Journal of Wildlife Diseases, 48(2), 2012, pp. 523–525

Disseminated Pulmonary Adiaspiromycosis in a Crested Porcupine (*Hystrix cristata* Linnaeus, 1758)

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ABSTRACT: Adiaspiromycosis is primarily a necrotizing granulomatous pneumonia caused by a dimorphic fungus of the genus *Emmonsia*. A young crested porcupine (*Hystrix cristata*) found dead showed multiple fractures, chronic pleuritis, and granulomatous pneumonia. Microscopically, cystic structures were consistent with adiaspiromycosis by *Emmonsia crescens*. The diagnosis was confirmed using molecular methods.

Adiaspiromycosis is primarily a noncontagious pulmonary disease (i.e., characterized by necrotizing granulomatous pneumonia) caused by a dimorphic fungus belonging to the genus *Emmonsia*. The condition is distributed in most of the world and recently was described in several animals in Great Britain (Borman et al., 2009). The only report from Italy was by Splendore (1920), who wrote about eggs of a worm "vermi sconosciuti" in voles (*Microtus savii*), which were later recognized as adiaspores of *Emmonsia crescens* by Burek (2001). *Emmonsia crescens* is the main species involved in European cases of adiaspiromycosis, and, rarely, *Emmonsia parva* can also be found. Adiaspiromycosis has been described in small mammals, and it has been found in several wild species, particularly in Mustelidae and Rodentia (Burek, 2001), including an American porcupine (*Erethizon dorsatum*; Barigye et al., 2007), but never in crested porcupine (*Hystrix cristata*). It is sporadically detected in domestic animals and in humans, in which it has been recently considered an emerging disease (Mendes et al., 2009), affecting immunocompromised patients in particular (Pelegrín et al., 2011). Considered a possible source of diagnostic doubts, it radiologically mimics widespread pulmonary malignancy (Denson et al., 2009), and, in many cases, it has been diagnostically approached by biopsy and/or more aggressively treated by surgery (Pelegrín et al., 2011). We describe a case of disseminated pulmonary adiaspiromycosis in a crested porcupine, the first in this species and in Italy.

A 3.7-kg, young male crested porcupine was found dead in apparently poor nutritional condition during the winter of 2010–11 in Loc. Zola Predosa (province of Bologna, Emilia-Romagna region, 44°30′N, 11°13′E). Necropsy was performed, and samples of lung tissue were fixed in 10% buffered formalin, paraffin-embedded (FFPE), and stained with hematoxylin and eosin and Grocott's methenamine silver techniques. For biomolecular testing, several FFPE 4-μm-thick sections were cut to collect 20 mg of tissue. The sections were dewaxed by lavage with 1.5 ml of xylene (100%) followed by two lavages with 1.2 ml of ethanol (96–100%). After incubating the tissue at 37 C to evaporate the remaining ethanol, DNA was extracted using the QiAmp Tissue DNA Mini Kit (Qiagen, Izasa, Madrid, Spain) following the manufacturer's instructions. Fifty microliters of phosphate-buffered saline buffer were used to elute. A 2-μl sample of DNA extract was used for the polymerase chain reaction (PCR).

Macroscopically, the subject showed minimal subcutaneous and abdominal fat deposits. The pelvis and posterior legs presented multiple fractures with diffuse edema and hemorrhage. In the thorax, multifocal, mild chronic adhesive pleuritis was observed, together with a few ecchymoses in the

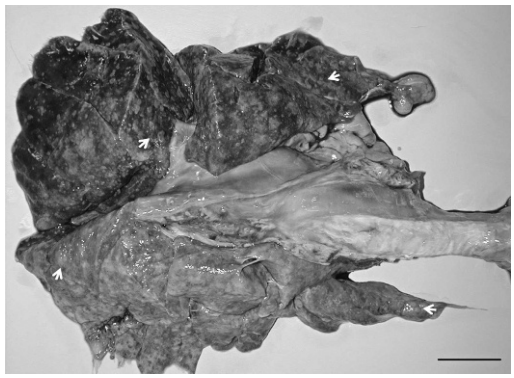

FIGURE 1. Lungs from a crested porcupine (*Hystrix cristata*) from Italy consistent with adiaspiromycosis by *Emmonsia crescens*. Note the presence of multiple disseminated grayish nodules (arrowheads) on the surface that are indicative of granulomas by *Emmonsia crescens*. Bar=3 cm.

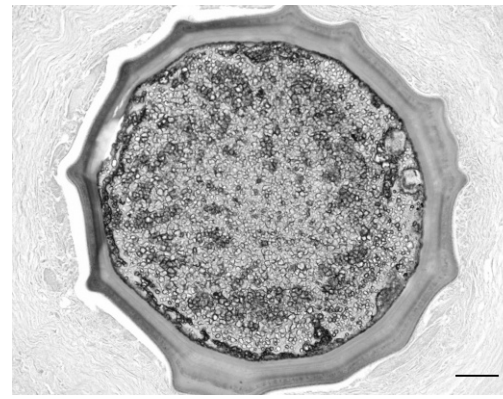

FIGURE 2. Lung tissue from a crested porcupine (*Hystrix cristata*) showing granuloma containing adiaconidia (Grocott's technique). Bar=50 μm.

ventral areas of each caudal lobe and disseminated, severe, granulomatous pneumonia (as whitish-gray scattered nodules, 1–2 mm in diameter with a tendency to conglomerate; Fig. 1).

Microscopically, the lungs displayed severe, diffuse, chronic interstitial pneumonia with multifocal necro-granulomas surrounding cystic structures. These latter (about 150–450 μm in diameter), generally empty or rarely containing basophilic, unstructured clod-like floccular material, had an eosinophilic, amorphous wall about 85–100 μm thick (often crenated or scalloped) surrounded by epithelioid macrophages and rare giant cells, with neutrophils and some lymphocytes scattered in afibrous capsules. The inflammatory infiltrate was more severe and centrally necrotic where cystic structures were damaged. The walls of the cystic structures stained black (positive argentaffin reaction) with Grocott's technique (Fig. 2).

The described macro- and microscopic characteristics were consistent with pulmonary adiaspiromycosis: the first reported in Italy and in a crested porcupine. A panfungal PCR assay confirmed that *E. crescens* was involved. DNA extracted from FFEP samples was employed to amplify the ITS1 region of ribosomal DNA using the primers described by White et al. (1990). Amplified fragments were sequenced and compared with the nucleotide sequence database available in the Micology Laboratory (Majadahonda, Madrid) and with the GenBank database (http://www.ncbi.nih.gov/GenBank/). The sequence matched those of *Emmonsia crescens*. The percentage of identity with both databases was 99%.

Conidia (spores) of *Emmonsia* spp. are commonly present in the environment as saprophytic soil fungi (Burek, 2001), and hosts generally become infected by inhaling the dustborne spores. The severity of the disease in the lungs depends on the immune response and on the amount of dust-borne conidia inhaled. The conidia do not replicate or diffuse in the host organisms, but they progressively enlarge to form thick-walled spherules (adiaconidia or adiaspores; Mörner et al., 1999; Seixas et al., 2006). The adiaspores of *E. parva* grow to up to 20 to 40 μm (Burek, 2001), whereas *E. crescens* adiaspores reach diameters of up to 500 μm (Simpson and Gavier-Widen, 2000). Despite cases of fatal adiaspiromycosis being reported in humans (Peres et al., 1992) and wild animals (Simpson and Gavier-Widen, 2000), this case, though severe, would not seem to be directly responsible for death. The multiple fractures of pelvis and

posterior legs with severe and diffuse edema and hemorrhages suggest a traumatic event, probably by motor vehicle. Underlying disease conditions (intoxications and diseases provoked by viruses, bacteria, parasites, or prions) can cause behavioral changes (Gagnaire et al., 2011), increasing the incidence of traumatic death (Cooper, 1996; Krumm et al., 2005). The case is especially important in light of a recent report of an environmentally linked outbreak of conjunctivitis in humans (Mendes et al., 2009), suggesting the possibility of similar epidemiologic behavior for wild species living in contaminated environments like burrows and tunnels where the crested porcupine could be.

We thank D. C. Cabellos and I. Pelegrín (IDIBELL-Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona) for their helpfulness and LIPU (Italian League for Bird Protection) of Bologna for referring the subject. We also thank S. Ghiotto for excellent laboratory work.

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Submitted for publication 9 August 2011.
Accepted 13 November 2011.