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SHORT PAPER

Pathology of Striped Dolphins (*Stenella coeruleoalba*) Infected with *Brucella ceti*

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Summary

Seventeen striped dolphins (*Stenella coeruleoalba*) displaying swimming disorders compatible with neurological syndromes were investigated for *Brucella* infection. Sixteen dolphins had meningoencephalomyelitis. Serum antibody against *Brucella* antigen was detected in all 14 animals tested and *Brucella ceti* was isolated from eight out of nine animals. *Brucella* antigen was detected in the brain by immunofluorescence, but not by immunohistochemical labelling. By contrast, *Brucella* antigen was demonstrated by immunohistochemistry in the trophoblast of animals with severe placentitis and in the mitral valve of animals with myocarditis. The microscopical lesions observed in the tissues of the infected dolphins were similar to those of chronic brucellosis in man. The severity of brucellosis in *S. coeruleoalba* indicates that this dolphin species is highly susceptible to infection by *B. ceti*.

Keywords: *Brucella*; *Brucella ceti*; endocarditis; neurobrucellosis; placentitis; *Stenella coeruleoalba*; striped dolphin

Members of the genus *Brucella* are intracellular pathogens of marine and terrestrial mammals, including man (Meador *et al.*, 1989; Ewalt *et al.*, 1994; Foster *et al.*, 2002; Pappas *et al.*, 2005; Groussaud *et al.*, 2007). The infection may pass unnoticed in non-pregnant natural hosts and in ill animals in which specific diagnostic procedures are not performed (Barquero-Calvo *et al.*, 2007). However, in gravid females the infection generally causes abortion and in males epididymitis and orchitis. In contrast, in secondary accidental hosts such as man, the infection commonly causes a severe and obvious illness with a broad spectrum of symptoms that may become grave if not treated (Pappas *et al.*, 2005).

Brucellosis in cetaceans is caused by *Brucella ceti*, a species that is predominant in dolphins and whales (Groussaud *et al.*, 2007). The *B. ceti* group, which may comprise at least two distinct strains (dolphin and porpoise types), is phenotypically similar to smooth *Brucella abortus* and *Brucella melitensis*, possessing the same surface antigens that are commonly used for the serological diagnosis of brucellosis in infected cattle (Baucheron *et al.*, 2002; Groussaud *et al.*, 2007). Moreover, marine *Brucella* strains have been described causing lesions in both cetaceans and man (Ewalt *et al.*, 1994; Brew *et al.*, 1999; Miller *et al.*, 1999; González *et al.*, 2002; Sohn *et al.*, 2003; McDonald *et al.*, 2006; Hernández-Mora *et al.*, 2008) and experimental infection with these strains may induce seroconversion and abortion in cattle (Rhyan *et al.*, 2001).

The isolation and characterization of *B. ceti* strains from the cerebrospinal fluid of striped dolphins (*Stenella coeruleoalba*) stranded on the Pacific shoreline of Costa Rica has been described previously (Hernández-Mora *et al.*, 2008). The present report extends these findings and describes pathological lesions in 17 affected striped dolphins and one fetus.

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Between 2001 and 2009, 17 striped dolphins were stranded on the Pacific shorelines of Costa Rica (Table 1). All of these animals displayed swimming disorders compatible with neurological syndromes before death. Necropsy examinations were performed by the Pathology Unit of the Veterinary School at the National University, Costa Rica. Blood samples were taken from the arterial plexus of 14 dolphins and serum was separated from the clot by centrifugation. Serum samples were tested for the presence of antibody to *Brucella* by the Rose Bengal test and indirect enzyme-linked immunosorbent assay (ELISA) (Hernández-Mora *et al*., 2009). All 14 animals were seropositive (Table 1).

During the gross necropsy examination samples were collected from a range of organs and tissues and fixed in 10% neutral buffered formalin. These were subsequently embedded in paraffin wax and sections were prepared for staining by haematoxylin and eosin (Kiernan, 2003). Smears taken during the gross examination were stained by the Wright–Giemsa method. Bacterial isolation and characterization was performed as described by Hernández-Mora *et al*. (2008). *B. ceti* was isolated from the brain and tissues of eight out of nine dolphins and the fetus (Table 1). Bacterial isolation was not attempted in the seven dolphins collected before 2005.

The major gross and microscopical findings are presented in Figs. 1 and 2. Many of the general pathological findings were not related to brucellosis, but the changes detected in the central nervous system, female reproductive system and heart were associated with *Brucella* infection. The most significant findings in the brain and meninges have been described previously (González *et al*., 2002; Muñoz *et al*., 2006; Hernández-Mora *et al*., 2008). Sixteen of the 17 animals had meningoencephalomyelitis with little or no involvement of the neural tissue. One juvenile male had hydrocephalus involving the lateral ventricles (Fig. 3A). Hyperaemia of the meninges and brain and cloudiness of the cerebrospinal fluid with increased cellularity was noted in 16 cases. Widespread periventricular encephalitis involving mononuclear cell infiltration was principally found around the third and fourth ventricles. Non-suppurative meningitis affected the spinal cord, medulla oblongata and cerebellum, but this lesion was milder in the meninges overlying the cerebral cortices. In most cases there was perivascular mononuclear infiltration of the white and grey matter of the cerebrum, cerebellum and brainstem, as previously reported (Hernández-Mora *et al*., 2008). Moderate to severe non-suppurative choroiditis and major loss of ependyma was also present. Plasma cells, small lymphocytes and macrophages dominated the cellular infiltrates. One juvenile male displaying meningoencephalomyelitis also had fibrinopurulent osteoarthritis with severe infiltration of the synovial fluid by macrophages and neutrophils affecting the right scapulohumeral joint. This change has been described previously in cetaceans with brucellosis (Dagleish *et al*., 2007). Nine dolphins with meningoencephalomyelitis (six with positive serology and four with positive *B. ceti* cultures) also had non-suppurative interstitial pneumonia and five others displayed periportal lymphocytic hepatitis.

Table 1
Meningoencephalomyelitis, detection of anti-*Brucella* antibodies and *B. ceti* isolation in 17 stranded *S. coeruleoalba* dolphins

Sex	*Age*	*Meningoencephalomyelitis*	*Positive serology*[*]	B. ceti *isolation*
Female	Adult	6/6	5/5	4/4
Female	Juvenile	2/2	1/1	1/1
Female	Calf	0/1	1/1	0/0
Male	Adult	2/2	2/2	1/1
Male	Juvenile	6/6	5/5	2/3
Total		16/17	14/14	8/9

[*]Rose Bengal test and indirect ELISA.

Detection of *Brucella* antigen in smears was undertaken by immunofluorescence (Hernández-Mora *et al*., 2008). Detection of *Brucella* antigen in tissue was undertaken by immunohistochemistry (IHC) by use of the streptavidin–biotin–horseradish peroxidase (HRP) method with rabbit anti-*Brucella* lipopolysaccharide antibody as primary reagent (Boenish 2001; Hernández-Mora *et al*., 2008, 2009). The presence of morbillivirus antigen in the brain was explored by IHC (Domingo *et al*., 1992) with sections of brain from a dolphin with known morbillivirus encephalitis as positive control. The presence of helminths was estimated by macroscopic and microscopic examination of tissues, and of *Toxoplasma* parasite infections by serology, or histological examination (O'Shea *et al*., 1991; Dubey *et al*., 2007).

Brain impressions and smears of cerebrospinal fluid were positive for *Brucella* by immunofluorescence in the nine animals tested (data not shown). *B. ceti* was cultured from eight of these animals (Table 1). In contrast, immunohistochemical examination of brain, medulla, cerebellum and spinal cord tissues failed to demonstrate *Brucella* or morbillivirus antigen in the 17 dolphins tested.

The only pregnant dolphin had severe placentitis with multiple necrotic foci and a dead fetus. The dolphin was estimated to have been in the seventh month of gestation (Hernández-Mora *et al*., 2008). Microscopical examination of the placenta confirmed severe and widespread necrosis (Fig. 3B) with abundant

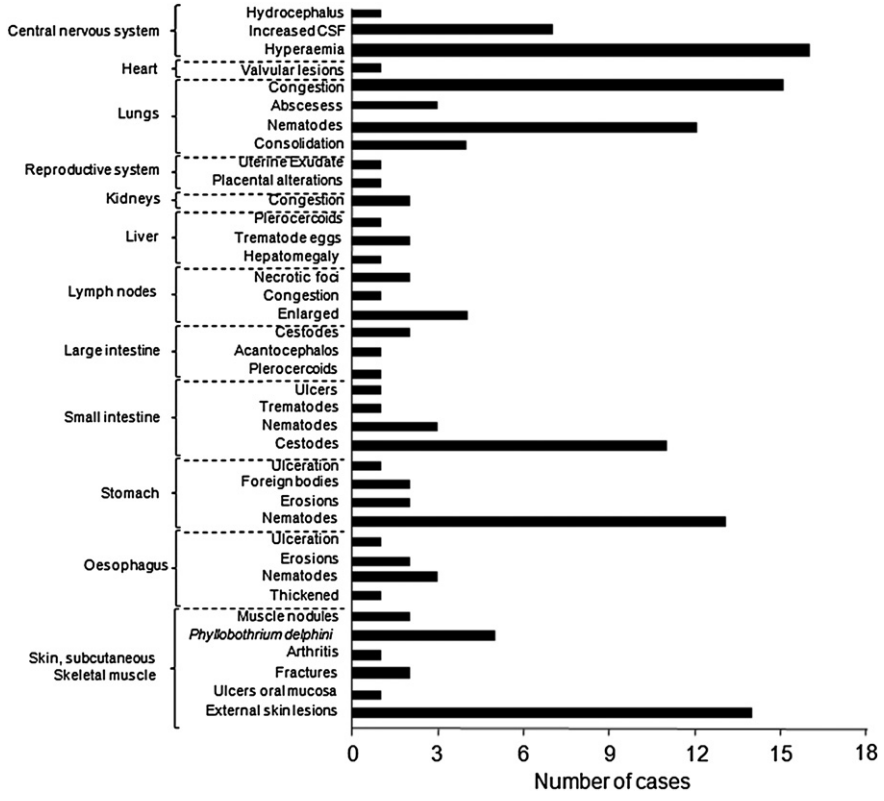

Fig. 1. Gross pathological findings detected in 17 *S. coeruleoalba* dolphins.

mixed mononuclear and polymorphonuclear infiltration of the trophoblast (Fig. 3C). *Brucella* antigen was detected by IHC within the inflammatory infiltrate as well as in some chorionic cells in these necrotic regions, the intensity of labelling consistent with the presence of large numbers of bacteria (Fig. 3D). Despite the placental lesions, no significant pathological changes were detected in the fetus.

One adult female had severe endocarditis with thickening and a prominent vegetative nodule of the mitral valve (Fig. 3E). The endocarditis was characterized by the presence of fibrin adjacent to the surface of the mitral valve, with a predominantly non-suppurative infiltration of lymphocytes, macrophages, plasma cells and multinucleate giant cells (Fig. 3F–H). Some scattered necrotic areas with

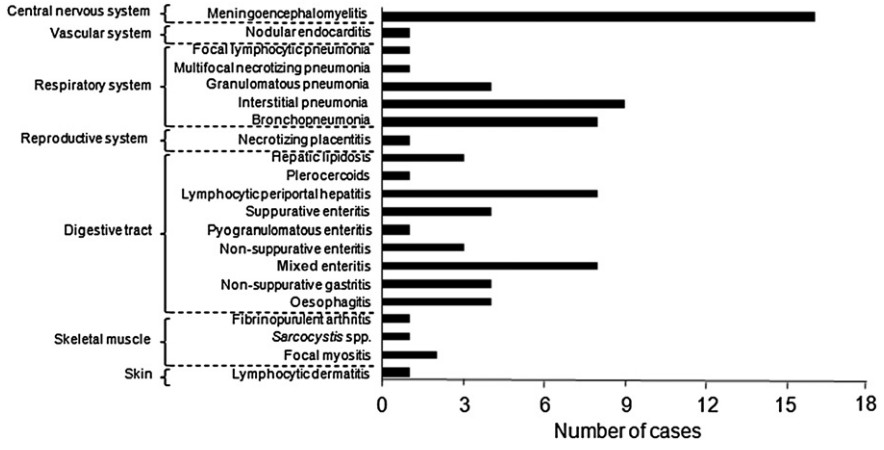

Fig. 2. Microscopical findings detected in 17 *S. coeruleoalba* dolphins.

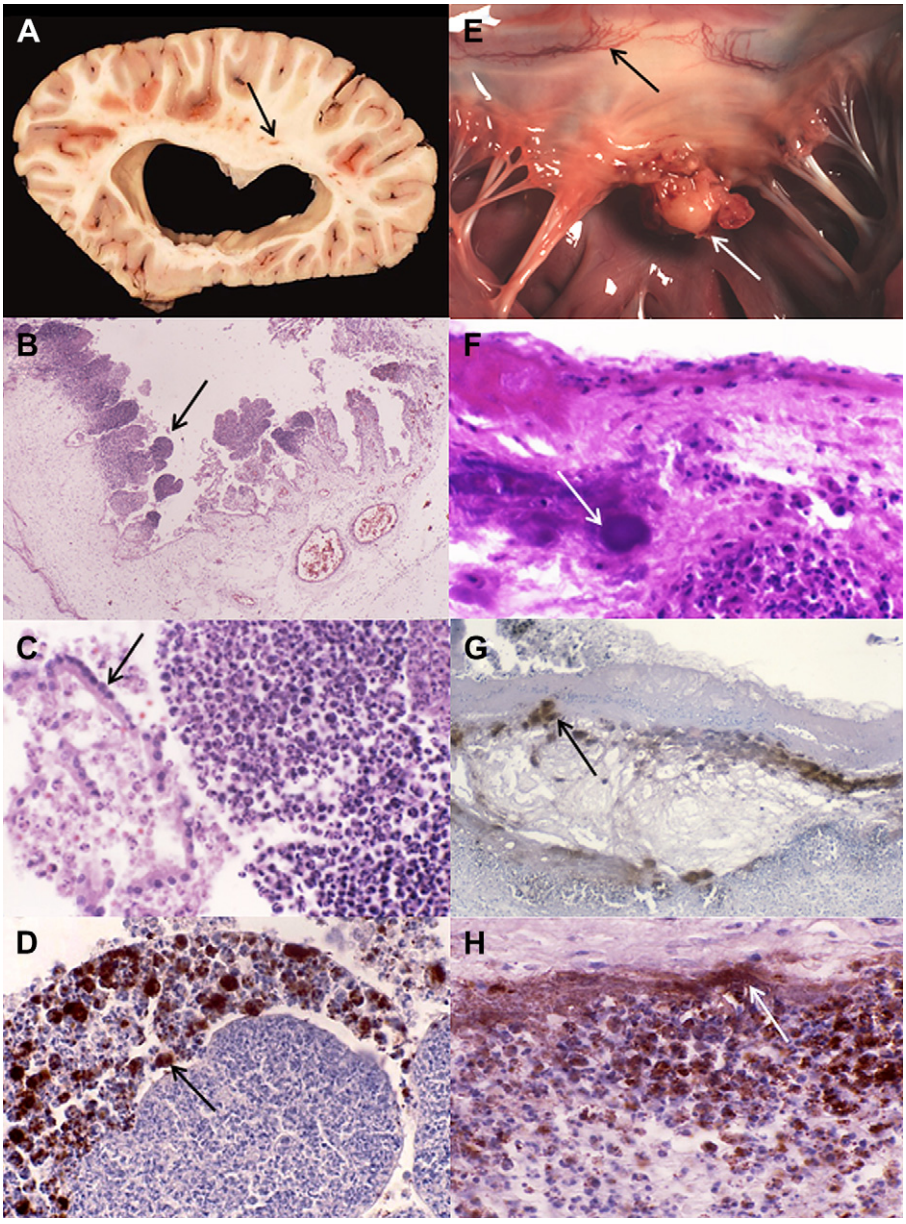

Fig. 3. Pathological findings in *B. ceti*-infected *S. coeruleoalba* dolphins. (A) Coronal transverse section of the lateral caudal region of the left hemisphere of the brain. There is internal hydrocephalus with enlargement of the lateral ventricles due to cerebrospinal fluid accumulation secondary to inflammation surrounding the ventricular system. Arrow indicates a hyperaemic blood vessel. (B) Necrotizing placentitis showing marked infiltration of inflammatory cells into the fetal placental villi (arrow), submucosal oedema and hyperaemic blood vessels. HE. ×4. (C) Placental villi with inflammatory infiltrate and some detached placental epithelial cells (arrowed). HE. ×40. (D) Labelling of *Brucella* antigen within inflammatory cells invading the placental villi (example arrowed). IHC. ×40. (E) Vegetative nodule in the mitral valve (white arrow) of the heart and hyperaemic blood vessels in the dorsal region of the valve (black arrow). (F) Microscopical appearance of the mitral valve shown in (E) with bacterial colonies (arrow) and fibrin deposition HE, ×40. (G) Section from the mitral valve shown in (E) demonstrating *Brucella* antigen (arrow) and labelled bacterial aggregates. IHC. ×10. (H) Section from the mitral valve shown in (E) demonstrating *Brucella* antigen within phagocytic cells and some probable bacterial aggregates (arrow). IHC. ×40.

dystrophic calcification and bacterial colonies surrounded by polymorphonuclear cells were also observed in this area (Fig. 3F). There was also focal degeneration of myocardial fibres that were surrounded by a mild lymphocytic infiltrate and perivascular oedema. Pericardial fibrosis with infiltration of lymphocytes and plasma cells was also present. *Brucella* antigen was detected by IHC associated with the infiltrating inflammatory cells and the bacterial colonies (Fig. 3G, H).

The results of the present study suggest that the observed stranding of striped dolphins may be directly

associated with meningoencephalomyelitis caused by infection with *B. ceti*. Although similar pathological changes have been observed in man and in other dolphin species infected with *Brucella* spp. (Foster *et al*., 2002; Pappas *et al*., 2005), these changes are seldom recorded in terrestrial hosts such as cattle, goats, sheep or pigs. In these hosts the main symptoms are related to abortion, placental retention, interstitial mastitis, epididymitis and, in some cases, hygromas (Hagemoser *et al*., 1988; Meador *et al*., 1989; Musa *et al*., 1990). It is notable that neurological or cardiac diseases associated with *Brucella* are not documented in these domestic animals. The microscopical lesions caused by *B. ceti* were strikingly different from encephalitis caused by morbillivirus, trematode parasites or *Toxoplasma*, all infections reported in *S. coeruleoalba* (O'Shea *et al*., 1991; Domingo *et al*., 1992; Dubey *et al*., 2007). In fact, the neuropathology recorded was similar to that described in meningoencephalomyelitis associated with *Brucella* infection in man (Shakir *et al*., 1987; Vinod *et al*., 2007).

Although *B. ceti* was isolated from many of the affected dolphins and *Brucella* antigen was detected by immunofluorescence in the brain and cerebrospinal fluid, it was not possible to detect *Brucella* antigen in the central nervous system by IHC. Brucellosis has been diagnosed by immunohistochemical labelling of the brain of one infected dolphin (González *et al*., 2002), but the lower sensitivity of this technique for identifying *Brucella* antigens in tissues is recognized (Seidel *et al*., 2003).

The lesions observed in the heart, liver, lungs, joints and placenta of animals in the presents study suggest that *B. ceti* has the ability to cause chronic infection of multiple organs before it crosses the blood–brain barrier. Similarly, mitral valve lesions have been reported in chronic brucellosis of man (Gon-Je and Song, 2008). The placentitis observed in one dolphin was similar to that reported in two previous cases (Miller *et al*., 1999); however, despite the placental lesions no significant pathological changes were detected in the fetus. This is noteworthy, as severe placentitis in bovine brucellosis is associated with abortion and these fetuses display severe central nervous system and pulmonary pathology with significant inflammation (Hong *et al*., 1991).

Descriptions of pathological findings due to natural brucellosis in secondary accidental hosts such as man are sparse (Hunt and Bothwell, 1967; Pappas *et al*., 2005). Therefore, the severity of the disease observed in the striped dolphins reported here may serve to increase understanding of the natural course of brucellosis in both man and animals.

Conflict of Interest

The authors do not declare any conflict of interest.

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