Pacific Marine Mammal Center

Case Reports in Marine Mammal Pathology
Volume 1, Number 2, December 2010

Bacterial Granulomatous Encephalitis in a Short-Beaked Common Dolphin (*Delphinus delphis*)

Annie Page, University of Georgia, College of Veterinary Medicine, Athens, Georgia, USA *

Abstract

An adult, female, short-beaked common dolphin (*Delphinus delphis*) was stranded and in *status epileptics* at Newport Beach, California in March of 2009. ELISA testing of cerebrospinal fluid, urine and serum failed to reveal any evidence of domoic acid. Gross postmortem examination revealed a large, left cerebral, frontal-lobe granuloma, multifocal pulmonary granulomata and bilateral tracheobronchiolar lymphadenopathy. Histologic and cytologic examinations of the cerebral lesion revealed a mixed to granulomatous inflammatory response associated with several morphotypes of gram-negative bacilli. *Citrobacter freundii, Enterococcus sp., Enterobacter cloacae, Pseudomonas putida* and *Pseudomonas flourescens* were isolated from the necrotic caseous material in the center of the granuloma. The route of infection was not determined, but may have occurred via hematogenous spread from pneumonic nematodiasis.

Case Report

On March 22, 2009, Newport Beach Animal Control officers responded to the stranding of an adult female short-beaked common dolphin (*Delphinus delphis*) in Newport Beach, California. The animal was observed to be in *status epilepticus* and was impounded and transported to the Pacific Marine Mammal Center (PMMC) in Laguna Beach, California. Observations by the veterinary team at PMMC (Figure 1) revealed the dolphin to be semi-comatose with intermittent seizuring and markedly diminished neurological signs including non-responsive right pupillary light reflex. Because of a poor prognosis for rehabilitation and the suspicion of domoic acid intoxication, the animal was euthanized. Urine, blood (serum) and cerebrospinal fluid were taken for domoic acid analysis (Marine Biology-Biological Oceanography Department, University of Southern California, Los Angeles) and a complete postmortem examination was conducted.

Figure 1. Adult female short-beaked common dolphin (*Delphinus delphus*)

In addition to marked depletion of visceral and subcutaneous adipose stores, gross pathology was observed in the lungs, tracheobronchiolar lymph nodes and brain. Externally, the brain appeared normal, but palpation revealed a sigmoid-shaped cavitation (4.5cm x 2-4cm) in the left cerebral frontal lobe, covered by a thin (5-10mm) layer of normal-appearing cerebral cortex. The cavitation was filled with a large amount of foul-smelling, cheese-like, tan-colored material. In addition, multiple small pockets filled with a thick, cream-colored liquid were noted along the interior of the cavitation (Figure 2, arrow). Detailed examination of the nasal passages, the cribiform area and portions of skull apposed to the lesion failed to reveal any pathology consistent with a focus of origin. Over the dorsal and dorsolateral aspects of both lungs, numerous 2-8 mm, tan-colored foci surrounded by a 1-2mm 'dirty-white' layer were noted. Careful dissection of these foci suggested bronchiolitis. The tracheobronchiolar lymph nodes were moderately enlarged.

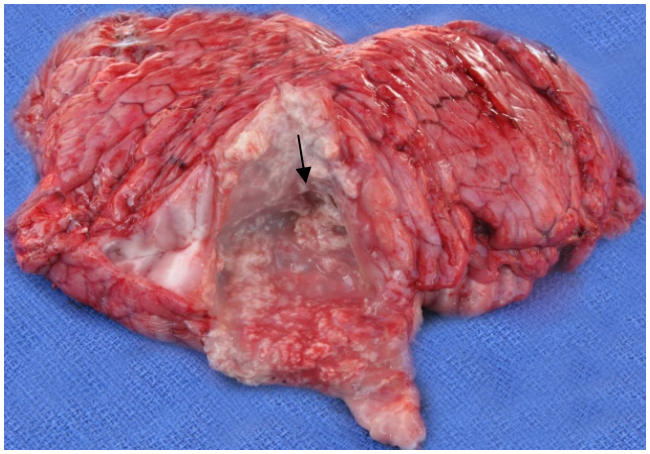

Figure 2. Brain of short-beaked common dolphin with a large cavitation in the frontal lobe of the left cerebral hemisphere.

Tissues were fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (Excalibur Pathology, Moore, Oklahoma). Histologically, the brain lesion was found to be a granuloma consisting of several morphologically distinct, coalescing layers around a liquid necrotic core. The inner layer was composed of 10-15 sublayers of densely-packed histiocytes, many undergoing necrosis, calcification and sloughing either individually or in rows into the liquid central portion of the granuloma (Figure 3, a). The outer layer was composed of thick, dense sheets of histiocytes extending deep into the neuropil (Figure 3, b) and blending into a thick terminal layer (Figure 3, c) of moderate to marked perivascular lymphoid infiltration with occasional, scattered foci of keratinized debris.

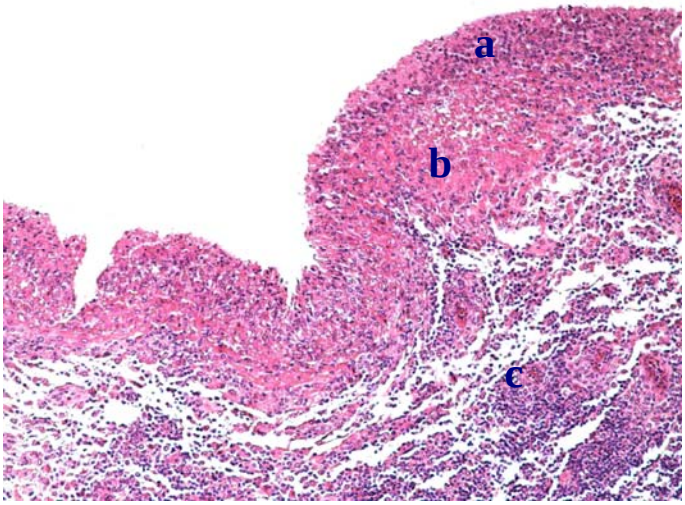

Figure 3. Full-thickness section of wall of cerebral granuloma, see text for description (Hematoxylin & Eosin, 10x).

Lung sections contained multiple small granulomata composed of a dense, variably-sized outer layer of fibrous tissue surrounding an active, inner layer of fibroplasia with perivascular lymphocyte cuffing and scattered multinucleate giant cells. In turn, these layers surrounded a central core of necrotic cellular debris, irregular sheets of keratin, and a few scattered structures with a thin keratinized outline, suggestive of a nematode (Figure 4).

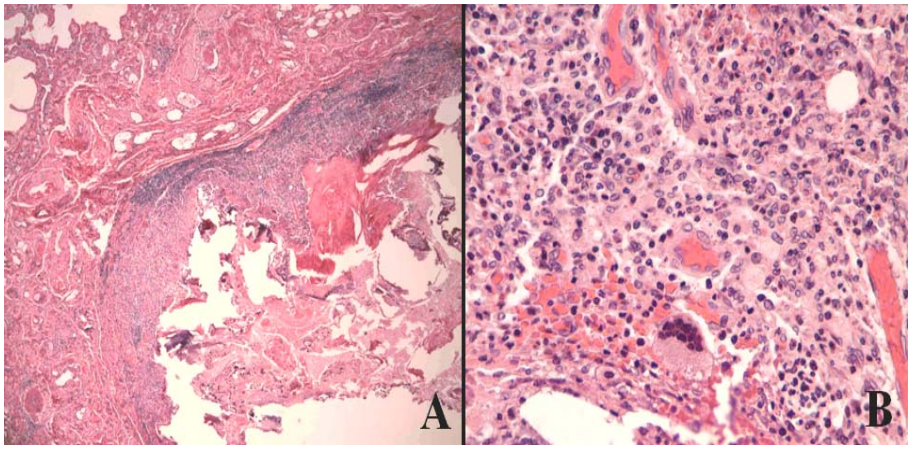

Figure 4. Histologic sections of the pulmonary granuloma, see text for description.
A. Full-thickness section of lung granuloma wall (Wright's Stain, 10x)
B. Higher magnification of the granuloma wall with numerous histiocytes and scattered multinucleate giant cells. (Hematoxylin & Eosin, 40x)

Sections of the tracheobronchiolar lymph node revealed diffuse proliferation of moderately- to-markedly enlarged lymphoid follicles with central proliferation of abundant, large, lymphoid cells having round to ovoid nuclei. The medullary cords contained moderate numbers of reactive lymphocytes, plasma cells, and moderate to marked numbers of eosinophils. In the liver, there was one small, elongated perivascular focus of hepatocellular degeneration and necrosis with abundant infiltration of lymphocytes, active macrophages containing yellow pigmented material, and occasional eosinophils.

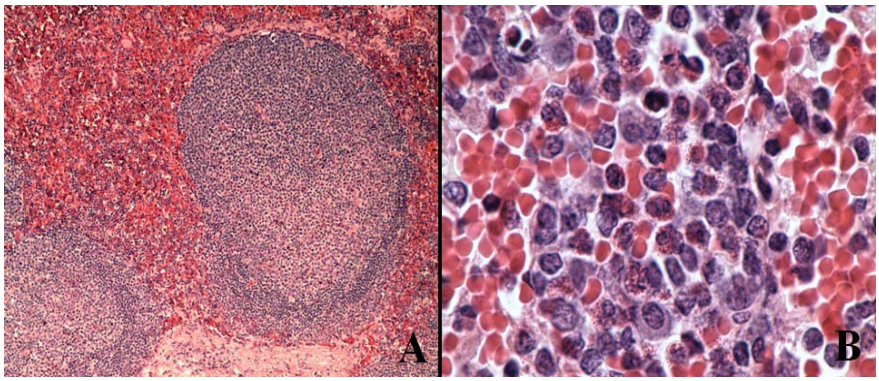

Figure 5. Histologic section of tracheobronchiolar lymph node, see text for description.
A. Enlarged lymphoid follicles (Hematoxylin & Eosin, 40x).
B. Higher magnification of medullary cords containing plasma cells and eosinophils (Hematoxylin & Eosin, 100x).

Impression smears of the tissue surrounding the left cerebral lesion were stained with Wright's-Giemsa stain (Vol-u-sol, Salt Lake City, Utah) and examined. Broad, dense sheets of necrotic cellular debris, 'ghost cells' and necrotic leukocytes, as well as small numbers of morphologically normal appearing neutrophils, macrophages, lymphoid cells and abundant red blood cells were present (Figure 6). Additionally, Gram's-stained sections (Vol-u-sol, Salt Lake City, Utah) revealed small numbers of several morphotypes of gram-negative bacilli.

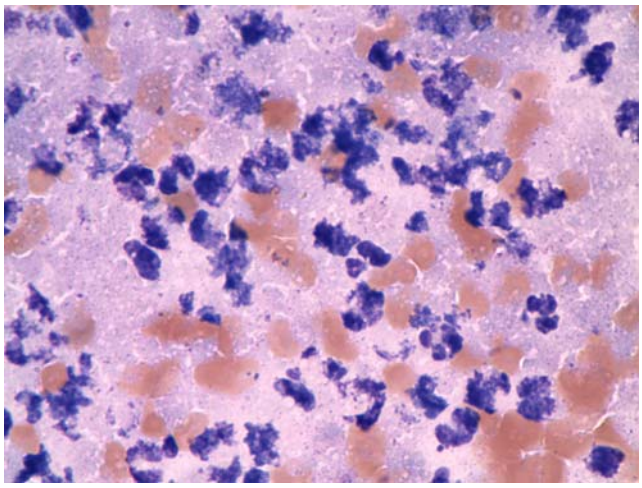

Figure 6. Cytology of cerebral granuloma, see text for description (Wright's Stain, 10x).

Swabs of aseptically collected cerebral granuloma exudate were submitted for bacterial culturing (IDEXX Laboratories, Irvine, California). *Citrobacter freundii, Enterococcus sp., Enterobacter cloacae, Pseudomonas putida*, and *Pseudomonas flourescens* were cultured from the exudate. Three milliliters of cerebrospinal fluid were extracted, centrifuged, and the resulting pellet was smeared and stained with Wright's-Giemsa and Gram's stains (Vol-u-sol, Salt Lake City, Utah). Scattered, small clusters of degenerate leukocytes were noted, with some identified as neutrophils and mononuclear cells, including plasma cells, lymphocytes, and active macrophages. Occasionally, bacilli were noted in the macrophages.

Urine, cerebrospinal fluid and serum samples submitted to the Marine Biology-Biological Oceanography Department, University of Southern California, Los Angeles, failed to reveal evidence of domoic acid.

Discussion

Citrobacter freundii, Enterobacter cloacae, Pseudomonas putida and *flourescens*, and *Enterococcus sp*. are ubiquitous in the environment and mammalian digestive tract (Willey *et al*, 2008). *Citrobacter freundii* is noteworthy, in that it has been associated with vertically-acquired neonatal brain abscesses in humans. *Citrobacter* invades and survives in epithelial cells, and also carries risk of subsequent inflammation of the cerebral white matter corresponding to the end zones of the small penetrating arteries of the brain (Agrawal and Mahapatra, 2005).

While the portal of entry of these bacteria into the dolphin's brain was not established, bacteremic 'seeding' via vascular-lymphatic sources, perhaps secondary to bacterial tissue-organ infectious processes, should be considered (Colegrove and Migaki, 1979). One could argue that the minor inflammatory lesions noted in the lung and liver of this dolphin were evidence of such a process. In addition, the nasal cavity-cribriform area should not be overlooked as a transmission route to the cerebrospinal fluid and/or brain directly. In fact, a case of meningoencephalitis associated with moderate to marked trauma, inflammation and several species of gram-negative bacteria, has been noted in the nasal-cribriform area of a California sea lion (*Zalophus californicus*) at this facility.

Lesions of focal, inflammatory, pneumonic nematodiasis (Metastrongyloidea) are not uncommon in wild cetaceans and pinnipeds and are thought to perhaps act as nidae for septicemia and subsequent multi-organ pathology (Measures, 2001). Further, the diffuse follicular lymphoid proliferation with eosinophilia, noted in the tracheobronchiolar lymph nodes is consistent with an immune response to nematodes.

This case illustrates several important points. First, it is necessary to conduct thorough gross postmortem examinations on all mortalities. In this case, had a postmortem examination and toxicology not been done, an erroneous presumptive clinical diagnosis of domoic acid intoxication, a not uncommon cause of neuropathy in cetaceans off southern California might have been rendered. Secondly, generalized body wasting, lethargy, and neurologic signs are not specific findings, but rather are found in a myriad of subacute to chronic organ system diseases. Finally, cytological examination is a simple and straightforward procedure with significant diagnostic utility. In this case, Wright-Giemsa and Gram's staining of the cerebral lesion readily revealed a severe, bacterial-associated inflammatory disease process (Fravel and Evans, 2008).

Acknowledgments

I would like to thank Erica L. Suebert at the University of Southern California, Los Angeles for testing urine, cerebrospinal fluid and serum for Domoic acid.

* This publication is in partial fulfillment of a preceptorship at Pacific Marine Mammal Center by Annie Page in 2010.

References

Agrawal D. and Mahapatra, A.K. Vertically acquired neonatal *Citrobacter* brain abscess- case reports and review of the literature. *J. Clin. Neurosci.* 12(2): 188-190, 2005.

Colegrove, G. S. and Migaki, G. Cerebral abscess associated with stranding in a dolphin. *J. Wildl. Dis*., 12: 271-274, 1979.

Fravel, V. and Evans, R. H. Staphylococcal pyelonephritis and cystitis in a California sea lion (*Zalophus californianus*). *J. Marine Animals and Their Ecology*, 1 (1): 11-13, 2008.

Measures, L. *Lungworms of Marine Mammals*. In: Parasitic Diseases of Wild Mammals, 2nd Ed. W.M. Samueal, *et al*., Iowa State University Press, Ames, IA. 279-300, 2001.

Willey, J.M., Sherwood, L.M., and Woolverton, C.J., In: Prescott, Harley, and Klein's Microbiology, 7th Ed. McGraw-Hill, New York, NY. 557-561, 2008.