Case Report

*Emmonsia crescens* infection in a British water vole (*Arvicola terrestris*)

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*Emmonsia crescens*, a dimorphic fungus of the order *Onygenales*, is primarily a pathogen of lower animals and rarely humans. Inhaled conidia of *E. crescens* fail to germinate in the lungs, and instead simply enlarge in lung tissue to become giant adiaspores. We present here the case of fatal *Emmonsia crescens* infection in a wild-caught British water vole (*Arvicola terrestris*). Histopathological examination of the animal, which died in captivity, revealed a multifocally extensive granulomatous reaction containing oval adiaspores scattered irregularly throughout the lungs. Mycological examination of fungus cultured from lung tissue and PCR amplification and sequencing of rDNA gene fragments of the cultured organism confirmed the diagnosis of massive infection by *E. crescens*.

**Keywords**  *Emmonsia crescens*, water vole, adiaspores, infection

Introduction

Adiaspiromycosis due to *Emmonsia* spp. is a mycotic condition of small mammals and rarely humans, caused by the dimorphic fungi, *Emmonsia parva* and *Emmonsia crescens*. *Emmonsia parva* has been found to be widespread in some xerothermic regions, including parts of the Americas, Central Asia and Africa. Conversely, *Emmonsia crescens* is the main causative agent of adiaspiromycosis in Europe [1]. Inhaled dustborne spores of *Emmonsia crescens* fail to germinate in the host, and instead increase dramatically in volume (up to 1 million-fold) to become thick-walled adiaspores. Thus, in tissues, the fungus is usually present as giant adiaspores, the pathological effects of which range from asymptomatic infection to necrogranulomatous pneumonia and death, depending on adiaspore load and host immunocompetence [2].

Case report

In November 2003, a wild-caught adult female water vole (*Arvicola terrestris*) from the North of England Zoological Society, Chester Zoo, died and was submitted for necropsy that day. The vole, one of 36 water voles captured using Sherman live-traps from an Essex nature reserve that was drained in winter 2003/2004, had been housed in captivity since October 2003 (1 month). The vole was found dead in its cage one morning without showing any clinical signs. Two other animals died whilst in captivity, but post mortem examination was not performed in those cases. No other voles exhibited any external lesions or clinical signs suggestive of fungal infection.

The water vole was severely emaciated and weighed only 93 g (normal weight 70–320 g depending on age and season). Widely distributed across the whole of the lung fields, there were multifocal, beige <1 mm diameter firm nodules which extended throughout the parenchyma (Fig. 1). The lungs were denser than would normally be expected. There was a moderate amount of blood stained pleural fluid and multifocal small fibrous adhesions between the pulmonary and thoracic pleura. Randomly scattered throughout the pulmonary...
interstitial tissue and alveolar spaces were multifocal oval, 600–800 μm encapsulated structures with a variably thick, trilaminar hyaline, periodic acid-Schiff reagent-positive wall (50–100 μm) and containing basophilic foamy amorphous material (adiaspores; Figs. 2 and 3). The surrounding lung tissue had a multifocal to coalescing moderate to severe infiltrate of macrophages with lesser numbers of lymphocytes and plasma cells and associated cell debris, multifocal mild haemorrhage and vascular congestion. In some areas, the bronchial epithelium was moderately hyperplastic. Focally, at the pleural surface and extending locally into the alveolar spaces and airways, there were branching fungal mycelia with 8–15 μm diameter, non-parallel walled, septate hyphae which branched dichotomously (Fig. 4). Gross and histological features lead to the diagnosis of multifocal to coalescing, severe, chronic, granulomatous pneumonia with multiple intralesional adiaspores and a focal moderate to severe necrotising pleuritis with septate fungal hyphae.

Culture of small sections of lung on glucose-peptone agar containing chloramphenicol (0.05 mg/ml) at 30°C yielded moderately spreading, white floccose to powdery colonies of mould. Microscopic examination of fungal cultures revealed numerous small, single-celled conidia borne singly on hyaline slender terminal or lateral stalks or inflations (Fig. 5A). Conidia were round to sub-spherical, 2–4 μm in diameter, smooth, thin-walled, and with narrow basal scars. These morphological features were consistent with *Emmonsia* spp. [3,4]. When maintained on brain-heart blood media at 37°C for several weeks, mould growth arrested and colonies became waxy and cerebriform. Under these
conditions, blastic micro-conidia swelled rapidly to yield spherical thick-walled adiaspores with diameters exceeding 100 μm (Fig. 5B). These adiaspores, which were morphologically indistinguishable from those observed in lung sections, are consistent with *Emmonsia crescens*, rather than *Emmonsia parva* [3,4]. Effectively, *Emmonsia parva* produces adiaspores only above 40°C, and adiaspore size rarely exceeds 20–40 μm, whereas *E. crescens* adiaspores are produced at temperatures in excess of 30–37°C (depending on the isolate; [3]) and routinely reach diameters of up to 500 μm [4]. That the organism was indeed *E. crescens* was definitively proved by PCR amplification and sequencing of a region of the nuclear rDNA repeat region, which generated a DNA sequence 100% identical to sequences of *E. crescens* in the published databases (data not shown). This strain of *E. crescens* has been included in the National Collection of Pathogenic Fungi (NCPF) housed at the Health Protection Agency Mycology Reference Laboratory, Bristol as NCPF 4268.

**Discussion**

*Emmonsia* species are ubiquitous filamentous fungi isolated most commonly from soil and occasionally from mammalian species, such as small rodents and insectivores [1,5–7], otters [8], stoats, weasels [9], moles [10] and ground squirrels [11]. Previous reports of *E. crescens* in water voles, mainly emanating from central and Eastern Europe, have suggested that between 10% to in excess of 50% of examined animals are infected [1,12–16]. In certain reports, as in the case presented here, macroscopically visible granulomas were observed in the lungs and adiaspore burdens were extremely high (more than 1000 adiaspores per animal [1,15]). In this respect, the large size of the individual adiaspores observed in the vole examined in the present study and the fact that the animal had been in captivity only for one month certainly argue that the animal acquired the infection in the field, prior to capture.

*Emmonsia crescens* is most frequently encountered as a dimorphic fungus due to its ability to produce adiaspores at 37°C and mycelia at lower temperatures. *Emmonsia* is also an occasional cause of human infections [2,19]. *Emmonsia crescens* has been reported world-wide and is the main source of human infection causing adiaspiromycosis, an asymptomatic pulmonary infection which may disseminate in immunocompromised hosts, such as patients with AIDS [17,18]. Adiaspiromycosis develops following inhalation of conidia of *Emmonsia* spp., which enlarge to form adiaspores in the alveoli and subsequently hinder regular pulmonary functions. In immunocompetent humans, adiaspores do not reproduce and remain localized at their primary implantation site, which can eventually become calcified and lead to a minimal reaction in the host tissue [2] or develop into a granulomatous pneumonia [19].

It is interesting that the lungs of this animal were apparently co-infected with *Emmonsia crescens*, and another, non-identified fungus. We believe that the septate hyphae evidenced in a localized part of the animal lung are extremely unlikely to be the mycelial phase of *Emmonsia crescens*. Firstly, the strain of *E. crescens* infecting this water vole stops growing in the mycelial phase and produces only adiaspores at temperatures little in excess of 30°C (unpublished data). Secondly, the vole was submitted to necropsy on the day of death, ruling out the possibility that...
Adiaspores could have germinated and invaded the animal’s lung as mycelium after death. As we were able only to culture *E. crescens* (and an unidentified yeast) from the animal’s lungs, the nature of this second fungal pathogen remains unidentified. However, our failure to culture this second species may well reflect the fact that it was causing only a localized infection of a small part of the lung, as compared to the adiaspiromycosis, which was widespread throughout the entire lung tissue. Given the extremely high adiaspore load in this animal, and the resulting widespread disruption of lung tissue (Fig. 2), we believe that adiaspiromycosis due to *Emmonsia crescens* was indeed the cause of death.

Finally, since several reports have now demonstrated isolated cases of *Emmonsia crescens* infection in British mammals (5,6,8,10; this report), it will be important to determine the prevalence of this potential human pathogen in the UK fauna.

**References**
