Isolation of *Brucella* species from a bottlenosed dolphin (*Tursiops truncatus*)

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Brucella was first recovered from marine mammals in 1994 (Ross and others 1994). Since then, there have been numerous further accounts of serological evidence and isolations of *Brucella* species originating from marine mammals inhabiting many of the world's oceans. This short communication describes the first confirmed case of *Brucella* species infection in a wild bottlenosed dolphin (*Tursiops truncatus*) in the UK.

In December 2004, a male bottlenosed dolphin was found dead on a beach at Gwithian Hayle, Cornwall. It was taken to the Veterinary Laboratories Agency (VLA) – Truro for postmortem examination. The animal, considered to be in moderate condition with moderate autolysis, was 258 cm long and weighed 160 kg. There were numerous deep rake or bite marks present over the entire body (Fig 1), possibly caused by other bottlenosed dolphins. There had been some scavenger damage to the left eye and left pectoral flipper. Several long-standing cestode (*Phyllobothrium delphini*) cysts were present in the blubber around the vent; abscesses associated with these cysts were similar to the abscess from which *Brucella* species was later isolated. Postmortem examination revealed numerous nematodes in the bronchi and bronchioles, which were associated with a parasitic pneumonia. All three sections of the stomach were empty, and chyle was not present in the mesentery, indicating that the animal had not fed recently. The rake or bite marks were not associated with any additional pathology and the cause of death was not established. The exact age of the animal had yet to be determined.

Samples of heart blood, lung, liver, kidney and intestine were prepared for routine bacteriological culture on blood agar and MacConkey's agar. A mixed growth of *Edwardsiella tarda* and a non-haemolytic *Staphylococcus* species was recovered from the heart blood, lungs and intestine. Analysis of pericardial fluid by the rose bengal plate test revealed antibodies to *Brucella* species.

Tissue samples of lung, liver, kidney, spleen, testes and brainstem, together with samples of pericardial and abdominal fluid, were therefore submitted to VLA – Weybridge to examine for the presence of *Brucella* species. Nematode worms recovered from the cardiac section of the stomach and from the lung were also subjected to examination by culture.

FIG 1: Rake or bite marks on the body of a bottlenosed dolphin (*Tursiops truncatus*) found dead on a beach in Cornwall

Brucella was isolated from a purulent abscess within blubber taken from around the vent, and a fine bacterial growth was produced after seven days of incubation in enrichment broth and two days after subculture on Farrell's media (Farrell 1974). The isolate was initially confirmed as *Brucella* species by classical biotyping methods (Alton and others 1988). It did not require additional carbon dioxide for growth and produced lysis by phages BK_2 and Wb (Table 1).

Amplification by PCR of an IS*711* element downstream of the *bp26* gene (Cloeckaert and others 2000) confirmed that the isolate possessed this unique feature specific to marine mammal strains of *Brucella* species. Molecular characterisation of the outer membrane protein of the strain using a selection of restriction enzymes (Cloeckaert and others 2001) revealed the type to be N-(K), as is found in common dolphins (*Delphinus delphis*) and striped dolphins (*Stenella coeruleoalba*).

A competitive ELISA (cELISA) and two indirect ELISAs (iELISAs) were used to screen the pericardial and abdominal fluids for the presence of *Brucella* antibodies. The cELISA (MacMillan 1990) uses a lipopolysaccharide (LPS) *Brucella melitensis* antigen and a monoclonal antibody conjugate. For the two iELISAs, *B melitensis* 16M antigen was used for the first, as described for the cELISA, and for the other a *Brucella abortus* LPS antigen was used. The iELISAs require an anti-globulin conjugate with specificity for the immunoglobulin isotypes of the species under test; however, protein A has been shown to bind to the immunoglobulin G of a range of marine mammals (Eliasson and others 1989, Sikkema 1989). 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 Britain for brucellosis (Table 2). Samples of both pericardial and abdominal fluids were found to be positive for *Brucella* antibodies by all three assays.

A previous report described the characterisation of *Brucella* species isolated from an aborted fetus of a captive

TABLE 1: Characteristics of *Brucella* species isolated from a bottlenosed dolphin (*Tursiops truncatus)* compared with other isolates of *Brucella* species

	Growth on media containing					Agglutination with monospecific		Lysis by phage at RTD				
	Urea	H_2S production	CO_2 requirement	BF*	Th*	A	M	Wb	Tb	BK_2	Fi	R/C
Bottlenosed dolphin VLA05/4	+	+/–†	–	+	+	+	–	CL	NL	CL	PL	NL
Brucella melitensis (1)	+	–	–	+	+	–	+	NL	NL	CL	NL	NL
Brucella abortus (1)	+	+	+	+	–	+	–	CL	CL	CL	CL	NL
Brucella suis (1)	++‡	+	–	–	+	+	–	CL	NL	CL	PL	NL

* At a concentration of 1/50,000 w/v,
† Hydrolysation of urea
‡ Slight H_2S production
H_2S Hydrogen sulphide, CO_2 Carbon dioxide, BF Basic fuchsin, Th Thionin, RTD Routine test dilution, + Positive, – Negative, CL Confluent lysis, NL No lysis, PL Partial lysis

TABLE 2: Immunological results for the presence of *Brucella* antibodies using a competitive ELISA (cELISA) and two indirect ELISAs (iELISAs) from samples of pericardial and abdominal fluid from a bottlenosed dolphin (*Tursiops truncatus*)

	iELISA (AS7)	iELISA (16M)	cELISA	Interpretation
Pericardial fluid	69	11	36	Positive
Abdominal fluid	71	19	31	Positive

iELISA >10 per cent positive, cELISA <60 per cent positive
AS7 *Brucella abortus* antigen, 16M *Brucella melitensis* antigen

bottlenosed dolphin in the USA (Ewalt and others 1994). Cases of *Brucella*-induced abortion and placentitis in bottlenosed dolphins have also been reported in the USA (Miller and others 1999). Bricker and others (2000) elucidated on the molecular characterisation of *Brucella* strains isolated from marine mammals, including three isolates from bottlenosed dolphins in the USA. However, to the authors' knowledge, this is the first confirmed account of *Brucella* species infection in a wild bottlenosed dolphin in the UK. Although the biochemical analysis of phage typing was not directly comparable with previous accounts, the present isolate differs in that the A antigen was dominant whereas in those previously described the M antigen was dominant. Further investigation of the prevalence, distribution and zoonotic potential of this disease is needed, including the implications for marine mammals worldwide.

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