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NOTE

Omphalitis, urachocystitis and septicemia by *Streptococcus dysgalactiae* in a southern right whale calf *Eubalaena australis*, Brazil

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ABSTRACT: Southern right whales *Eubalaena australis* (SRW) use the southern coast of Brazil as a wintering and calving ground. Other than anthropogenic threats, there is limited knowledge on health and disease aspects for this species. We report the gross and microscopic findings and microbiological identification of streptococcal septicemia in a SRW calf. Main gross findings included fibrinosuppurative omphalitis and urachocystitis, suppurative cystitis, valvular endocarditis and myocarditis, embolic pneumonia, suppurative myositis and osteoarthritis, and lymphadenomegaly. Histological examination confirmed the above inflammatory processes and indicated disseminated Gram-positive coccoid septicemia. PCR analysis, based on the 16S rRNA gene from bacteria isolated on blood agar, identified *Streptococcus dysgalactiae*. Pathologic and microbiologic analysis indicated that β-hemolytic *S. dysgalactiae* septicemia, presumably initiated as ascending omphalic infection, was responsible for stranding and death in this individual. These results further confirm pathogenicity of streptococci in cetaceans and add to the limited health and disease related pathology knowledge for this species.

KEY WORDS: Cetacean · Bacteria · Streptococcosis · Sepsis · β-hemolytic · Marine mammal

INTRODUCTION

Southern right whales (SRW) *Eubalaena australis* typically reach the southern coastline of Brazil to mate, give birth and nurse their calves from June through November (Groch & Flores 2011). These populations are slowly increasing, suggesting healthy population dynamics despite many anthropogenic threats (Peters & Barendse 2016). Nevertheless, mortality rates for this species vary widely among years, and recent studies have reported high numbers of deaths in calves of the year compared to other age classes (Sironi et al. 2014, McAloose et al. 2016). Moreover, health assessments and pathology-based studies have failed to find common etiologies for the increased mortalities (Rowntree et al. 2013, McAloose et al. 2016). Several studies have addressed potential causes of stranding and death in *E. australis* in differ-

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ent geographic locations, such as South Africa (Best et al. 2001), Australia (Kemper et al. 2008), Argentina (Rowntree et al. 2013, Wilson et al. 2016) and Brazil (Greig et al. 2001); however, confirmed natural diseases in this species, specifically of infectious nature, are very limited (McAloose et al. 2016).

Streptococcus species are some of the most commonly reported pathogens of pinnipeds and cetaceans, and may act as commensal, primary or opportunistic pathogens. The infection often occurs in association with cutaneous lesions, with subsequent septicemia (Tryland et al. 2018). High mortality rates have been reported in fishes infected by *Streptococcus dysgalactiae* (Abdelsalam et al. 2013), and this infection has been linked to systemic disease in harbor porpoises *Phocoena phocoena* (Swenshon et al. 1998). Herein, we described the pathologic, microbiological and molecular findings of omphalitis, urachocystitis and septicemia by *S. dysgalactiae* in an *E. australis* calf in Brazil.

MATERIALS AND METHODS

A 6 m long female *Eubalaena australis* calf, estimated as 1–2 mo old (Rowntree et al. 2013), in good nutritional status and mild autolysis was found stranded dead in Xangri-lá, Rio Grande do Sul state, Brazil (29° 80′ 84″ S, 50° 03′ 75″ W). An immediate external examination was performed using biometrics and photography, followed by a standard complete necropsy. Representative samples of main organs were collected and fixed in 10% formalin. All tissues were routinely processed for histology and stained with hematoxylin and eosin. Selected tissue sections (heart, umbilicus and urachus) were also Gram-stained to classify the bacteria.

Swab and tissue samples of urachus, urinary bladder, heart (vegetative material) and synovial fluid were inoculated on MacConkey agar (Kasvi®) and on 5% sheep blood agar plates (Kasvi®), followed by aerobic incubation at 37°C for 24 h.

Since the isolated bacterium from all inoculated samples had the same morphological and biochemical characteristics, the bacterium was further identified by molecular analysis of the 16S rRNA gene from the heart sample. Three colonies were randomly chosen from blood agar and incubated on brain heart infusion (Kasvi®) broth at 37°C for 24 h. Total DNA was extracted employing QIAamp DNA Mini Kit (Qiagen) and lysozyme (40 mg). The reaction to amplify and sequence a partial 16S rRNA region was performed as described by Hammerschmitt et al. (2017). DNA fragments were aligned to other sequences by MUSCLE, and the phylogenetic tree was constructed using the genetic distance model of Tamura-Nei by UPGMA method with 1000 replicates, including *Escherichia coli* as an outgroup.

RESULTS

Grossly, the calf had an incompletely healed umbilicus expanded by abundant purulent material that extended into the urachus and formed a tubular cast of fibrin in the lumen (Fig. 1A). The urinary bladder mucosa was hyperemic with multifocal hemorrhage, and a moderate amount of purulent exudate was present in the lumen (Fig. 1B). In addition, systemic septicemic lesions included vegetative mitral, tricuspid and aortic valvular endocarditis (Fig. 1C) and myocarditis, embolic pneumonia, suppurative generalized myositis (Fig. 1D), suppurative spondylitis and costal diaphyseal and epiphyseal osteomyelitis (Fig. 1E), fibrinosuppurative polyarthritis, and generalized lymphadenomegaly. Bilaterally, the adrenal glands contained diffuse hemorrhage (Fig. 1F), suggesting septic shock.

Microscopically, the umbilical and urachal epithelium was diffusely and severely ulcerated and covered by fibrin and myriad basophilic coccoid bacteria associated with a marked infiltrate of degenerate neutrophils, lymphocytes and macrophages (Fig. 2A). The connective tissue of the umbilicus and urachus was thickened by moderate fibroplasia. The endothelial lining of the valves was severely ulcerated and covered by a thick layer of fibrin with intralesional myriad basophilic coccoid bacteria (Fig. 2B). The endothelium of the endocardium and myocardium were infiltrated by numerous neutrophils accompanied by multifocal thrombosis, fibrinoid necrosis of blood vessels and hemorrhage (Fig. 2C). In the lung, there were multifocal abscesses centered on thrombosed vessels with countless coccoid bacteria (Fig. 2D). There was also embolic necrotizing myositis with vasculitis and innumerable coccoid bacteria in multiple segments of epaxial and hypaxial muscles. The brain displayed multifocal microabscesses often involving numerous similar bacteria in both the grey and white matter (Fig. 2E). Additional findings included widespread septic thromboemboli with intravascular bacteria and varying degrees of associated parenchymal suppurative inflammation (lymph nodes, spleen, stomach, liver, kidneys, spinal cord, bone marrow, small and large intestine). Both

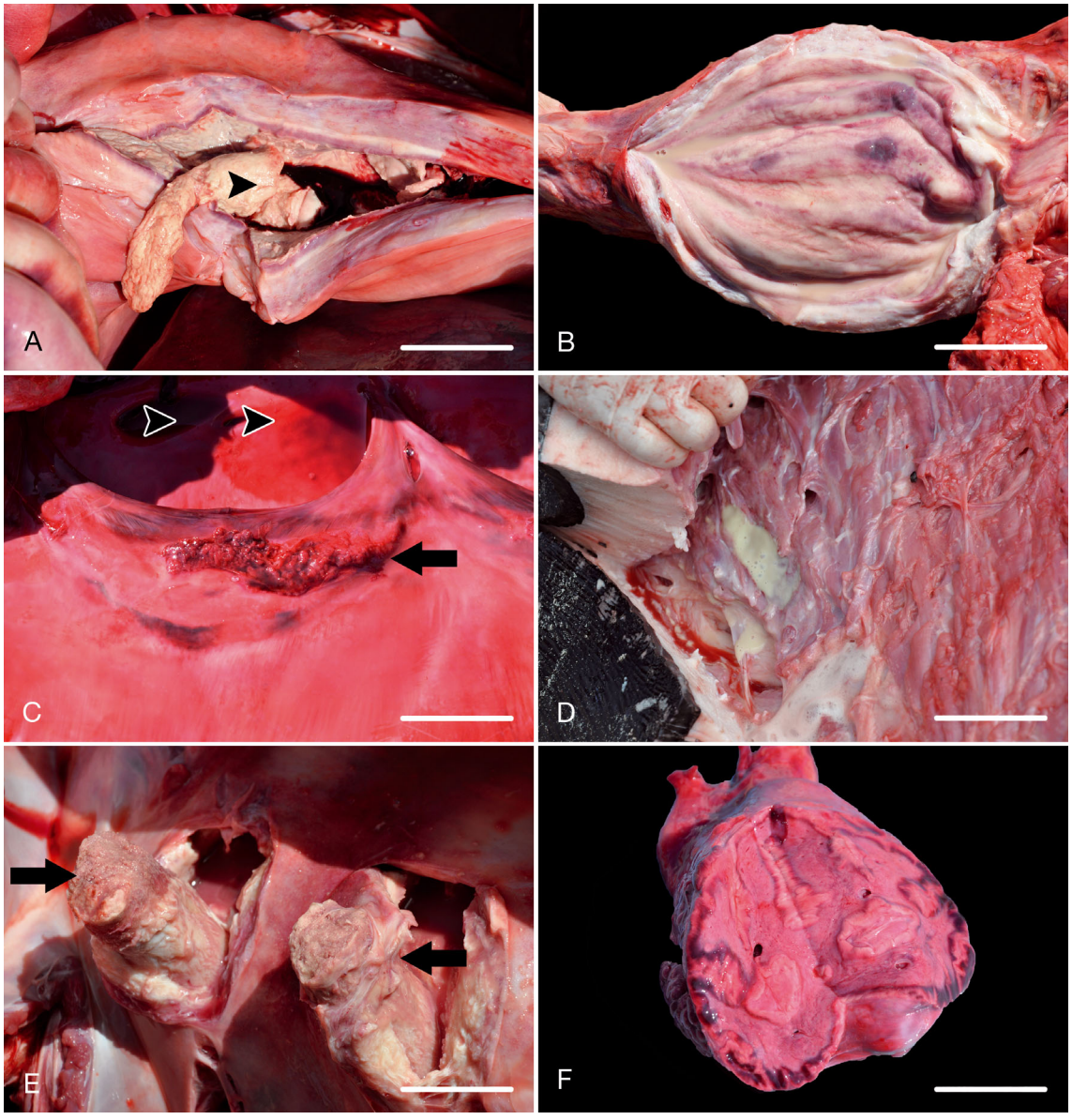

Fig. 1. Macroscopic lesions found in a southern right whale calf *Eubalaena australis*. (A) Incompletely healed proximal umbilicus with large fibrin cast in the lumen (arrowhead). Scale bar = 3 cm. (B) Urinary bladder mucosa is hyperemic, with multifocal hemorrhage, and contains a moderate amount of purulent exudate. Scale bar = 4 cm. (C) Ventral aspect of aortic valve, showing fibrinopurulent reddish vegetative lesion (vegetative valvular endocarditis, arrow). Note multifocal adjacent hemorrhages in the endocardium/intima and 2 right coronary ostia (arrowheads). Scale bar = 3 cm. (D) Periscapular musculature with multifocal sac-like and track-like areas filled with purulent exudate, consistent with a purulent myositis. Scale bar = 4 cm. (E) Severe costovertebral osteoarthritis and costal epiphyseal suppurative osteomyelitis characterized by yellow and friable material covering the articular surfaces and extending into the bone marrow (arrows). Scale bar = 6 cm. (F) Hemorrhage along the cortico-medullary region of the adrenal gland. Scale bar = 6 cm

adrenal glands had severe and diffuse hemorrhagic necrosis and intravascular bacteria. Numerous Gram-positive coccoid bacteria were highlighted with Gram staining.

Small, grey β-hemolytic and catalase-negative bacterial colonies were observed in all inoculated samples on blood agar plates (Fig. 2F); no growth was observed on MacConkey medium. Gram staining revealed long chains of Gram-positive cocci. Further bacteriological identification was confirmed by molecular characterization where the isolate was subjected to 16S rRNA gene analysis. DNA sequences

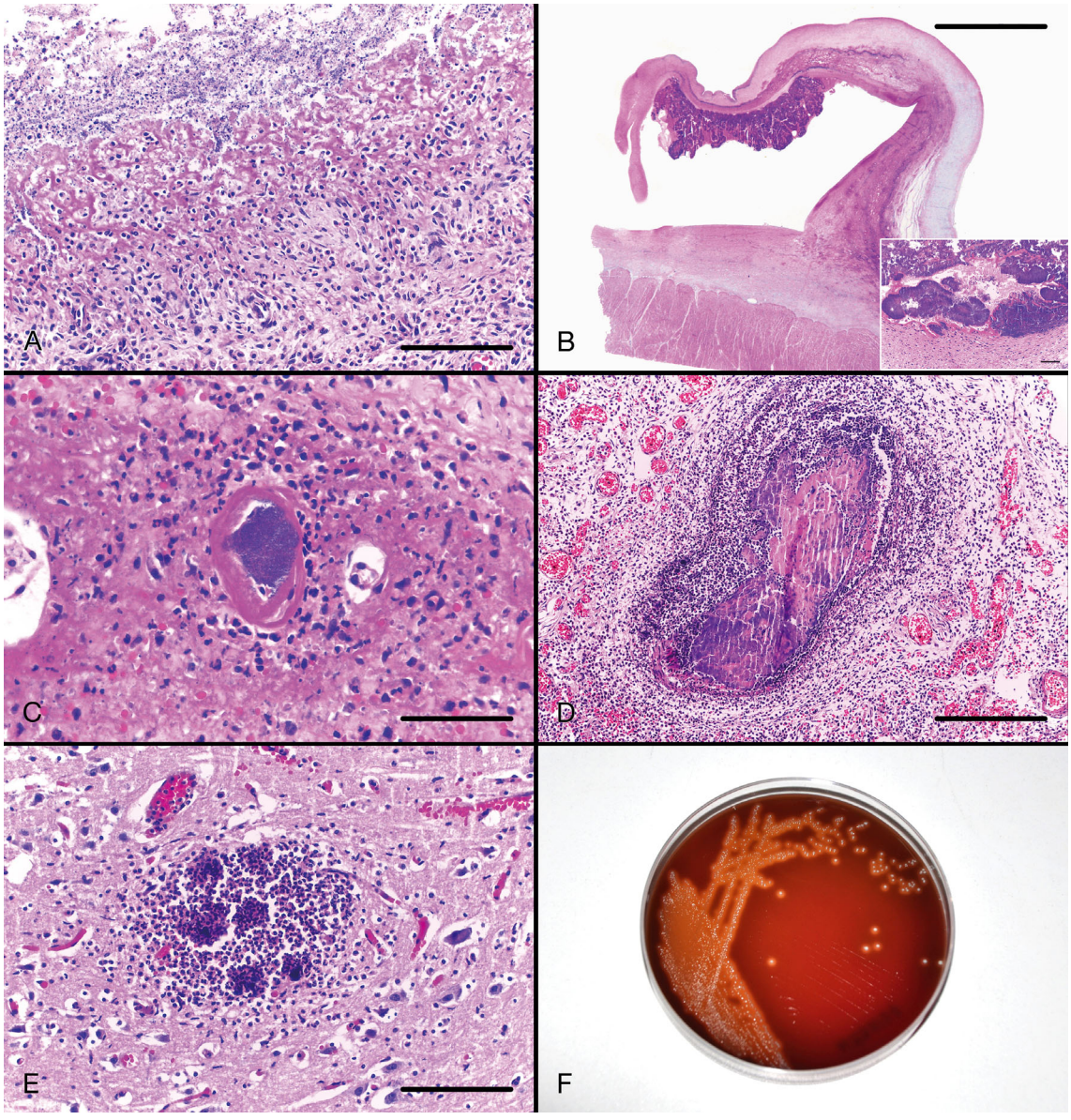

Fig. 2. (A–E) Microscopic lesions and (F) bacteriological findings in a southern right whale calf *Eubalaena australis*. (A) The umbilical epithelium is ulcerated and covered by abundant fibrin and myriad basophilic coccoid bacteria associated with numerous degenerate neutrophils, lymphocytes and macrophages. H&E, 20×; scale bar = 100 µm. (B) Subgross view of the mitral valve. The luminal/ventral endothelial lining is covered by a thick layer of fibrin with numerous basophilic coccoid bacteria. Scale bar = 1 cm. Inset: detail of basophilic coccoid bacteria in vegetative mitral valve. H&E, 20×; scale bar = 100 µm. (C) Mural fibrinoid vascular necrosis of small arterioles with septic embolus of basophilic coccoid bacteria in the myocardium underlying the endocarditic focus. H&E, 40×; scale bar = 50 µm. (D) Focal pulmonary abscess centered on septic thromboembolus with numerous basophilic coccoid bacteria. H&E, 10×; scale bar = 200 µm. (E) Focal microabscess in cerebral cortex. H&E, 20×; scale bar = 100 µm. (F) Small, grey and β-hemolytic bacterial colonies on blood agar plate

from overlapping strands generated a consensus sequence (1410 nucleotides) that was analyzed by a BLAST search following a multi-sequence alignment to 16S rRNA sequences available at GenBank/NCBI database. The newly isolated sequence (MG973080) showed 99% identity with the available *Streptococcus dysgalactiae* sequences, and by phylogenetic analysis clustered exclusively with this species, confirming its identity as *S. dysgalactiae*.

The combination of severe pathologic findings and molecular results supports that *S. dysgalactiae* was the likely cause of the death in this *E. australis* calf.

DISCUSSION

Herein, we report the pathologic findings and molecular identification of fatal septicemia by *Streptococcus dysgalactiae* in an *Eubalaena australis* calf. The combination of suppurative omphalitis, urachocystitis and multiorgan septic inflammation suggested an ascending process through the umbilicus. A similar condition was recently listed as the second most common infectious disease process leading to stranding and/or death in Brazilian humpback whale *Megaptera novaeangliae* calves (Groch et al. 2018). Stranding and death of calves of the year are common in Península Valdés (PV), Argentina, and in South Africa (Best et al. 2001), with calves estimated between 1–2 mo of age as the most affected in PV (McAloose et al. 2016), coinciding with this case. The high mortality rates in this age class may be related to social reasons (separation from the mother), as well to neonatal or perinatal diseases (Groch et al. 2018). If suckling is prevented early perinatally, there is potential for failure of passive transfer (FPT) and agammaglobulinemia. FPT has not been well studied in cetaceans, particularly in free-living individuals, and may be a relevant hypothesis to explain high mortality rates in *E. australis* calves of the year in various geographic locations.

Postmortem investigations of whales are typically limited by a series of inherent factors, such as climatic, geographic and postmortem autolysis. Thus, determining causes of stranding and/or death is challenging, and primary pathologies and comorbidities may go undiagnosed (Groch et al. 2018). The most common causes of mortality include harmful algal toxins, human interaction, gull predation and infectious diseases (Rowntree et al. 2013, Sironi et al. 2014). Anthropogenic incidents are less common in *E. australis* (Kemper et al. 2008); although there is considerable marine traffic in the area where the calf was found (Greig et al. 2001), no evidence of ship strike was noted. Furthermore, we did not find any skin lesions associated with kelp gull parasitism, which has been speculated as a cause for high mortality rates among calves (Sironi et al. 2014), and poses a major entry point for systemic bacterial infections in *E. australis* (Marón et al. 2015, McAloose et al. 2016).

The wound resulting from umbilical rupture after birth may have provided a route for invasion and systemic *S. dysgalactiae* spread in this calf, as reported in other marine mammal streptococcal infections (Bartlett et al. 2016, Díaz-Delgado et al. 2017, Taurisano et al. 2018). The gross and microscopic lesions in the present case resembled previous cases of streptococcosis where septicemia ensued and led to demise (Bartlett et al. 2016, Díaz-Delgado et al. 2017, Taurisano et al. 2018, Tryland et al. 2018). Microscopically, the umbilical cord and urachus had subacute primary lesions, characterized by necrotizing and proliferative omphalitis and urachocystitis, as in *M. novaeangliae* calves (Groch et al. 2018). Besides that, the calf had vegetative endocarditis, similar to a septicemia by *Streptococcus phocae* in a short-beaked common dolphin *Delphinus delphis* (Díaz-Delgado et al. 2017), and embolic pneumonia. This pneumonic pattern clearly differed from the bronchopneumonia observed in 2 *E. australis* calves (McAloose et al. 2016), in that the current case was not airway-oriented.

In conclusion, this *E. australis* calf had pyogenic lesions involving the umbilicus and urachus, and it was septicemic. Microbiological and molecular analysis identified *S. dysgalactiae* as the cause of these lesions, and the most plausible pathogenesis involved ascending umbilical infection and systemic spread. The severity and extent of the lesions could explain stranding and death in this calf. These results further attest to the pathogenic potential of streptococci in marine mammals. It also contributes to the body of knowledge on cetacean pathology, and widens the spectrum of natural causes of disease in *E. australis*, a species for which limited health knowledge exists.

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