

***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 Stranding Scheme, ST26, UK.

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



FIGURE 1. Adult female common minke whale (*Balaenoptera acutorostrata*) found stranded in Aberdeenshire, Scotland, UK, September 2014 with large swelling on throat.

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 balaenopterae*; 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 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, modified Ziehl-Neelsen-positive coccobacillus, and was not CO₂ dependent. The isolate was lysed by phages Berkeley, Weybridge, and 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 identified



FIGURE 2. Throat swelling of stranded common minke whale (*Balaenoptera acutorostrata*) in Aberdeenshire, Scotland, UK, September 2014, after incision showing large volume of yellow fluid. Inset: Close-up of abscess and necrotic material (arrow). Ruler=30 cm.

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

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

<i>Brucella</i> species	Hydrolyzation of urea	H ₂ S produced	CO ₂ required	BF	Th	Agglutination with monospecific antiserum ^b		Lysis by phage at routine test dilution ^c				
						A	M	Wb	Tb	BK2	Fi	R/C
Minke whale M251/14	+	-	-	+	+	+	-	CL	NL	CL	CL	NL
<i>B. melitensis</i>	+	-	-	+	+	-	+	NL	NL	CL	NL	NL
<i>B. abortus</i>	+	+	+	+	-	+	-	CL	CL	CL	CL	NL
<i>B. suis</i>	+	+	-	-	+	+	-	CL	NL	CL	PL	NL
<i>B. ceti</i> ^d	+	-	(-)	(+)	(+)	+	(-)	Lβ	NL _f	Lβ	NL/PL	NL
<i>B. pinnipedialis</i> ^d	+	-	(+)	+	+	(+)	(-)	Lβ	NL _f	Lβ	NL/PL	NL

^a H₂S = hydrogen sulfide; CO₂ = carbon dioxide; BF = basic fuchsin at 20 mL/mL (1/50,000 w/v); Th = thionin at 20 mL/mL (1/50,000 w/v); + = positive; - = negative; (-) = most strains negative; (+) = most strains positive.

^b A = *B. abortus* antigen; M = *B. melitensis* antigen.

^c 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.

^d Characteristics consistent with Foster et al. (2007) and Whatmore (2009).

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 et al. 2008).

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 multi-locus sequence typing (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 isolate in this study (M251/14/1) belonged

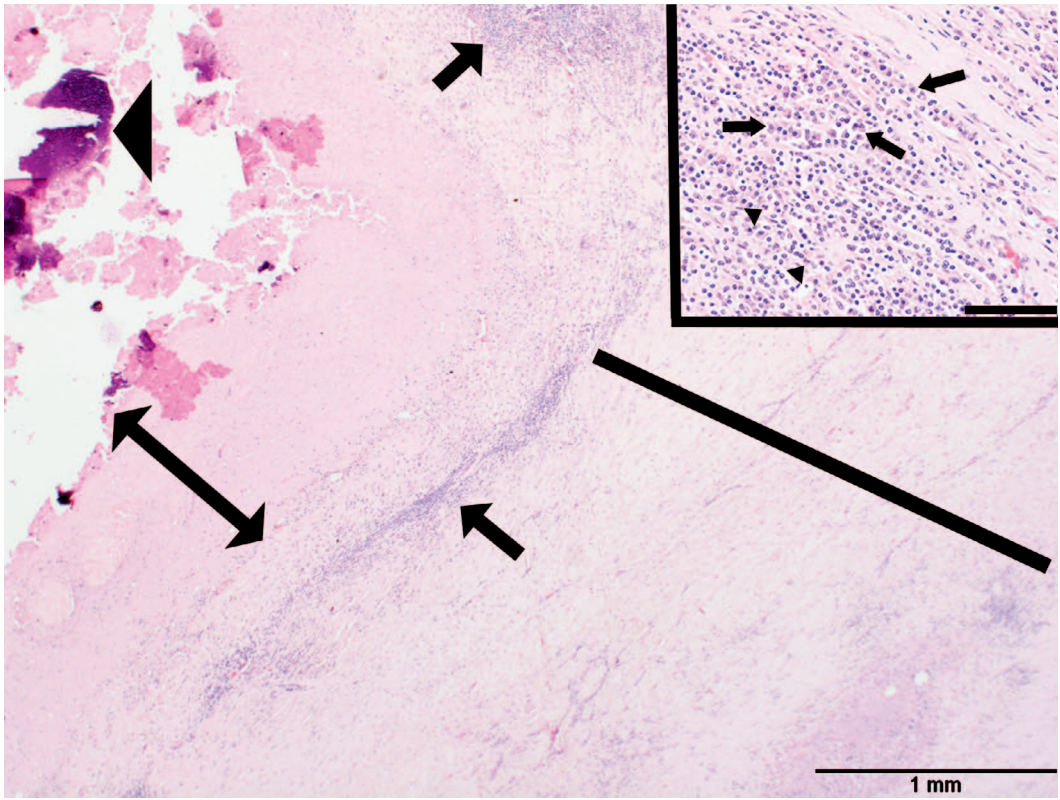


FIGURE 3. Histologic section of subcutaneous abscess wall of stranded common minke whale (*Balaenoptera acutorostrata*) found in Aberdeenshire, Scotland, UK, in September 2014. Note mineralized necrotic material (arrowhead), necrotic tissue (double-ended arrow), band and aggregation of inflammatory cells (arrows), and mature fibrous tissue (black line). H&E. Bar=1,000 μ m. Inset: Higher magnification of inflammatory cells showing macrophages (arrows), lymphocytes, and very occasional polymorphonuclear neutrophils (arrowhead). H&E. Bar=100 μ m.

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 (*Mesoploden 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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