

Spontaneous Primary Right Tibiotarsal Osteosarcoma with Pulmonary Metastasis in a Free-ranging Cory's Shearwater (*Calonectris diomedea*)

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ABSTRACT: We report the pathologic features of a primary right tibiotarsal productive osteoblastic osteosarcoma with pulmonary metastasis, a unique finding in a Cory's Shearwater (*Calonectris diomedea*).

The Order Procellariiformes comprises four families of pelagic seabirds: the albatrosses, petrels and shearwaters, and two families of storm petrels. Approximately 60% of the species in this order are threatened by anthropogenic factors such as intentional use or ingestion of marine debris (IUCN 2018). The Cory's Shearwater (*Calonectris diomedea*) is a migratory procellariiform that breeds on islands of Portugal and Spain. During austral summer, this pelagic bird migrates to the Southern Hemisphere and is commonly found stranded dead along the coast of Brazil (Mariani 2016). The causes of such stranding events are still poorly understood; however, human-related and natural causes are likely involved.

Neoplasia is common in poultry and in some companion birds, specifically in Psittaciformes (Robat et al. 2017). Skeletal tumors are infrequently reported in avian species. Here we describe an appendicular osteosarcoma with lung metastasis in a Cory's Shearwater.

A 610-g, 52-cm-long adult female Cory's Shearwater was found dead in fresh preservation status in Ilha Comprida, São Paulo State (24°56'06"S, 47°47'59"W; Brazil) in March 2017. A complete necropsy was performed and representative tissue samples of tumor lesion, skin, pectoral and tibiotarsus

muscle, tongue, oropharynx, esophagus, proventriculus, ventriculus, small and large intestines, liver, pancreas, larynx, trachea, lung, heart, great vessels, spleen, kidney, thyroid, cerebrum, cerebellum, spinal cord, eye, and salt gland were collected and fixed in 10% neutral buffered formalin. These tissues were processed routinely, embedded in paraffin wax, and 5- μ m-thick sections were stained with H&E for microscopic analysis. For parasitologic analysis, ectoparasites and endoparasites were preserved in 70% alcohol. Identification relied on morphologic features.

On necropsy, the main gross finding was a 5.5×4.0-cm-diameter firm mass centered on the diaphysis of the right tibiotarsus spanning from proximal to distal metaphysis (Fig. 1). On cut surface, the mass was multinodular with hard linear particles and foci of necrosis. It had effaced the medullary and cortical bone, encroached local tendons and ligaments, and was coated by atrophied muscle. Additional gross findings were generalized muscle wasting and fat depletion; cutaneous pediculosis by *Halipeurus* sp.; pulmonary hemorrhage and edema; proventriculitis by *Serautia shipleyi*; intestinal cestodiasis; and bilateral pododermatitis.

Microscopically, the tibiotarsal mass consisted of a well-demarcated, moderately cellular, expansive and focally infiltrative (overlying periosteum, deeper fasciae, and deeper muscles) neoplasm composed of mesenchymal tumor cells (osteoblasts) arranged haphazardly or in variably thick rows that delineated trabeculae of osteoid matrix (Fig. 1A). Tumor cells were pleomorphic,

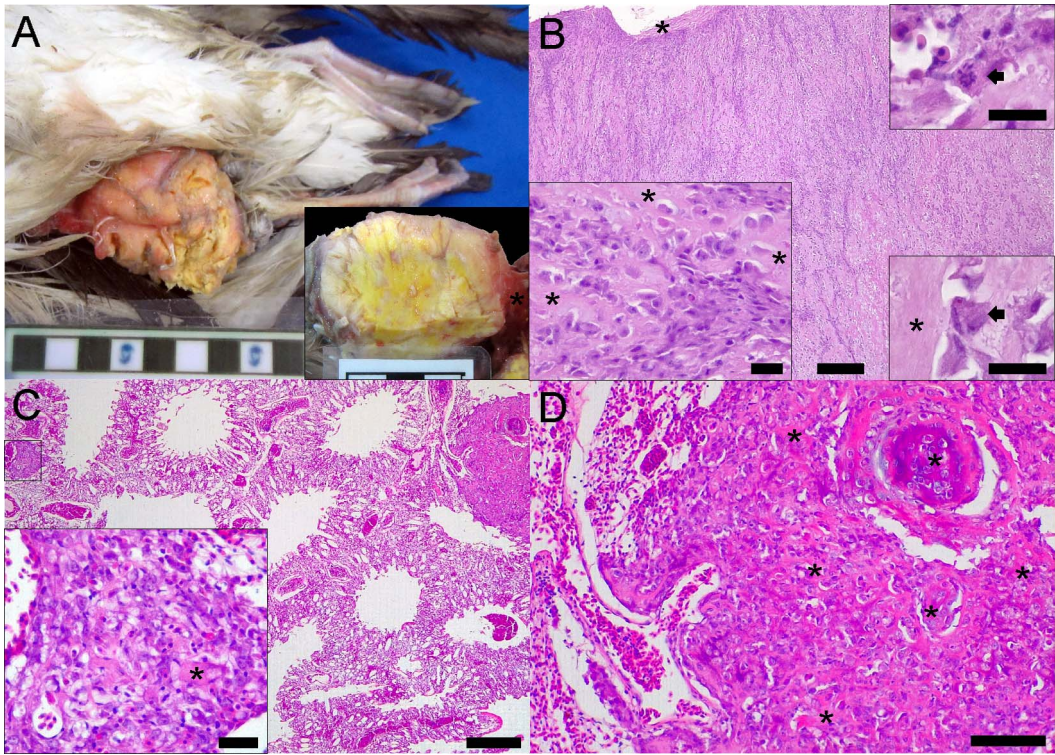


FIGURE 1. Cory's Shearwater (*Calonectris diomedea*) found in São Paulo State, Brazil in March 2017, with right tibiotarsal osteosarcoma and lung metastasis. (A) Right tibiotarsus is effaced and expanded by an oval, firm, yellow-tan mass that irradiates circumferentially and is coated by atrophied muscle. Bar=1 cm. Inset: mass is pale tan and lobulated with white-yellow gritty areas and has entrapped tendon and nervous structures. Asterisk indicates proximal epiphysis of right metatarsus. Bar=1 cm. (B) Subperiosteum is markedly expanded by the neoplastic growth. Asterisk indicates periosteum. H&E. Bar=300 μ m. Left inset: neoplastic cells progressively embed in communicating trabeculae of osteoid matrix (asterisks). H&E. Bar=50 μ m. Right upper inset: neoplastic cell displaying bizarre mitosis (arrow). H&E. Bar=50 μ m. Right lower inset: focal binucleated neoplastic cell (arrow) near an osteoid trabecula (asterisk). H&E. Bar=50 μ m. (C) Two osteosarcoma metastatic nodules. H&E. Bar=300 μ m. Inset (squared area in main figure): early metastatic focus is largely composed of neoplastic cells with little osteoid (asterisk). H&E. Bar=50 μ m. (D) Advanced osteosarcoma metastatic growth markedly distorts and replaces the pulmonary parenchyma and contains greater amount of variably mineralized osteoid (asterisks). H&E. Bar=200 μ m.

polygonal to oval to fusiform, had a moderate amount of eosinophilic cytoplasm with demarcated borders, and paracentral to eccentric hyperchromatic nuclei with one to two nucleoli. Anisocytosis and anisokaryosis were moderate, and the mitotic count was 12 per 10 high-power fields (400 \times). Occasional single tumor cell necrosis-apoptosis, binucleation, multinucleation, and bizarre mitoses were noted (Fig. 1B). There was intratumor necrosis associated with thromboses, and rare chondroid metaplasia. In the lungs, multifocal metastatic nodules with identical cytomorpho-

logical features to those observed in the right tibiotarsal neoplasm, including osteoid production (Fig. 1C, D), were observed typically associated with hemorrhage, primarily involving parabronchial arteries and adjacent parenchyma. On the basis of these features, a productive osteoblastic osteosarcoma with pulmonary metastasis was determined. Additional microscopic findings confirmed multi-etiological endoparasitism involving the digestive and urinary systems, and necrofibrinoheterophilic pododermatitis with bacteria and hyphae.

Here we reported the pathologic features of a spontaneous primary right tibiotarsus osteosarcoma with pulmonary metastasis. Various osteosarcoma subtypes are currently recognized in humans as low-grade central osteosarcoma, conventional osteosarcoma, telangiectatic osteosarcoma, small cell osteosarcoma, parosteal osteosarcoma, periosteal osteosarcoma, and high-grade surface osteosarcoma (Rosenberg et al. 2013). These subtypes may bear differential behavior, prognosis, and treatment responses (Rosenberg et al. 2013; Schott et al. 2018). The cytohistomorphologic features and metastasis observed in this case recapitulated previous stage III reports in humans and veterinary species (Rosenberg et al. 2013; Schott et al. 2018).

Primary bone tumors are rarely reported in avian species other than poultry. Overall, osteosarcoma and chondrosarcoma predominate, and typically arise from long bones (Robat et al. 2017). Osteosarcomas have been reported in psittacines (Fordham et al. 2010), galliformes (Reece 2008; Dittmer et al. 2012), columbiformes (Lamb et al. 2014), a Blue Crane (*Anthropoides paradiseus*; Churgin et al. 2013), an American Robin (*Turdus migratorius*), a Panama Boat-billed Heron (*Cochlearius cochlearius panamensis*; Liu et al. 1982), an Eastern White Pelican (*Pelecanus onocrotalus*; Molenaar et al. 2015), a Domestic Goose (*Anser domesticus*; Mawdesley-Thomas and Solden 1967), and a Powerful Owl (*Ninox strenua*; Sladakovic et al. 2017).

Osteosarcoma in birds is considered of low metastatic potential: in 29% (14/48) of the reported cases with necropsy, six tumors metastasized, with only two to the lungs (Dittmer et al. 2012; Churgin et al. 2013; Lamb et al. 2014; Robat et al. 2017; Sladakovic et al. 2017). Soft-tissue infiltration by tumor cells and lung metastasis accounted for malignancy in the present case, contrasting with less aggressive behavior in previous reports, and paralleled metastatic pneumotropic predilection in humans (Rosenberg et al. 2013) and dogs (Schott et al. 2018). The severity and extent of the primary tumor likely led to locomotor difficulties and progressive

debilitation during a highly demanding migratory phase. Such compromised status could have facilitated ecto- and endoparasitism and cutaneous opportunistic infections. Finally, metastatic lung lesions associated with hemorrhage could have potentially contributed to respiratory dysfunction and death. An etiology in this case remains unknown. We did not investigate a potential infectious cause so it cannot be discarded. Noninfectious causes including a previous traumatic event were not readily apparent.

In conclusion, we presented a spontaneous primary right tibiotarsal osteosarcoma with pulmonary metastasis in a Cory's Shearwater. The severity and extent of this malignant neoplastic process likely accounted for general debilitation and the pulmonary metastatic nodules could have potentially interfered with lung function, leading to morbidity and death in this case. Primary bone tumors in Procellariiformes have not been previously reported. Bone neoplasms should be included in the differential diagnosis of masslike lesions in the axial and appendicular skeleton of Procellariiformes.

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