Brucella ceti Infection in a Common Minke Whale (Balaenoptera acutorostrata) with Associated Pathology

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ABSTRACT: There are three major lineages of marine mammal strains of Brucella spp.: Brucella ceti ST23, found predominantly in porpoises; B. ceti ST26, in pelagic delphinids and ziphiids; and Brucella pinnipedialis ST24/25, predominantly in seals. The isolation of Brucella spp. in mysticetes has been described only in common minke whales (Balaenoptera acutorostrata) in Norway and Scotland. We report a third case of Brucella infection and isolation in a minke whale associated with a large abscess. In contrast to the two previous reports that involved isolates of B. pinnipedialis ST24 or the porpoise-associated B. ceti complex ST23, this case was associated with the dolphin-associated B. ceti ST26. Thus, minke whales can be infected naturally with members of all the distinct major lineages of Brucella associated with marine mammals. This report is unique in that the B. ceti ST26 did not originate from a pelagic delphinid or a beaked whale.

Key words: Balaenoptera acutorostrata, Brucella ceti, isolation, pathology, Scottish Marine Animal Strandings Scheme, ST26, United Kingdom.

An adult female common minke whale (Balaenoptera acutorostrata) was found stranded in Aberdeenshire, Scotland, UK (57°40′N, 2°34′W) in September 2014. A standardized cetacean necropsy was performed on site (Kuiken and Hartman 1991). The carcass was 830 cm from the tip of the rostrum to the tail notch, the mean of three standard blubber thickness measurements was 65 mm, and the nutritive condition of the animal was very good. The animal was pregnant with a grossly normal female fetus, 133 cm long, with a girth of 52 cm, and weighing 39.5 kg, in the left horn of the uterus. Uterus and placenta were grossly normal with no indication of inflammation or infection. There were numerous excoriations to the ventral abdomen extending caudally from the navel to the tailstock and fluke and cranially to a large swelling in the throat region (Fig. 1) which extended from the pharyngeal region to the thoracic inlet area. The swelling was a large abscess, approximately 1 m long, containing several liters of watery yellow fluid and necrotic material (Fig. 2). No obvious foreign body or trauma was associated with the abscess. The retropharyngeal lymph nodes (RPLNs) were fibrous and contained caseous, pleomorphic yellow lesions. All sections of the stomach contained watery fluid and a moderate nematode burden (presumed Anisakis sp.); no digesta were present. The liver was swollen with areas of fibrosis associated with the bile ducts. Numerous trematodes (Brachycladium goliath) were present in the bile ducts. There was a large amount of pericardial fat but the heart appeared normal. The lungs were asymmetric with the right hyperinflated and the left congested but otherwise grossly normal. Other organs were unremarkable.

Bacterial culture of the abscess contents, RPLN, lung, liver, and kidney was on Columbia sheep blood agar (CSBA; Oxoid, Basingstoke, UK) and Farrell’s medium (Animal and Plant Health Agency, Weybridge, UK), incubated at 37 C in air with 5% CO2 and examined daily for 14 d. All tissues were inoculated onto MacConkey agar without salt (Oxoid) and incubated in air at 37 C for 48 h. Anaerobic culture was undertaken on the abscess contents and RPLNs using fastidious anaerobe agar with 5% horse blood (Oxoid).
and anaerobic blood agar with naladixic acid and vancomycin (Oxoid) at 37°C and examined at 48 h. Culture of the lung produced a mix of bacterial flora overgrown with *Proteus* sp. The liver produced a mixed growth of *Edwardsiella tarda* and *Granulicatella balaneopterae*; the latter was also isolated from the kidney. Anaerobic cultures on the abscess contents and RPLN were negative after 48 h of incubation. Colonies typical of *Brucella* spp. were visible on CSBA and Farrell’s medium after 3 d. Subcultures of the suspect *Brucella* sp. were incubated aerobically at 37°C on CSBA. Bacterial colonies were subjected to Gram and modified Ziehl-Neelsen (MZN) stains and agglutination with *Brucella abortus*–positive control serum (Remel/Thermo Dartford, UK; Davison et al. 2015). The isolate (M251/14/1) agglutinated *B. abortus*–positive control serum, was a gram-negative, MZN-positive coccobacillus, and was not CO2 dependent. The isolate was lysed by phages BK2 (Berkeley), Wb (Weybridge), and Fi (Firenze) (Corbel et al. 1979; Table 1).

Molecular characterization of the outer membrane protein 2 (*omp2*) using a selection of restriction enzymes revealed the type to be N(K), found previously in short-beaked common dolphins (*Delphinus delphis*) and striped dolphins (*Stenella coeruleoalba*; Dawson et al. 2008). Multi-locus sequence typing (MLST) identified the isolate as genotype ST26, which has previously been associated only with delphinids and ziphiids in the Northeast Atlantic Ocean (Groussaud et al. 2007; Foster et al. 2015).

A wide range of tissue samples were taken for histopathology, fixed in neutral buffered formalin, processed routinely, sectioned, and stained with H&E. Histologically, the abscess wall showed mature fibrous tissue lined by necrotic material containing degenerate leukocytes, primarily neutrophils, and more peripherally surrounded by large numbers of macrophages and occasional multinucleate giant cells and eosinophils (Fig. 3). Lymphocytes were present in large numbers in the next layer with fewer plasma cells. The morphologic diagnosis was severe, chronic, focally extensive abscessation of the subcutis. There was also a moderate-to-severe, subacute, generalized hepatic fatty change and bile stasis, probably due to lack of recent feeding and a mild-to-moderate, subacute, multifocal lymphocytic hepatitis.

To our knowledge, this is the first report of *B. ceti* ST26 genotype isolated from a minke whale or any mysticete. There have been reports of isolation of *B. ceti* from odontocetes
in many parts of the world, including nine species in the UK (Foster et al. 2015). Except for two reports from the Northeast Atlantic Ocean (Clavareau et al. 1998; Foster et al. 2002), reports of Brucella spp. in mysticetes have relied on serology or molecular techniques (Tryland et al. 1999; Ohishi et al. 2003, 2005, 2008). Antibodies were found in common minke, sei (Balaenoptera borealis), and fin whales (Balaenoptera physalus) in the study from the North Atlantic Ocean although no pathology was reported and no isolates were recovered from the latter two species (Tryland et al. 1999). Ohishi et al. (2003) found antibodies to Brucella sp. and gross pathology and histopathology suggestive of Brucella infection in the reproductive organs of common minke whales and Brydes whales (Balaenoptera edeni). In the same study, no evidence of infection was found in Antarctic minke whales (Balaenoptera bonaerensis), suggesting this may be a naive population. Lesions like those produced by Brucella infection in terrestrial animals were observed in 37% (13/35) of mature male common minke whales of the western North Pacific Ocean. The authors were unable to isolate Brucella sp., but 10 testes samples were positive for Brucella DNA containing the IS711 element downstream of bp 26, characteristics of Brucella sp. from marine mammals (Ohishi et al. 2004, 2008). Other molecular data were most consistent with the rare genotype ST27 (Whatmore 2009).

The two reports of Brucella recovery from mysticetes originate from common minke whales. The first isolation (B202R) was from the spleen of an animal caught during commercial whaling off the Norwegian coast in 1995 (Clavareau et al. 1998; Tryland et al. 1999). The second (M192/00/1) was from the spleen and mesenteric lymph node of an animal that stranded in Scotland in 2000, entangled in fishing gear (Foster et al. 2002). Both isolates were assessed using molecular techniques including omp typing, multiplex PCR, and MLST (Dawson et al. 2008; Whatmore 2009). These approaches determined that isolate M192/00/1 was B. pinnipedialis ST24, predominantly isolated from seals (Foster et al. 2007). The isolate B202R represented a minor variant of B. ceti ST23, isolated predominantly from porpoises (Groussaud et al. 2007). In contrast, the

Table 1. Phenotypic characteristics of Brucella ceti isolated from a minke whale (Balaenoptera acutorostrata) found stranded in Aberdeenshire, Scotland, UK (57°40′N, 2°34′W) in September 2014, compared with other Brucella species. a

<table>
<thead>
<tr>
<th>Brucella species</th>
<th>Hydrolyzation of Urea</th>
<th>H2S Produced</th>
<th>CO2 Required</th>
<th>BF</th>
<th>Th</th>
<th>A</th>
<th>M</th>
<th>Wb</th>
<th>Tb</th>
<th>BK2</th>
<th>Fi</th>
<th>R/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minke whale M251/14</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>M</td>
<td>C</td>
<td>NL</td>
<td>CL</td>
<td>CL</td>
<td>NL</td>
</tr>
<tr>
<td>B. melitensis</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NL</td>
<td>NL</td>
<td>CL</td>
<td>NL</td>
<td>NL</td>
</tr>
<tr>
<td>B. abortus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>CL</td>
<td>CL</td>
<td>CL</td>
<td>CL</td>
<td>NL</td>
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<tr>
<td>B. suis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>CL</td>
<td>NL</td>
<td>CL</td>
<td>PL</td>
<td>NL</td>
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<tr>
<td>B. ceti d</td>
<td>+</td>
<td>-</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>(-)</td>
<td>Lβ</td>
<td>NL</td>
<td>NL</td>
<td>NL/PL</td>
<td>NL</td>
</tr>
<tr>
<td>B. pinnipedialis d</td>
<td>+</td>
<td>-</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>(-)</td>
<td>Lβ</td>
<td>NL</td>
<td>NL</td>
<td>NL/PL</td>
<td>NL</td>
</tr>
</tbody>
</table>

aH2S = hydrogen sulfide; CO2 = carbon dioxide; F = Basic fuchsin at 20 mL/mL (1/50,000 w/v); H = thionin at 20 mL/mL (1/50,000 w/v); RTD = routine test dilution; + = positive; - = negative; (-) = most strains negative; (+) = most strains positive.

bA = Brucella abortus antigen; M = Brucella melitensis antigen.

Wb = weybridge; Tb = tibilisi; BK2 = berkeley; Fi = firenze; R/C = phage for identifying rough strains of Brucella; CL = confluent lysis; NL = no lysis; Lβ = lysis occurs in most strains; NL/f = lysis occurs in a few strains; PL = partial lysis.

dCharacteristics consistent with Foster et al. (2007) and Whatmore (2009).
isolate in this study (M251/14/1) belonged to ST26, the second B. ceti group, primarily associated with pelagic delphinids (Groussaud et al. 2007). Thus, minke whales can be infected naturally with representatives of all three major Brucella strain groups previously associated predominantly with seals (B. pinnipedialis ST24), porpoises (B. ceti ST23), or pelagic delphinids (B. ceti ST26). There are reports of genotypes infecting atypical hosts, particularly for B. ceti ST23, which has been recovered from Atlantic white-sided dolphin (Lagenorhynchus acutus), white-beaked dolphin (Lagenorhynchus albirostris), short-beaked common dolphin, and harbor seal (Phoca vitulina) in the UK (Dawson et al. 2008). Additionally, B. pinnipedialis was isolated from a European otter (Lutra lutra; Ross et al. 1994; Dawson et al. 2008). There is potential that that these reports reflect spillover of infection from another host. Foster et al. (2015) isolated ST26 from a Sowerby’s beaked whale (Mesoplodon bidens), demonstrating that this genotype is present in another group of odontocetes. The abscess formation noted in our study is similar to pathologies seen in odontocetes, particularly pelagic delphinids infected with B. ceti (ST 26) genotype.

In conclusion, the excoriations to the abdomen, hyperinflated right lung, hemorrhage within the blubber, and preservation of the carcass suggest the animal was live-stranded. The large abscess was long-standing.
was significant as a nidus of infection, and probably hampered foraging and swallowing. Ultimately it may have been responsible for the live stranding and death of this otherwise apparently healthy whale. The limited number of isolations of Brucella spp. on both a host and geographic scale suggests the possibility of yet undiscovered lineages in cetacean populations in remote areas or in little-studied species. Further attempts to isolate Brucella spp. from cetaceans, including minke whales and other mysticetes, would provide more insight into the pathogenicity and host preference of this and other Brucella genotypes. Unlike the two previous reports of Brucella spp. isolation in minke whales, the B. ceti ST26 genotype we isolated was associated with pathology.

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LITERATURE CITED


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