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Distribution of *Chlamydia trachomatis* genotypes in neonatal conjunctivitis in Hungary

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Abstract

The objective of the present study was to determine the frequency and age distribution of different *Chlamydia trachomatis* (CT) genotypes causing ophthalmia neonatorum (ON) in Hungary. Using PCR, we tested 76 conjunctival samples from symptomatic infants up to 3 months old in the National Centre for Epidemiology, Budapest between 2008 and 2016. CT tested positive in 30 of 76 conjunctival samples (39.5 %). The sequencing of the positive samples was successful in every case but one, and resulted in 48 % dominance for genotype E (14/29), followed by 24 % for genotype G (7/29), 10 % for J (3/29), 6.9 % for K and F (2/29), and 3.4 % for H (1/29). CT must still be regarded as a common pathogen causing ON in Hungary. Routine screening and treatment of pregnant women can be recommended to prevent these conditions. Chronic ON cases can be reduced by early diagnosis. Further research is needed to explain the dominance of genotypes E and G.

Chlamydia trachomatis (CT) is a leading urogenital pathogen that causes a wide range of sexually transmitted infections (STIs) in an estimated 146 million patients per year globally. It is most prevalent in young people aged 15–24 years [1]. The risk of infection is not confined to the sexually active adult population; due to vertical transmission from the cervix, it also affects the neonates of infected mothers [2]. The transmission rate varies between 50 and 70 %. Ophthalmia neonatorum (ON) develops in about 30–50 % of the infected newborns, while respiratory tract infections (RTIs) develop in 10–20 % of them [3, 4]. Conjunctivitis is present in about half of the RTI cases, and its presence should therefore increase awareness and prompt investigation for early RTI [5].

Despite postpartum prophylactic procedures, such as the instillation of Credé drops, ON still remains a common pathology in Hungary. Due to its toxic side-effects, the original silver nitrate prophylaxis was replaced with silver acetate, or with topical antibiotic ointments (erythromycin or tetracycline) or, more recently, povidone–iodine [6]. Although all of these approaches are very effective in reducing neonatal gonorrhoeal eye infections [3, 7], other bacterial threats have become more prominent, including CT infection.

The literature suggests that the prevalence of CT ON can increase to 40 % [6, 7]. Other non-sexually transmitted bacteria, such as staphylococci, streptococci, *Haemophilus* spp., *Escherichia coli*, *Pseudomonas aeruginosa* etc., deriving from the normal/transient vulvovaginal flora, account for 30–50 % of this condition [7]. The efficacy of eye prophylaxis to prevent these infections is less well studied. CT ON itself is usually a mild illness. However, if left untreated, it may lead to chronic sequelae, and in rare cases even to blindness [7]. Diagnosis is based on the presence of CT DNA or other direct detection techniques, such as culturing or immunofluorescence microscopy. Of note, serology is not informative in these local epithelial infections.

Our aim was to assess the potential role of different CT genotypes in infants with symptomatic ON.

During the period from May 2008 until August 2016 conjunctival samples from 76 neonates suffering from symptomatic ON were collected. The age of the enrolled subjects (35 males, 41 females) varied between 1 and 11 weeks. Age,

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Abbreviations: CT, *Chlamydia trachomatis*; ON, ophthalmia neonatorum; STI, sexually transmitted infection; PCR, polymerase chain reaction.
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The GenBan accession numbers for the genotypes of *Chlamydia trachomatis* are as follows: for the E genotype sequence, JX559522; for the F genotype sequence, JX564244; for the G genotype sequence, JX564245; for the H genotype sequence, JX564246; for the J genotype sequence, JX648604; and for the K genotype sequence , JX564248.

sex and hospital admission were analysed. Swab scrapings were taken by an ophthalmologist from the mucosal surface of the eyelids of neonates with symptoms of suggestive ON – principally discharge, hyperaemia and swelling of eyelids. The samples were sent for CT testing to the Bacterial STI Laboratory of the National Centre for Epidemiology, a referral centre for Hungary, located in Budapest.

The conjunctival swabs were rinsed in 1 ml of phosphate-buffered saline and subsequently centrifuged at 10 000 ***g*** for 10 min. DNA was extracted from the pellet using the High Pure DNA template preparation kit (Roche Diagnostics). Chlamydial DNA was detected by an in-house CT PCR targeting the plasmid gene as described previously [8]. In brief, the sequencing of chlamydial DNA was performed on a 1070 bp fragment of the amplified *omp1* gene. Fragments with a length of approximately 900 base pairs were sequenced in order to identify the genotypes of *C. trachomatis* by alignment with reference sequences from GenBank [E (JX559522), F (JX564244), G (JX564245), H (JX564246), J (JX648604), K (JX564248)].

Fifty-seven of the 76 conjunctival samples (75 %) were collected at the Department of Ophthalmology of Semmelweis University, and a further 12 % (9/76) were collected from paediatric centres in Budapest. The rest of the samples (13 %) came from outside the capital area. CT DNA was detected in 30 of the 76 (39.5 %) conjunctival samples. The CT-positive cases (30/76) showed a 50–50 % male–female distribution, indicating that the onset of ON was not influenced by the gender of the baby (P=0.6).

Based on the clinicians' data, 24 of the 76 (31.5 %) ON patients received inpatient care because of the severity of their symptoms. Of these, two patients (8.3 %) had a proven CT eye infection at ages of 2 and 3 weeks, respectively. That means that 93.3 % of the confirmed chlamydial ON cases (28/30) did not require hospitalization.

The distribution of the positive samples by age and genotype is given in Table 1. One sample could not be sequenced because of the low DNA copy number. In total, six different genotypes were discovered: E, G, J, F, K and H. Genotypes D and I were not present in these samples. Genotypes E (48.3 %) and G (24.1 %) were the most prevalent and were present in 72.4 % of the positive samples, and they were followed in descending order by genotypes J (10.3 %), F and K (both 6.8 %), and H (3.4 %). The majority of the positive samples originated from 2- (53.3 %) or 3-week-old (24.1 %) babies (Table 1, highlighted in grey).

Table 1. Distribution of identified *C. trachomatis* genotypes by postpartum age of neonates (n=29)

Postpartum age of neonates	Identified genotypes						All
	E	G	J	F	K	H	
1 week	2	–	–	–	–	–	2
2 weeks	10	2	2	1	–	–	**15**
3 weeks	1	3	–	1	1	1	7
4–6 weeks	1	2	–	–	–	–	3
10 weeks	–	–	–	–	1	–	1
11 weeks	–	–	1	–	–	–	1
All	14	7	3	2	2	1	29

The observed 39.5 % rate of CT in symptomatic ON is consistent with the rates published elsewhere [9]. The high positivity rate of the conjunctival scrapings in our series may be due to some selection bias, as the clinical samples may have been preselected by ophthalmologists for further CT testing. This, together with the underreporting to our reference laboratory from remote areas of the country, would imply underreporting of the real prevalence. As 87 % of the ocular samples were received from the capital area, it might not reflect the countrywide prevalence correctly.

According to the literature, the mean incubation time of CT is 1 week, so diagnosis becomes evident 1 week after delivery [10]. In our series, however, we found a peak of chlamydial ON in 2-week-old newborns. This can be explained by the fact that the first-line empirical therapy with topical eye-drops is insufficiently effective against CT, similar to the other prophylactic ocular agents (silver acetate, povidone-iodine etc.) [6]. Indeed, although these treatments may not eradicate the infection, they may delay the onset of symptoms and diagnosis [11]. After another week, if symptoms are still present, the parents usually seek medical help, and so most conjunctival samples are sent to the laboratory after a week of delay. We even identified two misdiagnosed infants at ages of 10 and 11 weeks, which emphasizes the importance of early recognition.

Chlamydial ON is usually a mild disease, which is indirectly confirmed by the fact that the majority of the CT-positive ON patients only received outpatient care. Clinical treatment was more often necessary in other non-chlamydial ON cases, but unfortunately we have no detailed information about the aetiology or severity of these cases. To have a full idea of the pathogenesis, it would have been important to reveal the non-CT origin of the other ON cases, especially as a larger proportion of them were severe enough to require hospital admission.

The sequencing of the 30 positive samples was successful in 29 DNA samples and the results reflected a clear 48 % dominance of genotype E. This high percentage of genotype E is in accordance with the scarce data found in the literature: Fermepin *et al.* reported 71 % dominance for genotype E among the neonates suffering from ON [12], Gallo Vaulet and colleagues reported a similar 72.4 % prevalence [13], and Kese *et al.* found a prevalence of 63.3 % in adult conjunctival samples [14]. In Hungary, where CT was detected in 21.6 % (53/245) of adult conjunctival samples, genotype E (30 % prevalence) was also the most common of the seven genotypes found [8]. The dominance of genotype E may be due to the different tissue tropism of these strains for the conjunctival mucosa of neonates, or it may just reflect the actual dominance of circulating urogenital strains among

humans [13]. To clarify this hypothesis, new typing methods, such as multilocus sequence typing, must be applied, while a larger sample size is needed.

The second most common genotype was type G. It clustered a week later than type E, at week 3 after birth, with two cases as late as 4 to 6 weeks after birth. One wonders if in these late cases postnatal infection could still be the cause, potentially misdiagnosed or mistreated, or whether postnatal infection followed by chronic inflammation could have occurred. Together, groups E and G made up the majority of cases (72 %). Interestingly, genotype D was completely missing from the CT-positive neonatal conjunctival samples, although it has been shown in several studies to be one of the most frequent genotypes among adults [8, 13–15]. Our small study sample size for ON may be one reason for this, but another explanation might be the negative association of genotype D with ON, which Gallo Vaulet *et al.* assumed in their study [13].

As CT is obviously still an important cause of CT ON in Hungary, one should not focus exclusively on adolescents and adults as risk groups when dealing with STIs, but also pay attention to the potential infection of newborns. As CT ON results from vertical transmission, the CT of the mother and her sexual partner(s) is implicated. In order to enable treatment for all affected people to prevent the chronic sequelae of infection and further transmission, one should consider extending the laboratory testing to genitourinary screening of the parents (and possible sexual partners). It would have been interesting to compare the ON samples with the cervical samples of the mothers, especially in the late-onset ON cases, to clarify whether the mode of transmission was vertical (during birth, maternal) or horizontal (postnatal via non-maternal contacts). It would have been informative to look for the distribution of the maternal chlamydial genotypes and compare them with the neonatal spectrum.

This extended screening would ideally imply the involvement of a team including ophthalmologists, paediatricians, gynaecologists and family physicians.

It should also be discussed whether systematic CT screening and treatment of pregnant women is desirable. As CT also seems to be involved in the causation of chorioamnionitis [16], preeclampsia [17] and preterm delivery [18] (although not in all studies [19]), routine screening and treatment for CT could be an option to try to prevent CT ON in newborns. Cost–benefit analysis and proper treatment efficacy studies would be required to back up such a generalized approach to reduce the number of infected pregnant women and thereby the rate of vertical transmission. Moreover, neonatologists and neonatal nurses should also be warned of the risks of chlamydial infection and informed about the possibilities of correct sampling and laboratory diagnosis. They have a crucial role in the early recognition of ocular chlamydial infections, especially where no routine prenatal *C. trachomatis* screening exists, as in Hungary.

Our data reflect the fact that CT is a leading pathogen among ocular neonatal infections in Hungary, with nearly 40 % of the tested conjunctival samples being CT-positive. To the best of our knowledge, this is the first report about the distribution of neonatal ocular CT genotypes to show the dominance of genotypes E and G in ON in Hungary. Further research on the immunological and virulence factors for these genotypes is necessary to clarify the background of this finding.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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