DOI: 10.7589/2015-07-182

Journal of Wildlife Diseases, 52(2), 2016, pp. 407–410

Pulmonary Mast Cell Tumor and Possible Paraganglioma in a Free-ranging Pacific Walrus (*Odobenus rosmarus divergens*), Barrow, Alaska, USA

Mauricio Seguel,[1] Raphaela Stimmelmayr,[2,3] Elizabeth Howerth,[1] and Nicole Gottdenker[1,4] [1]Department of Pathology, College of Veterinary Medicine, University of Georgia, 501 D. W. Brooks Dr., Athens, Georgia 30602, USA; [2]North Slope Borough Department of Wildlife Management, PO Box 69, Barrow, Alaska 99723, USA; [3]Institute of Arctic Biology, University of Alaska Fairbanks, 902 N Koyukuk Dr., PO Box 757000, Fairbanks, Alaska 99775-70, USA; [4]Corresponding author (email: gottdenk@uga.edu)

ABSTRACT: We describe a pulmonary mast cell tumor in a subsistence-harvested free-ranging Pacific walrus (*Odobenus rosmarus divergens*). Neoplastic cells effacing a focal area of pulmonary parenchyma were characterized by rare metachromatic granules and positive staining for C-kit. We also report co-occurrence of a peribronchial mass with a morphologic and immunohistochemical profile compatible with paraganglioma.

Neoplasia is rarely reported in most free-living marine mammals. Exceptions are some populations of California sea lions (*Zalophus californianus*), with a high prevalence of urogenital carcinoma (Gulland et al. 1996), and St. Lawrence estuary beluga whales (*Delphinapterus leucas*), with up to 30% prevalence of sarcomas and carcinomas (Martineau et al. 2002). The most frequently described round cell sarcoma in marine mammals is lymphoma, with sporadic reports in cetaceans (Diaz-Delgado et al. 2015), mustelids (Tanaka et al. 2013), and pinnipeds (Venn-Watson et al. 2012). Fibrohistiocytic thoracic neoplasia was reported in a wild Pacific walrus (*Odobenus rosmarus divergens*; Fleetwood et al. 2005).

Mast cell tumors are common in dogs (*Canis lupus familiaris*), occasional in cats (*Felis catus*), and rare in horses (*Equus caballus*), cattle (*Bos taurus*), and pigs (*Sus scrofa domesticus*; Goldschmidt and Hendrick 2008). There are rare reports of mast cell tumors in ferrets (*Mustela putorius furo*; Parker and Picut 1993) and hedgehogs (*Atelerix albiventris*; Raymond et al. 1997). We describe gross, microscopic, and immunohistochemical features of two lung neoplasms in a walrus: a mast cell tumor and a possible paraganglioma.

A 15- to 20-yr-old (on the basis of tusk length; Fay 1982) 2.72-m female Pacific walrus in good nutritional condition was subsistence harvested with a rifle in July 2013 at Barrow, Alaska (71°17′44″N, 156°45′59″W). As part of the North Slope Marine Mammal Health Research Program, postmortem inspection followed a standard protocol (Dierauf 1994) to assess the harvested individual's health and collect baseline data (e.g., body measurements, reproductive status, nutritional condition). The protocol was modified to align with the traditional Inupiaq butchering process whereby pinnipeds are skinned and chest and abdominal organs are removed together after careful in situ evaluation. On gross examination, three coalescing, 3-cm-diameter white, firm masses elevated the pleura and effaced a small portion of the pulmonary parenchyma in the posterior margin of right caudal lung lobe.

Tissues with suspected or confirmed gross abnormalities (lung, heart, thyroid, parathyroid, pancreas, spleen, liver, and small intestine) were fixed in 10% buffered formalin, routinely processed for histopathology, and stained with H&E. Selected sections of lung were stained with Giemsa, toluidine blue, Luna and Gram stains, and periodic acid Schiff reaction. Immunohistochemical staining was performed in the lung tissue using several antibodies (Abs) following protocols (Table 1). As internal positive controls we used peribronchial/peribronchiolar leukocytes for CD18, bronchial epithelium for pancyto-

TABLE 1. Antibodies and methods used for immunohistochemical stains during an investigation of lung neoplasms in a free-ranging Pacific walrus (*Odobenus rosmarus divergens*), Barrow, Alaska, USA, 2013. Diaminobenzidine was used for immunocytochemical visualization.

Antibody[a]	Antibody clone	Antigen retrieval method	Primary antibody dilution[b]
Chromogranin A (DK)	Rabbit, anti-human, polyclonal	Citrate pH 6.0	1:6,000
CD18 (PM)	Mouse, anti-dog, monoclonal	Citrate pH 6.0	1:50
C-kit (CD117) (CM)	Rabbit, anti-human, monoclonal	Citrate pH 6.0	RTU
Cytokeratin AE1/AE3 (CM)	Mouse, anti-human, monoclonal	Citrate pH 6.0	RTU
Cytokeratin Lu5 (BC)	Mouse, anti-human, monoclonal	Pepsin	1:100
S-100 (CM)	Mouse, anti-human, monoclonal	No retrieval	RTU
Synaptophysin (BG)	Mouse, anti-human, monoclonal	Citrate pH 6.0	1:600
Vimentin (BG)	Mouse, anti-human, monoclonal	Citrate pH 6.0	1:3,000

[a] Manufacturers listed in parentheses: DK = Dako, Carpinteria, California, USA; PM = Dr. Peter Moore; CM = Cell Marque, Rocklin, California, USA; BC = Biocare, Concord, California, USA; BG = Biogenex, Fremont, California, USA.

[b] RTU = ready-to-use antibody, no dilution needed.

keratins AE1/AE3 and Lu-5, pericytes and bronchial/bronchiolar smooth muscle for S-100, and endocrine cells of the bronchial gland epithelium for chromogranin-A. No synaptophysin immunoreactivity was detected in normal and affected areas of lung. Other tissues were not evaluated.

A highly cellular, unencapsulated, infiltrative neoplasm effaced large portions of the pulmonary parenchyma (Fig. 1A) and compressed adjacent alveoli. Sheets of round neoplastic cells were supported by fine fibrovascular stroma and admixed with moderate numbers of viable and degenerate eosinophils (Fig. 1B). Neoplastic cells had an amphophilic granular cytoplasm that occasionally contained small metachromatic granules in Giemsa- and toluidine blue-stained sections and usually a centrally located nucleus with a magenta nucleolus (Fig. 1C). Anisocytosis and anisokaryosis were mild to moderate and the mitotic index averaged two per 10 400× fields. There were rare neoplastic cells with bizarre nuclei. Neoplastic cells had marked, diffuse, membranous staining with C-kit Ab (Fig. 1D) and marked, diffuse, cytoplasmic staining for vimentin, whereas they were negative for CD18, pancytokeratin AE1/AE3, S-100, and chromagranin-A immunostains. We classified this neoplasm as a mast cell tumor.

A 3-mm-diameter second neoplasm occupied part of a large bronchus, expanded the peribronchial tissue, and compressed adjacent small bronchi (Fig. 1E). The unencapsulated, expansile neoplasm was composed of lobules and nests of polygonal cells delineated by fine fibrovascular stroma and sometimes dissected by thick collagenous trabecular areas. The neoplastic cells had eosinophilic cytoplasm that occasionally contained 1–2-μm perinuclear eosinophilic globular inclusions (Fig. 1F). Anisocytosis and anisokaryosis were mild, the mitotic rate was seven in 10 400× fields, and single-cell necrosis was rare. Neoplastic cells were negative for vimentin, CD18, C-kit, and chromagranin-A. No immunoreactivity for synaptophysin was detected for normal or neoplastic tissue. With pancytokeratin AE1/AE3 and Lu-5, occasional groups of small cuboidal cells (probably trapped bronchial epithelial cells) had moderate, diffuse, cytoplasmic staining, but neoplastic cells were negative. With S-100, occasional stellate to spindle cells within the supporting stroma showed moderate cytoplasmic staining. A diagnosis of neuroendocrine neoplasia, such as bronchial paraganglioma, seemed most likely, but a carcinoid could not be ruled out. Additional histologic findings included a moderate interstitial lymphoplasmacytic pancreatitis, moderate interstitial fibrosis in the

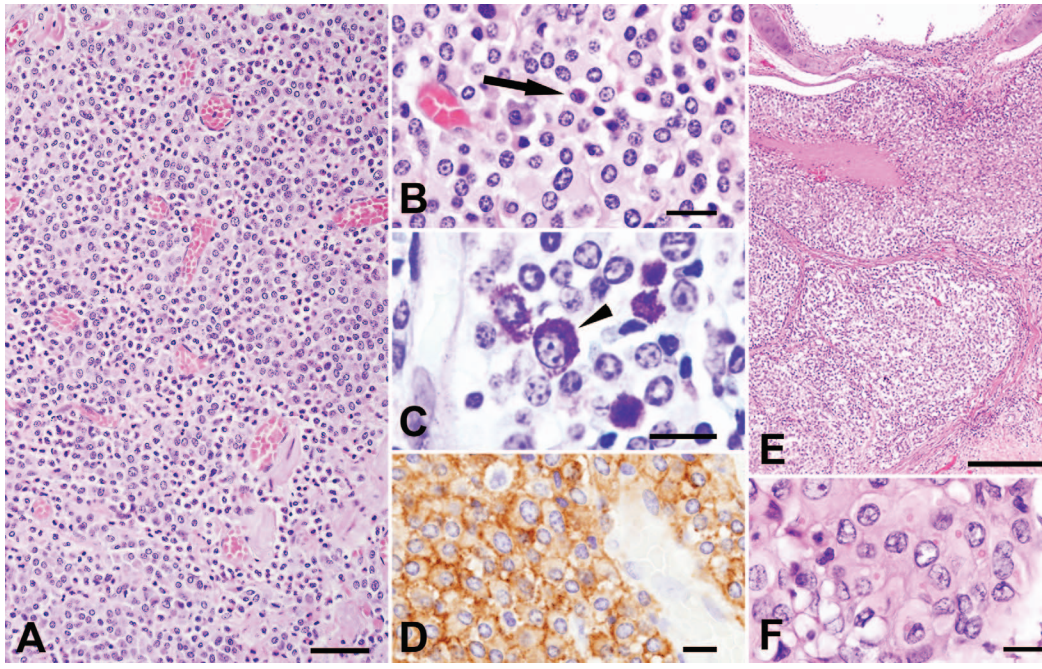

FIGURE 1. Mast cell tumor from a Pacific walrus (*Odobenus rosmarus*) in Barrow, Alaska, USA, 2013. (A) The pulmonary parenchyma is effaced by a round cell neoplasm supported by fine fibrovascular stroma. H&E stain. Bar=50 μm. (B) Neoplastic mast cells have moderate pale eosinophilic granular cytoplasm and are admixed with occasional eosinophils (arrow). H&E stain. Bar=15 μm. (C) Some neoplastic mast cells have metachromatic cytoplasmic granules (arrowhead). Toluidine blue stain. Bar=15 μm. (D) Neoplastic cells have moderate membranous and minimal cytoplasmic positive staining for C-kit (CD 117). Immunohistochemistry for C-kit antigen counterstained with hematoxylin. Bar=10 μm. (E) Pulmonary neoplasm from the same Pacific walrus. The highly cellular neoplasm expands the peribronchial tissue and compresses the adjacent pulmonary parenchyma. H&E stain. Bar=400 μm. (F) Polygonal neoplastic cells of the pulmonary neoplasm have moderately distinct cell borders and moderate eosinophilic cytoplasm that occasionally contains eosinophilic globular material or clear vacuoles that slightly displace the nucleus. H&E stain. Bar=10 μm.

thyroid and parathyroid with occasional thyroid cysts, and mild multifocal perinuclear vacuolation of cardiomyocytes.

The fact that the walrus was in apparently good health and the hunter did not report any abnormal behavior, with the small size of both neoplasms and lack of any evident metastases, suggest that these were incidental findings. Paragangliomas are rare neuroendocrine tumors that can be negative for common pancytokeratins and show variable immunoreactivity for synaptophysin and chromogranin A but have a S-100-positive stroma (Aubertine and Flieder 2004), as this walrus peribronchiolar neoplasm.

The origin of the neoplastic mast cells is uncertain, as no additional masses were identified in other tissues. Furthermore, these animals' skeletal muscles, subcutis, and skin are harvested for food and all internal organs carefully examined by the official veterinarian. The absence of any grossly evident distinct nonpulmonary mass with the gross and histologic pattern of the pulmonary involvement suggests that the mast cell tumor could be of primary pulmonary origin. Mammalian lungs normally harbor large numbers of a specific subset of mast cells that can potentially undergo neoplastic transformation (Abid et al. 2014). Primary mast cell tumors have been rarely reported in humans (Abid et al. 2014) and there are a few reports of primary thoracic involvement of disseminated visceral mast cell disease in dogs (Pollack et al. 1991). Neoplasia was found in 18 of 107 harvested Pacific walruses over a 10-yr period, but mast-

cell tumors were not observed (Fleetwood et al. 2005).

To our knowledge, this is a novel description of a pulmonary mast cell tumor in a wild marine mammal. Its temporal coexistence with a presumed pulmonary paraganglioma increases the body of knowledge on marine mammal pathology.

We thank the hunters and the community of Barrow for allowing us to conduct the study using subsistence-harvested walrus. Funding for this project is provided by Coastal Impact Assessment Program Marine Mammal Health (F12AF01265) and North Slope Borough Department of Wildlife Management. Marine mammal tissue collection was conducted under US Fish and Wildlife Service MA135907-1. We thank Abbie Butler and Patricia Rowe of University of Georgia Veterinary Diagnostic Laboratory for immunohistochemistry.

LITERATURE CITED

Abid N, Ayedi L, Makni S, Bahri I, Frikha I, Boudawara TS. 2014. Solitary mastocytoma of the lung: A case report and a brief review of the literature. *J Afr Cancer/Afr J Cancer* 6:107–110.

Aubertine CL, Flieder DB. 2004. Primary paraganglioma of the lung. *Ann Diagn Pathol* 8:237–241.

Díaz-Delgado J, Sierra E, Arbelo M, Suárez-Bonnet A, Suárez-Santana C, Grau-Bassas E, Fernández A. 2015. Primary uterine T-cell lymphoma with metastasis in an Atlantic spotted dolphin (*Stenella frontalis*), Canary Islands, Spain. *J Wildl Dis* 51:538–541.

Dierauf LA. 1994. *Pinniped forensic necropsy and tissue collection guide*, Wilkinson D, Driscoll C, editors. National Oceanic and Atmospheric Administration, Washington, DC, 80 pp.

Fay FH. 1982. Ecology and biology of the Pacific walrus, *Odobenus rosmarus divergens* Illiger. *N Am Fauna* 74:1–279.

Fleetwood M, Lipscomb TP, Garlich-Miller J. 2005. Summary of pathological findings from subsistence hunting of the Pacific walrus (*Odobenus rosmarus divergens*) in Alaska from 1995–2004. In: *Proceedings of the 36th annual meeting of the International Association Aquatic Animal Medicine*, IAAM, Seward, Alaska, 14–19 May, p. 172.

Goldschmidt MH, Hendrick MJ. 2008. Tumors of the skin and soft tissues. In: *Tumors in domestic animals*, 4th Ed., Meuten DJ, editor. John Wiley and Sons, Ames, Iowa, pp. 45–117.

Gulland FMD, Trupkiewicz JG, Spraker TR, Lowenstine LJ. 1996. Metastatic carcinoma of probable transitional cell origin in 66 free-living California sea lions (*Zalophus californianus*), 1979 to 1994. *J Wildl Dis* 32:250–258.

Martineau D, Lemberger K, Dallaire A, Labelle P, Lipscomb TP, Michel P, Mikaelian I. 2002. Cancer in wildlife, a case study: Beluga from the St. Lawrence estuary, Quebec, Canada. *Environ Health Perspect* 110:285.

Parker GA, Picut CA. 1993. Histopathologic features and post-surgical sequelae of 57 cutaneous neoplasms in ferrets (*Mustela putorius furo*). *Vet Pathol* 30:499–504.

Pollack MJ, Flanders JA, Johnson RC. 1991. Disseminated malignant mastocytoma in a dog. *J Am Anim Hosp Assoc* 27:435–440.

Raymond J, White TMR, Janovitz EB. 1997. Malignant mast cell tumor in an African hedgehog (*Atelerix albiventris*). *J Wildl Dis* 33:140–142.

Tanaka N, Izawa T, Kashiwagi-Yamamoto E, Kuwamura M, Ozaki M, Nakao T, Yamate J. 2013. Primary cerebral T-cell lymphoma in a sea otter (*Enhydra lutris*). *J Vet Med Sci* 75:1667–1669.

Venn-Watson S, Benham C, Gulland FM, Smith CR, St Leger J, Yochem P, Nollens H, Blas-Machado U, Saliki J, Colegrove K, et al. 2012. Clinical relevance of novel Otarine herpesvirus-3 in California sea lions (*Zalophus californianus*): Lymphoma, esophageal ulcers, and strandings. *Vet Res* 43:85.

Received for publication 13 July 2015.
Accepted 12 September 2015.