

Papers

Infection with *Brucella ceti* and high levels of polychlorinated biphenyls in bottlenose dolphins (*Tursiops truncatus*) stranded in south-west England

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Eight bottlenose dolphins (*Tursiops truncatus*) that stranded in Cornwall, south-west England, between June 2004 and December 2007 were examined using standardised postmortem examination and bacteriological methods. Evidence of *Brucella* species infection was found in four of these dolphins on culture. In addition, of the eight dolphins, four were positive and two were weakly positive for antibodies to *Brucella* species on serological analyses of pericardial and other fluids using a competitive ELISA and two indirect ELISAs. High or very high levels of the sum of 25 individual chlorobiphenyl congeners (Σ 25CBs) were also determined in blubber samples from two of the dolphins (45.5 and 446.6 mg/kg lipid weight).

Brucella species were first reported in marine mammals in the UK in Scotland in 1994 (Ross and others 1994). Subsequently, evidence of *Brucella* infection in marine mammals has been reported in many species globally since the early 1990s (Ewalt and others 1994, Jepson and others 1997, Dawson 2005, Tachibana and others 2006, Aguirre and others 2007, Ohishi and others 2007). The first isolation of *Brucella* species from marine mammals off the coast of Cornwall, UK, was recorded in 1998 from the liver of a by-caught harbour porpoise (*Phocoena phocoena*) (Dawson and others 2004). In 2007, the name *Brucella ceti* was proposed for *Brucella* strains isolated from cetaceans, and *Brucella pinnipedialis* for strains isolated from pinnipeds (Foster and others 2007).

Evidence of *Brucella* infection in bottlenose dolphins (*Tursiops truncatus*) throughout the world is particularly scarce, and there have been few isolations of *Brucella ceti* from this species. Ewalt and others (1994) and Miller and others (1999) reported the isolation of a *Brucella* species from aborted fetuses of captive bottlenose dolphins. In free-living

bottlenose dolphins, there has been only a single reported isolation of *Brucella* species, from a purulent blubber abscess in an animal stranded in Cornwall (Dawson and others 2006).

Serological evidence of exposure to *Brucella* species in bottlenose dolphins in the UK has so far been reported in only two animals, one from Wales (Jepson and others 1997) and the other from Cornwall (Dawson and others 2006). Serological evidence of exposure to *Brucella* species has also been reported in free-living bottlenose dolphins from Peru, the Mediterranean coast of Spain (Van Bressemer and others 2001), Costa Rica and Florida (Hernández-Mora and others 2009). There is also serological evidence of exposure to *Brucella* species for Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in the Solomon Islands (Tachibana and others 2006). In captive animals, there is serological evidence of *Brucella* species exposure in bottlenose dolphins in the USA (Miller and others 1999) and Black Sea bottlenose dolphins (*Tursiops truncatus ponticus*) in Russia (Aleksiev and others 2007).

Since 1990, the Animal Health and Veterinary Laboratories Agency (AHVLA) – Polwhele, Cornwall, has been involved in the UK Cetacean Strandings Investigation Programme (CSIP) to establish the cause of death of cetacean species stranded along the coast of south-west England, using standardised methodologies for postmortem examination, microbiological analysis and tissue sampling (Kuiken and Hartman 1991, Jepson 2005, Law 2006). This paper summarises the cultural and serological evidence for *Brucella* species infection and levels of polychlorinated biphenyls (PCBs) within the small population of semi-resident bottlenose dolphins inhabiting the coastal waters of Cornwall.

Materials and methods

Between January 2002 and December 2007, eight bottlenose dolphin carcasses were received for postmortem examination at AHVLA – Polwhele. They were from various locations covering both the north and south coasts and included one animal from the Isles of Scilly.

All animals were examined postmortem within 36 hours of receipt; carcasses that were not examined on the day of receipt were stored at 4°C. A standardised cetacean postmortem examination

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protocol was used to examine the carcasses (Kuiken and Hartman 1991, Jepson 2005, Law 2006). Standardised microbiological culture of heart blood, lung, liver and kidney, in addition to any lesions of suspected bacterial aetiology, was conducted in animals that were considered to be in a good state of preservation. The cultures were prepared as described by Davison and others (2009). Tissue samples including lung, liver, spleen, kidney, reproductive tissue, pericardial fluid, blood and any tissues with pathological lesions were prepared for specific *Brucella* culture by a method similar to that described by Foster and others (2002). Isolates were confirmed initially as *Brucella* species by phenotypic methods (Alton and others 1988). Amplification of an IS711 element downstream of the *bpp26* gene by PCR (Cloekaert and others 2000) was carried out to assess whether the isolates possessed the marker of *Brucella* species unique to marine mammals. Molecular characterisation of the outer membrane protein (*omp2*) locus of the strains using a selection of restriction enzymes was used to further characterise the isolates (Cloekaert and others 2001).

Samples of whole blood, milk/mammary fluid, pericardial fluid, and pleural and abdominal fluid were collected aseptically and stored at -20°C for serological analyses. These fluids were analysed by a competitive ELISA (cELISA) and two indirect ELISAs (iELISAs) to detect the presence of antibodies to *Brucella* species. The cELISA (MacMillan 1990) uses a lipopolysaccharide (LPS) *Brucella melitensis* antigen and a monoclonal antibody conjugate (Greiser-Wilke and others 1985). Of the two iELISAs, *B. melitensis* 16M antigen was used for the first, as described for the cELISA, and in the other a *Brucella abortus* LPS antigen was used. The iELISAs require an antiglobulin conjugate with specificity for the immunoglobulin isotypes of the species under test. However, protein A has been shown to bind to IgG of a range of marine mammals (Eliasson and others 1988, Sikkema 1989). Positive and negative thresholds for these assays were set with some uncertainty, but were based on those used for testing a wide range of terrestrial mammals in the UK for brucellosis (McGivern and others 2003).

A range of tissues from four of the dolphins were fixed in 10 per cent neutral buffered formalin. Due to autolysis, only reproductive tissue (testis or ovary) was preserved in 10 per cent buffered formalin in the four remaining animals in order to determine sexual maturity. All histological samples were routinely processed and stained with haematoxylin and eosin before microscopic examination.

Finally, two of the eight bottlenose dolphins were subjected to toxicological examination using internationally standardised methodologies (Jepson and others 2005, Law 2006). Blubber samples were examined for PCBs using the method and quality control procedures outlined by Jepson and others (2005). Briefly, 25 individual chlorobiphenyl congeners were determined in blubber samples using gas chromatography with electron-capture detection, and certified and/or laboratory reference materials were included within each sample batch as verification of the method's effectiveness. Concentrations were converted from a wet weight to a lipid weight (lw) basis as required using a blubber lipid content value that was derived following hexane extraction.

Results

Eight bottlenose dolphins, comprising four adults (two males and two females), three juveniles (all male) and a female calf, were submitted for postmortem examination. One of the adult females (case 8) was pregnant. One adult male dolphin was in a good nutritional state but the others were in a moderate or poor nutritional state; case 3 was in particularly poor condition (Fig 1). The cause of death could not be established in four animals (cases 1, 2, 4 and 5), partly due to the advanced state of autolysis; in one of these animals (case 5), bycatch could not be completely ruled out. Three animals (cases 3, 6 and 8) had relatively high parasitic burdens in both lungs and the cardiac stomach, and one of these animals (case 6) had fibrinous adhesions between the liver, diaphragm and peritoneum, and peritonitis. The cardiac stomach in case 6 also appeared to be compromised by the accumulation of a large number of fish bones and a fishing hook.

One aged dolphin (case 3) that stranded alive and was euthanased had evidence of previous entrapment and entanglement in fishing



FIG 1: Bottlenose dolphin (*Tursiops truncatus*) (case 3) that live-stranded off the coast of Cornwall in poor body condition

gear (not visible in Fig 1). Entanglement in fishing gear (bycatch) was confirmed as the cause of death in only one animal (case 7) (Table 1).

Due to autolysis, histological examination was either not possible or limited to reproductive tissue in cases 1, 4, 5, 6 and 7. Cases 5 and 7 were sexually immature.

In case 2, histological examination of the blubber pus lesion was not possible. There was mild lymphoid hyperplasia of the spleen associated with very rare megakaryocytes within the section. The mesenteric lymph nodes showed mild to moderate multifocal diffuse chronic granulomatous lymphadenitis. The pulmonary-associated lymph node had congestion of the medulla and only sparse distribution of lymphocytes and small lymphoid follicles within the cortex (appearance of lymphoid depletion). Examination of sections of the brain showed focal thickening/fibrosis of the cerebrum leptomeninges. The outer part of the cerebral cortex appeared to be mildly oedematous. Abnormalities were not detected in the rest of the brain. The testis was sexually immature and appeared normal.

In case 3, the testis was sexually mature but seasonally inactive with no abnormalities. Sections of mesenteric lymph node showed sparse lymphoid tissue with multifocal diffuse interstitial fibrosis. The pulmonary-associated lymph node had sparse lymphoid tissue and small lymphoid follicles in the cortex, with mild focal congestion in the medulla (appearance of lymphoid depletion). The kidney had diffuse, acute and mild congestion of the renal cortex. Sections of lung tissue revealed granulomatous bronchitis and interstitial pneumonia (subacute to chronic, mild to moderate multifocal diffuse) associated with nematode infection. There was also multifocal, moderate and acute pulmonary haemorrhage. Sections of the brain (cerebrum and cerebellum) showed a minimal focus of mononuclear cells within the cerebrum leptomeninges and some artefactual disruption to the tissue architecture. There was also minimal focal thickening of the leptomeninges of the cerebellum due to fibrosis and two very small foci of mononuclear cell infiltration.

In case 8, sections of the kidney showed congestion with some haemorrhagic foci within the renal cortex. Skeletal muscle had variable eosinophilia and vacuolation of some skeletal muscle fibres. Examination of the lymphatic system showed marked depletion of thymic lymphoid tissue and the appearance of lymphoid depletion in both the mesenteric and pulmonary-associated lymph nodes. There was mild granulomatous lymphadenitis within the mesenteric lymph nodes. Sections of the lung showed that the alveolar spaces were flooded with mucoproteinaceous exudate and occasional foci of granulomatous cellular infiltration were noted in the alveolar interstitium. Abnormalities were not detected in the uterus, liver, bladder or myocardium (Table 1).

Bacteriology

Four of the eight animals (cases 1, 4, 5 and 7) were not subjected to routine cultures due to autolysis. Tissue samples and body fluids were

TABLE 1: Causes of death and immunological results for the presence of antibodies to *Brucella* species in blood and body fluids from eight bottlenose dolphins (*Tursiops truncatus*) stranded in south-west England

Case	AHVLA – Polwhele reference	Immunological results for the presence of <i>Brucella</i> antibodies				Culture	PCB levels	Histology	Body condition	Sex	Age	Cause of death
		iELISA (A57)	iELISA (16M)	cELISA	Interpretation*							
1	22/M139/06/04 PCF	0	0	91	Negative	Negative	NT	ND	Moderate	F	Calf	Not established
2	22/M151/12/04 PCF	69	11	36	Positive	Positive (blubber pus)	87 mg/kg lw†	Lymphoid depletion	Moderate	M	Juvenile	Not established
3	Abdominal fluid 22/M39/11/05† PCF	71 8	19 8	31 44	Positive Weak positive	Positive (lung and kidney)	888 mg/kg lw‡	Lymphoid depletion	Poor	M	Adult	Parasitic pneumonia and live-stranding
4	22/M128/04/06 PCF	0	0	53	Weak positive	Positive (milk and mammary tissue)	NT	Ovaries only examined (autolysis)	Moderate	F	Adult	Not established
5	Milk/mammary fluid 22/M100/12/06 PCF	0 47	0 49	63 8	Negative Positive	Negative	NT	Testes only examined (autolysis, NAD)	Poor	M	Juvenile	Not established, possible bycatch
6	22/M14/04/07 PCF	20	6	31	Positive	Positive (lung, lungworms and testes)	NT	Testes only taken; not examined due to autolysis	Poor	M	Adult	Severe parasitic bronchitis, parasitic gastroenteritis, fibrinous adhesions between the liver, diaphragm and peritoneum, peritonitis and cardiac stomach obstruction
7	Pleural fluid 22/M40/10/07 PCF	20 0	9 0	35 91	Positive Negative	Negative	NT	Testes only examined (autolysis, NAD)	Good	M	Juvenile	Bycatch
8	22/M30/12/07 blood	85	84	6	Positive	Negative	NT	Lymphoid depletion	Moderate	F	Adult	Parasitic pneumonia
	PCF	86	84	6	Positive						(pregnant)	

* A positive result in only one of the three ELISAs is interpreted as weak positive. iELISA: >10 per cent positive, cELISA <60 per cent positive, A57 *Brucella abortus* antigen, 16M *Brucella melitensis* antigen

† This animal had evidence of entanglement in fishing gear before live-stranding

‡ Total PCB concentration (PCB as Aroclor 1254) levels

AHVLA Animal Health and Veterinary Laboratories Agency, F Female, lw Lipid weight, M Male, NAD Nothing abnormal detected, ND Not done due to autolysis, NT Not tested, PCB Polychlorinated biphenyl, PCF Pericardial fluid

taken from all eight animals for specific *Brucella* culture and serology. Of the four animals that were subjected to routine culture, only one (case 3) did not produce bacterial growth from any of the tissues sampled. *Edwardsiella tarda* was isolated in two of the remaining three animals: from the heart blood of case 2 and from the liver and intestine of case 8.

Haemolytic *Escherichia coli* was isolated from a liver lesion in case 6. None of these isolates was considered significant enough to cause death.

Four of the eight animals were positive for *Brucella* species infection by culture (Tables 1, 2). These included the first confirmed isolation in a free-living bottlenose dolphin, from blubber pus associated with long-standing cestode (*Phyllobothrium delphini*) cysts in a juvenile male (case 2). *Brucella* species were not recovered from the remaining tissues (lung, liver, kidney, spleen, testes), lungworms or stomach worms of this animal (Dawson and others 2006). *Brucella* species were also isolated from the lung and kidney but not from the liver, spleen or testes of an adult male (case 3), and from abnormal honey-coloured milk and mammary tissue but not from the lung, kidney, spleen, liver, uterus or brain of a female (case 4). In the remaining adult male (case 6), *Brucella* species were isolated from the lung, lungworms (*Halocercus* species) and grossly normal testes but not from the kidney, spleen or liver. *Brucella* species were not recovered from any of the tissues from the four remaining animals (cases 1, 5, 7 and 8), including the placenta, uterus, fetus, mammary tissue and milk of the pregnant female (case 8). All isolates were recovered as a light pure growth after seven to 15 days of incubation in Brodie and Sinton's broth (Brodie and Sinton 1975) and subsequent subculture on to Farrell's medium (Farrell 1974), with the exception of case 2, in which *Brucella* species were recovered as a light pure growth after six days of incubation directly on Farrell's medium.

The isolates were confirmed as *B. ceti* using phenotypic methods (Alton and others 1988, Foster and others 2007). Amplification of an IS711 element downstream of the *bp26* gene by PCR (Cloeckert

and others 2000) confirmed that the isolates possessed the unique feature specific to marine mammal strains of *Brucella* species. Molecular characterisation of the *omp2* locus of the strains using a selection of restriction enzymes revealed the type to be N(K), as found in common dolphins (*Delphinus delphis*), striped dolphins (*Stenella coeruleoalba*) and Atlantic white-sided dolphins (*Lagenorhynchus acutus*) (Cloeckert and others 2001).

Serology

The body fluids of all eight animals were assessed using a cELISA and two iELISAs. Six of the eight animals tested were interpreted as having antibodies to *Brucella* species on the basis of thresholds set for a range of terrestrial mammals from the UK for brucellosis (Table 1).

Toxicology

The sum of 25 chlorobiphenyl congeners (Σ 25CBs) in blubber from two animals was 45.5 mg/kg lw in case 2 and 446.6 mg/kg lw in case 3. The equivalent total PCB concentration (PCB as Aroclor 1254) levels were 87 and 888 mg/kg lw, respectively. Samples from all six remaining animals were collected but analysis could not be carried out due to financial constraints.

Discussion

Of the eight bottlenose dolphins examined in the present study, four (50 per cent) were positive for *B. ceti* on culture. These included the isolates from two reproductive tissues (cases 3 and 6), and also from lungworms removed from one animal (case 6), suggesting that lungworms may have a role to play in the transmission of *B. ceti*, as described by Dawson and others (2008a) in harbour porpoises. In addition, six of the animals (75 per cent) were either positive (n=4) or were weakly positive (n=2) for antibodies to *Brucella* in a range of fluids (including pericardial fluid, milk/mammary fluid, abdominal fluid, pleural fluid and blood) by cELISA and iELISA. One animal (case 4), which was considered weakly positive overall, was weakly positive for antibod-

TABLE 2: Characteristics of *Brucella* species isolated from bottlenose dolphins (*Tursiops truncatus*) that stranded on the south-west coast of England, compared with other *Brucella* species

Identity	Biotype	Urease	CO ₂ requirement	H ₂ S production	Growth on media containing		Agglutination with monospecific antisera				Lysis by phage at RTD				
					Thionin*	Fuchsin*	A	M	R	Tb	Wb	Bk ₂	Fi	R/C	Tb10 ⁴
22/M151/12/04	NA	+	-	-	+	+	+	-	NT	NL	L	L	PL	NL	NT
22/M39/11/05	NA	+	-	-	+	+	+	-	NT	NL	PL	L	L	NL	NT
22/M128/04/06	NA	+	-	-	+	+	+	-	NT	NL	PL	L	PL	NL	NT
22/M14/04/07	NA	+	-	-	+	+	+	-	NT	NL	PL	L	PL	NL	NT
Lungworms	NA	+	-	-	-	+	+	-	NT	NL	PL	L	L	NL	NT
Testes	NA	+	-	-	-	+	+	-	NT	NL	PL	L	NL	NL	NT
<i>Brucella abortus</i>	1	(+)†	(+)	+	+	+	+	-	-	L	L	L	L	NL	L
	2	+	(+)	+	+	-	+	-	-	L	L	L	L	NL	L
	3‡	+	(+)	+	+	+	+	-	-	L	L	L	L	NL	L
	4	+	(+)	+	+	+	-	+	-	L	L	L	L	NL	L
	5	+	-	-	+	+	-	+	-	L	L	L	L	NL	L
	6‡	+	-	(+)	+	+	+	-	-	L	L	L	L	NL	L
	7	+	-	(+)	+	+	+	+	-	L	L	L	L	NL	L
	9	+	-	+	+	+	-	+	-	L	L	L	L	NL	L
	<i>Brucella suis</i>	1	+	-	+	+	-	+	-	-	NL	L	L	PL	NL
2		+	-	-	+	-	+	-	-	NL	L	L	PL	NL	L
3		+	-	-	+	+	+	-	-	NL	L	L	PL	NL	L
4		+	-	-	+	(-)	+	+	-	NL	L	L	PL or L	NL	L
5		+	-	-	+	-	-	+	-	NL	L	L	PL	NL	L
<i>Brucella melitensis</i>	1	+	-	-	+	+	-	+	-	NL	NL	L	NL	NL	NL
	2	+	-	-	+	+	+	-	-	NL	NL	L	NL	NL	NL
	3	+	-	-	+	+	+	+	-	NL	NL	L	NL	NL	NL
<i>Brucella ovis</i>		-	+	-	+	(+)	-	-	+	NL	NL	NL	NL	L	NL
<i>Brucella canis</i>		+	-	-	+	-	-	-	+	NL	NL	NL	NL	L	NL
<i>Brucella neotomae</i>		+	-	+	-	-	+	-	-	NL or PL	L	L	L	NL	L
Newly described strains															
<i>Brucella ceti</i>		+	(-)	-	(+)	(+)	(+)	(-)	-	NLa	Lb	Lb	NL or PL	NL	
<i>Brucella pinnipedialis</i>		+	(+)	-	+	+	(+)	(-)	-	NLa	Lb	Lb	NL or PL	NL	
<i>Brucella microti</i>		+	-	-	+	+	-	+		NL	L		NL		L
<i>Brucella inopinata</i>		+	-	(-)	+	+	(-)	(-)		NL					NL

* Concentration 1/50,000 w/v

† Reference strain is negative but most field strains are positive

‡ For more certain differentiation of biotypes 3 and 6, thionin at 1/25,000 (w/v) is used in addition. Type 3 +, type 6 -

§ Some strains of this biotype are inhibited by basic Fuchsin

¶ Some isolates may be resistant to basic fuchsin, pyronin and safranin O

+ Positive, - Negative, (+) Most strains positive, (-) Most strains negative

Bk₂ Berkeley, Fi Firenze, L Confluent lysis, NL No lysis, NLa Most strains no lysis, NT Not tested, Lb Most strains lysis, PL Partial Lysis, R/C Rough strains, RTD Routine test dilution, Tb Tbilisi, Wb Weybridge, Tb10⁴ RTD x 10⁴

ies to *Brucella* in pericardial fluid but negative in milk/mammary fluid. In the same period (January 2002 to December 2007) and geographical region (Cornwall, south-west England), seven of 78 (8.9 per cent) stranded harbour porpoises, five of 72 (6.9 per cent) stranded common dolphins and one of eight (12.5 per cent) stranded striped dolphins submitted to VLA - Polwhele for postmortem examination were positive for *B. ceti* on microbiological culture (N. J. Davison, unpublished observations; Dawson and others 2008b). It is worth noting that there were no other isolations of *Brucella* species from 15 bottlenose dolphins submitted for postmortem examination between 1989 and 2008 in the rest of England and Wales, and only one isolation (in 2007) from the 36 bottlenose dolphins examined in Scotland during the same period (CSIP, unpublished observations).

Although no histological changes were seen in the gonads of the *Brucella*-positive bottlenose dolphins in the present study, *B. ceti* was isolated from reproductive tissues of both sexes (cases 4 and 6), which suggests that *B. ceti* may cause reproductive disease in free-living bottlenose dolphins, as described in captive bottlenose dolphins (Ewalt and others 1994, Miller and others 1999). The isolation of *B. ceti* from abnormal milk and mammary tissue of case 4 in the present study may also indicate persistent shedding of the organism, which is a characteristic of the disease in terrestrial mammals (Radostits and others 2007).

In cetaceans, *B. ceti* infection has been implicated in a number of disease processes. Epididymitis, splenic coagulative necrosis and blubber abscessation have been reported in harbour porpoises (Foster and others 2002). Chronic active mastitis, focal necrosis of the liver, spleen and mesenteric lymph node and endometritis have been reported in Atlantic white-sided dolphins (Foster and others 2002). Blubber abscessation, mammary gland granuloma and meningitis have been

reported in striped dolphins (Foster and others 2002, González and others 2002, Davison and others 2009). *B. ceti* was also recovered from the diseased atlanto-occipital joint of an Atlantic white-sided dolphin (Dagleish and others 2007) and from testicular abscessation in a harbour porpoise (Dagleish and others 2008). Since the first isolation of *Brucella* species in a harbour porpoise in Cornwall, in 1998 (Dawson and others 2004), *B. ceti* has been confirmed as the cause of meningitis in a striped dolphin (Davison and others 2009) from the same region. *B. ceti* has also been isolated from the uterus and testes of two common dolphins and the testes and milk of two harbour porpoises (Jepson and others 2009; N. J. Davison, unpublished observations).

In the USA, abortion and placentitis in captive bottlenose dolphins were attributed to *Brucella* species based on the isolation of the organism and pathological observations in the placenta (Ewalt and others 1994, Miller and others 1999). Muñoz and others (2006) also described meningitis in a striped dolphin from Spain, as did Hernandez-Mora and others (2008) in Costa Rica. Recently, Jauniaux and others (2010) reported *B. ceti* infection associated with genital ulceration and mammary gland acini in a harbour porpoise found stranded on the coast of Belgium, suggesting bacteraemia. *B. ceti* can cause disease in cetaceans, including conditions of the reproductive organs similar to those seen in terrestrial mammals, such as reproductive failure, abortion or weakness and mortality of offspring in the female, and orchitis, epididymitis and sterility in the male (Radostits and others 2007). Infection can have an adverse effect on reproduction in cetaceans (Miller and others 1999, Dagleish and others 2008, Jauniaux and others 2010).

In a recent review of chemical pollutants in marine mammals in the North Atlantic and Arctic oceans, high levels of PCBs were highlighted as the greatest toxicological threat to some marine top predators such as bottlenose dolphins and killer whales (*Orcinus orca*)

(International Council for the Exploration of the Sea [ICES] 2010). Although levels of PCBs in UK-stranded harbour porpoises declined gradually from 1990 to 1998, levels subsequently reached a plateau from 1998 to 2005 (Law and others 2010). A case-control study of UK-stranded harbour porpoises demonstrated a statistically significant association between higher PCB concentrations in harbour porpoises dying from infectious disease, compared with a control group that died of acute physical trauma (Jepson and others 2005). A second case-control study found an increased risk of infectious disease in harbour porpoises when the differences of $\Sigma 25$ PCB exceeded 45 mg/kg lw, and there was a twofold increased risk of death due to infectious disease when concentrations exceeded 80 mg/kg lw (Hall and others 2006a). Both bottlenose dolphins (cases 2 and 3) tested in the present study had PCB concentrations in excess of the levels that have been associated with infectious disease mortality in UK-stranded harbour porpoises. In fact, one of these bottlenose dolphins (case 3) had the highest level of PCBs (446.6 mg/kg lw) recorded in this species in the UK in the past 20 years. The equivalent total PCB concentration (PCB as Aroclor 1254) levels were 87 mg/kg lw for case 2 and 888 mg/kg lw for case 3, which also greatly exceeded a proposed toxicity threshold of 17 mg/kg lw for blubber concentrations of total PCBs in marine mammals (Kannan and others 2000). Both of these animals were culturally and serologically positive for *B. ceti*. Neither of these animals was considered to be in a good nutritional state, with case 3 in particularly poor condition.

Histological examination of lymphoid tissue from three bottlenose dolphins (cases 2, 3 and 8) in the present study showed the appearance of generalised lymphoid depletion (including the two dolphins [cases 2 and 3] with high PCB levels and *Brucella* infection). High PCB exposure has been linked to reproductive impairment (Wells and others 2005) and modelled to depress the population growth rate of some coastal bottlenose dolphin populations in the USA (Schwacke and others 2002, Hall and others 2006b), and may influence the susceptibility of individual bottlenose dolphins to infection with a range of pathogens, including *B. ceti*. High PCB exposure continues to pose a threat to the conservation status of small bottlenose dolphin populations in UK and other industrialised waters (ICES 2010).

The SCANS II survey estimated the total population of bottlenose dolphins in the European Atlantic and North Sea to be 12,700 (Hammond and others 2007). This would include both offshore and inshore resident populations, making it difficult to assess the status of the resident inshore population. However, the status of bottlenose dolphins within the UK stranding record has undergone a marked change, with many more animals stranding before 1970 (Jepson 2005). Between 1940 and 1960, this species was the second or third most commonly reported cetacean in the UK, but more recently it had been ranked only 10th or 11th (Jepson 2005). From 1948 to 1966, bottlenose dolphin strandings were reported in most areas of the UK, including the English Channel, south-east coast and East Anglia (Fraser 1974). Since 1990, most of the strandings in the UK have been restricted to west Wales and the Moray Firth, Scotland (Jepson 2005). These data trends would suggest that the UK inshore resident bottlenose dolphin populations have declined markedly from historic levels. Tregenza (1992) described a 90 per cent decline in smaller cetacean sightings in Cornwall (including bottlenose dolphins) between 1935 and 1985. However, in south-west England, there has been a small population of bottlenose dolphins resident in Cornish waters since 1991 (Tregenza 1992, Wood 1998). This group would appear to have replaced an earlier population lost in the 1970s (Jepson and others 2008). The loss of any individuals from such a small population will have an impact on its survivability.

In conclusion, the present study has reported both a high proportion of *B. ceti* infection and serological evidence of *Brucella* species exposure in bottlenose dolphins in south-west England. These comprise some of the first reported cases of *Brucella* infection caused by *B. ceti* in free-living bottlenose dolphins and include the first report of the isolation of this organism from *Halocercus* species lungworms. Further research is necessary to ascertain whether the relationship between brucellosis and high PCB exposure in bottlenose dolphins in south-west England is causal or coincidental. Continued exposure to high levels of legacy pollutants (specifically PCBs) may pose an ongoing

threat to many coastal bottlenose dolphin populations in industrialised regions (ICES 2010) despite the pollutants being completely banned for nearly two decades.

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Infection with *Brucella ceti* and high levels of polychlorinated biphenyls in bottlenose dolphins (*Tursiops truncatus*) stranded in south-west England

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