A prospective study of chlamydial, mycoplasmal, and viral infections in a neonatal intensive care unit

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SUMMARY In a prospective study of non-bacterial infection in a neonatal intensive care unit in north west London, *Chlamydia trachomatis* infection was identified in 4 of 280 babies (1.4%) and was the most common cause of neonatal ophthalmia. One of the four developed pneumonitis. *Ureaplasma urealyticum* was found to colonise the nasopharynx in 53 of 235 babies (22.6%), with *Mycoplasma hominis* present in 6 of 235 babies (2.6%). There was a statistically significant association between *U. urealyticum* colonisation and preterm birth or prolonged rupture of membranes. Colonisation occurred more commonly in babies with apnoea.

Viral infection was detected in 16 of 280 babies (5.7%). Rotavirus was identified in 5 of 170 babies (2.9%) and was associated with necrotising enterocolitis in two infants and with bloody diarrhoea in another. Respiratory syncytial virus, which was identified in 4 of 280 babies (1.4%), was not associated with lower respiratory tract infection.

Chlamydia trachomatis and the mycoplasmas, *Ureaplasma urealyticum* (ureaplasmas) and *Mycoplasma hominis*, are acquired sexually and transmitted from the genital tract of the mother to her infant. *C. trachomatis* is a cause of neonatal ophthalmia and pneumonitis which, although self-limiting, is a notable cause of hospital admission in American infants with respiratory infections.¹ Although chlamydial infection may occur in 1 to 2% of newborn term infants in the United States,²⁻⁴ the few prospective studies carried out in England have shown a lower rate of infection. Watson and Gairdner⁵ detected chlamydia ophthalmia in 4 of 2700 babies delivered in the maternity hospital in Cambridge. More recently, this condition was seen in only 1 of 450 consecutive births in Southampton.⁶ *C. trachomatis* was not isolated from the cervix of 107 patients attending an antenatal clinic in north west London.⁷ Recent national increases in the incidence of sexually transmitted disease and the probability that these were relevant to our area led us to suspect that *C. trachomatis* was an important cause of neonatal conjunctivitis in our population.

Isolation of *U. urealyticum* from the genital tract during preterm labour has been shown to be associated with chorioamnionitis.⁸ Isolation is directly related to preterm labour as well as to prolonged rupture of membranes.⁹ While there is

little information regarding the effect of ureaplasmas on the preterm neonate, Stagno *et al*¹⁰ isolated *U. urealyticum* from a number of infants admitted to hospital with pneumonitis in Birmingham, Alabama. *M. hominis*, which is transmitted less commonly to the newborn,⁹ has been isolated from infants with meningitis¹¹ and cerebral abscess.¹²

Neonatal viral infection may present very differently from infection in later life.¹³⁻¹⁵ While rotavirus infection has been reported to cause mild or asymptomatic infection in the newborn,^{16 17} a recent report suggests an association with more severe gastroenteritis, including necrotising enterocolitis.¹⁸

We aimed to establish the incidence of chlamydial, mycoplasmal, and viral infections among infants on the neonatal unit, and to assess the contribution of *U. urealyticum* and *C. trachomatis* to respiratory disease in the preterm infant.

Patients and methods

This research project was approved by the hospital ethical committee and informed consent was obtained from parents. All babies admitted to the neonatal intensive care unit between late August 1981 and December 1982 were eligible for the study. Of these 355 babies, 75 were excluded because they

Table 1 Characteristics of patients

Number of patients	280
Median gestation (weeks)	32
Median birthweight (g)	1620
<i>Gestation groups</i>	<i>n</i>
25–28 weeks	53
29–32 weeks	110
33–36 weeks	82
37–42 weeks	35
<i>Socioeconomic groups</i>	
I	32
II	34
III	19
IV	73
V	34
Unsupported mother	26
Unemployed father	19
Not known	43
<i>Race</i>	
Caucasian	179
Asian	62
Negro	33
Mixed	6
<i>Clinical features</i>	
Idiopathic respiratory distress syndrome	82
Oxygen for more than 5 days	115
Apnoea	39
Necrotising enterocolitis	9*
Purulent conjunctivitis	14
Prolonged rupture of membranes	54

* Eight cases of necrotising enterocolitis during period of stool collection.

either died (n=35) or left the neonatal intensive care unit (n=40) before specimens could be taken. The characteristics of the 280 infants studied are given in Table 1.

Clinical details were recorded on a pro forma and transferred to a computer at the end of the study. Prolonged rupture of membranes was defined as membranes ruptured for more than 24 hours before delivery. Criteria for idiopathic respiratory distress syndrome were two of the following signs after four hours: respiratory rate greater than 60/minute, intercostal recession, and grunting. Apnoea was defined as three or more episodes of duration 10 seconds or longer, associated with brachycardia (pulse less than 100/minute) or cyanosis in a 24 hour period. Any baby with a purulent exudate and inflammation of the conjunctiva was said to have conjunctivitis. The criteria for a diagnosis of necrotising enterocolitis were bloody diarrhoea, abdominal distension, and pneumatosis intestinalis on radiographs. Gestational age was estimated using maternal history, ultrasound, and Dubowitz assessment.¹⁹ Logistic regression analysis was used to determine the effects of certain variables on *U urealyticum* colonisation and χ^2 tests were used to observe differences between social and racial groups.

Nasopharyngeal aspirates, conjunctival swabs, stools, and urine specimens were collected at weekly intervals from the babies. Nasopharyngeal aspirates were collected using a size 5 feeding tube connected to a sputum trap, to which 50 mm Hg suction was applied. The conjunctival specimens collected at the same time were obtained by pulling a cottonwool tipped plastic swab across the everted lower eyelid of one eye. Both specimens were separately immersed in 0.2 M sucrose phosphate before the immediate inoculation into mycoplasma growth medium and transport to the laboratory in wet ice. Where clinically indicated, cerebrospinal fluid was examined. The nasopharyngeal aspirates and conjunctival specimens were collected over 16 months for chlamydial and viral isolation. *U urealyticum* and *M hominis* cultures were begun in November 1981, two months after the study began, so that only 235 of the 280 babies studied were screened. Stools and urine were collected for a 12 month period only, to include 209 of the 280 babies.

Laboratory methods

The mycoplasma medium was inoculated at the time of collection; slides were prepared for respiratory syncytial virus immunofluorescence on the day of collection, and the test was completed in batches later. For the first half of the study the nasopharyngeal aspirates and conjunctival swabs were frozen at -70°C before *C trachomatis* and virus isolation; during the second half these were inoculated on the same day. Isolation was performed as follows:

C trachomatis. Aliquots (1 ml) of McCoy cells (4×10^5 cells) in growth medium (Minimal Essential Medium—Gibco MEM with 10% fetal bovine serum—Flow Laboratories) were pipetted into plastic, flat bottomed tubes (Redhill Surgical, E 5/R), each containing a 13 mm glass cover slip (Slaughter Ltd, No. 3).²⁰ These were incubated at 37°C for 24 hours, by which time a monolayer had formed. They were then inoculated with the clinical specimens and underwent centrifugation at 3000 g at 35°C in an MSE Mistral 6L for one hour. The medium was then replaced with 1 ml aliquots of maintenance medium (Gibco MEM with 5% fetal bovine serum). Cultures were then incubated at 37°C for 72 hours. Specimens were fixed with 100% methanol and stained with buffered Giemsa pH 6.8.²¹ Cover slips were dried and mounted on microscope slides before examination under darkground illumination using an Olympus microscope. Chlamydial inclusion bodies showed bright green against the dull, dark purple cell monolayer. Control specimens were set up with

each batch. The effect of freezing on inclusion counts was studied on control specimens and was found to be negligible when stored at -70°C in 0.2 M sucrose phosphate.

Mycoplasmas. Isolation in broth and in agar was undertaken. Selective medium containing urea (1%) in broth at pH 6.3 for *U urealyticum*²² and arginine (1%) broth at pH 6.7 for *M hominis* was used (Oxoid formulations). Lincomycin (15 $\mu\text{g/ml}$) was added to urea broth, but because of the high rate of bacterial contamination of cultures, vancomycin HCl (5 $\mu\text{g/ml}$) was added to both types for the last 6 months of the study because it was found to reduce bacterial contamination without inhibiting mycoplasmal growth. Clinical specimens were inoculated into urea and arginine broth in screw capped glass bijou bottles as well as on agar. The specimens were incubated at 37°C , and agar plates were incubated in a $\text{CO}_2\text{-H}_2$ (Becton Dickinson) anaerobic jar. The broths were observed daily, and agar plates were examined under the low power of a microscope on alternate days. Cultures were discarded after 7 days if negative.

In the presence of *U urealyticum* the urea broth turned from yellow to magenta; a similar colour change was seen with *M hominis* in arginine broth. Identification was confirmed by the presence of characteristic dark staining *U urealyticum* colonies on agar after either primary isolation or subculture; *M hominis* gave the typical 'fried egg' appearance and showed growth inhibition in the presence of paper discs impregnated with *M hominis* antiserum.

Virus isolation

Nasopharyngeal aspirates, urine, stool, and cerebrospinal fluid specimens were inoculated into monolayers of human fetal lung fibroblasts and kidney cells, cynomolgous monkey kidney cells, and Hep 2 cells, as appropriate. Cell lines were propagated in Medium 199 (Gibco) with 10% fetal bovine serum and maintained in Medium 199 with 1% fetal bovine serum. Stools were homogenised in phosphate buffered saline; the supernatant was divided and one aliquot was frozen at -70°C for use in the rotavirus enzyme linked immunosorbent assay (ELISA) and electron microscopy studies. The rest was treated with chloroform, and the supernatant was inoculated on the cell monolayer. Urine was stored in an equal volume of 70% sorbitol before inoculation on fibroblast cells. After a one hour period of adsorption the medium was changed. The tubes were rolled at 33°C and 37°C according to cell type and examined every three days for evidence of viral cytopathic effect. On the 10th day kidney

cultures were haemadsorbed with human 'O' red blood cells. Urine cultures were observed for 28 days and other cultures for 21 days. Identification of isolates was carried out using neutralisation tests in compatible cell lines using standard antiviral antisera raised in animals.

Respiratory syncytial virus immunofluorescence.

Slides with 2×5 mm spots of epithelial cells from the nasopharyngeal aspirates were prepared using a cytospin technique (Shandon). After fixation in acetone and storage at -20°C , an indirect immunofluorescence technique was used,²³ taking care to incorporate adequate controls. Slides were examined with a Leitz UV microscope.

Rotavirus ELISA. The Rotazyme system (Abbott Laboratories) was used according to the manufacturer's recommendations. Positive samples were confirmed by electron microscopy using the negative stain phosphotungstic acid, pH 6.8.

Rubella serology. Selected sera was examined by the haemagglutination inhibition test for rubella antibodies in serum and, if more than 24 IU/ml, rubella specific IgM studies were performed after physical separation of the IgM by sucrose density fractionation.

Results

A summary of all isolations is given in Table 2. *C trachomatis* was identified in 4 of 280 babies

Table 2 Agents identified from the different specimens

Agent	No of babies screened	No of babies infected	Nature of specimen positive
<i>Chlamydia trachomatis</i>	280	4	Conjunctival swab—3 (NPA positive in 1/3) <i>C trachomatis</i> specific IgM positive in fourth baby (serum)
<i>Mycoplasma hominis</i>	235	6	NPA
<i>Ureaplasma urealyticum</i>	235	53	NPA
Rotavirus	170	5	Stool
Coxsackie B2	280	2	NPA
Cytomegalovirus	280	2	NPA
Respiratory syncytial virus	280	4	NPA
Parainfluenza 1	280	1	NPA
Parainfluenza 3	280	1	NPA
Rubella	280	1	Serum. Rubella specific IgM

(1.4%). The agent was isolated from the conjunctivae of three babies with neonatal ophthalmia; identification was confirmed by serology in a fourth baby who developed pneumonitis after treatment of an early eye infection with topical sulphacetamide alone. *C trachomatis* specific IgM in serum was detected in this case by the microimmunofluorescence test. *C trachomatis* was isolated from a nasopharyngeal aspirate in one of the babies with conjunctivitis but with no evidence of pneumonitis. Pneumonitis may have been prevented by the prompt use of systemic erythromycin in three infected babies. Bacterial infection accounted for only three of the 14 cases of conjunctivitis during the study. In 7 no agent was isolated.

M hominis was isolated from the nasopharyngeal aspirates in 6 of 235 babies (2.6%); in four of these there had been prolonged rupture of membranes, and two of the mothers had clinical signs of amnionitis. One baby had persistent pneumonia after operation for tracheo-oesophageal fistula. In another, prolonged oxygen dependence followed idiopathic respiratory distress syndrome and *Haemophilus influenzae* septicaemia. *U urealyticum* was isolated from 53 of 235 infants (22.6%). Specimens were taken from some of their mothers in a separate study, (Lamont R, Taylor Robinson D. Personal communication 1983) and it seemed that 40% of colonised mothers transmitted this organism to their infants. Certain factors were considered in relation to colonisation, and they are given in Table 3. The relation between gestation, prolonged rupture of membranes, and colonisation reached statistical significance. Thirteen of 29 babies with apnoea were colonised, but this did not reach significance.

Evidence of viral infection was found in 5.7% of infants. Rotavirus was identified in 5 of 170 babies (2.9%); in two of these on more than one occasion. In 8 of the 170 babies who developed necrotising enterocolitis, two excreted rotavirus in faeces; one of them after the signs of illness had appeared. Another baby developed bloody diarrhoea, while a further two had subclinical infections.

Table 3 Factors considered in relation to *U urealyticum* isolation (n=235)

	P value
Gestation	0.01
Prolonged rupture of membranes	<0.01*
Apnoea	0.06*
Idiopathic respiratory distress syndrome	0.3*
Oxygen for more than 5 days	0.3*
Social class	NS
Racial group	NS

* Logistic regression analysis (a form of multiple regression) used to eliminate the effect of gestation on colonisation from the calculation.

Respiratory syncytial virus was identified in 4 of 280 babies (1.4%). In three, excessive mucoid secretions from the respiratory tract were observed, but no evidence of pneumonitis or bronchiolitis was found. Nasopharyngeal infection with parainfluenza viruses was seen in two babies with no clinical illness and serotypes 1 and 3 were identified. Cytomegalovirus was isolated in two babies: excretion began after a period of four weeks in both cases and indicated neonatal as opposed to fetal acquisition. Both babies had received multiple blood transfusions. In one baby, infection was associated with a transient but severe neutropenia. Coxsackie B2 infection occurred in two babies within the same week. One of these developed aseptic meningitis but recovered without further sequelae. Rubella infection was diagnosed in one infant with thrombocytopenia and birth asphyxia who died at three weeks of age. Rubella specific IgM antibody was found in the baby's serum, confirming congenital rubella infection.

Discussion

Few data have been collected on non-bacterial infection in the neonate in this country, partly because investigations of the nature described are not routinely available, and also because they are both time consuming and expensive.

The prevalence of chlamydial infection in this series, 4 of 280 (1.4%), is comparable with that recorded by Schachter in San Francisco, among term infants.² *C trachomatis* was the most common cause of conjunctivitis in our population, and it is likely that this agent is equally prevalent in other urban centres in this country, where the incidence of sexually transmitted disease is particularly high. Because chlamydial ophthalmia usually occurs towards the end of the first week of life, many babies must develop this condition after discharge from maternity units to the community, where laboratory investigations are not readily available. If untreated, or even if partially treated with inappropriate antibiotics, infected babies may develop pneumonitis or perhaps eye damage.²⁴ It follows that chlamydial pneumonitis is not being recognised, especially when it occurs as a primary manifestation of infection.²⁵ If the chlamydial isolation rate among term infants in other areas of the country were comparable to that observed in our population, there might be justification for the routine identification of women infected with *C trachomatis* around the time of delivery. Their newborn infants could then be treated with systemic erythromycin, which has been shown to prevent conjunctivitis in many cases, as well as reducing nasopharyngeal

infection that is often associated with pneumonitis.²⁶ The provision of a routine chlamydial isolation service, as recommended recently for adults,²⁷ should be extended to include the care of the newborn.

Ureaplasmas do not seem to be associated with prolonged oxygen dependence; however, their increased incidence in the preterm infant may be related to chorioamnionitis, which occurred most commonly in the mothers of preterm babies. An increased number of babies with apnoea were colonised, even after adjustment for the effect of gestation on apnoea. Although statistical significance was not reached in our study, the findings of Stagno *et al*¹⁰ suggested an association with lower respiratory tract infection. Of 104 infants with pneumonitis, *U. urealyticum* was isolated from 8, and in two of these *U. urealyticum* was the only organism present in the nasopharyngeal aspirates. It is possible that follow up over a longer period would have shown a relation between respiratory tract infections and *U. urealyticum* colonisation in our babies since in vitro studies have shown that these organisms produce fetal trachea epithelial cell damage.²⁸ It is not known, however, whether infected infants produce a serological response to these organisms and serological studies to evaluate *U. urealyticum* and *M. hominis* antibodies are currently being undertaken.

As in other studies¹⁴ the respiratory viral pathogens, respiratory syncytial virus and parainfluenza viruses 1 and 3, were not associated with lower respiratory tract infection in these preterm infants. Because the two cases of Coxsackie B2 virus infection occurred in the unit within the same week, concern about nosocomial spread was aroused. No further cases were found in the unit, however, and recourse to special control measures were not undertaken.¹³ The use of 'walking' donors for the blood transfusion of the neonate should take into account the risks of cytomegalovirus infection and the debility that this infection can bring to preterm infants.¹⁵

The rotavirus results were unexpected. While there were too few babies for statistical analysis, rotavirus infection was associated with gastrointestinal disease. There is an urgent need for further virological studies to elucidate the role of this virus in necrotising enterocolitis and bloody diarrhoea.

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