Colonisation of pregnant and puerperal women and neonates with Chlamydia trachomatis

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SUMMARY Chlamydia trachomatis was cultured from cervical specimens of 14 (16·1%) of 231 women applying for legal abortion and from 23 (8·7%) of 273 puerperal women. The chlamydial isolation rate was related to the women's age. Of the pregnant and puerperal women under 20 years C trachomatis was isolated in 10% and 24% respectively; in those aged between 20 and 24 years the rates were 8·7% and 10·2% respectively whereas in those over 24 years the rates were 4·2% in both groups. Chlamydia were isolated more frequently from cervical specimens than from urethral specimens. However, if a cervical specimen alone had been examined the diagnosis would have been missed in three (17%) of 18 women.

IgG antichlamydial antibodies (titre >1/32) were detected by a micro-immunofluorescence test in samples of cord blood from 35 (25%) of 139 infants of the puerperal women. Of the 23 infants born to mothers harbouring chlamydia in the cervix C trachomatis was isolated from the conjunctival folds in five (22·5%). The chlamydial isolation rate from the eyes of the neonates was related to the time of sampling. None of the 108 infants examined between 6 and 7 days old was chlamydia-positive whereas chlamydia could be recovered from the conjunctival folds of four of them when re-examined from three to 23 days later.

Introduction

Chlamydia trachomatis is one of the most common pathogens recovered from the genital tract. The organism is generally transmitted by sexual intercourse. It is a frequent cause of serious complications following primary genital infections both in women (namely, acute salpingitis) and in men (namely, acute epididymitis). Asymptomatic female carriers of C trachomatis occur at least as frequently as those of Neisseria gonorrhoeae. However signs of inflammation are generally present in women with genital chlamydial infection.

Chlamydia may be transmitted from mother to infant at delivery. C trachomatis has been isolated from the eyes, the nasopharynx, the larynx, the lungs, the vagina, and from faecal specimens of infants born to mothers harbouring the organism in the cervix. Such infection of the infant may result in conjunctivitis, which generally runs a mild clinical course. However severe exudative ophthalmitis may occur, and rarely conjunctival scarring and pannus develop as sequelae. In recent years, C trachomatis has also been established as one of the most common causes of neonatal pneumonia. Neonates may also harbour this organism without showing signs of infection.

The present study was performed to: (a) study the incidence of infections with C trachomatis and N gonorrhoeae in different age groups of pregnant (women applying for legal abortion) and puerperal women; (b) determine the presence of IgG antichlamydial antibodies in cord blood of infants of the mothers studied; and (c) investigate chlamydial colonisation in the eyes of infants born to mothers infected with the organism.

Patients and methods

STUDY POPULATION AND SAMPLING

Group 1

Two hundred and thirty-five women applying for legal abortion (group 1) in the cities of Landskrona and Helsingborg between the eighth and thirteenth week of pregnancy were examined for cervical...
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infection with *C. trachomatis*; specimens for culture for *N. gonorrhoeae* were collected from the urethra, the cervix, and the rectum. All the 235 women studied had abortions performed.

**Group 2**
Cervical cultures for *C. trachomatis* were also obtained from 293 puerperal women (group 2) from the city of Landskrona; samples were taken on the sixth or seventh day after delivery. Specimens for chlamydial isolation were also collected from the urethra in 169 of these women. Specimens for isolation of *N. gonorrhoeae* were collected from the urethra, the cervix, and the rectum in 185 women in this group.

**Group 3**
Specimens were collected from the conjunctival folds of the infants born to the first 108 puerperal women studied (group 3) at six or seven days after birth. Of the remaining 185 puerperal women, conjunctival specimens were collected only from those neonates whose mothers had been found to be infected with chlamydia; samples were collected from these infants between 10 and 30 days after birth.

**CULTURE TECHNIQUES**
Specimens for chlamydial isolation were collected with a calcium-alginate swab (Inolex Corp, Glenwood, Illinois) by gentle rotation before withdrawal of the swab from the sampling site. The swabs were transported in a sucrose-phosphate buffer (2-SP), containing 5% fetal calf serum, 10 μg gentamycin, 100 μg vancomycin, and 2.5 μg amphotericin B per ml. Specimens were transported chilled (14°C) to the laboratory, where approximately 95% of them were stored at 4°C for less than 48 hours before inoculation on to tissue cultures. The remaining 5% of specimens had been stored at -20°C before being tested. Chlamydial isolation was made on cycloheximide-treated McCoy cells according to a technique described by Ripa and Mårth, although RPMI 1640 was used as tissue culture medium.

The sampling technique and the methods used for the isolation and identification of *N. gonorrhoeae* were those described elsewhere.

**SEROLOGICAL TESTS**
Specimens of cord blood from 139 of the neonates were tested for IgG antichlamydial antibodies with a modified micro-immunofluorescence (micro-IF) test using pools of antigens as described by Treharne and co-workers.

**TREATMENT**
Women in group 1 found to be infected with chlamydia were given doxycycline 200 mg (Vibramycin, Pfizer) daily for five days followed by 100 mg daily for 10 days; their sexual consorts received the same treatment. Women in group 2 infected with the organism received doxycycline 100 mg daily for 14 days; the same treatment was prescribed for their husbands. Infants with positive chlamydial isolation from the eyes were given erythromycin 20-25 mg/kg body weight (Abboticin, Abbott) twice daily for 10 days and chloromycetin ointment (Chloromycetin, Parke-Davis) locally.

**Results**

**CULTURAL STUDIES**

**Group 1**
In four (1.7%) of the 236 women in group 1 the cervical culture results were inconclusive owing to a non-specific cytopathogenic effect. *C. trachomatis* was isolated from 14 (6.2%) of the remaining 232 women (Table 1). Women of less than 20 years had the highest rate of positive culture results (10%) (Table 1).

*N. gonorrhoeae* was recovered from two (0.9%) women in group 1, one of whom also harboured chlamydia.

**Group 2**
Chlamydial cultures from 20 (6.8%) of the 293 women in this group gave an inconclusive result. *C. trachomatis* was isolated from 23 (8.4%) of the remaining 273 women. Also in this group the isolation rate was related to age (Table 1). Of the women under 20 years 24% harboured the organism compared with 4.4% of those of 25 years or older.

*N. gonorrhoeae* was not isolated from any of the 185 puerperal women.

**TABLE 1 Isolation of Chlamydia trachomatis from the cervix in different age groups of 231 pregnant (group 1) and 273 puerperal (group 2) women**

| Age (yrs) | Group 1* | | Group 2* |
|-----------|----------| |----------|
|           | No | % | No | % |
|≤19        | 2/22 | 10·0 | 6/25 | 24·0 |
|20-24      | 6/69 | 8·7 | 11/108 | 10·2 |
|25-29      | 3/49 | 6·0 | 3/87 | 3·4 |
|≥30        | 3/91 | 3·9 | 3/53 | 5·7 |
|Total      | 14/231 | 6·1 | 23/273 | 8·7 |

* The four women in group 1 and the 20 women in group 2 with inconclusive culture results have been excluded.
Comparison of cervical and urethral cultures (group 2)
The culture results for the 169 puerperal women from whom specimens for chlamydial isolation were collected from both the cervix and the urethra are shown in Table II. Eighteen patients harboured chlamydia—in most instances in the cervix (15 cases), although specimens from the cervix most often caused a non-specific cytopathogenic effect in the tissue cultures.

<table>
<thead>
<tr>
<th>Culture result</th>
<th>Cervix</th>
<th>Urethra</th>
<th>No of positive results from both sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>134</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>151</td>
</tr>
</tbody>
</table>

+ Positive – negative
* The culture result was inconclusive in cervical specimens from 10 patients, in urethral specimens from three patients, and in both specimens from four patients. In one patient the urethral culture was spoiled by bacterial overgrowth but the cervical specimen yielded chlamydia.

Group 3
*C_ trachomatis_ was not isolated in samples from any of the infants of the first 108 puerperal mothers studied when examined at six or seven days after birth. Nine of their mothers harboured chlamydia in the cervix. The infants of these nine mothers were re-examined between 10 and 30 days after birth as were a further 14 infants of the remaining 185 mothers who were infected with *C_ trachomatis_. At this time five infants harboured chlamydia in their conjunctival folds, four of whom were not found to be infected when examined at six or seven days after birth (Table III).

SEROLOGICAL STUDIES
The reciprocal titres of IgG antichlamydial antibodies in the 139 cord blood samples studied are shown in Table IV. Antibodies at a titre of 1/32 or greater were found in 25% of the specimens tested.

Table IV

<table>
<thead>
<tr>
<th>IgG antichlamydial antibodies in cord blood from 139 neonates tested by a micro-immunofluorescence test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antichlamydial antibody (reciprocal of titre)</td>
</tr>
<tr>
<td>≤16</td>
</tr>
<tr>
<td>No of neonates</td>
</tr>
</tbody>
</table>

Discussion
In this study *C_ trachomatis_ was recovered from 6.1% of the pregnant (women applying for legal abortion during the first trimester) and from 8.4% of the puerperal women studied. All these women were inhabitants of the cities of Helsingborg and Landskrona or of the surrounding rural areas (a total of approximately 200 000 inhabitants). These cities are located by the strait of Öresund in a mixed industrial and agricultural area with close contacts with the metropolitan region of Copenhagen. Both groups of women studied comprised consecutive cases treated at the gynaecological departments in these two cities. In Landskrona, which has 51 000 inhabitants, about 400 children are born annually, and during recent years about 600 women have applied annually for legal abortion in the Helsingborg-Landskrona region.

The chlamydial isolation rate in the women in the present study is similar to that reported in pregnant women in the United States (5-13%). These figures are somewhat higher than those for healthy, non-pregnant women who have served as controls in studies of women attending venereal disease clinics; the incidence has usually been less than 5%.

Moreover, we have isolated the organism in 10-30% of women attending venereal disease and gynaecological clinics in the same county (Skåne) as the women in the present study (Mårth et al, unpublished data).

As shown by the present study, the age distribution of the individuals studied influences the isolation rate of *C_ trachomatis_. In our series of sexually active persons the younger the women the more frequently the organism was isolated. This was also true in an earlier study of patients attending a gynaecological clinic in the same hospital catchment region and in a recent study of pregnant women in the United States. The comparatively low prevalence of genital chlamydial infection in the older age groups suggests that such infections are often self-limiting or that these women are less sexually active.

In non-pregnant, non-puerperal women with clinical signs of infection of the lower genital tract *C_ trachomatis_ may be found significantly more often...
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than in those without such signs. However, whether or not a pregnant or puerperal woman harbours chlamydia is extremely difficult to establish merely by clinical examination alone. The high incidence of asymptomatic female carriers of *C trachomatis* indicates that the cervix is an important reservoir for the spread of the organism in the community, as is shown in the present study. None of the chlamydia-positive puerperal women had complained of symptoms suggesting genital infection; this was also true of most of the women who had applied for abortion and who harboured *C trachomatis*.

It has been claimed that *C trachomatis* is a more common cause of sexually transmitted disease than is *N gonorrhoeae*. This claim is supported by the findings in the present study.

A comparison of the results of the cervical and urethral cultures in the puerperal women showed that *C trachomatis* could be recovered most often from cervical specimens. The cervix must therefore be recommended as the sampling site if lack of laboratory facilities restricts the number of specimens that can be investigated. However, a urethral specimen should also be taken if possible from puerperal women, as *C trachomatis* was isolated from the urethra alone in three (17%) of 18 culture-positive women.

The micro-IF test detects species-specific antibodies to *C trachomatis*. Since IgG antibodies pass through the placental barrier it should be possible to study their production in the mother by examining samples of cord blood. The result of our serological study indicated that at least one-third—or 5-6 times as many women as shown by the culture studies—had been exposed to *C trachomatis*. In principle, similar findings have been reported by others.

The risk of a newborn baby acquiring an eye infection due to *C trachomatis*, when the mother is harbouring the organism in the cervix, has been estimated to be between 23 and 60%. In our study, only 22% of the babies of chlamydia-positive mothers harboured chlamydia in their conjunctival folds. Frommel *et al.*, in a study of neonates, reported that nasopharyngeal specimens gave a positive result for chlamydia more frequently than specimens from the eyes. In the present study conjunctival specimens were collected from five of the 23 infants of chlamydia-positive mothers, but all gave a negative result.

The results of our study indicate that the time of sampling influences the isolation rate of chlamydia from the eyes of neonates. Four children in our study were chlamydia-negative at six to seven days after birth but harboured chlamydia in their conjunctival folds when re-examined three to 23 days later. From other studies, the incubation period of ophthalmia neonatorum caused by chlamydia can be as short as a few days. All the infants in our study had been treated prophylactically with silver nitrate on the day of delivery. Although it is known that Crede prophylaxis does not inhibit the growth of *C trachomatis*, it might have prolonged the incubation period and possibly also modified the clinical course.

None of the neonates harbouring chlamydia in their eyes showed such signs of infection as discharge, reddening, bulging, or oedema. This is in contrast to Rees and his co-workers, who found clinical signs of infection in all of 33 infants infected with chlamydia in their eyes. Because the infection was detected early and treatment was begun as soon as the result of the cultures was known, signs of conjunctivitis may have been prevented from developing.

An increasing number of reports from North America and from Europe have shown that *C trachomatis* may cause pneumonia in neonates. This type of neonatal pneumonia, which may occur with or without signs of conjunctivitis, was not diagnosed in any of our children.

Martin and his co-workers recently reported that the relative risk of stillbirth or neonatal death after 20 weeks’ gestation was significantly increased (approximately ten-fold) among chlamydia-positive women compared with chlamydia-negative controls who were matched for age, race, socioeconomic status, and number of pregnancies. Frommel *et al.*, however, did not find any correlation between genital chlamydial infection in pregnant women on one hand and difficulties of labour and delivery, initial condition of the neonate, and postpartum complications on the other.

The incidence of post-abortion and puerperal complications of primary genital chlamydial infections could not be established in the present study, as all carriers of *C trachomatis*, as well as their sexual consorts, were treated with doxycycline. In venereal disease clinic patients, this treatment is known to eradicate *C trachomatis* effectively. However, in another study (Møller *et al.*, unpublished observations) of women who underwent legal abortion and who received no antibiotic treatment, we found retrospectively that acute salpingitis had developed in several patients whose cervical specimens collected before abortion had yielded chlamydia. In some of these patients, we have evidence that the salpingitis was caused by *C trachomatis*. Thus, the diagnosis of genital chlamydial infection in women in whom abortion is performed and treatment with antibiotics if the women are chlamydia-positive are clearly important.
The high incidence of genital chlamydial infection in pregnant women suggests that steps should be taken routinely to detect such infections, particularly in young women. At present, however, limited laboratory resources make this difficult. Further studies are also needed before any recommendations can be made as to whether or not antibiotic treatment should be given to all pregnant women who are found to be carriers of *C. trachomatis*.

The infants harbouring chlamydia in their eyes were treated with oral erythromycin and chloromycetin ointment to prevent bacterial secondary infections as soon as the culture results were known. No control cultures were performed, but none of the infants developed signs of conjunctivitis or pneumonia during the follow-up period of three to 18 months. Although chlamydial conjunctivitis and pneumonia seem generally to be self-limiting the risk that the infant might develop severe eye infection, or even sequelae, seems to justify general treatment with erythromycin in infants in whom *C. trachomatis* has been recovered. Viral pneumonia during infancy holds a high risk of respiratory-tract complications during later life,123 whether or not this is also true of pneumonia caused by *C. trachomatis* is not known. Antibiotic treatment may also prevent the transmission of chlamydia to other members of the family.24

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References

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