Cryptococcosis in captive cheetah (*Acinonyx jubatus*): two cases

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ABSTRACT

Cryptococcus neoformans is a yeast-like organism associated with pulmonary, meningoencephalitic, or systemic disease. This case report documents 2 cases of cryptococcosis with central nervous system involvement in captive cheetah (*Acinonyx jubatus*). In both cases the predominant post mortal lesions were pulmonary cryptococcomas and extensive meningoencephalomyelitis. Both cheetahs tested negative for feline immunodeficiency virus and feline leukaemia virus. The organism isolated in Case 2 was classified as *Cryptococcus neoformans* var. *gattii,* which is mainly associated with disease in immunocompetent hosts.

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

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INTRODUCTION

Cryptococcus neoformans is the only one of 19 species of *Cryptococcus* that is pathogenic to animals and humans. It is an encapsulated yeast-like fungus with a worldwide distribution that is associated either with soil contaminated with pigeon faeces or with trees (various *Eucalyptus* spp.)[4,5,9,10,17,22]. Disease occurs via inhalation of yeast cells, resulting in primary infection in the respiratory tract with localisation in the lungs and/or haematogenous dissemination, especially to the central nervous system, skin, bones, joints, lymph nodes, as well as other internal organs[4,5,10,19,22,24]. The outcome of cryptococcal infection depends on the immune status of the host, the subspecies of *Cryptococcus* and virulence factors including capsule thickness[10,13,16,19,22]. Classic cryptococcal infection in humans involves 2 clinical forms; pulmonary and cerebromeningeal, by haematogenous spread from the primary pulmonary focus[5,10,24]. Rare manifestations are cutaneous, including cellulitis, and osseous[2,4,5,19,21,24]. In domestic cats, the respiratory form is characterised by nasal granulomas with chronic nasal discharge and regional lymph node involvement[4,22]. The primary focus of infection is usually the lungs with dissemination to the skin, lymph nodes, bones and meninges[1,19]. The cutaneous form is characterised by firm nodules over the face, head and neck, 30 % of which ulcerate. In systemic cryptococcosis, lesions are found in the lungs, lymph nodes, kidneys and skeletal muscles[4,22]. The occurrence of cryptococcal infection in domestic cats may be due to feline leukaemia virus (FeLV) or feline immunodeficiency virus (FIV) infections, both of which result in immunosuppression[1]. Cryptococcosis has been previously reported in 3 cheetahs, with lesions involving the nasal cavity in one case and osteomyelitis in another, both of which were successfully treated, and 1 fatal case with central nervous system involvement[2,3]. In South Africa, cryptococcosis has been previously reported in domestic cats and dogs, and a captive cheetah[3,4].

In this report, 2 cases of cryptococcosis with central nervous system involvement in captive cheetahs in South Africa are described.

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CASE HISTORIES

Case 1

A 4-year-old male king cheetah (*Acinonyx jubatus*) was presented with a soft tumour, approximately 8 × 4 cm in size, overlying the right zygomatic arch, that had grown rapidly over a period of 3 weeks. The ipsilateral submandibular lymph node was enlarged. Skull radiographs showed soft-tissue swelling over the right zygomatic arch containing irregular soft-tissue densities and apparent cystic spaces (Fig. 1). Encapsulated yeast-like organisms were identified on fine-needle aspirate cytology of the mass. Biopsy of the mass revealed severe pyogranulomatous cellulitis with intralesional, encapsulated yeast-like fungi (4–20 μm). A tentative diagnosis of cryptococcosis was made. Complete blood count showed an inflammatory leukogram with mild neutrophilia, regenerative left shift, and monocytosis. On serum biochemistry, there was a mild azotaemia (urea 10.4 mmol/ℓ, normal <8.9, creatinine 215 mol/ℓ, normal <133). Serum protein electrophoresis revealed hypogammaglobulinaemia and hyper-alpha-globulinaemia, consistent with immunosuppression and possible tissue necrosis respectively[11]. Fungal culture from the biopsied area isolated *Cryptococcus neoformans* (unfortunately not serotyped). The latex agglutination test for cryptococcal antibodies was negative, as may be the case where the cryptococcal polysaccharide capsule binds host antibodies, preventing their detection[4]. Both FeLV and FIV (Cite-combo test, IDEXX) were negative.

Fluconazole (Diflucan, Pfizer) treatment was instituted at 300 mg once a day *per os* for the 1st day and then 150 mg once a day *per os*. It is, however, uncertain whether the cheetah, whose appetite was depressed, actually ingested the medication. Three months after the onset of treatment, the animal's condition had worsened and the cheetah was presented recumbent. Repeat skull radiographs revealed osteomyelitis of the right zygomatic arch, characterised by irregular lytic lesions and adjacent soft-tissue swelling (Fig. 1). Cerebrospinal fluid (CSF) and blood for haematology and

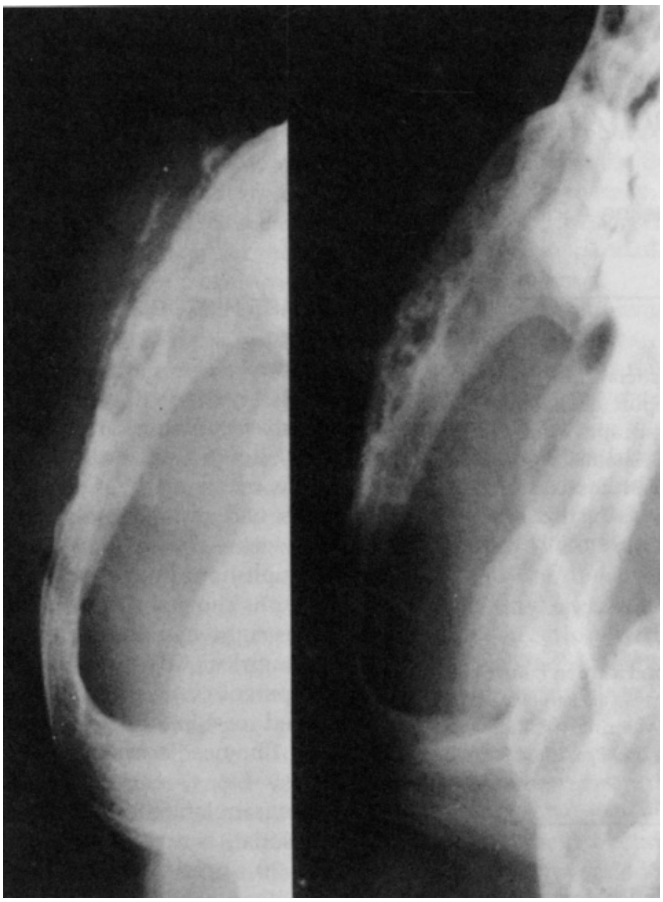

Fig. 1: **Radiographs of the cheetah's skull in Case 1. The progression of cystic osteomyelitis in the right zygomatic arch from the 1st examination** (on left) **to 3 months later** (on right)**, is noticeable**.

serum chemistry were collected. CSF cytological examination showed abundant budding cryptococccal organisms and a normal nucleated cell count, when compared to domestic cats. CSF protein content was not measured owing to an insufficient volume of fluid. A complete blood count revealed a marked inflammatory leukogram with leukocytosis, neutrophilia and regenerative left shift. Serum biochemical abnormalities were azotaemia (urea 25.2 mmol/ℓ, creatinine 216 mol/ℓ) and elevated aspartine transaminase, AST (59 IU/ℓ, normal <23), alanine transaminase, ALT (35 IU/ℓ, normal <23) and creatinine kinase, CK (507 IU/ℓ, normal <83). The prognosis was poor and the animal was euthanased.

At necropsy there was severe emaciation, dehydration and alopecia with multiple pressure sores complicated by secondary myiasis. The right eye was closed by a large swelling ventral to it. The right submandibular and prescapular lymph nodes were enlarged. Granulomatous pleuritis characterised by multiple soft, white grape-like lesions (averaging 1 cm in diameter) was evident. The right cranial lung lobe was swollen, firm and dark red on cut surface. Pin-head-sized dull white foci were disseminated over the pleural surface of the lung, consistent with endogenous lipid pneumonia[18,24]. Impression smears of the lymph nodes, tracheal mucus and pleural nodules revealed cryptococcal organisms. The liver was increased in consistency but normal in size and colour. The spleen was atrophic. Bilaterally, the adrenal glands were enlarged (diameter of 2 cm) and the ratio of cortex to medulla was 3:1. The stomach mucosa was irregularly thickened. No macroscopic lesions were present in the brain and spinal cord, which were fixed *in toto* for histopathological examination.

In affected organs the cryptococcal organisms were identified microscopically as monomorphic, yeast-like organisms 4–20 μm in diameter with a thick capsule that increased the diameter to 20–30 μm. The cell walls stained with periodic acid-Schiff (PAS) stain and the capsules with mucicarmine. There was granulomatous meningitis and multifocal granulomatous encephalomyelitis. Within the parenchyma of the brain were 2 foci of granulomatous inflammation involving the ventricular surface of the hippocampus. There was perivascular cuffing with lymphocytes and plasma cells in the adjacent brain parenchyma. Both the grey and white matter were multifocally affected in the spinal cord.

Diffuse granulomatous pleuritis and locally extensive granulomatous interstitial pneumonia were present. There was diffuse granulomatous lymphadenitis of the prescapular and bronchial lymph nodes. The tumorous facial swelling was characterised by severe locally extensive granulomatous cellulitis, dermatitis and osteitis of the zygomatic arch. These lesions were associated with the presence of cryptococcal organisms in all instances.

Lesions not directly related to the cryptococcal infection included severe diffuse membranoproliferative glomerulonephritis with multifocal glomerulosclerosis and mild glomerular amyloidosis. Cryptococcal organisms were seen within the blood vessels in the kidney, but no involvement of renal parenchyma was evident. There was diffuse, chronic lymphocytic and hyperplastic gastritis with many intraglandular spiral bacteria that stained with modified Steiner's stain. Diffuse splenic lymphoid atrophy and diffuse adrenocortical hyperplasia were present. All the non-cryptococcal-related lesions have previously been reported in captive cheetahs[7,18]. There were multiple foci of subpleural endogenous lipid pneumonia, a common finding in large wild felids[18].

Case 2

A 4-year-old female cheetah was examined for acute onset of generalised weakness, incoordination and possible change in behaviour. Clinical examination, performed under ketamine anaesthesia (Anaket-V, Centaur), revealed no abnormalities. Chemical pathology and haematology were normal except for elevated basal ammonia (54 μmol/ℓ, normal <30). This was, however, attributed to a delay in processing the sample. The animal was initially treated with amoxycillin (Clamoxyl RTU, Pfizer) given at 20 mg/kg twice a day, and when no improvement occurred, the amoxycillin was changed to enrofloxacin (Baytril 5 %, Bayer) given at 5 mg/kg once a day. Over the next few days the cheetah developed proprioceptive deficits of the forelimbs in particular. Fourteen days after the initial examination the animal was again anaesthetised with ketamine. Clinical examination, abdominal ultrasonography, FIV and FeLV titres, biochemical profile (including basal ammonia) and fine-needle aspirate cytology of the liver were all within normal limits. CSF analysis revealed pleocytosis

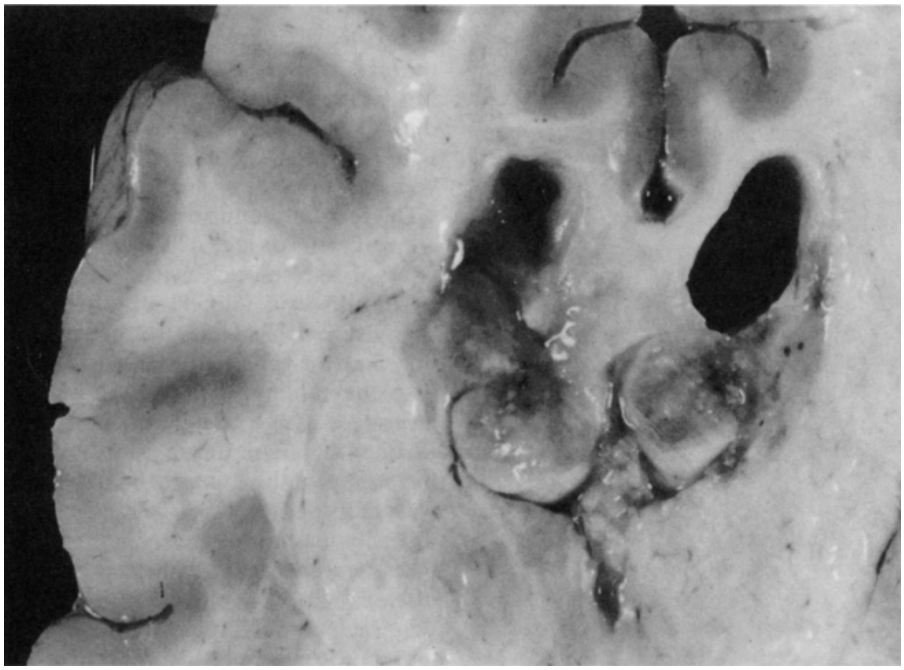

Fig. 2: **Parenchymal lesion in the brain in Case 2**.

and *C. neoformans* organisms. CSF protein content was not determined as there was insufficient CSF available. The animal died without recovering consciousness.

At necropsy the specific lesions were restricted to the lower respiratory tract and central nervous system (CNS). The pulmonary lesion consisted of a single, round, raised, tan-coloured nodule, 10 mm in diameter, on the subpleural surface near the hilus of the diaphragmatic lobe of the left lung. The nodule was well-demarcated and compressed the unaffected contiguous lung tissue. On section, cystic spaces containing greyish-white mucoid material were present within the solid lymphoid-like component.

Although disseminated throughout all sectors of the CNS, the lesions were particularly prominent in the thalamic region immediately cranial to the level of the hippocampus in the brain and focally in the mid-thoracic segment of the spinal cord. The thalamic lesion was bilateral and characterised by unilateral dilatation of the left ventricle with periventricular malacia of the adjacent parenchyma; the malacic areas were distinguished by greyish-white discolouration and mucoid dissolution (Fig. 2). The spinal lesion was similar in many respects, with greyish-white dissolution and petechiation of the parenchyma lateral and ventral to the central canal. The meninges appeared unaffected on macroscopic examination. Imprint smears from the lesions in the CNS revealed the presence of PAS-positive, encapsulated, yeast-like fungal microorganisms 5–20 μm in diameter and exhibiting occasional single, narrow-based budding consistent with *C. neoformans.* Additional findings included atrophy of the lymphoid component of the spleen and lymph nodes; atrophy of red bone marrow; bilateral diffuse adrenocortical hyperplasia, and the presence of infantile genitalia. *C. neoformans* var. *gattii* was isolated from specimens of both brain and spinal cord.

The histopathological features of the lung lesion were consistent with an active mycotic granulomatous interstitial pneumonia (Fig. 3). The reactive focus was encapsulated with attenuated fibrovascular connective tissue in which aggregates of lymphocytes and plasma cells were present. Lymphoplasmacytic foci also occurred within the granuloma in which macrophages, most laden with encapsulated cryptococcal elements, comprised the dominant cell type. The granuloma was interspersed with 'cystic spaces', known as cryptococcomas, comprising focal accumulations of encapsulated and occasional non-encapsulated fungi to create a 'soap bubble' appearance (Fig. 3). In the CNS the lesions were distributed in a predominantly periventricular location involving white matter in the brain and were present in both gray and white matter in the vicinity of the central canal of the spinal cord. However, the reaction was similar regardless of the site in the CNS. Malacic areas infiltrated by numerous macrophages and lesser numbers of neutrophils were the dominant feature. Cryptococcal elements were present both free and within macrophages. The parenchymal lesion was accompanied by focal and segmental infiltration of equal numbers of lymphocytes, plasma cells and macrophages together with occasional to rare multinucleated giant cells. Fungal microorganisms were less readily discernible in the reaction sites. Perivascular cuffing, predominantly lymphocytic, was a prominent finding in the less affected contiguous tissue (Fig. 4).

The histopathological findings in the other organs and tissues were essentially confirmatory of the macroscopical features.

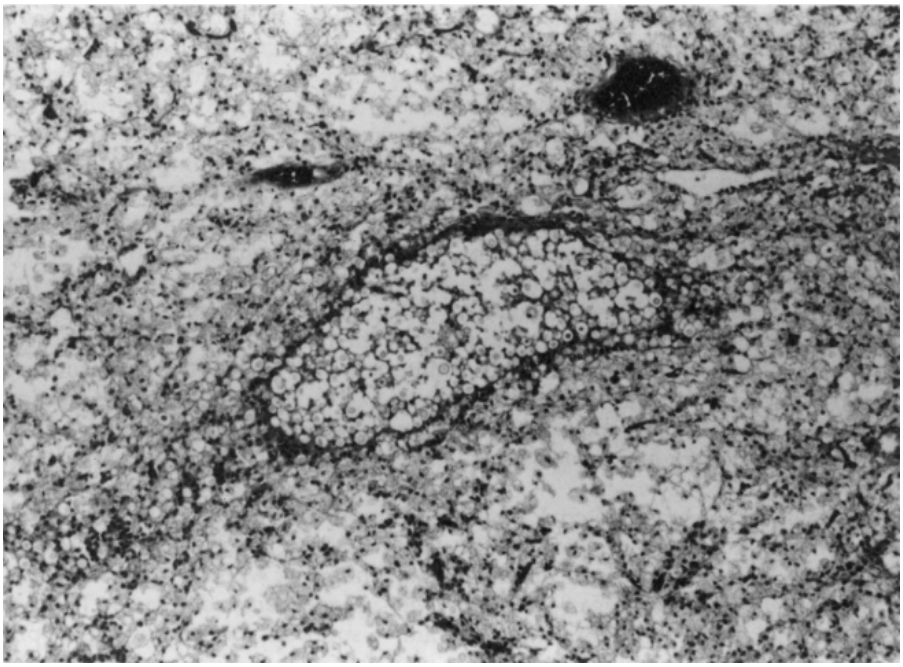

Fig. 3: **Micrograph of the pulmonary lesion in Case 2**. HE, ×200.

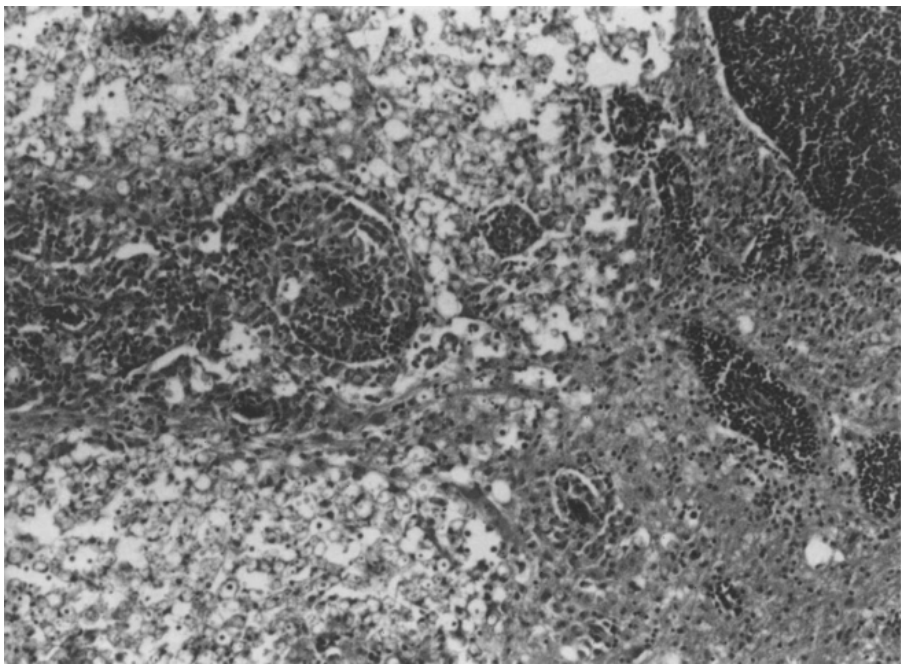

Fig. 4: **Micrograph of the lesion illustrated in Fig. 2 of the contiguous brain parenchyma with marked perivascular cuffing**. HE, ×400.

DISCUSSION

Cryptococcus neoformans is a saprophytic, spherical to oval, thin-walled budding yeast of variable diameter (2.5–20.0 μm) with a thick polysaccharide capsule[13,22]. Daughter cells are present, and are usually single, budding from the parent cell by a thin stem[22]. Four serotypes of *C. neoformans*, namely A, B, C and D, have been identified on the basis of the capsular polysaccharide. Biochemical tests have allowed the differentiation of 2 varieties: *C. neoformans* var. *neoformans* (serotypes A and D), and *C. neoformans* var. *gattii* (serotypes B and C). The source of infection to mammalian hosts is most commonly dust contaminated by bird faeces (mainly pigeon)[4,22], although in Australia an association between species of gum tree (*Eucalyptus camaldulensis* and *E. tereticornis*) and *C. neoformans* var. *gattii* has been reported[8,9,17]. In another recent study in Australia, *C. neoformans* was cultured from nasal washings from 14 % of dogs and 7 % of cats, selected randomly. Serum cryptococcal latex agglutination tests were negative in all these animals. The evidence was that they were potential carriers of this fungus and a possible source of infection for other animals and humans[14].

Inhalation of infective material is the predominant route of infection, with localisation in the lungs and/or dissemination to one or multiple organ systems. The course of the disease may be fulminant and fatal within 2 weeks or subclinical for months or years[13]. Unlike aspergillosis and other fungal diseases that affect mainly debilitated or immunosuppressed patients, cryptococcosis occurs in both immunocompetent and immunocompromised human patients[5,17].

The immune status of the host, subspecies of *Cryptococcus* and capsule thickness of the yeast-like fungus determine the course of the disease. Cryptococcal organisms within the CNS of immunocompromised patients provoke a mild inflammatory response[5,13]. In immunocompetent hosts, the presence of cryptococcal organisms is marked by infiltration of lymphocytes, histiocytes, epithelioid macrophages and multinucleated giant cells, often accompanied by an acute suppurative and necrotising reaction[5,13]. There is increasing evidence that *C. neoformans* var. *gattii* is responsible for cryptococcosis in immunocompetent hosts, while *C. neoformans* var. *neoformans* is generally cultured from infections in immunocompromised patients[19]. The cryptococcal polysaccharide capsule has anti-phagocytic, immunosuppressive and chemotactic properties that may interfere with the normal inflammatory response[10,22]. The inflammatory reaction in cryptococcosis is the result of close cooperation between the cellular and humoral arms of the immune system[10]. The importance of cellular immunity was emphasised in a recent report: 2 healthy adult humans, who had lymphopaenia with a proportionate decrease in T-helper and T-suppressor cells, developed primary cutaneous cryptococcosis, a rare variation of the disease[19]. T-lymphocytes and various serum factors are reported to be necessary for monocytes and macrophages to kill *Cryptococcus*[5,10]. Neutrophils have been shown to be capable of killing *C. neoformans* organisms either by direct cytotoxicity or in combination with extracellular anti-cryptococcal antibodies[10].

The immune status of the cheetah is a controversial issue[16]. The devastating effect of diseases such as feline infectious peritonitis and feline herpesvirus on captive cheetah colonies led to investigations into the genetic diversity of the captive cheetah population. The results indicated a high degree of monomorphism at the isozyme and major histocompatibility complex (MHC) loci. It was proposed that the lack of genetic diversity may have resulted in impaired immune responses to challenges by these diseases[20]. In work published by Miller-Edge *et al.*[16], the ability of peripheral blood mononuclear cells in captive cheetah to mount a response to a challenge by feline herpesvirus-1 (FHV-1) and *C. neoformans* was investigated. The tests included evaluation of the proliferative responses of peripheral blood mononuclear cells to the selected infectious agents and mitogens, assessment of the effect of administration of exogenous interleukin-2 (IL-2) on this proliferative response and the humoral immune response to FHV-1. The results showed that there was a variation in immune responses between individual cheetahs, even full siblings. Interleukin-2, an antigen-nonspecific lymphokine involved in T cell proliferation, enhanced the proliferative response of mononuclear cells in individual cheetahs. There were no significant differences between the responses of the cheetahs and those of a group of domestic cats[16]. The conclusion drawn from these experiments was that the immune status of cheetahs depends on mechanisms not solely related to their lack of genetic diversity[16].

The primary clinical lesion in Case 1 appeared to be cutaneous, although a small pulmonary lesion could have passed unnoticed on radiographs. In the 2nd case it is likely that the primary lesion was pulmonary, with secondary spread to the CNS. In classic cases of meningoencephalitic cryptococcosis the parenchymal lesions involve direct spread from the meninges[5]. In both cases, the predominant lesion in the CNS was meningitis with periventricular foci of malacia and inflammation. The periventricular distribution in these cases may indicate separate haematogenous spread to meningeal and periventricular parenchymal locations.

The outcome of cryptococcal infection

of the 2 cheetahs described here differed. In Case 1 the course of disease was chronic with locally extensive cellulitis and osteitis of the zygomatic arch and extensive involvement of the pleura, brain and spinal cord. An assumption was made that this cheetah was immunocompromised on the basis of the low immunoglobulin levels, poor inflammatory response to the cryptococcal organisms as seen on tissue sections, and the chronic course of the disease. Case 2, however, did not appear to be immunosuppressed, evidenced by the presence of a satisfactory inflammatory response, absence of gammopathy, the acute fulminating course of the disease and the culture of *C. neoformans* var. *gattii*. The progression of the disease in Case 2 was similar in all respects to that previously described in a captive king cheetah[3].

A common necropsy finding in captive cheetahs is diffuse adrenocortical hyperplasia (L Munson, University of Tennesee, 1996, pers. comm.). Diffuse adrenal hyperplasia has been described in many other wild animals in captivity[12,15,18,23]. The presence and extent of diffuse adrenocortical hyperplasia has been correlated to stress of captivity in species such as the nine-banded armadillo, the platypus and the harbour porpoise[12,15,23]. Hypercortisolaemia may occur in animals with diffuse adrenocortical hyperplasia and this, together with evidence that cortisone depresses monocyte function against *Cryptococcus*, may predispose to cryptococcal infections in captive cheetah[6,10]. This effect may be enhanced by the immunosuppressive role of the polysaccharide capsule of *C. neoformans*[10,22].

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