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CASE REPORT Open Access

First report of cryptococcuria in a bitch from India: a case report

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Abstract

Background: Cryptococcuria has not been reported in dogs although few cases have been described in humans and cats. Cryptococcuria is often seen as a manifestation of systemic or disseminated cryptococcosis. Early diagnosis and treatment is crucial as systemic infections are usually fatal. To the best of author's knowledge, this is the first reported case of cryptococcuria in a bitch from India.

Case presentation: An 8-month-old bitch was presented with anorexia, urinary incontinence and dysuria for the last 10 days. Urinalysis showed the presence of yeast cells, and *Cryptococcus neoformans* was confirmed by both fungal culture and gene sequencing. Antifungal susceptibility testing revealed resistance against ketoconazole, fluconazole and amphotericin B, while sensitivity towards itraconazole, voriconazole and terbinafine. Unfortunately, the bitch expired before the antifungal results were made available. Histopathological changes were observed in lungs which revealed several budding and encapsulated *C. neoformans* cells.

Conclusion: This report highlights the importance to conduct routine urine cultures for yeast infections along with antifungal susceptibility testing to identify and initiate proper antifungal therapy to improve clinical outcome.

Keywords: Antifungal susceptibility testing, Bitch, Case report, Cryptococcuria, *Cryptococcus neoformans*, Histopathology, India, Urine

Background

Cryptococcosis, most commonly caused by *Cryptococcus neoformans* and *Cryptococcus gattii*, is a fatal fungal disease in immunocompromised hosts (Hurtado et al. 2019). In animals, the clinical presentation of cryptococcosis often depends on the host and site of the infection (Refai et al. 2017). In dogs, the respiratory tract is the most common site of infection followed by the central nervous system, as opposed to cats, which mainly show localized or cutaneous lesions (Vorathavom et al. 2004).

Cryptococcuria is a relatively uncommon presentation, and only a few sporadic cases have been described in humans suffering from underlying conditions like HIV, diabetes mellitus and systemic lupus erythematosus (SLE) (Kiertiburanakul et al. 2004; Bae et al. 2011). While there are no reported cases of cryptococcuria in dogs, it has been described in cats from manifestation of disseminated infection (Chapman and Kirk 2008).

This study describes the first report of cryptococcuria in a bitch which was diagnosed by urinalysis, fungal culture and through internal transcribed spacer (ITS) gene sequencing of the ribosomal RNA (rRNA) region.

Case presentation

An 8-month-old, 7.5 kg, adopted stray bitch was presented with anorexia, depression, urinary incontinence and dysuria for 10 days. Approximately one month before presentation, the bitch was treated against anaemia, staphylococcal and mange infection at a local veterinary clinic. The treatment consisted of amoxicillin–clavulanic acid (Tab Clavam 375 mg; Alkem Laboratories Limited®) half tablet, twice daily for 15 days and was given a single dose of fluralaner

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(Tab Fluralaner 250 mg; Bravecto®). She also received a one-month supplementation of vitamin and chelated minerals (2.5 ml twice daily, aRBCe PET syrup; Vetoquinol®), omega fatty acids (4 ml once daily; Nutricoat Advance®) and immunol liquid (2 ml twice daily; Himalaya®) as immunomodulator.

The bitch was emaciated, dehydrated and had sunken eyes; nasal and ocular discharge was observed; and the body temperature was 103.9°F. Physical examination of the body revealed distended urinary bladder and abdominal pain. Pulse and heart rate were normal, and respiratory rate was 22–24 breaths per minute (bpm). A complete blood profile revealed low haemoglobin (9 g/dl; reference value 12–14 g/dl), leukocytosis (23,200/cu.mm; reference value 8000–18,000/cu.mm), neutrophilia (92%; reference value 62–80%) and lymphopenia (5%; reference value 10–28%). Blood biochemical parameters, however, were within normal limits.

Urinalysis revealed few epithelial and budding yeast cells without pseudohyphae (Fig. 1). Characteristic brown-coloured mucoid colonies were isolated on Bird Seed Agar (Fig. 2), and India ink staining showed encapsulated budding yeast cells suggestive of *C. neoformans* (Fig. 3). Further confirmation was determined by sequencing the ITS region of the rRNA with the help of primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC). Gene sequence of the isolate revealed 98.96% similarity with *C. neoformans* (strain PWQ2291, accession no. KP132173), and the sequence has been submitted in the NCBI database bearing accession no. MT941007.

Fig. 1 Epithelial and budding yeast cells in urine of a bitch under microscope (magnification 40 ×)

Fig. 2 Brown-coloured mucoid colonies of *Cryptococcus neoformans* on Bird Seed Agar (BSA)

Antifungal susceptibility testing (AFST) was performed using broth micro-dilution assay (CLSI 2017). Minimum inhibitory concentration (MIC) values for fluconazole and amphotericin B were 32 µg/ml, while that of ketoconazole was 16 µg/ml. MIC values for itraconazole, terbinafine and voriconazole were 2, 1 and 0.13 µg/ml, respectively. The bitch had expired before the AFST

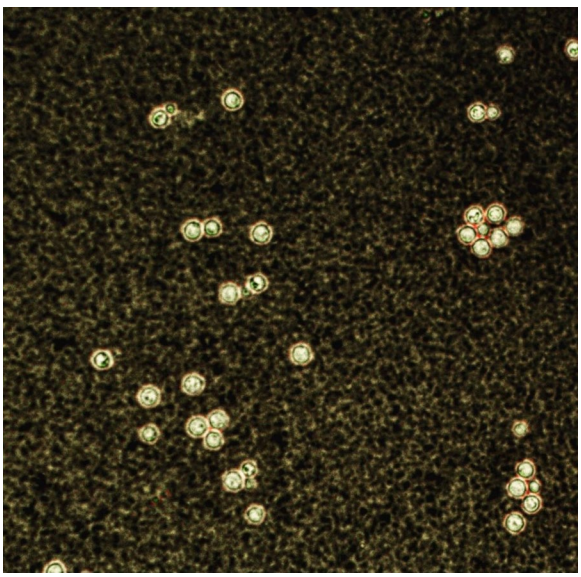

Fig. 3 India ink staining of *C. neoformans* showing encapsulated and budding yeast cells under phase-contrast microscope (magnification 100 ×)

results were available. After consent for post-mortem from the owner, tissues from lungs, kidney, heart, intestine and brain were collected, fixed in 10% neutral buffered formalin and were stained with Gomori's methenamine silver (GMS). Numerous dark-brown-stained round spores with budding and encapsulated yeast cells were noticed in the lungs (Fig. 4).

Discussion

C. neoformans is a ubiquitous fungal pathogen which can persist in the environment for years. Pigeons and their droppings are the important reservoirs of this fungus, and dogs have been known to get infected through inhalation of the spores (Vorathavorn et al. 2013). After establishment in the lungs, it spreads via the haematogenous route throughout the body (Maliehe et al. 2020). Thus, cryptococcuria is often reported secondarily to systemic or disseminated infections in humans and cats (Chapman and Kirk 2008; Severo et al. 2011). In the present case, prior to adoption of the bitch, she lived near the beach where numerous feral pigeons come for feeding. This frequent heavy exposure to *Cryptococcus* spores, suffering from *Staphylococcus* and mange, might have led to the establishment of *Cryptococcus* infection inside the lungs and finally dissemination through the urine.

As the routine examination of urine involves microscopic identification and not through growth in cultures or molecular tests, cryptococcuria in animals can be missed or misdiagnosed as *Candida* sp. (Bae et al. 2011). This will lead to improper treatment, antifungal resistance, and prolonged evolution of the disease. In this case, pulmonary involvement by *C. neoformans*, along with weakness, emaciation, severe skin infection, and failure to report to the veterinary clinic immediately after urinary symptoms might have increased the disease severity and death. Disseminated cryptococcosis is often fatal, and cryptococcuria's, being an early indicator of dissemination, early diagnosis proves crucial to improve clinical outcomes (Severo et al. 2011; Maliehe et al. 2020).

The most commonly used antifungals against *Cryptococcus* infections are amphotericin B and fluconazole (Chapman and Kirk 2008; Severo et al. 2011; Vorathavorn et al. 2013) against which the animal showed high resistance during AFST. If the treatment would have started randomly, it would have led to a non-responsive disease and ultimately further dissemination. In the present case, the antifungal treatment could have been started if the owner was not negligent in reporting the animal's urinary symptoms on time. It is therefore necessary to create awareness among the animal/pet owners regarding cryptococcosis and its consequences.

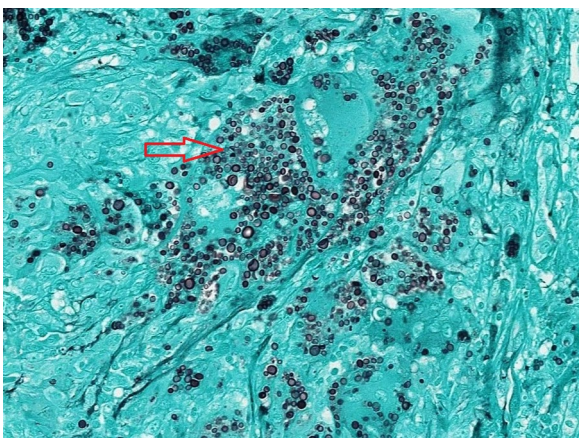

Fig. 4 Gomori's methenamine silver (GMS) staining of lungs showing numerous dark-brown-stained round spores (magnification 40 ×)

Conclusions

Routine urine cultures for yeast infections along with AFST should be carried out as a standard protocol to identify and initiate proper antifungal therapy. Laboratory technicians should also keep *Cryptococcus* sp. in the list of the differential diagnosis for urinary yeast infections in animals. To the best of the authors' knowledge, this is the first reported case of cryptococcuria in a bitch from India.

Abbreviations

AFST: Antifungal susceptibility testing; bpm: Breaths per minute; cc.mm: Cubic millimetre; g/dl: Grams per decilitre; GMS: Gomori's methenamine silver; HIV: Human immunodeficiency virus; ITS: Internal transcribed spacer; kg: Kilogram; mg: Milligram; MIC: Minimum inhibitory concentration; ml: Millilitre; NCBI: National Center for Biotechnology Information; RNA: Ribonucleic acid; rRNA: Ribosomal ribonucleic acid; SLE: Systemic lupus erythematosus.

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Authors' contributions

ADS conceived and performed the research; AB and KB wrote the paper and revised the final version. All authors read and approved the final manuscript.

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Availability of data and materials

The gene sequence of the isolate can be found in NCBI database under accession no. MT941007.

Ethics approval and consent to participate

Ethics approval in this study was secured from the Institutional Animal Ethics Committee (IAEC) of Faculty of Veterinary and Animal Science, WBUAFS, Kolkata, having number: IAEC/190(VI)/14.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

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