Feline Histoplasmosis in Brazil: Clinical and Laboratory Aspects and a Comparative Approach of Published Reports

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Abstract The present study described clinical and epidemiological aspects of three cases of feline histoplasmosis and compared them to previously described cases. A detailed mycological identification and antifungal susceptibility profile of each isolate are presented. Secondly, a serological survey for anti-*Histoplasma* antibodies was performed with domestic and wild cats. Diseased animals presented nodular to ulcerated skin lesions and respiratory disorders as main clinical signs. *H. capsulatum* var. *capsulatum* was isolated and the strains showed to be susceptible to antifungal drugs. Considering that feline histoplasmosis is uncommonly observed in veterinary clinics, diagnosis, and clinical management in endemic areas should be improved.

Keywords Cats · Felids · *Histoplasma capsulatum* · Antifungal susceptibility profile · Serologic survey

Introduction

The causative agent of histoplasmosis, the dimorphic fungus *Histoplasma capsulatum* var. *capsulatum*, exists in the environment as a mycelium-producing saprophytic species in the soil and is widely distributed throughout the world. It infects many mammals, including dogs, cats, and humans, [1, 2] and the resulting disease is considered the second most common systemic mycosis in cats [1]. Although it has been reported that feline histoplasmosis in

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tropical, subtropical, and temperate areas is frequent [3, 4], little information is available on feline histoplasmosis in Brazil, where only one case of the disease was reported in a domestic cat [5]. Considering the importance of histoplasmosis for Veterinary Mycology, as well as the scarcity of case reports in small animals, three cases of feline histoplasmosis in Brazil are presented in this study, with emphasis on clinical and phenotypical findings. In addition, data of a serological survey conducted with domestic and wild felids from the region are presented.

Methods

Case 1

A 6-year-old Siamese castrated male cat, from the city of Sobral (3°41'S–40°20'W), Ceará State, Northeastern Brazil. The animal lived in house and was allowed free outdoor access; initially, the cat presented good general conditions, with normal body temperature and hydration status, but discrete lymphadenomegaly and a non-ulcerated nasal mass, with approximately 2 months of evolution. Complete blood count and serum chemical profiles were within normal ranges and no clinical or serological evidences for other diseases, such as FeLv (feline leukemia virus) or FIV (feline immunodeficiency virus) were observed. Biopsy fragments were obtained from the borders of the nasal mass, for microbiological and histopathological analyses, through a surgical procedure, under anesthesia with association of xylazine (1 mg/Kg) and ketamine (10 mg/Kg). Based on microbiological results, the cat was initially treated with itraconazole (10 mg/Kg), orally, every 24 h, for 21 days. Treatment was suspended because the animal started to vomit, leading to clinical and prognostic worsening. Finally, the owner chose to euthanize the animal.

Case 2

A 3-year-old mixed-breed male cat, native to the city of Baturité (4°19'S–38°53'W), Ceará State, was investigated. The animal lived in a house surrounded by fruit trees and native vegetation, where other animals were also raised, such as chickens, pigeons, goats, dogs, and other cats were also seen. The cat presented two ulcers of approximately 1.5×2.0 cm

on the tail, near the anus, with 4 months of evolution. The ulcers presented elevated borders separated by a fine skin tissue and erythematous granulation tissue in the bed. Complete blood count, serum biochemical analyses, and serological tests for FeLv and FIV were not performed. An excisional biopsy of the lesion was performed, for microbiological and histopathological analyses, while the animal was under anesthesia with the association of xylazine (1 mg/Kg) and ketamine (10 mg/Kg). To evaluate the occurrence of primary lung infection, a thoracic X-ray was taken, but no alterations were observed. Therapy with itraconazole (5 mg/Kg), orally, every 24 h, for 60 days, was attempted after mycological diagnosis, but the lesion did not regress. Twenty days after the end of the antifungal therapy, the cat suddenly became lethargic, apathetic, and unconscious and died. A necropsy was performed and the main findings were suggestive of feline urological syndrome (FUS).

Case 3

A 3-year-old mixed-breed female cat from Fortaleza (03°41'15'S–38°29'W), Ceará State, was examined for presenting an enlargement of the nasal region, with nodules and purulent secretion, which obstructed the upper airway, causing respiratory distress. Through palpation, a fluctuating cervical nodule was detected. Pulmonary auscultation revealed the presence of secretion and thoracic radiographs evidenced the occurrence of pneumonia. In addition, complete blood count was within normal range, but biochemical analyses and serological tests for FeLv and FIV were not performed. Fine-needle aspiration biopsy (FNAB) of the nasal lesion was performed for cytological examination, which resulted in unspecific findings. Excisional biopsy was not performed. Besides FNAB, purulent exsudate from the nasal cavity was collected with a swab for bacteriological and mycological processing. The animal was treated with itraconazole (20 mg/Kg) orally, every 24 h, for 3 months, which was efficient and caused remission of clinical signs.

Classical Mycological Examination

Mycological examination was performed at the Specialized Medical Mycology Center (CEMM) of Federal University of Ceará, Brazil. Clinical specimens

obtained from the skin lesion of the three analyzed cats were inoculated in tubes containing agar Sabouraud, agar Sabouraud supplemented with chloramphenicol, and agar Sabouraud supplemented with chloramphenicol and cycloheximide. Macro and micromorphological characteristics were analyzed after 15–30 days of incubation at 28°C, as previously described [6]. The strains were also grown on 10% Sabouraud blood agar and incubated at 35°C for reversion to yeast-like phase [7]. All procedures were performed within a class II biological safety cabinet in a biosafety level 3 laboratory.

Antifungal Susceptibility Test

Microdilution testing was performed in accordance with the guidelines of the document M38-A and M27-A2 [8, 9] for mycelial and yeast-like phases, respectively, following the adaptations proposed by Brilhante et al. [7]. The three *H. capsulatum* isolates were tested against amphotericin B, itraconazole, voriconazole, caspofungin, and sulfamethoxazole-trimethoprim, since the latter has been successfully used for treating African histoplasmosis [10]. The results were visually read and interpreted according to previously described methodologies [7, 11]. Control strains (*H. capsulatum* var. *capsulatum* CEMM 03-4-036, *Candida parapsilosis* ATCC 22019, and *C. krusei* ATCC 6258) were used in order to assure the quality of the assays.

Serological Survey

The pursuit for the serum detection of anti-*Histoplasma* antibodies in cats was approved by the ethics committee of Ceará State University (Process 07381395-8). From January 2009 to June 2010, 131 serum samples from domestic cats were investigated, out of which 17 were from Sobral Zoonosis Control Center and 114 from Fortaleza (34 cats from veterinary clinics and 80 captured stray cats kept at Fortaleza Zoonosis Control Center or private shelters). In addition, six serum samples from wild felids were also collected, from four oncillas (*Leopardus tigrinus*) and two jaguarundis (*Puma yagouaroundi*) that were captured in the State of Ceará. The obtained serum samples were tested for the presence of anti-

Histoplasma antibodies by immunodiffusion (ID), using the *Histoplasma* ID Antigen (H & M), according to the supplier's instructions (Immy Immunodiagnostics, Inc., USA). Positive serum samples were tested for cross-reactions with coccidioidal antibodies using *C. immitis* IDCF antigen, following supplier's instructions (Immy Immunodiagnostics, Inc., USA).

Results and Discussion

The mycological analyses confirmed the identification of *H. capsulatum* in the three analyzed cases, as demonstrated by the growth of filamentous colonies that were initially smooth and white, but became cottony and brownish as they grew older. Microscopically, they were composed of hyaline septate hyphae with micro and macroconidia, including tuberculate macroconidia. Additionally, reversion to yeast-like phase was achieved.

The present study reports the first cases of animal histoplasmosis in the state of Ceará, Northeastern Brazil. It is noteworthy that despite the scarcity of animal histoplasmosis in Ceará, this state has a high incidence of human disease, particularly among HIV-positive patients [2]. Thus, considering the endemicity of human histoplasmosis in Brazil [2], it is believed that this disease is also common among animals. However, few reports corroborate this belief, since, in Brazil, *H. capsulatum* has only been isolated from soil containing bird or bat excrement [12, 13] and from some animal species, such as dogs [14, 15], opossums (*Didelphis albiventris*) [16] and from one cat [5].

In cats, *H. capsulatum* infection is mostly inapparent or it may cause clinical pulmonary granulomatous or disseminated disease, involving many organic sites, including lymphnodes and integument [1]. Respiratory compromise was observed in cases 1 and 3, corroborating previously described data concerning the occurrence of respiratory alterations [17, 18], but only case 3 presented signs of pneumonia, associated with skin lesions, suggesting the occurrence of disseminated histoplasmosis. Curiously, the only case of feline histoplasmosis reported in Brazil, also described the occurrence of a nasal cutaneous mass associated with respiratory distress, but with no signs of pulmonary compromise [5]. In case 2, only skin lesions were observed, raising the possibility of the occurrence of primary cutaneous histoplasmosis. In

Table 1 Main clinical findings of feline histoplasmosis from Ceará State, Brazil, compared with cases worldwide

Main clinical findings	Cases from Ceará state	Published cases of feline histoplasmosis worldwide	
		Country	
Cutaneous lesions and pneumonia (disseminated histoplasmosis)	Case 3	[23]	Italy
		[24]	Japan
		[3], [4], [17], [25], [26], [27], [28], [29], [30], [31], [32]	USA
Cutaneous lesions with no signs of respiratory distress	Case 2	–	–
Cutaneous lesions with signs of respiratory distress	Case 1	[5]	Brazil

our study, younger cats were affected (mean age of 4 years) and no gender predisposition was observed, as described in previous reports [1]. Table 1 summarizes the clinical findings observed in the present reported cases and compares them to previously published reports of feline histoplasmosis.

In vitro susceptibility testing of *H. capsulatum* demonstrated that all strains were susceptible to all tested drugs, with MIC mean values of 0.12; 0.016; 0.12; and 6.67 µg/mL for amphotericin B, itraconazole, voriconazole, and caspofungin, respectively, for mycelial phase, and 0.12; 0.023; 0.002; and 2 µg/mL for amphotericin B, itraconazole, voriconazole, caspofungin, respectively, for yeast-like phase. MICs for sulfamethoxazole-trimethoprim ranged from 0.08/0.016 to 0.3/0.06 and from 0.005/0.001 to 0.02/0.004 mg/mL for mycelial and yeast-like form, respectively. These findings agree with those from other studies with human strains of *H. capsulatum* [7, 11, 19–21].

Concerning the serological survey, none of the tested felids were positive for *Histoplasma* antibodies. This survey was performed through immunodiffusion (ID), but this technique presents low sensitivity, when compared to other techniques, such as ELISA [22], which may have compromised the detection of positive animals, considering that human histoplasmosis is highly prevalent in the state of Ceará [2] and that these animals may be equally exposed to this fungus.

These represented the first reports of animal histoplasmosis in the state of Ceará, Brazil, and knowledge on its clinical signs and epidemiology might enable veterinary clinicians to achieve early diagnosis and prescribe an adequate therapy more quickly.

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