Incidence of neonatal chlamydial conjunctivitis and its association with nasopharyngeal colonisation in a Hong Kong hospital, assessed by polymerase chain reaction

Terri PP Yip, WH Chan, KT Yip, TL Que, MM Lee, NS Kwong, CK Ho

Objectives
Using polymerase chain reactions, this study aimed to evaluate the incidence of neonatal chlamydial conjunctivitis in our region of Hong Kong and explore any association between such conjunctivitis and nasopharyngeal colonisation with Chlamydia trachomatis.

Design
Prospective epidemiological study.

Setting
Regional hospital, Hong Kong.

Patients
Consecutive patients with neonatal conjunctivitis presenting to our hospital were recruited from May 2004 to April 2005 inclusive. Both eyes were investigated separately for Chlamydia trachomatis by polymerase chain reaction, direct immunofluorescent assay, and cell culture by two assigned ophthalmologists. Neonates diagnosed to have ocular Chlamydia trachomatis infection were subjected to additional nasopharyngeal investigations. Complete sets of ocular and nasopharyngeal investigations were also undertaken 1 week after oral erythromycin treatment to confirm complete eradication of Chlamydia trachomatis.

Results
Of 192 patients with neonatal conjunctivitis, 24 were diagnosed to have chlamydial conjunctivitis. Fifteen of them had nasopharyngeal colonisation with Chlamydia trachomatis. Among the 20 neonatal chlamydial conjunctivitis patients that completed the follow-up study, one suffered treatment failure. None had clinically diagnosed systemic Chlamydia trachomatis infection 3 months after oral erythromycin.

Conclusions
The incidence of neonatal chlamydial conjunctivitis in our region of Hong Kong was 4 in 1000 live births, of whom 63% had nasopharyngeal presence of Chlamydia trachomatis. Owing to the high rate of nasopharyngeal isolation and possibility of treatment failure, post-treatment ocular and nasopharyngeal polymerase chain reaction testing for Chlamydia trachomatis may be considered to confirm complete eradication.

Introduction
Ophthalmia neonatorum is the most common infection in the neonatal period. The most likely identifiable causative organism in the United States and Europe during the 1990s was Chlamydia trachomatis. Being the most prevalent sexually transmitted disease in developed countries, approximately 50% of babies born to women whose genital tracts harbour C trachomatis developed neonatal chlamydial conjunctivitis (NCC). This can result in conjunctival or nasopharyngeal (NP) colonisation, and subsequently give rise to neonatal conjunctivitis, otitis media, rhinitis, and pneumonia. Untreated C trachomatis ocular infection in the newborn can lead to focal corneal neovascularisation, scarring, pannus formation, and chronic inclusion conjunctivitis. Prophylaxis with topical silver nitrate, erythromycin, or tetracycline does not prevent the development of NCC. Therefore, early diagnosis and treatment with oral antibiotics is essential for the prevention of ocular and systemic sequelae.

The incidence of NCC in the United States was 8 per 1000 births in the 1990s. A clinical study in China reported that the prevalence of NCC was 51% among neonates with conjunctivitis...
in 1997 to 1998. In Hong Kong, there is no corresponding screening for pregnant women and few studies have been undertaken to determine the incidence of *C. trachomatis* neonatal conjunctivitis. Previous studies have shown that polymerase chain reaction (PCR) is a good alternative to cell culture for its detection in adult conjunctival scrapings, being more sensitive and more specific.\(^5\) We therefore aimed to evaluate the local NCC incidence using PCR. Nasopharyngeal specimens were also of interest to evaluate the risk of nasal colonisation in neonates with ocular infection. Such information is useful for ophthalmologists, paediatricians, primary health care personnel, and parents with a view to developing a suitable collaborative management strategy.

**Methods**

This study was conducted according to the International Conference on Harmonization–Good Clinical Practice (ICH GCP) guidelines, with approval from the New Territories West Cluster Clinical Research and Ethics Committee. Informed consent was obtained from parents of the neonates under study. Consecutive neonates with conjunctivitis were recruited from 1 May 2004 to 30 April 2005. They included babies with gestational age of 32 weeks or older with acute conjunctivitis acquired in hospital within the first 28 days of birth. Two assigned ophthalmologists assessed all the patients. Each eye was scored and graded according to clinical severity (Table 1). Specimens were collected from each eye according to guidelines set by the Centers for Disease Control and Prevention (CDC)\(^1\), from the less affected eye first. Neonates that had prior topical antibiotics, had their medication stopped for 24 hours before the investigation.

Since the performance of the PCR (COBAS AMPLICOR; Roche Diagnostics GmbH, Mannheim, Germany) in ocular specimens was still under evaluation, conventional *C. trachomatis* tests, including direct immunofluorescent stain (DIF) [Syva MicroTrak, Palo Alto, CA, US] and cell culture (Syva MicroTrak, Palo Alto, CA, US) were also performed as a routine on both eyes, to avoid missing an infection. In the present study, infection was assumed if either one of the three *C. trachomatis* tests was positive. All neonates diagnosed to have ocular *C. trachomatis* infection, had NP swabs taken for PCR, DIF, and cell culture.

Before taking ocular specimens, any purulent exudates, if present, were first removed from the eye using a cotton swab. A Dacron swab was then used to smear materials from the palpebral conjunctival epithelium onto a glass DIF slide. The slide was air dried and immediately fixed in methanol. A second conjunctival swab was collected for cell culture and PCR with a new Dacron swab. This swab was cut into chlamydia transport medium (sucrose-phosphate buffer [pH 7.2] supplemented with inactivated foetal calf serum, 100 μg/ml of vancomycin, 10 μg/ml of gentamicin, and 25 units/ml of nystatin) and transported to the laboratory that day on a cold pack.

In the laboratory, a 200 μl aliquot of each sample in transport medium was kept at -70°C until further processing for PCR. The remaining samples and the methanol-fixed DIF slides were sent for cell culture and DIF respectively. Direct immunofluorescent stain was performed using the MicroTrak *C. trachomatis* Direct

**TABLE 1. Scoring and grading system for neonatal conjunctivitis**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Sum of individual scores according to each of the following clinical signs: purulent discharge, conjunctival congestion, eyelid stung, oedema and erythema of lids (0=absent, 1=mild, 2=moderate, 3=severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>1-3 inclusive</td>
</tr>
<tr>
<td>Moderate</td>
<td>4-8 inclusive</td>
</tr>
<tr>
<td>Severe</td>
<td>Presence of either pseudomembrane, corneal invasion, dacryocystitis OR a score ≥9</td>
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\(^{5}\) See reference for details.
Polymerase chain reaction was performed using the COBAS AMPLICOR CT/NG test and the results interpreted according to the manufacturer’s instructions. In brief, a 100 μL aliquot of specimen was mixed with 100 μL of CT/NG Lysis Buffer and incubated at room temperature for 10 minutes. After brief centrifugation, 200 μL of CT/NG Specimen Diluent was then added to the mixture and the resultant further incubated at room temperature for 10 minutes. A total of 50 μL of the processed sample was added to 50 μL of the master mix. Amplification and detection were performed on a COBAS AMPLICOR system.

For the NP specimen, rayon-tipped swabs with aluminium shafts moistened with sterile normal saline were used. A swab was gently inserted into the nostril of the neonate until it reached the posterior wall of the nasopharynx. Slides for DIF were immediately prepared after NP specimen collection. Following the same collection procedures, another rayon-tipped swab was cut into chlamydia transport medium for preparing cell culture and PCR. These specimens were sent to the laboratory and processed as for the ocular specimens.

Oral erythromycin (50 mg/kg/day divided into 4 doses) was administered for 2 weeks when either one of the three C. trachomatis tests was positive. The response and compliance to oral erythromycin were recorded. To confirm complete eradication of C. trachomatis, 1 week after a course of oral erythromycin, complete sets of ocular and NP specimens were obtained as described above. Treatment failure was defined as C. trachomatis persistence (demonstrated by positive PCR, cell culture, or DIF) in an ocular or NP specimen after a complete course of the antibiotic.

During each follow-up session, parents were asked about any wheezing, cough, or fever. Neonates having these symptoms were to be admitted to hospital immediately and paediatricians consulted for possible systemic C. trachomatis infection. Hospital records were checked 3 months after oral erythromycin, to ascertain any persistence of systemic C. trachomatis.

## Results

### Local incidence

In this hospital-based study, 192 neonates with conjunctivitis were recruited from 1 May 2004 to 30 April 2005, of which 24 were diagnosed with NCC according to our predefined criteria. Thus, C. trachomatis accounted for 12.5% of local neonates with conjunctivitis; the incidence of NCC in this region being 4 per 1000 live births (24 of 5973 live births in our hospital). Of our 24 NCC cases, 15 (62.5%) had nasal colonisation of C. trachomatis. None was diagnosed with systemic C. trachomatis infection up to 3 months after oral erythromycin.

### Clinical presentation

The 24 NCC patients were all Chinese, except for one who was Indian. There were 11 males and 13 females. Their birth weights, gestational age, age of disease onset, age and severity of conjunctivitis at presentation are shown in Table 2. There was a wide range for age at presentation (as early as week 1 up to week 3 after...
TABLE 4. Results of Chlamydia trachomatis tests in relation to clinical severity of conjunctivitis

<table>
<thead>
<tr>
<th>Grade of neonatal conjunctivitis</th>
<th>No. of C. trachomatis positive set, n=32</th>
<th>Positivity rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>4</td>
<td>12.5</td>
</tr>
<tr>
<td>Mild</td>
<td>4</td>
<td>12.5</td>
</tr>
<tr>
<td>Moderate</td>
<td>18</td>
<td>56.3</td>
</tr>
<tr>
<td>Severe</td>
<td>6</td>
<td>18.8</td>
</tr>
</tbody>
</table>

birth) and 13 of them presented unilaterally. All NCC patients had at least moderate conjunctivitis in one eye at presentation (Table 3).

Microbiological results

Each set of ocular investigations entailed three C. trachomatis tests (PCR, DIF, and cell culture). Each neonate had one set of ocular investigations for each eye. In this study, 192 neonates were recruited and 384 sets of ocular investigations were performed on presentation. Thirty-two of 384 sets were classified as C. trachomatis positive according to the study criteria. We encountered positive C. trachomatis in the contralateral eye, whether asymptomatic or mildly inflamed; the positive rate of C. trachomatis tests in both situations was 12.5% (Table 4).

All post-treatment ocular C. trachomatis investigations in NCC patients were negative. Of the 24 NCC cases, four defaulted. One of the 20 who completed the study had a positive NP PCR at post-treatment investigation, though the NP DIF and NP cell culture were negative. A complete set of post-treatment NP investigations (PCR, DIF, and cell culture) was repeated for this special patient, and yielded positive results with all three tests. Because C. trachomatis persistence was confirmed, this patient was considered a treatment failure. A second course of oral erythromycin was then given, which cured the disease, as confirmed by negative results for all three NP C. trachomatis tests.

Discussion

The incidence of NCC in our study population was 4 in 1000 live births and corresponded to the incidence in industrialised countries, where it was estimated to range from 0.5 to 5% (depending on the rate of maternal infection).12,13 Though Hong Kong is in close proximity to Mainland China, among neonates with conjunctivitis there is still a discrepancy in the disease prevalence. The reported prevalence of NCC in China is 51.2% of all patients with neonatal conjunctivitis3,8 while it was only 12.5% in our region of Hong Kong.

The incubation period of C. trachomatis is typically 1 week, with a range from 5 to 14 days.3,8 The clinical signs and symptoms of C. trachomatis conjunctivitis begin to develop within a few days to several weeks after birth, most commonly at 2 weeks.14,15 In our study, the disease usually presented by day 6 after birth, which is not uncommon.

It is well known that the presentation of NCC can vary; from mild to moderate conjunctival erythema, and from scanty mucoid discharge to a copious purulent discharge. It can also cause ocular oedema, chemosis or pseudomembrane formation.1,4,8 These variations were also encountered in our study. About half (54%) of our NCC cases presented unilaterally (Table 3). Among the 13 asymptomatic eyes, four were C. trachomatis positive (Table 4). These represented the presence of ocular C. trachomatis and possible infection. Therefore, ocular investigations for C. trachomatis should be considered despite unilateral presentation of neonatal conjunctivitis.

In Sandstrom et al’s study,13 C. trachomatis was isolated from eyes of 33 out of 160 infants with neonatal conjunctivitis. Chlamydia trachomatis could also be isolated from nasopharynx in 58% of these infants. Using PCR, our study found a higher rate (63%) of C. trachomatis isolation from the nasopharynx in our NCC patients. Because of such a high rate of C. trachomatis presence in the nasal area and the possible systemic infections it might cause, ophthalmologists and paediatricians should undertake detailed, collaborative evaluation.

Preliminary studies in the use of PCR on ocular and NP specimens from neonates showed comparable results to cell culture for the detection of C. trachomatis.13 Polymerase chain reaction was therefore employed in the current study, with the expectation that it would provide a superior epidemiological tool. Thus, this study was the first to use PCR to evaluate NCC in Hong Kong.

In our study, ocular investigations at presentation (involving specimen collection) were performed after cessation of topical and oral antibiotics for 24 hours. This was to eliminate possible inhibitory effects of such medications on the growth of C. trachomatis. At the end of the study, no adverse consequences were noted from this planned procedure and all neonates were cured of bacterial or C. trachomatis conjunctivitis without incurring any long-term complications.

In the current study, post-treatment NP PCR was carried out on specimens obtained 1 week after completing the course of oral erythromycin. This time frame was selected to avoid false-positive PCR results from residual non-viable C. trachomatis DNA. All follow-up NP PCR tests in our study yielded negative results except in one neonate. The latter was proved to be positive by NP specimen tests based on PCR, cell culture, and DIF. Further study is warranted to evaluate the optimal timing of post-treatment NP PCR for detecting NCC treatment failure.

Our hospital is a regional hospital serving the largest population in Hong Kong. It is located near the border of Mainland China, and its patients belong mainly
to the middle and lower social classes. Therefore the results of the current study may not be representative of Hong Kong as a whole. Nevertheless, until suitable multi-centre cross-sectional studies are conducted, it provides the best available estimate of NCC in the population and the basis for future planning of health programmes.

Conclusion

In this hospital-based study using PCR to diagnose C. trachomatis infection, the incidence of NCC in our region of Hong Kong was 4 in 1000 live births. Owing to the high rate of NP isolation (63%) in these patients and possible microbiological persistence, post-treatment ocular and NP PCR for C. trachomatis can be considered a means of confirming complete eradication. A larger scale study in disease-prevalent regions is needed to evaluate the usefulness and timing of post-treatment NP PCR in managing NCC.

References