

First description of adiaspiromycosis in an Eurasian otter (*Lutra lutra*) in Italy

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Keywords

Adiaspiromycosis,
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Summary

Adiaspiromycosis is a pulmonary disease caused by the inhalation of the ubiquitous fungus *Emmonsia* spp., a common soil inhabitant. Information about the replication and dissemination of the fungus from the primary site is lacking. Members of the Family *Mustelidae* seem to be highly susceptible to this infection, which has been previously reported in otters (*Lutra lutra*) in Czech Republic/Slovakia, Finland and in the UK. In many cases, *Emmonsia*-associated lesions have also been reported as incidental findings during necropsies of otherwise healthy animals. A road-killed male Eurasian otter was submitted for the post-mortem examination on 21st December 2009 at the Veterinary Pathology Unit of the Faculty of Veterinary Medicine of Teramo, as part of the RECAL [REcovery and post-mortem Analysis of Eurasian otters (*Lutra lutra*) in the National Park of Cilento, Vallo di Diano and Alburni (Salerno, Italy), and surrounding areas] project. Histologically, multifocal round structures with a PAS-positive thick tri-laminar wall and a central basophilic granular mass were observed within the alveoli. The adiaspores were surrounded by a severe granulomatous reaction with high number of macrophages, multinucleated giant cells, eosinophils, neutrophils and fibroblasts. Numerous multifocal cholesterol granulomas were observed close to those fungal-induced. To the best of our knowledge, this is the first description of adiaspiromycosis in an Eurasian otter in Italy.

Prima descrizione di un caso di adiaspiromicosi in lontra eurasiatica (*Lutra lutra*) in Italia

Parole chiave

Adiaspiromicosi,
Granuloma colesterinico,
Lontra,
Polmone,
RECAL.

Riassunto

L'adiaspiromicosi è una malattia fungina polmonare causata dall'inalazione di spore di *Emmonsia* spp., funghi filamentosi ubiquitari frequentemente isolabili dal suolo, caratterizzati da assenza di moltiplicazione e disseminazione a partire dal sito primario di infezione. I Mustelidi sembrano particolarmente suscettibili, l'infezione è stata descritta nella lontra in Cecoslovacchia, Finlandia e Gran Bretagna. Nella maggior parte dei casi le lesioni sono state rilevate occasionalmente in esame necroscopici su animali morti per altre cause. L'adiaspiromicosi, generalmente, non ha significato patologico nella lontra, sebbene sia stato descritto un caso mortale. In Italia, nell'ambito del progetto RECAL [RECupero e Analisi post-mortem di esemplari di Lontra (*Lutra lutra*), nel Parco Nazionale del Cilento, Vallo di Diano e Alburni (Salerno) e aree contigue] un esemplare maschio subadulto di lontra eurasiatica, rinvenuto morto con evidenti lesioni traumatiche, è stato conferito per l'esame necroscopico il 21 Dicembre 2009, presso l'Unità di Ricerca di Anatomia Patologica della Facoltà di Medicina Veterinaria di Teramo. L'osservazione microscopica di campioni di tessuto polmonare, sottoposti a processazione istologica di routine, ha evidenziato negli spazi alveolari la presenza di formazioni tondeggianti multifocali con spessa parete trilaminare intensamente PAS positiva e presenza nella parte interna di materiale granulare basofilo amorfo. Le formazioni sono risultate circondate da zone con intensa reazione granulomatosa caratterizzata da numerosi macrofagi, cellule giganti multinucleate, granulociti eosinofili e neutrofili, fibroblasti. Nel tessuto adiacente sono stati rilevati numerosi granulomi colesterinici. Gli autori presumono che il lavoro rappresenti la prima segnalazione di adiaspiromicosi nella lontra eurasiatica in Italia.

Introduction

Adiaspiromycosis is primarily a mycotic pulmonary disease caused by the inhalation of the ubiquitous, common soil inhabitants, dimorphic fungi of the genus *Emmonsia*: *Emmonsia parva* (*E. parva*) and *Emmonsia crescens* (*E. crescens*) (Chantrey et al. 2006).

Emmonsia crescens has been isolated from over 96 species of animals (Sigler 1996) and it is the most common isolate in Europe, while *E. parva* has been isolated in few species of animals (Morandi et al. 2012, Sigler 1996). The laboratory diagnosis can prove to be difficult for the mycelial stages of *E. parva* and *E. crescens* that are morphologically similar and their differentiation requires the use of polymerase chain reaction (PCR) (Borman et al. 2009). Although at a first sight the histology image of adiaspore resembles a parasite, an accurate analysis the absence of organs allows for correctly identifying the organism (Mörner et al. 1999); which is in turn characterized by large globose, thick-walled, non-proliferating structures called adiaspores (Sigler 1996). The term Adiaspore comes from the Greek terms *adia* and *speirein* meaning negative and scattering, respectively, and therefore adiaspiromycosis describes an infection without replication or dissemination of the fungus from the original site (Emmons and Jellison 1960).

Adiaspores are distributed within the pulmonary alveoli, being surrounded by a granulomatous inflammatory reaction consisting of macrophages, multinucleated giant cells and eosinophils, which are represented by small gray nodules. Infections may be asymptomatic but characterized by a granulomatous pneumonia, and the severity depends on the number of adiaspores and the host immunity.

Adiaspiromycosis has been described as pulmonary infection due to spores inhalation in mammals (Hamir 1999), particularly in different wild species of rodents and mustelids that are highly susceptible to this infection (Burek 2001, Simpson et al. 2013). The disease has been previously described in otters (*Lutra lutra*) in Czech Republic/Slovakia (Krivanec et al. 1976), Finland (Rudback and Stjernberg 1998) and England (Simpson 1998). In most cases, *Emmonsia*-associated lesions represent incidental findings during necropsies of otherwise healthy animals, thus being considered of limited pathological significance. However, fatal adiaspiromycosis has been described in an otter as well (Simpson and Gavier-Widen 2000).

Materials and methods

A male Eurasian otter was found dead on the road SP430 (Latitude 40° 13' 47.17", Longitude 15° 10'

14.73"), near the National Park of Cilento, Vallo di Diano and Alburni (PNCVDA), most likely hit by a car, and submitted for post-mortem examination on 21st December 2009. The animal weighed 6,8 Kg and was 110 cm long from the nose to the tail tip. This case is one of a series of post-mortem examinations performed at the Veterinary Pathology Unit of the Faculty of Veterinary Medicine of Teramo, from 2009 to 2013, as part of the RECAL project established for investigating the causes of death, the overall health, biometric and demographic parameters and levels of contaminants in the Eurasian otter (*Lutra lutra*) population in the PNCVDA and surrounding areas, by means of post-mortem analysis.

Representative tissues of all major organs were fixed in 10% neutral buffered formalin, embedded in paraffin, cut in 5 µm-thick sections, stained with haematoxylin-eosin (H&E) and examined by light microscopy. Selected lung sections containing fungal spherules were also stained with periodic acid-Schiff (PAS) and Grocott's methenamine silver (GMS) stains.

Results

At necropsy, the carcass had multiple to diffuse subcutaneous hemorrhages associated with fractures of both shoulders. Multiple fractures of the thoracic vertebrae, multiple and bilateral rib fractures with laceration of the parietal pleura, rupture of the pericardium and right atrium and severe hemothorax were also detected. In the cardiac cavity, a heartworm (subsequently identified as *Dirofilaria immitis*) was also found, without associated lesions. Other gross findings were multifocal bilateral severe pulmonary hemorrhages and moderate hemoperitoneum with intestinal tract herniation into the thoracic cavity.

Histological examination of lung tissue revealed multifocal scattered round structures within the alveolar space, up to 250 µm in diameter with a 20-30 µm thick tri-laminar wall consisting of a basophilic outer-layer (3.4 µm in diameter), an eosinophilic mid-layer (11 µm in diameter), a pale inner layer (43 µm in diameter) and a basophilic granular retiform center (Figure 1). The adiaspores were surrounded by a severe granulomatous inflammation with high number of epithelioid macrophages, Langhans multinucleated giant cells, eosinophils, neutrophils and fibroblasts (Figure 2). The cell wall was strongly PAS- and GMS-positive (Figure 2a and 2b). Numerous multifocal granulomas containing cholesterol clefts (Figure 3).

Discussion

Based on the fungal shape, staining features and associated histological lesions, pulmonary

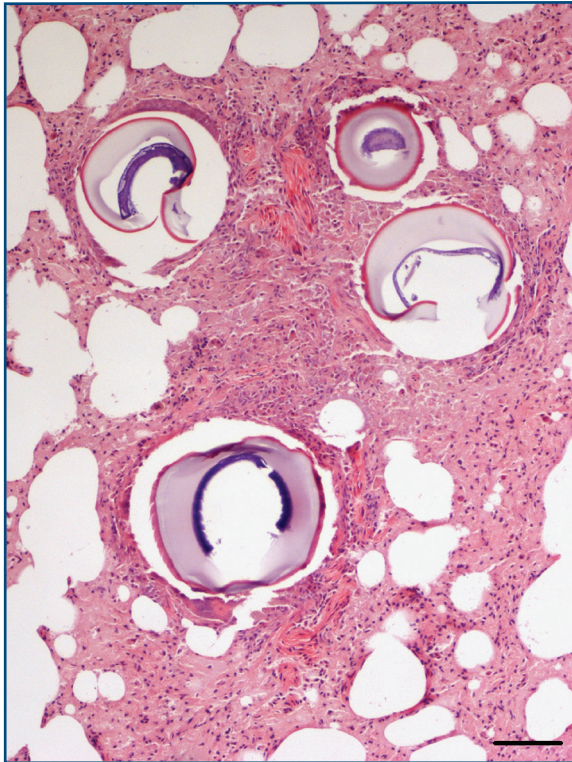


Figure 1. Lung of a male Eurasian otter found near the National Park of Cilento, Vallo di Diano and Alburni (Salerno, Italy; 21.12.2009): multiple variably sized adiaspores randomly scattered in the pulmonary parenchyma (Bar = 110 μ m) (H&E).

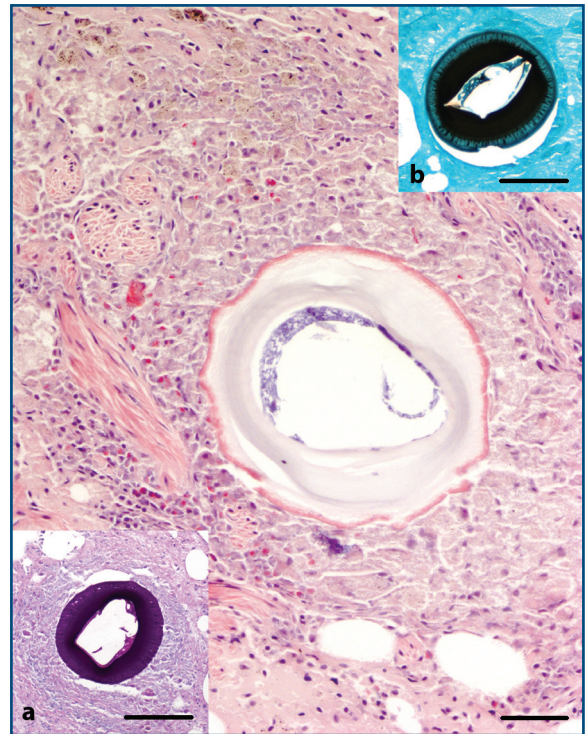


Figure 2. Lung of a male Eurasian otter found near the National Park of Cilento, Vallo di Diano and Alburni (Salerno, Italy; 21.12.2009): adiaspores surrounded by a severe granulomatous inflammation (Bar = 55 μ m) (H&E). Insets: strongly PAS- (a) and GMS- (b) positive adiaspores (Bar = 85 μ m).

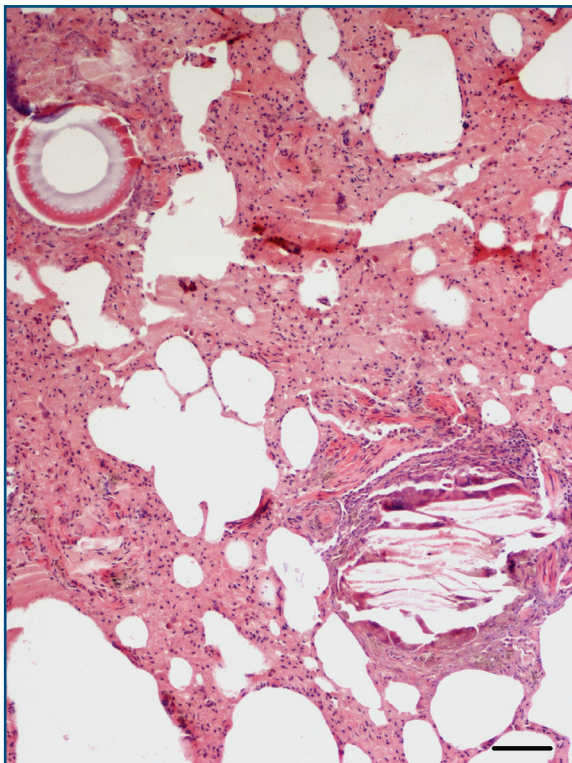


Figure 3. Lung of a male Eurasian otter found near the National Park of Cilento, Vallo di Diano and Alburni (Salerno, Italy; 21st December 2009): a focal granuloma containing cholesterol clefts, associated with adiaspore-induced granulomatous inflammation (Bar = 110 μ m) (H&E).

adiaspiromycosis, due to *Emmonsia* spp. infection was diagnosed (Malatesta et al. 2013). Unfortunately, we could not confirm the species involved, although, considering the worldwide distribution, *E. crescens* infection is the most likely (Borman et al. 2009).

Despite fatal adiaspiromycosis has been observed by Simpson and Gavier-Widen (Simpson and Gavier-Widen 2000) in an otter, in this case the primary cause of death would seem to be the traumatic lesions, due to a car accident. In fact, while the grossly-detectable lung hemorrhages were of traumatic origin, pulmonary lesions referable to adiaspiromycosis were only histologically observable and were not so severe and diffuse to cause clinical signs of respiratory failure. Furthermore, no other organs were affected by the fungal infection. The latter finding confirm the commonly accepted evolution and outcome of this infection in animals, since lesions of adiaspiromycosis are usually confined to the lungs, and the extrapulmonary involvement has been rarely observed only in immunocompromised human patients (Echevarria et al. 1993).

In Italy the adiaspiromycosis was described for the first time by Splendore (Splendore 1920) in voles and many years later in a wild rabbit (Rosmini et al. 1989), in small mammals (Gallo et al. 1962) and

in a Crested porcupine (Morandi *et al.* 2012). To the best of our knowledge, this is the first description of adiaspiromycosis in an Eurasian otter in Italy.

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