ORIGINAL ARTICLE

Reporting detection of *Chlamydia trachomatis* DNA in tissues of neonatal death cases

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Received 21 May 2013; accepted 12 August 2013
Available online 30 October 2013

**KEYWORDS**

*Chlamydia trachomatis*; Polymerase chain reaction; Newborn; Sepsis; Mortality

**Abstract**

**Objective:** to determine whether *C. trachomatis* was present in neonates with infection, but without an isolated pathogen, who died during the first week of life.

**Methods:** early neonatal death cases whose causes of death had been previously adjudicated by the institutional mortality committee were randomly selected. End-point and real-time polymerase chain reaction of the *C. trachomatis omp1* gene was used to blindly identify the presence of chlamydial DNA in the paraffinized samples of five organs (from authorized autopsies) of each of the dead neonates. Additionally, differential diagnoses were conducted by amplifying a fragment of the 16S rRNA of *Mycoplasma* spp.

**Results:** in five cases (35.7%), *C. trachomatis* DNA was found in one or more organs. Severe neonatal infection was present in three cases; one of them corresponded to genotype D of *C. trachomatis*. Interestingly, another case fulfilled the same criteria but had a positive polymerase chain reaction for *Mycoplasma hominis*, a pathogen known to produce sepsis in newborns.

**Conclusion:** the use of molecular biology techniques in these cases of early infant mortality demonstrated that *C. trachomatis* could play a role in the development of severe infection and in early neonatal death, similarly to that observed with *Mycoplasma hominis*. Further study is required to determine the pathogenesis of this perinatal infection.

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Introduction

Neonatal mortality has been defined as mortality occurring during the first 28 days of life. It is mostly associated to infections that are either congenital or acquired after birth. These infections strongly impact the causes of death and abortion in non-industrialized countries.\(^1\,2\) *Chlamydia trachomatis* is the cause of the most sexually transmitted bacterial infection worldwide.\(^3\) The prevalence *C. trachomatis* infection during pregnancy is variable: in the United States, it is between 2% and 13.7%; in Brazil, between 2.7% and 10%;\(^4\) and in Mexico, between 4% and 28%.\(^5\) It is related to premature rupture of membranes, chorioamnionitis, premature birth, and the development of neonatal ophthalmitis and pneumonitis. It has also been related to high rates of low birth weight and perinatal mortality.\(^6\) Variable clinical manifestations have been observed, including staccato cough, prodromal rhinorrhea, and history of conjunctivitis, tachypnea, and fever. The most frequent findings in chest radiographs are interstitial infiltration of bilateral lung fields, hyperinflation, and atelectasis.\(^6\)

The risk for vertical transmission of *C. trachomatis* is between 60% and 70%, and occurs during the infant’s passage through the birth canal; however, there is some evidence that vertical transmission can also occur in utero, since newborns delivered by cesarean sections have also been born infected and with intact membranes.\(^7\,8\)

Recently, Rours et al.\(^9\) demonstrated the presence of chlamydial DNA in the placenta of pre-term products, and found an association of the DNA with the degree and progress of tissue inflammation. Currently, there is no good experimental model available for the effects of *Chlamydia* infections during pregnancy and its association with neonatal death.

Aside from the pulmonary and conjunctival tissues of newborns, *Chlamydia* has also been found in intestinal, genitourinary, myocardial, and nervous system tissues.\(^10\,11\,12\) The presence of *Chlamydia* in these other tissues suggests that it has an invasive capacity. The present study aimed to detect chlamydial DNA in different tissues of neonates who were diagnosed with ‘‘infection without an isolated pathogen’’ and died during the first week of life. Neonates whose evident cause of death was not infectious were also studied. Additionally, it was sought to identify the *Chlamydia* genotypes involved in these neonates who developed early infection.

Methods

Ethics statement

This study’s protocol was reviewed and approved by the institutional Ethics Committee of the School Medicine at the National Polytechnic Institute, in Mexico City, Mexico.

Definitions

Infection

Severe neonatal infection was established by: a) finding of *C. trachomatis omp1* gene fragments in two or more different organs; b) clinical and laboratory data consistent with infection in the neonate during his lifetime; c) mother with antecedents of infection risk; and d) histopathological...
diagnosis of placental chorioamnionitis and pneumonitis in the cadaver.

*Preterm rupture of membranes (PROM).* Rupture of the fetal membranes prior to the onset of labor, regardless of gestational age.\(^{14}\)

*Early neonatal death.* Death occurring before the seventh day of extrauterine life.\(^{15}\)

**Inclusion criteria**

The inclusion criteria were: availability of all selected organs, samples, and reports; updated analysis by the perinatal mortality committee with final dictum; and complete records.

**Selection of autopsy samples**

A random selection of 20% of the cases of early neonatal deaths that occurred between January 1 and December 31, 2003 and fulfilled the inclusion criteria was performed. Cases were separated into two groups according to the judgment or final diagnosis of the institution’s perinatal mortality committee. Group 1 consisted of cases of death due to systemic infection\(^{16}\) without pathogen identification. Group 2 was formed by cases of death due to another cause that was not related with infection.

The autopsy organs, specifically lungs, kidneys, brain, liver, and bronchi, which were embedded in paraffin blocks for all participating cases, were located. The study included only cases that had all these organs available, as well as complete maternal and neonatal clinical records and histopathological studies of the autopsy and placenta. The pathologist in chief blindly restudied the histologic samples from the cadavers.

Placenta samples are usually routinely analyzed: hematoxylin and eosin-stained histologic sections are investigated for deciduitis, vasculitis, endometritis, or chorioamnionitis without special studies. Later, the entire material is discarded. For this reason, only the results of the single study of the placenta were included.

The rest of the plates were deparaffinized and processed for DNA extraction. All tests were performed in a blinded manner. Clinical and demographic data were obtained from hospital records.

**DNA extraction**

Tissue samples were deparaffinized with xylene at room temperature and washed with ethanol. The obtained tissue was then treated with proteinase K, and the DNA was obtained by the phenol-chloroform-isoamyl technique and ethanol precipitation as previously described.\(^{17}\)

**Chlamydia trachomatis detection**

End-point polymerase chain reaction and real-time polymerase chain reaction were performed using PTC-100 system (MJ Research, Inc. · Waltham, MA, United States) and StepOne (Applied Biosystems · Carlsbad, CA, United States), respectively. The primers used for end-point polymerase chain reaction are those proposed by Dutilh et al.\(^{18}\) and amplify a fragment of the omp1 gene of *C. trachomatis* (5’-GCCGCTTTGAGTCTGCTTCTC-3’; 5’-CCAAGTGTGCA AGGATCGCA-3’).

Each end-point polymerase chain reaction contained 1.75 mM MgCl\(_2\), 0.2 mM dNTPs, 25 pM of each proposed primer, 2.5 U Taq polymerase (GoTaq\(^{®}\) Flexi DNA polymerase Promega\(^{®}\) Madison, WI, United States), and 5 µL of the DNA sample, for a final volume of 25 µL. The reaction mixture was incubated for 5 min at 95 °C, followed by 35 cycles of 1 min at 95 °C for denaturation, 1 min at 59 °C for alignment, 1 min at 70 °C for extension, and a final elongation step of 5 min at 70 °C. The sample was considered positive when an amplification product of 129 bp was obtained.

The real-time polymerase chain reaction was performed using 3 mM of 2.7 mM MgCl\(_2\), 25 pM of each of the proposed primers, 2.5 U Taq polymerase (Applied Biosystems), and 5 µL of the DNA sample, for a final volume of 25 µL. The primers used were designed by Primer Express 3.0 Sequence program (Applied Biosystems), the amplified region is inside the same amplicon of 129 bp obtained with Dutilh’s primers Fw: 5’-CTCTGCTGAACCAACC TTATG-3’ and Rv: 5’-AGGATCTC GCGCAAAACC-3’; probe: 5’-TCGAGGAAATC TGT-3’. The reaction mixture was pre-heated at 95 °C for 20 s, followed by 40 cycles of 30 s at 95 °C, 20 s at 60 °C, and 20 s at 72 °C.

**Amplification and detection of Mycoplasma spp. for differential diagnoses**

End-point polymerase chain reaction was performed according to the protocol proposed by van Kuppeveld et al.\(^{19}\) The primers used were: MGSO: 5’-GCCCACTCTGTCATCTGTTA ACCTC-3’ and GPO-1: 5’-ACTCTCACGGGAGCAGCATAT III. Each polymerase chain reaction contained 20 pM/µL primers, 10 µM/µL dNTPs, 2 mM/µL MgCl\(_2\), 5 U/µL Taq polymerase, 3 µL DNA, and 43 µL water, for a final volume of 50 µL. The reaction mixture was incubated at 94 °C for 5 min, followed by 35 cycles of denaturing at 94 °C for 1 min, alignment at 60 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. A sample was considered positive if a 715 bp product was amplified.

To detect the species, the following primers were used with the same polymerase chain reaction protocol: Mychomp (5’-ATACAATGACGATCGAGCAG-3’) and Mychomn (5’-CATCTT TTAGGGGCCCTTAC-3’). Additionally, *M. hominis* was detected according to Grav et al.\(^{20}\) and *U. urealyticum* was detected according to Blanchard et al.\(^{21}\) with the primers US (5’-CAATCT GCTGGTGAAGTATTAC-3’) and U4 (5’-AGCACGTC CATAGCACAAT-3’).

**Analysis of restriction fragment length polymorphism**

A restriction fragment length polymorphism (RFLP) analysis was performed using a previously described method\(^{22}\) to genotype the samples with 129 bp amplicons. Briefly, a 1,142 bp fragment of the *C. trachomatis* omp1 gene was amplified. The primers used for the amplification were the same as those reported by Yang et al.\(^{22}\) OMP1 (5’-GCCGCTTTG AGTCTGCTTCTC-3’) and OMP2
Chlamydia trachomatis in tissues of neonatal death cases

(5'-ATTACGTGAGCAGCTCTCTCA T -3'). Samples positive for the 1,142 bp amplicon were subjected to a second polymerase chain reaction that generated an 879 bp fragment. The primers used were also described by Yang et al.22 P3 (5'-TGACCTTGTGGTTCGA CCGTGGTTT-3') and P4 (5'-TTTTCTAGATTTCCTTGTCAT/CTG-3'). The 879 bp fragment was then incubated with the endonuclease AluI (Invitrogen, Applied Biosystems) for 10 h at 37 °C. The resulting band pattern was compared with that of the corresponding type strain. In this study, that was uW-3Cx (ATCC VR-885D).

**Results**

Of the 73 cases available, 18 were chosen at random. However, it was not possible to locate all the organs of interest in three cases, and the placenta study results were not available for one case. Table 1 depicts results of the 14 infant mortality cases studied, the perinatal characteristics of the newborns, and the most relevant maternal data. Four cases fulfilled all of the infection or neonatal systemic infection criteria (cases 1, 3, 4, and 8). Cases 1 and 8 were negative for bacterial and fungal cultures performed both during their life or postmortem. On the first day of life for case 3, Staphylococcus haemolyticus was isolated through blood culture. In case 4, the newborn lived only one hour, and the corresponding microbiological cultures were not performed.

The intentional search for DNA from Mycoplasma and Chlamydia in postmortem tissues using polymerase chain reaction resulted in amplification of the 129 bp C. trachomatis omp1 gene in five neonates. It was amplified in two or more organs for cases 1, 2, 3, and 4, and only in the kidney for case 6. In case 8, a 715 bp product, corresponding to Mycoplasma, was amplified in lung and kidney tissues (image not shown).

Chlamydial DNA was found in tissues of cases 2 and 6, but with no clinical or histopathological correlation indicating infection. These cases only presented with prematurity, barotrauma, and pulmonary hemorrhage. Additionally, amplification of 1,142 bp and 879 bp fragments for RFLP analysis of samples positive for chlamydial DNA was only achieved for cases 1, 3, and 4 (Fig. 1). The RFLP analysis of the samples where amplification of the 879 bp fragment was achieved only allowed for the identification of genotype D of C. trachomatis (case 1; Fig. 2). In cases 2 and 6, it was not possible to amplify the 1,142 bp fragment, and the presence of chlamydial DNA was confirmed in liver tissue through real-time polymerase chain reaction only for case 2 (image not shown).

All cases were endotracheally intubated and received ventilatory assistance during their entire life. Six cases of premature membrane rupture were found. Of these, two neonates (cases 1 and 3) had evidence of chlamydial DNA and remained in utero with ruptured membranes for 8 and 6 days, respectively. The clinical characteristics of all cases are shown in Table 2.

The causes of death in the remaining cases (cases 5 to 7 and 9 to 14) were not associated with infection. Four (29%) died due to causes related with their premature birth, three (21%) due to structural congenital defects not compatible with life, one due to hydrops fetalis of non-immunological origin with severe preeclampsia, and one due to maternal-fetal isoimmunization to Rh. All these cases had negative polymerase chain reaction results for C. trachomatis and Mycoplasma spp., with the exception of case 6, previously mentioned.

**Discussion**

Severe infections are still the main cause of neonatal morbidity and mortality in developing regions. Three-fourths of infant mortality cases occur during the first week of life, and in many cases, the cause of death remains unknown.23 Chlamydial infection in the perinatal and neonatal stage can cause many diseases. These include conjunctivitis, nasopharyngitis, pneumonia, and less frequently, rhinitis, middle ear otitis, myocardiitis, and encephalitis.4 However, the discovery of genetic residues of this intracellular bacterium in organic tissues is quite rare in the medical
<table>
<thead>
<tr>
<th>Case</th>
<th>Maternal risk factor</th>
<th>Autopsy diagnosis</th>
<th>Placental diagnosis</th>
<th>Bacterial cultures outcomes</th>
<th>PCR</th>
<th>Previous diagnosis*</th>
<th>New diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PROM eight days</td>
<td>Congenital pneumonia</td>
<td>Chorioamnionitis</td>
<td>All negative</td>
<td>C. trachomatis in liver, brain, and bronchus</td>
<td>Neonatal sepsis UEA</td>
<td>Infection by C. trachomatis</td>
</tr>
<tr>
<td>2</td>
<td>Preterm delivery. Previous stillbirth</td>
<td>Moderate pneumothorax, 40% atelectasis</td>
<td>Normal</td>
<td>CSF PM P rettgeri + E gergoviae</td>
<td>C. trachomatis in liver and brain</td>
<td>Pneumothorax</td>
<td>Probable infection by C. trachomatis</td>
</tr>
<tr>
<td>3</td>
<td>PROM six days</td>
<td>Acute pneumonia, hyaline membrane</td>
<td>Chorioamnionitis</td>
<td>First day: hemoculture S haemolyticus</td>
<td>C. trachomatis in liver, kidney and brain</td>
<td>Neonatal sepsis UEA</td>
<td>Infection by C. trachomatis</td>
</tr>
<tr>
<td>4</td>
<td>PROM 26 h</td>
<td>Severe congenital pneumonia</td>
<td>Necrotizing chorioamnionitis, funisitis</td>
<td>Third day: CSF and hemoculture negative NM</td>
<td>C. trachomatis in liver and bronchus</td>
<td>Neonatal sepsis UEA</td>
<td>Infection by C. trachomatis</td>
</tr>
<tr>
<td>5</td>
<td>Previous abortion</td>
<td>Multiple major structural defects</td>
<td>Small</td>
<td>All negative</td>
<td>All negative</td>
<td>Multiple congenital structural defects</td>
<td>Multiple congenital structural defects</td>
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<tr>
<td>6</td>
<td>None</td>
<td>Renal right agenesis Pulmonary hemorrhage</td>
<td>Normal</td>
<td>NM</td>
<td>C. trachomatis in kidney</td>
<td>Surfaceant pulmonary hemorrhage</td>
<td>Complications of prematurity</td>
</tr>
<tr>
<td>7</td>
<td>PROM 26 h</td>
<td>33% Hyaline membrane 70% atelectasis</td>
<td>Normal</td>
<td>All negative</td>
<td>All negative</td>
<td>Hyaline membrane Pulmonary hypertension</td>
<td>Complications of prematurity</td>
</tr>
<tr>
<td>8</td>
<td>PROM 8 h</td>
<td>Congenital pneumonia</td>
<td>Severe chorioamnionitis and funisitis</td>
<td>All negative</td>
<td>Mycoplasma hominis in lung and kidney</td>
<td>Congenital neonatal sepsis UEA</td>
<td>Infection by Mycoplasma hominis</td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>Hydrops fetalis</td>
<td>Signs of hypoxia</td>
<td>Hemoculture: Staphylococcus spp. All the other negative</td>
<td>Nonimmune Hydrops fetalis</td>
<td>Nonimmune Hydrops fetalis</td>
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</tr>
<tr>
<td>10</td>
<td>Previous abortion</td>
<td>Immaturity systemic Erythroblastosis. Pulmonary emphysema</td>
<td>Normal</td>
<td>NM</td>
<td>All negative</td>
<td>Immaturity Obstetric trauma</td>
<td>Extreme prematurity</td>
</tr>
<tr>
<td>11</td>
<td>Two previous stillbirth and an infant death PROM 1 h</td>
<td>Multiple major structural defects</td>
<td>Normal</td>
<td>All negative</td>
<td>All negative</td>
<td>Hydrops fetalis from Rh isomunization</td>
<td>Extreme prematurity</td>
</tr>
<tr>
<td>12</td>
<td>None</td>
<td>Immaturity Mild subchorionitis and deciduiitis</td>
<td>Normal</td>
<td>All negative</td>
<td>All negative</td>
<td>Multiple major structural defects</td>
<td>Extreme prematurity</td>
</tr>
<tr>
<td>13</td>
<td>An infant death</td>
<td>Thanatophoric dysplasia</td>
<td>Normal</td>
<td>All negative</td>
<td>Severe bone dysplasia</td>
<td>Severe bone dysplasia</td>
<td></td>
</tr>
</tbody>
</table>

CSF, Cerebrospinal fluid; CT, Chlamydia trachomatis; NM, not made; PCR, polymerase chain reaction; PM, post-mortem; PROM, Preterm Rupture of Membranes (18); UEA, unknown etiologic agent.

* Determined by the Perinatal Mortality Committee.
Table 2  Clinical characteristics (mother and child) of the infant mortality cases.

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
<th>Case 7</th>
<th>Case 8</th>
<th>Case 9</th>
<th>Case 10</th>
<th>Case 11</th>
<th>Case 12</th>
<th>Case 13</th>
<th>Case 14</th>
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<tr>
<td>Age</td>
<td>3d 7h</td>
<td>2d 1h</td>
<td>1h 2m</td>
<td>5d 20h</td>
<td>3h 1m</td>
<td>1d 20h</td>
<td>1d 12h</td>
<td>1d 2h</td>
<td>20m</td>
<td>18h</td>
<td>5d 19m</td>
<td>41m</td>
<td>1h 20m</td>
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</tr>
<tr>
<td>Maternal age (years)</td>
<td>33</td>
<td>28</td>
<td>35</td>
<td>26</td>
<td>26</td>
<td>15</td>
<td>23</td>
<td>36</td>
<td>23</td>
<td>30</td>
<td>27</td>
<td>18</td>
<td>23</td>
<td></td>
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<tr>
<td>Duration of PROM</td>
<td>8d</td>
<td>0</td>
<td>6d</td>
<td>26h</td>
<td>0</td>
<td>0</td>
<td>1d 2h</td>
<td>8h</td>
<td>0</td>
<td>0</td>
<td>1h</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td>Delivery</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>ED</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>ED</td>
<td>C</td>
<td>C</td>
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<tr>
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<td>Female</td>
<td>Female</td>
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<td>Male</td>
<td>Male</td>
<td>Female</td>
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<tr>
<td>Apgar (1 and 5 minutes)</td>
<td>2-5</td>
<td>5-4</td>
<td>3-7</td>
<td>1-2</td>
<td>7-8</td>
<td>4-7</td>
<td>4-9</td>
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<td>3-7</td>
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<tr>
<td>Weight (g)</td>
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<td>740</td>
<td>710</td>
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<td>910</td>
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<td>34</td>
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<td>43</td>
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<td>30</td>
<td>38</td>
<td>45</td>
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<tr>
<td>Gestational age (weeks)</td>
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<td>29</td>
<td>27</td>
<td>26</td>
<td>41</td>
<td>26</td>
<td>32</td>
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<td>25</td>
<td>27</td>
<td>32</td>
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<tr>
<td>Maternal diagnostic</td>
<td>Hyp</td>
<td>PD</td>
<td>PROM Ch</td>
<td>PROM Ch</td>
<td>IGR</td>
<td>UTI Ch</td>
<td>PROM Ch</td>
<td>PROM Ch</td>
<td>Severe Pre-eclam</td>
<td>PD UTI</td>
<td>Isoimm</td>
<td>Hydrops fetalis</td>
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<tr>
<td>Neonatal antibiotic treatment</td>
<td>Ampi</td>
<td>Amika</td>
<td>Ami</td>
<td>Ami</td>
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<td>Ami</td>
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<tr>
<td>Growth for gestational age</td>
<td>SGA</td>
<td>AGA</td>
<td>SGA</td>
<td>AGA</td>
<td>SGA</td>
<td>AGA</td>
<td>AGA</td>
<td>AGA</td>
<td>AGA</td>
<td>LGE</td>
<td>AGA</td>
<td>AGA</td>
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</tr>
</tbody>
</table>

AGA, appropriate for gestational age; Amika, amikacin; Ampi, ampicillin; C, Cesarean section; Ch, chorioamnionitis; d, days; DD, dystotic delivery; Dicloxa, dicloxacillin; ED, eutotic delivery; h, hours; Hyp, hypothyroidism; IGR, intrauterine growth retardation; Isoimm, isoimmunization; LGE, large for gestational age; m, minutes; PD, preterm delivery; Pre-eclam, pre-eclampsia; PROM, premature rupture of membranes; SGA, small for gestational age; UTI, urinary tract infection.
Additionally, there is suspicion of congenital infection, since it covers almost all etiological possibilities of neonatal infection acquired in utero. However, the Chlamydia and Mycoplasma genera are not included in their antimicrobial spectrum. Ocular prophylaxis can fail to prevent neonatal chlamydial conjunctivitis, and does not prevent colonization or infection in the lungs; the only means of preventing chlamydial infection in the newborn is treating the infected mother.

Case 1 did not have bacterial or fungal development in the performed cultures, and case 2 was classified as contaminated because the cerebrospinal fluid sample was obtained postmortem and developed the polymicrobial species Providencia retgeri and Enterobacter gergoviae, which are not known as producers of neonatal infections. Case 3 was delivered by cesarean section with six days of premature membrane rupture and maternal chorioamnionitis. At birth, a sample of umbilical cord blood was obtained for culture, which showed the development of Staphylococcus haemolyticus. This bacterium is part of the cutaneous flora and is not a pathogen that produces congenital neonatal infections. Additionally, treatment with ampicillin and amikacin does not cover Staphylococcus haemolyticus, which is characteristically multiresistant. These premises support the conclusion that those samples were contaminated.

The serotypes of C. trachomatis with high invasive capacity can invade diverse tissues of adult individuals and cause lymphogranuloma (L1, L2, L2a, and L3). In this study, it was shown that the C. trachomatis infection in case 1 was due to genotype D, which is one of the highest prevalence serotypes worldwide in genitourinary infections of the sexually active population. Genotype D is one of the few C. trachomatis serotypes with a cytotoxic that has great homology to the family of large cytoxins (LCTs) produced by Clostridium difficile. These LCTs cause diverse cytopathic effects in their host cells because their glucosyltransferase activity modifies the intracellular regulatory molecules such as the GTP binding molecule of the Ras superfamily. Additionally, LCTs generally interfere with the organization and dynamics of actin in both the cytoskeleton and intracellular trafficking. The cytopathic effect produced by the cytotoxic of C. trachomatis serotype D results in the rounding of infected cells due to depolymerization of actin. This could help explain, in part, the capacity of serotype D to infect and disseminate to diverse organs in those newborns in whom chlamydial DNA was found. However, the mechanisms through which C. trachomatis infection is disseminated to different organs are yet to be identified.

This study presented the possibility of infection by C. trachomatis to various organs of fetuses and newborns, and that this might have been associated with infant mortality. However, further studies are needed to confirm this finding. The finding of chlamydial DNA in more than one organ of autopsy may not be the most accepted way for diagnosing systemic infection or establishing cause of death. However, in the authors’ opinion, the discovery of three different C. trachomatis DNA sequences by end-point and real-time polymerase chain reaction and identification in one case of the C. trachomatis genotype involved provides sufficient evidence for further investigations using additional and improved resources. Immunohistochemistry would
strengthen the evidence presented here, but unfortunately, immunohistochemical stains were not performed in the present samples.

Conflicts of interest

The authors declare to have no conflict of interests.

References