ORIGINAL ARTICLE

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

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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.

Key words
Chlamydia infections; *Chlamydia trachomatis*; Conjunctivitis, bacterial; Nasopharyngeal diseases; Polymerase chain reaction

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Introduction

Ophthalmia neonatorum is the most common infection in the neonatal period.[1-3] The most likely identifiable causative organism in the United States and Europe during the 1990s was *Chlamydia trachomatis*.[3-5] Being the most prevalent sexually transmitted disease in developed countries,[6] approximately 50% of babies born to women whose genital tracts harbour *C trachomatis* developed neonatal chlamydial conjunctivitis (NCC).[3] This can result in conjunctival or nasopharyngeal (NP) colonisation, and subsequently give rise to neonatal conjunctivitis, otitis media, rhinitis, and pneumonitis. 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.[4,7,8] 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.[6] A clinical study in China reported that the prevalence of NCC was 51% among neonates with conjunctivitis

in 1997 to 1998.[9] 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 scrappings, being more sensitive and more specific.[10] 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.

以聚合酵素連鎖反應，估計香港新生嬰兒出現衣原體性結膜炎的情況，以及此結膜炎與移生至鼻咽是否有關

目的 是次研究希望以聚合酵素連鎖反應，評估香港新生嬰兒出現衣原體性結膜炎的情況，和探討這種結膜炎與砂眼披衣菌移生至鼻咽是否有關。

設計 前瞻性流行病學研究。

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患者 2004年5月至2005年4月期間，出現結膜炎而到該醫院求診的新生嬰兒。病嬰雙眼由兩名指定的眼科醫生，以聚合酵素連鎖反應、直接免疫熒光檢定和結胞培植方式，分別檢查是否有砂眼披衣菌。如發現受感染，病嬰會再接受額外的鼻咽檢查。而在口服紅黴素一星期後，上述的眼睛和鼻咽的檢查會再次進行，確定砂眼披衣菌已全部被消除。

結果 192名患結膜炎的新生嬰兒中，24人被診斷患上衣原體性結膜炎。他們之中有15人出現砂眼披衣菌移生至鼻咽。而20名完成跟進研究的衣原體性結膜炎的嬰兒，一人治療失敗。在口服紅黴素3個月後的臨床檢查，沒有病人被診斷有系統性砂眼披衣菌感染。

結論 香港新生嬰兒中，每1000名便有4名感染衣原體性結膜炎，當中有63%病人有砂眼披衣菌移生至鼻咽。鑑於砂眼披衣菌移生至鼻咽的比率很高，並且有治療失敗的可能，須要考慮在治療後以聚合酵素連鎖反應，確定所有砂眼披衣菌已全部被清除。

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[11]), 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

Grade	Sum of individual scores according to each of the following clinical signs: purulent discharge, conjunctival congestion, eyelid stuck, oedema and erythema of lids (0=absent, 1=mild, 2=moderate, 3=severe)
Mild	1-3 inclusive
Moderate	4-8 inclusive
Severe	Presence of either pseudomembrane, corneal invasion, dacryocystitis OR a score ≥9

Specimen Test as recommended by the manufacturer. The slides were stained with fluorescein-conjugated monoclonal antibody specific to *C trachomatis* major outer membrane protein, and examined for the presence of elementary bodies under a fluorescent microscope.

Cell culture was performed on cycloheximide-treated monolayer McCoy cells in shell vials. Briefly, 150 µL of specimen in transport medium was inoculated and centrifuged onto the cell monolayer at 3000 X g at 37°C for 1 hour. The inoculum was discarded and replaced with 1 mL of pre-warmed growth medium. The shell vials were then incubated at 37°C in a humidified incubator in the presence of 2.5% CO_2. After 48 hours of incubation, the coverslip was examined for the presence of inclusion bodies by immunofluorescence (using the MicroTrak *C trachomatis* Culture Confirmation Test).

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*.

TABLE 2. Characteristics of neonates with *Chlamydia trachomatis* conjunctivitis (n=24)

Characteristic	Mean	Range
Birth weight (kg)	3.1	2.5-3.5
Gestational age (weeks)	38.6	34.0-40.0
Age (SD) at disease onset (days)	5.6 (2.2)	2-11
Age (SD) at presentation (days)	10.9 (4.8)	6-27
Time (SD) of delay in presentation (days)	5.0 (4.3)	1-21
Score (SD) of conjunctivitis severity at presentation	7.2 (1.7)	5-12

TABLE 3. Severity of conjunctivitis at presentation in neonates testing positive for *Chlamydia trachomatis*

Severity of conjunctivitis at presentation	No. of *C trachomatis* positive cases, n=24	% of neonatal chlamydial conjunctivitis represented
Unilateral disease (n=13)		
Mild	0	0
Moderate	8	33
Severe	5	21
Bilateral disease (n=11)		
Both eyes—mild	0	0
One eye—mild; one eye—moderate	8	33
Both eyes—moderate	2	8
One eye—moderate; one eye—severe	0	0
Both eyes—severe	1	4

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

Grade of neonatal conjunctivitis	No. of *C trachomatis* positive set, n=32	Positivity rate (%)
Asymptomatic	4	12.5
Mild	4	12.5
Moderate	18	56.3
Severe	6	18.8

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 conjunctivitis[9] 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.[3,4,8] 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.[3,4,8] 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,[14] *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*.[15] 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.

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