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Short communication

Pulmonary adiaspiromycosis in the Eurasian beaver (*Castor fiber*) inhabiting Poland

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Abstract

Adiaspiromycosis is a rare fungal infection caused by saprophytic fungi *Emmonsia* spp. (type *Ascomycota*) occurring especially in small free-living mammals. The aim of this study was to evaluate the occurrence of histopathological lesions asscociated with adiaspiromycosis in the Eurasian beaver inhabiting Poland. In order to evaluate the presence of natural adiaspiromycosis we systematically investigated beaver populations from north-eastern Poland for adiaspores in the lungs. This study reveals for the first time the presence of pulmonary adiaspiromycosis of Eurasian beaver in Poland. As far as we know, there is no published data regarding pulmonary adiaspiromycosis in human patients in Poland.

Key words: adiaspiromycosis, *Castor fiber*, beaver, Poland

Introduction

Adiaspiromycosis is a rare pulmonary mycosis caused by saprophytic fungi of the genus *Emmonsia* (family *Ajellomycetaceae*, type *Ascomycota*) *Emmonsia parva* and *Emmonsia crescens* (formerly *Chrysosporium parvum* var *crescens* and *C parvum* var *parvum*). Recently a new species *Emmonsia pasteuriana* has been described in an HIV-infected patient (Schwartz et al. 2015). This infection is distributed worldwide, especially among small free-living mammals eg. mice, rats, hedgehogs, otters, ground squirrels, skunks, armadillos, rabbits, mink, weasels and raccoons (Seyedmousavi et al. 2015). To date, only one study has demonstrated adiaspiromycosis in a Eurasian beaver (Morner et al. 1999). *E. crescens* is found in Europe and the United Kingdom, whereas *E. parva* is mainly widespread in some xerothermic regions (Africa, Central Asia, the Americas). Pulmonary adiaspiromycosis has also been reported in humans, especially in immunocompromised hosts, though it is a very rare and unusual disease. The term adiaspiromycosis originated from *adia-* being the negative with the Greek verb *speirein* for scattering which refers to the adiaconidia, the conidia of this fungus, which have the unusual feature of progressive

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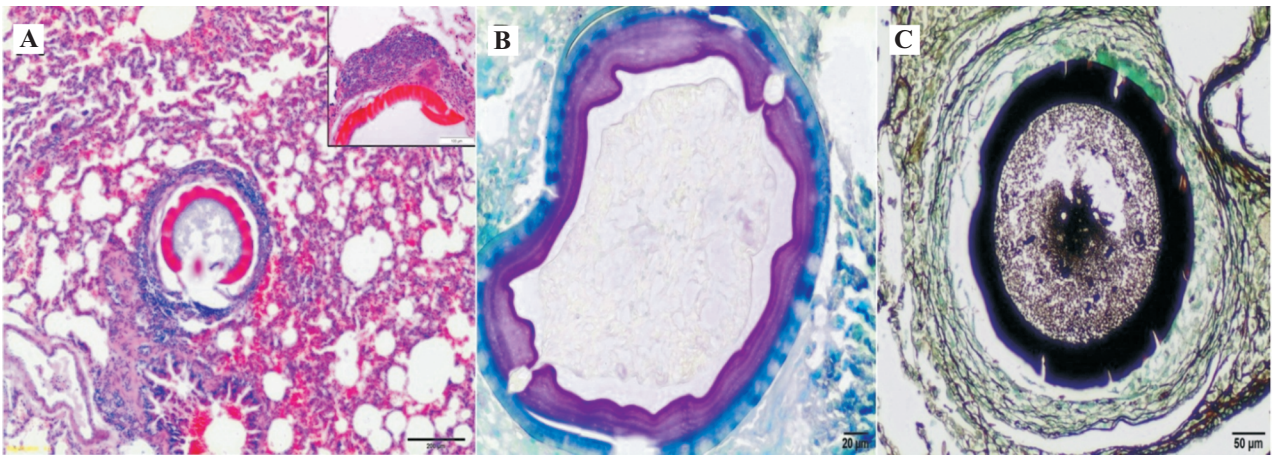

Fig. 1. Histopathological section of beaver lung showing adiaspores. (A) H-E stain. Scale bar = 200 µm. Inset: Adiaspore wall surrounded by granulomatous inflammation. Scale bar = 100 µm. (B). PAS stain. Scale bar = 20 µm. (C) GMS stain. Scale bar = 50 µm.

Table 1. Biometric and haematological data for beaver with adiaspiromycosis.

	Case 1	Case 2
Sex	Male	Female
Age (year)	>2.5	>2.5
Body weight [kg]	22.6	21.9
Body length (with tail) [cm]	114.0	121.0
Body length (without tail) [cm]	87.5	92
Head length [cm]	19.5	19.0
Head width [cm]	13.0	12.0
Thoracic perimeter [cm]	82.0	81.0
Fur color	Black	Black
WBC [10^9/l]	14.83	12.18
RBC [10^{12}/l]	2.25	2.24
Hb [g/dl]	9.5	9.2
Ht [%]	27.8	25.8
MCV [fl]	124	115
PLT [10^9/l]	87	139

WBC – White blood cell, RBC – Red blood cell, Hb – Haemoglobin, Ht – Haematocrit, MCV – Mean cell volume, PLT – Platelets.

enlargement without replication. The fungi are most commonly isolated from soil, and inhalation of dust-borne adiaspores is the main route of infection (Watts and Chandler 1990). The aim of this study was to verify the hypothesis whether adiaspores occur also in Eurasian beaver inhabiting Poland.

Materials and Methods

Twenty five beavers of both sexes (13 females and 12 males), from juveniles to sexually mature individuals were collected in north-eastern Poland (54°23'31''N; 22°55'26''E) with the approval of the Regional Nature Conservation Authority and the Local Ethics Committee. The anesthetized beavers were weighed, morphometric measurements and blood sampling for haematology analysis were performed. After sacrifice by decapitation, tissue samples (lungs, liver, spleen, cardiac muscle, kidneys) were collected and fixed in 10% formalin. Samples were then submitted to the Department of Pathology and Veterinary Diagnostics for histopathology by using hematoxylin-eosin staining (HE). Lung specimens were also stained with Periodic acid Schiff (PAS) and Grocott-Gomori methenamine silver (GMS). The largest diameter of adiaspores were measured using a BX43 microscope with Olympus Cell software.

Results and Discussion

The histopathological examination revealed the presence of adiaspores in 2 out of 25 beavers (8%). These fungal cystic structures were scattered throughout the lungs, round to oval in shape (some cases collapsed), ranging from 163.4 µm to 437.1 µm (mean 312.8 µm) with a thick wall (Fig. 1A). The wall of the spherules appeared trilaminar (or bilaminar), the outer layer was most visibly stained with eosin, the inner layers were slightly eosinophilic or not stained. The wall was stained purple by PAS reaction (the inner layer was most strongly stained) and black by GMS stain (Fig. 1B-C). The spherules were empty or contained fine granular or globular, amphophilic to basophilic material, but without proliferating endospores. We diagnosed these spherules as adiaspores caused by *Emmonsia* sp. infection, based on their morphology, and histopathological and histochemical findings, which were similar to previous reports (Morner et al. 1999, Malatesta et al. 2014, Matsuda et al. 2015). Unfortunately, we could not confirm the species (because of the lack of fresh-frozen lung tissue samples); however, considering the worldwide distribution, *E. crescens* infection is the most probable (Seyedmousavi et al. 2015). The adiaspores were surrounded by chronic granulomatous inflammation with macrophages, Langhans multinucleated giant cells, lymphocytes, some plasma cells, neutrophils, single eosinophils and fibroblasts. The lungs also revealed interstitial inflammation, emphysema, and focal thickening of pleura. The pulmonary lesions were more severe in the case with more adiaspores, which was consisted with the observations that the degree of infection is determined by the number of spores inhaled (Seyedmousavi et al. 2015). Severe pulmonary lesions were observed in the male European beaver, which was in poor physical condition, with sparse subcutaneous and visceral adipose tissue. There were no adiaspores in the beaver's other organs. The spleen demonstrated mild lymphoid depletion, and cardiomyocytes and hepatocytes showed atrophy and degeneration; mild myocarditis and focal calcium deposits were also observed. Haematological parameters for both animals showed low RBC counts, HB, Ht levels and PLT (Girling et al. 2015). Biometric and haematological results are presented in Table 1. Diagnosis of adiaspiromycosis is difficult, because this disease is mostly restricted to the lungs, patients are often asymptomatic, fungal culture is time-consuming and often ineffective and, no reliable serological tests are currently available. Therefore, histopathological examination of lung specimens is considered to be the gold standard. Differential diagnosis should include parasitic pneumonia, inhalation of foreign material, and other fungi, such as *Coccidioides immitis* and *Rhinosporidium seeberi*. Although they both stained with PAS and GMS, they are usually smaller than *Emmonsia* sp., have thinner capsules and contain endospores (Watts and Chandler 1990). To the best of our knowledge, this study reveals for the first time the presence of pulmonary adiaspiromycosis involving Eurasian beavers in Poland, and emphasizes the importance of determining the prevalence of this potential human pathogen in Polish wildlife fauna in the future.

Ethical standards

The research complied with the protocols approved by the Regional Nature Conservation Authority (opinion No. RDOŚ-28-OOP-0007-638/09/10pj 2010) and the Local Ethics Committee (opinion No. 11/2010), and it was conducted in observance of Polish law.

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