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CASE REPORT
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Cats with Central Nervous System Cryptococcosis

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ABSTRACT

***Background*:** Cryptococcosis is a serious fungal infection contracted by humans and animals, and the most common systemic mycosis found in cats. This disease is often contracted through inhalation of fungal propagules. The Central Nervous System (CNS) may be infected through local extension (nasal and frontal sinuses) or via hematogenous route. Similarly to CNS bacterial infection, the clinical signs of neurological dysfunction may be attributed to mass effect (gelatinous mass of fungal microorganisms and fungal granuloma formation) or to a more disseminated inflammatory response to invading microorganisms. The objective of this study is to report one case of a patient with cryptococcal granulomas in the central nervous system and one case of a patient with neurological signs associated to a cryptococcosis.

***Cases*:** *Case 1*. A 3-year-old male mixed breed feline was admitted to a veterinary clinic, located in Porto Alegre, RS, Southern Brazil. The patient presented unsourced behavioral changes, vestibular ataxia and dysphagia caused by inability of coordination. The following tests were performed: complete blood count test, biochemical analysis, computed tomography scan (CT scan), fluid analysis, radiography and toxoplasmosis test. The following medicine were administrated for treatment: fluconazole, dexamethasone, mannitol, phenobarbital and levetiracetam. Fluid therapy was also part of the treatment. Immediately after death, the cat was submitted for necropsy, and a fungal granulomatous meningoencephalomyelitis was diagnosed. *Cryptococcus* sp. was identified as the causal agent through pathological findings, fungal culture and PCR analysis. *Case 2*. One year later, another feline was admitted to the same clinic (a 2-year-old female mixed breed) presenting hypersalivation, tremors and excessive vocalization. The patient had contact with the deceased feline. The following tests were performed: complete blood count test, biochemical analysis, computed tomography scan (CT scan), cerebrospinal fluid analysis, and radiography. The following drugs were administrated for treatment: fluconazole, prednisolone, phenobarbital, potassium citrate and cefalotine. This patient is frequently monitored by a veterinarian and presents adequate health conditions after the occurrence. *Cryptococcus* sp. was identified as the causal agent through fungal culture and cytology (cerebrospinal fluid).

***Discussion*:** Cats are the most frequently infected animals with the involvement of the upper and or lower respiratory tract, subcutaneous granuloma, and disseminated infections. These animals present a higher quality of life when the disease is diagnosed and treated early. Peripheral enhancement of intracranial cryptococcal granulomas has been demonstrated in felines. These fungal granulomas often present evidence of significant perilesional edema. The most substantial evidence for diagnosis of cryptococcosis is the identification of the microorganism in Cerebrospinal fluid (CSF) samples. The mechanism of the lesion is cellular death, probably caused by secondary atrophy from distortion and tissue compression due to the expansion of *Cryptococcus* cysts in the cerebral parenchyma. There are several reports of long-lasting remission or cure of CNS cryptococcosis treated with drug combinations, including flucytosine and/or triazole antifungal agents (itraconazole and fluconazole.) The isolated use of flucytosine may contribute to development of drug resistance. One controversial alternative that may be beneficial to the patient is the administration of low prednisolone doses in order to avoid perilesional edema in the initial stages of treatment. The utilization of glucocorticoids after diagnosis was associated to higher survival rates in animals.

Keywords: cryptococcosis, *Cryptococcus* sp., felines, central nervous system, CNS.

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INTRODUCTION

Cats are the most frequently infected animals with the involvement of the upper and or lower respiratory tract, subcutaneous granuloma, and disseminated infections [8]. The most common species causing disease are *C. neoformans* and *Cryptococcus gattii* in humans and animals [1]. According to the literature, feline infection caused by *C. neoformans* is rare [11] and infection caused by *C. gattii* is more common in tropical and subtropical climates [9,11]. Cryptococcosis is contracted through inhalation of airborne fungal propagules microorganisms such as yeast cells desiccated by environmental exposure. The pathogenicity of yeasts depends of factors such as inoculum size, virulence of the cryptococcal strain and the state of defense of the host [1]. The infection occurs in the lungs, but it is believed that the microorganism suffers hematogenous dissemination to other places (such as the central nervous system) through macrophages [11]. The clinical signs of animals with cryptococcosis are anorexia, weight loss, lethargy, nasal secretions, sneeze, cough, skin lesions and ocular disease (uveitis, granulomatous chorioretinitis and exudative retinal detachment), observed through neurological signs [5]. Neurological signs related to cryptococcal infection in felines are obnubilation, behavioral and temperamental changes, hyperesthesia, contractions or tremors, seizures, circular motion, head pressure, ataxia, paresis, head inclination and other vestibular signs, anosmia and blindness [8,11,12]. Cryptococcosis in central nervous system (CNS) is diagnosed through computed tomography scan (CT scan), Magnetic Resonance Imaging (MRI scan), medical history, clinical exams and cytologic evaluation of the cerebrospinal fluid, where neutrophilic or mononuclear (mixed) pleocytosis may be observed. The most common occurrence in felines is the neutrophilic pleocytosis [5,12]. The objective of this study is to report one case of a patient with cryptococcal granulomas in the central nervous system and one case of a patient with neurological signs associated to cryptococcosis.

CASES

Case 1. A 3-year-old male mixed breed feline was admitted to a veterinary clinic, located in Porto Alegre, RS, Southern Brazil, in April 2019. The feline tested negative for FIV and FeLV and presented unsourced behavioral changes and vestibular ataxia. The symptoms persisted for 4 months, according to the tutor. The following tests were performed: complete blood count test, biochemical analysis, fluid analysis, radiography, toxoplasmosis test (indirect immunofluorescence assay (IFA): antibodies IgM and IgG negatives) and computed tomography scan (CT scan). The blood count test presented alteration of lymphocytes and superior platelet aggregation. The biochemical analysis presented results of albumin 2.51 g/dL, AST/ GOT - aspartate aminotransferase 56.00 U/L, creatinine 1.8 mg/dL, alkaline phosphatase 25.0 U/L, Gamma-glutamyl transferase (GGT) 0.00 U/L and urea 54.8 mg/dL with moderate hemolyzed serum. The fluid analysis with medical history of somnolence presented 69.0 mg/dL of proteins and sample composed of 65.8% of mononuclear and 34.1% polymorphonuclear, with the presence of erythrocytes. The radiography test presented pneumonia pattern (Figure 1). The head CT scan presented a discrete amorphous region in the hypophysis, captured in contrast; standard homogeneous brain parenchyma and cerebellar in pre and post contrast, with absence of mass effect; median line, cribriform plate and other bone structures intact; non-apparent lateral, third and fourth ventricles (diffuse edema); preserved left and right ears and normal head structures. The CT scan suggested neoplastic (adenoma) and infectious or noninfectious inflammatory processes. The following drugs were administrated for treatment: fluconazole[1] [0.5 mg/kg VO BID], mannitol[2] [10 mL/kg VI], Phenobarbital[3] [1 mg/kg BID], dexamethasone[4] [0.125 mg/kg SID], levetiracetam[5] [20 mg/kg PO TID] and fluid therapy[2] [70 mL/kg/day]. The patient was unresponsive to treatment and died.

Immediately after death, the cat was submitted for necropsy. Multiple samples of tissues were collected, fixed in 10% neutral buffered formalin, routinely processed for histology, and stained with hematoxylin and eosin (HE). *Post mortem* examination showed multifocal petechiae in the right cerebellar hemisphere, extending to the vermis and the oblong medulla. On the surface cut, gelatinous, pale, yellow to brown masses, which measured 0.5 to 1 cm in diameter were identified randomly in a couple of regions of the brain, including the parietal and frontal cortex, thalamus, and the cerebellar parenchyma (Figure 2). In addition, the leptomeninges of the brain were mildly edematous, thicken and slightly opaque. Microscopically, several fungal structures were observed in the cortex and leptomeninges of the frontal and parietal lobes of the brain (Figure 3), thalamus, cerebellum, sections of the spinal cord (cervical, thoracolumbar and sacral segments) and optic nerve. These organisms measured 10 to 20 μm in diameter, were ovoid to spherical, thick-walled, yeast-like structures that were surrounded by negatively-stained

halos of variable thickness compatible with *Cryptococcus* spp. These yeasts were often associated with a mild inflammatory infiltrate of macrophages, lymphocytes, and neutrophils. Additionally, there were multifocal areas of marked hemorrhage in the leptomeninges of the cerebellum. In the lungs and mucosa of the nasal cavity, a few scattered yeast forms were identified, similar to those observed in the nervous system, often associated to a mild inflammatory infiltrate of macrophages and lymphocytes.

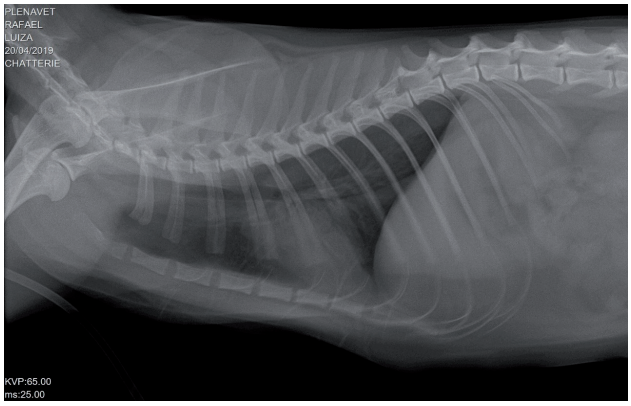

Figure 1. Case 1: the radiography test presented pneumonia pattern.

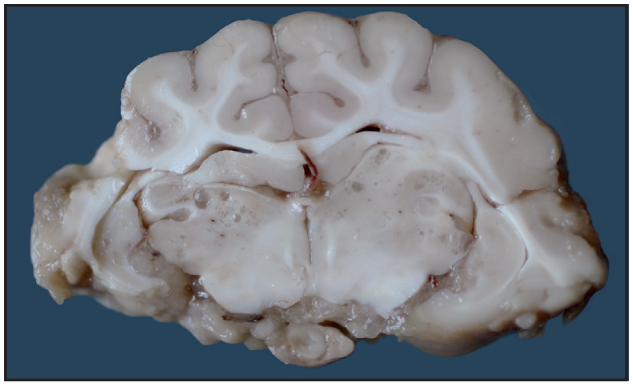

Figure 2. Case 1: transverse section of the brain. Numerous intracerebral gelatinous, pale masses measuring 0.5 to 1 cm in diameter.

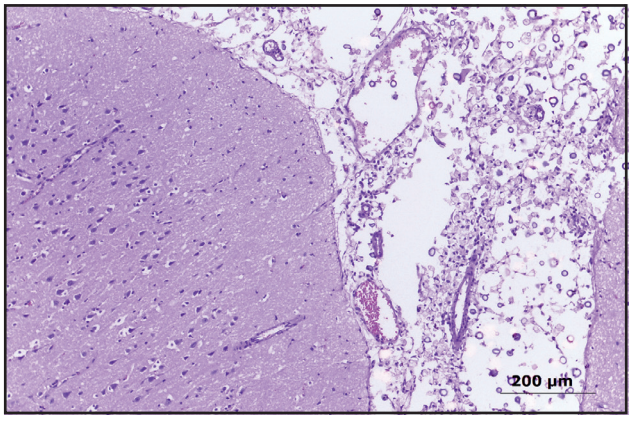

Figure 3. Case 1: granulomatous leptomenigitis by *Cryptococcus* spp. Leptomeninge of the brain markedly thickened by large amounts of cryptococcal yeast in a middle of a mild inflammatory infiltrate [HE; Bar= 200 µm].

Case 2. In April 2020, another feline was admitted to the same clinic (a 2-year-old female mixed breed). The animal had been presented unsourced behavioral changes (hypersalivation, tremors and excessive vocalization) for 7 days. The patient lived in the same residence of the deceased feline of case 1. This feline was not tested for FIV and FeLV. The following tests were performed: complete blood count test, biochemical analysis, radiography, fluid analysis and computed tomography scan. The presence of several red blood cells was detected in microscopic analysis of cerebrospinal fluid sample, 14% regular-sized lymphocytes, 56% neutrophils (rare degenerate), 17% eosinophils, 13% monocytes, absence of platelets and mitosis figures, phagocytic activity. The result of fluid analysis presented high levels of protein (57 mg/dL), which suggested an infectious/inflammatory process, tissue necrosis, alteration in CSF absorption and flow. The positive result in Pandy test[6] suggested alteration in blood-brain barrier permeability. The relation of fluid and blood glucoses was 66.65% and needs cautious evaluation due to the high glycemic levels of the patient. The sample presented mixed pleocytosis, which may be associated to meningoencephalitis, feline infectious peritonitis, diseases caused by protozoa and fungi, discopathy, ischemia and neoplasia. The CT scan showed preserved encephalic and cerebellar sulci; average morphology encephalic parenchyma, with focus of unenhanced hypersignals post-contrast in the pyriform lobes and average ventricular system and preserved ears.

The following drugs were administrated for treatment: potassium citrate[7] [250 mg/kg VO SID], phenobarbital[3] [3 mg/kg IV BID], cefalotine[8] [22 mg/kg IV TID], fluconazole[1] [0.5 mg/kg VO BID], prednisolone[9] [2 mg/kg VO SID], and fluid therapy[2] [150 mL/kg/day]. This patient is frequently monitored by a veterinarian and presents adequate health conditions after treatment.

Tissue fragments and cerebrospinal fluid from cases 1 and 2 were plated onto Sabouraud Dextrose Agar[10] and incubated at 30°C for 48 h. In addition, the isolate was discriminated by a color reaction developed on bird seed agar. Spherical-to-oval yeast cells surrounded by a mucopolysaccharide capsule were observed. Based on macro and micromorphology features, the isolate was consistent with *Cryptococcus* spp. Additionally, DNA was extracted

from these colonies using the Qiagen DNeasy® plant mini DNA extraction kit[11], according to the manufacturer instructions. DNA extracted was detected with panfungal polymerase chain reaction (PCR) using ITS1-F and ITS4-R primers [14] for amplification of internal transcribed spacer 1 and 2 regions. This reaction was performed in a 25 μL mixture containing 1 μL of DNA extract, 12.5 μL Taq PCR master mix (Qiagen)[11] and 0.5 μL of each primer (for a 0.2 μM final concentration of each primer). After a preincubation at 94°C for 15 min, amplification was performed for a total of 35 cycles, as it follows: denaturation at 94°C for 30 s, annealing at 51°C for 45 s, extension at 72°C for 1 min, and a final extension step of 10 min at 72°C. PCR product was separated onto 2% agarose, purified using PureLink® PCR Purification Kit[12] and sequenced to confirm the identity of the fungal isolate.

DISCUSSION

Diagnosis of cryptococcosis was based on the clinical, pathological, and identification of the fungus through mycological and molecular techniques. Early diagnosis and treatment improve health conditions and provide a higher quality of life to the animal. The identification of cryptococcal microorganisms in samples is the strongest evidence of diagnosis. Central nervous system (CNS) involvement in cats results from either cryptococcal meningitis or multifocal granulomatous encephalomyelitis [10]. Fungal infections in CNS induce to mixed pleocytosis and higher levels of protein in CSF [2], as observed in cases 1 and 2 (69 mg/dL and 57 mg/dL, consecutively). The nature of pleocytosis is highly variable, but generally presents high proportions of mononuclear cells and neutrophils [2]. Color and clarity are important diagnostic characteristics of CSF. Turbid CSF indicates alterations such as increased number of cells and proteins. The CSF sample in case 1 presented high levels of neutrophils and a colorless, turbid aspect that indicates infections. The CSF sample in case 2, presented a pink, turbid aspect. The color pink indicates blood contamination during sampling or trauma hemorrhage, while the turbid aspect indicated high levels of proteins and infection [3].

The pathological findings observed in case 1 were compatible with a fungal granulomatous meningoencephalomyelitis. The meninges are often involved in cases of neurological cryptococcosis in cats; however, the granulomatous reaction is usually minimal [10]. The thick yeast capsule is composed of polysaccharides, of which masks the yeast from recognition by phagocytes and may suppress T-cell responses, which explains the decrease in the inflammatory host response [4].

The visualization of yeasts in the nasal mucosa in the present case corroborates with the hypothesis that the nasal cavity is the initial site of infection in cats [12]. *Cryptococcus* propagules penetrate the nasal cavity and primarily infect the respiratory system, extending to the lungs, and can spread via the bloodstream to other tissues, or can infected the CNS by local extension [4,13]. In the case 1, the marked amount of yeasts cell observed in the optic nerve suggests that the infection in the CNS was secondary to extension from the optic nerve, of which probably was infected secondary to nasal cavity infection.

Despite the marked amount of yeasts cell numbers observed in the histopathology of case 1, these cells were not seen in the CSF analysis, similar to reported in another study [10]. Thus, it is important always to perform fungal cultures on all animals in which cryptococcosis is suspected, specially cats with neurological signs.

Cryptococcus species can be isolated readily in the laboratory on standard media. Fungal culture is considered as diagnostic method pattern of reference for the identification of *Cryptococcus* sp. and associated with microscopic examination of the tissue and fluid and PCR technique confirmed the etiological agent in both cases.

The treatment and prognosis are not clearly defined due to the scarcity of data about antifungal treatment in felines with CNS cryptococcosis. The most effective drug for treatment is fluconazole. The main disadvantage of fluconazole is the high cost. Few antifungal drugs are capable of traversing the blood-brain barrier effectively, even when inflamed. Flucytosine (5-fluorocytosine) and fluconazole (triazole agent) can easily traverse the blood-brain barrier [2,6,7]. The effectiveness of fluconazole was comproved in case 2, in which the patient presented an adequate recovery.

Cryptococcal infection in cats can results in multifocal granulomatous encephalomyelitis; however, animals with cryptococcosis in CNS can present adequate survival with frequent medical supervision, as exemplified in case 2. Supervision is necessary for at least 2 months in order to improve elimination of

cryptococcal granulomas from the organism, until the patient presents reduction of clinical signs and/or serum levels appear negative. The investigation of any unsourced behavioral changes followed by therapy in early stages of the disease are essential to achieve treatment success, even though there is not a consensus regarding treatment. Moreover, it can be concluded that imaging methods are also essential to conduct a precise and correct diagnosis.

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