First case of feline cryptococcosis in Bosnia and Herzegovina

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Abstract

Case summary A 2-year-old domestic longhair crossbred female cat was referred for a second opinion on a non-healing surgical wound after left eye enucleation. In addition to the left orbital lesion, ulcerative granular masses protruding from the left nostril and on the base of the left ear were noted. A diagnosis of cryptococcosis was established using histopathological examination and a latex cryptococcal antigen agglutination test. The cat was successfully treated with itraconazole.

Relevance and novel information Cryptococcosis, commonly reported in Australia, western Canada and the western USA, is rarely reported in companion animals in Europe. This marks the first report of cryptococcosis in cats in Bosnia and Herzegovina, emphasising the need to raise awareness within the veterinary community, both local and regional, about this disease.

Keywords: Azole therapy; *Cryptococcus*; cytology; Europe; yeast

Introduction

Cryptococcosis occurs in humans and a wide range of animals globally, including terrestrial placental mammals, marsupials, marine mammals, avian species and reptiles.[1,2] It is considered a relatively rare disease of domestic animals, apart from usually sporadic cases in dogs and cats. In reported cases, cryptococcosis is 6–8 times more prevalent in cats than in dogs.[3,4]

Cryptococcus species can be found in the environment, often on plant materials, soil and dust.[4] Small infectious propagules such as basidiospores (<2 μm) and desiccated yeast cells (<3 μm) are easily dispersed by air flow. Therefore, the primary route of infection is by inhalation.[3,5,6] *Cryptococcus neoformans* and *Cryptococcus gattii* are the main causative agents of cryptococcosis.[7] Heterogeneity of the isolates and the use of molecular typing methods have demonstrated that *C neoformans* and *C gattii* comprise genetically diverse monophyletic clades.[8,9] It has therefore been proposed that the seven clades identified should be designated as seven new species, dividing *C neoformans* into two and *C gattii* into five species.[10] Because the taxonomy continues to be in a state of flux, the terms *C neoformans* species complex and *C gattii* species complex have been used.[1,11] *C neoformans* species complex and *C gattii* species complex exhibit apparent niche differentiation in their environmental habitats.[2] *C neoformans* can be found in the droppings of pigeons or other birds, in soil enriched with their guano and in decaying vegetation worldwide,[12] while *C gattii* is strongly associated with the hollows of eucalyptus[13] and

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fig trees in Australia and fir trees in Canada.[14] This association was thought to contribute to its restricted geographical distribution, but based on the literature, *C gattii* is also present in North and South America, Africa, the Indian subcontinent and Europe.[2,3,15] Cryptococcosis caused by either species cannot be clinically distinguished.[16] Infected animals do not represent a direct threat to public health as the infection is acquired from a contaminated environment. Therefore, animals may serve as sentinels for human cryptococcosis.[6]

In Bosnia and Herzegovina (B&H), cryptococcosis has not yet been reported in companion animals. Here, we describe, to the best of our knowledge, the first clinical case of cryptococcosis in cats in B&H and the Western Balkan region.

Case description

A 2-year-old domestic longhair crossbreed female cat was presented to the clinic of Surgery, Anaesthesia and Resuscitation at the Veterinary Faculty of the University of Sarajevo for a non-healing wound following left eye enucleation performed elsewhere for suspected ocular neoplasia. The patient was an adopted stray cat with unknown medical history. The wound failed to heal despite frequent bandage changes for 45 days postoperatively. One week before presentation, the cat had become inappetent. Negative retroviral status was determined through the testing of a blood sample for feline leukaemia virus (FeLV) antigen and feline immunodeficiency virus (FIV) antibodies (IDEXX).

At clinical presentation, the cat was lethargic but responsive with normal vital parameters. The nasal planum was distorted with ulcerative grey-white to tan granular masses protruding from the left nostril and the base of the left ear. The non-healing surgical wound in the left orbital region was deep and covered by a layer of brown discharge (Figure 1).

The involvement of the cutaneous tissues contiguous with the nasal planum and the absence of signs of neurological disorders suggested that the disease was localised. A thorough physical examination was performed under anaesthesia; medetomidine (Sedastart; Dechra) and ketamine (Ketaminol 10; Intervet International) were administered intramuscularly at a dose of 80 µg/kg and 5 mg/kg, respectively. Haematology and biochemistry analysis before the procedure were declined by the owner owing to financial constraints. Subsequently, specimens for further diagnosis were collected. These included wound discharge swabs, fine-needle aspirate (FNA) and incisional biopsy of the affected tissues. The collected material was submitted for microbiology, cytology and histopathology. The cat was discharged with symptomatic therapy – a broad-spectrum antibiotic (Synulox RTU; Zoetis, 12.5 mg/kg) and an analgesic (Meloxidolor; Dechra, 0.3 mg/kg) – applied subcutaneously.

Figure 1 The first presentation of the case during the clinical examination. An unhealed deep surgical wound with distorted nasal planum. Surrounding tissue was oedematous with swelling extending over the bridge of the nose

Microscopic examination of the FNA of intraorbital proliferative tissue revealed numerous free and clustered yeasts characterised by 3–15 µm central to slightly eccentric spherical light basophilic nuclei surrounded by a thin wall and a thick clear capsule. Numerous neutrophils and fewer yeast-laden macrophages were also present (Figure 2).

In addition, histopathology of biopsy material revealed severe distension of the interstitial tissue with myriad yeast bodies. Moderate numbers of neutrophils and lesser numbers of lymphocytes and macrophages were infiltrating the remaining interstitial islands and often extending into the walls of blood vessels. Multiple blood vessels were occluded with homogeneous eosinophilic material (thrombi). Periodic acid–Schiff stain emphasised positive red to violet yeast nuclei and a thick clear capsule. Rare narrow budding structures were also visible (Figure 3).

On further diagnostic work-up, the patient's serum tested positive on latex cryptococcal antigen agglutination test (LCAAT) (Remel; Laboklin). The cat was treated according to a previously published protocol for feline cryptococcal infection with itraconazole oral solution (Itrafungol; Elanco) at a dosage of 10 mg/kg q24h with food.[17] One month after initiation of antifungal therapy, the clinical signs were slowly resolving,
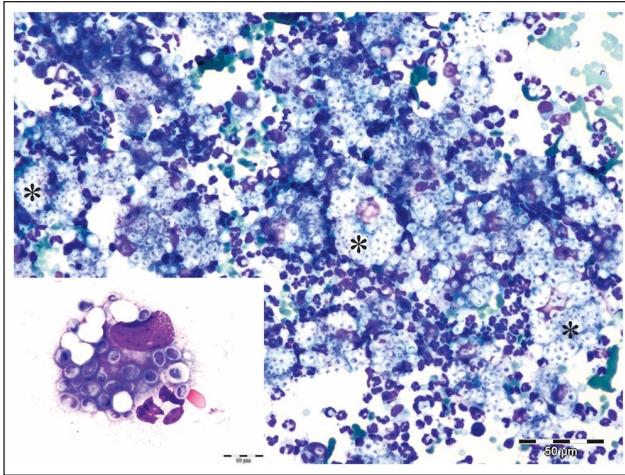
Figure 2 Fine-needle aspirate of intraorbital proliferative tissue from the cat infected with *Cryptococcus* species. Myriad free and clustered yeast forms in the mucinous matrix (asterisks), numerous neutrophils and few macrophages are visible. Diff–Quick, scale bar 50 μm. Insert: A macrophage laden with *Cryptococcus* species. May Grünwald Giemsa, scale bar 10 μm
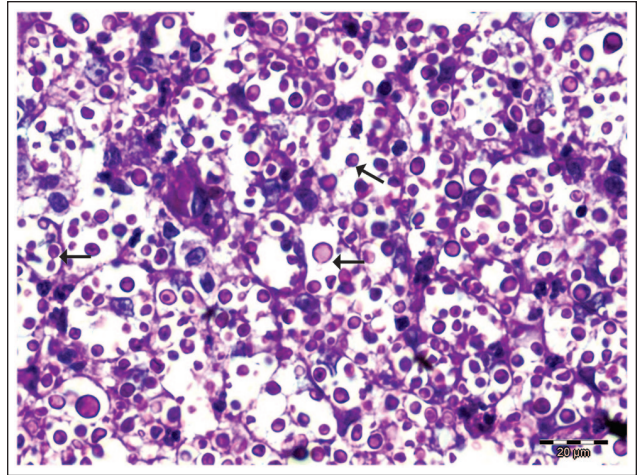

Figure 3 Intraorbital proliferative tissue from the cat infected with *Cryptococcus* species. Numerous red-violet yeast cells with thick capsules are present in the interstitial tissue. Note rare narrow buddings (arrows). Periodic acid–Schiff, scale bar 20 μm

with the wound gradually reducing in diameter (Figure 4a). Further improvement was observed 10 months later (Figure 4b). Continued treatment was recommended until the LCAAT results became negative.

Discussion

Larger retrospective studies of cryptococcosis in pets are available from Canada, Australia and California.[2,3,14] In Europe, since the disease usually occurs sporadically, data are derived from single case reports or small case series.[16–18] In B&H and neighbouring countries (Croatia, Serbia), there are only a few reports of cryptococcosis in human patients[19–21] and cases of companion animal cryptococcosis have not been reported in this region.

Cryptococcosis affects cats more commonly than dogs[4] with no sex predisposition.[3] A broad age range of cats with cryptococcosis has been reported;[2,3,6] in contrast, over 80% of dogs with cryptococcosis were aged less than 5 years at the time of diagnosis.[2] It has been postulated that most cats are infected while young, with their immune system restricting the microorganism to a quiescent focus from which it may activate later in life as a result of comorbidities and stress.[3] Stray cats can also be asymptomatic carriers. Danesi et al[22] have shown that the nasal vestibule of stray cats can be colonised by *Cryptococcus* if environmental niches favourable to its development are present in an area. Consequently, the incubation period varies from months to years, with the source of infection often remaining unknown.[16,23,24] The same applies to our patient, which was a 2-year-old

adopted stray cat and its general and travel history could not be determined.

The route of infection seems to be similar among host species, with *Cryptococcus* species being acquired from the environment primarily through the inhalation of basidiospores or more rarely by ingestion or direct cutaneous inoculation.[3] The upper respiratory tract is the primary site of cryptococcal infection in dogs and cats.[3,16,25] It is considered that in animals with a smaller tidal volume, spores are better captured by the respiratory mucosa of the nasal turbinates as a result of specific filtration and turbulent flow, compared with large and athletic animals, such as horses and cheetahs, which are more likely to have tracheal or pulmonary disease.[1] After inhalation, yeast cells adhere to and invade the epithelial mucosal cells. In cases of mucosal invasion, disease can develop locally and/or systemically. Accordingly, there are several forms of the disease: nasal, central nervous system (CNS), cutaneous and systemic.[16] Clinical signs depend on the extent of the infection.

The nasal form is the most common form of the disease in cats. It occurs as a chronic sinonasal disease, with or without involvement of local skin, subcutis, bones and regional lymph nodes.[16,26] The most common clinical signs reported are rhinitis, chronic nasal discharge, facial swelling, skin ulcerations, deformation of the nasal cavity and the presence of nasal planum granulomas, as seen in our case.[16,18,24,26,27] Affected patients do not show signs of pruritus.[16] Because the clinical signs resemble bacterial infections and neoplastic processes, the diagnosis of fungal disease can be delayed. This is why signs of inappetence, lethargy and weight loss usually

Figure 4 Reduction of clinical signs after initiation of antifungal therapy: (a) 1 month after treatment; and (b) 10 months after treatment with marked reduction of nasal and base of the ear masses.

accompany the primary signs of the disease. Complications that might arise are local dissemination through the cribriform plate to the CNS, with occurrence of neurological signs, and haematogenous dissemination, with clinical signs of both neurological and multi-organ involvement.[16] These complications can be life threatening in the chronic course of the disease.

It has been postulated that retroviral infections in cats may predispose them to cryptococcal infections;[28] however, our patient presented with a negative retroviral status. This finding is in accordance with studies that have not found an increased incidence of FIV and FeLV infections in cats with cryptococcosis.[2,3,15,26]

Cytology and histopathology remain a rapid, inexpensive and sensitive means of diagnosing cryptococcosis in animals.[15,16,25,29] Samples can be collected from lesions and submitted either for cytology and histopathology and/or for microbiology and molecular identification.[6,30] Isolation and molecular identification give the opportunity to identify the species and its genotype. In this study, only a bacteriological analysis was conducted, which did not lead to fungal microorganism detection or identification; however, in reports of human cryptococcosis in the region, the identified species was *C neoformans*.[19–21] This could imply that the species encountered in our case could, in fact, be *C neoformans*, which has predominantly been isolated from cats in Europe.[17,18,22,31]

In the present case, the cat was initially treated symptomatically, until the results of cytology and fungal antigen detection enabled definitive diagnosis. Regarding cryptococcosis, the choice of appropriate antifungal drug depends on many factors, such as the extent and location of the disease, especially the presence or absence of CNS involvement.[4] Different antimycotic medications have been administered to treat cats.[16,32] There are no data suggesting one drug choice is better than another. Our treatment of choice was itraconazole because of the localised nature of the disease and the availability of the drug in our region. The treatment should be continued until LCAAT results come back negative.[33]

The prognosis is favourable in most cases of feline cryptococcosis provided that the diagnosis is established early and that the owner demonstrates strong commitment to treatment. According to O'Brien et al,[4] disease severity does not impact therapy outcomes; however, the presence of CNS involvement does, although a complete recovery has been documented even in such cases.[34,35]

Conclusions

It is important to highlight that this case of feline cryptococcosis was not readily diagnosed by the primary veterinarian and a referral was necessary. Given the extent of the tissue destruction, it is plausible that the surgical intervention may have spread the infective agent deeper into the tissue and surrounding facial structures.

Because cryptococcosis is rare in Europe and has not been described in veterinary patients in this region before, this study draws attention to and advocates for diagnostic vigilance for fungal disease in cats presented with relevant clinical signs in this part of Europe.

Ethical approval The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognised high standards ('best practice') of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in *JFMS Open Reports*. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

Informed consent Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). For any animals or people individually identifiable within this publication, informed consent (verbal or written) for their use in the publication was obtained from the people involved.

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