



Phaeohyphomycosis in a Snow Leopard (*Uncia uncia*) due to *Cladophialophora bantiana*

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Summary

Phaeohyphomycosis caused by *Cladophialophora bantiana* was diagnosed in a 5-month-old snow leopard with spastic paralysis of the hind legs and inability to defaecate or urinate. At post-mortem examination, a greenish soft mass resembling an abscess was found on one side of the epidural space at the fourth lumbar vertebral body. Histological examination revealed a purulent meningitis with myelomalacia. Dematiaceous fungal hyphae, present within the inflammatory infiltrate, were identified as *C. bantiana* by culture and sequence analysis of the 18S ribosomal RNA gene. This neurotropic fungus rarely affects organs other than the brain in human beings and cats, and has been reported only occasionally in Europe. The case described suggests that phaeohyphomycosis due to *C. bantiana* infection may be recognized more frequently in the future and the possible involvement of organs other than the brain should be borne in mind.

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Cladophialophora bantiana is a pigmented fungus with a worldwide distribution. The fungus is also known under the names *Cladosporium bantiana* and *Xylohypha bantiana* (Matsumoto and Ajello, 1998). In human beings, infection with *C. bantiana* usually causes brain abscesses, but a recent review of the literature referred to a single case in which the spinal cord was affected (Revankar *et al.*, 2004). Phaeohyphomycosis due to *C. bantiana* has repeatedly been observed in cats and occasionally in the dog (Shinwari *et al.*, 1985; Abramo *et al.*, 2002; Bouljihad *et al.*, 2002; Mariani *et al.*, 2002; Eliesi *et al.*, 2003; Schroeder *et al.*, 2004). Until recently, the disease in cats was observed only outside Europe, usually with involvement of the central nervous

system (CNS), especially the cerebrum, as has been described in human beings (Mariani *et al.*, 2002; Shields and Castillo, 2002). Cases of cutaneous and systemic phaeohyphomycosis, the latter without CNS involvement, were reported in cats from Italy and France (Abramo *et al.*, 2002; Eliesi *et al.*, 2003). This paper describes an unusual case of *C. bantiana* infection of the spinal cord in a captive snow leopard (*U. uncia*).

A female snow leopard aged 5 months from the Zoologischer Garten Basel developed sudden onset spastic paralysis of both hind legs. The animal was unable to defaecate or urinate. Haematology revealed a leucocytosis with a left shift. The urine contained low concentrations of glucose and acetone. X-rays of the vertebral column showed no abnormalities. The animal was treated with fluids, antibiotics and glucocorticoids; there was no

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clinical improvement and due to the poor prognosis euthanasia was performed 4 days after the onset of signs. A post-mortem examination was carried out about 12 h later at the Zentrum für Fisch-und Wildtiermedizin, Institut für Tierpathologie, Universität Bern, Switzerland.

The carcass was fresh and in good bodily condition. The spinal canal at the fourth lumbar vertebral body contained greenish-yellow liquid material and greyish material covered the dura on one side and ventrally. Cross-section of the spinal cord revealed a red and white mottled appearance. The urinary bladder was markedly dilated with urine of normal appearance, but the urethra and other organs were without any pathological changes. The rectum contained hard dry faeces.

Samples of major parenchymal organs, including lung, heart, liver and kidneys, as well as the entire spinal cord, were collected, fixed in buffered 10% formalin, dehydrated and embedded in paraffin wax. Sections were cut and stained with haematoxylin and eosin for routine histopathology. Significant lesions were present only in the spinal cord. Unilaterally, the epidural fat was massively infiltrated by neutrophilic granulocytes (often of a degenerate appearance), lesser numbers of macrophages, and occasional eosinophilic granulocytes. Lymphocytes and plasma cells were less common. Inflammation extended into the dura, which contained multifocal infiltrates of lymphocytes and plasma cells with rare eosinophilic granulocytes. Clumps of slender, elongated, septate, brown hyphae with parallel walls were present within the inflammatory infiltrate (Fig. 1). The changes in the adjacent white matter of the spinal cord were characterized by swelling of axonal sheaths and axons with axonophagia; these changes, which

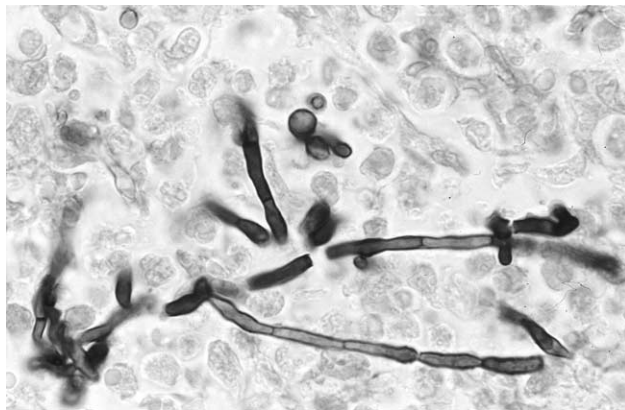


Fig. 1. Grocott-stained section showing pigmented fungal hyphae of *Cladophialophora bantiana* in the spinal cord meninges. $\times 400$.

were moderate and multifocal, affected the lateral and the ventral funiculi of L₄. Multifocal small areas of haemorrhage were present in the grey and white matter. Grocott staining enhanced the visibility of fungal hyphae, none of which were present in multiple sections of other areas of the spinal cord. There were no significant lesions in other organs.

Samples collected at necropsy for bacteriological and mycological testing included liver, spleen, kidney, lung, small and large intestine and spinal cord. No pathogenic microorganisms were isolated from liver, spleen, kidney or lung, but the intestine contained *Salmonella enterica* subsp. *enterica*. Pus from the epidural abscess was cultured at 37 °C on sheep blood agar and bromophenol blue lactose agar, and in thyoglycollate broth. After 48 h numerous tiny colonies of hyphal growth were observed. Incubation was continued further and pus was then also cultured at 25 °C on Sabouraud dextrose agar containing chloramphenicol. Dark grey to black fungal colonies with a short greenish aerial mycelium, resembling colonies of *Cladophialophora* sp., grew readily on all media. Direct smears of the purulent material mixed with KOH (20%) showed numerous brown hyphae. For further identification of the species, samples were sent to the Institut für Medizinische Mikrobiologie, Universität Zürich. There they were subjected to a polymerase chain reaction (PCR) amplification with ITS (internal transcribed spacer) 1 and ITS4 primers (White *et al.*, 1990). The amplicon was sequenced on an ABI PRISM™ 310 Genetic Analyser sequencer (Applied Biosystems, Rotkreuz, Switzerland); the resulting sequence was analysed with the FASTA program and compared with other sequences of the *Cladophialophora* genus published in GenBank (NCBI) and SeqWeb2.1.0. The sequence was identical with a sequence submitted under accession number AF131079 and only one mismatch was detected at base pair 36 when the sequence was compared with sequences AF397182 and AB091211, which code for the ITS of the ribosomal RNA gene of *C. bantiana*.

A diagnosis of a purulent spinal meningitis with myelomalacia due to infection with *C. bantiana* at the level of L₄ was made.

This case of phaeohyphomycosis in a snow leopard was unusual as it affected the lumbar spinal cord. So far, myelitis due to *C. bantiana* infection is documented in human beings only, in whom usually the brain is affected (Shields and Castillo, 2002; Revankar *et al.*, 2004). Disease in domestic cats also affects the brain, and only occasionally is there cutaneous or systemic disease

(Shinwari *et al.*, 1985; Abramo *et al.*, 2002; Bouljihad *et al.*, 2002; Mariani *et al.*, 2002; Eliesi *et al.*, 2003). The route of infection in phaeohyphomycosis is unknown but is thought to be haematogenous in cases of brain abscess, whereas wound infection was assumed in a cat with a cutaneous lesion (Abramo *et al.*, 2002; Revankar *et al.*, 2004). Additional possible routes discussed include the eye (Eliesi *et al.*, 2003). The reason for the location of the fungus in the vertebral canal of the snow leopard remains unclear, but previous damage to the spinal cord, for example by an in-coordinated jump, might have led to localization of a blood-borne infection. The pathological changes in the spinal cord itself were probably due to a combination of pressure from the epidural infiltrate, cytokine release from the inflammatory cells and haemorrhage. The extent and severity of the lesions readily explained the clinical signs.

There is debate as to whether *C. bantiana* is an opportunistic agent or a true pathogen (Revankar *et al.*, 2004). In human beings it demonstrates neurotropism and more commonly affects immunocompetent than immunocompromised persons (Brandt and Warnock, 2003). In contrast, cases of systemic phaeohyphomycoses in immunocompromised persons are usually not due to *C. bantiana* but to other fungi (Revankar *et al.*, 2002). The exact immune status of the affected snow leopard is unknown; however, it had no signs of other diseases and had been a healthy animal from birth until the onset of the paralysis, suggesting that it was not suffering from an immune system dysfunction. In contrast, in dogs, *C. bantiana* infection seems to manifest itself as a systemic disease, possibly secondary to other diseases or to a lymphopenic state (Lobetti, 1996; Anor *et al.*, 2001; Schroeder *et al.*, 2004).

Sequencing often results in a more accurate identification than that provided by the morphological analysis alone. The sequences of morphologically similar fungi may show striking differences. It is therefore possible to distinguish *C. bantiana* unambiguously from other fungi by means of the ITS region as the target for sequencing. There is only 0–1% variability in sequences within the species *Cladophialophora*, but more than 10% variability when compared with sequences from other species of the same genus.

C. bantiana survives in the soil and other feline cases have been reported from areas with a humid, warm climate (Shinwari *et al.*, 1985; Abramo *et al.*, 2002; Mariani *et al.*, 2002). The snow leopard in this report was born in Switzerland at the beginning of the warm summer season, which may have favoured

propagation of the fungus and led to the infection. The distribution of *C. bantiana* in continental Europe is at present unknown and reported cases are few. It is possible, however, that feline and canine cases may be seen more frequently in the future.

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