“Malta fever” in a minke whale; the first confirmed report of the isolation of *Brucella ceti* in a minke whale (*Balaenoptera acutorostrata*) with associated pathology.

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Introduction

"Malta fever", the disease now called *Brucellosis* was first documented by British medical officers in the 1850s in Malta during the Crimean War. Jeffery Allen Marston (1831-1911) described his own case of the disease in 1861. The causal relationship between organism and disease was first established in 1887 by David Bruce. This organism is now known as *Brucella melitensis.* Since then 11 species of *Brucella* have been discovered including *Brucella ceti* in cetaceans (Foster et al 2007). This infection has been reported in cetaceans from many parts of the world; however infection in mysticetes is almost unknown and the isolation of Brucella in mysticetes has only been described in two minke whales (*Balaenoptera acutorostrata*) in Norway and Scotland respectively. Here we report a third case with associated pathology.

Stranding Summary and Methods

- A pregnant adult female minke whale found stranded at Whitehills, Aberdeenshire, Scotland UK (57° 40' N 2° 34' W) in September 2014. (Fig 1). The animal was reported to have possibly live-stranded but when visited by an officer from Scottish Society for the Prevention of Cruelty to Animals (SSPCA) it was found to be dead.
- Routine bacterial cultures inoculated directly onto Columbia blood agar (Oxoid Basingstoke, UK), MacConkey agar (Oxoid) and Farrell’s medium and incubated at 37ºC in a capnophilic (10% CO_2) atmosphere and examined daily for 14 days.
- Isolates were confirmed by classical bio-typing methods. By amplification of an IS*711* element downstream of the bp26 gene by PCR (Cloeckaert *et al*., 2000). Molecular characterisation of the outer membrane protein (*omp2*) of the strain using a selection of restriction enzymes was also performed (Cloeckaert *et al*., 2001).
- MLST was performed as described by Whatmore 2009.
- Histology was carried out using routine H&E staining.

Fig 1: Adult female minke whale with large swelling on throat (arrow)

Results

- There were numerous excoriations to the ventral abdomen extending caudally from around the navel to the tailstock and fluke and cranially to a large swelling in the throat. This swelling extended from the pharyngeal region to the thoracic inlet area. Upon incision this swelling was shown to be a very large abscess approximately 1 metre in length and full of yellow fluid and necrotic material. (Fig 2) The associated retropharyngeal lymph nodes were fibrous and contained caseous yellow lesions 1mm to 3cm in diameter. There was no obvious associated foreign body or trauma associated with this. The excoriations, haemorrhage within the blubber and preservation of the carcase would suggest the animal had live stranded.
- Cultures from the abscess fluid produced a pure growth of *Brucella.ceti*.
- The isolate produced lysis of phages BK2 (Berkeley), Wb (Weybridge) and Fi (Firenze).
- Amplification of an IS*711* element by PCR confirmed that the isolates possessed this unique feature specific to marine mammal strains of *Brucella* species.
- Molecular characterisation of the outer membrane protein 2 (*omp2*) revealed the type to be N(K), found previously in oceanic delphinids.
- MLST showed a 9 loci profile was ST26 a sequence type associated with pelagic delphinids in the north east Atlantic
- Histology on the abscess wall showed mature fibrous tissue lined by necrotic material containing large numbers of macrophages and smaller numbers of eosinophils. Lymphocytes were present in large numbers in the next layer. This was a severe, chronic, focally extensive abscessation of the sub-cutis. The liver showed moderate patchy congestion throughout parenchyma and a large number of hepatocytes with one or two large cytoplasmic vacuoles (presumed fatty change) and containing small to medium sized green/brown pigmented granules (presumed bile salts). A medium number of medium sized foci of primarily, lymphocytes were randomly scattered throughout the parenchyma and had replaced the hepatocytes with some necrotic remnants remaining. A small number of medium sized foci of bacterial cocci were present but with no associated inflammatory cell reaction. Very mild bile duct proliferation and hypertrophy which contained cocco-bacilli within their lumina.

References

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Acknowledgements

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Fig 2: Large swelling after incision showing large volume of yellow fluid.

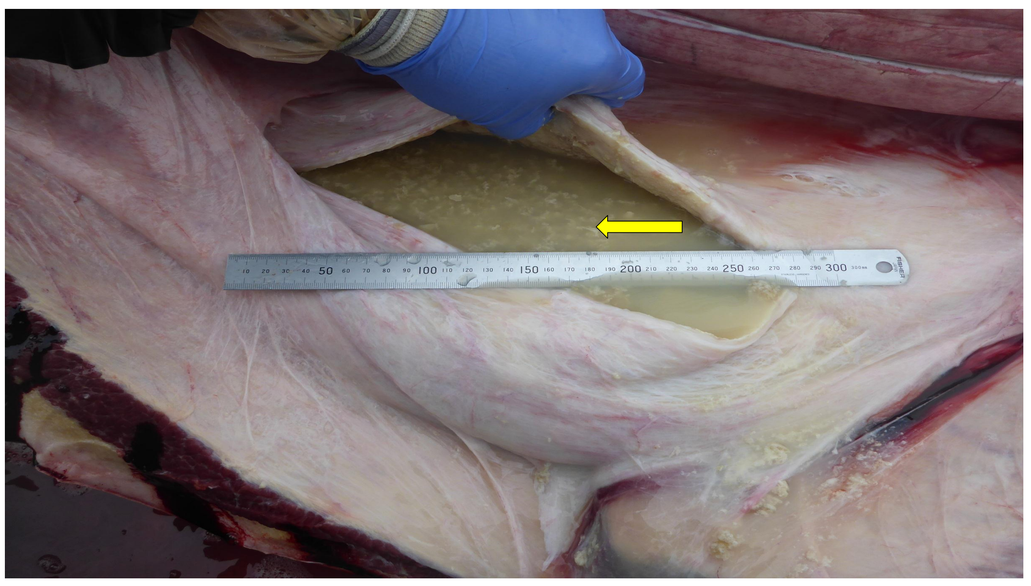

Fig 3: Close up of abscess showing necrotic material (arrow).

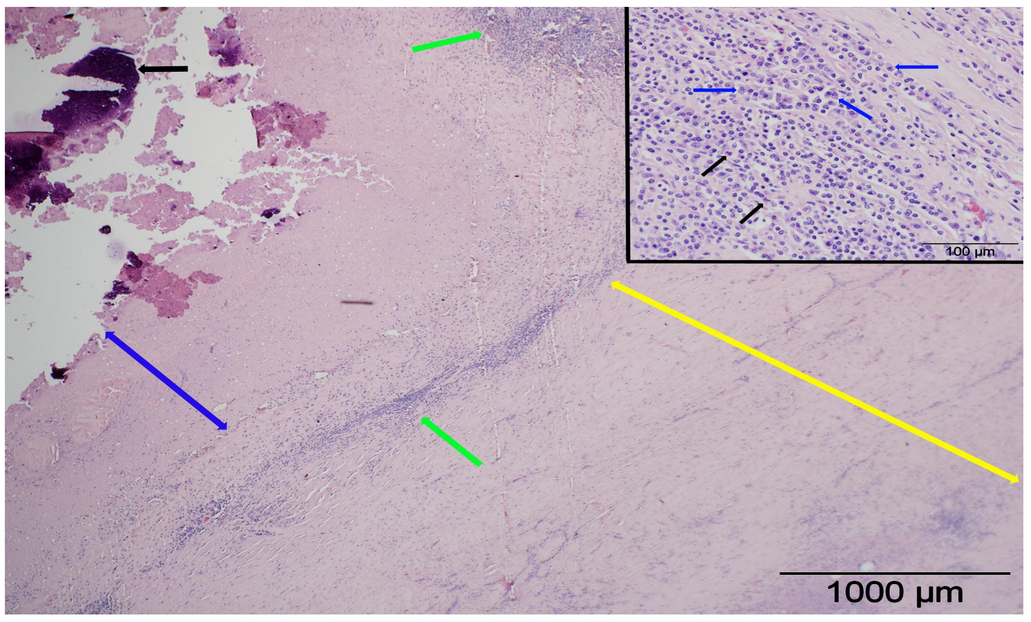

Fig 4: Histological section of subcutaneous abscess wall. Note mineralised necrotic material (black arrow), necrotic tissue (blue band),and aggregation of inflammatory cells (green arrows) and mature fibrous tissue (yellow band).
Insert: higher magnification of inflammatory cells showing macrophages (blue arrows), lymphocytes and very occasional polymorphonuclear neutrophils (black arrows).
Stain: haematoxylin and eosin.

Conclusions

There have previously been two reported isolations of *Brucella* from minke whales. Clavereau in 1998 reported it as an incidental finding in an animal caught as part of whaling operations of Norway in 1995. The second isolate was recovered from an animal that stranded in Scotland in 2000 (Foster 2002) Both of these isolates have since been subjected to further molecular characterisation of the outer membrane protein 2 (*omp2*) (Dawson 2008) and MLST based on 9 loci (unpublished) and are now typed as *B.ceti* (omp2 type M(J) & ST23) the type found predominantly in porpoises and *B.pinnipedialis* (omp2 type O(I) & ST 24) the type found in seals respectively. Neither of these cases had pathology associated with the isolation of either organism. The former being caught as part of a commercial hunt and the latter a case of entanglement. The large abscess found in the animal in the present study was long-standing but there was evidence suggestive of septicaemia spread in the liver. The presence of this large abscess in the animal is significant both as a source of infection but may also have hampered recent foraging by making swallowing difficult. This evidence for lack of feeding was born out by the fatty change seen in the liver histologically. It may also suggest a reason for the live stranding and acute death in this otherwise healthy pregnant female animal. This is the first time *B.ceti* ST26 omp2 N(K) has been isolated from a minke whale and means that all 3 of the major groups of marine mammal Brucella (B.pinnipedialis, B.ceti ST23 (porpoise type) and B.ceti ST26 (dolphin type)) have now been isolated from this species. We need more *Brucella* isolates from minke whales before we can comment on which type (if any) prefers this species as a host. We can say that unlike the two previous isolates Brucella ceti ST26 is associated with pathology in minke whales.