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Atypical Morphology and Disparate Speciation in a Case of Feline Cryptococcosis

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Abstract A 6-year-old, spayed female cat was presented with acute respiratory signs and pleural effusion. Computed tomography scan revealed a large, lobulated mass effect in the ventral right hemithorax with concurrent sternal lymphadenopathy. A cytologic sample of the mass contained pyogranulomatous inflammation, necrotic material, and abundant yeast structures that lacked a distinct capsule and demonstrated rare pseudohyphal forms. Fungal culture and biochemical testing identified the yeast as *Cryptococcus albidus*, with susceptibility to all antifungal agents tested. However, subsequent 18S PCR identified 99% homology with a strain of *Cryptococcus neoformans* and only 92% homology with *C. albidus*. The patient responded well to fluconazole therapy unlike the only known previous report of *C. albidus* in a cat. The unusual cytologic morphology in this case underscores the need for ancillary testing apart from microscopy for fungal identification. Though *C. albidus* should be considered as a potential feline pathogen, confirmation with PCR is recommended when such rare non-*neoformans* species are encountered.

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Introduction

Cryptococcosis is the most common fungal infection of cats worldwide and is caused by dimorphic yeast in genus *Cryptococcus*, with two major species affecting cats—*C. neoformans* and *C. gattii* [1]. There are eight major genotypes between these species, as well as several subtypes, each with unique geographical distribution and clinical characteristics [2]. Other species have also been reported very rarely in the literature including a single case report of *C. albidus* in an immunosuppressed cat which died of systemic cryptococcosis [3]. *C. magnus* was incidentally isolated from the ear canal of a clinically healthy cat [4] and found to cause dermal lesions and lymphadenopathy in a young, apparently immunocompetent cat [5]. These, and other more obscure cryptococcal species, were relatively commonly isolated from asymptomatic feral cats in Italy [6]. Therefore, non-*neoformans*/*gattii* species of *Cryptococcus* appear to be widely distributed but rarely pathogenic.

Case Description

A 6-year-old, spayed female, indoor-only domestic shorthair cat residing in Virginia, USA, was presented

to a veterinary internist for evaluation of acute respiratory distress, pleural effusion, and a cranial mediastinal mass effect. Computed tomography (CT) scan was taken to evaluate the origin of the mass and to determine surgical options (Fig. 1). CT scan demonstrated the presence of a large, lobulated, heterogeneously contrast-enhancing mass effect in the ventral right hemithorax. The mass was causing cardiac displacement to the left and extended into what appeared to be the mediastinum caudal to the heart. There was concurrent enlargement of the sternal lymph node. Neoplasia was highly suspected. A fine needle aspirate of the mass was obtained and submitted for cytological evaluation at the Clinical Pathology Laboratory at Colorado State University. The cytologic sample contained significantly increased numbers of macrophages and many non-degenerate neutrophils. There were many ovoid, approximately 8–10 μm, pale and eosinophilic, yeast structures with internal basophilic stippling and a thin clear external halo. They were observed both extracellularly and within macrophages. Low numbers of these structures extended into elongated tube-like forms (pseudohyphae). Often, the structures had smudged cytomorphology with aberrant staining including pale pink or clear structures. Blue–grey amorphous granular material was distributed throughout the smear. The cytologic interpretation was pyogranulomatous inflammation with poorly encapsulated, pseudohyphae-forming yeast and necrotic debris (Fig. 2). The cytologic appearance of these yeast was not considered pathognomonic for any commonly encountered dimorphic fungi; differentials included histoplasmosis, sporotrichosis, cryptococcosis, blastomycosis, candidiasis, and emmonsiosis. Fungal culture was recommended for identification to guide therapy.

The patient was hospitalized and empirically treated with oral fluconazole (50 mg, equal to 9.65 mg/kg, every 12 h) and intermittent thoracocentesis, while awaiting the results of a fungal culture of pleural fluid that had been submitted to the Bacteriology and Mycology Laboratory at Auburn University's College of Veterinary Medicine. The patient improved on therapy and was discharged on long-term oral fluconazole (same dose as above). The patient responded very well and was asymptomatic with no pleural effusion on radiographs at a follow-up

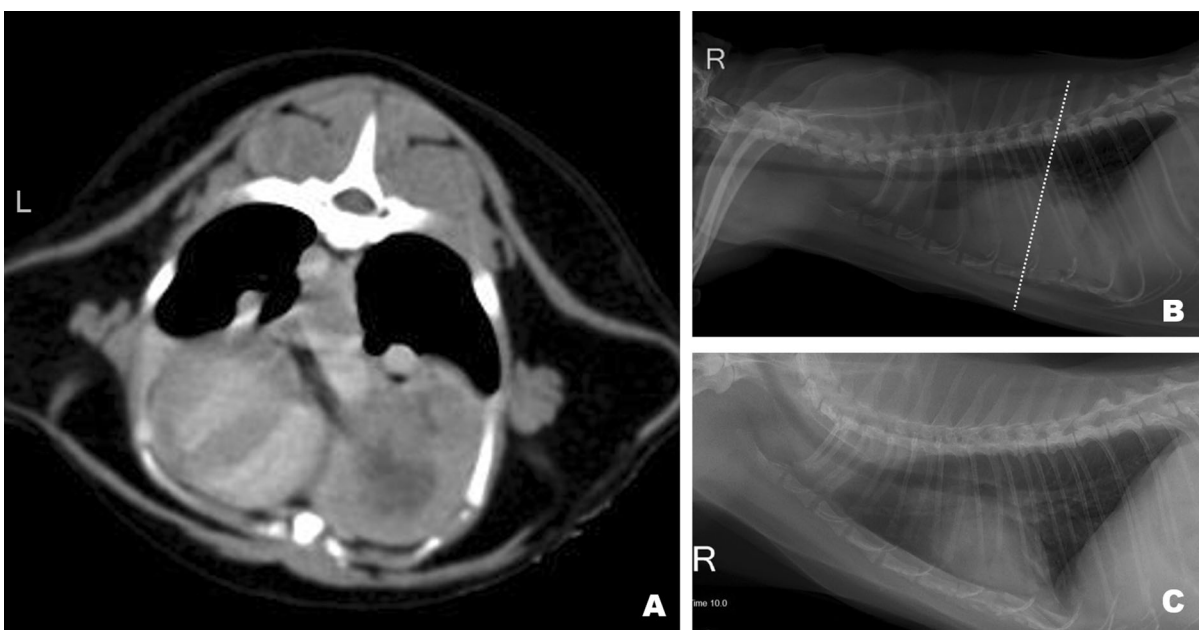

Fig. 1 Post-contrast transverse commuted tomography (CT) image of a 6-year-old spayed female cat at the level of the 8th thoracic vertebrae (*dotted white line* in B) obtained at the time of diagnosis of cryptococcosis demonstrating a large, cavitated mass in the *right ventral hemithorax* with *left-sided* cardiac displacement (**a**). *Right lateral* thoracic radiograph obtained following thoracocentesis at the time of diagnosis with scant pleural effusion and a nodular soft tissue opacity present both cranial and caudal to the cardiac silhouette (**b**). *Right lateral* thoracic radiograph obtained after 5 months of treatment with fluconazole demonstrating resolution of nodular mass effect and pleural effusion (**c**)

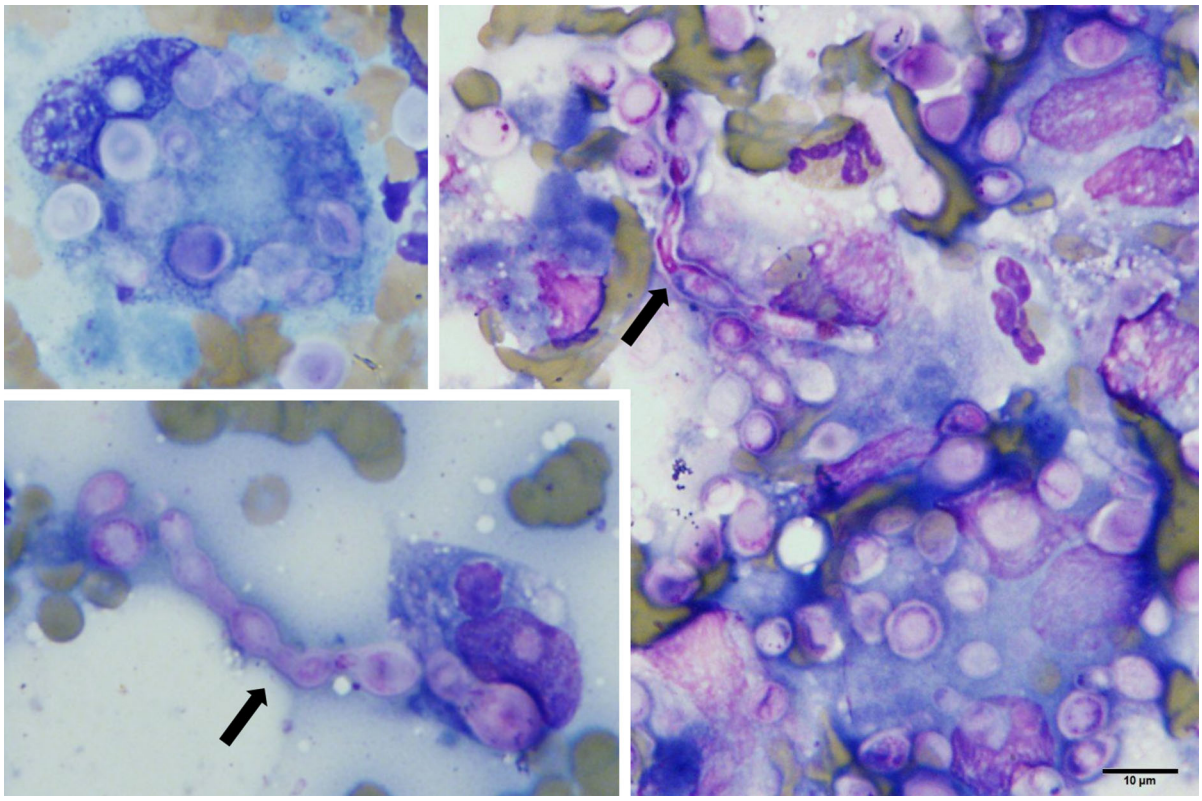

Fig. 2 Fine needle aspirate of mediastinal masses in a 6-year-old, spayed female cat. Photomicrographs demonstrate pyogranulomatous inflammation, necrotic debris, and abundant intra- and extracellular yeast organisms in varying stages of degradation/necrosis. Formation of pseudohyphae is observed (*black arrows*). Wright–Giemsa stain, ×1000 magnification

appointment 5 months after initial presentation (Fig. 1).

The fungal culture showed heavy and rapid growth with colonies noted at 48 h of culture. The isolate grew as a yeast form at 30 °C with typical colony size and appearance for *Cryptococcus*. The isolate had no evidence of capsule production even in capsule-stimulating media. For identification, the RapID Yeast Plus by Remel (Thermo Fisher Scientific, product #R8311007) was used, with resulting 99.8% probability of *C. albidus* and 0.19% probability of *C. neoformans*. Since a rare organism was identified, ancillary biochemical testing was used for confirmation. Select culture and biochemical test results are summarized in Table 1 and are contrasted with expected results for both *C. albidus* and *C. neoformans*. Subsequent susceptibility results revealed the isolate to be susceptible to fluconazole (3 µg/mL), ketoconazole (0.064 µg/mL), itraconazole (0.094 µg/mL), voriconazole (0.032 µg/mL), and amphotericin B (0.023 µg/mL). Values provided are mean inhibitory concentrations (MICs) based on epsilometer testing (*E*-test).

The clinical response to therapy contrasted with the previously reported cases of *C. albidus* in a cat [3]. Therefore, the authors submitted a cytologic preparation to confirm the identification made by traditional biochemical assays to the University of Illinois Veterinary Diagnostic Laboratory for molecular identification by polymerase chain reaction (PCR) and sequencing for 18S rRNA. The methods and primers used for amplification are previously described [7, 8]. The resulting 550-base pair amplicon was compared with sequences in the NCBI GenBank using a megablast search. It was found to be 99% identical to *C. neoformans* (accession KY102839.1). When compared with all GenBank sequences for the organism *C. albidus* (taxid:100951), the highest total alignment score was 92% identical (accession KC295589.1). The 604-base pair amplicon was submitted to GenBank as accession #KY967697.

Table 1 Selected culture and biochemical results for the patient sample compared with expected characteristics of two cryptococcal species

Characteristic	Patient sample	Expected for *C. neoformans*	Expected for *C. albidus*
Germ tube	Negative	Negative	Negative
India ink prep capsule formation	Negative	Positive	Positive
Urease	Positive	Positive	Positive
Citrate utilization	Positive	Positive	Positive
Growth with cycloheximide	Negative	Negative	Negative
Growth at 37 °C	Negative*	Positive	Variable
Nitrate assimilation	Positive*	Negative	Positive
Caffeic acid disc	Negative*	Positive	Negative/slight

* Denotes results which are more consistent with *C. albidus* than *C. neoformans*

Discussion

Here we describe a case of cryptococcosis in the thoracic cavity of a cat with unusual cytologic morphology and discordant speciation results. At the time of viewing, *Sporothrix* or *Candida* were thought most likely due to the presence of pseudohyphal structures and lack of a distinct clear polysaccharide capsule which is commonly expected of *Cryptococcus* species. After the initial cytologic review, the sample was reviewed by dozens of board-certified clinical pathologists during an online international rounds for the American Society of Veterinary Clinical Pathology[1]; polling demonstrated that the majority of participants considered the morphology of this sample most consistent with *Sporothrix* (a minority chose *Cryptococcus*). Pseudohyphal forms of *Cryptococcus* are not normally found in vivo. They have very rarely been observed in clinical samples of *C. neoformans* [9], but to the authors' knowledge this has not reported in *C. albidus*.

Non-*neoformans*/*gattii* cryptococcal infections of humans have most often been associated with immunological impairment; prevalence rose with HIV/AIDS pandemic [10]. The case described here was not associated with any known immunological disorder, and the patient tested negative for FIV/FeLV infection.

The only known report of *C. albidus* in a cat in the veterinary literature was identified using both biochemical testing and 28S sequence analysis queried against the DNA Data Bank of Japan (DDBJ). This is in contrast to 18S analysis queried with GenBank (which encompasses the DDBJ). The cat in the current report responded well to therapy, in contrast to the cat in that report which died after 3 days of voriconazole treatment [3]. The rapid resolution of a severe infection in this cat may suggest lower pathogenicity compared with typical cryptococcal infections. Antigen testing in serum such as latex cryptococcal antigen agglutination test (LCAT) was unfortunately not performed in this case due to the initially very low suspicion of fungal infection. This information would have been useful to monitor response to therapy, document infection eradication, and provide indirect data about the cryptococcal species.

There was a disparity in this case between PCR results and biochemical testing for the purpose of fungal speciation. Traditionally, fungal species identification has been made exclusively on phenotypic characteristics, and this is still largely the case in clinical microbiology laboratories [11]. The RapID Yeast Plus identification method has been shown to be greater than 95% accurate in identification of clinical yeast isolates [12]. A previous report has found a high degree of genetic variability among non-*neoformans* isolates from veterinary species [13], though the implications for pathogenesis has not been investigated. A report of meningitis in a human patient was originally identified as *C. albidus* based on biochemistry results and later identified as *Cryptococcus adeliensis* based on DNA sequencing [14]. One potential explanation for the discrepancy in that report

[1] American Society of Veterinary Clinical Pathology online rounds, August 18th, 2016. Proceedings published at https://www.asvcp.org/membersonly/rounds.cfm.

as well as the present one is the largely unknown rates of evolution for morphological, biochemical, and reproductive traits of fungi in nature [11]. Although there is no official consensus on which is most correct, DNA sequencing and other molecular-based techniques are increasingly accepted as the truest means of microbiological speciation. The distinction between *C. neoformans/gattii* and non-*neoformans* species is important for both epidemiological monitoring of emerging pathogens, and may hold implications for antifungal susceptibility. Specifically, fluconazole has been shown to have poor in vitro activity against *C. albidus* [15], but is a common clinical choice for other cryptococcal infections and was the chosen treatment in this case.

The fungal isolate in this case was unfortunately not stored by the isolating laboratory for future use, which would have been of considerable benefit for the investigation of sequence homology of various cryptococcal gene targets (other than 18S rRNA), susceptibility to additional antifungal agents, mass spectrometric profile (e.g. MALDI-TOF), and other attributes. This information would have contributed greatly to our understanding of this unusual isolate; thus, preservation of all atypical fungal isolates is recommended as routine practice in the clinical microbiology setting.

Conclusions

The case of feline cryptococcosis presented here had an unusual cytologic morphology which prevented definitive identification of the genus of yeast by microscopy; *Cryptococcus* was considered unlikely. This underscores the need for ancillary testing for fungal identification. The cause of the disparate biochemical and 18S PCR analysis results is unknown. Molecular identification makes this case most likely *C. neoformans* infection, which is supported by the presence of pseudohyphae and positive response to fluconazole therapy. Though *Cryptococcus albidus* should be considered as a potential feline pathogen, confirmation with PCR is recommended when rare non-*neoformans* species are encountered.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

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