Cryptococcus neoformans granuloma in the lung and spinal cord of a free-ranging cheetah (*Acinonyx jubatus*). A clinical report and literature review

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ABSTRACT

A 6-year-old, male, wild-born, free-ranging cheetah (*Acinonyx jubatus*) was evaluated for acute onset of progressive lameness in the right hind limb. Survey radiographs were unrewarding and myelography indicated an intramedullary compressive mass at the L3–L4 region. A fine needle aspirate of the lesion indicated the presence of *Cryptococcus* organisms. Necropsy confirmed the presence of granulomas (cryptococcoma) in the lung and the spinal cord (meningomyelitis) caused by *Cryptococcus neoformans* var. *gattii*. *Cryptococcus neoformans* is a yeast-like organism that is a potential pathogen to many species. Initial infection is thought to be of respiratory origin and then it commonly disseminates systemically from the nasal cavity or lungs to the skin, eyes and central nervous system in particular. The cheetah tested negative for both feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV), as have all the previously reported cheetah cases. *C. neoformans* is a non-contagious, opportunistic organism and is the most common systemic mycoses in domestic cats and the cheetah.

Key words: *Acinonyx jubatus,* cheetah, cryptococcoma, *Cryptococcus neoformans* var. *gattii,* meningomyelitis.

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INTRODUCTION

Cryptococcus neoformans is a saprophytic yeast that is normally found in the environment associated with organic material such a pigeon droppings and leaf litter. However, *C. neoformans* is a potential pathogen of both humans and animals.

The infectious particle, the basidiospore, is adapted for air dispersal and has properties that allow it to adhere to and penetrate the respiratory epithelium of the infected host[10,16].

Primary infection occurs most commonly *via* the respiratory route[5,21,25], the infection then spreads through either direct extension or by the haematogenous route to the central nervous system (CNS), eye, skin, bone, lymph nodes and multiple other organs. *Cryptococcus neoformans* does display some degree of neurotropism[17,23].

In humans cryptococcal infections occur most commonly in immunocompromised individuals[16]. It has been suggested that the outcome of a cryptococcal infection may depend on the immune status of the host, the subspecies of the *Cryptococcus* and the virulence factors such as capsular thickness[5]. All of the cheetah cryptococcal infections reported in South Africa to date have been FeLV and FIV negative[3,5,22].

In domestic cats there may be a genetic susceptibility to *Cryptococcus* infection[16] and it has been postulated that the cheetah may also have a similar genetic predisposition[22] owing to their lack of genetic diversity[18]. Other factors, such as chronic stress in captive cheetah may also be a component in this increased susceptibility.

C. neoformans var. *gattii* is found most commonly in tropical areas such as Australasia, South America and Africa. *C. neoformans* var. *neoformans* occurs worldwide but is the predominant subspecies causing infection in North America[22].

As with domestic felids, adult male cheetahs appear to have the highest incidence of *Cryptococcus* infection[1,3,5,9,22].

This report describes a cheetah displaying CNS symptoms due to *C. neoformans* var. *gattii* and is the first reported case of *C. neoformans* in a wild born, free-ranging cheetah. All previous reported cases have been in captive cheetah[1–3,5,22].

CASE HISTORY

A 6-year-old, male, wild-born, free-ranging cheetah (*Acinonyx jubatus*) was presented for an acute onset of right hind limb lameness. The lameness had started 36 hours previously and had progressed from an initial slight limp to an apparent paresis of the right hind limb. Prior to this, the animal had not shown any problems and was reported to have killed a Zebra (*Equus burchelli*) with his coalition partner just 2 days before the onset of the symptoms.

Zolazepam 100 mg/ℓ (Zoletil, Virbac RSA, Halfway House, South Africa) was used to induce general anaesthesia and the animal was then maintained on Isoflurane (Isofor, Safe Line Pharmaceuticals, Johannesburg, South Africa) gaseous anaesthesia to facilitate safe handling and radiology. Physical examination showed the cheetah to be in good body condition. A moderate amount of muscle wasting was noted in the right thigh muscles (10–15 % of muscle bulk). Orthopaedic examination revealed no abnormalities, and the spinal reflexes: patella (L4–L6), cranial tibial reflex (L6–S1/2), gastrocnemius reflex (L6–S1/2) and perineal reflex (S1–S3) were all within the normal limits for an animal under general anaesthesia.

Radiology and ancillary tests

Survey radiographs of the right hind limb, pelvis and spine were within normal limits. Analysis of cerebrospinal fluid revealed a mild neutrophilic pleocytosis and a mild elevation of proteins. Sample size prevented further analysis.

Lumbar myelography revealed an intramedullary swelling extending from the mid-body of the 3rd lumbar vertebrae to the mid-body of the 4th lumbar vertebrae (Fig. 5). Cord compression was great-

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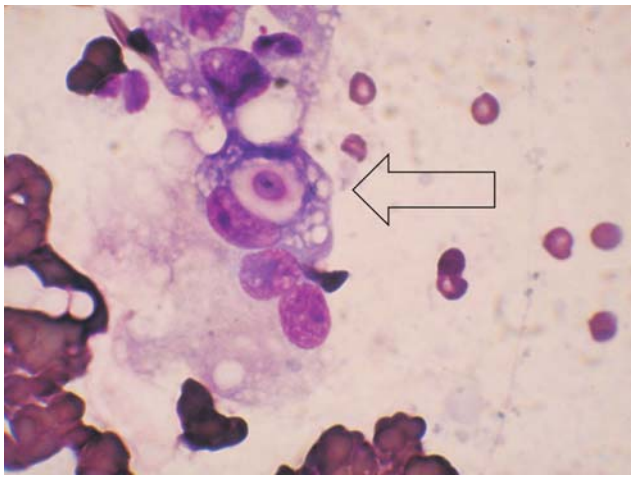

Fig. 1: **Phagocytosed cryptococcal organism** (open arrow).

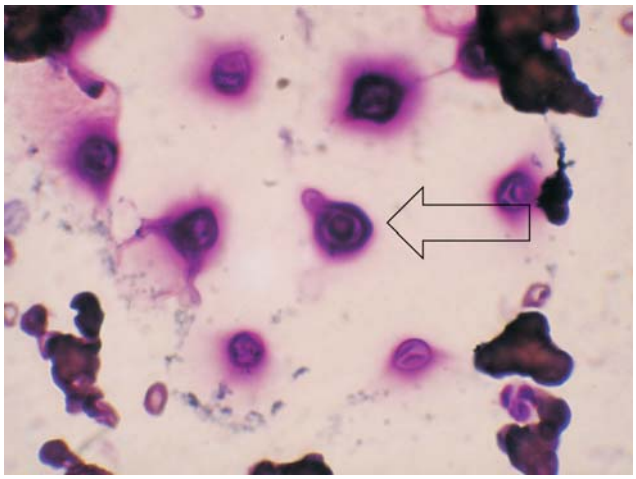

Fig. 2: **Free cryptococcal organism, showing asexual budding** (open arrow).

est ventro-laterally on the right. A fine-needle aspirate was taken from the intramedullary lesion, *via* the interarcuate ligament with fluoroscopic guidance. This revealed the presence of active macrophages, fibroblasts and a number of free and phagocytosed *Cryptococcus* organisms (Figs 1, 2).

Haematological values were within the normal ranges.

The cheetah was both FeLV and FIV (Cite-combo test, IDEXX) negative.

A diagnosis of spinal cryptococcal granuloma was made, euthanasia was performed and the animal underwent necropsy.

Necropsy findings

At the level of the 4th lumbar vertebra the spinal cord showed a marked swelling. The swelling was about 20 mm long and increased evenly, from both ends, to reach about twice the diameter of the unaffected adjacent spinal cord at the mid-point of the swelling (Fig. 6). Within the swelling the parenchyma of the cord had been replaced by a well-demarcated focus of proliferative, greyish yellow material with a gelatinous consistency (Fig. 5). There was a small focus of haemorrhage and malacia in the spinal cord just caudal to the cord swelling, probably caused during the myelogram procedure.

The ventral border of the cranial lobe of the right lung contained a tan coloured, well-demarcated, discoid nodule 15 mm in diameter and 7 mm thick (Fig. 4).

There was a linear splenic scar and scattered, 1 mm diameter, acquired accessory spleens, indicative of a previous non-fatal splenic rupture.

Also moderate hyperplasia of the mesenteric lymph nodes and mild hepatosis were noted.

Microscopically the focal lesion in the spinal cord was sharply demarcated from the surrounding normal nervous tissue

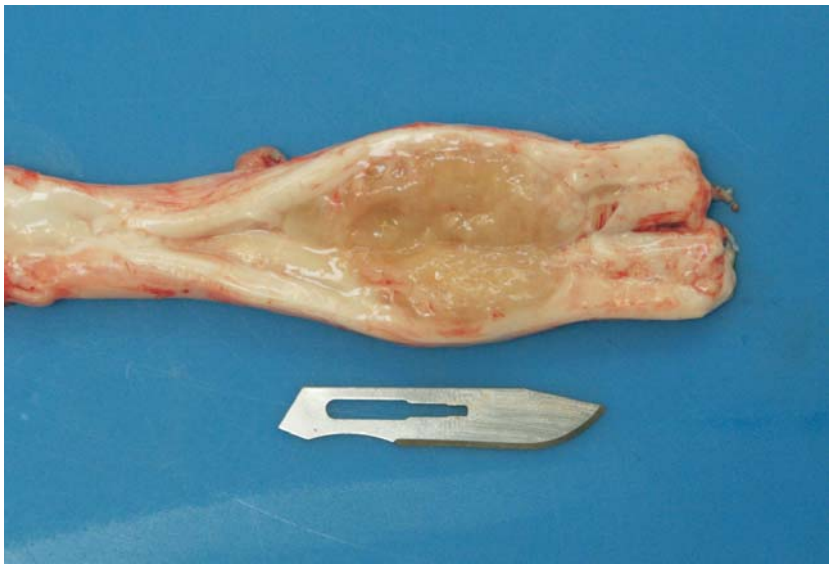

Fig. 3: **Cryptococcal granuloma within the cord parenchyma.**

but there was no evidence of compression or fibrosis at the interface. The lesion consisted of an irregular network of fibres, with masses of necrotic macrophages in the interstices. The network of fibres contained numerous arterioles, each of which was surrounded by a thick cuff of lymphocytes and lesser numbers of plasma cells. Peripheral to the lymphocytic cuff there was a band, of variable

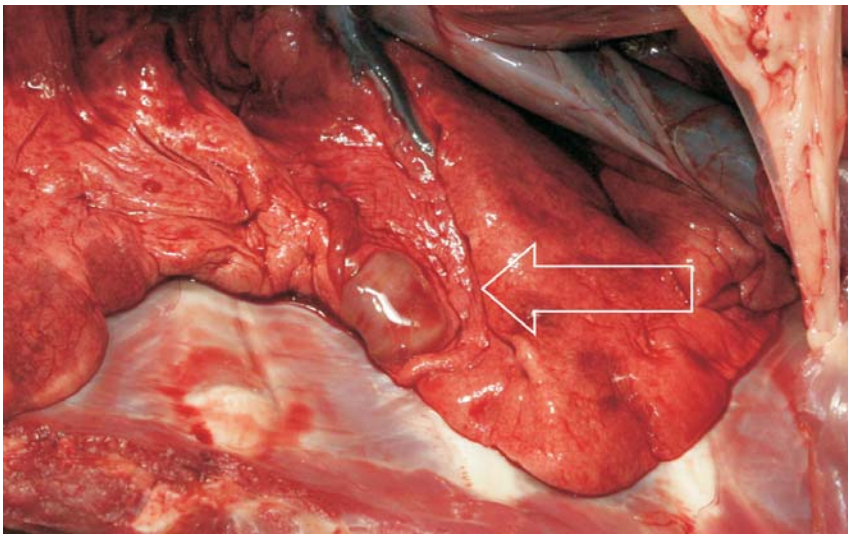

Fig. 4: **Cryptococcal granuloma in the ventral border of the right cranial lung lobe** (open arrow).

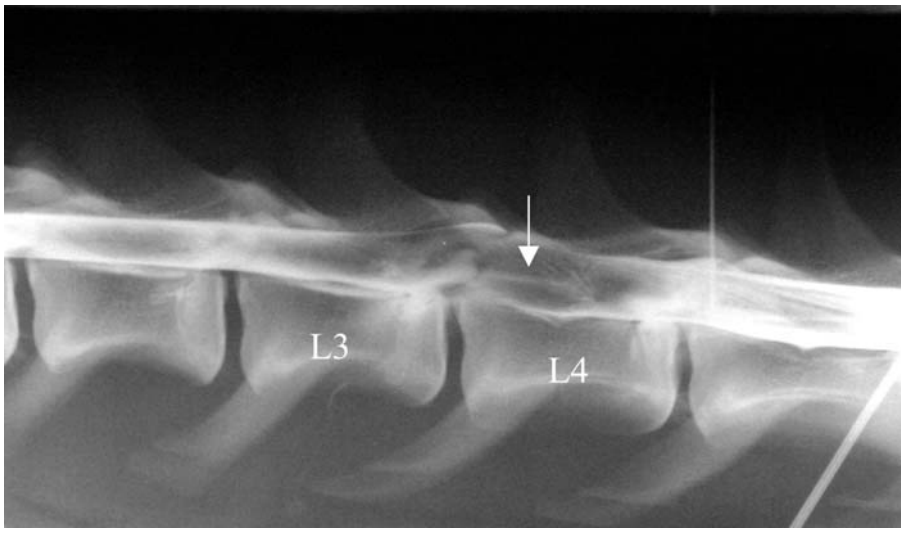

Fig. 5: **Appearance on myelogram. Note the divergence, with internal attenuation, of dorsal and ventral contrast columns. Also a central canalogram is evident in the L4 segment but becomes obliterated at the L3–L4 intervertebral space** (white arrow).

thickness, consisting of tightly packed, viable epithelioid macrophages. A few giant cells were noted in this layer. Still more peripherally, the macrophages showed progressively severe injury until they gave way to necrotic macrophages, with pycnotic nuclei and coagulated or dissolute cytoplasm. Blood vessels adjacent to the main lesion, showed moderate lymphocytic cuffing. Typical thickly encapsulated yeast-like organisms, with morphology typical of *Cryptococcus* species, were present within and between the macrophages.

The focal pulmonary lesion was similarly well demarcated from the normal lung parenchyma and without evidence of an interface reaction. The lesion consisted of densely packed groups of necrotic and dying macrophages of varying size and numbers of mostly non-viable cryptococcal organisms, separated by well-vascularised fibrous stroma. A few of the macrophages were bi-nucleate but fully developed giant cells were not seen. Scattered, mostly small, lymphoid foci were present in the lesion. Within the lesion 1 large lymphoid aggregate surrounded a central accumulation of viable epithelioid macrophages and viable cryptococcal organisms.

The capsules of the yeasts, in both the spinal and pulmonary lesions, were readily demonstrated by both Gomori's methanimine silver (GMS) and periodic acid Schiff (PAS) stains.

Following culture and isolation, the *Cryptococcus* was serotyped on canavanine-glycine-bromthymol blue agar as *C. neoformans* var. *gattii* by the Mycology Section of the South African Medical Research Council (J Picard, University of Pretoria, pers. comm., 2005).

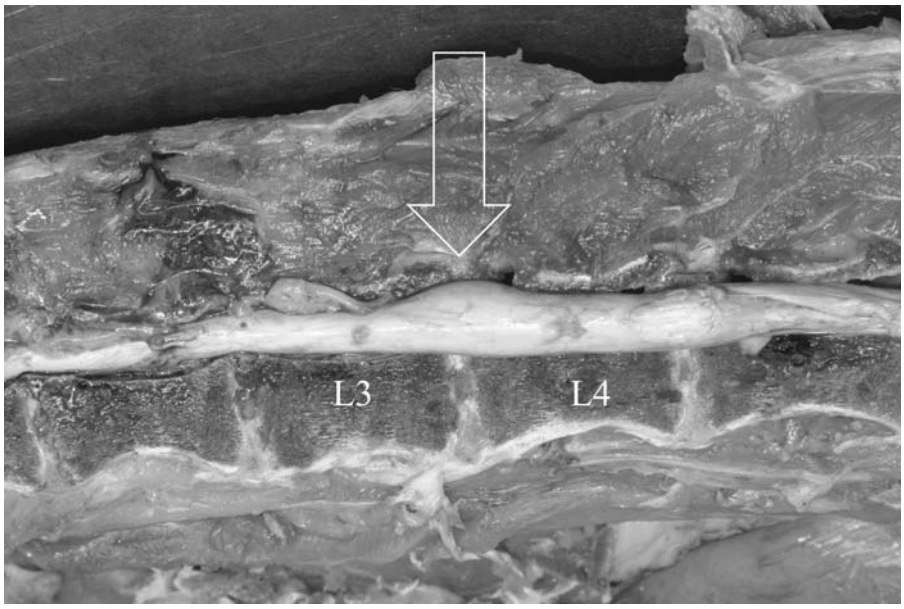

Fig. 6: **Appearance of spinal cord at necropsy. Note the cord enlargement in the L3 to L4 spinal segment** (open arrow).

DISCUSSION

The lesions in mycotic infections are frequently mistaken for neoplasms and only upon histopathology is an accurate diagnosis made[17]. Radiology of this individual revealed an intramedullary mass in the lumbar spine and neoplasia was on the list of differential diagnoses until fluoroscopic guided fine-needle aspirate revealed the presence of cryptococcal organisms.

Although *C. neoformans* is normally a saprophytic basidiomycetous yeast in the environment, it can behave as a pathogen to both human and animals. Although the sexual mycelial phase of *C. neoformans* var. *neoformans* has been produced in the laboratory it has never been found in nature[10,25] but recent evidence indicates that *C. neoformans* var. *gattii* reproduces sexually in nature[10,16]. The asexual yeast phase of both subspecies is found in nature and in infected tissues (Figs 1, 2).

C. neoformans var. *neoformans* is found mainly in temperate climates and is associated with pigeon faeces while *C. neoformans* var. *gattii* is typically found in tropical and subtropical climates[13] and has been associated with bark and leaf litter from certain eucalyptus (*Eucalyptus* spp.)[5,17,25], fig (*Ficus* spp.)[10,16] and resin (*Ozoroa* spp.) trees[22]. *Cryptococcus* requires a high nitrogen and creatinine level for growth[10] and these elements are provided by decomposing wood, organically rich soils and bird or bat guano[22].

Infections caused by *C. neoformans* var. *gattii* are typically more severe and more refractory to antifungal drugs than infections caused by *C. neoformans* var. *neoformans*[10,16].

The infectious particle, the basidiospore, is adapted for air dispersal and has properties that allow it to adhere to and penetrate the respiratory epithelium of the infected host[10,16]. The organism has a mucoid polysaccharide capsule, which appears to aid in its survival in the host and environment. It can survive in faeces for up to 2 years if it does not become desiccated[9,22] and the capsule seems to act as a virulence factor, helping it to minimise the host immune response once the organism enters the body[2,5,25].

Primary infection occurs most commonly *via* the respiratory route[5,21,25,26] and establishment and spread is dependent on cell-mediated immunity[4]. Asymptomatic carriage of *C. neoformans* is not uncommon in the nasal cavity of dogs and cats[15]. Once established, the infection spreads *via* direct extension or through the haematogenous route, to the CNS, eye,

skin, bone, lymph nodes and multiple other organ systems. *C. neoformans* does display some degree of neurotropism[4,17,23]. This may be explained in that it produces the enzyme laccase, which catalyses the formation of melanin from phenolic compounds. The CNS is rich in catecholamines, which can act as a substrate for this enzyme[23]. Also, the cerebrospinal fluid lacks the alternate pathway complement components, which normally bind to the carbohydrate-based capsule and facilitate phagocytosis and killing by polymorph nuclear cells[24].

In humans, cryptococcal infections tend to occur in immunosuppressed individuals[10,17] and are almost exclusively *C. neoformans* var. *gattii*[10,16]. Some authors have postulated that in cats, FeLV and FIV infection may facilitate cryptococcal infections[8,14,15,17]. Others have found that FeLV or FIV infection does not appear to predispose to infection with *C. neoformans*[10], and that infection can occur in both normal and immunocompromised patients[12,17]. However, concurrent FeLV or FIV infection may result in more severe clinical signs[9,25] and cases are more likely to have ophthalmic or neurological signs[25] and have poorer prognosis with treatment[10,11].

All of the cheetah cryptococcal infections reported in South Africa to date have been FeLV and FIV negative and have all been due to *C. neoformans* var. *gattii*[3,5,22]. FeLV has not been detected in free-ranging cheetahs[19] and although FIV antibodies have been detected in 26 % of cheetahs from the Serengeti National Park in Tanzania[6], FeLV and FIV virus have never been a problem in captive cheetahs in South Africa[7]. FIV has not been associated with immunological impairment in non-domestic felines[6].

In domestic cats there may be a genetic predisposition to cryptococcosis and Siamese cats are over represented in the clinical reports[10]. This genetic predisposition also seems to apply to dogs, where purebred dogs (especially the American Cocker Spaniel) are more likely to develop cryptococcosis than mixed breed dogs[4]. It has been suggested that the cheetah is also overly susceptible to cryptococcal infections[22] and that this may be due to the narrow range of genetic diversity, concurrent disease or chronic stress. The limited genetic diversity has been demonstrated through cheetah having a high degree of monomorphism at the isoenzyme and major histocompatability complex loci[18]. However, when the immune response of the cheetah was compared with that of the domestic cat there was no significant difference between the 2 species, but there was a high degree of variation in response between individual cheetah. Thus it was concluded that the immune status of cheetahs depends on mechanisms not solely related to their lack of genetic diversity[18]. Further investigation may be warranted into this genetic predisposition as of the 7 reported cases, 4 (57 %) were in king cheetah, which are derived from a considerably narrower gene pool. This high percentage of cases being in king cheetahs indicates an over representation in comparison with their numbers in the general cheetah population and certainly king cheetah appear to be somewhat more susceptible to diseases in general (L Holm, De Wildt Cheetah and Wildlife Centre, pers. comm., 2005).

Diffuse adrenocortical hyperplasia has been a common finding in the cheetah (85 % prevalence)[20] and other wild animals in captivity and has been correlated to the stress of captivity in these species[5]. Hyperadrenocortisolaemia may occur in animals with adrenocortical hyperplasia. Cortisone depresses monocyte function against *Cryptococcus* and so may predispose to cryptococcal infections in captive cheetah. A lymphocytic depletion of the spleen has also been noted in a number of captive cheetah cases[20]. These immunosuppressive effects would be enhanced by the polysaccharide capsule of *C. neoformans,* which aids in inhibiting phagocytosis, plasma cell function and leukocyte migration[4,5,25].

Dogs appear to show no sexual predilection[4] while adult male cats appear to show the highest prevalence of *Cryptococcus* infection among all of the domestic species. It has been postulated that this may be due to their roaming behaviour[9] and possibly also their scent marking and smelling behaviours. Males represented 68 % of the domestic cats reported in 1 study[9]. This sexual predilection also applies to the cheetah where, of the reported cases, 71 % are male[1,2,5,22].

This is the first reported case in a wild-captured, free-ranging cheetah. Previous cases have all been in captive cheetahs[1–3,5,22].

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