Molecular detection of chlamydia-like organisms in cattle drinking water

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Abstract

A substantial proportion of the causes of infectious bovine abortion remain largely undiagnosed, potentially due to the presence of previously unrecognised infectious agents. Recently, several reports have demonstrated the presence of Parachlamydia sp. in placental and foetal tissues derived from bovine abortions of unknown aetiology but the route of transmission remains undefined. The drinking water from one such recent case study was analysed for the presence of Parachlamydia sp. as a potential source of infection. Chlamydiales sp. 16S rRNA genes were PCR-amplified from the drinking water and a 16S rRNA gene clone library constructed. DNA sequencing of thirty-one clones indicated the presence of organisms belonging to the Parachlamydiaceae, specifically the genera Parachlamydia and Neochlamydia. Seven 16S rRNA gene sequences were identical to a Parachlamydia sp. sequence obtained from placental tissue from an abortion case originating from the same farm. These results raise the possibility that the drinking water is a source of Parachlamydia, which may play a role in infectious bovine abortion.
Short Communication

Molecular detection of *Chlamydia*-like organisms in cattle drinking water

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\textsuperscript{1}Equal contributions
Abstract

A substantial proportion of the causes of infectious bovine abortion remain largely undiagnosed, potentially due to the presence of previously unrecognised infectious agents. Recently, several reports have demonstrated the presence of *Parachlamydia* sp. in placental and foetal tissues derived from bovine abortions of unknown aetiology but the route of transmission remains undefined. The drinking water from one such recent case study was analysed for the presence of *Parachlamydia* sp. as a potential source of infection. *Chlamydiales* sp. 16S rRNA genes were PCR-amplified from the drinking water and a 16S rRNA gene clone library constructed. DNA sequencing of thirty-one clones indicated the presence of organisms belonging to the *Parachlamydiaceae*, specifically the genera *Parachlamydia* and *Neochlamydia*. Seven 16S rRNA gene sequences were identical to a *Parachlamydia* sp. sequence obtained from placental tissue from an abortion case originating from the same farm. These results raise the possibility that the drinking water is a source of *Parachlamydia*, which may play a role in infectious bovine abortion.

Keywords

*Parachlamydiaceae*; cattle; abortion; water
1. Introduction

Bovine abortion is a significant animal welfare issue and a cause of significant economic loss, yet despite this, investigations leading to no definitive diagnosis remain high (Cabell, 2007). In Great Britain, figures from the 2002-09 Veterinary Investigation Surveillance (VIDA) Report show that 77% (6,560 of 8,549 samples) of diagnosed bovine fetopathies resulted from an infectious cause, whereas no diagnosis was reached for 80% (34,462 of 43,011 samples) of all cases of bovine fetopathy (http://www.defra.gov.uk/vla/reports/docs/rep_vida_cattle_2009.pdf). This could in part be explained by the presence of unidentified infectious abortifacient agents. While Chlamydophila abortus is recognised as a known aetiological agent of ruminant abortion (Longbottom and Coulter, 2003), several novel species of Chlamydia-like organisms have also emerged as putative ruminant abortifacients. These species include Waddlia chondrophila, isolated originally from an aborted bovine fetus in the USA (Dilbeck et al., 1990) and subsequently identified in a stillborn calf in Germany (Henning et al., 2002). Recent studies carried out on aborted bovine placentas in Switzerland, using both molecular and immunohistochemical techniques, identified the presence of other non-characterised chlamydia-like organisms and Parachlamydia sp. (Borel et al., 2007; Ruhl et al., 2009). These findings were supported by the identification of Parachlamydia, Rhabdochlamydia and Neochlamydia species in foetal and placental tissue samples from the UK (Deuchande et al., 2010; Wheelhouse et al., 2010). However, although several reports have demonstrated the presence of these organisms in aborted bovine tissues, no studies have investigated possible sources of infection. Many of the identified strains of Parachlamydia were discovered as intracellular parasites of amoeba isolated from a range of environmental niches.
including water supplies (Greub and Raoult, 2002). This study was carried out to examine whether contaminated drinking water could be a potential source of transmission of *Parachlamydia* on a farm that experienced an abortion storm in which parachlamydial involvement was confirmed (Deuchande et al., 2010).

### 2. Materials and Methods

#### 2.1 Isolation of DNA

Samples were obtained from a Scottish farm on which suspected parachlamydial involvement in cases of bovine abortion were previously reported (Deuchande et al., 2010). Water samples were taken from each of the cattle drinking troughs in the calving sheds and also directly from the bore hole supplying them. Prior to DNA extraction 35ml of each sample was centrifuged at 20,000 rpm for 60 min using an SW32Ti rotor in a Beckman Coulter Optima™ L-90K ultracentrifuge (Beckman Coulter (UK) Ltd., High Wycombe, Buckinghamshire, UK). DNA was extracted from the subsequent pellet using the QIAamp DNA stool mini kit (Qiagen, Crawley, UK).

#### 2.2 PCR, cloning and sequencing

A pan-*Chlamydiales* PCR targeting the 16S rRNA gene was performed using forward primer 16S FOR2 (5’-CGTGGATGAGGCATGCAAGTCGA-3’) and reverse primer 16S REV2 (5’-CAATCTCTCAATCCGCTAGACGTCTTAG-3’) to generate amplicons of approximately 260bp (Ossewaarde and Meijer, 1999). Negative-control reactions contained DNA-free water instead of extracted DNA. PCR products were purified (Qiaquick® PCR purification kit, Qiagen) prior to cloning into the pGEM-T® vector (Promega, Hampshire UK). Plasmids were transformed into *Escherichia coli* JM109 competent cells (Promega) and successfully transformed cells were selected by
ampicillin resistance and blue-white colony selection, according to standard procedures. The insertion of PCR products was confirmed by colony PCR using the 16S rRNA gene pan-Chlamydiales primers. Positive colonies resulting from each water trough were grown in overnight broths, the plasmid DNA extracted using a Qiagen Miniprep kit (Qiagen), and sequenced. Sequencing was achieved using primers directed against the T7-promoter sequence of the plasmid by dideoxy chain termination / cycle sequencing on an ABI 3730XL DNA sequencer (Eurofins MWG Operon, Ebersberg, Germany).

2.3 Sequence analysis

16S rRNA gene sequences were aligned in ClustalW2 (Larkin et al., 2007) and the Phylip program DNAdist (Felsenstein, 1989) was used to calculate a distance matrix using a Jukes Cantor model of nucleotide substitution (Jukes and Cantor, 1969). The software package MOTHUR was used to assign sequences to operational taxonomic units (OTUs) and generate rarefaction curves to assess diversity and sampling intensity (Schloss et al., 2009). The phylogenetic classification of a representative sequence from each OTU was determined using the Ribosomal Database Project (RDP) Classifier program with a bootstrap cut-off of 50% (Wang et al., 2007) and by comparison with sequences in the NCBI DNA databases. All sequences were submitted to GenBank.

3. Results

PCR analysis revealed the presence of chlamydial DNA in each of the drinking troughs but not in the original bore-hole water source. The 31 16S rRNA gene sequences (GenBank accession HQ702405-HQ702435) were grouped into 12 OTUs using a cut-off of ≥99% sequence identity (Table 1). Rarefaction curves appeared to be close to saturation indicating that the 31 clones sequenced were representative of the
diversity present in the water sample and that further sampling was unlikely to reveal additional diversity (Figure 1).

Fifteen and sixteen of the cloned 16S rRNA gene sequences each representing 6 OTUs originated from organisms belonging to the genera *Neochlamydia* and *Parachlamydia* respectively (Table 1). BLAST analysis revealed that representative sequences from each OTU were closely related to Parachlamydiaceae sequences obtained from environmental samples and clinical samples taken from bovine abortion, ovine ocular infections and fish epitheliocystis. Seven (22.5%) of the 16S rRNA gene sequences obtained from the water troughs and allocated to OTU 1 were identical to a 16S rRNA gene recovered from a placental sample originating from a case of bovine abortion from the same farm (Deuchande et al., 2010).

4. Discussion

Parachlamydiaceae have now emerged as potential causes of bovine abortion both in the UK (Deuchande et al., 2010; Wheelhouse et al., 2010) and in Switzerland (Borel et al., 2007; Ruhl et al., 2009). Despite this there have been no studies investigating the possible sources of infection. This study was carried out to determine whether drinking water could be a possible medium for parachlamydial transmission in cattle in which *Parachlamydia* had been detected in aborted placental tissue (Deuchande et al, 2010). Given that the water at source was negative, yet the water in the drinking troughs was positive by PCR for the presence of parachlamydial DNA, suggests that contamination of the trough water occurred after extraction from the borehole in this group of animals. Moreover, the similarity in sequence of many of the samples with one obtained from a placenta on the same farm suggests that the water is a potential source for transmission of the infection from one animal to another. However,
although the source of this contamination is unclear at present, *P. acanthamoebae* has been demonstrated to exist as an endosymbiont of *Acanthamoeba* sp. that commonly inhabit water samples. Furthermore, while *P. acanthameoba* exists as an endosymbiont at environmental temperatures it is lytic to its protozoan host at physiological temperatures, exposing the animal to the organism and promoting infection (Greub et al., 2003). This could suggest that thorough cleaning and disinfection of the troughs before the re-introduction of animals into housing should be considered to reduce the risk of infection.

In addition to bovine abortion, *Chlamydia*-like organisms have also been reported to play possible roles in other pathogenic conditions, such as ocular infections in sheep (Polkinghorne et al., 2009). Indeed, for 16 of the 31 submitted sequences the top DNA database matches were sequences obtained from ocular swabs from sheep with ocular disease, possibly indicating that the same organisms may cause a range of conditions in different host species. As well as their potential roles in animal disease there is a possible threat to human health, with evidence supporting a role for *Parachlamydia* sp. in human pneumonia and ocular disease (Greub, 2009). There is also evidence that *P. acanthamoebae* can cross the human placenta to the unborn fetus (Baud et al., 2009) and be a cause of neonatal morbidity and hospitalisation (Lamoth et al., 2009). Its isolation from human nasal mucosa (Amann et al., 1997), the presence of it within guinea-pig (Lutz-Wohlgroth et al., 2006) nasal secretions and its detection in ocular secretions of both sheep (Polkinghorne et al., 2009) and domesticated cats (Richter et al., 2010) suggests that contamination of the drinking water by ocular or nasal secretions may act as a potential source for transmission to livestock, which requires further more detailed investigation.
In summary, this report demonstrates the presence in the environment of Parachlamydia sp. that have been linked to cases of bovine abortion and the possible role of drinking water as a source of infection. These findings support the need for further investigations to determine the role of Parachlamydiaceae sp. in bovine abortion and their potential environmental reservoirs.

Acknowledgments

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References


Figure 1. Rarefaction curve showing the observed diversity of *Chlamydiales* 16S rRNA genes retrieved from water trough samples. The average number of OTUs retrieved at each sampling is indicated by (●). Upper and lower 95% confidence intervals are indicated by dashed lines. A theoretical 1:1 sampling is represented by the solid line.
Table 1. OTU grouping and phylogenetic assignment of *Parachlamydiaceae* 16S rRNA gene sequences retrieved from water trough samples.

<table>
<thead>
<tr>
<th>Operational taxonomic unit (OTU)</th>
<th>Clones assigned to OTU</th>
<th>Genus assignment by RDP Classifier (% confidence)</th>
<th>Top BLAST match, (% similarity), source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WT16, WT20, WT15, WT45, WT48, WT19, WT17</td>
<td><em>Parachlamydia</em> (70%)</td>
<td>FJ160739. Uncultured <em>Chlamydiales</em> bacterium clone USC7 (99%) Sheep with mild ocular disease</td>
</tr>
<tr>
<td>2</td>
<td>WT2, WT10, WT9, WT8</td>
<td><em>Neochlamydia</em> (73%)</td>
<td>FJ160741. Uncultured <em>Chlamydiales</em> bacterium clone USC9 (90%) Sheep with severe ocular disease</td>
</tr>
<tr>
<td>3</td>
<td>WT30, WT23, WT27, WT24</td>
<td><em>Parachlamydia</em> (68%)</td>
<td>FJ160733. Uncultured <em>Chlamydiales</em> bacterium clone USC1 (93%) Sheep with mild ocular disease</td>
</tr>
<tr>
<td>4</td>
<td>WT34, WT36, WT31</td>
<td><em>Neochlamydia</em> (52%)</td>
<td>AF097198. Uncultured <em>Chlamydiales</em> bacterium clone CRG15 (94%) Human respiratory tract infection</td>
</tr>
<tr>
<td>5</td>
<td>WT4, WT21, WT3</td>
<td><em>Neochlamydia</em> (64%)</td>
<td>AY013469. Uncultured <em>Chlamydiales</em> bacterium clone CRG93 (99%) Human respiratory tract infection</td>
</tr>
<tr>
<td></td>
<td>WT44, WT47, WT46</td>
<td>Neochlamydia (84%)</td>
<td>Soil</td>
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<td>6</td>
<td></td>
<td>AY326518. Uncultured soil bacterium clone 4-1 (94%)</td>
<td></td>
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<tr>
<td>7</td>
<td>WT41, WT13</td>
<td>Parachlamydia (70%)</td>
<td>Human respiratory tract infection</td>
</tr>
<tr>
<td>8</td>
<td>WT42</td>
<td>Parachlamydia (74%)</td>
<td>Sheep with mild ocular disease</td>
</tr>
<tr>
<td>9</td>
<td>WT22</td>
<td>Parachlamydia (83%)</td>
<td>AM408789. Endosymbiont of Acanthameoba sp. E12 (88%)</td>
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<tr>
<td>10</td>
<td>WT14</td>
<td>Parachlamydia (54%)</td>
<td>Human respiratory tract infection</td>
</tr>
<tr>
<td>11</td>
<td>WT6</td>
<td>Neochlamydia (69%)</td>
<td>FJ376381. Uncultured Chlamydiales bacterium clone UFC3 (91%)</td>
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<tr>
<td>12</td>
<td>WT7</td>
<td>Neochlamydia (55%)</td>
<td>FJ532292. Parachlamydiaceae bacterium clone CRIB39 (93%)</td>
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<td></td>
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<td>Fish with epitheliocystis</td>
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<td>Water</td>
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Figure