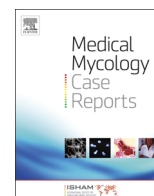




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## Disseminated histoplasmosis in a domestic cat imported from the USA to Austria



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### ABSTRACT

We present a case of disseminated histoplasmosis in a domestic cat imported from the USA to Austria. Histopathological examination revealed a systemic mycosis with most severe involvement of the lungs suggestive of *Histoplasma (H.) capsulatum*-infection. Molecular confirmation was based on polymerase chain reaction (PCR) and sequence analysis of a fungal culture from liver samples. This is the first case of feline histoplasmosis proven by molecular diagnostic technique in Europe and reported in Austria, etc.

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## 1. Introduction

Histoplasmosis, a fungal disease caused by the dimorphic fungus *Histoplasma (H.) capsulatum* can affect both human beings and animals worldwide, with highest prevalence in the Midwest and South of the United States and the Near East. The organisms are especially found in soil contaminated by bird and bat droppings. Infections occur by inhalation of conidia and may be subclinical, but can also cause pulmonary disease and lymphogenous and hematogenous spread to different organs with affinity to the reticuloendothelial system resulting in spleno- and hepatomegaly [1]. The clinical signs in cats such as weakness, lethargy, emaciation, ocular signs, skeletal involvement, fever, anorexia, and respiratory signs, are often chronic and nonspecific despite disseminated disease [2].

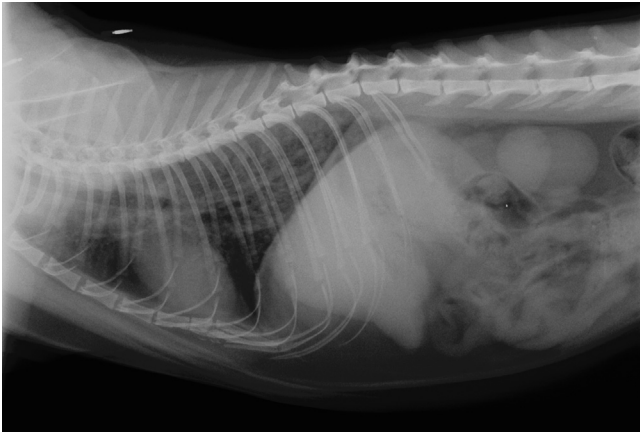
Histoplasmosis is comparatively rare in Europe. According to the European Confederation of Medical Mycology Working Group Italy, Germany and Turkey are considered to be endemic regions for this disease [3]. In Austria reports on histoplasmosis in animals include one badger (*Meles meles*) [4] and one chinchilla (*Chinchilla chinchilla*) [5]. To our knowledge reports on *H. capsulatum* concerning cats in Europe are restricted to one case in Italy [6] and Turkey [7]. Similar is the situation of human histoplasmosis in Europe, where most patients are immigrants or returning from endemic areas [8].

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## 2. Case

A six-year-old, female domestic shorthair cat had originally been a stray kitten found in Texas, USA with severe flea infestation and emaciated. Until the age of six months the animal used to live outdoors settled in the wood around opossums. Then the cat was taken to the household, but still moved outdoors until the owners removed to Austria at the age of four years. After importation the animal was kept strictly indoors. Two years later, at the age of six years, the cat's owner was hospitalized four weeks at day-30. During this period relatives took care of the pet. On day 0 the owner returned home and noticed that the cat from that time demonstrated lethargic behavior. On day +160 the owner observed dyspnea of the cat, which was presented for clinical examination to the veterinarian on day +161. Auscultation of the lungs revealed increased lung sounds and a physiological body temperature of 38.4 °C. Ventrodorsal and laterolateral thoracic radiography was performed and revealed a generalized interstitial pattern of the lung (Fig. 1). 5 mg/kg enrofloxacin (Baytril<sup>®</sup>), 0.08 mg/kg dexamethasone (Voren<sup>®</sup>), and 0.5 ml 10% butaphosphan plus cyanocobalamin (Catosal<sup>®</sup>) were administered once subcutaneously. The cat was presented again at the veterinarian on day +162. At that time the dyspnea was much worse. The animal was placed into an oxygen tent and died within 30 min after intravenous application of 10 mg furosemide (Lasix<sup>®</sup>).

Subsequently necropsy was performed and tissue samples were fixed in 4% neutral buffered formalin, paraffin embedded, sectioned and stained with hematoxylin and eosin (HE) and

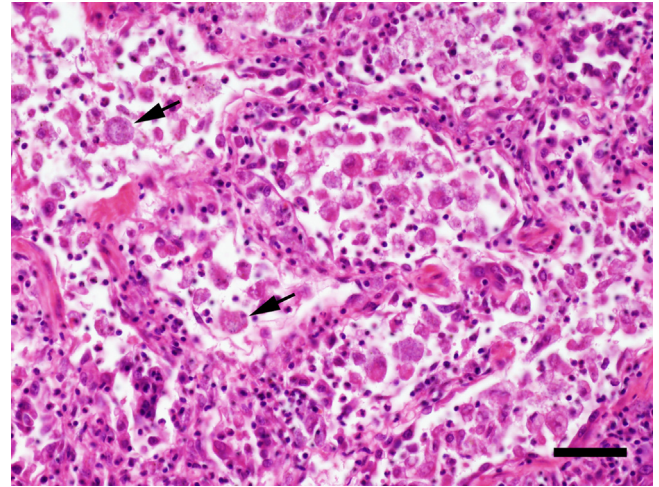


**Fig. 1.** Lateral radiography showing generalized peribronchial nodular pattern of the lung.

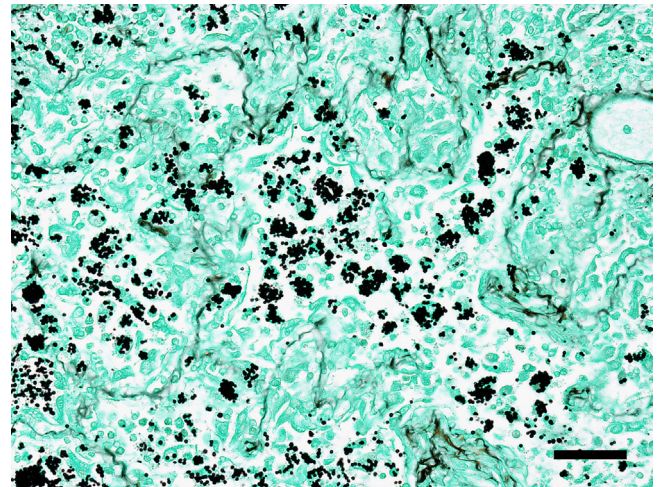
Grocott. The cat was in a good body condition. The lung showed a moderate alveolar edema, was moderately enlarged, inhomogenous and firm. The liver was moderately enlarged with multiple subcapsularly localized sinusoidal dilations. The spleen showed a severe swelling with erythrophagocytosis and histiocytosis. There was a small amount of serohemorrhagic transudate in the thoracic cavity. Histological examination of the lungs revealed a severe diffuse interstitial to suppurative pneumonia (Fig. 2). Hyperplasia of type II pneumocytes and alveolar histiocytosis were further prominent findings. Additionally there was a moderate interstitial edema. Numerous fungal elements suggestive of *H. capsulatum* were present within alveolar macrophages (Fig. 3) and to a lesser extent in brain, liver, kidneys, spleen and adrenal glands. Further findings include a moderate lymphocytic interstitial nephritis and tubulonephrosis, lymphocytic adrenalitis and periadrenitis and lymphoplasmocytic choroid plexitis.

Samples of the liver were cultivated at 28 °C on Sabouraud dextrose agar (SAB) (Becton Dickinson, Heidelberg, Germany), subcultivated on Columbia CNA Agar with 5% Sheep Blood, Improved II (Becton Dickinson, Heidelberg, Germany) and incubated at 35 °C for 10 days. A thermally dimorphic fungal isolate (t975\_12) resembling *H. capsulatum* was recovered from liver samples by cultivation on SAB (Fig. 4) and Columbia CNA Agar with 5% Sheep Blood for 17 days.

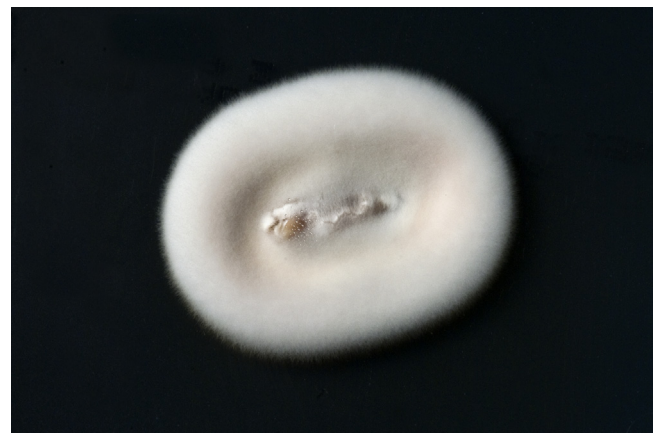
Finally, DNA extraction and PCR amplification of the 18S rRNA were performed as described elsewhere [9]. A part of ITS1, the 5.8S rRNA gene, and ITS2 were amplified as published previously [10]. BLASTn with default settings (<http://www.ncbi.nlm.nih.gov/BLAST>) [11], was used to compare both obtained sequences to the non-redundant DNA database sequences. The sequence was aligned with the 50 most similar sequences deposited in GenBank in order to define the levels of relatedness among fungal isolate t975\_12 and isolates from other countries. by using the Mega version 5 [12]. The same software was used for phylogenetic analyses. For multi-locus sequence analysis (MLSA) of the four loci *arf*, *H*-anti, *ole*, and *tub1* primer and PCR conditions were used as described elsewhere [13]. Sequence alignments of 80 unique haplotypes of *H. capsulatum* isolates were downloaded from TreeBase (<http://www.treebase.org/treebase-web/search/study/summary.html?id=1063>) and the sequences of t975\_12 were aligned manually. Phylogenetic analyses were performed as described above. The size of the small-subunit rDNA was approximately 1700 bp. Comparison with corresponding sequences showed that the t975\_12 sequence shared high similarity scores (> 99%) with *Ajellomyces (A.) capsulatus* (teleomorph of *H. capsulatum*) strain ATCC 11408 (X58572). An approximately 560 bp fragment of ITS from t975\_12 shared 100% identity with three



**Fig. 2.** Diffuse severe interstitial to suppurative pneumonia with multiple alveolar macrophages laden with *Histoplasma capsulatum* organisms (arrows), Hematoxylin and Eosin. Bar=80 µm.



**Fig. 3.** Numerous fungal elements within airways and alveolar macrophages, Grocott staining. Bar=80 µm.



**Fig. 4.** Colony of *Histoplasma capsulatum* on Sabouraud dextrose agar, incubated at 28 °C for 17 days.

sequences originated from *A. capsulatus* USA strains H9 (AF322378), IFM41329 (AB055228), and H79 (AB071770) (Fig. 5). In order to analyze the phylogenetic placement of t975\_12, MLSA

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