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Chlamydia trachomatis-associated respiratory disease in the very early neonatal period

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Of 103 preterm neonates admitted consecutively to the neonatal intensive care unit soon after birth for respiratory distress, 8 were found to be *Chlamydia trachomatis*-positive as early as within the first 24 h of life . All these patients required mechanical ventilation and supplemental oxygen. Six infants had evidence on chest radiographs of hyaline membrane disease, one of pneumonia, and one of slight bilateral parenchymal changes. Our results suggest that the presence of *C. trachomatis* in preterm infants with neonatal respiratory distress is probably not an infrequent event. □Chlamydia trachomatis, *preterm infant, respiratory distress*

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Knowledge of the importance of *Chlamydia trachomatis* in human respiratory disease has expanded greatly in the past 20 years, beginning with the description in 1975 by Schachter of a $7\frac{1}{2}$-week-old infant who had previously had conjunctivitis and who had an afebrile interstitial pneumonia associated with isolation of *C. trachomatis* from a throat culture (1). During this time period the organism has been recognized to be a common cause of afebrile pneumonia in early infancy (2, 3). However, the extent and place of *C. trachomatis* in the neonatal respiratory disease is incompletely understood (3). Yet, much less is known about *C. trachomatis*-associated disease of the respiratory tract in the very early neonatal period (4, 5). We report here on the detection of *C. trachomatis* as early as within the first 24 h of life from the respiratory tract of a number of preterm infants with respiratory distress.

During the course of an ongoing prospective study on the pathogenic potential of *Ureaplasma urealyticum* in the respiratory tract of preterm infants (6), 103 preterm neonates with birth weights of 2000 g or below and acute respiratory distress who were consecutively admitted soon after birth to the neonatal intensive care unit (NICU) at the University of Rome from February 1993 to June 1995 were evaluated for *C. trachomatis*. Our NICU is the major referral centre for high-risk premature infants born in the Rome area. Data on maternal and perinatal risk factors (ruptures of membranes, maternal fever, perinatal asphyxia and Apgar scores) were obtained by chart abstraction. The gestational age of all infants was determined using the Dubowitz score. The study population included 74 preterm infants weighing 1300 g or less, and 29 weighing 1301–2000 g. Nasopharyngeal, ocular and tracheal samples for detection of *C. trachomatis* were obtained from all 103 infants within the first day of life, during the first postnatal week, and whenever the patient's respiratory condition deteriorated. Thin wire-stemmed calcium alginate swabs, pretested for lack of toxic effects to *C. trachomatis*, were used to sample both lower conjunctivae,and the posterior nasopharynx through both nostrils. Swabs were placed in 2-SP transport medium. Tracheal aspirates were collected by mechanical suction through a catheter passed into the distal trachea through an endotracheal tube, and immediately placed into the transport medium. Specimens were assessed by both the direct immunofluorescence test and cell culture isolation. For direct immunofluorescent staining (7) of freshly collected specimens, fluorescein isothiocyanate was conjugated with monoclonal antibodies raised against the major outer membrane protein present in all 15 known human serovars of *C. trachomatis* (Syva MicroTrak). The test was considered positive for *C. trachomatis* if at least five fluorescing elementary bodies were found in the specimen (7). For isolation of chlamydiae, cyclohexiimide-treated McCoy cells were used according to the method of Ripa and Mardh (8). Cultures were incubated for 48–72 h at 37 °C, and the findings were read after staining with the same fluorescein-conjugated monoclonal antibody.

Tracheal aspirates were cultured for mycoplasmas, viruses, aerobic and anaerobic bacteria. Nasopharyngeal samples were cultured for viruses and mycoplasmas. In

addition, blood was cultured for aerobic and anaerobic bacteria, and mycoplasmas. For isolation of mycoplasmas, specimens were inoculated into urea and arginine broths, and subcultured into A7 agar medium. *Mycoplasma hominis* and *U. urealyticum* were respectively identified by the hydrolysis of arginine and urea, and their characteristic colonial morphologies. Monolayers of primary rhesus monkey kidney cells and human embryonic lung fibroblasts were inoculated for isolation of viruses. Blood samples for aerobic and anaerobic bacteria were placed into Isolator 1.5 microbial tubes (Merck) and processed according to the manufacturer's instructions. Tracheal samples for aerobic and anaerobic bacteria were respectively inoculated into brain heart infusion broth and enriched thioglycolate broth.

C. trachomatis was detected by both the direct immunofluorescence test and cell culture isolation in 15 (14.5%) of the 103 investigated preterm neonates. Eight of these 15 infants appeared *C. trachomatis*-positive as early as within the first 24 h of life, and the remaining 7 at the age of 12–60 days (mean age, 31 ± 21 days). Of the eight neonates with very early detection of *C. trachomatis*, two were found to be positive in both tracheal and nasopharyngeal specimens, two in tracheal aspirates alone, three in nasopharyngeal specimens alone, and one in tracheal as well as in nasopharyngeal and ocular specimens. Of these eight infants one (case 4, Table 1) had associated tracheal isolation of *U. urealyticum*, but none had positive blood cultures for bacteria as well as positive tracheal and nasopharyngeal cultures for bacteria and viruses.

The demographic and clinical characteristics of these very early detected cases are shown in Table 1. Six infants were delivered by Caesarean section, and two of them with intact fetal membranes. Premature (>12 h) rupture of membranes occurred in two infants. One infant (case 7, Table 1) was exposed to antenatal steroids. Exogenous surfactant was used in five of the eight infants. On admission, all these patients presented with acute respiratory disease requiring mechanical ventilation and supplemental oxygen. Four of them developed later severe apnoeic spells. Six infants had evidence on chest radiographs of hyaline membrane disease, one of pneumonia (9), and one of slight bilateral parenchimal changes. Laboratory findings included a normal white blood cell count in all of these patients,and an increase in the number of eosinophils (>600 mm^3) in three of them. Among these eight cases there were three infants who died within postnatal day 2 because of intraventricular haemorrhage ($n = 2$) and hypertensive pneumothorax ($n = 1$). No attempts were made to demonstrate *C. trachomatis* in the lungs of these infants at autopsy. Of the five surviving infants,who were tested for *C. trachomatis* by both immunofluorescence and culture methods 1 week after completion of a 14 day oral course with erythromycin (50 mg kg^{-1} day^{-1} in four divided doses), four had subsequent clinical improvement and eradication of the organism. The last one required a retreatment with a 14 day oral course of rifampin (10 mg kg^{-1} day^{-1} in two divided doses) owing to persistent respiratory symptoms and positive *C. trachomatis* cultures following treatment with erythromycin. This infant developed severe chronic lung disease.

In the seven infants who were found to be positive for *C. trachomatis* at the age of 12–60 days, the most striking clinical correlates were the occurrence of severe apnoeic spells and a prolonged requirement for supplementary oxygen. All these seven infants survived.

Very few cases with *C. trachomatis*-associated disease of the respiratory tract have been seen in the very early neonatal period (4, 5). In 1984, Mardh et al. demonstrated, by means of the immunofluorescence monoclonal antibody staining test, the presence of *C. trachomatis* in the postmortem lung tissue of a 2-day-old infant (4). The autopsy findings in this infant were more consistent with hyaline membrane disease. In the series reported by Attenburrow et al., out of the five low-birthweight neonates who developed a severe pneumonia, one (case 5) was found to be culture-positive for *C. trachomatis* in the ocular, throat and nasopharyngeal specimens obtained at birth (5). In this premature infant the initial radiographic appearances were those of hyaline membrane disease.

From our additional eight cases it is difficult to draw conclusions and recommendations about the pulmonary involvement of *C. trachomatis* in the very early neonatal period of preterm infants. The respiratory distress syndrome of the newborn ("hyaline membrane disease" or "idiopathic respiratory distress syndrome") has many features similar to those of the respiratory distress due to early onset infection with some microorganisms (10). The situation is further complicated by the fact that the two diseases may coexist in some infants (11). Most of our cases were in fact diagnosed radiologically as hyaline membrane disease. Though our results do suggest that the presence of *C. trachomatis* in preterm infants with neonatal respiratory distress is probably not an infrequent event, more prospective studies are needed to further elucidate the true incidence and significance of this association.

The polymerase chain reaction has been recently recognized as a rapid, specific and more sensitive assay than the traditional methods for *C. trachomatis* detection in ocular specimens of neonates (12). In terms of future directions, the next task will be also to assess whether this method may further help to identify in the period immediately after birth those neonates who might benefit from earlier specific treatment.

In utero transmission of *C. trachomatis* is not definitively known to occur, and infants born by Caesarean section are considered to have a very low risk of acquiring chlamydial infection unless there has been premature rupture of membranes (3). In our study,

Table 1. Clinical and laboratory findings in *C. trachomatis* (CT)-positive preterm infants.

Case	Sex	Gestational age (weeks)	Birth weight (g)	Delivery/ rupture of membranes	Time of sampling after birth (h)	Site of CT detection[a]	Clinical features	Chest radiograph findings	CT eradication	Duration of oxygen therapy (days)	Outcome
1	F	27	960	Vaginal/ at birth	10	T, P	RDS, apnoeic spells	HMD	Yes	38	Mild CLD
2	F	31	1250	Caesarean/ at birth	24	P	RDS, apnoeic spells	Slight pulmonary changes	Yes	4	Uneventful
3	M	29	1590	Caesarean/ 48 h	24	T, P, E	RDS	HMD	–	–	IVH, death
4	M	28	1000	Vaginal/ at birth	10	P	RDS	HMD	–	–	IVH, death
5	F	29	1270	Caesarean/ 8 h	24	T,P	RDS, apnoeic spells	HMD	No	182	Severe CLD
6	M	31	1685	Caesarean/ 10 h	1	P	RDS, apnoeic spells	HMD	Yes	8	Uneventful
7	F	28	1165	Caesarean/ 48 h	24	T	RDS	HMD	–	–	PNX, death
8	M	31	1750	Caesarean/ at birth	8	T	RDS	Pneumonic changes	Yes	3	Uneventful

[a] All samples were found to be positive by both direct immunofluorescence and culture methods.
RDS, respiratory distress syndrome; HMD, hyaline membrane disease; IVH, intraventricular haemorrhage; CLD, chronic lung disease; PNX, pneumothorax; T, trachea; P, pharynx; E, eye.

C. trachomatis was also detected within 24 h of life from preterm infants born by Caesarean section without premature rupture of membranes. Accordingly, anecdotal detection of *C. trachomatis* has been reported soon after birth in the lung tissue, tracheal aspirates, or ocular and pharyngeal specimens of preterm infants born by Caesarean section with no premature rupture of membranes (4, 5, 13, 14). Furthermore, in a recent study Pao et al. detected *C. trachomatis* DNA sequences in the amniotic fluid of a substantial proportion of women with urogenital chlamydial infection, thus suggesting an in utero mechanism by which chlamydiae can be transmitted before birth (15).

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