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The frequency and the role of *Chlamydia trachomatis* infection in premature labour - A multicentre epidemiological study in Hungary

PhD Thesis

BY

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CONTENTS

ABBREVIATIONS

CB	Confidence bounds
DNA	Deoxyribonucleic acid
EB	Elementary body
ELISA	Enzyme-linked immunosorbent assay
HIV	Human immunodeficiency virus
HUF	Hungarian forint
Ig	Immunoglobulin
IUGR	Intrauterine Growth Retardation
IVF	In vitro fertilisation
LCR	Ligase chain reaction
LGV	Lymphogranuloma venereum
MOMP	Major outer membrane protein
NICU	Neonatal intensive care unit
NGU	Non-gonococcal urethritis
PCR	Polimerase chain reaction
PGU	Post-gonococcal urethritis
PID	Pelvic inflammatory disease
PROM	Premature rupture of membranes
PUA	Premature uterine activity
RB	Reticulate body
STD	Sexually transmitted diseases
SPSS	Statistical Package for the Social Sciences
TWAR	Taiwan acute respiratory agent
UK	United Kingdom
USA	United States of America

SUMMARY

A multicentre survey was carried out in order to determine the prevalence and risk factors of *Chlamydia trachomatis* infection in the pregnant population in Hungary and the importance of *Chlamydia trachomatis* infection in the aetiology of premature labour was examined and to assess the cost-effectiveness of identifying and treating asymptomatic female carriers of *Chlamydia trachomatis*.

The nucleic acid hybridization method (PACE 2 Gen-Probe) was applied for the examination of *Chlamydia trachomatis*. An interviewer-administered standardized questionnaire was completed. Data management was carried out with self-developed software, and statistical analyses with SPSS software package. To compare groups Chi-square tests and Student's t-tests were employed. For the examination of trends in the monthly frequencies of infection, the moving average and linear regression methods were used. To obtain an overview of the risk, multiple logistic regression analysis was performed. A probability level of $p<0.05$ was considered statistically significant.

The overall average prevalence of *Chlamydia trachomatis* cases during an eighteen-month survey on 6161 pregnant women was 5.9%. There were significant differences in the proportions of chlamydial infection in the centres, and also in the different age groups and the different family status groups. The perinatal mortality rate exhibited a significantly higher prevalence (8.5%) among *Chlamydia trachomatis*-positive than among negative patients (2.0%). In the anamnestic histories of *Chlamydia trachomatis*-infected patients, the frequency of premature uterine activity was 8.1%, in contrast with 5.2% in the non-infected group ($p<0.05$).

In our analysis of cost-effectiveness, the cost of the sequelae of untreated *Chlamydia trachomatis* infections is slightly less than the costs of using the ELISA method for screening of the age group between 15-19 years. Since the majority of the *Chlamydia trachomatis*-infected cases were asymptomatic, we suggest to use the ELISA method for screening of all women with evidence of mucopurulent cervicitis and all women of the age group between 15-19 years. We also recommend testing women between 20 to 24 years of age who have not consistently used barrier contraception and testing the pregnant population before delivery and an abortion procedure and after spontaneous abortion. Furthermore, cases with a poor obstetric history and/or socially high-risk patients should be screened for *Chlamydia trachomatis* infection, and in positive cases treatment is recommended.

PUBLICATIONS OF THE AUTHOR

This thesis is based on the following papers:

I Nyári T, Deák J, Nagy E, Veréb I, Kovács L, Mészáros Gy, Orvos H and Berbik I: Epidemiological study of *Chlamydia trachomatis* infection in pregnant women in Hungary. *Sex Transm Inf* 1998;**74**:213-15.

II Deák J, Nagy E, Mészáros Gy, Kovács L, Nyári T, Berbik I. Prevalence of *Chlamydia trachomatis* in low risk gravid population. *Sex Transm Dis*. 1997;**24**:538-42.

III Kovács L, Nagy E, Berbik I, Mészáros Gy, Deák J, Nyári T. The frequency and the role of *Chlamydia trachomatis* infection in premature labor. *Int J Gynec Obst*. (In press).

Publications related to the thesis:

IV Nyári T, Deák J, Veréb I, Nagy E, Kovács L, Mészáros Gy, Orvos H. Epidemiological study of *Chlamydia trachomatis* infection in pregnant women in Hungary. *Magyar Venerológiai Archivum*. (In press, in Hungarian).

V Deák J, Nagy E, Veréb I, Mészáros Gy, Kovács L, Nyári T, Berbik I. Diagnostical methods for screening *Chlamydia trachomatis* infection in low risk population. *Klin-Kíserl Lab Med*. 1997;**24**:35-44, (In Hungarian).

VI Kovács L, Nagy E, Berbik I, Mészáros Gy, Deák J, Nyári T. The frequency and the role of *Chlamydia trachomatis* infection in premature labor - A multicentre study in Hungary. *Magyar Nőorvosok Lapja*. 1996;**59**:353-59, (In Hungarian).

1. INTRODUCTION

1.1. Historical perspective

The eye disease trachoma, described five thousand years ago in China, is the earliest known human disease entity caused by *Chlamydiae*. The Ebers papyrus (c.1500 bc) refers to an affliction that was almost certainly trachoma and to its alleviation with copper salts, a form of treatment that persisted well into the twentieth century. Trachoma was known by the ancient Greeks and Romans. Accounts of military campaigns from the Crusades to the Napoleonic wars refer to severe ophthalmic infections acquired in the Middle East, and it is quite possible that trachoma was disseminated to Europe and elsewhere by returning soldiers. The disease did not spread to the general population and eventually disappeared from most of Europe, even before the introduction of specific treatment.

In 1907, Halberstaedter and von Prowazek, working in Java, described the transmission of trachoma from humans to orangutans by experimental infection [55]. These newly discovered organisms were called Chlamydozoa (from the Greek chlamys, a mantle). Similar inclusions were described subsequently in the conjunctival cells of babies with non-gonococcal ophthalmia neonatorum in cervical epithelium from some of their mothers and in urethral epithelium from male patients with non-gonococcal urethritis [38]. Thus, trachoma, inclusion conjunctivitis of the neonate, and infection of the adult genital tract were caused by similar infective agents, all of which were capable of passing filters that otherwise generally retained bacteria. This latter property, coupled with the inability of these agents to grow in artificial media, led to the erroneous belief that they were "viruses".

In 1929-30, Levinthal, Coles and Lillie independently described minute basophilic particles in the blood and tissue from birds and human patients with psittacosis. Bedson et al. soon proved their etiologic relationship with psittacosis and subsequently defined the characteristic chlamydial developmental cycle. With great foresight, Bedson referred to this agent as "an obligate intracellular parasite with bacterial affinities", a concept not generally accepted for another 30 years [4, 5]. In 1934, Thygeson drew attention to the similarities between the agents of trachoma, of inclusion conjunctivitis and of psittacosis [170]. The related agent of

lymphogranuloma venereum (LGV) was propagated first in monkey brain in 1931 [124]. The agent of LGV was isolated by Macchiavello in yolk sac in 1944 [93].

In 1950, Harkness, a British venereologist, published a monograph on non-gonococcal urethritis, in which he reported that he had identified chlamydial inclusions in urethral specimens. The search for inclusions was laborious and insensitive procedure [166].

The first isolations of *Chlamydia trachomatis* (*C. trachomatis*) from a neonate with inclusion conjunctivitis and from the mother of another such infant were reported by Jones et al. [73]. Furthermore, Jones drew attention to the close association between adult genital infection and adult inclusion conjunctivitis [75].

In 1965 a cell culture method was devised which was both sensitive and easier to perform than the yolk sac technique [47], and with this method and an immunofluorescent test reported in 1970 the modern era of the study of genital chlamydial infection began. In 1977, a causal role for *Chlamydia* in acute epididymitis was shown, and in the same year, came the first of many studies, which established chlamydial infection as a major cause of salpingitis and subsequent infertility [8,129]. During the 1970's, the spectrum of neonatal chlamydial infection expanded to include a pneumonia syndrome, chronic respiratory disease, and otitis media [34, 74].

In recent years, the evidence has accumulated that atypical *Chlamydiae* known as TWAR (Taiwan acute respiratory) strains cause outbreaks of acute infection of the lower respiratory tract in man. The first outbreaks have been reported from Finland and the USA [50, 51, 138]. These agents form a genetically and serologically homogeneous group, which resembles *Chlamydia psittaci* more than *C. trachomatis* but which differs sufficiently from both to be classified as a third species of *Chlamydia*, for which the name *Chlamydia pneumoniae* (*C. pneumoniae*) was proposed by Grayston et al. [49]. Note that *C. pneumoniae* is distinguished sharply from *Chlamydia psittaci* (*C. psittaci*) by being spread from man to man and by the fact that as yet no avian or other mammalian host has been identified. In 1992 *Chlamydia pecorum* (*C. pecorum*) is the fourth species of the genus *Chlamydia* on the basis of the results of a genetic analysis of *Chlamydia* strains that were isolated mostly from cattle and sheep [39, 40].

1.2. Biology

Chlamydia are spherical or ovoid obligately intracellular bacteria and have an unique mode of replication that undergoes a characteristic and well-defined dimorphic life cycle within eukaryotic host cells that distinguishes them from all other groups of bacteria. This developmental cycle consists of two main forms of the micro-organism. The infective form is the elementary body (EB), 200-300 nm in diameter, which develops within the host cell into the intracellular replicative form, the reticulate body (RB), 600-1000 nm in diameter. The RB divides by binary fission, each RB eventually producing one or more EBs within an enlarged endocytic vacuole termed the inclusion. The mechanical strength of the chlamydial cell envelope derives from the major outer-membrane protein (MOMP) and other cysteine-rich proteins which are extensively cross-linked by disulphide bonds in the EB but much less so in the RB. *Chlamydia* lack peptidoglycan in their cell walls so are relatively resistant to beta-lactam antibiotics but susceptible to macrolides and tetracyclines. Chlamydial development is reversibly inhibited by interferon-gamma.

1.2.1. Taxonomy (Phenotypic classification)

The order *Chlamydiales* consists of one family, the *Chlamydiaceae* contains one genus, *Chlamydia*. There are currently four species known within the genus *Chlamydia*: *C. trachomatis, C. psittaci, C. pneumoniae* and *C. pecorum,* and they resemble each other in their morphology. This classification into four species is moderately satisfactory, but it takes no account of the many reports of *Chlamydia*-like organisms living in invertebrate hosts, and it is based solely on phenotypic characters. *C. trachomatis* and *C. pneumoniae* have been considered strictly human pathogens without animal reservoirs [52]. *C. psittaci* is a common pathogen in avian species and mammals and, although capable of causing human disease, does so as a zoonosis with very little person-to-person transmission being documented. *C. pecorum*, the fourth species, is not known to infect humans, but it is a common pathogen for cattle and sheep, and also causes encephalomyelitis, pneumonia and arthritis [76]. The characteristics of these four species are summarized in Table 1.

Table 1. Characteristics of the four chlamydial species.

	C. trachomatis	*C. pneumoniae*	*C. psittaci*	*C. pecorum*
Natural hosts	Humans, mice, pigs	Humans, horses	Birds, mammals, occasionally humans	Cattle and sheep
EB morphology	Round	Round or pear shaped	Round	Round
Inclusion	Oval, vacuolar	Oval, dense	Variable, dense	Oval, dense
Iodine staining	Yes	No	No	No
Sulphonamide sensitive	Yes	No	No	No
No. of serovars	at least 18 A,B,Ba,C D,Da,E,F,G H,I,Ia,J,K L1,L2,L2a,L3	1	Undefined	3
Characteristic infections	Genital and ocular mucosa; often inapparent; intermittent shedding; Rarely systemic	Chronic respiratory tract infections; Possible association with heart disease	Frequently systemic: pneumonia, abortion, etc.	CNS, respiratory and gut; often inapparent; prolonged carriage

1.3. Diseases caused by *C. trachomatis*

In humans, the syndromes caused by *C. trachomatis* fall into three groups, each of which tends to be associated with a particular set of serotypes:

1. trachoma (mainly serotypes A, B, Ba and C)
2. oculogenital and, occasionally, more general infections (mainly serotypes D-K, although serovar B has been recovered from both genital disease and endemic trachoma) and
3. lymphogranuloma venereum (serotypes L1, L2, L2a and L3).

1.3.1. Trachoma

Trachoma is the most common preventable form of blindness and affects about 360 million people worldwide of whom up to 7 million are blind [20, 22, 23, 171]. Trachoma is endemic primarily in tropical and subtropical countries; those worst affected are North Africa, the Middle East and the northern part of the Indian subcontinent. The disease is also prevalent in Sub-Saharan Africa, the Far East, Austral-Asia and Latin America. Its prevalence and severity vary considerably from country to country and in different areas within the same country. Adult inclusion conjunctivitis is primarily a sexually transmitted disease, in contrast to the eye-to-eye spread of trachoma. The former may be acquired by direct or indirect contact with genital secretions from an infected person, or by autoinfection. Trachoma is associated with poor living standards and hygiene and thus tends to be more prevalent in rural than in urban settings. Inadequate chlorination of swimming baths has in the past been implicated in the transmission of eye infection, presumably from contamination of the water with genital secretions.

1.3.2. Genital infections of men

Non-gonococcal urethritis

In most communities, non-gonococcal urethritis (NGU) is far more common than gonococcal urethritis. Urethral irritation and dysuria are followed by the appearance of a mucopurulent discharge. *C. trachomatis* is the only agent firmly implicated in the causation of NGU. The organism is isolated from 20-30% of cases, compared to 3-5% of matched controls without urethritis [117].

Post-gonococcal urethritis

The persistence or recurrence of symptoms and signs following effective treatment of gonococcal urethritis is almost always due to concurrent infection with an agent or agents of NGU. *C. trachomatis* is isolated from 11-30% of men with gonococcal urethritis and probably accounts for 80-90% of post-gonococcal urethritis (PGU) cases [116].

Other infections of the male genital tract

Epididymitis in men under 35 years old is associated with *C. trachomatis* in approximately 50% of cases, compared with 15% of older men [154]. Diminished fertility is an accepted consequence of epididymitis, but to what extent *C. trachomatis* per se contributes to male infertility is unknown. The role of the organism in acute or chronic prostatitis is more controversial. The balance of evidence suggests that *C. trachomatis* is not causally linked to prostatitis. C. trachomatis has been isolated from the rectum of 4-8% of randomly selected homosexual males, most of whom admitted unreceptive intercourse [46, 122, 125].

1.3.3. Genital infections of women

Sexually acquired chlamydial infection in women may involve not only the cervix and urethra, but also the endometrium, fallopian tubes and rectum. Extension of infection into the peritoneal cavity may result in perihepatitis and periappendicitis.

Lower genital tract infection

Chlamydial infection of the cervix is found in 15-30% of women attending clinics for sexually transmitted diseases (STD) [135, 141]. Carrier rates for this organism are relatively high in women because up to 70% of the infections may have neither signs nor symptoms of infection [67, 123]. With no obvious need for evaluation or treatment, prevalence increases. Thus, a wide range of infection rates may be found in women having routine pelvic examinations at family planning clinics or settings where they are having annual physical examinations. Lower socio-economic classes and young age are the most readily identifiable risk factors for chlamydial infection. The organism is present in the cervix of over 80% of primary contacts of men with chlamydial urethritis. The 35-45% of women with cervical gonorrhoea have concurrent chlamydial infection, i.e., approaching twice the prevalence in men with gonorrhoea [121].

There are few distinctive symptoms or signs. Brunham et al. [12] demonstrated a relationship between chlamydial infection and mucopurulent cervical discharge in the absence of *Neisseria gonorrhoea* (*N. gonorrhoea*). Predictive values associating this sign and infection are often less than 30% [33].

Urethral infection

This may occur in the absence of cervical infection in up to one-third of infected women. Although infections with *C. trachomatis* may cause urinary symptoms, its role in the urethral syndrome remains controversial [77, 144].

C. trachomatis may cause vaginitis in prepubertal girls and also in hysterectomized women. Davies et al. isolated *Chlamydiae* from Bartholin's ducts in 9 women, of whom 7 had concurrent gonorrhoea [21]. The true prevalence of chlamydial infection at this site is unknown.

Upper genital tract infection

Ascending chlamydial infection of the female genital tract is well reviewed by Mardh [98]. Mid-cycle bleeding is often the only abnormal sign associated with chlamydial infection. This possibility should be considered in the differential diagnosis of irregular menstrual bleeding. Post vaginal delivery and post abortal endometritis are well documented.

The association of *C. trachomatis* with salpingitis has been recognized for many years. Less clear are the frequency of a chlamydial aetiology and the inter-relationship of *C. trachomatis* with other organisms associated with salpingitis, including *N. gonorrhoea, Mycoplasma hominis* (*M. hominis*) bacteria. Mardh et al. [99] establish that 8-20% of women with cervical chlamydial infection will experience salpingitis as a complication. The organism has been recovered from the fallopian tubes at laparoscopy [177]. Opinions differ as to the relative frequencies of chlamydial and gonococcal salpingitis [10, 36]. This may reflect the milder and more protracted symptoms of the former, the imprecision of clinical diagnosis of salpingitis, or a geographical variation in the distribution of the two organisms. Thus, identification rates for *C. trachomatis* vary between 5 and 20% in the USA, to 25-40% in Europe [85]. Bevan et al. [9] recently reported a United Kingdom (UK) cohort study on 104 laparoscopy confirmed cases of pelvic inflammatory disease. *C. trachomatis* was identified (culture, antigen detection or serology) in 55%.

Characteristically, a patient with chlamydial salpingitis has abdominal pain of longer duration and the erythrocyte sedimentation rate is higher than is usual in gonococcal salpingitis. A modest pyrexia is present (rectal temperature 38°C or higher). At laparoscopy, the tubal damage seen may be more severe than expected from the milder onset. Involuntary infertility is

an important complication in 12-13% of women following a first attack, rising to 75% after more than 2 episodes [178].

Tubal factor infertility may also be more likely after a severe episode than a mild one. Ectopic pregnancy is another common outcome of chlamydial salpingitis reflecting the subsequent tubal damage. Ectopic pregnancy rates increase 7-10-fold after episodes of salpingitis [128].

Many of women who have tubal damage and high levels of antibody to *Chlamydia* have no prior clinical history of salpingitis. The silent salpingitis may be more common than clinically apparent salpingitis [9, 153].

The extension of infection into the peritoneal cavity results in perihepatitis (Curtis-Fitz-Hugh syndrome). At laparoscopy, thin fibrous adhesions ("violin string") are seen between the fallopian tubes and the liver capsule. A similar condition may occur with non-chlamydial salpingitis. The role of *C. trachomatis* in abortion, stillbirth and prematurity remains uncertain. Little interest has been shown in rectal chlamydial infection in women [34, 156].

1.3.4. Neonatal and childhood infections

Babies born vaginally to mothers with cervical chlamydial infection are at risk of becoming colonized or infected with an organism. Estimates of the risk are as high as 70% [143]. Conjunctival infection occurs in 30-40% and pneumonia in 10-20% of these babies [139]. Other sites infected (either alone or concurrently) include the middle ear, nasopharynx and the rectum. The association with overt disease at some of these sites is uncertain. Infection of the vagina, rectum and pharynx may be delayed for up to 7 months after birth [7, 53, 56]. True congenital infection has not been reported.

Ocular infection

Chlamydial infection of the neonate eye is more common than gonococcal ophthalmia. Estimates in the USA range from 1.6% to 12% of neonates [126]. Onset is from 6 to 21 days after birth. Physical signs can be florid conjunctivitis with pronounced conjunctival and periorbital oedema and a purulent or mucopurulent discharge. Untreated, the condition resolves over 2-3 months; conjunctival scarring occurs in some cases [65].

Pneumonia

This syndrome was described by Schachter et al. in 1975 [142]. Onset occurs characteristically 4-12 weeks after birth. The main features are dyspnoea, a staccato cough and sometimes a nasal discharge. There may be evidence of otitis media. Untreated, the disease runs a benign clinical course, leading to apparent recovery. Although the number of children who have had long-term follow-up after an episode of chlamydial pneumonia in infancy is quite small, in one study [139], two-thirds of them had long-term consequences. Approximately one-third developed asthma, whilst the rest had abnormal respiratory functions tests [158].

1.3.5. Lymphogranuloma venereum

Lymphogranuloma venereum differs considerably from other syndromes caused by *C. trachomatis*; it is caused by members of serotypes L1, L2, L2a and L3, which attack lymphatic and subepithelial rather than epithelial tissues and there may be extensive fibrosis in the later stages [104]. Although less prevalent than other STDs in industrialized countries, it has a wide geographical distribution, prevalence being highest in the tropics and subtropics.

1.4. Epidemiology

C. trachomatis infection is an STD that is common worldwide and have surpassed gonorrhoea as the number one in the United States (USA), the UK and the Scandinavian countries [6, 44]. Sweden was the first country in the world to establish a national laboratory service for detecting chlamydial infections. In the Netherlands in 1987, about 90 000 individuals were estimated to be infected with *C. trachomatis*. The annual medical costs were estimated to be $20 million of which 75 % was attributed to sequelae of chlamydial infections [169]. The annual cost of chlamydial infection and its sequelae was estimated at $2.2 billion in 1990 in USA [174].

Female cases have generally dominated in those countries that have introduced screening programmes, e.g. in women attending for contraceptive device, cancer-screening and pregnancy termination [13, 160]. In the Scandinavian countries infections by *C. trachomatis* have been included in laws involving contagious diseases, which in various ways have meant an economic support to detect, survey and treat such infections.

Table 2. Incidence/prevalence of *C. trachomatis* in Europe [3, 25, 35, 61, 83, 90, 119, 134, 148, 149, 168, 173].

Country	*Year*	*%*	*Asymptomatic*	*Diagnosed*
Austria	1995	7.2	Pregnant women	
Bulgaria	1995	6.1	Female	
Croatia	1995	17.1		Female and male
Denmark	1995	4.8		Female and male
France	1993-1995	6-11		Female and male
Germany (East)	1995	4.7-6.5		
Germany (West)	1995	2.71		
Greece	1995	4-7	Female	
Hungary	1995	14.5		Female and male
The Netherlands	1994	5.1-12.3		STD patients female
Norway	1995	4.4	Female	
Switzerland	1994	4.6		Female and male
United Kingdom	1995	3-12		

Table 3. The incidence of *C. trachomatis* cases in Hungary [25].

Year	*No of total*	*No of positive*	*%*
*1986	102	42	41.2
*1987	134	71	53.0
*1988	138	60	43.5
*1989	336	177	52.7
1990	177	53	29.9
1991	263	71	27.0
1992	329	75	22.8
1993	4636	433	9.3
1994	8913	1515	17.0
1995	9633	1401	14.5
1996	13981	1495	10.7

*nonbacterial and *Mycoplasma hominis*, *Ureaplasma urealyticum*-negative cases

The changes of screening methods for genital chlamydial infections have had a great impact on the registered epidemiological trends [95, 106]. The incidence of *C. trachomatis* frequencies in some European countries are summarised in Table 2.

The nationwide registration of *C. trachomatis* in Hungary has been performed since 1995 in ten clinical, hospital and public health laboratories. In some research centres and hospital laboratories *Chlamydia* diagnostic methods have been carried out since 1985. The incidence of *C. trachomatis* cases in the past 11 years are shown in Table 3. The *C. trachomatis* examinations were carried out only in the group of symptomatic genital infections without bacterial, *Mycoplasma hominis*, *Ureaplasma urealyticum* infections between 1986 and 1989.

1.5. Diagnosis

Diagnosis can be made by a variety of techniques. For many years, culture has been considered the diagnostic test of choice [91]. Antigen detection methods [88] - either direct fluorescent antibody tests or enzyme-linked immunosorbent assay (ELISA)- and nucleic acid detection tests are now more commonly used [109, 161]. Specimens from the urethra require insertion of a swab 3-4 cm, having first removed any excess discharge. There is an increasing evidence that urine samples may provide a satisfactory alternative. Polymerase chain reaction (PCR) and ligase chain reaction (LCR) tests for chlamydial DNA may well prove to be suitable techniques for use on urine specimens from men, providing a more acceptable alternative to swab insertion, particularly in asymptomatic men [15, 72].

To increase the yield of *Chlamydiae* from the lower genital tract of women, at little additional cost, the pooling of urethral and cervical specimens from a patient has its advocates [97]. It has been demonstrated that LCR tests on urine may be a substitute for cervical specimens [92].

1.6. Treatment

Today the drugs of choice in medicine are tetracycline and macrolide antibiotics, compounds, which are effective in the treatment of intracellular organisms such as *Chlamydiae* [54, 68, 94]. An alternative first-line antibiotic is erythromycin used especially for children and pregnant women. The recently developed azithromycin is a third alternative, with its advantageous

single-dose regimen for genital infections, in contrast to the conventional treatment of 7-14 days [37, 89, 117, 131].

1.7. Screening for *C. trachomatis* in asymptomatic women

Chlamydial infections of the genital tract do not invariably cause symptoms that would prompt a person to seek medical aid. However, identification and treatment of infected persons is important not only for the individuals but also to prevent the spread of *C. trachomatis* in the society [80, 133]. Screening asymptomatic women for chlamydial infection is the cornerstone of effort to reduce the burden of the disease, since chlamydial cervicitis is not associated with specific complaints [123]. The absence of symptoms may also be common in those with upper genital tract invasion. An endometrial biopsy revealed endometritis in 40% of women and *Chlamydia* was isolated from endometrial cultures in 41% of women with chlamydial cervicitis but no suspicion of pelvic inflammatory disease (PID) [78, 118].

Since the medical costs rise, a new attention to quality and cost of care has become apparent. The key to the prevention of chlamydial infections and their sequelae is screening using a high performance diagnostic test.

Diagnostic tests for detection of *C. trachomatis* have been shown to be cost-effective in the group of non-pregnant women attending sexually transmitted disease clinics and are not treated with empirical therapy, and in asymptomatic women at moderate risk for infection [45, 57, 111]. In general, the age-based screening provided the greatest cost savings. However, universal screening is desirable in some situations [110]. In most cases, screening done by using any criteria and a highly sensitive diagnostic assay should be part of any STD prevention and control program or health plan.

The frequencies of PID and male STDs are poorly documented in Hungary [27, 162], and the annual cost of chlamydial infection and its sequelae was not estimated yet. In order to determine the prevalence and some of the risk factors of genital *C. trachomatis* infections, we have performed a study among pregnant women attending health centres in different regions of Hungary. Furthermore, a cost-effectiveness analysis of screening of chlamydial infection and its sequelae has been carried out.

2. AIMS OF THE INVESTIGATION

This investigation was carried out with the following aims:

1. to study the epidemiology of genital *C. trachomatis* infections in Hungary;

2. to determine the prevalence, risk factors of cervical chlamydial infection in asymptomatic pregnant women;

3. to examine the role of *C. trachomatis* with preterm delivery, premature rupture of membranes, and intrauterine growth retardation ;

4. to study the connections of perinatal mortality and maternal chlamydial infection;

5. to determine the effectiveness of antibiotic therapy to treat *C. trachomatis* infection;

6. to assess the cost-effectiveness of identifying and treating asymptomatic female carriers of *C. trachomatis*.

3. MATERIALS AND METHODS

3.1. Study population

The study started in January 1994 and terminated in June 1995 in seven different centres: two in Budapest, and one each in Debrecen, Miskolc, Nyíregyháza, Szeged and Szombathely. The envisaged sample size was around 600 women at each centre [145]. Pregnant women were recruited in a complaint-free condition and also when complaints arose during pregnancy: intrauterine growth retardation (IUGR), premature rupture of membranes (PROM), spontaneous preterm labour, threatening abortion and premature delivery. The background data relating to social and marital status and age were recorded.

3.2. Laboratory tests and treatment

The PACE 2 Gen-Probe based on non-amplified nucleic acid hybridization was applied to detect *C. trachomatis* in each centre. In addition, in the Szeged centre comparative examinations were performed on a low number of samples with cultivation on the McCoy cell line, and with a rapid ELISA diagnostic method (SYVA MicroTrak) for the detection of *C. trachomatis* antigen [42, 48]. Samples were taken from the squamocolumnar region of the endocervix, placed in appropriate transport tubes, and processed within 1-2 days [11]. In three of the seven centres (Miskolc, Szombathely, Szeged) *C. trachomatis-positive* pregnant patients received roxithromycin treatment (150mg x 2 daily 10 days).

3.3. Data collection

An interviewer-administered standardized questionnaire was completed. There were four kinds of questionnaire, in which age, social and marital status, anamnestic and obstetrical history and neonatal data were recorded in order

Type A: to screen *C. trachomatis* infection only at delivery

Type B1: to screen *C. trachomatis* infection during pregnancy

Type B2: to screen *C. trachomatis* infection after treatment (If the test of screening during pregnancy is positive)

Type C: to screen *C. trachomatis* infection at delivery to complete B1 sheet with obstetrical history and neonatal data. All personal data were protected from illegal use.

3.4. Statistical analysis

Data management was carried out with self-developed software (CLIPPER 5.0). Statistical analyses were performed with SPSS for Windows (6.1.2 version) software packages. Taking the Gen-Probe results as standard sensitivity, specificity, and positive and negative predictive values were calculated. To assess the prevalence of genital chlamydial infections, women positive in at least one test were considered to be infected. To compare groups Chi-square tests and Student's t-tests were employed. For the examination of trends in the monthly frequencies of infection the moving average and linear regression methods were used. A moving average seeks to remove seasonality by averaging over a period, so that the effects of extreme values within the period cancel out [105]. To obtain an overview of the risk, the data were cross-tabulated in several ways and multiple logistic regression analysis was performed to differentiate between subgroups with various degrees of risk. A variety of risk factors may be contributing to the occurrence of disease and logistic regression allows one to calculate odds ratios for individual risk factors. These odds ratios represent the risk of disease for those with the exposure being studied, relative to the risk of disease among those without the exposure - adjusted to remove the effects of all other risk factors used in the logistic regression [181]. Results are presented as percentages, means and odds ratios with 95% confidence intervals. A probability level of $p<0.05$ was considered statistically significant.

3.5. The analysis of cost-effectiveness

Table 4. The direct cost of different diagnostic methods in HUF.

Method	Cost
ELISA method for detection of *C. trachomatis*	1,400
Amplified nucleic acid hybridization Gen-Probe method for detection of *C. trachomatis*	2,800
PCR method for detection of *C. trachomatis*	4,200
Isolation of *Ureaplasma urealyticum*	1,400
Isolation of *Mycoplasma hominis*	1,400
Isolation of *Trichomonas vaginalis*	490
Isolation of bacteria	630
Isolation of *Candida* species	210

Costs were based on local charges of the Hungarian Health Insurance [29]. Only direct costs were calculated in Hungarian Forint (HUF). The costs of inpatient and outpatient treatment are defined by scores. Thus, these were multiplied by the average exchange rate of 0.7 to get the costs in HUF in case of outpatient treatment . The costs of inpatient care have included the full cost of treatment and hospitalisation. These costs were calculated by using the current basis amount, which was currently 61,000 HUF. Table 4-8 present the base costs. Total cost of screening the *C. trachomatis* infection using the ELISA method includes a gynaecological examination too (Table 6).

Table 5. The laboratory costs of screening for most common pathogens of genital infections in HUF.

Method	Cost	Total cost
The direct cost of diagnostic methods for isolations of bacteria, *Trichomonas vaginalis and Candida* species	1,330	
If ELISA method is used for detection of *C. trachomatis*	1,400	**2,730**
If Gen-Probe method is used for detection of *C. trachomatis*	2,800	**4,130**
If PCR method is used for detection of *C. trachomatis*	4,200	**5,530**

Table 6. Total cost of screening the *C. trachomatis* infection using the ELISA method in HUF

Woman	Cost	Total cost
Visit to physician	350	
Cervical smear	35	
Cytology	490	
Colposcopy	105	
Bacterial smear	70	
Laboratory diagnostic test to detect *C. trachomatis* infection	2,730	
Total		**3,780**
Man		
Visit to physician	350	
Sperm collection	112	
Laboratory diagnostic test to detect *C. trachomatis* infection	2,730	
Total		**3,192**

In Table 7, the cost of doxycyclin therapy (100mg x 2 daily 14 days) for treatment of chlamydial infected patients and the cost of control laboratory tests were calculated. The *Mycoplasma hominis* and *Ureaplasma urealyticum* isolations are only included in the control diagnostic method.

Table 7. Cost of treatment and control laboratory tests in HUF.

Woman	Cost	Total cost
Two physician visits	700	
Doxycyclin therapy	1,160	
Bacterial smear	70	
Ureaplasma urealyticum isolation	1,400	
Mycoplasma hominis isolation	1,400	
Cost of using ELISA method for detection of *C. trachomatis*	2,730	
Total		**7,460**
Man		
Two physician visits	700	
Sperm collection	112	
Doxycyclin therapy	1,160	
Ureaplasma urealyticum isolation	1,400	
Mycoplasma hominis isolation	1,400	
Cost of using ELISA method for detection of *C. trachomatis*	2,730	
Total		**7,467**

Treatment of infertility is not involved in the cost-benefit analysis since there are poorly documented data of treatment of infertility in Hungary. However, the estimated rate of 10% of infertile couples were treated using in vitro fertilisation (IVF) technology. The cost of IVF, the expensive medical high technology is approximately 200,000 HUF for a treatment. In general, more treatments are necessary for a successful pregnancy, but this method has only at most 30% rate of efficiency.

Table 8. Outpatient cost of infertility examination in HUF.

Women	Cost	Total cost
Total cost of screening of a woman using ELISA method	3,780	
Hormone profile for women	3,640	
Hysterosalpingography	1,400	
***Total**		**8,820**
Man		
Total cost of screening of a man using ELISA method	3,192	
Hormone profile for men	2,380	
Sperm analysis	322	
Total		**5,894**
Couple		**14,714**

*The cost of laparoscopy is calculated in the cost of institutional care

Age stratified demographical data of Hungarian women population were available from the Hungarian National Statistical Office [31]. Approximately 400,000 women belonged to the age group between 15 and 19 years on January 1, 1996 (Figure 1).

Figure 1. Age stratified demographical data of Hungarian women on January 1, 1996.

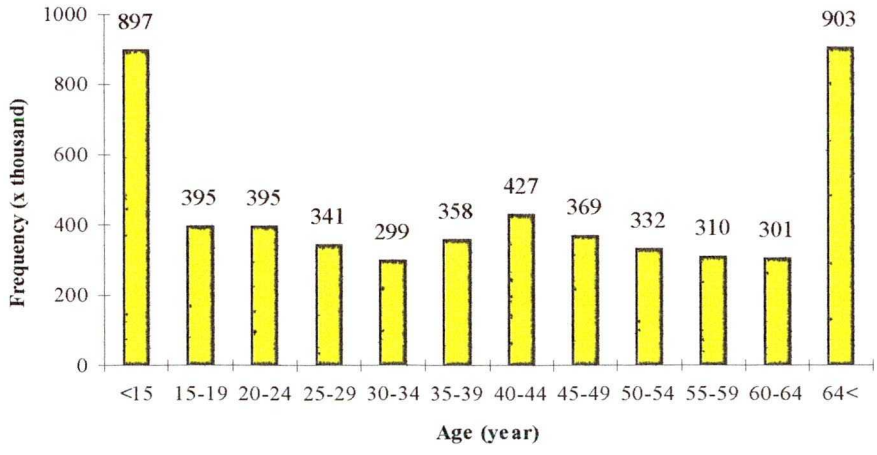


Table 9. The frequency of *C. trachomatis* infection in the seven centres.

Centre	*Type of questionnaire*									Total		
	A and/or C			B1			B2					
	No	*C. trachomatis*-positive	%	N	*C. trachomatis*-positive	%	No	*C. trachomatis*-positive	%	No	*C. trachomatis*-positive	% infection
Budapest I	188	15	8.0	176	13	7.4	-	-		364	28	7.7
Budapest II	683	20	2.9	32	-	0.0	-	-		715	20	2.8
DOTE	471	6	1.3	1	-	0.0	-	-		472	6	1.3
Miskolc	2114	209	9.9	206	13	6.3	13	2	15.4	2327	227	9.8
Nyíregyháza	1416	76	5.4	287	16	5.6	-	-		1703	92	5.4
Szombathely	292	4	1.4	462	6	1.3	6	1	16.6	757	12	1.6
SZOTE	997	32	3.2	151	9	6.0	9	1	11.1	1157	42	3.6
Total	6161	362	5.9	1315	57	4.3	28	4	14.3	7495	427	5.7

No Number of cases

% % infection of *C. trachomatis*-positive cases

Type A: to screen *C. trachomatis* infection only at delivery

Type B1: to screen *C. trachomatis* infection during pregnancy

Type B2: to screen *C. trachomatis* infection after treatment (If the test of screening during pregnancy is positive)

Type C: to screen *C. trachomatis* infection at delivery to complete B1 sheet with neonatal data.

4. RESULTS

4.1. The frequency of *C. trachomatis* infection

During the 18 months study 7495 examinations of 6161 patients were performed in the seven centres. Table 9 presents the rates of the infection in the individual centres.

4.2. Epidemiology

A total of 6161 pregnant women were examined for the occurrence of *C. trachomatis*. The overall average *C. trachomatis* infection rate was 5.9%, the data varying in the range 1.3-9.9%. The monthly relative frequencies of *C. trachomatis* exhibited linear growth (R=0.937) (Figure 2). There was statistically significant difference ($p<0.001$) between the yearly frequency of the *C. trachomatis*-infected patients (Table 10). A significantly higher *C. trachomatis* infection rate ($p<0.01$) was identified in the Miskolc region (9.9%), where the highest rate of unemployment occurred in Hungary in the last few years (Table 11).

Figure 2. The monthly relative frequencies of *C. trachomatis* infection in Hungary between January 1994 and June 1995. The trend was fitted by using moving averages.

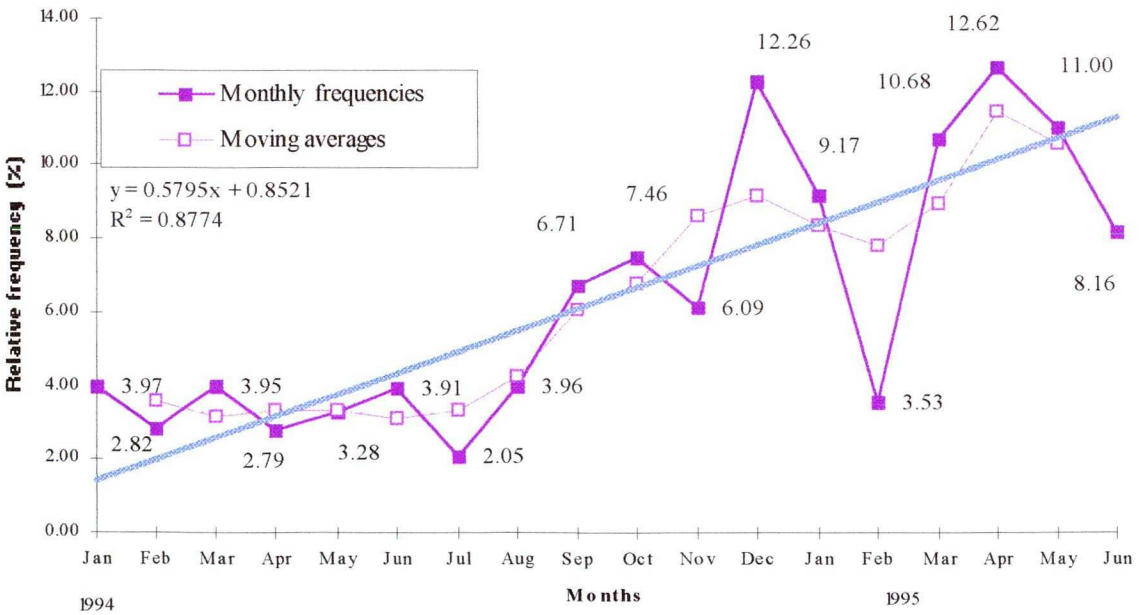


Table 10. The yearly frequencies of *C. trachomatis*.

Year	*C. trachomatis* Positive	Negative	Total	Relative frequency
1994	193	4105	4298	4.5%
1995	168	1695	1863	9.0%
Total	361	5800	6161	5.9%

Table 11. Risk factors for *C. trachomatis* infections among 6161 pregnant women. Odds ratios and p values from multivariate logistic regression analysis. An odds ratio of 1.0 indicates the reference category. 95% CB: 95% confidence bounds. Data missing in up to 74 women.

	No	*C. trachomatis*-positive	%	Odds ratio (95% CB)	Probability level of significance
Centre					p<0.01
Budapest I	188	15	8.0	2.0(1.3-3.1)	
Budapest II	683	20	2.9	0.9(0.7-1.3)	
Debrecen	471	6	1.3	0.5(0.2-1.0)	
Miskolc	2114	209	9.9	3.0(2.1-4.5)	
Nyíregyháza	1416	76	5.4	1.2(1.1-1.4)	
Szombathely	292	4	1.4	0.5(0.2-1.2)	
Szeged	997	32	3.2	1.0	
Age (years)					p<0.05
<20	743	85	11.4	1.6(1.3-1.9)	
20-28	3243	176	5.4	1.0(0.9-1.1)	
≥29	2175	101	4.6	1.0	
Marital status					p<0.05
Unmarried	1055	89	8.4	1.4(1.2-1.8)	
Married	5032	273	5.4	1.0	
Previous pregnancies					p<0.01
Primigravida	2515	178	7.1	1.4(1.1-1.7)	
Multigravida	3625	184	5.1	1.0	

The difference between the ages of the *C. trachomatis*-infected patients (24.5±7.4 years) and the non-infected women (26.3±6.9 years) was statistically significant ($p<0.01$). The mean number of pregnancies in the positive cases was 1.2±1.7, as compared to 1.3±1.7 among the negative cases.

4.3. Social and age related factors and *C. trachomatis* infection rate

Certain potential risk factors of the infection were examined. As shown in Table 11, young age ($p<0.05$), unmarried status ($p<0.05$) and the case of no previous pregnancies ($p<0.01$) were statistically significant predictors of the infection. Furthermore, the age group under 20 years displayed a very high rate of infection (11.4%). Both univariate and multivariate analysis indicated that treatment with antibiotics during pregnancy (to treat infections different from *C. trachomatis*, upper or lower respiratory tract infections) did not significantly influence the risk of infection.

The infection rate was significantly ($p<0.01$) higher in single than in married patients. Only 5.4% of the married women were *C. trachomatis*-positive. In the groups of the 949 unmarried and 117 divorced patients, the *C. trachomatis* infection rates were 10.5% and 10.2%, respectively. There was no significant difference between unmarried and divorced patients.

4.4. Pregnancy outcome and *C. trachomatis* infection

4.4.1. Premature uterine activity

The association of clinical symptoms and signs with the probability of chlamydial infection was also examined. During the study period, 319 patients (5.2%) were admitted because of premature uterine activity (PUA). In the anamnestic history of *C. trachomatis*-positive patients the PUA was 8.0% opposite to the 5.0% frequency of *C. trachomatis*-negative group and the difference was statistically significant ($p<0.05$). In cases requiring intravenous beta-sympathomimetic tocolysis *C. trachomatis* infection rate was similar to the average (5.2%).

Cervical cerclage operation was performed in 0.8%. The frequency of the operation was higher in the *C. trachomatis*-positive (1.1%) than in negative patients (0.7%). Because of the low case number, no statistical analysis was performed.

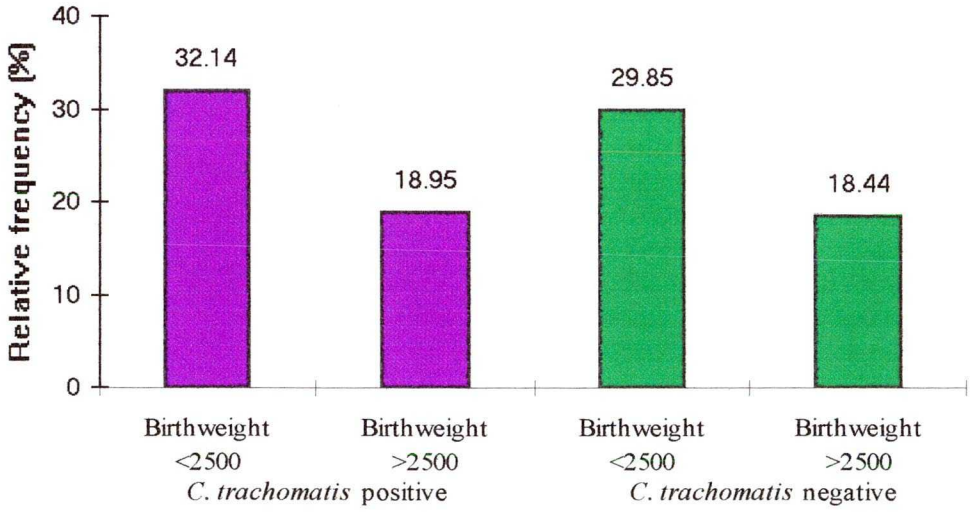
4.4.2. Complications at delivery

Premature rupture of the membranes occurred in 20.0%. Differentiation of the *C. trachomatis*-positive and negative groups revealed that the rate of PROM was 21.0% in the infected group, whereas it was 19.9% in the non-infected group. The difference is not significant. Figure 3 demonstrates the frequency of PROM in case of low and normal birth weight in *C. trachomatis-positive* and negative patients. Although PROM was significantly higher in the low birth weight group ($p<0.05$) than in term deliveries, there was no significant connection between low birth weight and maternal *C. trachomatis* infection.

Uterine inertia occurred in 17.5% of the deliveries. 48 of the 1076 cases (4.5%) were *C. trachomatis-positive*.

Intrauterine distress occurred in 8.6% of all deliveries. The rate of *C. trachomatis*-positive patients was 5.5% in this group. The difference was not significant.

Figure 3. The frequency of premature rupture of the membranes among *C. trachomatis*-positive and negative patients in normal and low birth weight groups.



4.4.3. Neonatal data

The average prenatal mortality rate was 2.1%. in the seven centres. There was a significantly higher ($p<0.05$) mortality rate (3.6%) in the group of *C. trachomatis*-positive patients than in the group of negative patients (2.0%). In the group of 131 perinatal deaths, the maternal *C. trachomatis* infection rate was 9.9% that is higher than that in the total study population.

Table 12 reveals the connections between perinatal mortality and maternal *C. trachomatis* infection.

Table 12. The distribution of perinatal mortality and maternal *C. trachomatis* infection.

	C.trachomatis positive	*C.trachomatis* negative	*Total*
Living	349	5681	6030
Dead	13	118	131
Total	362	5799	6161

With regard to the *C. trachomatis* infection and perinatal mortality rates in the normal and low birth weight groups, among the 40 infants under 2500 g, the maternal *C. trachomatis* infection rate was 17.5%, whereas in the group of 91 infants above 2500 g the maternal infection rate was only 5.5%. The difference was significant ($p<0.05$).

A significantly higher number of *C. trachomatis*-positive newborns (17.1%) were treated in neonatal intensive care units (NICU) as compared with negative ones (6.3%). Congenital pneumonia was identified in 7.1% of the chlamydial infected newborns.

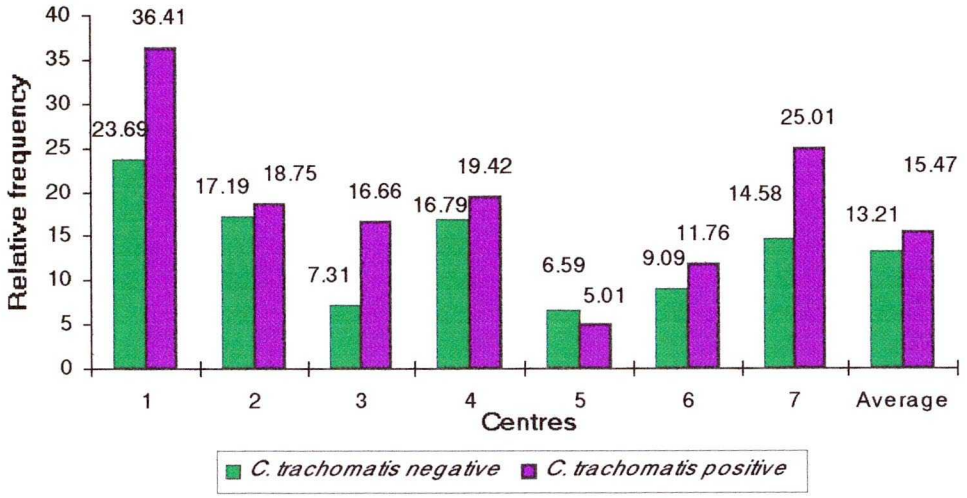
4.4.4. Low birth weight and *C. trachomatis* infection

Low birth weight (birth weight<2500 g) occurred in 13.3% of the parturients. In this group, the maternal infection rate was 6.8%. As compared with the general infection rate (5.7%), the difference was not statistically significant.

The frequency of low birth weight in the *C. trachomatis*-positive patients was 15.5%, while it was 13.2% in the group of *C. trachomatis*-negative patients. The difference between the two groups was not significant. Figure 4 depicts the rates of low birth weight newborns of *C. trachomatis*-positive and negative patients in the individual centres.

Intrauterine growth retardation occurred in 5.3% of the cases. The *C. trachomatis* infection rate was 7.3% in this group. The difference (1.6%) between the general infection rate (5.8%) and that one in IUGR was not significant.

Figure 4. The frequency of low birth weight newborns in the *C. trachomatis*-positive and negative groups.



4.5. Results of treatment

According to the study protocol, in three of the seven centres a roxithromycin therapy was administered in those patients who proved to be *C. trachomatis*-positive during their pregnancy. 28 of the 819 patients (3.4%) were *C. trachomatis*-positive in the treated group. 9 of 28 were treated with roxithromycin during hospital observation. 8 of them had successful outcome, with term deliveries, healthy newborns and negative *C. trachomatis* results at delivery. One patient had a preterm delivery on the 6th day of antibiotic treatment; the endocervical sample showed a *C. trachomatis*-positive result. 19 patients were treated as out-patients. Control samples could be collected in all of cases, and 4 of these samples gave *C. trachomatis*-positive results. In the group of patients receiving completed antibiotic therapy as outpatients, there were neither premature deliveries nor maternal or neonatal drug-related complications or side-effects. All the examined women were *Treponema pallidum* and *HIV*-negative. *N. gonorrhoea* infections were not registered. In 22 cases, *Trichomonas vaginalis* (4 coinfections with *C. trachomatis*) infections were detected and treated.

4.6. Comparison of the diagnostic methods

Culturing of *C. trachomatis* (n=562) and the ELISA (n=95) method were carried out in parallel with the PACE 2 Gen-Probe for the detection of *C. trachomatis* only in the Szeged centre. Comparison of the culture technique with the PACE 2 Gen-Probe method demonstrated that the sensitivity of the former was 45.4%, its specificity was 98.3%, its

demonstrated that the sensitivity of the former was 45.4%, its specificity was 98.3%, its positive predictive value was 35.7%, and its negative predictive value was 98.9%. For ELISA was performed in parallel with the PACE 2 Gen-Probe method, the sensitivity was 50.0%, the specificity was 98.9%, the positive and negative predictive values were 50.0% and 98.3%, respectively.

4.7. Cost-benefit analysis

The age group between 15-19 years displayed a very high rate of infection (11.4%). The number of chlamydial infected cases were estimated approximately 45,600 of 400,000 women of this group (Fig 1). The total costs of screening of 400,000 women between 15-19 years are presented in Table 13 and Table 14 for using the ELISA method for detection of *C. trachomatis* and the amplified Gen-Probe method for detection of *C. trachomatis*, respectively.

Table 13. Total cost of screening using the ELISA method for detection of *C. trachomatis* in HUF.

	Cost
400,000 women	1,512,000,000
45,600 infected women	340,176,000
45,600 infected men	340,495,200
Total	**2,192,671,200**

Table 14. Total cost of screening the using amplified Gen-Probe method for detection of *C. trachomatis* in HUF.

	Cost
400,000 women	2,072,000,000
45,600 infected women	404,016,000
45,600 infected men	404,335,200
Total	**2,880,351,200**

The cost of the sequelae of untreated *C. trachomatis* infections is detailed in Table 15. An estimated 10% to 40% of women with untreated chlamydial infections develop pelvic inflammatory disease [130, 146,155]. We attempted to correct for this diagnostic uncertainty

Table 15. The direct cost of the sequelae of untreated *C. trachomatis* infections in HUF.

PID	No of cases	Individual cost	Cost
Outpatient treatment of women	8,208	11,240	92,257,920
Outpatient treatment of men	9,120	10,659	97,210,080
Inpatient treatment of women	912	36,738	33,505,485
Total			***222,973,485***
Infertility			
Outpatient investigation of couples	4,560	15,414	67,095,840
Inpatient investigation of women	912	71,287	65,013,744
Inpatient investigation of men	228	50,247	11,456,316
Total			***143,565,900***
Ectopic pregnancy			
Total	*4,560*	*79,165*	***360,992,400***
Cause of treatment in NICU			
[a]Pneumonia (7.1%)	3,192	227,069	724,805,684
Other (10%)	4,560	156,668	714,403,891
Total			***1,439,209,575***
Total cost of treatment of sequelae			**2,166,741,360**

[a]The syndromes of pneumonia occurs 4-12 weeks after birth, since this frequency exceeds 10%

by using a lower frequency of PID (20%, n=9120), which was suggested by Skjeldestad et al. [150]. 8208 cases (90%) and 912 cases (10%) of the 9120 were estimated outpatient and inpatient, respectively. Approximately 8-17% of women treated for PID will be infertile [36, 178, 179]. Here we used a probability of infertility of 10%. Another 10% of pregnant women with untreated chlamydial infections will have an ectopic pregnancy [81, 175, 176]. Neonatal data based on the our results of our survey are described in section 4.4.3. [87, 114]. Neonatal conjunctivitis was not separated from the other serious illness of neonates. The cost of the sequelae of untreated *C. trachomatis* infections without the cost of treatment of infertility is slightly less than the costs of screening using the ELISA method for detection of *C. trachomatis*.

5.DISCUSSION

The results of studies of gynaecological and obstetrical infectious diseases indicate that the frequency, outcome and consequences of the diseases all vary in different continents, in different countries, and even within the same country. *C. trachomatis* infection as a sexually transmitted disease is one of the best examples. Its frequency and morbidity are influenced by socio-economic factors among others. The controversial data in the large number of studies on *C. trachomatis* frequency, its role in obstetrical pathologies and consequences, and the possible cost-benefit effect of treatment support the necessity of a large, prospective, representative study on the infection rate in the pregnant Hungarian population. Until now, there have been very few data in Hungary regarding the role played by *C. trachomatis* in obstetrical pathology [127, 163].

In the present study, examinations were carried out on 6161 pregnant women during the 18-month period between January 1994 and June 1995. The overall rate of *C. trachomatis* infections was 5.9%. The monthly frequency of *C. trachomatis* infection varied between 2.1 and 12.6%. The moving average method was used to smooth these data and reveal any underlying trend in the time series. A moving average seeks to remove seasonality by averaging over a period, so that the effects of extreme values within the period cancel out. The moving averages then estimate the trend. Because of the increasing trend in the monthly frequencies of positive cases, we consider that the true prevalence of chlamydial infection is higher [113]. A methodological failure can be excluded because the same laboratory method and evaluation scheme were applied and regular technical staff consultations were held. The differences may be due in part to the same causes, which explain the considerable differences found in conditions the various studies: 3.4% [132] and 35.9% [71], and in part to the variations in socio-economic and ethnical differences between the populations examined in the different centres. The prevalence of chlamydial infection was significantly higher in the less developed north-eastern region of Hungary where the unemployment rate is higher than in the more developed western region.

Numerous surveys have been carried out to study the incidence of urogenital *C. trachomatis* infections. The results of these studies reflect various frequencies in the different countries. Del-Piano et al. carried out *C. trachomatis* screening in 5270 women in North Italy,

and reported an infection rate of 5.8% [30]. Humphreys et al. found that the rate of *C. trachomatis* infection in women in the State of Colorado was 7.7% (n=11,793) [69], but a rate of 21.8% has also been reported (n=11,544) [151]. Compared to other countries the *C. trachomatis* infection rate of asymptomatic women is moderate in Hungary.

In our survey the age group under 20 years exhibited a very high risk of infection. Herrmann et al. carried out a survey (n=863) in Nicaragua to determine the prevalence, risk factors and clinical chlamydial infection rate in different groups of women [62]. A young age, the use of oral contraceptives and commercial sex work were identified as statistically significant risk factors for chlamydial infection. Hillis et al. examined age as one of the *C. trachomatis* infection risk factors [63, 64], and established that in the age groups <16, 16-19 and 20-29 years, infection recurred 8 times, 5 times and 2 times more often than in the age group 30-44 years.

Significant differences between married and unmarried women were found in the examined Hungarian population as regards *C. trachomatis* infection. Hayashi et al. reported that in married pregnant women on Hokkaido island the infection rate was 6.1-7.3%, while in unmarried pregnant women it was 15.7-22.9%. Furthermore, in the group of unmarried women admitted for artificial interruption in Hokkaido (n=1,792), a 15.2% *C. trachomatis* infection rate [58] and among 10,980 pregnant married women, 5.6% *C. trachomatis* infection rate was found [84], respectively.

Preterm birth is the most important perinatal problem in Hungary. The underlying cause is not known in most cases, and physicians are left with marginally effective methods to delay delivery by arresting labour. A breakthrough in the prevention of this problem requires more data about the causes leading to premature uterine contractions. A large number of observations suggest that infections may have a causative role in the origin of preterm birth [17, 18, 59, 60]. *C. trachomatis* is regarded as one of the most frequently occurring micro-organisms in cases of genital infections leading to preterm birth and perinatal pathologies. In the last three decades, more than 6000 studies have dealt with *C. trachomatis* in different branches of medicine, but the urogenital infections seemed to have the most important consequences. Non-gonococcal urethritis in males, and infertility caused by salpingitis and pelvic inflammatory diseases in females are well documented, but there are controversial data about the importance of *C. trachomatis* infection in perinatal pathologies [112, 120].

The results of a number of studies suggest that bacterial vaginosis [14, 56, 103] bacterial infections and especially *C. trachomatis* infection can cause PROM or PUA leading to preterm birth. The mechanism of action however, is not clear. Close correlations between the *C. trachomatis* infection rate and PROM have been described in some studies [16, 32, 82, 108]. The data from our study, similar to those from other trials [136, 164], did not support this. In our observations, there was a significant correlation between the *C. trachomatis* infection rate and PUA. Premature uterine activity occurred in 8% of the infected group, but in only 5% of the non-infected group. In cases of PUA, examination for *C. trachomatis* is recommended. As the incidence is low, screening [115], but at least vaginal pH measurement [60], is suggested only in high-risk pregnancies such as those involving an unfavourable obstetric history, a poor socio-economic situation and a young age [86, 87].

Similar to others, our results suggest that *C. trachomatis* infection is of great importance in perinatal pathologies [172]. Low birth weight as a consequence of *C. trachomatis* infection could not be proved in our study, although the maternal infection rate was higher than the average in the low birth weight group.

A significantly higher number of *C. trachomatis*-positive newborns were treated in NICU as compared with negative ones. The newborns were generally transferred to the NICU because of congenital pneumonia. The 7.1% incidence of congenital pneumonia among chlamydia-infected newborns was less than the result found by Smith and Ryan et al. [137, 152].

C. trachomatis infection results in an elevated prostaglandin production [140]. Thus in cases of infection, a decreased frequency of uterine inertia ought to be observed. Ismail [70] did not find a correlation between *C. trachomatis* infection and uterine activity during labour. Our results suggested a small (1.3%) decrease of uterine inertia in cases of *C. trachomatis* infection.

The perinatal mortality rate, as one of the most important indicators of the quality of perinatal care, was significantly higher in the *C. trachomatis*-positive patients. The difference was more marked in low birth weight group (17.5%) than in the normal birth weight cases (5.5%).

Germain et al. [43] conducted a large-scale survey (n=13,994) with the aim of determining the roles of different vaginal pathogens in IUGR. Whereas *Bacteroides*, *Prevotella*,

Porphyromonas spp., *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Trichomonas vaginalis* did show a close correlation with IUGR, *C. trachomatis* infection had no role in IUGR. Our examinations did not reveal a significant connection between IUGR and *C. trachomatis*-positive cases.

The amplified methods can detect a very low number of pathogens with high sensitivity and specificity [1, 2, 28, 151, 165, 167]. The Gen-Probe method was applied to detect *C. trachomatis* in each centre, which is a nucleic acid hybridization method without amplification, and it is generally suitable in cases of moderate *C. trachomatis* incidence [27].

Cultivation and ELISA testing were carried out in parallel with the PACE 2 Gen-Probe only in the Szeged centre. The triple sampling and the processing of the different samples by three methods involved the risk that in those cases where a low number of pathogens were present, one of the samples would not contain bacteria. Because of the low number of ELISA examinations, no reliable statistical conclusions can be drawn. The cultivation of *C. trachomatis* requires special laboratory conditions. Therefore, on the basis of these diagnostic techniques we suggest the introduction of the ELISA or Gen-Probe method in non-STD centres for the sensitive detection of *C. trachomatis* [24, 26].

In many parts of the world, a substantial proportion of cases of PID, infertility and ectopic pregnancy can be attributed to *C. trachomatis* [13, 180]. Thus, in Northern and Western Europe, over half of all cases of salpingitis are caused by *C. trachomatis* as shown by isolation of the microbal agent from tubal and cervical specimens or by serology. Today *C. trachomatis* infections are much more important cause of PID than *N. gonorrhoea* [101, 155]. In contrast, results from North American studies on the aetiology of PID have been more diverse, with chlamydial infection rates varying by study setting [159]. The situation in Hungary is poorly documented. Both tubal infertility and ectopic pregnancy are developed from "silent" salpingitis, which is probably accounting for a major proportion of cases. This association is supported by a few cohort studies [66, 96]. Declining rates of chlamydial infection should be followed several years later by a decrease in the incidence of tubal sequelae.

The major goals of chlamydial infection control are the prevention of overt and silent salpingitis and their sequelae, and of perinatal and postpartum infections. Primary prevention strategies, aimed at the prevention of chlamydial infection, are similar to those for other STDs and HIV infection, and they include behaviour change for risk reduction and the usage of

condoms. The estimated protective effect of barrier methods in case control studies was 0.34 in a meta-analysis [19]. The effectiveness of the diaphragm appears to be at least as high, if not higher, than the male condom, for the prevention of chlamydial infection [182]. In addition, identification and treatment of infected individuals before they infect their sex partners or newborns is an important primary prevention strategy, as well as a secondary prevention intervention to prevent complications and sequelae [100].

As a major proportion of women and perhaps men with chlamydial infection are asymptomatic and as silent infection may have destructive consequences, control programmes cannot rely only on improved services for symptomatic patients or on promoting better health care seeking behaviour [102, 136]. Therefore, and much more than is the case for other treatable STDs, case finding on a thorough screening is the cornerstone of *Chlamydia* control programmes. In theory, detection and treatment of asymptomatic cases should have a major impact on the incidence of chlamydial infection as these asymptomatic individuals are an active source of new infections [107, 133].

In the developed countries, the debate on *Chlamydia* control has centred around the cost-effectiveness of different approaches for identifying infected persons [157]. Studies have concluded that among women case findings using laboratory tests is cost-effective at *C. trachomatis* prevalence rates as different as 2% to 16% [147].

Because the frequencies of PID and male STDs are poorly documented in Hungary [27, 79, 162, 163] the prevalence of sequelae is derived from the literature in our analysis of cost-effectiveness. Financial resources are insufficient to offer *Chlamydia* testing to all women in Hungary. Therefore, selection criteria for testing should be constructed for a specific setting that includes young age between 15-19 years. The results of our analysis revealed that the cost of the sequelae of untreated *C. trachomatis* infections is slightly less without the cost of infertility treatment than the costs of using the ELISA method for detecting of *C. trachomatis* of the age group between 15-19 years, which displayed a 11.4% rate of infection.

Since the majority of the *C. trachomatis*-infected cases were asymptomatic, we suggest for use the ELISA method for screening of all women with evidence of mucopurulent cervicitis and

1) all women of age group between 15-19 years who have had sexual intercourse. We also recommend testing

2) women between 20 to 24 years of age who have not consistently used barrier contraception or have had a new sex partner or more than one sex partner during the past six months and testing

3) the pregnant population before delivery and

4) before an abortion procedure and

5) after spontaneous abortion.

6) Furthermore, we suggest the testing of women after stillbirth using the PCR (with high sensitivity) method for detection of *C. trachomatis* and complete an extra screening for Cytomegalo-Virus, Varicella-Zoster-Virus, Herpes-Simplex-Virus and Parvo-B19-Virus.

7) In every case of detected *C. trachomatis* infection, we suggested the control and possible antibiotic treatment of the partner.

The screening for *HIV* and *Treponema pallidum* is ordained before delivery in Hungary however, the prevalence of these infections are low. The introduction of the screening of *C. trachomatis* can reduce the sequelae of untreated *C. trachomatis* infections and can detect other STDs. This prevention may reduce the number of expensive diagnostic and therapeutic treatment methods [41].

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Orvosi Informatikai Intézet

Tisztelt dr. Nyári Tibor!

Örömmel értesítem, hogy a Szent-Györgyi Albert Orvostudományi Egyetem Doktori és Habilitációs Bizottsága 1998. október 22-én hozott határozata alapján Önt

Ph.D. tudományos fokozattal doktorrá

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A doktori (Ph.D.) oklevél átadására későbbi időpontban, ünnepélyes keretek között kerül sor.

Szeged, 1998. október 29.

Tisztelettel

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