Coxiella burnetii Infection of a Steller Sea Lion (Eumetopias jubatus) Found in Washington State

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A pregnant sea lion stranded in the State of Washington was found to have placentitis caused by a unique strain of Coxiella burnetii. This is the first description of coxiellosis in a sea lion and suggests that exposure to sea lions may be a risk factor for contracting Q fever.

CASE REPORT

A pregnant adult female Steller sea lion (Stranding number WDFW2008-058) was found dead on the beach at Westhaven State Park in Westport, WA (lat 46.8981, long 124.1307), on 9 June 2008. The adult female had an estimated weight of 250 to 300 kg and was in fair body condition. Based on the condition of the body and the frequency of beach surveys in the area, the time since death was estimated at approximately 3 days. The animal was in moderate postmortem condition. The abdominal cavity was distended by the enlarged uterus, a near-term fetus, and approximately 2 liters of creamy, brown-pink exudate. Fish bones were dispersed throughout the omentum, and there was pronounced thickening and opacification of the omentum and mesentery. The spleen was moderately enlarged, and there was pronounced injection of the meningeal vasculature, with scattered hemorrhage throughout the surface of the brain. Gross necropsy of the fetus disclosed a near-term male with a weight of 19 kg. The fetus was in good body condition. The fetal heart by PCR (13).

Genomic DNA was purified from the infected sea lion placenta using the QIAamp DNA minikit (Qiagen, Valencia, CA), and PCR was performed to test for multiple Coxiella burnetii (data not shown).
Coxiella \textit{djIA} gene was different from the Nine Mile strain sequence at 20 sites (97.2\% identity). Among the 37 isolates mentioned above, none of the \textit{djIA} gene sequences differed from the Nine Mile sequence at more than 4 bases (16). Thus, the sequence of the \textit{com1} and \textit{djIA} genes reveals a strain of \textit{Coxiella} that is remarkably distinct from the characterized strains of \textit{C. burnetii}.

The sequences of 16S rRNA genes among the five fully sequenced strains of \textit{C. burnetii} differ from the Nine Mile reference strain at only 0 bases, 1 base, or 2 bases. The 16S rRNA gene from the sea lion strain of \textit{C. burnetii} was sequenced, and it matched the Nine Mile reference sequence at 1,478/1,482 nucleotides (99.7\% identical). A phylogenetic tree was constructed using the 16S rRNA sequences of the sea lion strain, 5 sequenced strains of \textit{C. burnetii}, and the near-neighbor species \textit{Legionella pneumophila} and \textit{Pasteurella multocida} (Fig. 2). When the 16S rRNA genes of these other species are compared to known \textit{C. burnetii} strains and the strain infecting the sea lion, all of the \textit{C. burnetii} strains, including the sea lion strain, fall into a closely related group. Although it is distinct from other strains of \textit{C. burnetii} by having 4 nucleotide substitutions instead of 1 or 2, this 16S rRNA sequence is much more like \textit{C. burnetii} than other near-neighbor species.

\textit{Coxiella burnetii} causes Q fever, a worldwide zoonosis. Acute Q fever is a febrile illness, with hepatitis or pneumonia found in more severe cases (6). Chronic Q fever usually presents as a culture-negative endocarditis. Recent outbreaks of Q fever have been associated with exposure to \textit{C. burnetii} via inhalation of aerosolized bacteria from infected livestock (cows, sheep, or goats) (1, 14, 15). \textit{C. burnetii} can replicate to high levels in the placentas of infected animals and is an established endemic abortifacient in goats and sheep (2, 9). A recent case of chronic Q fever in Greenland in a resident whose primary animal exposure was to sled dogs and seals raises the question of whether marine mammals expose humans to \textit{C. burnetii} (7). The only previous description of \textit{C. burnetii} infection in a marine mammal was a report of \textit{C. burnetii} placentitis in a Pacific harbor seal found in California (10). The findings for this Steller sea lion expand the geographic and host range of \textit{C. burnetii} and show that \textit{C. burnetii} can replicate to high levels in the placentas of marine mammals, just as it does in terrestrial mammals. This case therefore suggests that persons working with or living near populations of pregnant Steller sea lions could be exposed to concentrated aerosols of \textit{C. burnetii} and be at risk for Q fever.

Although the precise source of bacterial exposure could not be determined in this case, the uniqueness of this sea lion \textit{Coxiella} strain makes it possible that this bacterium has a unique and novel marine cycle. Further studies are needed to determine the impact of \textit{C. burnetii} on the overall health of the marine mammal population. For this sea lion, it is not known if this infection resulted in any clinical symptoms or if the infection contributed to its stranding. Concurrent detection of mild encephalitis and detection of the protozoan pathogens \textit{T. gondii} and \textit{S. neurona} may also be significant. Serological studies of marine mammal populations will be...
Molecular characterization of \textit{C. burnetii} strain isolated from marine mammals. DNA sequencing was performed on purified PCR products. 16S rRNA gene sequences obtained for this study have the following GenBank accession numbers: for sea lion (MucZ), GU797243.

\textbf{FIG. 2.} Phylogenetic tree of 16S rRNA gene sequences from 5 isolates, sea lion \textit{C. burnetii} strain isolated from marine mammals. *FAM, 6-carboxyfluorescein; Gel, ethedium bromide-stained agarose gel.*

**TABLE 1.** Gene targets and primer sequences for PCR

<table>
<thead>
<tr>
<th>Gene target</th>
<th>Gene product</th>
<th>Primers and probe</th>
<th>Oligonucleotide sequence</th>
<th>Detector*</th>
<th>Cycling conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>com1</td>
<td>27-kDa outer membrane protein</td>
<td>COM1 TaqMan fwd and rev</td>
<td>5'-TTGGCAGCGTATTGCGATT-3'</td>
<td>FAM</td>
<td>95°C, 10 min</td>
<td>This study</td>
</tr>
<tr>
<td>CBU_678</td>
<td>Putative ADP heptose synthase</td>
<td>CB1F</td>
<td>5'-TTAACACGCCAAGACGTATCGCTG-3'</td>
<td>Gel</td>
<td>30 cycles of 94°C, 30 s; 55°C, 30 s; 72°C, 30 s</td>
<td>This study</td>
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<tr>
<td>CBU_686</td>
<td>Pyruvate dehydrogenase</td>
<td>686F and 686R</td>
<td>5'-CATGTAGCCATCGAGCAGATC-3'</td>
<td>SYBR green</td>
<td>94°C, 3 min</td>
<td>This study</td>
</tr>
<tr>
<td>IS1111a</td>
<td>Multicopy insertion sequence</td>
<td>IS1111F and IS1111R</td>
<td>5'-CCGATATTGGGCGCT-3'</td>
<td>FAM</td>
<td>50°C, 2 min</td>
<td>11</td>
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<tr>
<td>16S rRNA gene</td>
<td>rRNA</td>
<td>16SUPF and 16SUPR</td>
<td>5'-TACATCGCAAGTGCAACGCG-3'</td>
<td>Gel</td>
<td>40 cycles of 94°C, 30 s, 55°C, 30 s, 72°C, 30 s</td>
<td>This study</td>
</tr>
<tr>
<td>16S rRNA gene</td>
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<td>CBIF and CBIR</td>
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<td>Gel</td>
<td>40 cycles of 94°C, 30 s, 55°C, 30 s, 72°C, 30 s</td>
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<td>16S rRNA gene</td>
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<td>40 cycles of 94°C, 30 s, 55°C, 30 s, 72°C, 30 s</td>
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<td>Gel</td>
<td>40 cycles of 94°C, 30 s, 55°C, 30 s, 72°C, 30 s</td>
<td>This study</td>
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</tbody>
</table>

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**REFERENCES**


