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Original Article

Chlamydial Infections in Term and Preterm Neonates

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SUMMARY: The aim of this study was to evaluate the incidence and morbidities of *Chlamydia trachomatis* infections in newborn infants. Tissue culture and direct immunofluorescence (DIF) tests were used to detect the presence of nasopharyngeal *C. trachomatis* infection in 35 preterm and 21 healthy term neonates. All infants were followed up clinically for 3 months, and enzyme-linked immunosorbent assay analysis for serum antichlamydial IgG and IgM was performed on day 15 and week 6. Tissue culture and/or DIF studies showed that 10 of the preterm infants (28.57%), but none of the term infants, were *C. trachomatis*-positive. The sensitivities of DIF and tissue culture were 40% and 70%, respectively, demonstrating the diagnostic superiority of tissue culture tests for detecting *C. trachomatis*. Only one asymptomatic preterm infant was found to be positive for antichlamydial antibodies at the 6th week. All *C. trachomatis*-positive infants were given macrolide antibiotics for 14 days. The study showed that male infants were more frequently infected, but types of delivery, mean gestational ages, mean birth weights, and the need for mechanical ventilation were similar in *C. trachomatis*-infected and uninfected preterm infants. However, the duration of oxygen treatment was longer in infected preterm infants. Clinical conjunctivitis was more frequent in *C. trachomatis*-infected infants (60%) than in uninfected infants (24%). *C. trachomatis*-positive infants had pneumonia more frequently; however, all patients with pneumonia were negative for antichlamydial IgM and IgG antibodies. Macrolide treatment for 2 weeks for nasopharyngeal *C. trachomatis* positivity may have prevented *C. trachomatis* related pneumonia, but it may not have significantly influenced the risk of pneumonia caused by other agents. Chlamydial infections may lead to early and late respiratory problems in preterm infants. Nasopharyngeal screening may help physicians detect *C. trachomatis* infections and provide a means of early diagnosis in this vulnerable patient group.

INTRODUCTION

Chlamydia trachomatis is probably one of the most prevalent sexually transmitted agents. The exact incidence of cervical infection caused by *C. trachomatis* is not known due to the frequently asymptomatic nature of the disease (1). A high prevalence of chlamydial antibodies was reported in pregnant, nonpregnant, symptomatic, and asymptomatic adult women in Bangladesh (2). In that study, the overall seropositivity was 21.6% (16/74) in symptomatic cases and 44.1% (15/34) in asymptomatic cases. Therefore, routine screening for chlamydial infections is suggested to prevent serious complications in all sexually active symptomatic and asymptomatic women, including pregnant women. A study conducted in our center reported a cervical *C. trachomatis* infection rate of 23% in asymptomatic expectant women (3).

Chlamydial infection in pregnancy may cause stillbirth and preterm delivery (4,5). Chlamydial infection is associated with intrapartum fever and late postpartum endometritis (6). Among infants born to mothers with untreated cervical *C. trachomatis* infection or colonization, 20–50% have clinical conjunctivitis and 10–20% have pneumonia (7). Very low birthweight preterm infants with respiratory distress syndrome are reported to show biphasic clinical features if infected with *C. trachomatis* (8). Perinatally acquired *C. trachomatis* pneumonia is mostly seen between 4–11 weeks, and *C. trachomatis* is responsible for 25–45% of all pneumonia cases in patients aged less than 6 months (9). In a previous study in our center, 21 of 51 pneumonia patients (41%) aged 1 month to 1 year had chlamydial antigen positivity, of which 15 were positive for chlamydial IgM (10).

The most common methods for diagnosing *C. trachomatis* infection are antigen detection (direct immunofluorescence [DIF] and enzyme immunoassay [EIA] methods), agent detection (tissue culture and polymerase chain reaction methods), and detection of specific IgM and IgG antibodies (serologic tests). DIF is reported to be more sensitive than EIA in antigen detection; both methods are cost-effective and rapid. Considering tissue culture as the gold standard, the sensitivity, specificity, and positive predictive value of DIF have been reported to be 93%, 98%, and 93%, respectively (11). Improper sampling, transportation, and previous antibiotic therapy may negatively affect tissue culture results (12). Serological results are negative in superfi-

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cial infections such as conjunctivitis and nasopharyngeal infections, but positive in pneumonia and in gastrointestinal infections (13).

We studied 21 term and 35 preterm infants within the first 3 months of life to evaluate the incidence and morbidities of *C. trachomatis* infection in this patient group.

MATERIALS AND METHODS

Patients: In this 3-month prospective study in 2004, 35 preterm infants, admitted to Ege University Faculty of Medicine Neonatal Intensive Care Unit, and 21 healthy term infants were recruited after obtaining parental consent. Infants with congenital malformations were excluded. Demographic characteristics and clinical features during hospitalization were recorded, and all infants enrolled in the study were closely followed up for 3 months to screen for the development of conjunctivitis and pneumonia.

Infants with positive-nasopharyngeal *C. trachomatis* results were treated with macrolide antibiotics for 2 weeks.

Diagnosis: (i) Sample collection and transport to laboratory: Nasopharyngeal swabs were taken between the 48th and 72th hours of life and inoculated on chlamydia transport media consisting of 2 ml phosphate buffer with fetal bovine serum, aminoglycoside, and amphotericin B to prevent bacterial and fungal contamination. Specimens were rapidly transferred to the laboratory. The swabs were taken out rapidly, and centrifuged samples were protected at $-80°C$ until further studies and were subsequently used for shell-vial culture and preparation of slides for DIF.

(ii) DIF test: All samples were evaluated with DIF before inoculation for cell culture. The presence of epithelial cells and mucus was evaluated to determine if the sample was obtained correctly. A commercial DIF antibody test (MicroTrak; Syva Co., Palo Alto, Calif., USA) was used to detect chlamydial antigens using a high-performance fluorescence microscope. This technique depends on special chlamydial monoclonal antibodies fixed with fluorescein isothiocyanate.

(iii) Cell culture: Freshly trypsinized McCoy cells treated with cycloheximide were used for shell-vial culture. Cells were stained with the same fluorescence-labeled antibodies as above after incubation for 72 h and were evaluated for the presence of chlamydial inclusions and elementary bodies. Positive and negative controls were used.

(iv) Serology: Blood samples were taken from preterm infants on the 15th day and the 6th week of life and used for detection of antichlamydial IgM and IgG antibodies. If pneumonia was diagnosed, new blood samples were obtained from patients after diagnosis. Sera from blood samples were conserved at $-20°C$ until further study.

Species-specific antichlamydial IgM antibodies were tested with an enzyme-linked immunosorbent assay (Euroimmun AG, Lübeck, Germany) using native major outer membrane protein antigen purified from cells infected with *C. trachomatis* serotype K. IgG antibodies were similarly detected with antihuman IgG (enzyme conjugate), and the color change was evaluated by a photometric method.

Statistical analysis: Statistical analysis was performed with the SPSS 10.0 statistical software program. Data sets were compared using the Mann-Whitney U, chi-square, and Fischer exact tests.

RESULTS

Patients: The demographic data of preterm and term infants are given in Table 1. A total of 56 infants (35

Table 1. Demographic characteristics of study group patients ($n = 56$)

	Term infant ($n = 21$)	Preterm infant ($n = 35$)
Maternal age (y)[1)]	26.42 ± 2.70	29.41 ± 5.20
Gestational age (week)[1)]	39.45 ± 0.73	31.45 ± 2.42
Birth weight (g)[1)]	3,268 ± 407	1,633 ± 463
Gender (M/F)	12/9	17/18
Vaginal delivery (%)	100 (21/21)	17.14 (6/35)
Membrane rupture time (h)[1)]	5.55 ± 2.14	25.71 ± 16.21
Tocolytic treatment (%)	0	42.85 (15/35)
Betamethasone treatment rate (%)	0	48.57 (17/35)
Duration of hospitalization (days)	0	13.71 ± 8.85
IUGR rate (%)	0	20 (7/35)
Oxygen treatment (%)[2)]	0	85.71 (30/35)
Duration of oxygen treatment (h)[1)]	0	93.26 ± 77.17
Mechanical ventilation (%)[2)]	0	45.71
Duration of mechanical ventilation (h)[1)]	0	71.07 ± 48.73
Conjunctivitis (%)[3)]	14.28 (3/21)	34.28 (12/35)
Pneumonia in infancy (%)[3)]	0	31.42 (11/35)
Exitus (%)	0	8.57 (3/35)

[1)]: mean ± SD.
[2)]: 45.71% (16/35) of preterm infants received both oxygen and mechanical ventilation.
[3)]: 17.14% (6/35) of preterm infants had both conjunctivitis and pneumonia.
IUGR, intrauterine growth retardation.

preterm, 21 term) were studied. There were 18 girls and 17 boys in the preterm group and 9 girls and 12 boys in the term group. The mean gestational age of the preterm infants was 31.45 ± 2.42 weeks and the mean birth weight was 1,633 ± 463 g. The mean gestational age and birth weight of term infants were 39.45 ± 0.73 weeks and 3,268 ± 407 g, respectively.

Diagnosis of *C. trachomatis* infection: The diagnostic criteria for *C. trachomatis* infection in infants are shown in Table 2. All 10 infants who were *C. trachomatis*-positive were preterm, and none of the term infants showed *C. trachomatis* contamination or infection, thus indicating a positive statistical relationship between preterm birth and *C. trachomatis* infection ($P < 0.05$). However, infected preterm infants (n = 10/35, 28.38%) were not different from uninfected preterm infants (n = 25/35, 71.42%) in terms of gestational age (31.62 ± 2.71 versus 31.44 ± 2.36 weeks) ($P > 0.05$).

Table 2. Diagnostic test results of infected infants

Case no.	DIF positivity at birth	Tissue culture positivity at birth	Antichlamydial antibody at 2 and 6 weeks
1	Positive	Negative	Negative/negative
2	Positive	Negative	Negative/negative
3	Negative	Positive	Negative
4	Positive	Positive	Negative
5	Negative	Positive	Negative
6	Negative	Positive	Negative
7	Positive	Positive	Negative
8	Negative	Positive	Negative
9	Negative	Positive	Negative
10[1)]	Negative/positive	Negative	Negative/positive

[1)]: Reevaluation of negative DIF test was performed at 6 weeks and found positive.

As shown in Table 2, 2 of these 10 infected infants showed *C. trachomatis* positivity in DIF analysis of nasopharyngeal samples, 2 infants showed *C. trachomatis* positivity in both the DIF analysis and the tissue culture test, and 5 infants showed *C. trachomatis* positivity in the tissue culture test alone. One infant, who was found to be positive for *C. trachomatis* IgG at 6 weeks, showed negative results for the nasopharyngeal swab test at birth and negative serological results at the 2nd week of life. The reevaluation of this asymptomatic infant's nasopharyngeal swab with a DIF test at the 6th week yielded a *C. trachomatis*-positive result. This result may be explained by the presence of antigen at levels below the detection limit in the earlier samples.

Two infants, who had negative tissue culture results but positive DIF results in the early time points, were treated with macrolides antenatally or soon after birth.

Clinical characteristics of infants with *C. trachomatis* infection: Clinical characteristics of the infected and uninfected groups of preterm infants are shown in Table 3. The incidence of intrauterine growth retardation (IUGR) was 0% in the term group (Table 1); but it was 20% (7/35) in the preterm group. However, the incidence of *C. trachomatis* infection was not related to the intrauterine growth status of the infants.

Among the 10 *C. trachomatis*-infected preterm infants, 8 (80%) were males and 2 (20%) were females ($P < 0.05$).

Maternal age, use of tocolytics and betamethasone, and type of delivery were not related to the presence of neonatal *C. trachomatis* infection (Table 1).

Thirty out of 35 preterm infants (85.71%) received oxygen treatment with a mean duration of 93.26 ± 77.17 h. The 10 infants with *C. trachomatis* infection needed oxygen treatment for a mean duration of 124.83 ± 93.12 h. There was no significant difference in oxygen requirement between infected and uninfected

Table 3. Demographic characteristics of infected and uninfected preterm infants (n = 35)

	Infected[2)] (n = 10)	Uninfected (n = 25)	P
Maternal age (y)[1)]	28.61 ± 3.62	29.80 ± 4.14	NS
Gestational age (week)[1)]	31.62 ± 2.71	31.44 ± 2.36	NS
Birth weight (g)[1)]	1,634.01 ± 556.58	1,620.04 ± 429.50	NS
Gender (M/F)	8/2	9/16	<0.05
Vaginal delivery (%)	10 (1/10)	20 (5/25)	NS
Membrane rupture time (h)[1)]	14.62 ± 5.11	30.68 ± 14.60	NS
Tocolytic treatment (%)	50 (5/10)	40 (10/25)	NS
Betamethasone treatment rate (%)	50 (5/10)	48 (12/25)	NS
Duration of hospitalization (days)	16.52 ± 8.68	13.29 ± 8.41	NS
IUGR rate (%)	20 (2/10)	20 (5/25)	NS
Oxygen treatment (%)	100 (10/10)	80 (20/25)	NS
Duration of oxygen treatment (h)[1)]	124.83 ± 93.12	84.48 ± 65.28	<0.05
Mechanical ventilation (%)	50 (5/10)	36 (9/25)	NS
Duration of mechanical ventilation (h)[1)]	86.17 ± 57.72	62.77 ± 43.81	NS
Conjunctivitis (%)	60 (6/10)	24 (6/25)	<0.05
Pneumonia in infancy (%)	60 (6/10)	20 (5/25)	<0.05
Exitus (%)	0	3	<0.05

[1)]: mean ± SD.
[2)]: Clarithromycin: a macrolide antibiotic was given to all infected patients. One patient antenatally had clindamycin due to maternal indication.
IURG, intrauterine growth retardation; NS, not significant statistically.

Table 4. Characteristics of preterm infants who had pneumonia at an early stage of infancy ($n = 11$)

Parameter	
Age at diagnosis (days)	21.63 ± 10.59
Apnea (%) ($n = 4$)	36.36
Conjunctivitis (%) ($n = 6$)	54.55
Rales (%)	100
X ray findings ($n = 11$)	
Bronchopneumonia (%)	54.55
Hyperinflation + infiltration (%)	36.36
Lober pneumonia (%)	9.09
Leukocyte count (/mm³) (mean ± sd)	8109.10 ± 3418.02
Eosinophilia (%)	0
Antichlamydial antibodies (%)	0

patients ($P > 0.05$). However, the duration of oxygen treatment was significantly longer in infected preterm infants (124.83 ± 93.12 h) than in uninfected preterm infants (84.48 ± 65.28 h) ($P < 0.05$). None of the term infants needed oxygen treatment (Tables 1 and 3).

The need and duration of mechanical ventilation were similar between infected and uninfected preterm infants. Five out of 10 (50%) infected infants and 9 out of 25 (36%) uninfected preterm infants received ventilation ($P > 0.05$); the duration of mechanical ventilation was 86.17 ± 57.72 h versus 62.77 ± 43.81 h, respectively ($P > 0.05$).

Eleven out of the 35 preterm infants (31.42%), but none of the term infants, showed clinical and radiological pneumonia-related findings at follow-up. The characteristics of the preterm infants who developed pneumonia at an early stage of infancy are shown in Table 4.

Six out of the 11 infants (54.54%) with pneumonia showed *C. trachomatis* positivity in nasopharyngeal samples, but none of them had *C. trachomatis* IgM or IgG antibodies at either 2 or 6 weeks. This superficial *C. trachomatis* infection was related to increased risk of pneumonia with other agents ($P < 0.05$). Only 3 pneumonia patients had *C. trachomatis*-positive blood cultures, and *Klebsiella pneumoniae* was isolated in all of them.

Clinical conjunctivitis was found in 12 (34.28%) preterm infants, 6 of whom had *C. trachomatis*-positive nasopharyngeal swabs. Three term infants had conjunctivitis, but none of them were *C. trachomatis* positive.

None of the preterm infants developed bronchopulmonary dysplasia (BPD).

Three preterm infants (8.57%) died in the first 2 weeks of life, 2 due to necrotizing enterocolitis, and 1 due to heart failure caused by patent ductus arteriosus unresponsive to treatment. Mortality was not found to be related to *C. trachomatis* infection.

DISCUSSION

In this prospective study, 35 preterm infants and 21 term infants were evaluated in order to determine the postnatal influence of *C. trachomatis* infection on term and preterm infants during the first 3 months of life. None of the term infants, but 10 of the 35 (28.57%) preterm infants, were found to be *C. trachomatis* positive using DIF, tissue culture, and IgG and IgM antibody tests. Our study results showed higher *C. trachomatis* positivity in preterm infants than in term infants, but the IUGR rates were not significantly higher in preterm infants, although maternal *C. trachomatis* infection has been reported to cause to IUGR (14).

Chlamydial infections have been reported in 23% of pregnant Turkish women in our region (3). *C. trachomatis* causes a sexually transmitted infection that may lead to urethritis, cervicitis, endometritis, pelvic inflammatory disease, acute urethral syndrome, preterm delivery, and neonatal conjunctivitis and pneumonia in young infants. Although young maternal age is reported to be a risk factor (15), our study did not find this correlation, a fact that can be attributed to Turkish social values, which shun adolescent extramarital sexual relations.

In our study group, the frequency of *C. trachomatis* infection was higher in males (male/female, 8/2). Chlamydial infection-associated pneumonia is more frequently reported in males (male/female, 2.6/1) (16), and male infants are known to be more prone to neonatal sepsis (17).

The rates of preterm premature rupture of membranes and preterm deliveries are reported to be higher (4,18,19). In comparison with the corresponding values in healthy mothers and term infants, maternal *C. trachomatis* anti-IgM positivity is much higher and the mean gestational age is shorter for infants hospitalized in neonatal intensive care units (20).

In another study analyzing 41 mothers with preterm labor and their 50 newborn infants, 12 mothers were found to be serologically positive (29%), and 5 mothers had *C. trachomatis* DNA in cervical specimens. Eighteen neonates were born to the 14 mothers with positive serological results and/or *C. trachomatis* DNA. Chlamydial DNA was detected in specimens from 10 of these 18 neonates (55.5%), and 3 infants had cord blood *C. trachomatis* IgM antibodies. The authors suggested that the mothers and their preterm babies may benefit from screening for active *C. trachomatis* infections (21).

We have not observed a relationship between the type of delivery and the incidence of *C. trachomatis* infection. This study group, although limited in number, had 2 *C. trachomatis*-positive infants born by Caesarean section to mothers with intact membranes, implying that antenatal infection with *C. trachomatis* may not be such a rare occurrence. Vaginal delivery and prolonged rupture of membranes (PROM) are reported to increase the risk of *C. trachomatis* transmission (22). However, there is data showing that infants born by Caesarean section without PROM may also be infected (23–25).

Preterm infants nasopharyngeally infected with *C. trachomatis* showed a 60% incidence of conjunctivitis, compared to an incidence of 24% in uninfected preterms; however, only 14.28% of term infants had conjunctivitis, and the causative agent in none of the cases was *C. trachomatis*. The incidence of conjunctivitis is reported to be 45–47% in infants born to mothers infected with *C. trachomatis*, as opposed to 14–18.3% in infants born to healthy mothers (26,27).

None of the *C. trachomatis*-infected preterm infants in our study developed BPD; however, despite similar durations of mechanical ventilation, these infants required oxygen treatment for a longer mean duration

than in uninfected preterm infants. The increased oxygen requirement in our infected preterm infants may indicate an increased degree of lung damage.

Biphasic neonatal respiratory distress syndrome is reported in very low-birth weight preterm infants with *C. trachomatis* infection (8). An increased incidence of BPD is reported in preterm infants whose mothers were infected with *C. trachomatis*, as also in those infected with *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma pneumoniae*, and adenovirus (28–30).

The duration of hospitalization and mortality in preterm infant group infected with nasopharyngeal *C. trachomatis* was similar to those in the uninfected preterm infant group. The similar outcomes in the infected and uninfected preterm infants may have resulted from the early diagnosis of *C. trachomatis* infection and the use of macrolide treatment over a 2-week period. One study on the morbidities of preterm infants born to *C. trachomatis* infected and uninfected mothers showed similar outcomes (31). In two other studies, the poorer mortality outcome of *C. trachomatis* infected neonates was reported to be associated with preterm delivery (32,33).

In our study, none of the term infants from the study group developed pneumonia during the study period. However, 11 prematurely born infants (6 of whom had been treated with macrolides) developed pneumonia in the first 3 months. Chlamydial IgM and IgG antibodies were not detected in any of the 11 pneumonia patients. All infants with nasopharyngeal *C. trachomatis* infections were *C. trachomatis* antibody-negative at the 2nd and 6th weeks. Two weeks of macrolide treatment for nasopharyngeal *C. trachomatis* positivity may have prevented *C. trachomatis* related pneumonia, but not pneumonia caused by other agents. In a study conducted in Nairobi, Kenya, a very high risk of chlamydial nasopharyngeal colonization and a high frequency of late-onset neonatal pneumonia were reported (34). Chlamydial infections are frequently detected along with infections of *U. urealyticum* and other agents. In one report, 29 of 61 infants with *C. trachomatis*-associated pneumonia also had co-infections (35). These authors also indicated that infants infected solely with *C. trachomatis* needed less mechanical ventilation support than infants infected with mixed infectious agents. Most infants with pneumonia had long-term respiratory problems, regardless of the cause.

DIF is a practical and commonly used rapid diagnostic tool; however, tissue culture is still accepted as the gold standard. In our study group, DIF sensitivity was 4/10 (40%) and tissue culture positivity was 7/10 (70%), showing the superiority of tissue culture over DIF in detecting *C. trachomatis* infection. Notably, 2 infected infants with negative tissue culture had been prescribed antibiotics (clindamicin and clarithromycin) prior to sample collection, which may have prevented the culture positivity as previously reported (12).

Chlamydial infection may lead to preterm delivery, and early and late morbidities are more prevalent in preterm infants than in term infants. Therefore, routine nasopharyngeal screening with sensitive and affordable rapid diagnostic tests may help clinicians diagnose *C. trachomatis* infection earlier, and help treat this high-risk infection in preterm infants.

Conflict of interest None to declare.

REFERENCES

1. Dereli, D., Ertem, E. and Serter, D. (1991): Urogenital *Chlamydia trachomatis* infections. Mikrobiyol. Bült., 25, 277–284 (in Turkish).
2. Mahmud, N.U., Hossain, M.A., Nahar, K., et al. (2011): Seroprevalence of genital *Chlamydia trachomatis* infection in women of reproductive age. Mymensingh Med. J., 20, 187–191.
3. Dereli, D., Serter, D., Ertem, E., et al. (1995): Investigation of servical *Chlamydia trachomatis* infection in pregnants. Mikrobiyol. Bült., 29, 261–264 (in Turkish).
4. Claman, P., Toye, B., Peeling, R.W., et al. (1995): Serologic evidence of *Chlamydia trachomatis* infection and risk of preterm birth. Can. Med. Assoc. J., 153, 259–262.
5. Gencay, M., Koskiniemi, M., Ammälä, P., et al. (2000): *Chlamydia trachomatis* seropositivity is associated both with stillbirth and preterm delivery. APMIS, 108, 584–588.
6. Wager, G.P., Martin, D.H., Koutsky, L., et al. (1980): Puerperal infectious morbidity: relationship to route of delivery and to antepartum *Chlamydia trachomatis* infection. Am. J. Obstet. Gynecol., 138, 1028–1033.
7. Smith, J.R. and Robinson, D.T. (1993): Infection due to *Chlamydia trachomatis* in pregnancy and the newborn. Baillière's Clin. Obstet. Gynaecol., 7, 237–255.
8. Sollecito, D., Midulla, M., Bavastrelli, M., et al. (1992): *Chlamydia trachomatis* in neonatal respiratory distress of very preterm babies: biphasic clinical picture. Acta Paediatr., 81, 788–791.
9. Tipple, M.A., Beem, M.O. and Saxon, E.M. (1979): Clinical characteristics of the afebrile pneumonia associated with *Chlamydia trachomatis* infection in infants less than 6 months of age. Pediatrics, 63, 192–197.
10. Dereli, D., Coker, M., Ertem, E., et al. (1996): Chlamydial infection in infants. J. Trop. Pediatr., 42, 233–236.
11. Paisley, J.W., Lauer, B.A., Melinkovich, P., et al. (1986): Rapid diagnosis of *Chlamydia trachomatis* pneumonia in infants by direct immunofluorescence microscopy of nasopharyngeal secretions. J. Pediatr., 109, 653–655.
12. Hammerschlag, M.R., Roblin, P.M., Cummings, C., et al. (1987): Comparison of enzyme immunoassay and culture for diagnosis of chlamydial conjunctivitis and respiratory infections in infants. J. Clin. Microbiol., 25, 2306–2308.
13. Schachter, J., Grossman, M. and Azimi, P.H. (1982): Serology of *Chlamydia trachomatis* in infants. J. Infect. Dis., 146, 530–535.
14. Germain, M., Krohn, M.A., Hillier, S.L., et al. (1994): Genital flora in pregnancy and its association with intrauterine growth retardation. J. Clin. Microbiol., 32, 2162–2168.
15. Ottesen, M., Sahl, I., Herbstman, M.M., et al. (1996): *Chlamydia trachomatis* in pregnant women in the county of Vestsjaelland. Prevalence, prevention of perinatal transmission and cost-effectiveness of screening. Ugeskr. Laeger., 158, 756–758 (in Danish).
16. Puthavathana, P., Habanananda, S., Wasi, C., et al. (1994): Incidence of *Mycoplasma pneumoniae*, *Chlamydia trachomatis*, and viral infections in pneumonia cases under six months of age, Bangkok, Thailand. Southeast Asian J. Trop. Med. Public Health, 25, 657–663.
17. Washburn, T.C., Medearis, D.N., Jr. and Childs, B. (1965): Sex differences in susceptibility to infections, 35, 57–64.
18. Sweet, R.L., Landers, D.V., Walker, C., et al. (1987): *Chlamydia trachomatis* infection and pregnancy outcome. Am. J. Obstet. Gynecol., 156, 824–833.
19. Martin, D.H., Koutsky, L., Eschenbach, D.A., et al. (1982): Prematurity and perinatal mortality in pregnancies complicated by maternal *Chlamydia trachomatis* infections. JAMA, 247, 1585–1588.
20. Gencay, M., Koskiniemi, M., Saikku, P., et al. (1995): *Chlamydia trachomatis* seropositivity during pregnancy is associated with perinatal complications. Clin. Infect. Dis., 21, 424–426.
21. Gencay, M., Koskiniemi, M., Fellman, V., et al. (2001): *Chlamydia trachomatis* infection in mothers with preterm delivery and in their newborn infants. APMIS, 109, 636–640.
22. Hammerschlag, M.R. (1989): Chlamydial infections. J. Pediatr., 114, 727–734.
23. Attenburrow, A.A. and Barker, C.M. (1985): Chlamydial pneumonia in the low birthweight neonate. Arch. Dis. Child., 60,

1169–1172.
24. Evsiukova, I.I., Patrusheva, E.N. and Savicheva, A.M. (1995): Current problems in the clinical course, diagnosis and treatment of *Chlamydia* infection in newborns. Akush. Ginekol. (Mosk), 1, 18–21 (in Russian).
25. Bell, T.A., Stamm, W.E., Kuo, C.C., et al. (1994): Risk of perinatal transmission of *Chlamydia trachomatis* by mode of delivery. J. Infect., 29, 165–169.
26. Shen, L., Wu, S. and Liu, G. (1995): Study on the perinatal infection caused by *Chlamydia trachomatis*. Chin. J. Obstet. Gynecol., 30, 714–717 (in Chinese).
27. Preece, P.M., Anderson, J.M. and Thompson, R.G. (1989): *Chlamydia trachomatis* infection in infants: a prospective study. Arch. Dis. Child., 64, 525–529.
28. Da Silva, O., Gregson, D. and Hammerberg, O. (1997): Role of *Ureaplasma urealyticum* and *Chlamydia trachomatis* in development of bronchopulmonary dysplasia in very low birth weight infants. Pediatr. Infect. Dis. J., 16, 364–369.
29. Couroucli, X.I., Welty, S.E., Ramsay, P.L., et al. (2000): Detection of microorganisms in the tracheal aspirates of preterm infants by polymerase chain reaction: association of adenovirus infection with bronchopulmonary dysplasia. Pediatr. Res., 47, 225–232.
30. Colaizy, T.T., Morris, C.D., Lapidus, J., et al. (2007): Detection of ureaplasma DNA in endotracheal samples is associated with bronchopulmonary dysplasia after adjustment for multiple risk factors. Pediatr. Res., 61, 578–583.
31. Alger, L.S., Lovchik, J.C., Hebel, J.R., et al. (1988): The association of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and group B streptococci with preterm rupture of the membranes and pregnancy outcome. Am. J. Obstet. Gynecol., 159, 397–404.
32. Goldenberg, R.L., Andrews, W.W., Yuan, A.C., et al. (1997): Sexually transmitted diseases and adverse outcomes of pregnancy. Clin. Perinatol., 24, 23–41.
33. Nyári, T., Woodward, M., Mészáros, G., et al. (2001): *Chlamydia trachomatis* infection and the risk of perinatal mortality in Hungary. J. Perinat. Med., 29, 55–59.
34. Were, F.N., Govedi, A.F., Revathi, G., et al. (2002): Chlamydia as a cause of late neonatal pneumonia at Kenyatta National Hospital, Nairobi. East. Afr. Med. J., 79, 476–479.
35. Brasfield, D.M., Stagno, S., Whitley, R.J., et al. (1987): Infant pneumonitis associated with cytomegalovirus, *Chlamydia, Pneumocystis*, and *Ureaplasma*: follow-up. Pediatrics, 79, 76–83.