Multifocal Granulomatous Panniculitis with Ceroid Pigment in Two Mediterranean Striped Dolphins (*Stenella coeruleoalba*)

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ABSTRACT: Two striped dolphins (*Stenella coeruleoalba*) were found stranded on the Catalonian Spanish coast. The main pathologic finding in both animals was the existence of multiple granulomatous lesions in the blubber, microscopically composed of macrophages and multinucleated cells containing vacuolar material. This material was identified as ceroid pigment due to its ultrastructural morphology, autofluorescence, and positive staining with periodic acid-Schiff and Ziehl-Neelsen techniques. The special stains and electron microscopy did not reveal any microorganisms associated with the lesions. These findings are very suggestive of "nutritional panniculitis," a well-defined entity associated with vitamin E deficiency that has been rarely described in free-living species.

Key words: Blubber, ceroid pigment, granulomatous panniculitis, nutritional panniculitis, *Stenella coeruleoalba*, striped dolphin, vitamin E deficiency.

Several processes have been described affecting the panniculus of free-ranging cetaceans (Cowan, 1993; Schulman and Lipscomb, 1999; Bowenkamp et al., 2001; Bonar and Wagner, 2003). Here we describe macroscopic, microscopic, and ultrastructural findings in two free-ranging Mediterranean striped dolphins (*Stenella coeruleoalba*) affected by a granulomatous panniculitis with intralesional ceroid pigment resembling nutritional panniculitis.

A 62 kg, 1.89 m adult female striped dolphin found dead on the coast of Roses-Girona (Catalonia, Spain, 42°15′N, 3°09′E) in April 2008, and a 98.5 kg, 2.24 m adult male striped dolphin that died shortly after being found stranded on the coast of Platja d'Aro-Girona (Catalonia, Spain, 41°48′N, 3°03′E) in February 2009, were transported to the Veterinary School in Barcelona, Spain. Complete necropsies were performed following the protocol of the Catalonian Mediterranean Morbillivirus Surveillance Program.

Samples of pharynx, lung, heart, liver, intestine, stomach, spleen, lymph node, pancreas, adrenal, kidney, urinary bladder, tongue, brain, skeletal muscle, and skin were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin and eosin (H&E). Skin sections were also examined under polarized light and stained with periodic acid-Schiff (PAS), Ziehl-Neelsen (ZN), Fite, Gram's, and Grocott's methenamine silver (GMS) techniques. Formalin-fixed blubber samples were processed for electron microscopy. Several brain sections and the prescapular, thoracic, and mesenteric lymph nodes were tested for morbillivirus by immunohistochemistry (IHC) using the avidin-biotin-peroxidase method (Thermo Fisher Scientific, Rockford, Illinois, USA). A mouse monoclonal antibody against canine distemper virus nucleoprotein (VMRD Laboratories, Pullman, Washington, USA), diluted to 1:200, and a goat antimouse IgG (Dako, Glostrup, Denmark) as secondary antibody, were used.

The most relevant macroscopic lesions were several irregular, well-defined, non-raised yellow-orange lesions, approximately 0.5–2.0 cm in diameter in the middle-to-deep blubber (Fig. 1). The lesions were distributed ventrally and dorsally in the
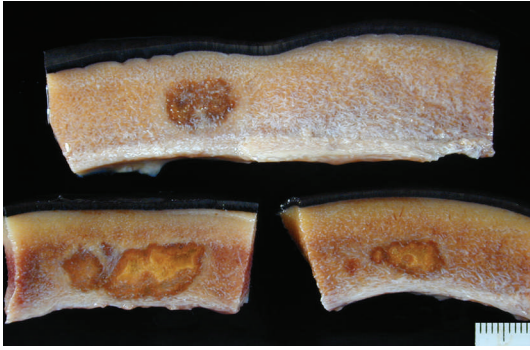
FIGURE 1. Three skin fragments containing well-defined, non-raised, irregular focal lesions in the deep blubber characterized by an intense yellow-orange color. Bar=1 cm.
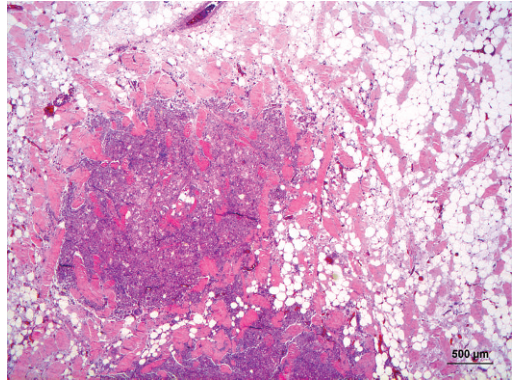

FIGURE 2. Skin blubber showing well-defined, coalescing granulomatous lesions. H&E stain. Bar=500 μm.

female and mainly in the abdominal area in the male. In both cases, up to 10% of the blubber layer was affected. Both had a normal external skin surface except for numerous conspecific bite marks and two tattoo lesions in the male. Both had a medium-to-high number of merocercoids of *Phyllobothrium delfini* in the blubber of the genital area. A few copepods (*Penella* spp.) in the fluke of the female were also found. The keratinized stomach was empty in both animals, with low quantities of sand in the male dolphin. The second stomach of the male contained one prominent parasitic nodule.

Microscopically, the blubber lesions corresponded to multifocal to coalescing, well-defined granulomas (Fig. 2) composed of numerous macrophages and multinucleated giant cells. The cytoplasm of these cells contained abundant round-to-oval, nonrefringent, colorless or pale yellow vacuolar material, varying from approximately 2–30 μm in diameter (Fig. 3). Scant foci with adipocyte necrosis and fat saponification were also observed, surrounded by macrophages and scattered karyorrhectic neutrophiles (Fig. 3, inset). An increased number of collagen bundles (fibrosis) was associated with the inflammatory infiltrate. No foreign material was visible under polarized light. The vacuolar material in the blubber lesions was intensely autofluorescent under blue light

and stained with PAS and ZN (Fig. 4). The PAS, ZN, Fite, Gram's, and GMS stains did not reveal microorganisms associated with the granulomatous skin lesions. Ultrastructurally, in the blubber lesions, numerous osmiophilic rounded or granular structures of variable size were observed in the cytoplasm of macrophages (Fig. 5). Additional microscopic findings in both dolphins included mild, multifocal lymphoplasmacytic nephritis with tubular mineralization and moderate neuronal lipopigment deposit. The male also had chronic nodular parasitic gastritis with intralesional trematode eggs. Results of IHC for morbillivirus were negative in both dolphins.

Several processes associated with granulomatous panniculitis have been described in cetaceans, including nocardiosis (Leger et al., 2009), mycobacteriosis (Bowenkamp et al., 2001), lobomycosis (Cowan, 1993), and fusariosis (Frasca et al., 1996). The absence of acid-fast bacteria (ZN and Fite stains) associated with the granulomas ruled out *Mycobacterium* and *Nocardia* as possible etiologies of the lesions. Mycotic infections are also an unlikely cause, as these organisms are usually easily detected in affected tissues using PAS and GMS stains. If these lesions were the normal result of a response to fat breakdown in marine
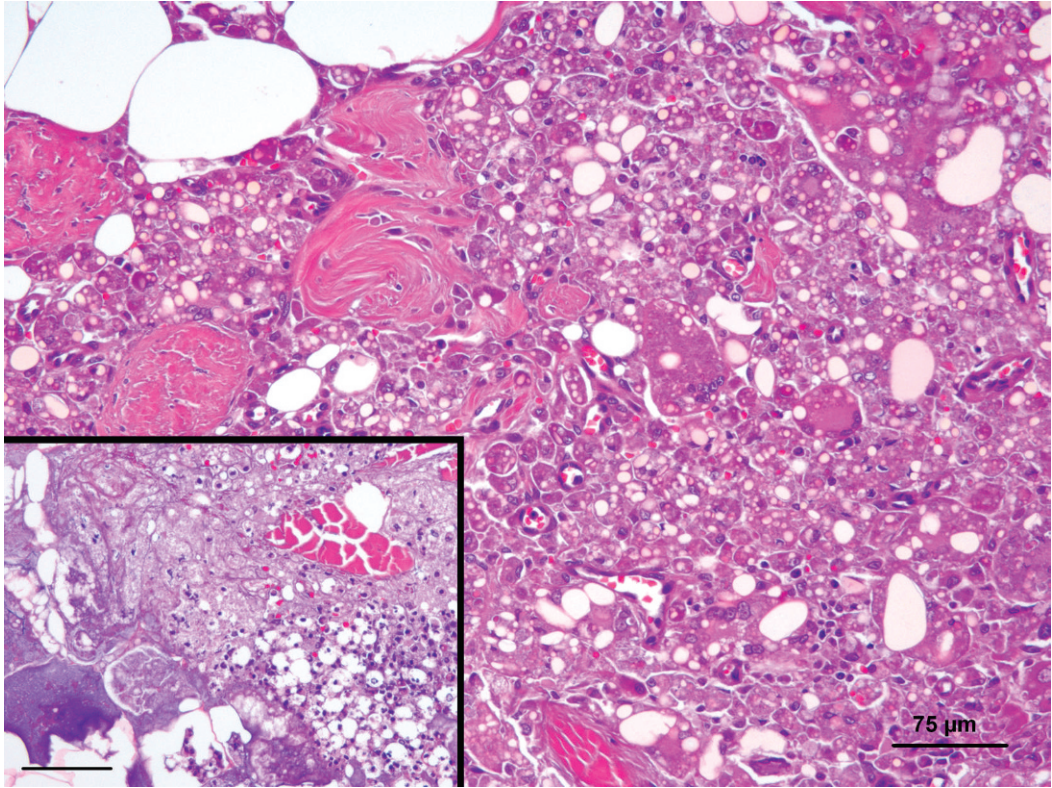
FIGURE 3. Locally extensive granulomatous panniculitis composed of abundant macrophages and multinucleated giant cells, showing abundant, cytoplasmatic, colorless or pale yellow vacuolar material. H&E stain. Bar=75 μm. Inset: Focal blubber fat necrosis with degenerated neutrophils and macrophages. Bar=75 μm.

mammals, they would have been described more often in routine necropsies. Conditions associated with granulomatous panniculitis in dogs and cats have included foreign-body reactions, trauma, pancreatic panniculitis, nutritional panniculitis, and idiopathic-nodular panniculitis (Gross et al., 2005). However, the absence of macroscopic and microscopic pancreatic lesions in these dolphins ruled out a pancreatic origin. The high number of lesions affecting the blubber, their scattered distribution, and the absence of associated foreign material made a foreign-body reaction unlikely. No lesions were evident in the epidermis associated with the granulomas, and fat necrosis was not a significant finding, making trauma an unlikely cause. Although idiopathic inflammation can still be considered, the

granulomas in both dolphins were characterized by the presence of abundant intralesional material with morphologic, physical, and staining properties consistent with ceroid pigment, including ultrastructural electrodense vacuolar structures with marked autofluorescence under blue light and positive staining with PAS and ZN techniques (Seehafer and Pearce, 2006; Ginn et al., 2007). This finding strongly suggested nutritional panniculitis, as observation of ceroid pigment is the characteristic histopathologic finding of this condition and, to our knowledge, has not been described in association with other causes of panniculitis (Gross et al., 2005).

Nutritional panniculitis in several animal species (Ginn et al., 2007) is associated with deficiency of vitamin E due to
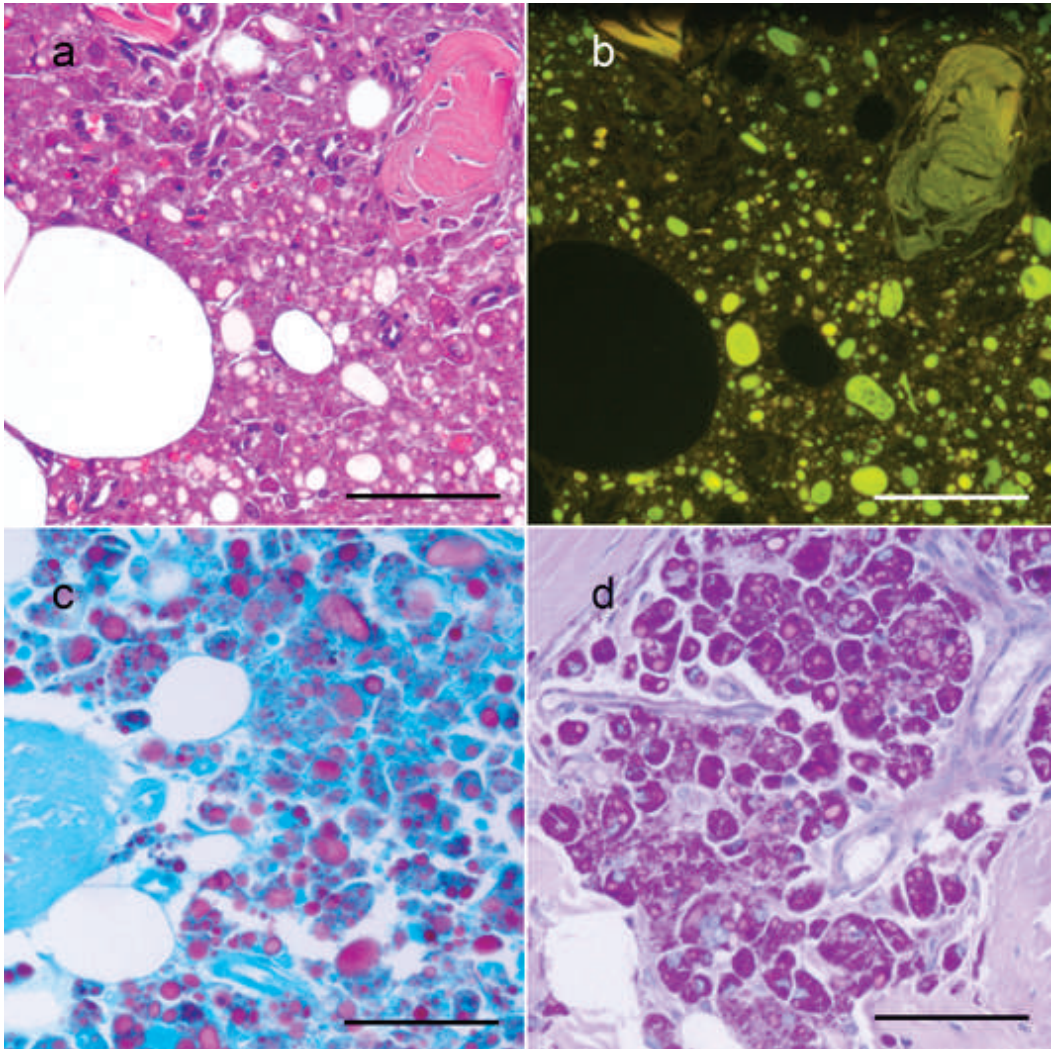
FIGURE 4. Photomicrographs showing characteristic staining and physical properties of ceroid pigment in the granulomatous lesion of the dolphin blubber: (a) Colorless or pale yellow vacuolar material with H&E stain. (b) Intense yellow-green autofluorescence under blue light. (c) Acid-fast red staining with Ziehl-Neelsen method. (d) Intense magenta staining with periodic acid-Schiff method. Bar in the four figures=75 μm.

inadequate intake of the vitamin, consumption of high levels of unsaturated fatty acids that deplete vitamin E, or both (Niza et al., 2003). The negative antioxidant status leads to peroxidation of susceptible lipids, with formation of free radicals that provoke the characteristic inflammatory reaction in response to formation of irritant soaps and ceroid pigment (Brown et al., 2007). The yellow color of the lesions is a consequence of the deposition of ceroid (Ginn et al., 2007).

Contrary to the systemic presentation of nutritional panniculitis in cats (Ginn et al., 2007), fat deposits in any organs other than the blubber, which in dolphins are confined to small quantities of adipose tissue surrounding organs such as lymph nodes and heart, were not affected in these two cases. One explanation could be that the process targets sites with high biochemical activity. The blubber is the primary site for energy storage, and the middle and deep blubber layers where the
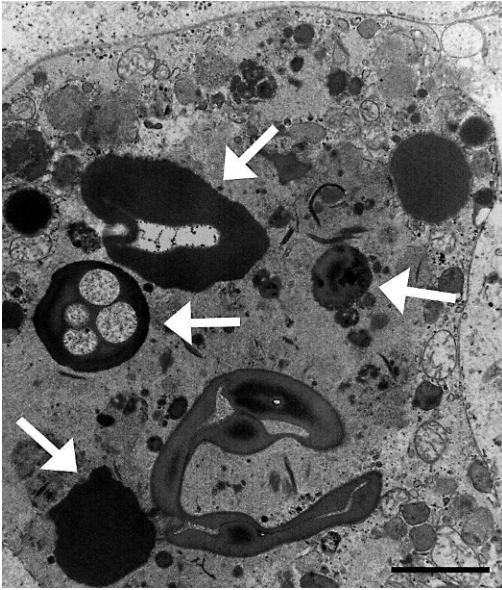
FIGURE 5. Electron micrograph illustrating intracytoplasmic, heterogenous black, osmiophilic rounded and granular structures compatible with lipopigments (arrow) within a macrophage. Bar= 2 μm.

granulomas were localized appear to be the most metabolically dynamic layers (Struntz et al., 2004). Skeletal muscle lesions, which could be expected with vitamin E deficiency, were not observed in either case. Dolphins may respond similarly to other carnivores in which nutritional myopathy due to vitamin E imbalance is unusual (Van Vleet and Valentine, 2007). Another adult striped dolphin, found on the Catalonian coast and affected by fatal sarcocystosis, had blubber granulomas similar to those we describe. The authors associated the lesion to a supposed vitamin E deficiency due to fat malabsorption associated with chronic parasitic pancreatitis (Resendes et al., 2002). In our cases, where no apparent pancreatic lesion was present, the cause of vitamin E deficiency could have been consumption of an abnormal quantity of oily fish with a high concentration of unsaturated fatty acids. Sardines and anchovies are normal prey for Mediterranean dolphins (Hammond et al., 2008).

The same cause has been implicated in nutritional panniculitis in domestic cats and mink farming (Niza et al., 2003). Because no serum or liver samples were available for the measurement of vitamin E in our dolphins, a nutritional origin for the granulomatous panniculitis could not be definitively shown.

Other processes affecting the panniculus have also been described in cetaceans, including bacterial infections (e.g., *Streptococcus* spp. [Bonar and Wagner, 2003], *Erysipelothrix rhusiopathiae* [Thurman et al., 1983], *Pseudomonas aeruginosa* [Diamond et al., 1979], and *Aeromonas hydrophila* [Cusick and Bullock, 1973]), as well as parasitic infections (e.g., ciliated protozoa [Schulman and Lipscomb, 1999], tetraphyllidean cysticerci [Norman, 1997], and nematodes [Dailey and Stroud, 1978]). The histopathologic findings described in association with these processes are not compatible with those observed in the blubber lesions we describe. In addition, no bacteria or parasites were detected during the histologic (routine examination and staining) and ultrastructural procedures, making these infections an unlikely cause of the lesions.

Finally, the differential diagnosis could include a long list of viruses such as coxsackie, influenza, cytomegalovirus, rabies, vaccinia, Ross River, and hantaviruses, all of which have been described as causes of inflammation or necrosis of fat deposits in rodents (Nisimura et al., 2000). Those viruses are an unlikely cause of the process affecting our dolphins due to the absence of a granulomatous response associated with these agents (Nisimura et al., 2000) and to the absence of viral structures in the electron microscopic examination of the blubber lesions.

In summary, generalized granulomatous panniculitis with intralesional ceroid pigment was the only pathologically relevant finding in the two striped dolphins reported on in this paper. These findings suggest nutritional panniculitis due to vitamin E deficiency. However, further work to

confirm the etiology of the lesions is needed.

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