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Hypertrophic Osteopathy Associated with Mycotic Pneumonia in a Roe Deer (*Capreolus capreolus*)

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ABSTRACT: *Aspergillus fumigatus* is one of the most common *Aspergillus* species causing disease in individual animals but it rarely affects deer species. We report a case of *A. fumigatus* infection causing mycotic pneumonia and hypertrophic osteopathy in a roe deer (*Capreolus capreolus*).

Roe deer (*Capreolus capreolus*) is one of the most widespread and important game species in Slovenia. Necropsies of 586 free-ranging roe deer were performed within the framework of a passive wildlife health surveillance program in the period 2000–17. Pulmonary infection due to *Aspergillus fumigatus* was the cause of death in four cases; however, only in one case were lesions characteristic of hypertrophic osteopathy (HO) found. We describe this case, observed in 2017, where we believe that *A. fumigatus* caused mycotic pneumonia and secondary HO. Also known as Marie's disease, HO is a syndrome characterized by diffuse, periosteal new bone formation along the diaphyses and metaphyses of certain limb bones, which occurs in association with a chronic inflammatory or neoplastic lesion, usually present in the thoracic cavity (Craig et al. 2016). This syndrome has not been documented in other Slovenian wildlife.

An adult, approximately 5-yr-old male roe deer was found dying and harvested in January 2017 at the Lukovica hunting area in central Slovenia (46°10′106′′N, 14°41′266′′W) by a local hunter and submitted for necropsy and radiography, necropsy, and histopathologic and bacteriologic examination to the Veterinary Faculty (University of Ljubljana, Ljubljana, Slovenia). The animal was in poor body condition with overgrown hooves. Marked bilateral enlargement of the metacarpo- and metatarso-phalangeal joints (Fig. 1A) regions, with a decreased range of motion of the effected joints, was also observed.

Radiography revealed multiple, variably sized pulmonary masses and diffuse periosteal proliferation (Fig. 1B) of the long bones of the extremities, tarsal, carpal, and metacarpal bones and phalanges. At necropsy, the animal was severely emaciated, with serous atrophy of adipose tissue and moderate muscle wasting. Macroscopic examination of the lungs revealed the presence of encapsulated, caseous nodules measuring from 4 to 6 cm; one was in the right lung and four were in the left. Intervening pulmonary parenchyma had multifocal areas of hemorrhage, consolidation, and peripheral emphysema, producing a mottled, irregular surface. There were multifocal, fibrous, pleural adhesions in the thoracic cavity. The bones of the forelimbs and hindlimbs were thickened and their outer surfaces were roughened (Fig. 1A, C). No pathology was noted on gross examination of other organs. Samples of lungs and of the mandibular, mediastinal, and mesenteric lymph nodes were taken for bacteriology, and samples of lungs and metacarpal bone were taken for histopathology.

Samples for histopathology were fixed in 10% buffered formalin, processed, embedded in paraffin, sectioned, and stained with H&E, Periodic acid–Schiff, and Grocott methenamine silver stains according to standard protocols. The bones were decalcified with OSTEOMOLL® (EMD Millipore Corporation, Billerica, Massachusetts, USA) before tissue processing. For bacteriology, culture from tissue samples was performed on Sabouraud dextrose agar (Oxoid, Basingstoke, Hampshire, UK) with chloramphenicol. The inoculated medium was incubated for 5 d at 37 C. Mycobacterial examinations of lungs and lymph nodes were conducted according to the protocol described by Kent and Kubica

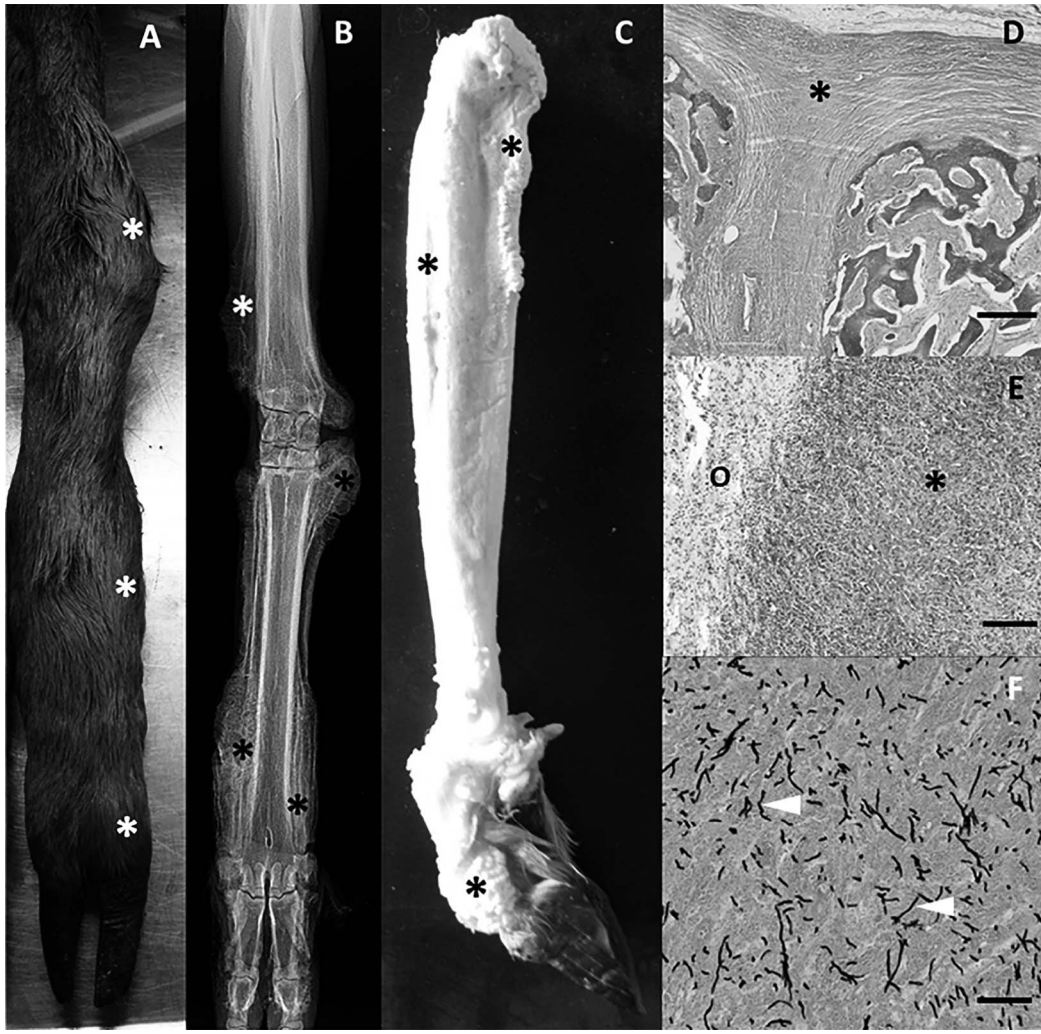

FIGURE 1. Gross, radiographic, and microscopic images of hypertrophic osteopathy and bronchopulmonary aspergillosis in a roe deer (*Capreolus capreolus*). (A–C) Front leg with marked lesions (*) characteristic of hypertrophic osteopathy: (A) Gross view; (B) radiograph; (C) macerated specimen; (D) severely thickened periosteum (*) that extends between irregular trabeculae of woven bone. H&E. Bar=200 μm; (E) pulmonary granuloma with large central caseoliquefactive necrosis (*) and a thick capsule (○). H&E. Bar=250 μm. (F) Numerous fungal hyphae (white arrowheads) in the pulmonary granuloma. Grocott methenamine silver stain. Bar=70 μm.

(1985) and inoculated onto the following selective media: Löwenstein-Jensen (one slant supplemented with pyruvate and one slant supplemented with glycerine), Stonebrink, Middlebrook 7H10 and BBL MGIT (Mycobacteria Growth Indicator Tube (Becton Dickinson, Franklin Lakes, New Jersey, USA).

On microscopic examination of the slide culture, hyphae with septa, domed vesicles, phyllites covering half of the upper portion, and conidia arranged in columns were observed and defined as *A. fumigatus*. Bacteriology of the lung tissue sample revealed no significant growth by aerobic culture. Lung and lymph node cultures were negative for mycobacteria.

On histopathologic examination of lung tissue, nodules with central caseoliquefactive

necrosis and numerous 3–6 μm wide, parallel walled, regularly septate, dichotomous branching hyphae were observed (Fig. 1E, F). The necrosis was demarcated by macrophages and degenerate neutrophils, which were further surrounded by a thick, fibrous capsule with multifocal, moderate infiltrates of lymphocytes at the periphery. Diffuse alveolar edema, multifocal emphysema, and hyperplastic bronchus-associated lymphoid tissue and vascular-associated lymphoid tissue were also noted in the pulmonary parenchyma while the pleura were multifocally, moderately infiltrated with lymphocytes and were diffusely edematous. The pulmonary lesions were diagnosed as multifocal granulomatous necrotizing pneumonia with fungal hyphae, etiology consistent with infection by *Aspergillus* sp.

The periosteum of the metacarpal bone was severely thickened and extended between irregular trabeculae of woven bone, which was multifocally perpendicularly oriented toward the surface (Fig. 1D). The lesions were diagnosed as diffuse, severe, periosteal new bone formation consistent with HO.

Hypertrophic osteopathy is a rare pathologic condition commonly associated with the presence of mass lesions in the thoracic or abdominal cavities including those induced by tumors and infections, particularly *Mycobacterium* sp. (Mair et al. 1996; Ogilvie 1998; Schleining and Voss 2004; Craig et al. 2016). There are many hypotheses concerning the pathogenesis of secondary HO. The initial mechanism probably involves an increased blood flow to the limbs and fluid retention, which are then followed by proliferation of vascular connective tissue and periosteum and finally bone deposition. Different etiologies such as hormones, hypoxia, and arteriovenous shunts, and neurologic mechanisms such as vagal stimulation, have been proposed as a cause of increased blood flow (Mair et al. 1996; Withers et al. 2015; Craig et al. 2016). Dickinson and Martin (1987) suggested that megakaryocytes and clusters of platelets in the distal vasculature of the limbs induce release of platelet-derived growth factor and vascular endothelial growth factor that leads to proliferation of connective tissue and periosteum. Furthermore, vascular endothelial growth factor has been identified as an osteogenic–angiogenic coupling factor involved in new bone formation, vascular hyperplasia, and edema, which are all typical symptoms of HO (Atkinson and Fox 2004). The most recent hypothesis suggests chronic activation of macrophages by a persistent irritant or hypoxia that leads to elevated levels of growth factors and formation of granulomas in an array of pulmonary pathologies (Toovey and Eisenhauer 2010).

Hypertrophic osteopathy has been recorded in various species, including a wide range of wild animals, mainly affecting single animals. In deer, HO was reported in farmed elk (*Cervus elaphus*; Ferguson et al. 2008), free-ranging roe deer (Schulze et al. 2005), and free-ranging white-tailed deer (*Odocoileus virginianus*; Madson et al. 2009) associated with disseminated granulomatous inflammation caused by *A. fumigatus* infection, chronic interstitial pneumonia, and systemic *Conidiobolus incongruous* infection, respectively. Bronchopulmonary aspergillosis in roe deer has been reported only by Vitovec et al. (1974) and Pewsner et al. (2017). Although the prevalence of *A. fumigatus* infection in roe deer is very low, we believe that additional cases may continue to occur sporadically.

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