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

I R Millward* and M C Williamsb

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: Cryptococcus jubatus, cheetah, cryptococcoma, Cryptococcus neoformans var. gattii, meningomyelitis.


ABSTRACT
Cryptococcus neoformans is a saprophytic yeast that is normally found in the environment associated with organic material such as 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.

Primary infection occurs most commonly via the respiratory route, 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.

In humans cryptococcal infections occur most commonly in immunocompromised individuals. 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. All of the cheetah cryptococcal infections reported in South Africa to date have been FeLV and FIV negative.

In domestic cats there may be a genetic susceptibility to Cryptococcus infection and it has been postulated that the cheetah may also have a similar genetic predisposition owing to their lack of genetic diversity. 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.

As with domestic felids, adult male cheetahs appear to have the highest incidence of Cryptococcus infection. 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.

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/t (Zoletil, Virbac RSA, Halfway House, South Africa) was used to induce general anaesthesia and the animal was then maintained on Isoflurane (Isofor, Safe Line Pharmaceutical, 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-
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 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

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

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

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

Fig. 4: **Cryptococcal granuloma in the ventral border of the right cranial lung lobe** (open arrow).
morbidity typical of encapsulated yeast-like organisms, with lymphocytic cuffing. Typical thickly cent to the main lesion, showed moderate dissolute cytoplasm. Blood vessels adjacent to pycnotic nuclei and coagulated or they gave way to necrotic macrophages, showed progressively severe injury until more peripherally, the macrophages giant cells were noted in this layer. Still viable epithelioid macrophages. A few 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 dissolve 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 methanamine silver (GMS) and periodic acid Schiff (PAS) stains.

Following culture and isolation, the yeasts, in both the spinal and pulmonary lesions, 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 methanamine silver (GMS) and periodic acid Schiff (PAS) stains.

The infectious particle, the basidiospore, requires a high nitrogen and creatinine level for growth and these elements are provided by decomposing wood, organically rich soils and bird or bat guano. 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. 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. 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 and the capsule seems to act as a virulence factor, helping it to minimise the host immune response once the organism enters the body.

Primary infection occurs most commonly via the respiratory route and establishment and spread is dependent on cell-mediated immunity. Asymptomatic carriage of C. neoformans is not uncommon in the nasal cavity of dogs and cats. Once established, the infection spreads via direct extension or through the haematogenous route, to the CNS, eye,
In humans, cryptococcal infections tend to occur in immunosuppressed individuals and are almost exclusively C. neoformans var. gattii. Some authors have postulated that in cats, FeLV and FIV infection may facilitate cryptococcal infections. Others have found that FeLV or FIV infection does not appear to predispose to infection with C. neoformans, and that infection can occur in both normal and immunocompromised patients. However, concurrent FeLV or FIV infection may result in more severe clinical signs and cases are more likely to have ophthalmic or neurological signs and have poorer prognosis with treatment.

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. FeLV has not been detected in free-ranging cheetahs and although FIV antibodies have been detected in 26% of cheetahs from the Serengeti National Park in Tanzania, FeLV and FIV virus have never been a problem in captive cheetahs in South Africa. HIV has not been associated with immunological impairment in non-domestic felines.

In domestic cats there may be a genetic predisposition to cryptococcosis and Siamese cats are over represented in the clinical reports. 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. It has been suggested that the cheetah is also overly susceptible to cryptococcal infections 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 histocompatibility complex loci. 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 cheetahs.

Infection can occur in both clinical reports. Siamese cats are over represented in the predisposition to cryptococcosis and infections has been suggested that the cheetah is more predisposed to cryptococcosis than mixed breed dogs (especially the American Cocker Spaniel) are more likely to develop purebred dogs (especially the American Cheetah in South Africa). Cryptococcus gattii have all been due to C. neoformans var. gattii. Some authors have postulated that in cats, FeLV and FIV infection may result in more severe disease 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) and other wild animals in captivity and has been correlated to the stress of captivity in these species. Hyperadrenocorticalism 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. These immunosuppressive effects would be enhanced by the polysaccharide capsule of C. neoformans, which aids in inhibiting phagocytosis, plasma cell function and leukocyte migration.

Dogs appear to show no sexual predilection 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 and possibly also their scent marking and smelling behaviours. Males represented 68% of the domestic cats reported in 1 study. This sexual predilection also applies to the cheetah where, of the reported cases, 71% are male.

This is the first reported case in a wild-captured, free-ranging cheetah. Previous cases have all been in captive cheetahs. The CNS is rich in catecholamines, which can act as a substrate for this enzyme. 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.

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REFERENCES


Animal diseases have always been one of the main constraints on animal production, especially in Africa with its plethora of tropical and subtropical diseases. Tough climatic conditions, the presence of wildlife carriers and the prevalence of arthropod vectors transmitting diseases exacerbate the problem on our continent. Knowledge of these diseases and ways to combat them is therefore highly relevant to the socioeconomic development of Africa and its fight against poverty. Furthermore, global developments are increasing the importance of animal diseases for the developed world. Globalisation, accompanied by the relaxing of trade barriers, results in increased trade in animals and animal products which in turn increases the risk of spreading diseases across international borders and establishing emerging or re-emerging diseases in developed countries. In addition, climatic changes, especially global warming, has led to the geographic expansion of the distribution of certain vectors and the diseases they transmit. Two recent examples of this phenomenon are the outbreaks of bluetongue in southern European countries and of Rift Valley fever in the Middle East. The knowledge contained in the volumes under review is therefore of value for, and will benefit, both the developing and developed world.

It is indeed a monumental piece of work. Published in a 3-volume format by the prestigious publishers Oxford University Press (SA), no less than 197 specialists from 24 countries have contributed 214 chapters covering most, if not all, of the infectious diseases of livestock. It constitutes a thoroughly revised and considerably expanded second edition of the groundbreaking text published in 1994, which was received with acclaim. Two areas of expansion are the inclusion of the majority of infectious livestock diseases occurring outside sub-Saharan Africa and also those of wildlife, where relevant. These additions transform the book into the most comprehensive text on infectious animal diseases presently available. It is primarily aimed at the needs of veterinarians, undergraduate and postgraduate veterinary students and veterinary libraries. It will, however, also be very useful as a reference work for animal scientists, regulatory authorities and anyone involved in animal production.

Eleven introductory chapters deal with factors influencing the occurrence of infectious diseases, including vectors, climate and husbandry practices. Beautifully illustrated reviews, in full colour, are presented of the ticks, tsetse flies, tabanids, midges and mosquitoes responsible for the transmission of so many of these diseases. This is followed by chapters on arthropod-borne viruses, the control of infectious diseases in different epidemiological and socioeconomic situations and on the wildlife/livestock interface. The rest of Volume 1 deals with protozoal diseases, babesioses, theilerioses, rickettsial and clamidial diseases and with anaplasmoses. A total of 37 chapters, most of them written by internationally recognised experts, cover these diseases.

Volume 2 is dedicated to viral diseases. Starting off with rinderpest, the socioeconomically most important disease in the history of Africa and perhaps the world, a total of 83 chapters deal with as many disease entities. It is of interest that the pandemic of rinderpest during the late 19th century was directly responsible for the establishment of both the OIE and the Onderstepoort Veterinary Institute. The viral diseases are dealt with according to the internationally accepted classification of viruses into families such as the paramyxoviridae, retroviridae, herperviridae, etc. It may be argued that, while scientifically sound, this approach requires some virological expertise to find a disease. Not so. The comprehensive ‘Contents’, which covers all 3 volumes and is included in each of them, serves well to address this potential problem. Diseases with virus-like aetiological agents, such as scrapie and bovine spongiform encephalopathy (‘mad cow disease’), are dealt with in the last four chapters of this volume. Volume 3 deals with bacterial diseases in 69 and mycoplasmal diseases in 6 chapters. In addition there are 3 chapters on mycotic and algal diseases, 2 on unclassified bacteria and 3 on disease complexes of unknown aetiology.

As can be expected from a compendium containing such a variety of subjects treated by so many authors, there is some variation in the quality of the contributions. The depth to which the various diseases are discussed is obviously also influenced by their economic importance. The editors have managed admirably, however, to ensure a consistent style and adherence to a set of high standards by requiring each contribution to deal with the aetiology, epidemiology, pathogenesis, clinical signs, pathology, diagnosis, differential diagnosis and control of each disease. A comprehensive list of references is also provided for each chapter and ample illustrations, many in full colour, as well as top-quality printing will ensure that the present edition will follow in the footsteps of its predecessor. It can be expected to remain the standard reference work in its field for many years and is highly recommended for everyone interested in the health of our livestock.

B L Penzhorn
Department of Veterinary Tropical Diseases
Faculty of Veterinary Science
Onderstepoort

D W Verwoerd
Faculty of Veterinary Science Research Coordinator
Onderstepoort

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