Heart pathologies in dolphins stranded along the northwestern Italian coast

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ABSTRACT: Nine striped dolphins *Stenella coeruleoalba* and 1 bottlenose dolphin *Tursiops truncatus* stranded along the Ligurian Sea coast of Italy were necropsied between February 2011 and April 2012. Macroscopic and histological findings were observed in the hearts of all animals and included saccular aneurysms of the pulmonary trunk (n = 3), cirsoid aneurysms (n = 1), right ventricular dilation (n = 1) associated with hypoplasia of the tricuspid chordae (n = 1), valvular fibrosis (n = 3), mitral leaflet thickening (n = 1), left ventricular hypertrophy (n = 1), lymphocytic myocarditis (n =1), and Lambl's excrescences (n = 4). To our best knowledge Lambl's excrescences, aneurysm of the pulmonary trunk, and cirsoid aneurysms have not previously been described in marine mammals, and some of these findings should be taken into account as possible causes of dolphin morbidity, mortality, and stranding.

KEY WORDS: Aneurism • Lambl's excrescences • *Stenella coeruleoalba* ■ *Tursiops truncatus*

INTRODUCTION

Little is known about the development of and pathology affecting cetacean hearts (Sedmera et al. 2003), since they have been poorly studied in marine mammals. The few cases of heart disease reported in cetaceans include infections (Guzman-Verri et al. 2012), parasites (Jardine & Dubey 2002), and malformations (Slijper 1961, Gray & Conklin 1974, Neurohr 1982, Troncone & Zizzo 1994, Powell et al. 2009). Bossart et al. (2007) reported cases of myocardial degeneration and moderate myocarditis in stranded sperm whales *(Kogia* spp.), and myocardial contraction band necrosis was identified by Turnbull & Cowan (1998) in 100% of 52 cetaceans of different species stranded between 1991 and 1996. A low incidence of abnormal cardiac development in cetaceans has been reported (Powell et al.

2009).

Congenital anomalies, including heart malformations, are rarely observed in Atlantic bottlenose dolphins *Tursiops truncatus* and other delphinids, and are mainly reported in stillborn or neonate cetaceans. A case of right ventricular hypertrophy, ventricular septal defect, aortic dilation, atrial septal defect, subvalvular pulmonic stenosis, and hypoplasia of the pulmonary artery and mitral valve were described in a stranded neonate *T truncatus* by Powell et al. (2009). Gray & Conklin (1974) described a transposition of the pulmonary artery and aorta associated with interventricular foramen in an unborn bottlenose dolphin fetus. Persistent ductus arteriosus, atrial septal defect, and right ventricular hypertrophy were reported in a 7 d old captive-born *T truncatus* by Neurohr (1982). While there have been individual observations, reports of cardiac pathologies in multiple animals have not been well documented. The aim of this study was to evaluate cardiac pathology in stranded dolphins.

MATERIALS AND METHODS

Nine striped dolphins *Stenella coeruleoalba* (7 males and 2 females) and 1 male bottlenose dolphin stranded along the Ligurian Sea coast of Italy between February 2011 and April 2012 were examined (Table 1). Age classes were determined (sub-adults: case nos. 6 and 10; adults: all other animals), and field necropsies were performed on all animals to ascertain the cause of death. Intact hearts submitted to the Department of Veterinary Science of the University of Turin (Italy) were fixed in 10% neutral buffered formalin (pH 7). The hearts were examined according to Virchow (1880) and Finkbeiner et al. (2009), and lesions were systematically described and graded. For histological investigations, tissue samples from the hearts were wax-embedded, sectioned at 4 pm using a microtome (Leica Microsystems), and stained with hematoxylin and eosin (HE) and Weigert-Van Gieson (WVG) stains.

RESULTS

No gross lesions were observed in case no. 6. In 6 animals, parasitic nodules were detected in different anatomic locations (peritoneum: cases 1 and 3; liver: case 1; skeletal muscle: case 2; omentum: cases 1 and 6; pericardium: case 2; intestine: case 6; lungs: cases 8 and 10). In case 4, the left lung had emphysema. In case 6, suppurative bronchopneumonia with suppurative mediastinal lymphadenitis was detected. Traumatic lesions were observed in 2 cases: hemorrhages of the left eye (case 2), and subcutaneous hematoma of the left side of the abdomen, hemorrhage in the left kidney and hemoperitoneum (case 3). Abdominal changes were observed in 4 cases: marble spleen and adrenal hypoplasia (case 1), splenomegaly (case 2), intestinal adhesions (case 7), and fibrinous peritonitis (case

9).

Gross cardiac pathology was observed in 6 of 10 animals, and histological lesions were found in 7 out of 10 dolphins. Findings are summarized in Table 1.

Macroscopic evaluation of the hearts in 3 cases (1, 7, and 9) showed a white, elevated, well demarcated, ovoid area on the pulmonary artery adjacent to the pulmonary ostium in the pulmonary artery. These findings were classified as aneurysms of the pulmonary trunk and aneurysms of the right sinus of Valsalva. Histopathology of the affected pulmonary artery revealed marked diffuse mural atrophy on comparison to healthy control animals. In all observed cases, the wall of the pulmonary artery was atrophic (Fig. la,b) compared to a normal artery (Fig. 2).

One dolphin (case 1) had a dilated right ventricle, hypoplasia of the tricuspid chordae, severe and diffuse fibrosis associated with pronounced thickening and retraction of the tricuspid leaflets, and consequent left ventricle dilation. Adjacent to the papillary muscle in the interventricular septum, an intramural, firm, white spot, 0.2 cm in diameter, corresponding to focal interstitial lymphocytic myocarditis on histological examination, was also observed. Mitral valvular changes were observed in 3 cetaceans and included mitral fibrosis (case 2), mitral leaflet thickening (case 4), and left ventricular hypertrophy (case 6). Case 4 also presented a serpiginous course of the left subepicardial coronary artery which, at the cut surface, revealed dilated arteries surrounded by connective tissue, consistent with cirsoid aneurysm (Fig. 3a).

Histologically, the aneurysms of the pulmonary trunk (cases 1, 7, and 9) showed a thinner wall (Fig. lc) compared to a normal vessel, and WVG staining revealed thick, shattered, and randomly arranged elastic fibers (Fig. Id).

The walls of a limited number of cirsoid aneurysms of coronary arteries revealed the presence of intimal digi-tations. Necrosis and pyknosis were detected in smooth muscle cells and nuclei of the tunica media in some arteries in the absence of an inflammatory process, probably related to terminal metabolic changes. We did not detect arteriovenous anastomosis (Fig. 3b).

In 5 animals, histological examination of the mitral leaflets (cases 2, 4-6) and of the tricuspid leaflets (case 1) showed thickening of the spongiosa characterized by proliferation of fibroblastic tissue, deposition of eosinophilic interstitial matrix, and degeneration of the fibrosa, with degeneration in the central fibrous core (Fig. 3c) consistent with endocardiosis. Four dolphins (cases 1, 2, 4, and 6) had small pointed projections from the edges of the valve cusps, comprised of connective tissue proliferations covered by endothelium, with variable aspect and shape that were identified as Lambl's excrescences (Fig. 3d).

DISCUSSION

Dolphin cardiac diseases show the same complexity as terrestrial mammals and humans, with interesting similarities and differences. In fact, fibrosis of the atrioventricular valves found in many of our stranded dolphins (cases 1, 2, 4-6) is comparable to that found in terrestrial mammals, and caused by previous injuries; the same applies for the hypoplasia of the chordae tendineae.

Lambl's excrescences represent a noteworthy finding. They were first described by Lambl (1856) and are represented by filiform fronds that form on the aortic surface atrioventricular valves, and anywhere in semilunar valves (Aziz & Baciewicz 2007). In human pathology, Lambl's excrescences are fairly rare (Aziz & Baciewicz 2007), while in veterinary medicine they have only been reported in horses (Else & Holmes 1972, Guarda et al. 1997), and the exact pathogenetic mechanisms are not yet fully understood (Liu et al. 2012). Pomerance (1961) considered that Lambl's excrescences have their origin in endothelial damage, followed by minor fibrin deposition and organization, which may result in papillary proliferations. In contrast, Sinapius (1955) assumed that these excrescences develop from small, mostlyfibrinous thrombi. Lambl's excrescences have the potential to embolize to distant organs and, although it has not yet been fully clarified (Melduni et al. 2008), a correlation between the presence of Lambl's excrescences and neurological signs (such as confusion and disorientation) has been hypothesized (Aziz & Baciewicz 2007). Although most patients with this pathology are asymptomatic, Lambl's excrescences can break apart and embolize to the brain, causing strokes and cerebrovascular lesions. We found Lambl's excrescences in 4 stranded dolphins, but embolization within the examined tissues was not observed. These findings should be taken into account as a possible cause of dolphin strandings.

The finding of aneurysms of the pulmonary trunk in 3 dolphins is significantly relevant. Martineau et al. (1986) previously described a case of rupture of a dissecting false aneurysm of the pulmonary trunk in a beluga wale *Delphinapterus leucas* associated with verminous pneumonia. Histologically, islets of red cells dissect the media, with fibrin, edema, and collagen fiber deposition, resulting in disorganized and fragmented elastic fibers in the media. In our study, the aneurysms of the pulmonary trunk are analogous to human aneurysms of the sinus of Valsalva, a rare cardiac anomaly in human pathology (Mohite et al. 2012), which can be acquired or congenital (Huh et al. 2012). The congenital variety is due to failure of fusion between the aortic media and the heart at the annulus fibrosus of the aortic valve. In fact, in humans, congenital absence of the tunica media of the aortic wall behind the sinus of Valsalva can cause an aneurysmal dilation (Edwards & Burchell 1957).

Another interesting finding presented in this study is the presence of cirsoid aneurysms in a dolphin. Cirsoid aneurysms of the coronary arteries are congenital malformations that have occasionally been observed in bovines and more rarely in horses and swine (Drommer 1991, Marcato

2002). The coronary arteries were dilated, intertwined in the outer zones of the myocardium, and concamerated.

Although not speciated, the parasitic nodules throughout the epicardium in one of our cases would likely not have contributed to dysrhythmias, cardiovascular compromise, or the death of this dolphin. Further investigations are needed to better evaluate their role in dolphin pathology.

To our best knowledge, Lambl's excrescences, aneurysms of the pulmonary trunk, and cirsoid aneurysms have not been previously described in marine mammals, and some of these findings should be taken into account as possible causes of dolphin strandings. Furthermore, the hearts of marine mam mals need to be studied more carefully to evaluate similarities and differences compared to lesions of terrestrial mammals and humans. Further studies should include the analysis of a greater number of cetacean hearts in order to obtain statistically significant numbers to correlate with the age of the subjects and other concomitant diseases, and to correlate heart lesions to other systemic changes (i.e. lung, liver, and central nervous system).

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No.	Species	Sex	Age	Length	Weight	Necropsy findings	Gross heart lesions	Microscopic heart lesions
1	*Stenella coeruleoalba*	M	Adult	194 cm	73 kg	Marble spleen; adrenal hypoplasia; multifocal, irregularly distributed, 2-mm parasitic nodules scattered throughout peritoneum, liver and omentum	Right ventricular dilation; aneurysm of right sinus of Valsalva; hypoplasia of tricuspid chordae; tricuspidal fibrosis; interventricular white spot	Aneurysm of right sinus of Valsalva; tricuspid endocardiosis; Lambl's excrescences; lymphocytic myocarditis
2	*Stenella coeruleoalba*	F	Adult	188 cm	68 kg	Abdominal muscle and pericardial parasitic nodules; hemorrhages of the left eye; splenomegaly	Mitral fibrosis	Mitral endocardiosis; Lambl's excrescences
3	*Stenella coeruleoalba*	M	Adult	203 cm	73 kg	Subcutaneous hematoma of left side of abdomen; hemorrhages in left kidney; hemoperitoneum; multifocal, irregularly distributed, 2-mm parasitic nodules scattered throughout peritoneum	Marked autolysis and putrefaction	Marked autolysis and putrefaction
4	*Stenella coeruleoalba*	M	Adult	194 cm	65 kg	Emphysema of the left lung	Cirsoid aneurysms; mitral leaflet thickening	Cirsoid aneurysm; mitral endocardiosis; Lambl's excrescences
5	*Stenella coeruleoalba*	F	Adult	203 cm	76 kg	Absence of gross lesions	Absence of gross lesions	Mitral endocardiosis
6	*Stenella coeruleoalba*	M	Subadult	136 cm	26 kg	Emaciation; suppurative bronchopneumonia; suppurative mediastinal lymphadenitis; intestinal and omentum parasitic nodules	Left ventricular hypertrophy	Mitral endocardiosis; Lambl's excrescences
7	*Stenella coeruleoalba*	M	Adult	208 cm	63 kg	Emaciation; intestinal adhesions	Aneurysm of the right sinus of Valsalva	Aneurysm of right sinus of Valsalva
8	*Stenella coeruleoalba*	M	Adult	205 cm	73 kg	Severe parasitic pneumonia	Absence of gross lesions	Absence of microscopic lesions
9	*Stenella coeruleoalba*	M	Adult	192 cm	70 kg	Fibrinous peritonitis	Aneurysm of right sinus of Valsalva; mitral fibrosis	Aneurysm of right sinus of Valsalva
10	*Tursiops truncatus*	M	Subadult	181 cm	NC	Severe parasitic pneumonia	Absence of gross lesions	Absence of microscopic lesions

Table 1. Necropsy findings, gross and microscopic heart lesions. NC = not collected

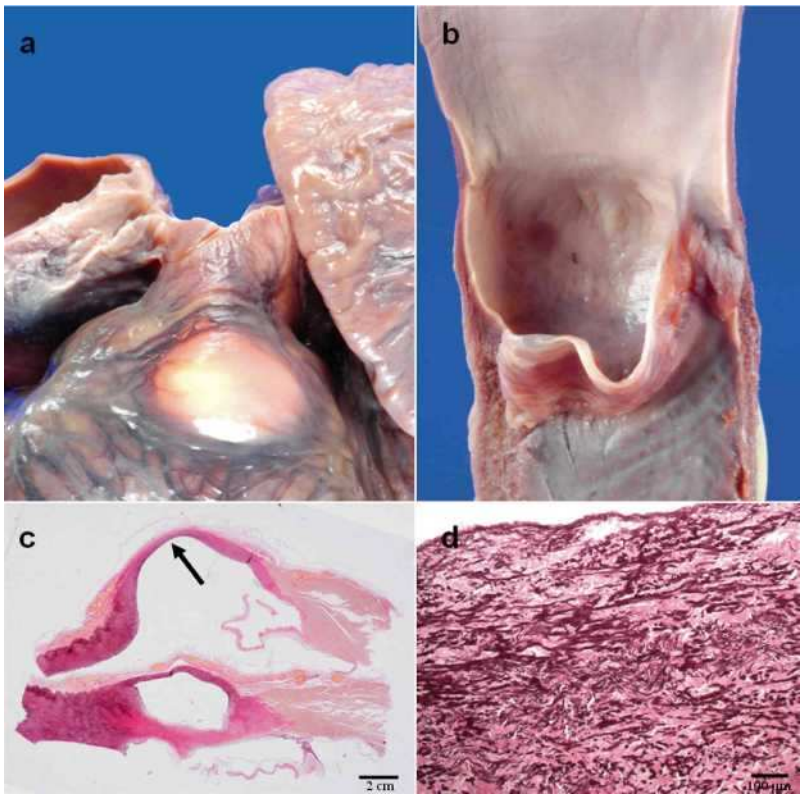

Fig. 1. *Stenella coeruleoalba*. Case no. 1, pulmonary artery: (a) white, elevated, well demarcated aneurysm of the right sinus of Valsalva; (b) endothelial surface of the aneurysm of the right sinus of Valsalva; (c) thinning of the pulmonary arterial wall (arrow) (Weigert-Van Gieson stain, WVG); (d) thick, shattered, and randomly arranged elastic fibers of the pulmonary arterial wall (WVG)

Fig. 2. *Stenella coeruleoalba.* Outline of a normal heart of a striped dolphin

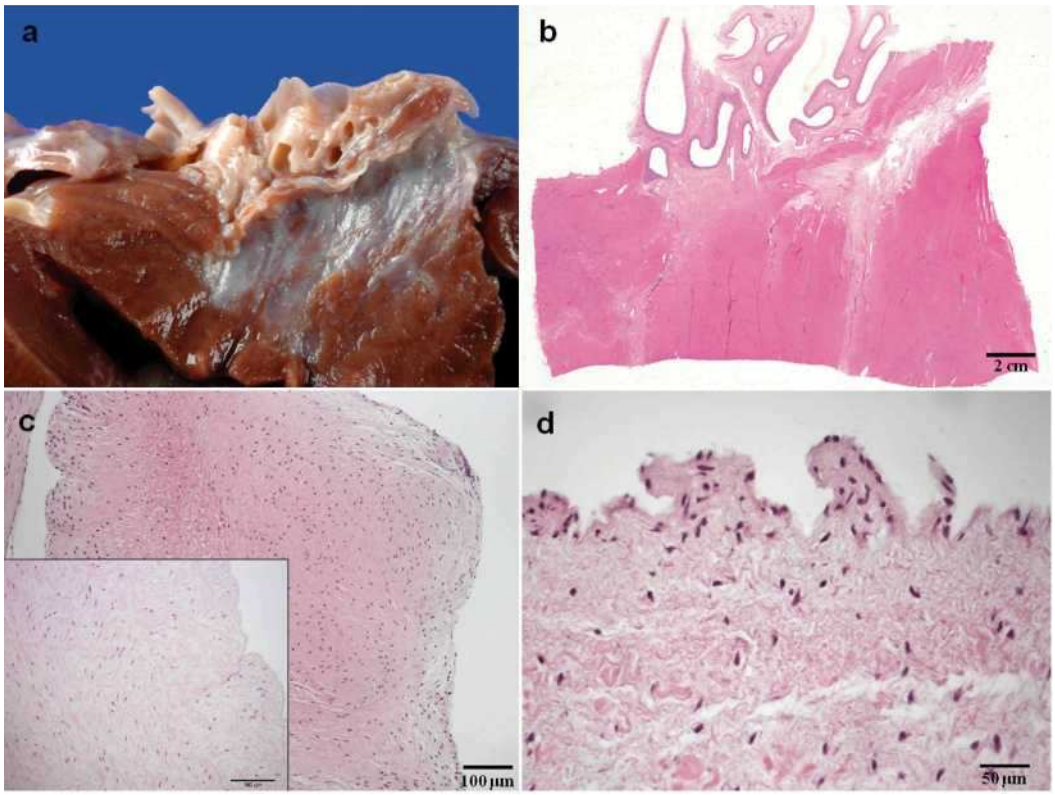

Fig. 3. *Stenella coeruleo-alba*. (a) Case no. 4: cut surface of the left subepicardial coronaric artery: cirsoid aneurysm; (b) case 4: histological features of cirsoid aneurysm: dilation, twisting, lengthening of the coronary artery surrounded by connective tissue; (c) case 5: thickening of the mitral spongiosa caused by myxoid degeneration in the central fibrous core (hematoxylin and eosin stain, HE); inset: higher magnification of the myxoid degeneration (HE); (d) case 6: Lambl's excrescences (HE)

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