Vol. 116: 237–241, 2015
doi: 10.3354/dao02926

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published October 27

NOTE

First report of *Brucella ceti*-associated meningoencephalitis in a long-finned pilot whale *Globicephala melas*

Nicholas J. Davison[1,*,], Andrew Brownlow[1,**], Barry McGovern[1,4,**], Mark P. Dagleish[2,**], Lorraine L. Perrett[3,**], Emma-Jane Dale[3,**], Mark Koylass[3,5], Geoffrey Foster[1,**]**

[1]Scottish Marine Animal Stranding Scheme, SAC Consulting, Veterinary Services, Drummondhill, Inverness, Scotland, IV2 4JZ, UK

[2]Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Edinburgh, Scotland EH26 0PZ, UK

[3]Department of Bacteriology and TB, Animal and Plant Health Agency (APHA), New Haw, Addlestone, Surrey KT15 3NB, UK

[4]*Present address:* Namibian Dolphin Project, Meersig Lagoon, Walvis Bay, Namibia

[5]*Present address:* Laboratory Services, Genomics, LGC, Unit 1-2 Trident Industrial Estate, Pindar Road, Hoddesdon, Hertfordshire EN11 0WZ, UK

ABSTRACT: Fatal *Brucella ceti* infection with histological lesions specific to the central nervous system has been described in only 3 species of cetaceans: striped dolphins *Stenella coeruleoalba*, Atlantic white-sided dolphins *Lagenorhynchus acutus* and short-beaked common dolphins *Delphinus delphis*. This paper describes the first report of a *B. ceti*-associated meningoencephalitis in a long-finned pilot whale *Globicephala melas*, showing the increasing range of species susceptibility. *Brucella* was recovered in larger numbers from cerebrospinal fluid than from brain tissue and is the sample of choice for isolation.

KEY WORDS: *Globicephala melas* · Isolation · *Brucella ceti* · Meningoencephalitis · UK

INTRODUCTION

Brucella ceti (Foster et al. 2007) has been isolated from multiple organs from 9 different species of cetacean in the UK: harbor porpoise *Phocoena phocoena*, short-beaked common dolphin *Delphinus delphis*, common bottlenose dolphin *Tursiops truncatus*, Atlantic white-sided dolphin *Lagenorhynchus acutus*, striped dolphin *Stenella coeruleoalba*, white-beaked dolphin *L. albirostris* (Foster et al. 2002), common minke whale *Balaenoptera acutorostrata* (Clavareau et al. 1998), long-finned pilot whale *Globicephala melas* and a Sowerby's beaked whale *Mesoploden bidens* (Foster et al. 2015). However, in association with central nervous system (CNS) histological lesions, *B. ceti* has been recovered most frequently from striped dolphins in Europe (UK, Spain and Italy) and Costa Rica (González et al. 2002, Muñoz et al. 2006, Hernández-Mora et al. 2008, Davison et al. 2009, Alba et al. 2013) and reported from an Atlantic white-sided dolphin (Dagleish et al. 2007) and a short-beaked common dolphin (Davison et al. 2013) in the UK.

*Corresponding author: nick.davison@sruc.ac.uk
**These authors contributed equally to this article

MATERIALS AND METHODS

A single, sub-adult, male long-finned pilot whale (Ref: M19/13) was found dead at Balmedie beach near Aberdeen, Scotland, UK (57° 17′ N, 2° 0′ W) in January 2013; the carcass exhibited only slight decomposition and was subjected to a standardized cetacean necropsy within 24 h of being reported (Kuiken & Hartman 1991). A wide range of tissue samples, including whole brain, thyroid, lungs, heart, liver, spleen, kidney, adrenal, mesenteric lymph node, urinary bladder, testis and skeletal muscle were taken for histopathology, processed routinely, and sections were stained with hematoxylin and eosin. As culture is considered the gold standard for *Brucella* diagnosis, samples for bacteriology (lung, liver, spleen, kidney, mesenteric lymph node, intestine, urine, brain, and cerebral spinal fluid [CSF] taken from the dilated lateral ventricles) were inoculated directly onto Columbia sheep blood agar (CSBA) (Oxoid) and Farrell's medium (Animal and Plant Health Agency), incubated at 37°C in a capnophilic (5% CO_2) atmosphere and examined daily for 14 d. Samples were inoculated directly onto MacConkey agar (Oxoid) and incubated at 37°C in air. Subcultures of suspect *Brucella* spp. colonies were made onto CSBA, incubated aerobically and in a capnophilic atmosphere at 37°C, subjected to Gram and modified Ziehl-Neelsen (MZN) stains and agglutination with *B. abortus* positive control sera (Remel). Serum was analyzed by 1 competitive and 2 indirect ELISAs (cELISA and iELISA, respectively) for the presence of anti-*Brucella* antibodies. Positive/negative thresholds for these assays were set with some uncertainty but were based on those used for testing a wide range of terrestrial mammals from the UK (McGiven et al. 2003). Urine was tested by Combur^{9}_Test® (Roche).

Isolates were typed by classical phenotypic-typing methods: amplification of an IS*711* element downstream of the *bp26* gene by PCR, molecular characterization of the outer membrane protein 2 (*omp2*) of the strain using a selection of restriction enzymes and multi locus sequence typing (MLST) as described previously (Whatmore 2009).

RESULTS

The carcase was 426 cm long, and had a girth at the level of the anterior insertion of the dorsal fin of 198 cm. The mean of 3 standard blubber thickness measurements (taken at the same location as the girth) was 31.3 mm, and the nutritive condition of the animal was poor (Kuiken & Hartman 1991). Blood was present in the anterior chamber of the left eye; focal bruising was present in the blubber in the left flank, and there was localized yellow gelatinous/proteinaceous fluid in the subcutis. The nasal cavity contained sand; stable foam and light brown fluid was present in both the trachea and bronchi of the left lung. There was hypostatic congestion of the right lung and hyperinflation of the left lung with small emphysematous bullae present. All compartments of the stomach were devoid of ingesta; bile-stained fluid was present in the duodenum and small intestine, and the large intestine contained green faeces. Both kidneys were enlarged and oedematous, and the urinary bladder contained dark brown/red urine with levels of hemoglobin/myoglobin ~250 Ery μl^{-1} when tested by dipstick. There was moderate enlargement of both mesenteric and pulmonary-associated lymph nodes. The brain had severely dilated ventricles and contained an excess (~150 ml) of cloudy, red-stained CSF (Fig. 1). No gross abnormalities of the cranial cervical spinal cord, other viscera or carcase were found. The pattern of asymmetric congestion, inflation in the upper lung, sand within the nasal cavity and hemoglobinuria/myoglobinuria is consistent with initial live-stranding prior to death.

Microscopic examination of the brain revealed moderate to severe generalised congestion. Large numbers of lymphocytes and a smaller number of macrophages were present in aggregations within the meninges (Fig. 2), the choroid plexuses and also in peri-vascular cuffs within the brain; the latter were more numerous in the para-ventricular areas. The severity of lesions in the meninges and choroid

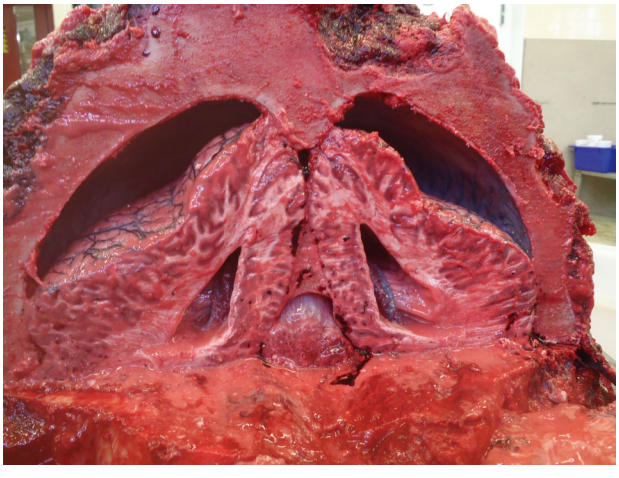

Fig. 1. Transverse section through cranium and midbrain: note dilated lateral ventricles

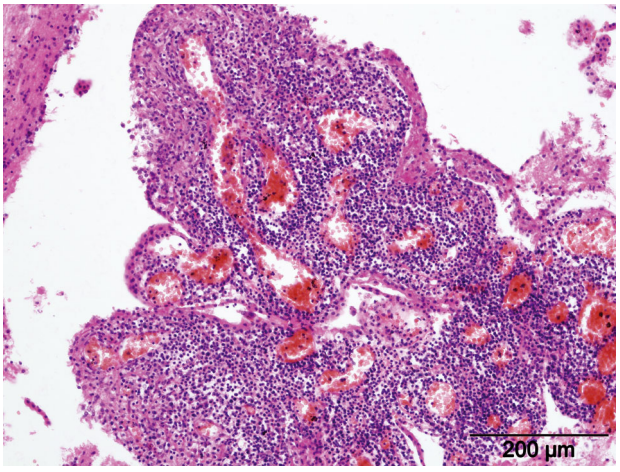

Fig. 2. Histological section of the choroid plexus from the fourth ventricle of the brain. Note the very large numbers of lymphocytes infiltrating the tissue distorting the normal anatomy. Lymphocytic chorio-meningoencephalitis is a frequent and characteristic lesion reported in cases of neurobrucellosis in other cetacean species. Haematoxylin & eosin

plexuses increased in a cranial to caudal manner, as did the number of macrophages present. The thyroid gland was moderately congested, and the follicles were lined by low columnar to cuboidal epithelium and filled with colloid. Severe, generalized congestion was present in the right lung, and the alveoli were collapsed; the left lung showed hyperexpansion of alveoli with disruption of alveolar septa and bullae formation. A single, medium-sized focus of, primarily, polymorphonuclear neutrophils with a few macrophages was present in a respiratory bronchiole which extended into adjacent alveoli. Perivascular protein-rich fluid (edema) was present in the epicardium and myocardium, and the liver had severe generalized centrilobular (peri-acinar) congestion with notable sparing of the peri-portal (centriacinar) regions. A mild lymphoplasmacytic hepatitis centred on the peri-portal areas was also present. Severe generalised congestion was present in the kidney, and mild to moderate autolysis of the epithelium of the proximal convoluted tubules, which hindered assessment. The corticomedullary junction of the adrenal was moderately congested, and the cortex mildly congested. The paracortex of the mesenteric lymph node was severely depleted of lymphocytes; no secondary follicles were present, and a very large number of plasma cells were present in the medullary sinuses. The spleen, testis (no spermatozoa present) and skeletal muscle were within normal limits.

The primary morphological diagnosis was a severe, sub-acute to chronic, generalised, predominantly lymphocytic, meningoencephalitis and a pattern of congestion in the lungs, liver, kidney and adrenal consistent with live-stranding prior to death.

Cultures of the CSF on CSBA produced, after 3 d, a profuse pure growth of non-haemolytic circular colonies 0.5–1 mm in diameter and pale honey-coloured colonies on Farrell's medium resembling *Brucella* sp. Cultures of brain tissue on the same media produced a scant pure growth, after 3 d, resembling *Brucella* sp. No bacterial growth was present on MacConkey agar. Cultures of the liver, spleen kidney and urine produced no bacterial growth after 14 d in a capnophilic atmosphere at 37°C. Culture of the lung, mesenteric lymph node and intestine produced a mixed growth of *E. coli* and *Photobacterium damselae*; neither isolate was thought significant. Subcultures of the *Brucella* sp. made onto CSBA and incubated aerobically at 37°C showed the isolates were not CO_2 dependent, agglutinated with *B. abortus*-positive control sera (Remel), were oxidase positive, Gram negative and modified ZN-positive coccobacilli; they were identified tentatively as *B. ceti*. The isolates were lysed by phages BK2 (Berkeley), Wb (Weybridge) and Fi (Firenze) (Table 1).

Amplification of an IS*711* element downstream of the *bp26* gene by PCR confirmed that all the isolates possessed this unique feature, which is specific to marine mammal strains of *Brucella* species. Molecular characterisation 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 and striped dolphins (Whatmore 2009). MLST identified a 9 loci profile consistent with genotype ST26, which is associated with delphinids in the North East Atlantic (Whatmore 2009).

Serum was positive for anti-*Brucella* antibodies in all 3 ELISAs (Table 1).

The lesion morphology and distribution, along with isolation of large numbers of *B. ceti* organisms from the CSF and a smaller number from the brain tissue in pure cultures, lead to a definitive diagnosis of neurobrucellosis.

CONCLUSIONS

To the authors' knowledge this is the first report of *Brucella ceti*-associated pathology in the CNS of a long-finned pilot whale, and the CNS lesions are highly consistent with those described in other delphinids with fatal neurobrucellosis (González et al. 2002, Muñoz et al. 2006, Dagleish et al. 2007, Hernán-

Table 1. (A) Phenotypic characteristics of *Brucella ceti* isolated from a long-finned pilot whale *Globicephala melas* (M19/13) compared with other *Brucella* species and (B) immunological results for the presence of *Brucella* antibodies in serum. +: positive; –: negative; (+): most strains positive; (–): most strains negative. BF: basic fuchsin at 20 µl ml^{-1} (1/50 000 w/v); TH: thionin at 20 µl ml^{-1} (1/50000 w/v); RTD: routine test dilution; Wb: Weybridge; Tb: Tibilisi; BK_2: Berkeley; Fi: Firenze; R/C: phage for identifying rough strains of *Brucella*; CL: confluent lysis; L: lysis; PL: partial lysis; Plq: plaques; NL: no lysis; *f*: lysis occurs in a few strains; β: lysis occurs in most strains; OD: optical density; indirect ELISA (iELISA) >10% positive; competitive ELISA (cELISA): <60% positive; AS7: *B. abortus* antigen; 16M: *B. melitensis* antigen

(A)	Urea	H_2S prod.	CO_2 req.	BF	TH	Agglutination with monospecific antiserum A	Agglutination with monospecific antiserum M	Lysis by phage at RTD Wb	Lysis by phage at RTD Tb	Lysis by phage at RTD BK_2	Lysis by phage at RTD Fi	Lysis by phage at RTD R/C
M19/13	+	–	–	+	+	+	–	CL	NL	CL	Plq	NL
B. melitensis (1)	+	–	–	+	+	–	+	NL	NL	CL	NL	NL
B. abortus (1)	+	+	+	+	–	+	–	CL	CL	CL	CL	NL
B. suis (1)	++	+	–	–	+	+	–	CL	NL	CL	PL	NL
B. ceti[a]	+	–	(–)	(+)	(+)	+	(–)	L β	NL*f*	L β	NL/PL	NL
B. pinnipedialis[a]	+	–	(+)	+	+	(+)	(–)	L β	NL*f*	L β	NL/PL	NL

(B)	iELISA (AS7)	iELISA (16M)	cELISA (16M)	Interpretation
Serum OD	52	44	14	Positive

[a]Characteristics consistent with Foster et al. (2007) and Whatmore (2009)

dez-Mora et al. 2008, Davison et al. 2009, 2013, González-Barrientos et al. 2010, Alba et al. 2013). The pattern and location of the lesions in the brain may be due to a combination of the blood supply to the brain and also to the breach in the continuity of the blood–brain barrier provided by the meninges in the area postrema, allowing bacteria to enter the CSF and adjacent medulla (Williams et al. 1995). Although a report exists of *B. ceti*-associated disease in a long-finned pilot whale involved in a mass stranding event (MSE) in Scotland in 2011, this individual had a severely infected shoulder joint, which was probably functionally compromised. However, it is unlikely that this infection caused the animal to live-strand, since it was in good body condition, suggesting it could forage successfully. Furthermore, no brain lesions were present (Foster et al. 2015).

The zoonotic potential of marine mammal *Brucella* has been well documented, particularly genotype ST27 (Brew et al. 1999, Sohn et al. 2003, McDonald et al. 2006). The expanding list of cetacean species affected by *B. ceti* suggests care should be taken when performing any cetacean necropsy to reduce zoonotic transmission and also highlights our lack of understanding of transmission mechanisms of the organism both within and between species. Additionally, this work shows CSF is the sample of choice for isolating the organism from the CNS. This sample can be relatively easily obtained through the foramen magnum even in field necropsies, but if not available, then samples of caudal brain stem would be the next appropriate sample.

Acknowledgements. The authors thank Clare Underwood and Val Forbes (Moredun Research Institute) for histological preparations. The post mortem investigation was carried out under the aegis of the UK Cetacean Strandings Investigation Programme, which is jointly funded by the Department for Environment Food and Rural Affairs (Defra) and the Scottish Government.

LITERATURE CITED

Alba P, Terracciano G, Franco A, Lorenzetti S and others (2013) The presence of *Brucella ceti* ST26 in a striped dolphin (*Stenella coeruleoalba*) with meningoencephalitis from the Mediterranean Sea. Vet Microbiol 164:158–163

Brew SD, Perrett LL, Stack JA, MacMillan A (1999) Human exposure to *Brucella* recovered from a sea mammal. Vet Rec 144:483

Clavareau C, Wellemans V, Walravens K, Tryland M and others (1998) Phenotypic and molecular characterization of a *Brucella* strain isolated from a minke whale (*Balaenoptera acutorostrata*). Microbiology 144:3267–3273

Dagleish MP, Barley J, Howie FE, Reid RJ, Herman J, Foster G (2007) Isolation of *Brucella* species from a diseased atlanto-occipital joint of an Atlantic white-sided dolphin (*Lagenorhynchus acutus*). Vet Rec 160:876–878

Davison NJ, Cranwell MP, Perrett LL, Dawson CE and others (2009) Meningoencephalitis associated with *Brucella* species in a live-stranded striped dolphin (*Stenella coeruleoalba*) in south-west England. Vet Rec 165:86–89

Davison NJ, Barnett JEF, Perrett LL, Dawson CE, Perkins MW, Deaville RC, Jepson PD (2013) Meningoencephalitis and arthritis associated with *Brucella ceti* in a short-beaked common dolphin (*Delphinus delphis*). J Wildl Dis 49:632–636

Foster G, MacMillan AP, Godfroid J, Howie F and others (2002) A review of *Brucella* sp. infection of sea mammals with particular emphasis on isolates from Scotland. Vet Microbiol 90:563–580

Foster G, Osterman BS, Godfroid J, Jacques I, Cloeckaert A (2007) *Brucella ceti* sp. nov. and *Brucella pinnipedialis* sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts. Int J Syst Evol Microbiol 57: 2688–2693

Foster G, Whatmore A, Dagleish MP, Baily JL and others (2015) First reports of the isolation of *Brucella* ceti from a long-finned pilot whale (*Globicephala melas*) and a Sowerbys beaked whale (*Mesoploden bidens*). J Wildl Dis (in press) doi:10.7589/2015-04-112

González L, Patterson IA, Reid RJ, Foster G and others (2002) Chronic meningoencephalitis associated with *Brucella* sp. infection in live-stranded striped dolphins (*Stenella coeruleoalba*). J Comp Pathol 126:147–152

González-Barrientos R, Morales JA, Hernández-Mora G, Barquero-Calvo E, Guzmán-Verri C, Chaves-Olarte E, Moreno E (2010) Pathology of striped dolphins (*Stenella coeruleoalba*) infected with *Brucella ceti*. J Comp Pathol 142:347–352

Hernández-Mora G, González-Barrientos R, Morales JA, Chaves-Olarte E and others (2008) Neurobrucellosis in stranded dolphins, Costa Rica. Emerg Infect Dis 14: 1430–1433

Kuiken T, Hartman MG (1991) Cetacean pathology: dissection techniques and tissue sampling. In: Proc European Cetacean Society Workshop, Leiden

McDonald WL, Jamaludin R, Mackereth G, Hansen M and others (2006) Characterization of a *Brucella* sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. J Clin Microbiol 44:4363–4370

McGiven JA, Tucker JD, Perrett LL, Stack JA, Brew SD, MacMillan AP (2003) Validation of FPA and cELISA for the detection of antibodies to *Brucella abortus* in cattle sera and comparison to SAT, CFT, and iELISA. J Immunol Methods 278:171–178

Muñoz PM, De Miguel MJ, Marín CM, Barberán M, Blasco JM (2006) Short communications isolation of *Brucella* species from a live-stranded striped dolphin (*Stenella coeruleoalba*) in Spain. Vet Rec 158:450–451

Sohn AH, Probert WS, Glaser CA, Gupta N and others (2003) Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. Emerg Infect Dis 9:485–488

Whatmore AM (2009) Current understanding of the genetic diversity of *Brucella*, an expanding genus of zoonotic pathogens. Infect Genet Evol 9:1168–1184

Williams PL, Bannister LH, Gray HA (1995) Gray's anatomy: the anatomical basis of medicine and surgery, 38th edn. Churchill Livingstone, New York, NY

Editorial responsibility: Michael Moore, Woods Hole, Massachusetts, USA

Submitted: February 23, 2015; Accepted: September 9, 2015
Proofs received from author(s): October 2, 2015