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## SHORT PAPER

## Spinal Cryptococcoma in an Immunocompetent Cat

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### Summary

This report describes an unusual case of primary cryptococcoma in the proximal thoracic spinal cord of an 11-year-old immunocompetent cat from a farm on which there were large numbers of pigeons. This animal was referred for examination with progressive paralysis and shown to be free from feline immunodeficiency virus, feline leukaemia virus, feline coronavirus and *Toxoplasma gondii*. It died 2 months later. At necropsy, the only lesion detected was a malacic area, 4 cm in length, in the spinal cord. Histopathological examination of the spinal cord revealed severe granulomatous inflammation associated with large numbers of encapsulated yeast cells. In addition to the granulomatous host response, necrosis, digestion chambers, Gitter cells, spheroids and lymphocytic perivascular cuffs were features of the malacic areas. Immunohistochemistry confirmed the presence of *Cryptococcus neoformans* var. *grubii* yeast cells.

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*Cryptococcus neoformans* is a dimorphic fungus. It has a worldwide distribution and has been recovered from numerous environmental sources, including soil and avian excreta (Bicanic and Harrison, 2004; Shoham and Levitz, 2005). Based on genetic characteristics and capsular antigen, two varieties have been defined, namely *C. neoformans* var. *neoformans* (serotype D) and *C. neoformans* var. *grubii* (serotype A), as well as a hybrid between serotypes A and D. The closely related *Cryptococcus gattii* (serotypes B and C) was formerly considered a variety of *C. neoformans* (Franzot *et al.*, 1999). Cryptococcosis is thought to result primarily from inhalation of infectious propagules (probably basidiospores) (Rodrigues *et al.*, 1999) with initial localization in the respiratory tract and then dissemination to other tissues, particularly brain (Rodrigues *et al.*, 1999; Shoham and Levitz, 2005; Caswell and Williams, 2007; Lopez, 2007). Grossly,

in central nervous system (CNS) tissue, multiple small lesions with a viscous, gelatinous appearance are often seen. Histological lesions generally consist of numerous yeast cells that present the classically described “soap-bubble” appearance, because of the unstained capsule. There is a variable, often minimal, inflammatory host response, consisting of macrophages, lymphocytes and plasma cells (Summers *et al.*, 1995; Caswell and Williams, 2007; Lopez, 2007). The severity of the inflammatory response is dependent on the balance between the yeast's virulence factors and the immunological status of the host. When the lesion is well delimited it is called a cryptococcoma.

Cryptococcosis is often fatal in man and is associated with immunosuppression. In animals, it is most common in adult intact cats and dogs, but it occurs occasionally in horses and rarely in other species (Summers *et al.*, 1995; Mandrioli *et al.*, 2002; Caswell and Williams, 2007; Lopez, 2007). Immunosuppression of cell-mediated immunity, caused by feline immunodeficiency virus (FIV) or feline leukaemia

virus (FeLV) in cats or by *Ehrlichia canis* or long-term glucocorticoid therapy in dogs, may increase susceptibility to cryptococcosis (Lopez, 2007); there is, however, some disagreement about the predisposing effect of immunosuppression in animals (O'Brien *et al.*, 2004).

CNS cryptococcosis without respiratory tract involvement has not previously been reported in animals. The present report describes an unusual case of spinal cryptococcoma in an apparently immunocompetent cat.

An 11-year-old male neutered cat was referred with a progressive paraparesis. On neurological examination, hindlimb conscious proprioceptive deficits with normal spinal reflexes were observed. A clinical diagnosis of upper motoneuron syndrome, probably with a lesion in the thoracolumbar spinal cord (T3–L3), was made. The most likely differential diagnoses for a lesion at this site were considered to be (1) a disorder of growth (e.g., a neoplasm such as lymphosarcoma), (2) an inflammatory lesion associated with an infectious disease (e.g., feline infectious peritonitis, toxoplasmosis, cryptococcosis or other mycosis), or (3) a traumatic lesion, such as that produced by disc prolapse. Lateral and dorsoventral radiography and myelography of the thoracolumbar vertebral region revealed no significant abnormalities. Complete blood count, serum biochemistry, serum protein electrophoresis, abdominal and cardiac ultrasound examination, coproscopy and FeLV antigen and FIV antibody assays gave negative results. Cerebrospinal fluid (CSF) analysis revealed a mild mixed pleocytosis with 2% of eosinophils and an increased total protein content (0.43 g/litre). A polymerase chain reaction (PCR) for feline coronavirus and *Toxoplasma gondii* and a *C. neoformans* antigen assay were performed on CSF, with negative results. Magnetic resonance imaging revealed no brain abnormality, but the cervical and thoracic regions of the spinal cord were heterogeneously hyperintense on T2-weighted-images and iso to hypointense on T1. A small syrinx could be seen in the cervical area. After contrast enhancement, an intramedullary mass with an irregular outline was detected (Fig. 1). These findings were interpreted as a tumour or a granuloma with secondary extensive oedema, or gliosis and syringohydromyelia. The cat died spontaneously 2 months later and a full necropsy was performed.

A focal brownish malacic area (4 cm in length) in the proximal thoracic spinal cord (Fig. 2) was the only macroscopical abnormality detected. Samples of the spinal cord were formalin-fixed, processed for histology by routine methods, sectioned (4 µm) and stained with haematoxylin and eosin (HE). In addition, some sections were stained with periodic

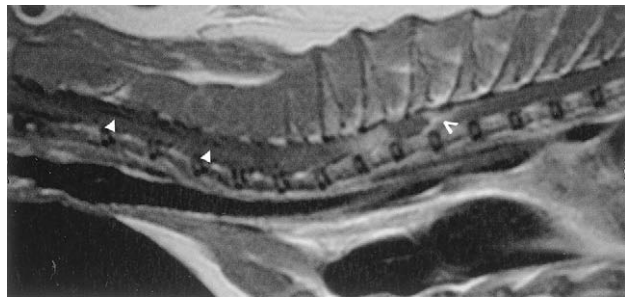


Fig. 1. Post-contrast sagittal T1-weighted image of the cervical and thoracic spine. The cryptococcoma was observed as an isointense mass with peripheral enhancement (open arrowhead). Secondary syringohydromyelia was also noted in the cervical spine (arrowheads).

acid-Schiff (PAS), Grocott or mucicarmine (Ganter and Jollès, 1970).

Sections (4 µm) were examined by immunohistochemistry (IHC) in an Autostainer (Autostainer Plus; Dakocytomation, Carpinteria, CA, USA) at room temperature. The three primary monoclonal antibodies (mAbs) used, kindly supplied by Prof. T. Kozel, were: mAb 471, which labels all strains of *C. neoformans* complex; 302, which labels *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* strains; and F10F5, which labels *C. neoformans* var. *grubii* and *C. gattii* strains (T. Kozel, personal communication). Tests were made in duplicate and two negative controls were included for each tissue examined, the method used being that described by Krockenberger *et al.* (2001). The avidin–biotin–horseradish-peroxidase (HRP) complex detection system was employed to detect antibody–antigen binding, with 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Immunodiagnosics, Camperdown, NSW, Australia). Specimens were lightly counterstained with haematoxylin, dehydrated in ethanol to xylol, and “coverslipped”.

Histopathological examination of the thoracic spinal cord revealed a discrete granulomatous lesion,



Fig. 2. Thoracic spinal cord, sagittal section: in the grey and the white matter, a 4 cm malacic lesion is present.



approximately 4 cm in length, effacing the grey and the white ventral matter (Fig. 3), with myriads of round, intra-cellular and extra-cellular yeasts enmeshed in abundant eosinophilic amorphous material and nuclear debris (necrosis). The yeasts were round in shape, 7  $\mu\text{m}$  in diameter and rimmed by a basophilic thin cell wall, which was surrounded by a halo of variable thickness (Fig. 4). Narrow-necked budding forms were also present. Yeast cells were surrounded by numerous macrophages and epithelioid cells and smaller numbers of lymphocytes and plasma cells rimmed the lesion. The parenchymal remnants contained digestion chambers in which Gitter cells, spheroids and myelinic debris were present. Perivascular lymphocytic cuffs were also observed. The yeast cell walls were stained positively with PAS (Fig. 4, inset), mucicarmine (Fig. 4, inset) and black with Grocott stain. The diagnosis made was that of a focally extensive, severe, chronic granulomatous myelitis, probably caused by a cryptococcoma. This aetiology was confirmed by positive immunohistochemical labelling with antibodies 471, 302 and F10F5. The antibody specificities (see above) indicated that the strain was *C. neoformans* var. *grubii* (serotype A) (Figs. 5 and 6).

To the authors' knowledge, this is the first reported case of spinal cryptococcoma in an immunocompetent cat with no apparent mycotic respiratory lesion. In animals, cryptococcosis is mainly reported in adult intact cats. Immunosuppression was formerly considered to be an important predisposing factor (Summers *et al.*, 1995; Caswell and Williams, 2007;

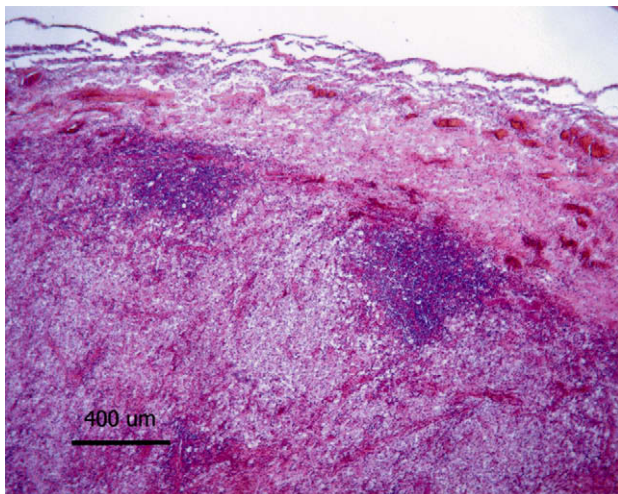


Fig. 3. Thoracic spinal cord: effacing the spinal cord, a discrete granulomatous lesion characterized by a large number of foamy macrophages is present. It is associated with lymphocytic perivascular cuffs, localized at the edges of the lesion, and vascular hyperaemia. HE. Bar, 400  $\mu\text{m}$ .

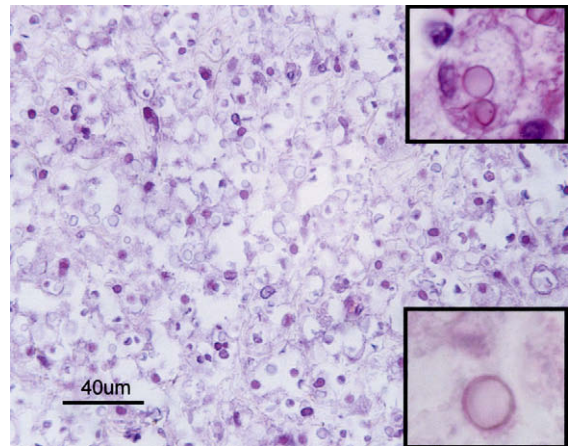


Fig. 4. Thoracic spinal cord: granuloma composed of many foamy macrophages and myriads of round, intra- and extra-cellular yeasts. HE. Bar, 40  $\mu\text{m}$ . Fungal capsule stained with PAS (top inset) and mucicarmine (bottom inset). Insets:  $\times 100$ .

Lopez, 2007), but evidence to support this hypothesis would seem to be lacking (O'Brien *et al.*, 2004, 2006). In the present case, the cat was neutered and, according to the owners, lived on a pigeon-breeding farm. Pigeons may be a natural source of cryptococcal yeasts, which are excreted in the faeces and can infect other animals by inhalation (Caswell and Williams, 2007; Lopez, 2007).

In cats, the respiratory tract is the usual site of primary infection, the nasal cavity being more often affected than the lungs (Summers *et al.*, 1995; O'Brien *et al.*, 2004; Malik *et al.*, 2006; Caswell and Williams, 2007; Lopez, 2007). From a nasal or sinus infection, the yeast may spread to the leptomeninges, subarachnoid space and brain. Alternatively,

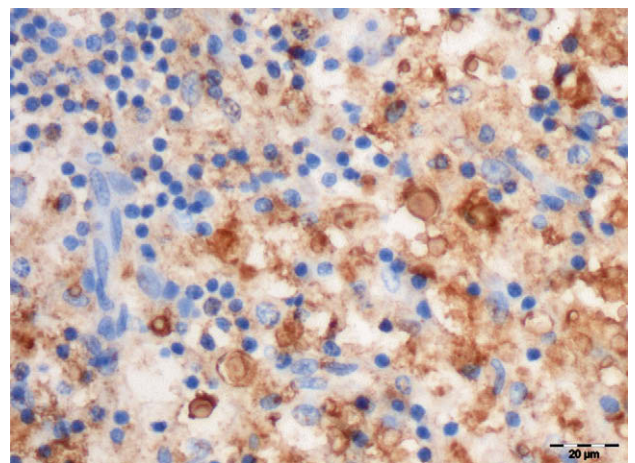


Fig. 5. Labelling of cryptococcal yeast cells with F10F5 antibody, against var. *grubii* and var. *neoformans* capsular antigens. IHC. Bar, 20  $\mu\text{m}$ .

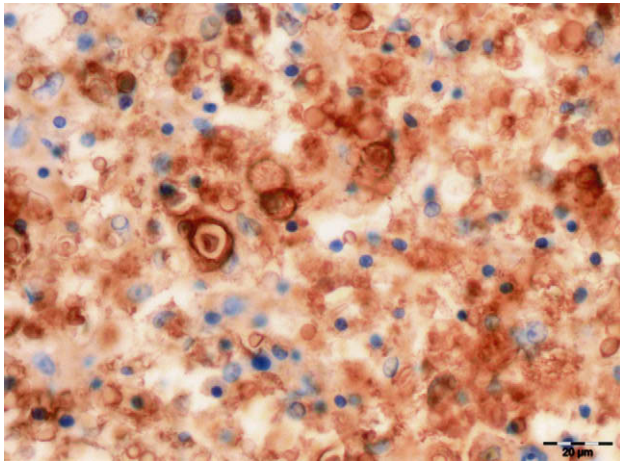


Fig. 6. Labelling of cryptococcal yeast cells with 302 antibody, against var. *grubii* and var. *neoformans* capsular antigens. IHC. Bar, 20  $\mu$ m.

following a pulmonary infection, the yeast may spread haematogenously by “leucocytic trafficking” (Garcia-Hermoso *et al.*, 1999; Bicanic and Harrison, 2004; Caswell and Williams, 2007; Lopez, 2007). In the present case, a single malacic lesion was found in the thoracic spinal cord, with no associated lesions in the respiratory tract or brain. The intramedullary localization without any meningeal lesion excludes the possibility of extension by a neural route from the nasal cavity. Despite the absence of macroscopical signs of pulmonary infection, it is possible that primary infection occurred in the lungs or tracheobronchial lymph nodes but was limited by the immune system (Baker, 1976); infection may then, nonetheless, have spread haematogenously into the spinal cord. This hypothesis is supported by the findings of Garcia-Hermoso *et al.* (1999), who reported systemic cryptococcosis, in the absence of gross pulmonary findings, 13 years after exposure to the causative organism.

The metastatic medullary lesion with no other sign of systemic dissemination may have been related to the neurotropic properties of the agent and the immunological state of the cat. In the CNS, the tropism of *Cryptococcus* spp. is probably due, at least in part, to the lack of the alternative complement pathway necessary for phagocytosis and for the lethal effect of binding to capsular glucuronoxylomannan (GXM) (Rodrigues *et al.*, 1999); it is perhaps also due to the presence of a high concentration of catecholamines, which are used by *C. neoformans* to produce a melanin-like pigment by means of the enzyme laccase. Melanins are scavengers of reactive oxygen intermediates generated by leucocytes, contributing to the resistance of *C. neoformans* to host immune defences (Rodrigues *et al.*, 1999; Shoham and Levitz, 2005).

For these reasons, the nervous system provides an ideal environment for the survival of *Cryptococcus* spp.

The cat in this report appeared to be immunocompetent, being serologically negative for FIV and FeLV and PCR negative for feline coronavirus and *T. gondii*. Bone marrow examination and serum protein electrophoresis showed no abnormality. Histologically, the cat showed a granulomatous lesion rich in inflammatory cells, indicative of an immune response, which may have confined the infection. In cats, cryptococcosis is sometimes associated with FIV or FeLV infection, both of which impair the CD4 T lymphocytes, which are necessary to control the infection (Rodrigues *et al.*, 1999; Shoham and Levitz, 2005). CD4 T lymphocytes produce interferon- $\gamma$  (INF- $\gamma$ ), which is a  $T_H1$  pathway cytokine responsible for the recruitment of macrophages in a cell-mediated immune response, forming multinucleated giant cells and releasing hydrolytic enzymes. Granuloma formation is associated with resistance to cryptococcal infection (Rodrigues *et al.*, 1999). Moreover, nitrites produced by macrophages activated by INF- $\gamma$ , tumour necrosis  $\alpha$  and interleukin-6 are fundamental in destroying yeasts (Rodrigues *et al.*, 1999; Bicanic and Harrison, 2004; Shoham and Levitz, 2005). Cryptococcosis has been reported in immunocompetent human patients (Krishnan and Corbett, 2004; Ecevit *et al.*, 2006; Marroni *et al.*, 2007). Such cases are generally associated with *C. gattii* infection, while infections by var. *grubii* generally occur in immunodeficient patients (Chen *et al.*, 2000). However, a study based on strains of var. *grubii* isolated from apparently immunocompetent human patients suggested that some such infections depended on mutations conferring increased virulence, or on underlying immunosuppressive disease, or on both (D’Souza *et al.*, 2004). Different degrees of virulence in var. *grubii* were demonstrated by Silva *et al.* (2006). The cat in the present report may have been infected by a strain of *C. neoformans* var. *grubii* whose virulence was sufficient to overcome the resistance of an apparently immunocompetent animal. An alternative hypothesis is that the cat was exposed to particularly high environmental levels of *C. neoformans* var. *grubii*, which overwhelmed the immune response. The cat was kept in close proximity to large numbers of pigeons, in whose faeces the concentration of the yeast may well have been high (Bicanic and Harrison, 2004; Shoham and Levitz, 2005).

*C. neoformans* var. *grubii* (serotype A) predominates in Europe, is heat tolerant, and is generally associated with systemic disease (Martinez *et al.*, 2001; Steen *et al.*, 2002; Tintelnot *et al.*, 2004). Heat tolerance and specific virulence factors are considered to be the reasons for its tendency to produce systemic



lesions, in contrast to var. *neoformans* (serotype D) (Tintelnot *et al.*, 2004; Bahn *et al.*, 2005).

The *C. neoformans* antigen assay was negative in CSF, probably because the inflammatory reaction in the spinal cord lesion prevented the spread of the yeast cells into the subarachnoid space. The pleocytosis detected was characterized by 2% of eosinophils and increased total protein, suggestive of a fungal or parasitic infection.

In conclusion, this report describes an unusual *C. neoformans* var. *grubii* infection in the spinal cord of an immunocompetent cat, in the absence of a primary respiratory lesion.

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