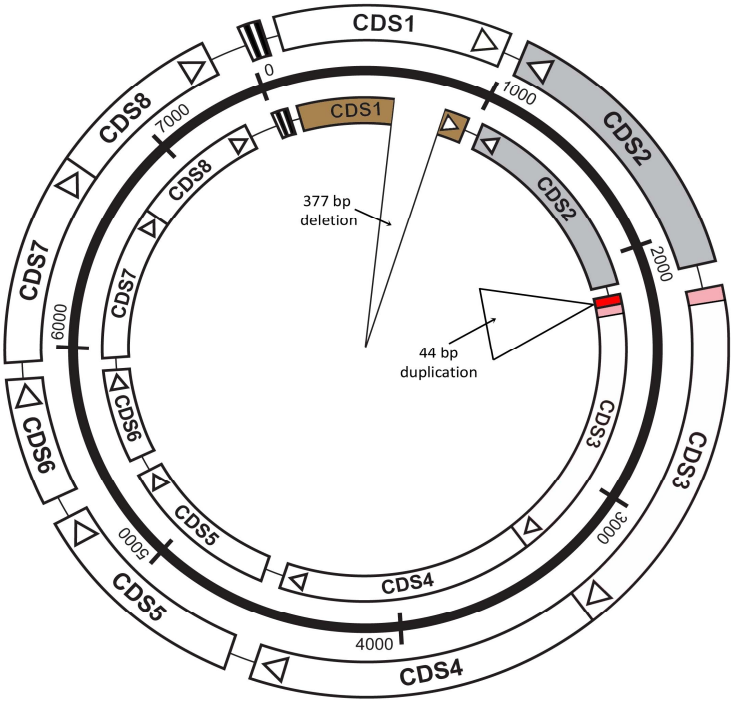

Figure 1. Comparison of plasmids pSW2 (inner circle) and pSW3 (outer circle), pSW2 carries a 377bp deletion in CDS1 (coloured brown for pseudogene) and a 44bp duplication immediately upstream of CDS3 (shown in red). CDS2 is transcribed in the opposite direction to the other CDSs and is shaded grey. The set of 22bp repeats upstream of CDS1 form the putative origin of replication.

A strain of nvCT was isolated in Malmö in 2006 and later sent to our collaborators in Southampton General Hospital, UK. Using immunofluorescence the nvCT is demonstrated (Figure 2)

A B

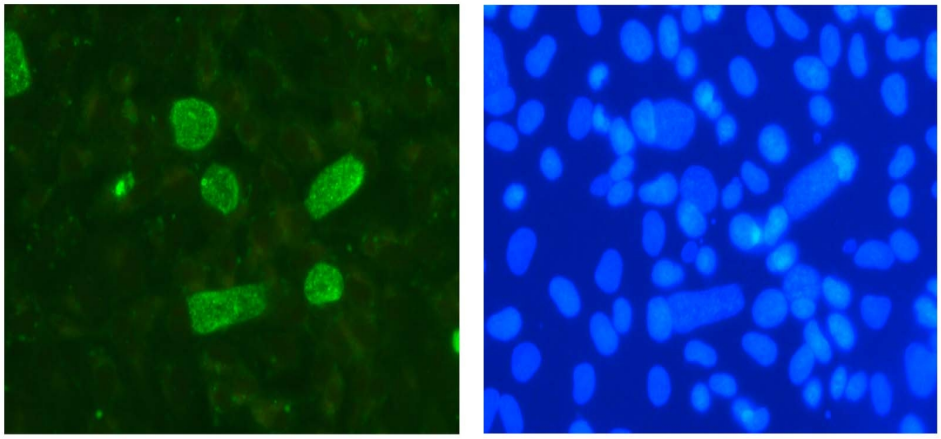

Figure 2 Immunofluorescence of nvCT (strain Sweden 2) isolated in Malmo in 2006. McCoy cells were infected with Sweden 2 and fixed at 48 hours post infection. In panel A chlamydial inclusions are green and have been stained with a monoclonal antibody to a chlamydial surface antigen. Panel B is the same field showing cell nuclei and DNA in chlamydial inclusions stained blue.

Immunopathology of chlamydial disease

C.trachomatis is an intracellular bacterial parasite that evokes a cellular and humoral immune response.

The most severe consequences of *C.trachomatis* infection (visual loss in trachoma; ectopic pregnancy (EP) or infertility in PID) are caused by fibrosis and scarring due to the repair of tissue damaged by chlamydial-induced inflammation.

Apart from the major differences between *C.trachomatis* associated with lymphogranuloma venereum, on the one hand, and trachoma and lower genital

tract infection on the other, there is little evidence for major differences in virulence between different *C.trachomatis* strains. There is, however, evidence that the host immune response may itself contribute significantly to tissue damage (immunopathology) as well as to immunity (Morrison, 1991; Hemmerich *et al.*, 1998). Some observations have suggested that the immune response may be part of an immunopathological process aggravating the clinical manifestations of chlamydial disease.

The eye disease trachoma may offer a case in point. This is an endemic infection to many underprivileged areas in Africa, Asia, Australia and the Americas. It is caused by serovars A-C of *C.trachomatis*. Children are infected at young age resulting in prolonged conjunctivitis. Recurrent or reinfection leads to progressive scarring of the eyelids. Deformation of the eyelids causes erosion of the cornea with resulting corneal damage and finally blindness.

Early trials to vaccinate against *C.trachomatis* had to be stopped as those receiving the vaccine had more severe disease than controls suggesting that immunity in some way contributed to the adverse reactions (Grayston *et al.*, 1963). It has later been shown that people in trachoma areas who resolve infection have a Th-2 dominated response to chlamydial antigens while those with progressive inflammation have a Th-1 response in vitro to *C.trachomatis* (Holland *et al.*, 1996). Again these findings suggest that the immune response may shape or at least reflect the clinical course (Ward, 1995).

Animal models have been used to further elucidate the mechanisms involved. Guinea pigs can be infected by the guinea pig inclusion conjunctivitis agent (GPIC-agent). In 1989 Morrison *et al*. (Morrison *et al.*, 1989) demonstrated that immunized but not naïve animals reacted with an extract of *C.trachomatis* inoculated in the eyes. The characterisation of the active component revealed that it belonged to a group of ubiquitous proteins called heat shock proteins (hsp). These proteins are well conserved and can be found in both prokaryotic and eukaryotic cells. They are transcriptionally upregulated in response to

physical or chemical stress and help to reconstitute intracellular proteins. The active GPIC extract contained Hsp60.

It has been suggested that similar immunological mechanisms may operate in chlamydial genital infection as well. It was shown by Wagar *et al*. in 1990 (Wagar *et al.*, 1990) that women with tubal damage had more antibodies to chlamydial hsp60 than women with PID, with normal Fallopian tubes. Several studies have since confirmed that women with tubal factor infertility (TFI) or EP have more antibodies to hsp60 than controls (Brunham *et al.*, 1992b; Witkin and Ledger, 1993; Ault *et al.*, 1998). An autoimmune reaction has been proposed but still remains unproven.

Men with non-gonococcal urethritis have more antibodies to hsp60 when symptoms persisted in contrast to patients where symptoms disappeared after treatment of *C.trachomatis* infection (Horner *et al.*, 1997).

It seems likely that the immune response may adversely affect the clinical course of *C.trachomatis* infection although the mechanisms are not yet fully known. Preventive immunity has been considered incomplete at best and short lived. Plans to develop an effective vaccine have long been frustrated but a change of focus towards a Th-2 response may have changed the situation.

Mycoplasma genitalium

M.genitalium is a small parasitic bacterium belonging to the class Mollicutes. Mollicutes are bacteria which have a small genome and that lack a cell wall; they are bounded only by a plasma cell membrane. There are more than 100 recognized species in the genus *Mycoplasma*.

Mycoplasmas are usually organ and tissue specific. Many mycoplasmal pathogens have features that mediate attachment to host target cells. Most mycoplasmas adhere to the epithelial linings of the respiratory or urogenital tract. Infections with pathogenic mycoplasmas are rarely of the fulminant type, but rather follow a chronic course (Razin *et al.*, 1998).

Several mycoplasma species have been isolated from humans, in total 16. The genital tract is the main site of colonization for six of them, *M. hominis, U. urealyticum, M. primatum, M. genitalium, M. spermatophilum* and *M. penetrans* (Taylor-Robinson and Furr, 1998).

M.gentialium has one of the smallest genomes, known so far, with 521 genes in one circular chromosome of 582,970 base pairs (Peterson *et al*., 1993, Fraser *et al*., 1995). The small size of the bacterium lies on the threshold of visibility under the light microscope. In electron microscopy *M.genitalium* has been shown to be not spherical but flask- shaped with a specialized protruding tip important for adhesion of the organism to cell surfaces. A similar structure has been identified in the closely related *M.pneumoniae* (Tully, 1983).

M.pneumoniae is found preferentially in the respiratory tract although findings in the genital tract have been reported (Goulet *et al.*, 1995). Reports of *M.genitalium* from the respiratory tract seem to be due to laboratory contamination of strains.

The tip is used for attachment to the host cell. The major attachment protein is MgPa, a 140kDa protein that differs from that of *M.pneumoniae* (P1) although they have extensive homology and share cross-reactive epitopes (Inamine *et al.*, 1989). The antigenic relationship between *M.genitalium* and *C.pneumoniae* has hampered diagnostic serology and made epidemiological studies difficult until the era of NAATs.

M.genitalium not only adheres to the host cell but also invades it and becomes intracellullar (Mernaugh *et al.*, 1993; Jensen *et al.*, 1994). *M.genitalium* also possesses the ability to be actively motile and the assumption is that motility is important as a means of penetrating the epithelial cell wall and helps in the invasion process (Taylor-Robinson, 1995).

Diagnostics

Chlamydia trachomatis and new variant Chlamydia trachomatis

Clinical diagnosis of *C.trachomatis* became feasible with the introduction of cell culture methods in the 1960s. This method was improved when cycloheximide was added to selectively inhibit the division of the target cells leaving *C.trachomatis* free to multiply within the inclusions (Ripa and Mardh, 1977). Iodine staining of the cell cultures was later replaced by specific immunofluorescence staining using a specific monoclonal antibody to *C.trachomatis* (Stephens *et al.*, 1982; Wang *et al.*, 1985).

During the 1980s commercial enzyme linked immunosorbent assay (ELISA) tests became available which could be used for large scale screening. During the 1990s the NAATs were introduced which have now completely replaced other methods for routine *C.trachomatis* testing. These new tests have increased sensitivity from 60–70 % of the cell culture and ELISA methods to better than 90 %.

New, less invasive samples can be used with the NAATs. Urine samples from males have therefore completely replaced urethral swabs and in females urine samples and vaginal swabs now offer alternative to urethral and cervical swabs used previously (Schachter *et al.*, 2005).

These new samples can easily be collected by the patients themselves which has made home-sampling possible. Websites are now offering test facilities for *C.trachomatis* in Sweden (www.klamydiatest.nu, www.klamydia.se).

The NAATs are now being performed on automated laboratory platforms which means high and fast throughput. On average more than half of the samples are tested within 24 hours and most of the samples within three working days. This is a marked improvement over cell culture which on average needed one week for completion.

The NAATs do not need living organisms in contrast to the traditional cell culture method. This has improved sensitivity of the test compared to culture but also means that *C.trachomatis* DNA may be detected several weeks after successful treatment of infection.

In 2006 a new variant of *C.trachomatis* was detected in Sweden. It had a 377-bp deletion in the cryptic plasmid and later complete sequencing of the plasmid also showed a 44-bp duplication downstream of the deletion. Unfortunately the deletion harboured the target sequences of two different commercial tests from Roche and Abbott, respectively. Laboratories that used one of these tests could not detect this new variant.

In Malmö the Roche test had been used for 10 years until it was replaced by a new test from Abbott in 2006. However, both these commercial tests were compromised and unable to detect the new variant and had to be modified. An in-house test was introduced in Malmö in November, 2006, which could detect the nvCT until a commercial modified test was available from Abbott in August, 2007. Thus, nvCT have been specifically detected since November, 2006, in our laboratory based on PCR typing of all positive samples.

Antibodies to *C.trachomatis* can be detected in the blood and in local secretions. IgG, IgM and IgA antibodies are found but not all patients with proven *C.trachomatis* infection will develop a detectable antibody response. *C.trachomatis* antibodies has traditionally been measured by micro immunofluorescence (MIF) described in 1970 (Wang, 1971).

This test is based on organisms grown in embryonated eggs. Small dots of yolk sac antigen material are placed on slides. Patient serum is placed on the dots. Antibodies present in the serum will react with the antigen. After washing these antibodies can be detected following incubation with an anti-Ig-antibody labelled with an immunofluorescent tag.

C.trachomatis antibodies can then be detected by fluorescence microscopy. Antibody studies have been immensely important to describe associations

between *C.trachomatis* and late sequelae such as EP and TFI. On the other hand antibody measurement is not used routinely for the demonstration of current *C.trachomatis* infection. There may be just one important exception in infants with *C.trachomatis* pneumonia. In this condition *C.trachomatis* IgM antibodies are considered diagnostic (Schachter *et al.*, 1986).

Mycoplasma genitalium

M.genitalium was first demonstrated in the 1980s in 2 of 13 men with NGU. Repeated attempts to cultivate this organism proved to be very difficult (Samra *et al.*, 1988; Taylor-Robinson, 2002) and even when successful it takes several weeks or even months for each isolate to grow. More extensive studies on *M.genitalium* epidemiology and its role as a STI could not be performed until the development of the PCR in the 1990s.

After the development of DNA methods based on the polymerase chain reaction (PCR) laboratory diagnosis has become possible (Jensen *et al.*, 1991; Palmer *et al.*, 1991). Different in-house tests have been reported based on the surface exposed protein, MgPa, or detection of the 16S RNA gene (Jensen *et al.*, 2003). No commercial test is yet available.

As *M. genitalium* and *M. pneumoniae* share antigens, giving rise to extensive cross-reactions in most serological tests this method of diagnosis is difficult. Serology in its more sophisticated forms may have a role in epidemiological studies but is not of value in diagnosis. Serological methods similar to the MIF or based on the ELISA format have been described but are still only research tools.

Epidemiology

Chlamydia trachomatis and new variant Chlamydia trachomatis

C.trachomatis is the most common sexually transmitted bacterial infection throughout the world with an estimated 92 million new cases each year (WHO, 2001).

When diagnostic tests for *C.trachomatis* became available during the 1950s and 1960s the focus was mainly on trachoma, a blinding eye infection affecting millions of people in underprivileged areas of the world. From the mid 1960s through to the 1970s genital *C.trachomatis* infection and its late sequelae were unravelled.

During the 1980s screening for genital *C.trachomatis* infection was introduced in Sweden and other Nordic countries. From 1988 it became a reportable disease with mandatory partner notification in Sweden. Before that time voluntary laboratory reports gave some idea of the magnitude of the problem in the population.

In the 1980s the reported rates in Sweden were initially increasing when testing for *C.trachomatis* became more widespread but during the second half of the 1980s and into the beginning of the 1990s a decline was seen. Some 30,000 cases were detected in Sweden in 1990. After that a gradual decline occurred until the middle of the decade when 12,000 cases were reported. Since then *C.trachomatis* infections have soared reaching a peak in 2007 with 47,000 reported cases. During 2008 the number declined after a new variant of *C.trachomatis* had been demonstrated in 2006. The proportion of tested positive males to females is 1:1.3 in our population.

The decline in the late 1980s and early 1990s was believed to be the result of opportunistic screening and partner notification (Herrmann and Egger, 1995). However, in 1995 a slow increase began and from 1997 a steep fourfold

increase up to 2005 was seen. Other European countries were reporting the same development (Amatu-Gauci, 2007).

This generated questions about the prevention strategies of *C.trachomatis*. It did not seem to be effective enough with the Swedish approach as Sweden faced the same rising trends as other European countries both with and without defined prevention strategies or mandatory partner notification.

The number of reported *C.trachomatis* infections decreased when opportunistic screening was introduced in Sweden (Herrmann and Egger, 1995), British Colombia (Brunham *et al.*, 2005) and Northwestern United States (CDC, 2004) but have since the mid 1990s, been increasing steeply.

In 2008 Low *et al*. (Low *et al.*, 2008) reported results from a systematic review of the effectiveness of *C.trachomatis* screening. Among six reviews and five randomized trials there were two register-based randomized trials that showed *C.trachomatis* screening to reduce the incidence of PID in women. One was performed in a high risk population of women and the other among high school male and female students. One randomized trial showed that opportunistic screening among women undergoing surgical termination of pregnancy reduced the postabortal rates of PID compared with no screening. They did not find any randomized trial showing a benefit of opportunistic screening in other populations.

Low *et al*. (Low *et al.*, 2008) conclude in 2008 that there is an absence of evidence supporting opportunistic *C.trachomatis* screening in the general population younger than 25 years, the most commonly recommended approach.

After ten years of long and steady increases in reported *C.trachomatis* infections a levelling out in 2005 to 2006 was seen in Sweden. In some counties there was a decline of as much as 25 %. The decline was shown to be illusory as a new genetic variant was discovered that had escaped detection by some of the NAATs used in Sweden at that time. Reports from different

counties in Sweden showed a proportion of the new variant *C.trachomatis* of 20–65 % (Herrmann, 2007).

When the tests were modified and were again able to detect the new variant of *C.trachomatis* the number of cases increased dramatically from 32,523 in 2006 to 47,099 in 2007. In 2008 the number of reported *C.trachomatis* infections had decreased to 42,001 although at levels still higher than in 2006 before the nvCT was detected (Figure 3).

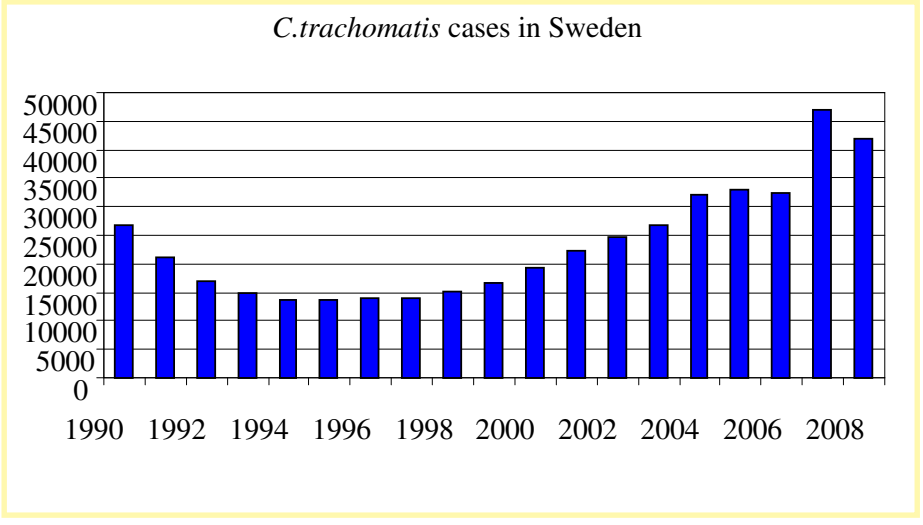

Figure. 3. Number of cases of *C.trachomatis* in Sweden

With nvCT emerging in our population in Sweden we have now a unique possibility to survey the development of the epidemiology and spread of this organism. Before 2007 the organism could spread without repression by antibiotic treatment.

Surveillance systems measuring the number of infections diagnosed in the community merely reflects the number of infections in the tested population, not in the general population. If we want to find out the rate of the spread and

change over time in the general population we need repeated point estimates from the general population.

These data do not exist and we have to interpret available surveillance data with this in mind. There are several factors important for either over- or -underestimation of the burden of infections over time.

Factors for underestimating the infection rate: data not fully reported to surveillance authorities, use of insensitive tests in the laboratory (other than gene amplification tests), use of incomplete sample material and changes in the infectious agent making it less detectable with gene amplification tests.

Factors that will tend to overestimate infection rate are: use of non-specific test and testing a community with higher prevalence than the general population, (Andersen and Ostergaard, 2008).

The only proven predictors for *C.trachomatis* in asymptomatic individuals are age and number of sexual partners but when applying these algorithms to limit the number of tests it results in too many missed *C.trachomatis* infections (van Valkengoed *et al.*, 2000; Andersen *et al.*, 2002).

Several studies from Manitoba, Canada, have reported the prevalence of *C.trachomatis* to be quite different in different social networks, demonstrating the uneven distribution of the infection (Jolly *et al.*, 2001). It is also recognized that so called 'bridgers' (individuals moving in between sexual networks) are the individuals of most interest for sustaining the infection (Nordvik *et al.*, 2007).

A deeper understanding of *C.trachomatis* epidemiology is needed and characterization of *C.trachomatis* strains is a helpful tool. The circulation and movement of different strains of *Neisseria gonorrhoeae* has proved valuable to broaden our understanding of sexual networks and to identify core groups (Berglund *et al.*, 2001; Unemo *et al.*, 2002).

Control and prevention of C.trachomatis infections

Opportunistic screening and mandatory contact tracing has been the strategy adapted to control *C.trachomatis* infections in Sweden. Contract tracing is mandatory after *C.trachomatis* became a reportable disease in 1988. One third of all *C.trachomatis* cases in Malmö are detected in contacts to index cases (40 % in males and 15 % in females) (data from Kenneth Persson, Department of Clinical Microbiology, Malmo, Sweden).

The impact of chlamydial infections on public health is large and comprises the direct and indirect costs of chlamydial disease including mental as well as physical and economic costs. In Europe an estimated prevalence of *C.trachomatis* infections in 2002 showed a range in prevalence of 1.7–17 % with prevalence depending upon the setting, context and country (Wilson *et al.*, 2002). The prevalence varies widely according to age, gender, geographic region, risk factors and diagnostic methodology. In general in developed countries the prevalence of *C.trachomatis* in sexually active women in 15–25 years of age will be in the order of 9 %.

Chlamydial infections in the community are dynamic. The benefits of screening can be reduced by high *C.trachomatis* incidence and repeat infection rates. In a retrospective cohort study on 3,568 patients in Colorado, USA that were tested repeatedly, 13.8 % had a positive result at their first visit (baseline infection) and 10.8 % had a positive test at a subsequent visit (incident infection).The incidence of repeat infections were 23.6 % and repeat infections accounted for 26 % of all incident infections (Rietmeijer *et al.*, 2002). In a home-based setting in Denmark it was found that the cumulative recurrence of urogenital *C.trachomatis* infections after antibiotic treatment was 29 % over a 24 week period, presumably by reinfection from sexual partners (Kjaer *et al.*, 2000).

To achieve an effective screening strategy rapid diagnosis and treatment, good sexual partner management and rescreening should be included. The burden of chlamydial genital tract infections for health services and individuals are large with women particularly disadvantaged as the major complications are

affecting women. It is estimated that 25–50 % of all PID cases is attributed to *C.trachomatis* infection (Bevan *et al.*, 1995; Schachter, 1999). In Malmö approximately 30–45 % of all PID cases in women below 35 years of age are associated with a *C.trachomatis* infection (Osser and Persson, 1982; Bjartling and Persson 2006).

A high number of studies have assessed the cost-effectiveness of screening for asymptomatic chlamydial infection in sexually active women in order to reduce the complication rate of PID. In 2002 Honey *et al*. (Honey *et al.*, 2002) reviewed these studies using systematic economic evaluation criteria. Only one randomized controlled trial was identified. In this study, women aged 18–34 years considered to be at high risk for chlamydial infection were identified by means of a questionnaire. They were then randomly assigned to undergo testing for *C.trachomatis* or receive usual care. The relative risk for PID in the screening group were only half of that in the usual care group (RR 0.44 CI 0.20–0.90) .This study provides the strongest evidence yet that a strategy of identifying ,testing and treating women at increased risk of cervical chlamydia infection can reduce the incidence of PID (Scholes *et al.*, 1996). In 2000 Østergaard *et al*. (Ostergaard *et al.*, 2000) compared the efficacy of a conventional screening strategy performed at the physician's office (control group) with a screening strategy based on home sampling (intervention group) and showed after 1 year follow up that the proportion of PID in the intervention group were significantly lower (2.9 %) than in the control group (6.6 %), p-value 0.026.

C.trachomatis infection is amenable for screening as it is mainly asymptomatic and widespread in the population. Several studies on cost-effectiveness have shown that screening could be recommended in populations with a prevalence of *C.trachomatis* infections of 3 % or more (Honey *et al.*, 2002). In 2002 Wang et al.,(Wang *et al.*, 2002) evaluated the cost effectiveness of a school based screening program using an estimated PID rate in *C.trachomatis* infection of 30 %. The basic assumptions for calculations of this kind have recently been questioned as complications seem to be less frequent then previously estimated

(van Valkengoed *et al.*, 2004; Low *et al.*, 2006). In a large population based study in the Netherlands a low prevalence of urogenital *C.trachomatis* infection was found and the author suggests that selective screening approaches are preferred (van Bergen *et al.*, 2006).

The major benefits of screening for asymptomatic infection in men and women lay in high risk populations, however cost-effectiveness of screening strategies are different in different settings and need to be considered against local factors.

The usefulness of screening has also been questioned in recent years due to the soaring numbers of *C.trachomatis* infections both in countries with and without extensive screening programmes. The introduction of the NAATs during the 1990s with a higher sensitivity than previous tests explains part of the increase but the increase has continued after that. The increase is also higher than could be explained by the observed increase in the number of persons in the appropriate age-groups. Thus, a genuine increase of *C.trachomatis* infections has most probably occurred since the mid 1990s.

In the last few years the situation has been compounded by the occurrence of the nvCT which was first reported in 2006. This new variant had not been detected by either of the two tests in use over the last few years in our county. It constituted at least 1/4 of the strains in 2006.

In archive material from 2000/2001 no nvCT was detected among 259 *C.trachomatis* culture positive samples from our laboratory. The nvCT has therefore appeared only during the last few years. It is mainly found in Sweden with only a few cases in neighbouring countries (Morre *et al.*, 2007; Savage *et al.*, 2007; Westh and Jensen, 2008). Since 2007 when diagnostic tests became available and an 'intervention programme' was introduced covering the nvCT, a gradual decline of the proportion of nvCT of all strains has been observed in our county. From 30 % of the strains it is now close to 15 % (Figure 4).

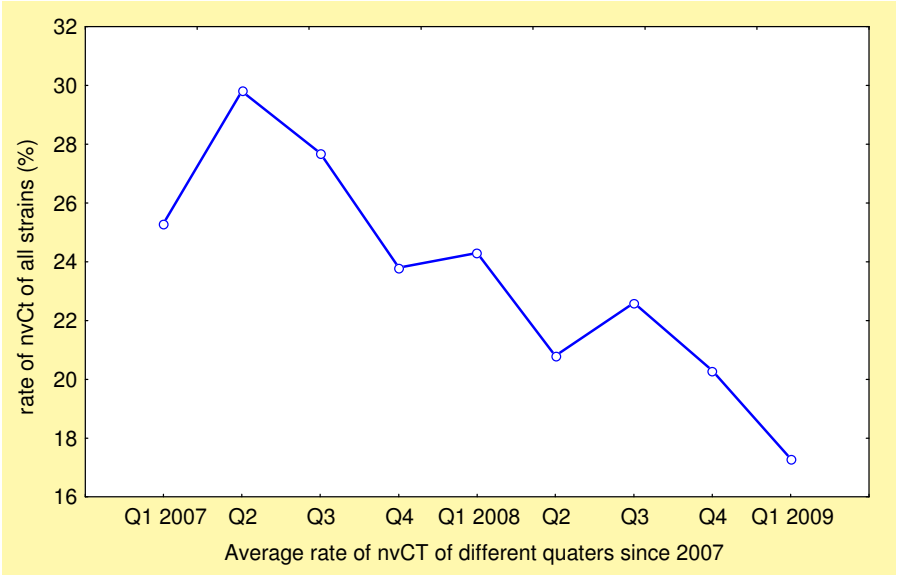

Figure 4. Proportion of nvCT of the total number of *C.trachomatis* cases in the county of Skåne from 2007–2008.

This selective decline of the nvCT in relation to the 'wild' type strains can best be explained as a result of intervention which was non-existent for the nvCT in 2006 and before. The nvCT will not disappear altogether but will find a new equilibrium with the wild types at some point. This balance point is being approached 'from below' in counties that use tests from Becton-Dickinson or Gene-Probe which have been able to detect the nvCT from the start. The true trend will be revealed in the coming years.

Recently, it was proposed that the screening activities may be part of the problem and not the solution. In the Vancouver area in British Colombia an increase of *C.trachomatis* infections since the mid 1990s has coincided with an increase in repeat infections. It has been suggested that detection and early treatment of *C.trachomatis* infection may lead to an arrested immunological response that could make patients more prone to reinfection (Brunham and

Rekart, 2008). This is still a hypothetical explanation and has recently been challenged (Low, 2008).

In Malmö the proportion of reinfection has not changed between 1990 (15 %) and 2003 (15 %) in spite of the same epidemiological development of *C.trachomatis* as in the Vancouver area.

The control of *C.trachomatis* infections also involves preventive measures. Recently circumcision of males has been associated with a lower risk for HIV infection. Circumcision has not been found to have a similar effect to reduce the risk of *C.trachomatis* infections.

Condom use will diminish the risk for STI transmission. Recently a self-instructive computer-based programme was reported to increase condom use and lower the risk of *C.trachomatis* infection (Grimley and Hook, 2009). Health education has sometimes been disappointing but some recent projects like SAFE and RESPECT have shown encouraging results (Shain *et al.*, 2002).

Mycoplasma genitalium

Information of the prevalence of *M.genitalium* in general populations not seeking health care because of symptoms are limited. In one study from Denmark 731 men and 931 women, 21–24 years old, who were participating in a population based *C.trachomatis* screening program, were tested. The prevalence was 2.3 % in women and 1.1 % in men (Andersen *et al.*, 2007). In another large population based adolescent health study from USA, Seattle, 1.1 % of 1,218 men and 0.8 % of 1,714 women were tested positive for *M.genitalium* (Manhart *et al.*, 2007).

In patients seeking care at STI clinics the prevalence is higher and frequencies of 4-8 % have been observed (Anagrius and Lore, 2002; Manhart *et al.*, 2003; Falk *et al.*, 2005; Edberg *et al.*, 2008; Moi *et al.*, 2009).

Recently, a study showed *M.genitalium* to be common in young women seeking legal abortion in New Zeeland (8.7 %) while another study from

Denmark reported a low prevalence in a similar population (0.98 %), (Lawton *et al.*, 2008; Baczynska *et al.*, 2008).

So far it seems that the prevalence of *M.genitalium* is approximately half that of *C.trachomatis* depending on population and setting. No data are available on long term development of *M.genitalium* prevalence.

Clinical manifestations

Chlamydia trachomatis

C.trachomatis is transmitted during vaginal, anal or oral sex and can be passed from the infected mother to the newborn child during vaginal delivery causing conjunctivitis and /or pneumonia. *C.trachomatis* infects mainly columnar or transitional epithelium in the urogenital sphere but can also infect the squamous epithelium in conjunctiva and pharynx.

In men it can cause urethritis, proctitis, prostatitis, epididymitis and possibly infertility. In women it can cause proctitis, urethritis, cervicitis and PID with long-term complications such as tubal factor infertility and ectopic pregnancy. In both men and women sexually acquired reactive arthritis (SARA) and Reiter´s syndrome can be seen as an extra genital manifestation of a primary genital infection in genetically predisposed individuals (Sieper and Braun, 1999).

Lower genital tract infection in men

Urethritis is an inflammation in the urethra which can be caused by a range of different bacteria.Sometimes no microbiological agent can be identified. Non specific urethritis or non gonococal urethritis (NGU) is the term for a sexually transmitted urethritis not caused by *N. gonorrhoeae*. In developed countries, *C.trachomatis* (serovars D to K) is the dominant pathogen, associated with 30-50 % of cases of symptomatic non specific urethritis in men. Various other microorganisms including *M. genitalium* and Ureaplasma species have also been incriminated (Horner *et al.*, 2001; Taylor-Robinson, 2002; Tait and Hart, 2002; Dixon *et al.*, 2002).

Common symptoms include urethral discharge and /or dysuria although asymptomatic infection is common. Chlamydial urethral infection is much

more likely to be asymptomatic than is gonococcal infection (Burstein and Zenilman, 1999; Stamm *et al.*, 1984).

The presence of 4 or more polymorphonuclear leukocytes (PMNL) in a Gram stained preparation of urethral discharge, examined at x 1000 magnification, high power field (HPF) in more than 5 HPFs are common used criteria for diagnosis of urethritis. The absence of gonococci in Gram stain or on culture establishes a diagnosis of, non gonococcal urethritis.

The sexually active male with asymptomatic urethritis is a significant reservoir of potential infection for women, in whom the consequences of lower genital tract infection are likely to be more severe. In one study, infection ratios in women exposed to male sex partners with chlamydia were 65 % and with gonorrhoea 73 % (Lin *et al.*, 1998).

Lower genital tract infection in women

Urethritis is an inflammation of the epithelium in the urethra and urinary symptoms include dysuria, or elevated frequency of passing urine. In most cases of chlamydial cervicitis, there is also associated infection of the urethra. However, it is not clear in all cases whether this is due to genuine chlamydial colonization or is contamination with chlamydial infected discharge from the cervix.

The prime target of chlamydial infection in the lower genital tract of women is the columnar epithelial cells outlining the endocervical canal. Cervicitis is an inflammation of the cervix. Cervicitis is defined clinically by the presence of either mucopurulent discharge or easily induced bleeding (friability) at the endocervical os. It is frequently asymptomatic but some women complain of symptoms such as abnormal vaginal discharge, intermenstrual vaginal bleeding and/or contact bleeding (e.g. after sexual intercourse), (CDC, 2006).

Chlamydial cervicitis is caused by *C. trachomatis* of serovars D to K. Serovar E is particularly common both in Sweden and in several other countries in Europe, Asia and Africa (Fredlund *et al.*, 2004).(

Up to the 1980s there were no established criteria for cervicitis. In 1984 the clinical syndrome of cervicitis was recognized as 'the ignored counterpart in women of urethritis in men' by Brunham *et al*. A combination of >10 PMNL per HPF (leucorrhea) and visible purulent discharge from the cervical os or friability was proposed as diagnostic signs (Brunham *et al.*, 1984).

Pathological vaginal wet smear (PMNL> than epithelial cells) in the absence of inflammatory vaginitis might be a sensitive indicator of cervical inflammation with a high negative predictive value (Marrazzo *et al.*, 2002).

Although few population-based data are available to estimate the true prevalence of cervicitis, it appears to be a common finding among women. The frequency is strongly dependent on the setting and population. In our study from Malmö in a gynaecological out-patient population the proportion of cervicitis among 5,519 women were estimated to be approximately 7 %.

C.trachomatis might act as a co-factor in cervical cancer. Various serological studies have suggested but not proven *C.trachomatis* to be a significant co-factor alongside HPV in the aetiology of cervical cancer (Anttila *et al.*, 2001; Naucler *et al.*, 2007). *C.trachomatis* is also associated with cervical cancer in prospective studies (Wallin *et al.*, 2002).

Pelvic inflammatory disease

PID occur when micro organisms ascend from the lower genital tract (vagina and cervix) to infect the upper genital tract, involving the endometrium (endometritis), the Fallopian tubes (salpingitis) and/or pelvic peritoneal cavity (pelvic peritonitis).

It is caused by ascending infection from the lower genital tract by bacteria associated either with sexually transmitted infections or various vaginal

anerobic bacteria. While it is clear that *C. trachomatis* and *N. gonorrhoeae* are common and important causes of PID, there is less information on the role of *M. genitalium*. This will be outlined in a following section.

The clinical spectrum of PID ranges from subclinical endometritis and salpingitis to severe salpingitis, pyosalpinx, tubo-ovarian abscess, pelvic peritonitis, and perihepatitis. The inflammatory response to PID may result in scarring and blockage of the Fallopian tubes, which can lead to infertility and/or EP.

Symptoms of PID include signs of cervicitis together with abnormal bleeding, dyspareunia, and lower abdominal pain.

There is a wide variation in signs and symptoms of acute PID and many women have subtle or mild symptoms. The clinical diagnosis of PID is imprecise and has a low sensitivity. Several studies have shown sensitivities between 30–75 % for clinical diagnosis compared to laparoscopy (Jacobson and Westrom, 1969; Wasserheit *et al.*, 1986; Burchell and Schoon, 1987; Sellors *et al.*, 1991; Westrom *et al.*, 1992; Tukeva *et al.*, 1999; Munday, 2000). Laparoscopy is considered the gold standard and can be used to obtain a more accurate diagnosis of salpingitis and a more complete bacteriologic diagnosis but is associated with high costs and in most settings not readily available.

As is the case with cervicitis many cases of PID associated with *C.trachomatis* appear to be silent or sub clinical (Osser *et al.*, 1989; Paavonen and Eggert-Kruse, 1999). A large number of sero-epidemiological studies in the 1980s have shown a positive correlation between chlamydial antibodies and tubal damage in infertile women and in women with EP, both in women with and without a self reported history of PID. In the studies of patients with tubal infertility fewer than half of the patients had a self reported history of PID (Punnonen *et al.*, 1979; Henry-Suchet *et al.*, 1981; Moore *et al.*, 1982; Jones *et al.*, 1982; Kane *et al.*, 1984; Brunham *et al.*, 1985; Cates and Wasserheit, 1991; Westrom *et al.*, 1992).

Most women with TFI have never been diagnosed as having chlamydial infection or PID and in EP a large proportion of *C.trachomatis* infections of the Fallopian tubes are asymptomatic or subclinical suggesting that silent infections are the most common cause of tubal infertility (Paavonen and Eggert-Kruse, 1999).

Criteria for the clinical diagnosis of PID are generally based on abdominal pain and cervical motion tenderness or uterine tenderness or adnexal tenderness together with either elevated oral temperature or elevated C-reactive protein or pathological saline prepared vaginal wet smear, following the guidelines from the Center of infectious disease control in Atlanta, USA, 2006 (CDC, 2006).

N. gonorrhoeae was considered to be the most 'important pathogen' for many years and cases of salpingitis were divided into gonococcal and non-gonococcal salpingitis (Curtis, 1921; Studdiford, 1938; Eischenbach, 1975). The proportion of gonococcal salpingitis was generally high reflecting the prevalence of *N.gonorrhoeae* in the population.

In 1976 Hamark *et al*. isolated *C.trachomatis* from a Fallopian tube of a patient with laparoscopically verified salpingitis and this observation was soon followed by others (Hamark *et al.*, 1976; Eilard *et al.*, 1976; Mardh *et al.*, 1977; Gjonnaess *et al.*, 1982).

During the 1980s the proportion of *C.trachomatis* caused salpingitis was reported to be approximately 30 % (Westrom, 1980; Munday, 2000).

The contribution of chlamydiae to PID varies depending on the particular population, setting and time. Identification rates for C. trachomatis in pelvic inflammatory disease range from about 20 % in the United States, to 25–55 % in Europe (Schachter, 1999; Bevan *et al.*, 1995). In a recent review from 2009 by Bébéar and de Barbeyrac the possible proportion of *C.trachomatis* PID is estimated to be 60 % in Europe (Bebear and de Barbeyrac, 2009).

The risk factors associated with PID are reported to be the same as identified for *C.trachomatis* infections, young age and number of sexual contacts (Simms

et al., 2006). There have been concerns as to whether the insertion of intrauterine devices (IUDs) increases the risk of pelvic inflammatory disease (Prager and Darney, 2007).

A review by Mohllajee *et al*. in 2006 (Mohllajee *et al.*, 2006) reported that none of the studies that examined women with STIs compared the risk of PID between those with insertion or use of an IUD and those who had not received an IUD. They reviewed indirect evidence from six prospective studies that examined women with insertion of a copper IUD and compared risk of PID between those with STIs at the time of insertion with those with no STIs. Women with chlamydial infection or *N.gonorrhoeae* infection at the time of IUD insertion were at an increased risk of PID relative to women without infection. However, the absolute risk of PID was low for both groups.

In an overview by Prager and Darney in 2007(Prager and Darney, 2007), studies of IUD in relation to PID and infertility were assessed. They concluded that the presence of an LNG-IUS does not increase the risk of PID or infertility in either parous or nulliparous women and the LNG may be protective against infection.

As for the copper releasing IUDs, results are more divergent but recent studies have shown an association between PID and infertility and cervical infection, not IUD use (Hubacher *et al.*, 2001).

Earlier studies in the 1980s showed that use of oral contraception was associated with a decreased risk of PID (Wolner-Hanssen *et al.*, 1985; Wölner - Hansen, 1987). Recent studies have been somewhat conflicting and in 2001 Ness *et al.* (Ness *et al.*, 2001)reported that no hormonal contraceptive were related to reduction in upper genital tract disease among 563 women with clinical pelvic inflammatory disease. A review in 2005 by Barett *et al*. (Barrett and Taylor, 2005) reported oral contraceptives to be a risk factor for PID masking the clinical severity of the disease.

Ectopic pregnancy and infertility

The major complications of PID are caused by acute and chronic inflammation of the Fallopian tubes (salpingitis). An infection can cause intramural fibrosis and scarring leading to tubal occlusion or impaired tubal function. Functional damage to the tubes may affect egg transport, leading to implantation of the fertilized ovum in the tube rather than in the womb resulting in ectopic pregnancy. Occlusion of the tubes by scar tissue prevents egg transport and fertilization, leading to infertility if it is bilateral.

Repeated episodes of salpingitis lead to a greatly increased likelihood of infertility (Westrom *et al.*, 1992; Rietmeijer *et al.*, 2002). Repeated genital tract infections are common. In women each episode of pelvic inflammatory disease roughly doubles the risk of permanent tubal damage (Westrom *et al.*, 1992; Paavonen and Lehtinen, 1994) irrespective of whether infection was silent or overt (Patton *et al.*, 1989).

In the female macaque monkey, repeated genital tract infection was necessary to produce the pelvic adhesions, tubal scarring and occlusion characteristic of severe pelvic inflammatory disease (Patton *et al.*, 1990). Thus repeated infection or severe infection at young age is associated with severe responses to *C. trachomatis* infection.

The necessity for rapid antimicrobial therapy to avoid tubal pathology is suggested by studies in the mouse which show that oviduct pathology and infertility due to chlamydial infection cannot be reversed by antibiotic treatment beyond about 12 days post infection (Tuffrey *et al.*, 1994).

In Sweden a series of classical studies was performed by Weström *et al*. where the impact of PID on infertility in women where established. During 1960–1984 the reproductive performance of 2,501 women who underwent laparoscopy was observed (1,844 with evidence of PID and 657 with no evidence of PID). In 1992 the results were reported. In the PID group 16 % failed to conceive vs. 2.7 % in the non-PID group and 9.1 % vs. 1.4 % respectively developed ectopic pregnancy. Total obstruction of the tube was

seen in 10.8 % of the PID group vs. 0 % in the non-PID group (Westrom *et al.*, 1992).

In another study using a smaller group of 708 patients and 100 controls the impact of repeated PID on fertility was shown. The first episode of PID resulted in 11.4 % tubal obstruction, the second 23.1 and the third (or more) in 54.3 % (Westrom and Mardh, 1983).

In these studies *C.trachomatis* was by far the most commonly identified cause of PID, although its contribution was most likely to have been underestimated because of the diagnostic methods used at that time.

The role of *C.trachomatis* particularly in EP was confirmed during the 1990s by a number of studies (Chow *et al.*, 1990; Sherman *et al.*, 1990; Osser and Persson, 1992a; Brunham *et al.*, 1992b) including a large case-control study from France by Coste *et al.* (Coste *et al.*, 1994)in 1994 that reported sexually transmitted diseases, in particular *C.trachomatis* to be the major cause of EP.

The rates of EP in Scandinavia and western industrialized countries increased steeply in the mid 1980s and then decreased until the late 1990s (Skjeldestad *et al.*, 1997; Makinen *et al.*, 1989). In 2004 Coste *et al.,* reported increasing incidence rates from 1992–2002 in France while reported rates for the whole of Sweden seem to be declining slightly although data is not complete (only cases of hospital inpatients are included in the national register). However, in Malmö, where the rates of EPs have been observed since the late 1960s (including outpatients), an increasing trend has been seen since the late 1990s up to 2005. During the last 3 years a slight decrease has been seen (Figure 5).

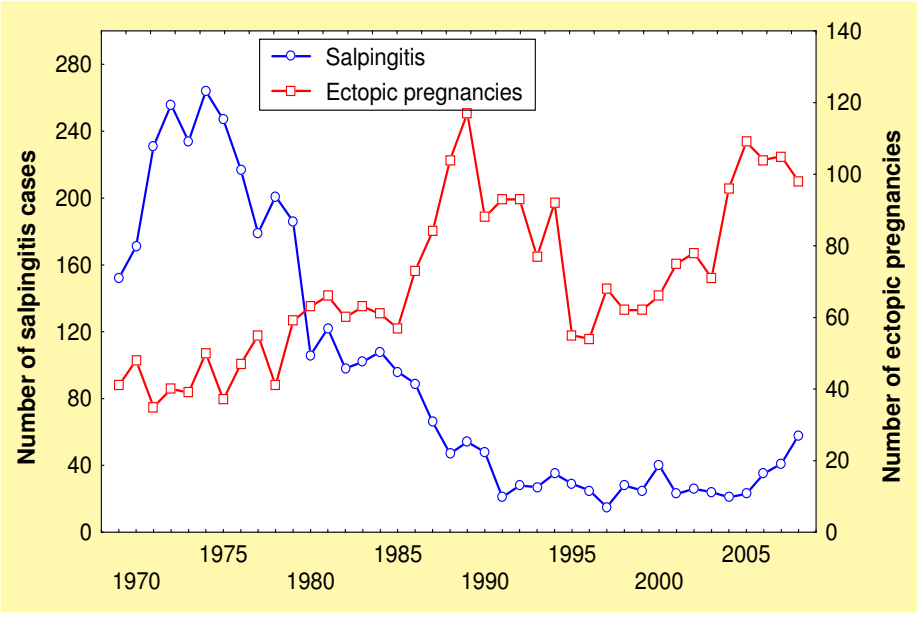

Figure. 5. Number of salpingitis cases and ectopic pregnancies in Malmö 1969- 2008

Pregnancy

One of the leading causes of perinatal mortality is prematurity. Uterine contractions may be induced by cytokines, proteolytic enzymes or prostaglandins released or induced by microorganisms. Asymptomatic bacteriuria, gonococcal cervicitis and bacterial vaginosis are strongly associated with preterm delivery, but the role of *C. trachomatis* is less clear (Locksmith and Duff, 2001; Cram *et al.*, 2002) although there are a substantial number of studies suggesting that maternal *C. trachomatis* infection in pregnancy is associated with premature delivery (Andrews *et al.*, 2000; Nyari *et al.*, 2001).

C.trachomatis has also been associated with intrauterine growth retardation and has been shown experimentally to induce pre-term birth in intravaginally infected mice (Blanco *et al.*, 1997; Pal *et al.*, 1999).

Termination of pregnancy (i.e. induced abortion) is a common procedure in the gynaecologic department. A large number of studies have shown that there is a high prevalence of *C.trachomatis* genital tract infection among women seeking termination of pregnancy. Postabortal pelvic inflammatory disease is a well recognized complication of termination of pregnancy, with its risks of tubal dysfunction and either infertility or subsequent ectopic pregnancy.

In the 1980s *C.trachomatis* was proven to be a substantial risk factor for postabortal infection with a proportion of more than 20 % in *C.trachomatis* positive patients (Moller *et al.*, 1982; Osser and Persson, 1984; Levallois *et al.*, 1987). Antibiotic treatment with doxycycline prior or in conjunction with the termination of pregnancy reduced the proportion of postabortal *C.trachomatis* infection from 23.2 % to 7.2 % (Osser and Persson, 1989).

New variant Chlamydia trachomatis

When nvCT was discovered in 2006 its prevalence was already quite high, a potential explanation for the rapid spread of the nvCT in the population (even in counties where it was always detected) could be that it causes reduced symptoms. Data on symptoms in nvCT infections will be discussed in the Results and comments-section.

Mycoplasma genitalium

M.gentalium has several similarities to *C.trachomatis* although its origin and structure is different. These bacteria are both host cell dependent for replication and they share the preference for epithelial and columnar cells in the urogenital tract. As well as *C.trachomatis*, *M.genitalium* has been recognized as the causative agent of a sexually transmitted infection (Keane *et al.*, 2000; Hjorth *et al.*, 2006).

In numerous studies *M.genitalium* has proved to be an important cause of urethritis in men, independent both of *C.trachomatis* and *N.gonorrhoeae*, and

in 2004 a meta analysis of published studies strongly confirmed this (Jensen, 2004).

Symptoms of urethritis in *M.genitalium* positive men with NGU are at least as common as in *C.trachomatis* positive NGU (Falk *et al.*, 2004; Anagrius *et al.*, 2005).

Whether *M.genitalium* can cause complications such as epididymitis and prostatitis remains unclear. Systematic studies linking the organism to these complications are lacking although *M.genitalium* DNA has been found in the urethra of men with epididymitis and in tissue from men with prostatitis (Krieger *et al.*, 1996; Eickhoff *et al.*, 1999).

Recent studies have shown that *M.genitalium* causes chronic infections in humans. Potential mechanisms of resistance have been reported. It seems that *M.genitalium* can undergo extensive gene sequence variation within persistently infected individuals (Hjorth *et al.*, 2006; Ma *et al.*, 2007).

In 2008 Jensen *et al*. showed that *M.genitalium* developed resistance to azithromycin trough mutations in region V of the 23S ribosomal RNA gene.

While *M.genitalium* is well documented as an agent of NGU in men, fewer studies has documented its role in women. In 1991 the first studies, applying PCR technique for detecting *M.genitalium*, showed the presence of the organism in both urethral and cervical specimens from women (Jensen *et al.*, 1991; Palmer *et al.*, 1991).

The correlation with lower genital tract infection including cervicitis has been reported by different groups (Uno *et al.*, 1997; Anagrius and Lore, 2002; Manhart *et al.*, 2003; Pepin *et al.*, 2005; Falk *et al.*, 2005) but an association between cervicitis and *M.genitalium* has not always been found, (Casin *et al.*, 2002; Cohen *et al.*, 2007; Tosh *et al.*, 2007; Huppert *et al.*, 2008).

Two studies from Nairobi, Africa and one from Pittsburgh, USA have shown *M.genitalium* in the endometrium of women with acute infection (Cohen *et al.*,

2002; Cohen *et al.*, 2005; Haggerty *et al.*, 2006). In one single case *M.genitalium* was detected in the Fallopian tubes of a patient with PID (Cohen, 2005). These studies have mostly referred to populations with concurrent HIV infection.

In a case-control study from the UK in 2003 Simms *et al*. found that in 45 PID cases 13 % had *M genitalium* infection and 27 % *C. trachomatis* infection compared to none of 37 controls. The association of these microorganisms with PID was significant ($p < 0.001$) and largely independent of each other.

Some serological studies have been able to link *M.genitalium* with PID (Moller *et al.*, 1984; Svenstrup *et al.*, 2008) but there are also negative results from studies not able to establish such an association (Lind and Kristensen, 1987; Jurstrand *et al.*, 2007). A serological association between *M.genitalium* and tubal damage in patients with tubal infertility has also been reported (Clausen *et al.*, 2001; Svenstrup *et al.*, 2008).

AIMS OF THE STUDIES

The purpose of the studies in this thesis was to explore the epidemiology, clinical manifestations and complications of *C.trachomatis* and *M.genitalium* infections in women.

Study I: To investigate whether the frequency of salpingitis and ectopic pregnancy could indirectly illustrate the epidemiological pattern of *C.trachomatis* during the time before testing for *C.trachomatis* became available and to elucidate the epidemiological pattern of *C.trachomatis* infections and ectopic pregnancies during the time after testing became available.

Study II: To investigate the persistence of *C.trachomatis* infection in ectopic pregnancy by looking for *C.trachomatis* DNA in fresh frozen tubal tissue of patients with ectopic pregnancy and to investigate the immunopathology of Fallopian tubal damage by assessing the correlation of different *C.trachomatis* antibodies, such as chlamydial IgG antibodies, chlamydial IgA antibodies, c-hsp60 antibodies and h-hsp60 antibodies in patients with ectopic pregnancy. using normal pregnant women as controls.

Study III: To investigate epidemiology and clinical manifestation of the new variant *C.trachomatis* in a high risk population and to assess the role of new variant *C.trachomatis* in ascending infection in women resulting in pelvic inflammatory disease.

Study IV: To investigate the prevalence, clinical manifestations, and complications of *M.genitalium* infection in women presenting at an outpatients service at a gynaecological hospital department, either with various acute/semi acute gynaecological problems or requesting termination of pregnancy.

SUBJECTS AND METHODS

Study population and study design

All studies were performed at the University Hospital, MAS, in Malmö, Sweden. *Study I, II* and *IV* were performed at the Department of Obstetrics and Gynaecology and *Study III* was performed at the Centre of Sexual Health (CSH), an interdisciplinary outpatient unit, affiliated to the University Hospital, MAS, Department of Venereology, Department of Infectious diseases and Department of Obstetrics & Gynaecology.

The city of Malmö is the third largest city in Sweden with approximately 290,000 inhabitants. The University Hospital is the only hospital serving the city and all patients included in the studies were hospitalized at the Department of Obstetrics and Gynaecology or visiting one of its outpatient's units. This setting is particularly suitable for epidemiological studies.

Study I was a retrospective observational study of 5,233 patients admitted to the University Hospital in Malmö between 1969 and 1996 with a diagnosis of either ectopic pregnancy, non-gonococcal salpingitis, or gonococcal salpingitis. Diagnoses were traced in the hospital registers, which are organized according to the WHO international numbering and nomenclature of diagnoses, International Classification of Diseases (ICD), 9th revision, (ICD-9) and 10th revision (ICD-10).

The study group included 1,794 ectopic pregnancies, 2,842 non-gonococcal salpingitis and 597 gonococcal salpingitis cases. Women diagnosed with ectopic pregnancy were stratified into four age-groups by four-year time periods. The age-specific frequencies of EPs were calculated for each consecutive 4-year period to demonstrate the development of ectopic pregnancy over time in age specific groups. The salpingitis and EP cases were correlated to the frequency of *N.gonorrhoeae* for the whole study period and to *C.trachomatis* from 1984–1996.

Study II was a case-control study on 55 women admitted to the University Hospital in Malmö diagnosed with EP and undergoing salpingectomy. Tissue from the Fallopian tubes and venous blood in patients and venous blood in controls were examined for *C.trachomatis* and other markers for *C.trachomatis* infection implicated in the immunopathology of tubal damage. The controls were age-matched women with normal pregnancy attending a mother health care unit in Malmö for a routine visit.

Patients with EP were divided into two groups, group I comprised 36 women with no previous PID or EP and group II comprised 19 women with a history of previous PID and/or EP. Venous blood samples were analysed for *C.trachomatis* antibodies including Ct-IgG, Ct-IgM and Ct-hsp60. Specimens from EP cases were also analysed for *C.trachomatis* DNA. Patients with EP were compared to women with normal pregnancies used as controls.

S*tudy III* was designed as a case-control study on sexual lifestyle and manifestations of uro-genital infection. Patients with infection by the new variant of *C.trachomatis* (nvCT) were compared to patients infected by the pre-existing *C.trachomatis* strains (wtCT) or to controls negative for *C.trachomatis*. All visitors at the CSH having a *C.trachomatis* test were invited to participate in the study and to complete a questionnaire. A *C.trachomatis* test is offered routinely to all visitors at the unit. During the study time a total of 8,365 patients were tested, 4,156 men (49.7 %) and 4,209 women (50.3 %). The questionnaire was completed by 84 % (7,020/8,365) of those invited to participate.

For each *C.trachomatis* positive case two negative controls were selected. They were matched for age, gender and medical examination. The controls had tested negative for *C.trachomatis* in the same month as the corresponding case.

All cases of PID in Malmö were studied to investigate the role of nvCT in PID, and all cases of *C.trachomatis* infections in women in Malmö were collated to assess the ratio of diagnosed PID in this group.

In *Study IV* 7,598 women presenting at the gynaecological outpatient service were tested for *M.genitalium* and *C.trachomatis* in a case control study. There were two different groups of patients tested; patients seeking care with acute gynaecological symptoms (5519) and patients presenting for termination of pregnancy (TOP), (2081). In patients presenting with acute gynaecological symptoms 108 *M.genitalium* positive cases, 143 *C.trachomatis* positive cases and 253 negative controls were included. Ten patients (3.9 %) were co-infected with *M.genitalium* and *C.trachomatis* and were excluded from the analyses.

In women presenting for TOP, 49 *M.genitalium* positive cases, 51 *C.trachomatis* positive cases and 168 negative controls were included. Four cases (4 %) were co-infected with *M.genitalium* and *C.trachomatis* and were excluded from the analysis

Controls were age-matched and randomly chosen among patients tested negative for *C.trachomatis* and *M.genitalium* infection in the same month. Patients presenting with acute gynaecological symptoms and *M.genitalium* infection had an average of 2.3 controls/case and *C.trachomatis* infected patients had 1.8 controls/case.

Controls for patients presenting for TOP (3.4 controls/ case in *M.genitalium* infected patients and 3.4 controls /case in *C.trachomatis* infected patients) were also matched for having a TOP either by a medical or a surgical method.

Data from medical records were evaluated and compared between cases and controls in patients presenting with acute gynaecological symptoms regarding gynaecological characteristics, self reported symptoms, clinical findings and clinical diagnoses. In patients presenting for TOP the postabortal infection frequency was compared between cases and controls.

Diagnosis of ectopic pregnancy and pelvic inflammatory disease

In Study I and II the diagnosis of ectopic pregnancy was based on the histological evidence of either embryo or chorionic villi in the material removed at surgery. Samples were histologically evaluated in the Department of Clinical Microbiology, Malmö University Hospital. In S*tudy II,* when salpingectomy was performed, a 2 cm piece of the tube including the pregnancy, was cut out and immediately sent to the Department of Clinical Microbiology where it was frozen at –70°C for later testing. One tubal tissue sample from each patient was also sent for routine histological examination and confirmation of tubal pregnancy.

Pelvic inflammatory disease refers to ascending infection from the cervix and includes endometritis, salpingitis, tubo-ovarian abscess, and general infection in the pelvis of the women.

In Study I, 80 % of the salpingitis cases were confirmed with laparoscopy. The remaining 20 % were diagnosed based on the following criteria; lower abdominal pain, cervical, uterine or adnexal tenderness at pelvic bimanual examination together with one of the following signs: pathological vaginal wet smear/ yellow pus from the endocervical canal/ pathological vaginal discharge, an elevated erythrocyte sedimentation rate level (≥15 mm), elevated C-reactive protein >8 or fever (oral temperature >38.0°C).The diagnostic criteria for PID were according to the CDC guidelines for sexually transmitted infections (CDC, 2002).

In patients with suspected salpingitis, specimens from the cervical ostium and urethra were taken for culture of *N. gonorrhoeae* with a charcoal-treated cotton swab and immediately sent to the laboratory in Stuart's transport medium. During the last 16 years of the study period (from 1980 to 1996) specimens were also taken for culture of *C. trachomatis*.

In Study III C.trachomatis PID was confirmed by laparoscopy in 70 % of the cases. Inflammation of the Fallopian tubes observed at laparoscopy, together with a positive test for *C.trachomatis* of the abdominal fluid, was considered proof of *C.trachomatis* PID. For the remaining cases of PID a clinical diagnosis was based on the guidelines from CDC (CDC, 2006)

In S*tudy IV* the clinical diagnosis of PID was based on the same criteria as in S*tudy I* except for an elevated erythrocyte sedimentation rate level (±15 mm) which is no longer used routinely. Patients presenting for TOP were assessed for postabortal infection. Postabortal infection was based on the same clinical criteria as in PID (CDC, 2006).

Diagnosis of urethritis and cervicitis

In Study III the diagnosis of urethritis was defined as ≥4 polymorph nuclear leukocytes per high- power field (x100) for both men and women and the diagnosis of bacterial vaginosis in women was based on Amsels´ciriteria who are proposing the presence of three out of four following criteria; 1) pH above 4.5 2) characteristic vaginal discharge 3) positive potassium hydroxide odor findings 4) clue cells on saline wet mount (Amsel *et al.*, 1983).

The diagnosis of cervicitis in S*tudy III* was defined as either a Gram stain showing ≥30 polymorphonuclear leukocytes per high-power field (x100) or an endocervical wet smear showing more polymorph nuclear leukocytes than epithelial cells per high power field (x100).

In S*tudy IV* the clinical diagnosis of cervicitis was based on either a pathological vaginal wet smear (more leucocytes than epithelial cells in the absence of clue-cells in high power field x 40-100) or a pathological vaginal discharge (yellow, purulent) together with yellow pus from the endocervical canal or friability or cervical motion tenderness.

Questionnaire

In S*tudy III* a questionnaire was used. The questionnaire consisted of 14 key questions (11 for men) regarding sexual lifestyle, uro-genital symptoms and diagnoses (Figure 6).

Uppgifter för undersökning av den nya klamydiabakterien

(Ska ifyllas och medfölja klamydiaremissen)

Namn ..... Personnummer……

Postnummer……

Antal partners senaste 6 månaderna	**1 ❑**	**2-5 ❑**	**>6 ❑**
Antal tidigare partners (totalt)	**1 ❑**	**2-5 ❑**	**>6 ❑**
Ny sexuell kontakt senaste 2 månaderna?		**Ja ❑**	**Nej ❑**
Sexkontakter utomlands senaste 2 månaderna?		**Ja ❑**	**Nej ❑**
Preventivmedel? **Inget ❑**	**Kondom ❑**	**P-piller ❑**	**Annat ❑**
Använde du kondom vid senaste samlaget?		**Ja ❑**	**Nej ❑**
Röker du?		**Ja ❑**	**Nej ❑**
Har du haft klamydia tidigare?		**Ja ❑**	**Nej ❑**

Ifylles om du är kvinna			**Ifylles om du är man**		
Har du haft några av följande besvär?	**Ja**	**Nej**	**Har du haft några av följande besvär?**	**Ja**	**Nej**
Sveda vid vattenkastning?	❑	❑	**Sveda vid vattenkastning?**	❑	❑
Vattenkastning oftare än normalt?	❑	❑	**Vattenkastning oftare än normalt?**	❑	❑
Blödning vid/efter samlag?	❑	❑	**Flytning från urinröret?**	❑	❑
Blödning mellan menstruationerna?	❑	❑			
Onormal flytning?	❑	❑			
Lågt sittande buksmärta?	❑	❑			

Rutorna nedan ifylles av undersökande läkare

Kvinnor	**Ja**	**Nej**	**Män**	**Ja**	**Nej**
Onormal flytning från cervix	❑	❑	**Onormal flytning från uretra**	❑	❑
Mikroskopiska tecken på vaginos	❑	❑	**Mikroskopiska tecken på uretrit**	❑	❑
Mikroskopiska tecken på cervicit	❑	❑			
Mikroskopiska tecken på uretrit	❑	❑			

Figure 6. Questionnaire regarding sexual lifestyle, uro-genital symptoms, and diagnoses.

The questions covered known risk factors and symptoms of *C.trachomatis* infections (Gotz *et al.*, 2005; Fenton *et al.*, 2005).

In most cases, patients were seen by a consultant triage nurse who referred the patient to a physician for examination when complaints of lower abdominal pain, irregular bleeding or any uro-genital symptoms were identified. A medical examination was performed in 22 % of the women and in 35 % of the men. In those patients the physician completed the last section of the questionnaire.

Data collection from medical records

In the medical records from the Department of Obstetrics & Gynaecology a diagnosis according to the Swedish version of International Statistical Classification of Diseases, tenth version (ICD-10) was recorded for each patient when clinically assessed.

In S*tudy IV* data were assembled from medical records. Gynaecological characteristics, self- reported symptoms, clinical findings and clinical diagnoses were collected in women seeking acute or semi acute care.

Gynaecological characteristics comprised age, pregnancy status, and previous pregnancies, phase of menstrual cycle, and use of contraception. Self reported symptoms included lower abdominal pain, abnormal vaginal discharge, (post) coital bleeding/irregular bloody shedding, painful urination and, a prolonged menstrual cycle (> 10 days).

Clinical signs included: pathological saline prepared wet smear (leucocytes >epithelial cells), pathological vaginal discharge, easily induced cervical bleeding (friability) and cervical tenderness. Elevated level of C-reactive protein (CRP >8) and fever (>38.0°C) were also assessed. An external genital and bimanual pelvic examination was performed in all participants.

In patients presenting for TOP, postabortal infection (endometritis and salpingitis, ICD, O04.1) were assessed. Postabortal infection was based on the same criteria as in PID. An infection was considered postabortal if occurring within four weeks following the abortion.

Ethical committee approvals

Studies I-IV were approved of by the local Research Ethics Committee at Lund university Medical faculty (LU 43998). All patients were informed and oral consent was obtained from all participants.

Microbiological diagnostics

Clinical specimens

For many years, swabs from the cervix and urethra in SPG medium were used as specimens to test for *C.trachomatis* in females. In men urethral swabs were used. When NAATs were gradually introduced during the 1990s first void/catch urine samples (FVU/FCU) replaced urethral swabs in men. Later urine samples were also accepted for screening in women. In 2005 the m2000 test platform from Abbott (Abbott Molecular, Des Plaines, IL, USA) replaced the Cobas Amplicor (Roche Molecular Systems Inc., Branchburg, NJ, USA).In this diagnostic system testing vaginal swabs is feasible. These swabs were often self-collected and placed together with a urine sample in the same plastic tube. Such dual samples almost completely replaced the cervical and urethral swabs in women. The same specimens were used for both *C.trachomatis* and *M.genitalium* detectrion.

In S*tudy II* tissue from the Fallopian tubes were analyzed for *C.trachomatis*. Frozen tissue was enzymatically digested and DNA purified using a commercial kit (High pure PCR template preparation kit, Roche Diagnostics GmbH, Mannheim, Germany).

Detection of Chlamydia trachomatis in clinical samples

In S*tudy I* specimens from the cervix and urethra were cultured in cycloheximide-treated McCoy cells for diagnosis of a genital chlamydial infection. The Cobas Amplicor test (Roche) for *C.trachomatis* gradually replaced cell culture during the 1990s. The Cobas Amplicor test was used in S*tudy II*. In addition a quantitative in-house real-time PCR (q-PCR) was used to test the tissue samples in S*tudy II*. The q-PCR was able to detect less than 10 copies of the plasmid in each reaction, which corresponds to 1-2 organisms of *C.trachomatis*.

The Cobas Amplicor test was used until 2005 when the m2000 test platform (Abbott) was introduced. This test features an integral DNA purification step after which *C.trachomatis* is detected by a real time PCR method. Both these commercial tests were used in S*tudy IV*.

Detection of the new variant of Chlamydia trachomatis

In 2006 a new variant of *C.trachomatis* was reported from Sweden by Ripa and Nilsson. This new variant had a deletion of 377 bps in the cryptic plasmid. Within this deletion the target sequences for the tests from both Roche and Abbott were situated. These two tests were thus unable to detect the new variant. An interim testing strategy was adopted from November, 2006. From this time all negative samples were retested by an in-house PCR method targeting a sequence outside the deletion in the cryptic plasmid. In August 2007 a modified version of the m2000 kit (Abbott) was introduced and replaced the makeshift arrangements with a double testing protocol for both genome and plasmid.

To distinguish the new variant of *C.trachomatis* from existing strains two different PCR methods were developed. One of these methods was a nested PCR based on primers flanking the deletion. In gel electrophoresis the nvCT and wtCT gave amplification products of different sizes that could easily be differentiated (Figure 7).

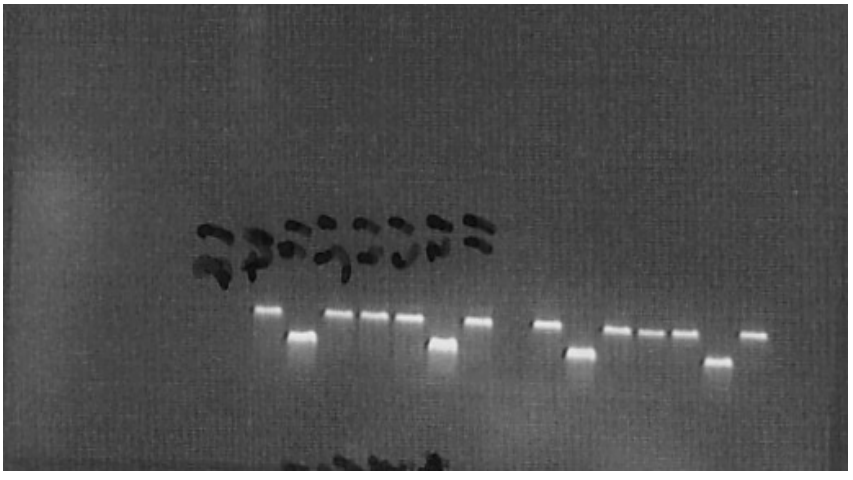

Figure 7. Gel electrophoresis followed by staining with ethidium bromide of the amplification products after PCR of the new variant of Ct and wild type strains.

The second method involved two separate real time PCR set-ups with one target completely within the deletion and another spanning the gap of the deletion of the new variant. Strains were positive by one of the real time PCRs only and could be positively identified as either new variant or wild type strains. All samples in S*tudy III* were analyzed according to the above strategy.

Detection of Mycoplasma genitalium in clinical samples

In *Study IV* urine or swab samples obtained for *C.trachomatis* testing were also used for *M. genitalium* PCR. Samples where pooled with 5-10 samples in each pool and then submitted to PCR. A seminested PCR method with primers towards the surface protein MgPa gene described by Jensen *et al.*, 2004 was used. Postive pools were resolved and individual samples were tested separately. The amplification product was identified after gel electrophoresis and staining by ethidium bromide (Figure 8).

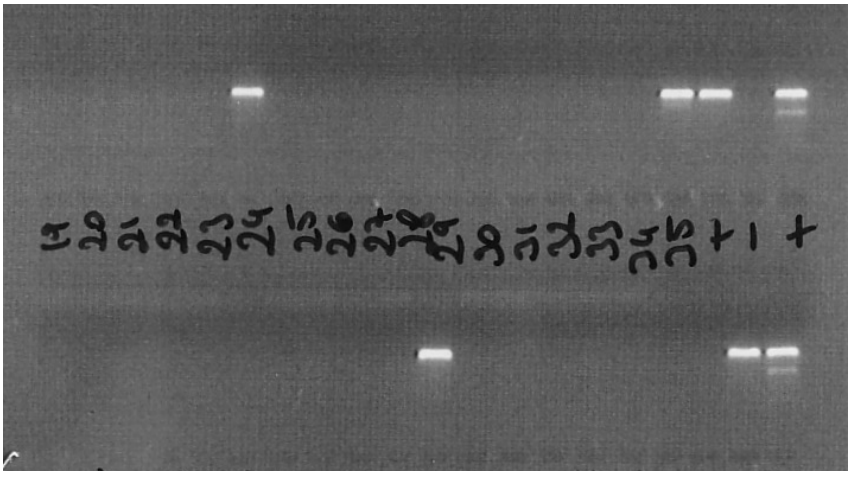

Figure 8. Gel electrophoresis followed by ethidium bromide staining of the amplification products after PCR of samples tested for *Mycoplasma genitalium.*

To further increase the specificity a semi-nested PCR was finally used with a combination of the MgPa-1 primer and the MgPa-2 primer. All *M.genitalium* strains detected in *Study IV* were positively identified by DNA sequencing of the amplification fragment

Detection of antibodies to Chlamydia trachomatis

Formalin-treated elementary bodies of *C.trachomatis* were used in an in-house microimmunofluorescence test (MIF) as described by Wang *et al.* in 1971. Three different antigen dots were used on microscopy slides. One dot consisted of a strain of *C.pneumoniae* (IOL-207) and one dot contained the *C.psittaci* strain 6-BC. The third dot consisted of a pool of *C.trachomatis* strains of serovars D-K usually associated with genital infection. Patient sera were tested at dilutions of 1/16, 1/32, 1/64, 1/128, 1/256 and 1/512. IgG and IgM antibodies were measured. The titre of a serum was designated as the inverse dilution step where activity could still be seen.

Antibodies to heat shock protein 60 of chlamydial or human origin (*study II*) were measured by commericial kits from medac (medac GmbH, Wedel, Germany) and used according to the kit insert.

Statistical analyses

The first step to conducting a study is to formulate a research hypothesis. The **null hypothesis** is a theoretical premise that states no difference between the groups investigated, any observed difference is the result of chance. The null hypothesis is used for possible rejection.

The **alternative hypothesis** is the opposite of the null hypothesis and states a possible difference between groups investigated.

The probability of the null hypothesis to be correct can be calculated by tests of significance generating a **p- value**. The p-value is the probability of obtaining a result at least as extreme as the one that was actually observed, assuming that the null hypothesis is true.

Two types of error can occur, **Type I** error (or α error) occurs when the null hypothesis is rejected even though it's true, i. e. the study findings indicate a statistically significant difference giving a false positive result.

The significance level, α level, is the level of maximum acceptable probability to make a type I error. The most commonly used α level is 0.05 (5 %) and this level is also used in the studies in this thesis. A p-value of 0.05 means the risk of making a type I error is 5 %. If tests of significance gives a p-value lower than the chosen α-level, the null hypothesis is rejected. Such results are referred to as 'statistically significant'.

Type II error (or β error) occurs when the alternative hypothesis is true but the study findings fail to reject the null hypothesis giving a false negative result. The probability of failing to detect a significant difference when it truly exists is denoted by β. The probability of detecting the difference (statistical power) is

the complement of β (1- β), i.e. the probability to not make a Type II error. Statistical power is also influenced by the sample size and use of statistical methods.

Student´s t test was used in S*tudy III* and S*tudy IV* to compare the significance of differences of means in two independent groups with assumed normally distributed populations. The *t-value* is calculated from the difference between two sample means divided by the standard error. Tables of the distribution of *t-value* has been generated and if the **null hypothesis** is true the probability of getting a *t- value* as large as the one calculated can be found for different degrees of freedom and α levels. At an α level of <0.05 the likelihood that the calculated difference in means should occur by chance is very low. The **null hypothesis** can be rejected and one can conclude that the difference in means is significant.

The **Mann Whitney –U test** was used in S*tudy III* to evaluate the significance of difference in groups where data had a skewed distribution. It´s a non-parametric test in which the raw value of the data is assigned a rank number and the mean of the rank sum is used when comparing the significance of the difference between the groups.

In *Studies II-IV* the Pearson **Chi-Square test** was used. When comparing categorical or nominal variables from two or more groups the data is presented in a contingency table (2x2). The Chi-square test can be used to determine how likely the result obtained could have occurred by chance. Using the null hypothesis an expected frequency can be determined.

Fisher´s exact test was used in *Studies II-IV* for categorical variables. This test is used to evaluate if differences between groups are significant when the expected frequency in the contingency table is <5 in one or more cells.

The odds of an event are calculated as the number of events divided by the number of non-events. The **odds ratio (OR)** is a way of comparing whether the probability of a certain event is the same for two groups. An odds ratio is

calculated by dividing the odds in the exposed group by the odds in the control group. An **OR** of 1 implies that the event is equally likely in both groups. An **OR** >1 implies that the event is more likely in the first group. An **OR** <1 implies that the event is less likely in the first group. If a 95 % confidence interval (CI) is calculated, statistical significance is assumed if the interval does not include 1.

A **confidence interval** (**CI**) is an interval estimate of a population parameter used to indicate the reliability of an estimate. The probability for an interval to contain the parameter is determined by the confidence level**.** In these studies a confidence level of 95% have been used which means that with a p-value of <0.05 the probability of the calculated parameter to be inside the CI is 95 %.

Regression analysis is used for the modeling and analysis of numerical data consisting of values of a dependent variable (response variable or measurement) and of one or more independent (explanatory variables or predictors).

Logistic regression is used for prediction of the probability of occurrence of an event by fitting data to a logistic curve. It can be used for binary outcome/response data (dependent variables) with one or more predictors (independent variables). In binomial regression, the probability of an event is related to independent variables. Logistic regression models work in terms of odds, and report effects as odds ratios. Multiple regression analysis was used in *Studies II-IV* for adjustment of possible confounders.

Statistical analyses were performed using SPSS for Windows version 16.0 and the Statistica package (Stat Soft Inc., Tulsa, OK, USA).

RESULTS AND COMMENTS

Study I

The frequency of salpingitis and ectopic pregnancy as epidemiologic markers of Chlamydia trachomatis

The incidence of gonorrhoea has been recorded in Sweden since the beginning of the last century while the incidence of *C.trachomatis* before the 1980s is uncertain. In this study the frequencies of non-gonococcal salpingitis, gonococcal salpingitis and EP were observed and correlated to the prevalence of *N.gonorrhoeae* and *C.trachomatis* in our population in Malmö.

Results

The mean age of patients with non-gonococcal salpingitis was 24 years for the whole study period ranging from a mean age of 22 years in 1980 to 27 years in 1992. The mean age of patients with EP was 29 years for the whole study period ranging from a mean age of 27 years in 1972 to 31 years in 1988, 1995 and in 1996.

The annual number of all cases of acute salpingitis (both non-gonococcal and gonococcal) as well as the number of cases of *N.gonorrhoeae* peaked in the early 1970s and then declined over the study time showing the same development (Figure 9).

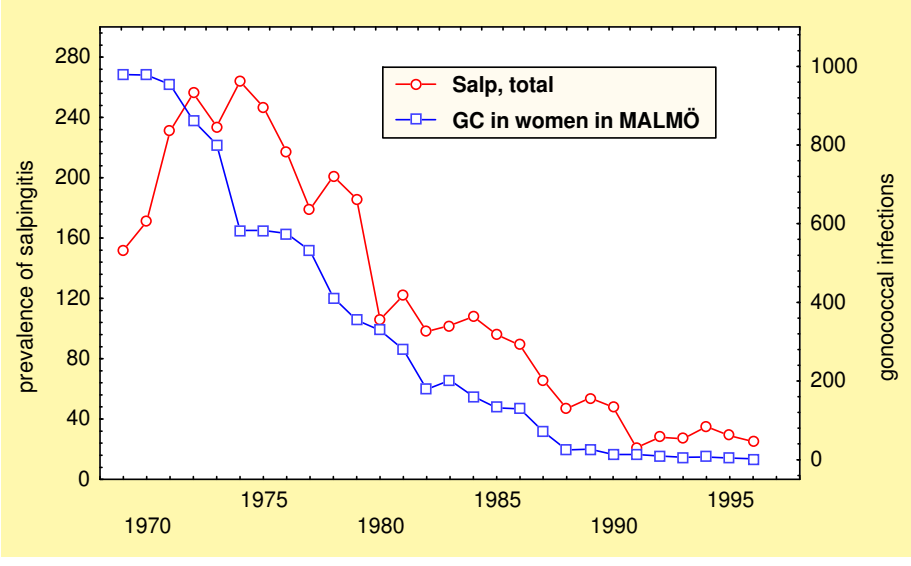

Figure 9. Annual number of cases of acute salpingitis and cases of *N.gonorrhoeae* in Malmö

The development of EPs showed a different pattern with a steady increase up to 1994 and then a decline. From 1985 to 1995 a rather steep increase was observed followed by a sharp decline. The peak of salpingitis cases in the 1970s was followed by a peak of EPs in the 1980s. When superimposing the peak of salpingitis cases over the peak of EPs cases the two curves had a similar form and spanned a similar time period. The interval between the start and end of the two peaks was 15 years (Figure 10).

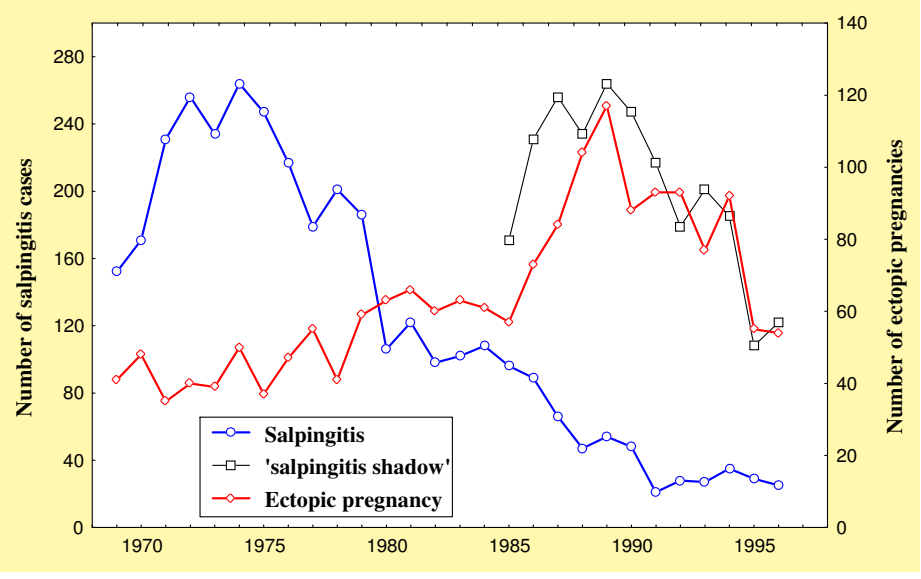

Figure 10. Annual number of cases of acute salpingitis and ectopic pregnancies in Malmö

When adjustments were made for changes in the number of deliveries in the population the basic curve for EPs did not change.

For each consecutive 4-year period an age-specific EP prevalence was calculated for the following age groups; group 1 (20–24 years), group 2 (25–29 years), group 3 (30–34 years) and group 4 (35–39 years). Age group 3 and 4 (women 30–39 years) showed the most marked increases during the 1980s. When comparing a low incidence period (1969–1976) to a period with high incidence (1985–1992) the increase in women of 20–29 years of age was 47 % and in women of 30–39 years of age 184 %.

During the low incidence period in 1969–1976, the two groups with younger women (20–29 years) constituted 64 % of the EPs, but only 47 % in the high incidence period 1985–1992. The major increase in EPs thus occurred in women who were about 20 years of age during the early 1970s.

Comments

After the peak of salpingitis cases in the 1970s the number declined during 20 years to one tenth of the highest number. The same development was seen for gonococcal infections in women over the same period. It seems that the frequency of acute salpingitis reflects the prevalence of STIs such as gonococcal infections.

Some other factors could also influence the change in frequency. In the beginning of 1970 the copper IUD was introduced and in several studies an increased risk of PID associated with IUD was documented (Senanayake and Kramer, 1980). This could account for a part of the increase in the 1970s.

Another explanation for the observed decline in hospitalized cases of acute salpingitis could be that more cases were treated as outpatients. A nationwide study in Sweden by Weström *et al*. in 1988 (Westrom, 1988)showed a decrease in the numbers of women treated for acute salpingitis in hospital to be 40 % during 1974–1984. However, the proportion of clinically mild cases increased instead of decreased as would have been seen if a larger proportion of such cases had been treated as outpatients, suggesting the decrease of acute salpingitis cases was genuine. Further support for this view is that in our catchment area most cases of suspected salpingitis are referred to this hospital which is the only hospital serving the area. Only a small proportion ought to have been treated as outpatients.

Gonococcal and non-gonococcal salpingitis have followed a similar trend over the years studied. Since the 1980s gonococcal salpingitis has almost disappeared. *C.trachomatis* is to a large extent associated with non-gonococcal salpingitis. In our material *C.trachomatis* has been detected in 25–40 % of the cases of non-gonococcal salpingitis in women below 25 years of age since 1985. During the first half of the 1980s almost 50 % of patients with salpingitis had *C.trachomatis* in our area (Osser and Persson, 1982).

The frequency of non-gonococcal salpingitis should reflect the prevalence of *C.trachomatis* and might be used to describe the epidemiological trends for such infections before diagnostic facilities became generally available.

A threefold increase was shown in the frequency of EPs from 1969 to the peak in the late 1980s. Since then a decrease in number of cases has been seen. The same observation as been made for the whole of Sweden (Thorburn, 1995). In Norway (Skjeldestad *et al.*, 1997) and Finland (Makinen *et al.*, 1989; Makinen, 1996) a similar trend have been reported.

Reason for this steep increase is probably manifold. Different fertility assistance methods were introduced during the study period, thus introducing patients who are generally at increased risk for EP (Alsunaidi, 2007). This might count for a proportion of the increase in the prevalence of ectopic pregnancies in the population. More sensitive pregnancy tests together with the development of more sensitive ultrasound examination methods could increase the proportion of diagnosed EP as spontaneous resolution of EP does take place (Ylostalo *et al.*, 1991; Elson *et al.*, 2004). Together these factors could increase the number of EP cases but the decline noted in the 1990s must have another explanation.

The association between EP and preceding *C.trachomatis* infection has been demonstrated in several serological studies (Walters *et al.*, 1988; Chow *et al.*, 1990; Osser and Persson, 1992b). EP thus represents late sequelae after an acute salpingitis in a proportion of cases. In this study the surge of EPs was preceded with a similar peak of acute salpingitis cases about 15 years earlier. When the peak of acute salpingitis cases were superimposed on the peak of EPs the two curves spanned a period of 10 years and showed the same shape.

The mean age of the salpingitis patients in our study was in the range of 20–25 years and in patients with EP around 30 years, which was also the mean age of women with normal pregnancies. The increase of acute salpingitis cases in the 1970s ought to be followed by an increase of EPs only with a time delay of 5–10 years.

The increase of EPs in women of 30–39 years of age was four times as high as in women of 20–29 years of age in our study. The women aged 30–39 where the risk increased the most were about 20 years of age during the first half of the 1970s when the peak of acute salpingitis cases occurred. It seems likely that the peak of EPs could be accounted for by the peak of acute salpingitis cases during the 1970s.

Demographic changes during the study time could influence the incidence of EPs. In Malmö about 30 % of the inhabitants are first generation immigrants. Finland has a very small proportion of immigrants, but has experienced the same development of EPs as has Sweden, suggesting that the influence on the incidence of EPs of a population with possibly another epidemiological background is small. The immigrants constitutes about half of all women of childbearing age but only 10 % of the EPs. EPs could also vary in relation to total number of deliveries. When EPs were adjusted for the number of deliveries the peak of EPs remained.

The prevalence of EPs seems to reflect the prevalence of acute salpingitis with an interval of 15 years. Additional factors such as improved means of diagnostics may have increased the level of EPs but cannot explain the decline of EPs observed at the end of the study period. This decline seems to reflect the decline in acute salpingitis cases since the mid 1970s.

In conclusion, the frequency of acute salpingitis seems to reflect the prevalence of circulating STI agents such as *N.gonorrhoeae* and *C.trachomatis*.

The frequency of acute salpingitis might be used to estimate the occurrence of *C.trachomatis* infections during the 1970s and early 1980s before diagnostic facilities became available.

It is likely that the steep increase in ectopic pregnancies in the middle of the 1980s and early 1990s was due to the steep increase of salpingitis cases during the 1970s.

The frequency of chlamydial pelvic inflammatory disease

Since 1996 we have continued the surveillance of EPs, salpingitis cases and the prevalence of *C.trachomatis*. Over the period of 1984 to 2004 all cases of *C.trachomatis infection*, all cases of salpingitis and all cases of EPs were studied The proportion of diagnosed salpingitis cases in relation to the number of *C.trachomatis* cases was assessed during two periods of the study time (Bjartling and Persson 2006).

From 1995 there was an increase in *C.trachomatis* cases. During the early part of this period the frequency of salpingitis cases decreased in the same way as the prevalence of *C.trachomatis* infections in the population but after 1995 the number of salpingitis cases remained stable (Figure 11).

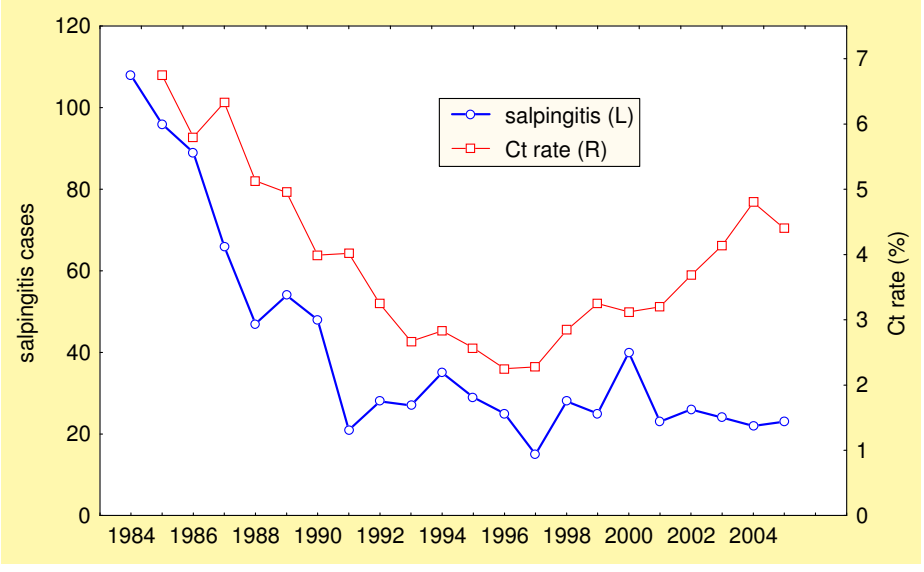

Figure 11. The frequency of *C.trachomatis* and salpingitis cases in Malmö

During the 1990s the number of EP cases decreased until 1997 when a new increase begun (Figure 12).

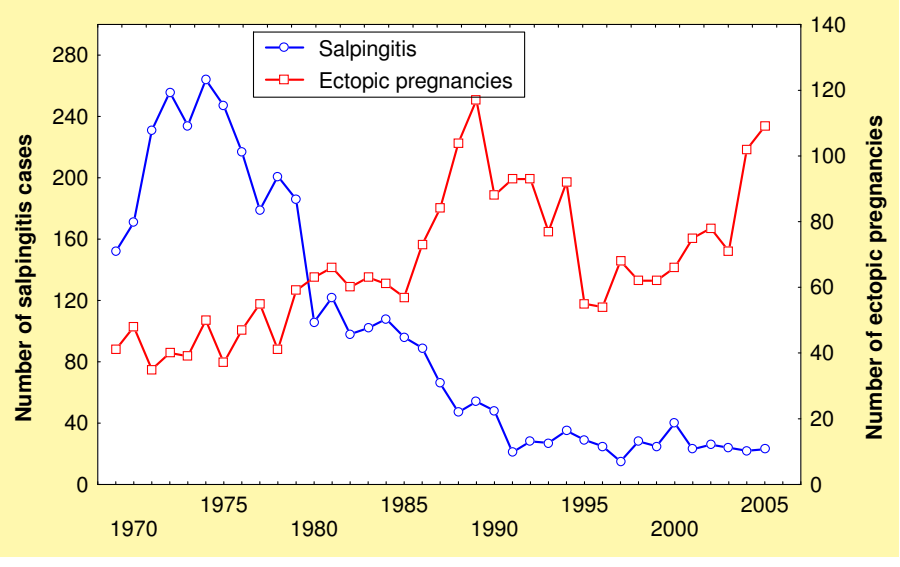

Figure 12. The number of salpingitis cases and ectopic pregnancies per year in Malmö

The number of *C.trachomatis* eye infections also followed the same trend as the frequency of *C.trachomatis* cases (data not shown).

During the study period from 1984 to 2004 the number of diagnosed salpingitis cases showed the same patterns as the number of *C.trachomatis* cases until the mid 1990s. From this time, the number of diagnosed salpingitis cases did not increase despite the large increase of *C.trachomatis* cases in the population (Fig 1). The same observations have been reported from other studies. In Norway there has been a 28 % reduction of hospitalised PID cases in the last decade

(Sorbye *et al.*, 2005) and a study from Australia reported the same experience in spite of a fourfold increase in *C.trachomatis* cases (Chen *et al.*, 2005).

Several different explanations are possible. In the period from 1989 to 1991 the proportion of diagnosed *C.trachomatis*- associated salpingitis cases in relation to the number of *C.trachomatis* positive cases was 2.6 % in our population. Between 2001 and 2004 the same proportion was only 0.4 %. The numbers of tests taken were comparable during these two periods.

The routine procedure for diagnosing PID in our hospital is by laparoscopy. However, in recent years the adherence to this routine has weakened and fewer patients than earlier are undergoing diagnostic laparoscopy. The declining acceptance for invasive diagnostic methods and limited resources could play an important role, leaving only the patients with severe symptoms eligible for diagnostic laparoscopy.

There are also studies showing that the diagnostic criteria for PID which were developed when the prevalence of *Neisseria gonorrhoeae* was very high, has a lower specificity and sensitivity in a population were *C.trachomatis* is the major agent (Simms *et al*., 2003).

Another possible explanation is that there is a true decline in the prevalence of PID. If so, other well-known complications would also decline. In our population we found that the number of EP cases has increased since 1996 although the number of salpingitis cases has decreased since 1985 and remained stable since 1996. The number of chlamydial conjunctivitis has also increased markedly during the last 10 years.

The increase of both EPs and chlamydial conjunctivitis cases reflect the increased prevalence of *C.trachomatis* infections in the population since the mid 1990s. In contrast, the number of confirmed cases with *C.trachomatis* salpingitis has remained unchanged during this period.

These epidemiological data may suggest that the true occurrence of *C.trachomatis* salpingitis is underestimated. Milder symptoms of *C.trachomatis* salpingitis and changes in clinical practice may explain this development.

Study II

Deoxyribonueclic acid of Chlamydia trachomatis in fresh tissue from the Fallopian tubes of patients with ectopic pregnancy

It has been suggested that the immune response to a persistent infection may lead to progressive injury of the tubal epithelium, possibly aggravated by autoimmune response (Witkin and Linhares, 2002). In patients with advanced PID and tubal damage circulating antibodies to the c-hsp60 protein is suggested to cross react with human hsp60 (h-hsp60) and thus initiate/or enhance the scarring process (Domeika *et al.*, 1998).

In this study we explored the possible presence of *C.trachomatis* DNA at the time of EP using freshly frozen tubal tissue and analyzing for *C.trachomatis* with PCR and a highly sensitive real time PCR test. We also investigated the correlation between c-hsp60 antibodies and h-hsp60.

Results

All tubal tissue samples from the 55 patients with EP tested negative for *C.trachomatis* by PCR (Cobas Amplicor). An inhibition control was used for each sample. All tissue samples were also tested by a quantitative real time PCR with negative result. The probability of detecting at least two positive cases was 0.90 with a significance level of 0.01 given the mean success *C.trachomatis* DNA discovered in earlier studies was 26 %.

When comparing all patients with EP with their controls, IgG antibodies to *C.trachomatis* were detected by MIF in 40 and 24 % respectively. This difference was not statistically significant. IgA antibodies to *C.trachomatis* were found in 20 % of the patients with EP and in 4 % of the controls. This difference was statistically significant (p-value 0.05).

When patients with EP and their normal pregnant controls were compared using logistic regression analysis no single antibody could predict EP but when combining IgG antibodies to *C.trachomatis* and chlamydial hsp60 a strong association with EP was found (OR 5.26, CI 1.31- 21.1).

The patients were divided into two groups, patients without previous history of prior PID or EP (group I) and patients with a history of PID or EP (group II). In *group II* a significantly higher proportion of Ct-IgG and Ct-hsp60 antibodies were seen compared to their controls. In group I there was no significant difference between patients and controls. There were no difference in human hsp60 antibodies either in group I or group II compared to their controls (Table 1).

Table 1. Antibodies in group I and II subdivided into patients and controls.

	Ct-IgG[1]	**Ct-IgA[1]**	**Ct-hsp60[2]**	**h-hsp60[3]**	**Cpn-IgG[1]**	**Ct-IgG x Ct-hsp60**
Group I, n=36						
Patients	8 (22)	3 (8)	7 (19)	4 (11)	23 (64)	3 (8)
Controls	8 (22)	0	12 (33)	5 (14)	21 (58)	3 (8)
p-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Group II n=19						
Patients	14 (74)	8 (42)	12 (63)	3 (16)	12 (63)	11 (58)
Controls	5 (26)	2 (11)	4 (21)	4 (21)	9 (47)	1 (5)
p-value	0.01	0.05	0.05	n.s.	n.s.	0.002

[1]MIF ≥ 64,[2]EIA-OD ≥ 0.6,[3]EIA-OD ≥0.4

When comparing antibodies to *C.trachomatis* between group I and II, patients in group II had significantly more Ct-IgG, 74 % vs. 22 % ($p < 0.001$) and Ct-hsp60, 63 % vs. 19 % ($p<0.003$). No difference was seen regarding human hsp60 (Table 2).

Table 2. Antibodies to *C.trachomatis* in group I and II.

	Group I n= 36 (%)	Group II n= 19 (%)	p-value
Ct-IgG (MIF ≥ 64)	8 (22)	14 (74)	< 0.001
Ct-IgA (MIF ≥ 64)	3 (8)	8 (43)	0.009
Ct-hsp60 (EIA-OD ≥ 0.6)	7 (19)	12 (63)	0.003
human-hsp60 (EIA-OD ≥ 0.4)	2 (6)	2 (11)	n.s.
Cpn-IgG (MIF ≥ 64)	23 (64)	12 (63)	n.s.
Ct-IgG x Ct-hsp60	3 (8)	11 (58)	0.0002

In group II the mean geometric titre of antibodies to *C.trachomatis* was higher than in group I ($p < 0.003$). No such difference was seen for human hsp60 antibodies to *C.trachomatis*.

In group II, antibody markers for EP were analyzed using a logistic regression model. Ct-IgG antibodies and Ct-hsp60 antibodies were both associated with EP, OR 7.84 CI 1.78-34.6, $p < 0.01$ and 7.00 CI 1.50-32.6, $p < 0.002$ respectively, but when including both of them in an adjusted logistic regression analysis none of them could independently predict EP (Table 3).

Table 3. Logistic analysis of different serological markers for ectopic pregnancy in group II including both patients and controls.

	Crude OR	p-value	Adjusted OR[1]	p-value
Ct-IgG (MIF ≥ 64)	7.84 (1.78-34.6)	0.01	5.23(0.96-28.4)	0.06
Ct- IgA (MIF ≥ 64)	6.18 (0.84-17.1)	0.08		
Ct- hsp60 (EIA-OD ≥ 0.6)	7.00 (1.50-32.6)	0.02	2.96 (0.54-2.79)	0.22
Human hsp60 (EIA-OD ≥ 0.4)	2.13 (0.14-31.6)	0.6		
Cpn- IgG (MIF ≥ 64)	1.90 (0.50-7.2)	0.35		
Ct-IgG x Ct- hsp60			34 (1.45-797)	0.035

[1]Ct-IgG (MIF) and Ct-hsp60 antibodies were included in this analysis

Comments

C.trachomatis DNA could not be detected in any of the tubal tissue specimens from our 55 patients with EP although highly sensitive diagnostic methods were used.

In a previous study of 33 patients with EP at this hospital, paraffin archived tubal tissue was used and tested for *C.trachomatis* DNA with an in house single step PCR method, this also gave a negative result (Osser and Persson, 1992). In the current study on fresh frozen material a commercial PCR kit (Cobas Amplicor) together with an in-house quantitative real-time PCR method was used, both with higher sensitivity than previoulsy. The quantitative real time PCR that was used was able to detect 1–2 organisms of *C.trachomatis* in each reaction as determined from standard curve with a cloned target sequence.

In spite of using methods with higher sensitivity and fresh frozen material we were unable to detect any *C.trachomatis* DNA in our patients. Other groups have also reported negative results, both in archive and fresh frozen material (Hartford *et al.*, 1987; Maccato *et al.*, 1992; Brunham *et al.*, 1992a). The groups that have found *C.trachomatis* DNA in patients with EP (five studies with detection ratio of 0.03-70%) have used both archive and fresh frozen material (Lan *et al.*, 1995; Gerard *et al.*, 1998; Toth *et al.*, 2000; Barlow *et al.*, 2001; Noguchi *et al.*, 2002) although the number of patients have been considerable lower than in the studies with negative result. There is no clear reason for these divergent results. Our study had sufficient power to detect at least 2 positive cases on basis of a mean detection rate of 26 % in the other studies.

It has previously been shown that chlamydial antibodies are associated with EP but only in a subgroup of patients with previous PID (Osser and Persson, 1992). In this study the subgroup with previous PID (group II) had a higher proportion of MIF chlamydial antibodies and hsp60 antibodies to *C.trachomatis* than age matched normal pregnant controls.

A combination of antibodies to *C.trachomatis* and chlamydial hsp60 was a predictor for EP both when all patients were compared with their controls and when patients in subgroup II were compared with their controls. This joint effect has also been reported by others (Claman *et al.*, 1997).

Increased proportions of antibodies to chlamydial hsp60 have been reported repeatedly in patients with tubal infertility and EP compared to controls (Brunham *et al.*, 1992a; Sziller *et al.*, 1998). It has been suggested that a persistent *C.trachomatis* infection of the Fallopian tubes (that would produce increased levels of Ct-hsp60) may lead to autoimmune responses directed against conserved epitopes in the human hsp60. Due to the homologous nature of hsps among species there may be a cross- reaction of the immune response to the chlamydial hsp60 with the human hsp60 of the host. Our findings do not support such a mechanism.

In our study prior EP /PID was associated with ct-hsp60 antibodies but not with human hsp60. Comparison of the antibody levels of chlamydial hsp60 and human hsp60 in our patients with EP showed no correlation.

Chronic persistent infection or reinfection by *C.trachomatis* is believed to play a key role in the process leading to Fallopian tubal damage (Patton *et al.*, 1990; Cappuccio *et al.*, 1994; Witkin, 2002). According to the divergence of results of earlier studies the prevalence of persistent infection in EP is unclear. A proportion of our patients (35%) had morphological changes to the Fallopian tube on the contra lateral side together with an increased proportion of antibodies to *C.trachomatis* suggesting *C.trachomatis* was a cause of tubal damage in these cases. Persistent infection could not be demonstrated in these patients or in any of the patients with EP. Therefore the tubal infection had probably resolved prior to the EP.

Patients with prior EP or PID had higher ratios of antibodies to *C.trachomatis* than both controls and patients without evidence of prior EP or PID.

We did not find any correlation in patients with EP with regard to Ct-hp60 and h-hsp60 antibodies. No association between patients with EP and antibodies to h-hsp60 was observed, thus, there is no evidence for cross reactions between Ct-hsp60 and h-hsp60. In patients with ectopic pregnancy persistent *C.trachomatis* infection in the Fallopian tubes was rare in our population.

Study III

Clinical manifestations and epidemiology of the new variant of Chlamydia trachomatis

In 2006 a new genetic variant of *C.trachomatis* (nvCT) was detected (Ripa and Nilsson, 2006). This new variant has a deletion of 377 base pairs in the plasmid. The function of the plasmid is largely unknown but recent studies showing the plasmid to be involved in virulence (Pickett *et al.*, 2005; O'Connell *et al.*, 2006; Carlson *et al.,* 2008, Li *et al*., 2008) implies an important role in infection.

In this study we compared clinical manifestations of infections with nvCT and wild type *C.trachomatis* (wtCT) in both men and women and estimated the frequency of ascending infections (PID) in women.

Results

Over the study period 8,365 patients were tested for *C.trachomatis*. The overall prevalence of *C.trachomatis* was 9.7 % (8.3% in women and 11.0 % in men) and 24 % were infected with nvCT. The ratio of nvCT in women was significantly higher than in men, 28.8 % and 20.3 % respectively ($p < 0.01$).

In total 1.878 patients were included, 626 cases and 1,252 controls, 345 *C.trachomatis* positive men (269 wtCT and 76 nvCT) and 281 *C.trachomatis* positive women (199 wtCT and 82nvCT).

Men with nvCT infection had less sexual contact abroad (p=0.003) and were less frequently smokers (<0.001) than men with wtCT infection. These differences remained significant after adjustment for age. No other differences in sexual lifestyle were noted in men. In women there were no observed differences regarding sexual lifestyle or smoking between nvCT and wtCT infected cases.

In women with nvCT painful urination (12.2 % vs. 25.8 %, p=0.02) and lower abdominal pain (13.4 % vs.27.8 %, p=0.02) was reported to a much lesser extent than in women with wtCT. This difference *remained statistically* significant after adjustment for age. Frequent urination, (post) coital bleeding, inter-menstrual bleeding and abnormal vaginal discharge were equally common (Table 4).

Table 4. Symptoms and clinical findings in women with *C.trachomatis* infection and negative controls.

Women	CT positive vs. CT negative			nvCT vs. wtCT		
	positive	negative	p-value	nvCT	wtCT	p-value
Symptoms (%)						
Painful urination	n=280 61 (21.8)	n=562 95 (16.9)	n.s.	n=82 10 (12.2)	n=198 51 (25.8)	0.02 *0.41 0.17-0.96
Frequent urination	n=280 63 (22.5)	n=562 88 (15.7)	0.02	n=82 16 (19.5)	n=198 47 (23.7)	n.s.
(Post) coital bleeding	n=280 44 (15.7)	n=562 54 (9.6)	0.01	n=82 13 (15.9)	n=198 31 (15.7)	n.s.
Intermenstrual bleeding	n=280 53 (18.9)	n=562 93 (16.5)	n.s	n=82 13 (15.9)	n=198 40 (20.2)	n.s
Abnormal vaginal discharge	n=280 111 (39.6)	n=562 172 (30.6)	0.01	n=82 27 (32.9)	n=198 84 (42.4)	n.s.
Lower abdominal pain	n=280 66 (23.6)	n=561 109 (19.4)	n.s.	n=82 11 (13.4)	n=198 55 (27.8)	0.02 *0.45 0.20-0.99
Clinical findings (%)						
Pathological cervical discharge	n=63 19 (30.2)	n=126 14 (11.1)	<0.01	n=18 6 (33.3)	n=45 13 (28.9)	n.s.
Diagnosis of cervicitis	n=63 17 (27.0)	n=126 15 (11.9)	0.02	n=18 5 (27.8)	n=45 12 (26.7)	n.s.
Diagnosis of urethritis	n=63 20 (31.7)	n=125 12 (9.6)	<0.001	n=18 2 (11.1)	n=45 18 (40.0)	[1]0.04
Diagnosis of bacterial vaginosis	n=63 12 (19.0)	n=126 22 (17.5)	n.s.	n=18 3 (16.7)	n=45 9 (20.0)	n.s.

CT = *C.trachomatis* ; n.s.=non significant; *results when adjusted for age by logistic regression analysis. [1] Fisher's exact test; [2] combined oral contraception, [3] intrauterine device, hormone implants and injections

Urethritis was less common among women with nvCT than in women with wtCT, 11.1 % vs. 40.0 %, p= 0.04, respectively. No differences were seen in the clinical findings of pathological cervical discharge, diagnosis of cervicitis or bacterial vaginosis (Table 4). Men with nvCT infection did not differ in either symptoms or clinical findings compared to men with wtCT infection.

From January 2007 through April 2008 3,063 cases of *C.trachomatis* infection occurred in Malmö, 1,762 women and 1,301 men. PID was diagnosed in 55 cases during this period. Ten cases were associated with wild type *C.trachomatis*. No case was associated with the new variant *C.trachomatis*. All cases except three were confirmed with laparoscopy and abdominal fluid analyses detected *C.trachomatis* in the abdomen at the time of diagnosis. The proportion of *C.trachomatis* positive PID cases among all PID cases was 18.9 % (10/53) and 29.0 % (9/31) when only including women below 35 years of age.

Among 1,762 *C.trachomatis* infected women detected during the study period ten women were diagnosed with PID. The proportion of *C.trachomatis* positive PID was 10/1,762 (0.6 %). *C.trachomatis* PID associated with wtCT infection was 0.8 % (10/1,307) and with nvCT infection 0 % (0/455) in these groups respectively. The observed difference did not reach statistical significance p=0.13 (Table 5).

Table 5. Pelvic inflammatory disease and prevalence of *C.trachomatis* in Malmo from January 2007 through April 2008.

	City of Malmoe		
	All (%)	Women (%)	Men (%)
CT	3063	1762	1301
nvCT	781	455	326
wtCT	2282	1307	975
nvCT ratio	781/3063 (25.5)	455/1762 (25.9)	326/1301 (25.1)
PID		53	
PID mean age/range		34.3/17-59	
CT PID, frequency		10/53 (18.9)	
CT PID, mean age/ range		24.3/17-40	
CT PID, age ≤35		8/10 (80.0)	
nvCT PID		0	
nvCT PID, mean age/ range		0	
wtCT PID		10	
wtCT PID mean age/ range		24.3/17-40	
Prevalence of CT PID		10/1762 (0.6)	
Prevalence of wtCT PID		10/1307 [1](0.8)	
Prevalence of nvCT PID		0/455 [1](0.0)	

CT = *C.trachomatis*; [1] Difference in prevalence of wtCT PID and nvCT PID non-significant, p=0.13 when using Chi square-test

Comments

In this study we report on the symptoms and clinical manifestations of nvCT infection in patients in a high risk population. We also assess the frequency of PID in wtCT and nvCT infected women in the general population in Malmö.

In one limited study reporting on the occurrence of the nvCT in a population of STI clinic visitors in Stockholm, Sweden no difference in symptoms of lower genital tract infection were seen when comparing patients with nvCT and wtCT infection (Marions *et al.*, 2008).

There is no certain knowledge of when the nvCT might have appeared in Sweden. We examined frozen archive material of 259 *C.trachomatis* positive strains, originally isolated by cell culture from 2000 and 2001 but no nvCT was

detected among these, which suggests that nvCT has been introduced or emerged in Sweden only recently and probably since 2001.

The role of the plasmid in *C.trachomatis* is unclear but recent studies have associated it with virulence in mice (Carlson et al., 2008). There are eight open reading frames (ORF) in the plasmid with a marked evolutionary preservation of its DNA (<1 % variability) which suggests key roles for at least some of the proteins coded for by the plasmid (Hatt *et al.*, 1988; Thomas and Clarke, 1992). The deletion situated in the first ORF may have caused changes in its biological properties and possibly in the clinical manifestations of the new variant host strain. Plasmid free strains are extremely rare and only a few plasmid free isolates have been described, found in isolated cases without secondary spread (Farencena *et al.*, 1997; Stothard *et al.*, 1998.

Comparisons of nvCT infected patients with wtCT infected patients regarding sexual lifestyle and smoking showed they were quite similar. The only differences noted were seen in men. Men with nvCT were less likely to smoke and less likely to have had a sexual contact abroad than men with wtCT infection. This was expected as the nvCT strains seems to be mostly confined to Sweden. A similar trend was noted for women but no significant difference was seen.

No differences regarding uro-genital symptoms or clinical findings were observed between men with nvCT or wtCT infection.

Women with nvCT infection had less uro-genital symptoms and clinical findings compared to women with wtCT infection. They reported painful urination only half as often as did wtCT infected women and urethritis was diagnosed at only 1/4 of the proportion diagnosed in wtCT infected women. The frequency of lower abdominal pain in nvCT infected women was half the frequency reported in wtCT infected women. In analysis of symptoms such as vaginal discharge, frequency of urination, post coital bleeding and inter menstrual bleeding there were no difference between nvCT and wtCT infected women.

A significant difference was seen in the clinical manifestations of the nvCT infection in women, mainly with respect to urethral inflammation. Both the symptom of painful urination and the diagnosis of urethritis reflect an inflammatory process in the urethra. The concurrent finding of a lower proportion of painful urination and urethritis in women corroborate each other.

From January 2007 to April 2008 all cases of PID were assessed. In women below 35 years of age the proportion of PID associated with *C.trachomatis* was 29 %. This is in the range of the proportion reported from this hospital and by others 20–30 years ago (Paavonen, 1980; Osser and Persson, 1982). The proportion of *C.trachomatis* associated PID in relation to all *C.trachomatis* positive cases was 0.6 % and to wtCT cases 0.8 %, which is much lower than has previously been estimated in the review by Paavonen and Eggert-Kruse, 1999. Some more recent studies have reported similar proportions to our results (van Valkengoed *et al.*, 2004; Low *et al.*, 2006).

Ten cases of wtCT associated PID were detected during the study period and no case of nvCT were found. The difference in proportions of nvCT PID (0 %) and wtCT PID (0.8 %) was not statistically significant and the numbers are too small to give definitive conclusion. It is not yet clear whether nvCT is as likely as wtCT to cause PID.

The clinical diagnosis of PID is difficult and has a low sensitivity, which may put some limitations to this study regarding PID. Several studies have reported sensitivities of 30–60 % for clinical diagnosis compared to laparoscopy. In this study 7/10 cases of *C.trachomatis* associated PID were confirmed by laparoscopy but the prevalence in the population remains uncertain.

The conclusions of this study were as follows: the new variant *C.trachomatis* was highly prevalent and is now endemic in the south of Sweden, representing approximately 25% of all infections detected. The mean age of patients with nvCT infection was slightly lower than in patients with wtCT infection although nvCT was distributed in all age groups. Men and women with nvCT or wtCT infection were similar with regard to sexual lifestyle parameters and

they had the same frequency of previous chlamydial infection. Asymptomatic infection seemed more common in women with nvCT infection than in women with wtCT infection. This would decrease the rate of detection for this organism, giving it a strong advantage to remain undetected and allowing more opportunities for transmission. Our findings suggest a difference in virulence between the nvCT and the wtCT.

Study IV

Mycoplasma genitalium is an independent risk factor for cervicits and is associated with pelvic inflammatory disease after termination of pregnancy

M.genitalium is associated with NGU in men (Horner *et al.*, 1993; Totten *et al.*, 2001) and is recognized as a sexually transmitted agent (Keane et al., 2000; Hjorth et al., 2006). In women manifestations of *M.genitalium* are less well documented. Results from studies on the association between *M.genitalium* and lower genital tract infection including cervicitis have been divergent (Manhart *et al.*, 2003; Anagrius *et al.*, 2005; Casin *et al.*, 2002; Huppert *et al.*, 2008).

Our study was performed to investigate the prevalence, clinical findings and complications of *M.genitalium* in women.

Results

In 7,598 women tested for *M.genitalium* and *C.trachomatis* the prevalence was 2.1 % and 2.6 % respectively. The population tested consisted of two groups, one group comprised women of various gynaecological symptoms seeking acute/semi acute care and the other group of women had presented for termination of pregnancy (TOP).

From the group of patients with acute gynaecological symptoms 108 *M.genitalium* positive patients, 143 *C.trachomatis* positive patients and 253 *M.genitalium* and *C.trachomatis* negative controls were included. There were no differences in mean age between patients with *M.genitalium* (25.6 years) and *C.trachomatis* (26.1 years). Younger women had higher proportions of both *M.genitalium* and *C.trachomatis*. The peak for *M.genitalium* infections was seen in women of 20–24 years and slightly earlier in *C.trachomatis* infections (below 19 years).

Cervicitis was significantly more common in patients with *M.genitalium* than in negative controls (21.6 % vs. 5.8 %, p <0.001). The proportion of PID in patients with *M.genitalium* was higher than in negative controls, 4.9 % and 1.0 % respectively, but this difference was not statistically significant (p=0.06).

The frequency of cervicitis in *C.trachomatis* positive patients was 35.7 % which was significantly higher than in negative controls (5.8 %, p <0.001) and also significantly higher than in *M.genitalium* positive patients (21.6 %, p=0.02). *C.trachomatis* positive patients had a PID frequency of 16.1 % which was significantly higher than both *M.genitalium* positive patients (4.9 %, p=0.01) and negative controls (1.0 %, p<0.001). Also in pathological discharge (44.4 % vs. 14.4%), cervical tenderness (37.8 % vs. 20.8 %), fever (17.8 % vs. 2.7 %) and abnormal vaginal discharge (54.3 % vs. 31 %) the frequency was significantly higher than in *M.genitalium* positive patients.

When using multivariate logistic regression analysis adjusting for age and *C.trachomatis* infection, *M.genitalium* was associated with cervicitis (OR 3.23 CI 1.71–6.10) but not with PID (OR 1.37 CI 0.41–4.57, p=0.61). *C.trachomatis* were strongly associated with both cervicitis (OR 7.05 CI 3.98–12.49, p <0.001) and PID (OR 8.75 CI 3.23–23.68, p <0.001) (Table 6)

Table 6. Association of significant variables in *M.genitalium* and *C.trachomatis* infection by logistic regression in women presenting with various gynaecological symptoms

	M. genitalium OR (95% CI)	p-value	***C.trachomatis*** OR (95% CI)	p-value
Clinical diagnose				
Cervicitis	3.23 (1.71-6.10)	<0.001	7.05 (3.98-12.49)	<0.001
Pelvic inflammatory disease	1.37 (0.41-4.57)	0.61	8.75 (3.23-23.68)	<0.001
Clinical sign				
Pathological vaginal wet smear	1.58 (0.82-3.02)	0.16	1.68 (0.94-3.01) 2.21(1.14-4.28)[1]	0.080.02[1]
Pathological vaginal discharge	0.45 (0.25-0.82)	0.008	2.21 (1.43-3.43)	<0.001
Friability	1.05 (0.62-4.71)	0.30	3.33 (1.40- 7.97)	0.007
Cervical tenderness	1.19 (0.67-2.11)	0.55	2.99 (1.84- 4.86)	<0.001
C-reactive protein >8	0.96 (0.42-2.16)	0.92	1.97 (1.40-7.94)	0.03
Fever (>38.0°C)	0.89 (0.27-2.88)	0.84	5.3 (2.12-12.90)	<0.001
Self reported symptom				
Abnormal vaginal discharge	1.11 (0.68-1.79)	0.68	3.0 (1.96-4.61)	<0.001
(Post) coital bleeding	1.64 (0.93-2.92) 1.82 (1.00-3.25)[2]	0.09 0.06[2]	2.51(1.51-4.16)	<0.001
Group of signs				
Pathological vaginal discharge Friability and (Post) coital bleeding	2.53 (1.00-6.48) 2.49 (1.03-15.16)[1]	0.05 0.04[1]	3.44 (1.79-5.16) 4.47 (2.09-.59)[1]	<0.001 <0.001[1]

NOTE. All variables adjusted for age, *M.genitalium* and *C.trachomatis* infection. OR indicates odd ratio and CI confidence interval. [1] Additionally adjusted for diagnosis of Candida vulvo- vaginitis and bacterial vaginosis. [2]Additionally adjusted for Candida vulvo-vaginitis

No clinical sign or self reported symptom was independently associated with *M.genitalium* but when using a group of signs (pathological vaginal discharge, friability and (post) coital bleeding) in multivariate analysis adjusting for age and *C.trachomatis* infection an association was seen (OR 2.53 CI 1.00–6.48, p=0.05).

In 2,081 patients presenting for termination of pregnancy (TOP) *M.genitialium* was detected in 52 (2.5 %) and *C.trachomatis* in 59 (2.8 %). Five patients were

infected with both *M.genitalium* and *C.trachomatis.* Overall 268 patients and controls were included. Postabortal infection (compatible with PID) was diagnosed in six of 49 (12.2 %) of the women with *M.genitalium* and in four of 168 (2.4 %) of the negative controls. This difference was statistically significant using Fishers' Exact test (p=0.02).

When adjusting for age and *C.trachomatis* infection in multivariate logistic regression analysis, *M.genitalium* was clearly associated with postabortal infection (OR 6.29 CI 1.56–25.2). No single postabortal infection was seen among *C.trachomatis* positive women (Table 7).

Table 7. Post abortal infection correlated to *M.genitalium* and *C.trachomatis* in 268 women undergoing termination of pregnancy using logistic regression

	M. genitalium				***C.trachomatis***		
Post abortal infection		**Controls**	**OR (95% CI)**	**p-value**		**OR (95 % CI)**	**p-value**
Medical and surgical termination	6/49 (12.2)	4/168 (2.4)	6.29 (1.56-25.2)	0.01	0/51 (0)	0.77 (0.11-6.71)	0.81
Surgical termination	3/15 (20.0)	3/70 (4.3)	5.78 (1.02-32.80)	0.05	0/27 (0)	0.00 (3.41-4.21)	1.00
Medical termination	3/33 (9.1)	1/98 (1.0)	8.68 (0.58-4.84)	0.12	0/24 (0)	5.48 (0.35-5.61)	0.22

NOTE. All variables are adjusted for age, *M.genitalium* and *C.trachomatis* infection. OR indicates odd ratio and CI confidence interval. Data is no. (%) of subjects.

Comments

During the study time two tests for detecting *C.trachomatis* were used, Cobas Amplicor and in the second half of the study m2000. Another study from this hospital showed Cobas Amplicor to be less sensitive than m2000 because the Cobas Amplicor procedure did not include a DNA purification step. It is likely that the PCR test for *M.genitalium* could be affected in a similar way. In order

to increase the sensitivity a semi nested setup was used. All strains were sequenced to avoid false positive results. Samples were pooled (5–8 samples in each pool). Pooling has been evaluated and results reported comparable with testing individual samples (Kacena *et al.*, 1998; Rours *et al.*, 2005).

The prevalence of *M.genitalium* was 2.1 % in women attending our gynaecological outpatient's service, a lower prevalence than in reports from STI clinics that have reported prevalence of 4–8% (Falk *et al.*, 2005; Edberg *et al.,* 2008; Moi *et al.*, 2009). In two population based studies the prevalence has been 0.8–2.3 % (Andersen *et al.*, 2007; Manhart *et al.*, 2007). *M.genitalium* was almost as prevalent as *C.trachomatis* (2.6 %) in this population.

In several studies cervicitis and symptoms of lower genital infection have been associated with *M.genitalium* (Manhart *et al.,* 2003; Anagrius *et al.,* 2005; Falk *et al.*, 2005) and in population based studies asymptomatic infection with *M.genitalium* has been shown (Andersen *et al.*, 2007; Manhart *et al.*, 2007). In our study *M.genitalium* was strongly associated with cervicitis. It is likely that both asymptomatic carriage and symptomatic infection may be seen in *M.genitalium* positive women.

M.genitalium is an independent predictor of cervicitis as cervicitis was strongly associated with *M.genitalium* infection even after adjustment for *C.trachomatis*.

There was an increased proportion of PID in patients with *M.genitalium* compared to negative controls but a statistically significant difference was not reached. This was probably due to a low number of PID cases in this group.

Clinical manifestations were similar between *M.genitalium* and *C.trachomatis* infected patients but less frequent in patients infected with *M.genitalium*. No clinical sign or symptom was independently associated with *M.genitalium* infection but when using a group of signs (pathological vaginal discharge, friability and (post) coital bleeding) a borderline association was seen. In contrast, all self reported symptoms and clinical signs were independently

associated with *C.trachomatis*. These findings suggest that *M.genitalium* is a less aggressive pathogen than *C.trachomatis* regarding clinical signs and symptoms.

In patients requesting TOP two studies beside ours have reported on the prevalence of *M.genitalium*. One study from Denmark showed a prevalence of 0.5 % (Baczynska *et al.*, 2008) and another from New Zealand 8.7 % (Lawton *et al.*, 2008) in women below 25 years of age. Complications were not assessed in these studies. In our study the prevalence was 2.5 % and the mean age in women were 25.6 years.

The proportion of complications associated with *C.trachomatis* following TOP is well documented (Rådberg and Hamberger, 1980; Westergaard *et al.*, 1982; Qvigstad *et al.*, 1982). The proportion of postabortal infections in *C.trachomatis* positive patients previously reported from this hospital have been more than 20 % (Osser and Persson, 1984). Similar results have been reported in other studies (Moller *et al.*, 1982; Westergaard *et al.*, 1982; Qvigstad *et al.*, 1982). All patients in this study were screened for *C.trachomatis* and if positive treated with doxycycline before TOP. In the present study no postabortal infection was detected in the *C.trachomatis* positive patients and treatment was therefore effective.

In patients that underwent TOP (involving both surgical and medical method), *M.genitalium* was strongly associated with postabortal infection both when using Fishers' exact test and when using logistic regression analysis adjusting for age and *C.trachomatis*. These results suggest that *M.genitalium* causes ascending infection in women undergoing TOP.

Among the controls, negative for both *M.genitalium* and *C.trachomatis* the proportion of postabortal infection was 2.4 % and among 2.5 % patients with *M.genitalium* 12.2 % developed postabortal infection which gives an estimated risk of 0.3 % for the total group. The proportion of postabortal infection associated with *M.genitalium* accounted for 12.5 % of the total proportion for non-infected patients. In our hospital all patients requesting TOP are screened

for *C.trachomatis* and a clinical sample is obtained. This sample could be used for analysing *M.genitalium* as well. Although no commercial test is yet available in house tests have been developed and scientifically evaluated in several laboratories. In many centres *M.genitalium* testing has been introduced for testing for NGU in men. If routine screening for *M.genitalium* in patients requesting TOP were introduced, PID associated with *M.genitalium* could possibly be prevented.

The present study has demonstrated that *M.genitalium* is associated with cervicitis and the observed association is independent of age and *C.trachomatis* infection.

The frequency of symptoms and clinical signs were higher in patients with *C.trachomatis* infection suggesting that *M.genitalium* is a less aggressive pathogen in terms of symptoms and clinical signs.

M.genitalium was clearly associated with PID in patients requesting TOP. In patients with acute gynaecological symptoms an increased proportion of PID was also seen although it did not reach statistical significance. Infection with *M.genitalium* is associated with clinical manifestations that are treatable and complications which are possibly preventable. To prevent PID, testing for *M.genitalium* should be considered before termination of pregnancy.

CONCLUSIONS

Study I

- The frequency of acute salpingitis reflected the prevalence of *N.gonorrhoeae* and *C.trachomatis* in our population
- It is likely that the steep increase in ectopic pregnancies in the mid of the 1980s and early 1990s was due to the steep increase of acute salpingitis in the 1970s.
- The frequencies of acute salpingitis and ectopic pregnancy may reflect the prevalence of preceding *C.trachomatis*, thus the prevalence of *C.trachomatis* did probably decline during the 1970s and early 1980s before diagnostic facilities became available.

Study II

- Patients with prior pelvic inflammatory disease or prior ectopic pregnancy had higher proportions of antibodies to Ct-IgG and Ct-hsp60 than both controls and patients without evidence of prior pelvic inflammatory disease or ectopic pregnancy
- No evidence of persistent infection of C.trachomatis was found in patients with ectopic pregnancy
- No support was found for an autoimmune response to human hsp60.

Study III

- The new variant of *C.trachomatis* is endemic in our population in the south of Sweden and represents about 25 % of all *C.trachomatis* infections detected

- Men and women with new variant *C.trachomatis* infection are slightly younger than those with wild type *C.trachomatis* infection however like wild type *C.trachomatis* infection, it is found in all age groups
- Men and women with new variant *C.trachomatis* and wild type *C.trachomatis* infections are similar with respect to sexual lifestyle parameters and have similar frequencies of previous chlamydial infection
- No case of pelvic inflammatory disease associated with new variant *C.trachomatis* was detected in this study, thus pelvic inflammatory disease associated with new variant *C.trachomatis* is rare in our population
- Asymptomatic infections were more common in women with nvCT infections and a difference in virulence between the new variant *C.trachomatis* and the wild type variant *C.trachomatis* is suggested.

Study IV

- *M.genitalium* is independently associated with cervicitis
- *M.genitalium* may be a less aggressive pathogen than *C.trachomatis*
- *M.genitalium* is clearly associated with postabortal infection
- *M.genitalium* is associated with clinical manifestations that are treatable and complications that are possibly preventable.

SUMMARY

C.trachomatis infection is the most common bacterial STI in the world and a major public health problem. Complications such as PID, EP and TFI have a large impact on women's reproductive health. The majority of infections with *C.trachomatis* are asymptomatic. Prevention of transmission in the population is difficult, depending on large scale screening programs and sexual health units offering individuals testing and treatment. The rate of complications following a *C.trachomatis* infection is of crucial importance when evaluating cost-effectiveness in screening programs.

M.genitalium infection has recently been associated with urethritis in men and lower genital tract infection, including cervicitis in women. A few studies have reported on *M.genitalium* in PID and TFI but a broader role in these conditions is still unclear.

The aim of this thesis was to elucidate developments in epidemiology, clinical manifestations and complications in *C.trachomatis* and *M.genitalium* infection with special reference to women.

Patients with a diagnosis of either ectopic pregnancy, non-gonococcal salpingitis, or gonococcal salpingitis were studied during 27 years from 1969 to 1996. The frequencies of these conditions were observed and correlated to the prevalence of *N.gonorrhoeae* and *C.trachomatis.* The mean age of patients with non-gonococcal salpingitis was in the range of five to ten years lower than the mean age of patients with EP. The annual number of all cases of acute salpingitis as well as the number of detected cases of *N.gonorrhoeae* peaked in the early 1970s and then declined over the study time showing the same trend. The peak of salpingitis cases in the 1970s was followed by a peak of EPs in the 1980s. When superimposing the peak of salpingitis cases over the peak of EP cases, the two curves had a similar form and spanned a similar time period.

The major increase in EP occurred in women who were about 20 years of age during the early 1970s.

After the peak of salpingitis cases in the 1970s the number declined over 20 years to one tenth of the highest number. The same trend was seen for gonococcal infections and in *C.trachomatis* infections (post 1984) in women in the same area. The frequency of acute salpingitis reflected the prevalence of *N.gonorrhoeae* and *C.trachomatis* in our population. The frequency of acute salpingitis and ectopic pregnancy might be used to estimate the occurrence of *C.trachomatis* during the 1970s and early 1980s before diagnostic facilities became available. It is likely that the steep increase in ectopic pregnancies in the middle of the 1980s and early 1990s was due to the steep increase of acute salpingitis in the 1970s.

Persistent *C.trachomatis* infection at the time of the ectopic pregnancy has been reported but not generally confirmed. In patients with advanced PID and tubal damage, circulating antibodies to the c-hsp60 protein has been suggested to cross react with a human hsp60 (h-hsp60) and thus initiate or enhance the scarring process. We explored the possible presence of *C.trachomatis* DNA at the time of EP using freshly frozen tubal tissue and analyzed for *C.trachomatis* by PCR and a highly sensitive real time PCR test in patients with EP. The correlation between c-hsp60 antibodies and h-hsp60 were also investigated. Chlamydial DNA was not detected in any of the tubal specimens. When all patients with EP and their normal pregnant controls were compared, no single antibody could predict EP, but when combining IgG antibodies to *C.trachomatis* and chlamydial hsp60 a strong association with EP was found. The patients were divided into two groups, patients without previous history of prior PID or EP (group I) and patients with a history of PID or EP (group II). In group II a significantly higher rate of Ct-IgG and Ct-hsp60 antibodies were seen compared to their controls. In group I there was no significant difference between patients and controls. There were no differences in human hsp60 antibodies, either in group I or group II compared to their controls. In group II Ct-IgG antibodies were more common in patients than in controls. Specific antibodies to hsp60 of chlamydial origin were strongly associated to ectopic pregnancy but not antibodies of human origin. No correlation between

antibodies to ct-hsp60 and h-hsp60 were seen. We did not find any support for cross reaction between chlamydial hsp60 and human hsp60 in our patients with ectopic pregnancy and no evidence of persistent infection of *C.trachomatis* in the Fallopian tubes at the time of ectopic pregnancy.

In 2006 a new genetic variant of *C.trachomatis* with a deletion of 377 bps in the plasmid was discovered in Sweden. We studied the epidemiology and compared sexual lifestyle and clinical manifestations between nvCT and wtCT in a high risk population and assessed the rate of ascending infection in women resulting in PID in the general population of Malmö. Over the study period 8,365 patients were tested for *C.trachomatis*. The prevalence of *C.trachomatis* was 9.7 %, 8.3 % in women and 11.0 % in men. A proportion of 24 % were infected with nvCT. The ratio of nvCT in women was significantly higher than in men.

When comparing nvCT infected patients with wtCT infected patients with regard to sexual lifestyle and smoking they were quite similar. Men with nvCT infection had less sexual contact abroad and were less frequently smokers than men with wtCT infection. In women there were no observed differences regarding sexual lifestyle or smoking between nvCT and wtCT infected cases. In women with nvCT, painful urination and lower abdominal pain was reported to a much lesser extent than in women with wtCT. Urethritis was less common among women with nvCT than in women with wtCT.

In women below 35 years of age the proportion of PID associated with *C.trachomatis* was 29 %. The proportion of *C.trachomatis* associated PID in relation to all *C.trachomatis* positive cases was 0.6 % and to wtCT 0.8 %. Ten cases of wtCT associated PID were detected during the study period, no case of nvCT was found. It is not yet clear whether nvCT is as likely as wtCT to cause PID. Asymptomatic infection seems more common in women with nvCT infection than in women with wtCT infection. These findings suggest a difference in virulence between the nvCT and the wtCT.

The epidemiology, clinical manifestations and complications of *M.genitalium* were assessed. In women tested for *M.genitalium* and *C.trachomatis* the prevalence was 2.1 % and 2.6 % respectively. The tested population consisted of two groups, one group with women of various gynaecological symptoms seeking acute/semi acute care and one group of women presenting for termination of pregnancy (TOP). Cervicitis was significantly more common in patients with *M.genitalium* than in negative controls. The proportion of PID in patients with *M.genitalium* was higher than in negative controls, but this difference was not statistically significant. Clinical manifestations were similar between *M.genitalium* and *C.trachomatis* but less frequent in patients infected with *M.genitalium*. These findings suggest that *M.genitalium* is a less aggressive pathogen than *C.trachomatis* in respect of clinical signs and symptoms.

Postabortal infection (compatible with PID) was diagnosed 12.2 % of the women with *M.genitalium* and in 2.4 % of the negative controls. When adjusting for age and *C.trachomatis* infection in multivariate logistic regression analysis, *M.genitalium* was clearly associated with postabortal infection.

This thesis has described the epidemiology and clinical manifestations of two important agents in STI, *C.trachomatis* and *M.genitalium*. The possible clinical use and application of conclusions drawn from these studies are presented below:

Epidemiological data over long time is a useful tool to evaluate the correlation between prevalence of a pathogen and complications associated with the pathogen. The frequency of salpingitis and ectopic pregnancy can be used as epidemiological markers for *C.trachomatis*.

The frequency of PID following a positive *C.trachomatis* test is important when evaluating the cost-effectiveness of screening programs. We have assessed the frequency of PID and found it to be lower than previously reported in cost-effective analyses.

Asymptomatic infection with nvCT is more common than with wtCT, which may further emphasize the need for screening. During the period of study the proportion of nvCT infections declined from 30 % to 15 % in women, beginning when detection of the nvCT started. The selective decline of nvCT in relation to wtCT can best be explained as a result of an intervention that was non-existent in 2006.

M.genitalium was associated with cervicitis among women presenting at an outpatients service at our gynaecological department. This knowledge might help to make decisions on whether *M.genitalium* should be tested for or not in this population.

A strong association was seen in *M.genitalium* and postabortal infection. Screening for *M.genitalium* in women requesting TOP might be considered.

M.genitalium was almost as common as *C.trachomatis* in our population. Infections with *M.genitalium* seem to be less aggressive than infections of *C.trachomatis* in terms of symptoms, clinical finding and complications. This has implications for future management and possible screening strategies.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Bakgrund

Genitala infektioner hos kvinnan är infektioner som inte bara behöver uppmärksamhet när de behandlas, de kan också utgöra ett hot mot kvinnans reproduktiva hälsa längre fram i livet. Många av infektionerna som drabbar kvinnans underliv är sexuellt överförbara. Bland de vanligaste sexuellt överförbara bakterierna är *Chlamydia trachomatis* (*C.trachomatis*) eller klamydiabakterien och *Neisseria gonorrhoeae* (*N.gonorrhoeae*) eller gonokocken.

C.trachomatis är den vanligaste sexuellt överförbara bakterien i världen med ca 92 miljoner nya fall/år globalt. I Sverige diagnostiserades 42 001 fall under 2008. Komplikationer i form av salpingit (äggledarinflammation), ektopisk graviditet (utomkvedshavandeskap) och infertilitet (ofruktsamhet) har stor inverkan på kvinnans reproduktiva hälsa.

Medan gonorré har minskat i många delar av världen kvarstår klamydia som ett allt mer växande problem. Infektioner med klamydia är särskilt svåra att begränsa då majoriteten av infektionerna löper utan eller endast med svaga symptom. Detta för med sig att en andel av befolkningen (de som inte testats och behandlats) utgör en reservoar för spridning av klamydia bakterien.

Begränsning av spridningen av klamydiainfektioner i befolkningen är beroende av storskaliga provtagningsprogram (screening) och hälso- och sjukvårdsenheter som ger möjlighet för individen att testa sig och få behandling. Kunskap om infektionen och dess komplikationsfrekvens är nödvändigt för att utvärdera kostnadseffektivitet vid olika screening strategier.

År 2006 upptäcktes en ny genetisk variant av *C.trachomatis* (nvCT). Flera av de vanligaste kommersiella analysmetoderna kunde inte upptäcka denna nya variant varför den snabbt kunde sprida sig i befolkningen, men även i landsting

som använde en analysmetod som kunde upptäcka nvCT var spridningen stor. En möjlig orsak till den snabba spridningen kan vara att en infektion med nvCT ger mindre symptom än den ”vilda” typen, wild type *C.trachomatis* (wtCT).

Mycoplasma genitalium (M.genitalium) är en bakterie som upptäcktes på 1980-talet men som först på senare tid har kunnat studeras i större sammanhang. Den har liksom *C.trachomatis* visat sig vara en betydelsefull sjukdomsframkallande mikroorganism med samma typ av smittspridning och symptom. *M.genitalium* är förknippad med urinvägsinfektion hos män och nedre genital infektion hos kvinnan. Ett fåtal studier har visat *M.genitalium* som en möjlig orsak vid salpingit och äggledarberoende infertilitet, men den vidare betydelsen vid dessa tillstånd är fortfarande oklar.

Den högsta förekomsten av klamydiainfektioner finns hos unga sexuellt aktiva män och kvinnor under 30 år. Komplikationer i form av ektopisk graviditet och infertilitet relaterad till äggledarskada upptäcks inte förrän långt senare. Det återstår att se om *M.genitalium* kommer att följa samma mönster som *C.trachomatis* i detta avseende.

Det övergripande syftet med denna avhandling är att utvärdera och belysa epidemiologisk utveckling, kliniska manifestationer och komplikationer vid infektioner med *C.trachomatis* och *M.genitalium* hos kvinnan.

Delarbete 1

Mellan åren 1969 och 1996 inkluderades sammanlagt 5 233 kvinnor som fått någon av diagnoserna gonokocksalpingit, non-gonokocksalpingit eller ektopisk graviditet. för att värdera om frekvensen av akuta salpingiter och ektopiska graviditeter indirekt kunde spegla det epidemiologiska mönstret för *C.trachomatis* innan detta var känt.

Utvecklingen av akuta salpingiter och förekomsten av *N.gonorrhoeae* visade samma mönster under studietiden. Antalet fall av både akut salpingit och *N.gonorrhoeae* ökade kraftigt under 1970-talet för att sedan sjunka och plana ut

på en låg nivå. Gonokocksalpingiterna utgjorde 21 % av det totala antalet akuta salpingiter. En betydande andel av non-gonokocksalpingiterna kan associeras till *C.trachomatis*. Från 1980 och framåt utgjorde *C.trachomatis* associerade salpingiter bland kvinnor under 25 år, 25–40 % i vårt material

Ektopiska graviditeter visade ett annat mönster med en kontinuerlig uppgång från 1970-talet fram till 1994 och sedan en nedgång. Uppgången av salpingitfall på 1970-talet följdes av en uppgång av ektopiska graviditeter under 1980-talet. När toppen av salpingitfall överlagrades på toppen av ektopiska graviditeter visade de två kurvorna samma form och omfattade samma tidsintervall. Den största ökningen av ektopiska graviditeter skedde bland kvinnor som var ca 20 år gamla under tidigt 1970-tal.

Frekvensen av akuta salpingiter speglade förekomsten av *N.gonorrhoeae* och *C.trachomatis* i vår population. Det är troligt att den kraftiga ökningen av ektopiska graviditeter i mitten av 1980-talet och tidigt 1990-tal berodde på den kraftiga ökningen av akuta salpingiter under 1970-talet. Frekvensen av akut salpingit och ektopiska graviditeter speglar troligen förekomsten av tidigare C.*trachomatis* infektioner och bör kunna användas för att uppskatta utbredningen av *C.trachomatis* infektioner under 1970-talet och tidigt 1980-tal innan diagnostiska faciliteter var tillgängliga.

Delarbete 2

C.trachomatis patogenes (sjukdomsframkallande process) vid ärrbildning i äggledarna är inte klarlagd. En persisterande (kvardröjande) latent infektion skulle kunna väcka ett immunförsvar med autoimmuna inslag. Forskning har hittills visat motstridiga resultat. Det har förslagits att en korsreaktion mellan 'humant heatshock protein 60' (h-hsp60), ett immunförsvarsprotein, och ”*C.trachomatis* heat shock protein 60” (Ct-hsp60) skulle kunna initiera eller förvärra ärrbildningsprocessen i äggledaren. Syftet med detta delarbete var att, med mycket känslig analysteknik (realtids PCR) och färskfrusna vävnadsprover, undersöka om *C.trachomatis* DNA fanns kvar i äggledarna hos

patienter med ektopisk graviditet som ett tecken på persisterande infektion, att undersöka olika antikroppar involverade i patogenesen vid äggledarskada samt särskilt undersöka om det fanns tecken till korsreaktion mellan h-hsp60 och Ct-hsp60 hos dessa patienter.

Från 55 patienter med ektopisk graviditet togs blodprover och vävnadsprov från äggledaren. Blodproverna jämfördes med blodprover från 55 kontroller med normal graviditet. *C.trachomatis* DNA kunde inte påvisas i något vävnadsprov från äggledarna hos patienterna med ektopisk graviditet. När alla patienter med ektopisk graviditet och deras kontroller med normal graviditet jämfördes kunde ingen enskild antikropp prediktera för ektopisk graviditet men när IgG antikroppar mot *C.trachomatis* och Ct-hsp60 kombinerades fanns en klar association till ektopisk graviditet.

Patienterna med ektopisk graviditet delades upp i två grupper, patienter utan tecken eller anamnes på tidigare salpingit eller ektopisk graviditet (grupp I) och patienter med anamnes eller tecken på tidigare salpingit eller ektopisk graviditet (grupp II). Hos patienterna i grupp II fanns en högre andel av Ct-IgG och Ct-hsp60 antikroppar än hos deras kontroller, denna skillnad fanns inte för grupp I och deras kontroller. Det fanns heller ingen skillnad i andelen h-hsp60 mellan patienter och kontroller, varken i grupp I eller grupp II.

Antikroppar mot Ct-hsp60 var associerade med ektopisk graviditet men ingen association fanns mellan antikroppar mot humant hsp60 och ektopisk graviditet. Ingen korrelation fanns mellan Ct-hsp60 antikroppar och humant hsp60 antikroppar hos patienter med ektopisk graviditet. I den här studien fann vi inget stöd för korsreaktion mellan Ct-hsp60 och humant hsp60 bland patienterna med ektopisk graviditet och ingen indikation på att persisterande *C.trachomatis* infektion fanns i äggledarna vid tiden för ektopisk graviditet.

Delarbete 3

År 2006 upptäcktes en ny genetisk variant av *C.trachomatis* (nvCT) i Sverige. Denna nya variant har bl.a. en 377 baspar lång skada i plasmidens DNA.

Plasmidens funktion är till stora delar okänd men det faktum att den evolutionära utvecklingen har bibehållit plasmiden samt att plasmidlösa klamydiabakterier är extremt ovanliga och inte ger upphov till större spridning antyder en biologiskt viktig roll. I det här arbetet jämförde vi sexuell livsstil och kliniska manifestationer mellan nvCT infektion och infektion med wtCT hos både män och kvinnor i en högrisk population av besökare på en sexhälsomottagning. Hos kvinnor undersökte vi också uppåtstigande infektioner (salpingiter) diagnostiserade på kvinnokliniken i Malmö.

Under studietiden testades 8.365 patienter för *C.trachomatis*. Förekomsten av C.trachomatis var 9,7 % (8,3 % för kvinnor och 11,0 % för män). Andelen nvCT av alla positiva prov utgjorde 24 %. Det var en signifikant skillnad mellan män och kvinnor (kvinnor 28,8 %, män 20,3 %).

När patienter med nvCt och wtCt jämfördes gällande sexuell livsstil fanns ingen större skillnad. Män med nvCT infektion rökte i mindre utsträckning och uppgav i mindre utsträckning sexuella kontakter utomlands. Denna skillnad syntes inte hos kvinnorna. Det fanns ingen skillnad i symptom eller kliniska manifestationer mellan nvCT och wtCT infektion hos männen.

Kvinnor med nvCT infektion rapporterade smärtsam vattenkastning och lågt sittande buksmärta i betydligt mindre omfattning än kvinnor med wtCT infektion. Diagnosen uretrit (inflammation i urinröret) var mindre vanlig bland kvinnor med nvCT infektion än med wtCT infektion.

Hos kvinnor under 35 år var 29 % av de diagnostiserade salpingiterna associerade med *C.trachomatis.* Proportionen av diagnostiserade *C.trachomatis* salpingiter av alla *C.trachomatis* positiva fall var 0,6 % och av wtCT 0,8 %.

Tio fall av *C.trachomatis* salpingiter upptäcktes under studietiden, de var alla wtCT, inget fall var nvCT. Denna skillnad var dock inte statistisk signifikant, sannolikt beroende på det låga antalet salpingiter. Det är fortfarande inte klart om nvCT orsakar salpingit i lika stor omfattning som wtCT.

Asymptomatisk infektion förefaller mer vanlig hos kvinnor med nvCT infektion än hos kvinnor med wtCT infektion. Våra resultat antyder att det finns en skillnad i virulens (sjukdomsalstrande förmåga) mellan nvCT och wtCT.

Delarbete 4

M.genitalium är kopplad till non-gonocock uretrit (NGU) hos män och är i likhet med *C.trachomatis* en sexuellt överförbar infektion. *M.genitalium* manifestationer hos kvinnor har inte dokumenterats i lika stor utsträckning. Resultat från studier gällande samband mellan cervicit (livmoderhalsinflammation) och *M.genitalium* har visat på olika resultat. Syftet med den här studien var att undersöka förekomst, kliniska fynd och komplikationer för *M.genitalium* hos kvinnan. Bland 7.598 kvinnor var 2,1 % *M.genitalium* positiva och 2,6 % *C.trachomatis* positiva. Den testade populationen bestod av två patientgrupper, en grupp som sökte till kvinnoklinikens akutmottagning med olika gynekologiska besvär och en grupp som sökte för legalt avbrytande av graviditet. Patienternas symptom och kliniska manifestationer jämfördes inbördes samt med kontroller som inte hade vare sig *M.genitalium* eller *C.trachomatis*. Gällande patienterna från akutmottagningen fanns ett klart samband mellan *M.genitalium* och cervicit. Andelen salpingiter var högre hos patienter med *M.genitalium* än hos kontroller men skillnaden var inte statistiskt signifikant.

De kliniska manifestationerna av *M.genitalium* och *C.trachomatis* var likartade men mycket mer frekventa hos patienter med *C.trachomatis* infektion. Dessa resultat antyder att *M.genitalium* är en mindre aggressiv patogen än *C.trachomatis* avseende symptom och kliniska fynd.

Bland kvinnor som sökte för avbrytande av graviditet fanns ett starkt samband mellan M.*genitalium* infektion och komplikation efter avbrytandet i form av uppåtstigande infektion i livmodern och äggledarna.

Avhandlingens nyhetsvärde och kliniska användbarhet

Avhandlingen belyser och beskriver epidemiologi och kliniska manifestationer för två viktiga sexuellt överförbara infektioner, *C.trachomatis* och *M.genitalium*.

Epidemiologiska data över lång tid är ett användbart verktyg för att utvärdera relationen mellan förekomst av en patogen och dess komplikationer. Vi har belyst den epidemiologiska utvecklingen för *C.trachomatis* och *N.gonorrhoeae* under en längre tid i vår population. Frekvensen av salpingiter och ektopiska graviditeter kan användas som epidemiologiska markörer för *C.trachomatis.*

Kunskap om frekvensen av komplikationer såsom salpingit och ektopisk graviditet är avgörande för att kunna värdera kostnadseffektiviteten för olika screening strategier. Vi har utvärderat den diagnostiserade salpingitfrekvensen bland *C.trachomatis* positiva kvinnor och funnit att den är lägre än man tidigare antagit. Sannolikt finns här ett mörkertal, men frekvensen av ektopiska graviditeter kan vägas in och medverka till förbättrad uppskattning av *C.trachomatis* komplikationer.

Persisterande infektion med *C.trachomatis* och dess roll för ärrbildning av äggledarna är ett mycket diskuterat problem. Vi har tillfört kunskap genom att visa att trots användande av de mest känsliga analysmetoderna har vi inte funnit några tecken på att *C.trachomatis* infektionen är kvar i äggledarna vid tidpunkten för ektopisk graviditet. Detta har betydelse för handläggningen av dessa patienter.

Avhandlingen har visat att asymptomatisk infektion är ännu vanligare vid infektion med nvCT än med wtCT vilket ytterligare understryker behovet av screening för *C.trachomatis*. Under observationstiden minskade andelen nvCT från ca 30 % till 15 % i populationen, minskningen skedde under samma tid som nvCT började upptäckas och behandlas, vilket visar att denna ”nya” provtagningsinsats påverkade förekomsten av nvCT.

M.genitalium var kopplat till cervicit bland kvinnor som sökte till akutmottagningen vid kvinnokliniken i Malmö. Dessa resultat kan vägleda vid övervägande om provtagning för *M.genitalium* i denna population.

I avhandlingen beskrivs för första gången sambandet mellan *M.genitalium* infektion och komplikation i form av uppåtstigande infektion efter avbrytande av graviditet. Sambandet var relativt starkt och screening för *M.genitalium* hos dessa patienter kan övervägas.

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