

# Use of Terbinafine in the Treatment Protocol of Intestinal *Cryptococcus neoformans* in a Dog

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## ABSTRACT

A 2.5 yr old sexually intact male vizsla was admitted to the Iowa State University Veterinary Teaching Hospital for persistent diarrhea, weight loss, and panhypoproteinemia. Examination revealed an emaciated condition and melena. Two masses were palpated in the cranial abdomen. Hematology and serum biochemistry exhibited a regenerative anemia and confirmed the presence of panhypoproteinemia, suggestive of a protein-losing enteropathy. Distinct areas of thickened intestinal wall and enlarged mesenteric lymph nodes were found on abdominal ultrasound. Cytology from those nodes showed the presence of suspected *Cryptococcus* spp., and infection was confirmed utilizing a cryptococcal antigen titer. Medical therapy with lipid-complexed amphotericin B and fluconazole was unsuccessful. Two surgical procedures were performed to remove the affected areas of intestine and lymph nodes, but the disease persisted as evidenced by a persistently elevated cryptococcal antigen titer. Terbinafine was prescribed, which resulted in complete resolution of clinical signs and a steadily decreasing cryptococcal antigen titer. Very few cases of intestinal cryptococcosis have been reported. In this case, infection resulted in a protein-losing enteropathy. In addition, this article describes the use of terbinafine in the treatment of intestinal cryptococcal infection in the dog, which has not been previously reported. (*J Am Anim Hosp Assoc* 2012; 48:216–220. DOI 10.5326/JAAHA-MS-5813)

## Introduction

*Cryptococcus neoformans* and *C. gatii* are fungal organisms that infect many domesticated species, as well as humans. It is thought that infection is acquired environmentally, following inhalation of basidiospores. This yeast is not generally considered transmissible from one infected animal to either another animal or to a human.<sup>1</sup>

The primary means of diagnosis of cryptococcosis is identification of the organism on aspirate or histopathology. An encapsulated basophilic organism with narrow-based budding is characteristic for *Cryptococcus* spp.<sup>1</sup> If the disease is suspected but no organisms are identified, fungal culture of the affected areas may yield diagnostic results. The organism has also been reported

on fecal cytology.<sup>2</sup> Otherwise, the latex agglutination test is used for identifying the capsular antigen in serum.<sup>3</sup> Although that test has been proven to be sensitive, it has also been shown that the antigen can persist in the serum for an extended period of time after clinical disease has subsided.<sup>4</sup> One additional method of identification is through DNA sequencing, as was the case with this patient.

Cryptococcosis has been documented in the nasal cavity, central nervous system, optic nerve, cutis, bone, lymph nodes, and (uncommonly) in the gastrointestinal tract of various species.<sup>1</sup> The case presented here describes a unique presentation of protein-losing enteropathy due to infection with *C. neoformans* located primarily within the small intestine and local lymph nodes, as well

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AMB amphotericin B; PO per os

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as utilization of a novel therapy, terbinafine, in the treatment protocol that resolved the enteropathy.

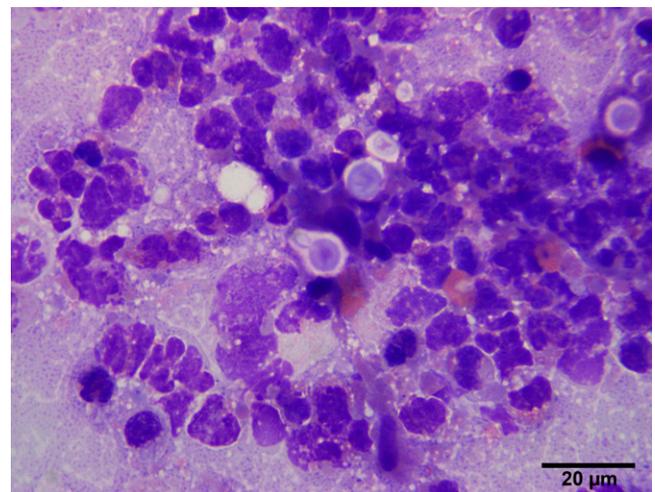
## Case Report

A 2.5 yr old male vizsla weighing 14 kg was referred to the emergency service of the Iowa State University Veterinary Teaching Hospital with a 4–5 mo history of weight loss and small bowel diarrhea. Initially, empirical therapy with metronidazole (12.5 mg/kg per os [PO] q 12 hr), cefpodoxime (10 mg/kg PO q 24 hr), and a bland diet had been prescribed by the referring veterinarian to alleviate the diarrhea. No improvement was noted. Repeated serum biochemical analyses performed over the 3 mo period prior to presentation showed only a panhypoproteinemia. Total protein was 4.9 g/dL (reference range, 5.0–7.4 g/dL) and albumin was 2.0 g/dL (reference range, 2.7–4.4 g/dL). Vomiting and melena developed 1 mo prior to referral. At that time, 1 mg/kg prednisone PO q 24 hr was prescribed. The metronidazole was continued, and sucralfate (1 g PO q 8 hr) was also added to the treatment regimen.

Upon presentation to the Internal Medicine Service of the Iowa State University Veterinary Teaching Hospital, the patient was quiet, alert, and responsive. Physical exam revealed severe emaciation (body condition score was grade 1/9). Mucous membranes were pale with a normal capillary refill time. There were no abnormalities on abdominal palpation, and peripheral lymph nodes were not enlarged. The rest of the physical exam was unremarkable. The initial complete blood count demonstrated a leukocytosis ( $25.87 \times 10^3/\mu\text{L}$ ; reference range,  $6.0\text{--}17.0 \times 10^3/\mu\text{L}$ ) characterized by a mature neutrophilia ( $19.40 \times 10^3/\mu\text{L}$ ; reference range,  $3.0\text{--}11.4 \times 10^3/\mu\text{L}$ ) and a monocytosis ( $3.62 \times 10^3/\mu\text{L}$ ; reference range,  $0.15\text{--}1.35 \times 10^3/\mu\text{L}$ ). A macrocytic, hypochromic, regenerative anemia was also noted. The red blood cell count was  $2.21 \times 10^6/\mu\text{L}$  (reference range,  $5.50\text{--}8.50 \times 10^6/\mu\text{L}$ ), hematocrit was 17.3% (reference range, 37.0–55.0%), hemoglobin was 5.4 g/dL (reference range, 12.0–18.0 g/dL), corrected reticulocyte count was 3.8% (reference range, <1.0%) mean corpuscular volume was 78.6 fL (reference range, 60.0–77.0 fL), and mean corpuscular hemoglobin concentration was 31.4 g/dL (reference range, 32.0–36.0 g/dL). Serum biochemistry abnormalities included hypokalemia (3.5 mEq/L; reference range, 3.9–5.3 mEq/L), hypocalcemia (8.6 mg/dL; reference range, 9.2–11.2 mg/dL), panhypoproteinemia (total protein was 3.3 g/dL; reference range, 5.2–7.1 g/dL and albumin was 1.6 g/dL; reference range, 3.2–4.3 g/dL), hypocholesterolemia (108.0 mg/dL; reference range, 140–270 mg/dL), and an elevated blood lactate (10.3 mmol/L; reference range, <2.0 mmol/L). Colloid oncotic pressure was decreased (7.1 mmHg; reference range, 20–26 mmHg). Fecal flotation did not identify any ova, and an

enzyme-linked immunosorbent assay for *Giardia* antigen was negative. Urinalysis uncovered no significant abnormalities. A neurologic exam and a dilated ophthalmic exam (performed by an ophthalmologist) were unremarkable.

Due to the panhypoproteinemia, decreased oncotic pressure, and worsening anemia, the patient was hospitalized and received packed red blood cells<sup>a</sup>, plasma<sup>b</sup>, and 6% hetastarch<sup>c</sup> transfusions. Abdominal radiographs were performed, which revealed decreased abdominal detail likely due to a combination of decreased fat for contrast and abdominal effusion. Thoracic radiographs were performed to investigate tachypnea. A mild amount of pleural effusion was noted, whereas the pulmonary parenchyma appeared normal. A cranial abdominal mass was also discovered upon repeated palpation. Abdominal ultrasound revealed two areas of thickened intestine (approximately 12 mm wall thickness; reference range,  $\leq 2.6\text{mm}$ ) with loss of normal wall layering. Additionally, two masses (both measuring approximately 80 mm  $\times$  30 mm in size) were present in the midabdominal region, which were presumed to be mesenteric lymph nodes based on their location, shape, and echogenicity. Cytologic examination of ultrasound-guided fine-needle aspirates obtained from those two masses displayed moderate numbers of red blood cells and neutrophils, with numerous extracellular, round-to-oval organisms ranging in size from 4  $\mu\text{m}$  to 20  $\mu\text{m}$  in diameter with a large clear capsule, a basophilic center, and rare narrow-based budding. These organisms were most consistent with *Cryptococcus* spp. (Figure 1).



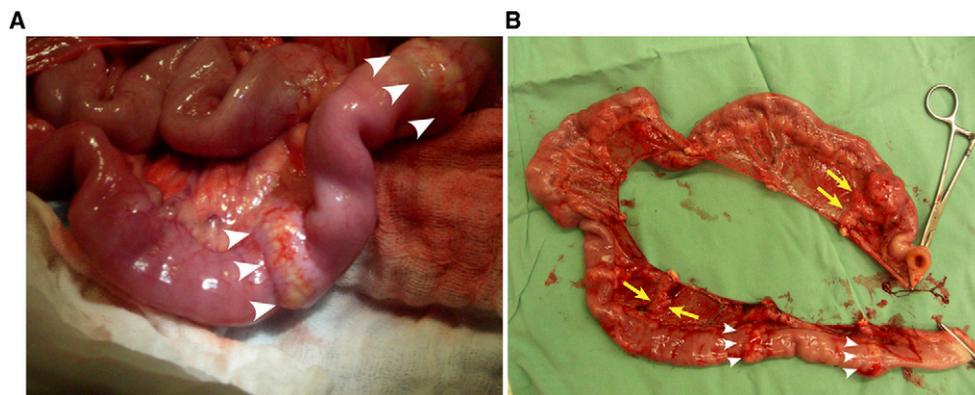
**FIGURE 1** Photomicrograph of a fine-needle aspirate of the mesenteric lymph node demonstrating neutrophilic inflammation (mostly degenerate neutrophils and nuclear streaming) with multiple yeast-like organisms surrounded by a clear capsule, including one exhibiting narrow-based budding. Wright's stain, original magnification  $\times 100$ .

Cryptococcal antigen titers via latex agglutination were submitted and fluconazole<sup>d</sup> (5 mg/kg PO *q* 12 hr) was initiated along with IV lipid-complexed amphotericin B (AMB)<sup>e</sup>. The first dose of AMB was 0.5 mg/kg, with subsequent doses incrementally increased to a maximum of 1.5 mg/kg. In total, 11 treatments were administered *q* 48 hr, resulting in a final cumulative dose of 14 mg/kg. Doses of AMB were given over a 30 min period and were preceded and followed with 4 hr of diuresis. Serial urinalyses and serum biochemistries were monitored throughout the AMB treatment period to monitor for any signs of renal damage (i.e., casts, increasing urea nitrogen or creatinine). The patient's renal values and urinary concentrating ability remained stable throughout the treatment period. At completion of the AMB treatment, the panhypoproteinemia persisted (total protein was 3.7 g/dL and albumin was 1.4 g/dL).

The original cryptococcal antigen titer was positive at the 1:420 dilution. After 2 wk of treatment with fluconazole and AMB, antigen titer was 1:462. While receiving antifungal treatment, the dog's attitude improved, along with appetite, fecal consistency, and body condition, with a weight gain of 1 kg. However, 2 wk after the AMB was discontinued, the patient began losing weight, his attitude declined, and melena recurred. Repeat ultrasonographic evaluation of the abdomen revealed that the presumed lymph nodes, as well as the thickened intestines, had not changed in either size or appearance. Surgical intervention was recommended at this time. Analysis of a complete blood count and serum biochemistry indicated a worsening anemia (packed cell volume was 12%) and decreasing total solids (periodic measurement of total solids had revealed improvement to a peak of 4.8 g/dL, but subsequently decreased to 3.6 g/dL). Albumin was 1.5 g/dL. Cross-matched-compatible packed red blood cell and plasma transfusions were administered immediately prior to surgery. Two mesenteric lymph nodes and approximately 60 cm of jejunum (and

associated mesentery) were removed (Figures 2A, B). The section of jejunum that was removed was orad to the ileoceocolic junction and aborad to the duodenum. Recovery from surgery was uneventful. Following surgery (within 24 hr), AMB was initiated. The cumulative dose was 16.0 mg/kg, with monitoring of renal values and urinalysis as previously described. After the AMB treatment was completed, fluconazole was reinitiated at the original dose. In addition, histologic examination of the excised intestinal and mesenteric lymph nodes confirmed the presence of fungal organisms consistent with *Cryptococcus* spp. Fungal cultures were submitted to multiple institutions, but yielded no growth upon multiple attempts.

One month after completion of the AMB, the patient was bright, alert, and responsive. A moderate weight increase was noted during the AMB treatment period (body weight increased from 15.9 kg to 20.4 kg and body condition score increased to grade 4/9). Cryptococcal antigen titers were rechecked and had markedly decreased to 1:69. Fluconazole was prescribed for an additional 6 mo with scheduled rechecks of the antigen titer *q* 3 mo. At the time of the first recheck, the antigen titer was 1:124, but by 5 mo postsurgically, the patient had lost weight and was vomiting. Ultrasound revealed enlarged mesenteric lymph nodes and multiple areas of thickened intestine, similar to findings previous to the surgery. Additionally, there appeared to be an intestinal stricture, which was considered to be the cause of the patient's clinical signs. A second surgery was therefore performed to remove the strictured intestine (measuring approximately 10 cm in length), effectively alleviating the obstruction. Histopathology of the strictured area of intestine was similar to the previous surgery, with fungal organisms and granulomatous inflammation predominating. Terbinafine<sup>f</sup> (30 mg/kg PO *q* 24 hr) was initiated in place of fluconazole. This medication was prescribed indefinitely to be re-evaluated based upon clinical response and



**FIGURE 2** A: Intraoperative photograph showing the encircling nature of the fungal growth in the wall of the jejunum (arrow heads). B: Photograph of the resected intestine. Note the encircling lesions (arrowheads) with associated lymphatic involvement (arrows).

cryptococcal antigen titers. After initiation of terbinafine, the patient improved continually. No clinical relapses were noted, and the cryptococcal antigen titer decreased steadily. At the time this report was written, the patient was doing well, maintaining both weight and appetite. A negative cryptococcal antigen titer was ultimately achieved. Two years after initiating terbinafine, no additional surgeries were required. Total protein and albumin returned to normal values (6.5 g/dL and 3.6 g/dL, respectively) within 1.5 mo after initiating terbinafine and remained normal for the duration of the follow-up period. Serial ultrasound examinations performed *q* 6 mo after the second surgery did not identify any additional areas of thickened intestine, and monitoring of mesenteric lymph nodes indicated resolution of the lymphadenomegaly by approximately 2 yr after initiation of terbinafine.

Although cryptococcal antigen titers confirmed the presence of the organism, failure of fungal growth precluded definitive identification of the organism. For this reason, polymerase chain reaction identification of the organism from paraffin-embedded intestinal tissue was attempted as previously described.<sup>5</sup> Primers ITS3/IT4, which amplify the ITS2 region of the 18S rDNA, were used for amplification. A single 376 base pair product was amplified and sequenced in both directions using the amplification primers. A Basic Local Alignment Search Tool using the Genbank database gave a 100% match to multiple isolates of *C. neoformans*, including strain ATCC MYA-4565. There were no acceptable matches to other species, confirming *C. neoformans* as the causative agent.

## Discussion

The patient in this case presented with a long history of weight loss associated with nonresolving diarrhea that had recently escalated to include vomiting and melena. Additionally, the dog had long-standing panhypoproteinemia and anemia. To the authors' knowledge, this is the first reported case of panhypoproteinemia with resolution following treatment of intestinal cryptococcosis. Furthermore, the authors of this report did not find any other reports describing terbinafine for the treatment regimen for cryptococcosis, emphasizing that this was a novel approach to the disease.

A previous report described cryptococcal infection of the gastric mucosa of a dog, which closely resembled a gastric mass in appearance.<sup>6</sup> Few other reports have described intra-abdominal infections as the sole location of the disease.<sup>7,8</sup> The present case is one of very few cases that the authors are aware of in which the primary route of infection appeared to be gastrointestinal, involving the mesenteric lymph nodes and circumferential intestinal

lesions. Reports in the human literature of primary intestinal cryptococcal disease appear to be limited to immunocompromised individuals, and lesions were noted in multiple different areas of the intestinal tract.<sup>9–11</sup> Medical therapy was attempted first in this case to provide a less-invasive treatment of the disease. However, after medical treatment failed to resolve the lesions, surgical therapy was pursued followed by a novel medical therapy, terbinafine.

There are occasional reports of the development of resistance to fluconazole during long-term therapy for cryptococcosis in humans.<sup>12</sup> This may explain why the lesions in the case described herein did not respond favorably to treatment with fluconazole, despite its excellent penetration of the gastrointestinal tract. However, once terbinafine was initiated, cryptococcal antigen titers dropped dramatically and the patient remained stable. Although response to treatment could not be definitively related to the patient's improvement (surgery coincided with the commencement of terbinafine), a previous surgery did not result in sustained improvement as was noted with the terbinafine. After beginning terbinafine, no additional intestinal lesions developed and lymphadenopathy resolved. Although it is possible that the lack of efficacy of fluconazole stems from the thickened intestine and the inability of the drug to penetrate the intestinal tissue and that surgical removal of the lesions and continuation of the fluconazole might have resulted in clinical remission, it also seems apparent that treatment with terbinafine resulted in resolution of clinical signs and decreasing antigen titers.

Terbinafine has been documented to enter the central nervous system of rats.<sup>13</sup> In addition, terbinafine has been investigated in the treatment of rabbit coccidioid meningitis, but it was not found in any appreciable concentration in the cerebrospinal fluid.<sup>14</sup> Additionally, there are a few reports indicating good *in vitro* activity of terbinafine against *C. neoformans*.<sup>15,16</sup> Uptake into the central nervous system appears to be species-dependent and needs to be further investigated for both humans and dogs as an inexpensive treatment alternative for cryptococcal meningitis, a disease often noted affecting immunocompromised individuals. There is little information in the literature regarding terbinafine in the treatment protocol of canines with intestinal fungal diseases. Two recent cases were reported out of Brazil. One of those indicated success, whereas the other reported failure with a treatment protocol that used terbinafine.<sup>17,18</sup> Another recent study investigated the pharmacokinetics of terbinafine in canines and revealed that plasma concentrations high enough to combat systemic fungal diseases can be achieved.<sup>19</sup>

Side effects of terbinafine have not routinely been encountered in the canine species, but mild gastrointestinal upset can

occur. In addition to the gastrointestinal upset, idiosyncratic events including liver failure and dermatologic abnormalities have been reported in humans.<sup>20</sup> No adverse effects were encountered in this patient during treatment with terbinafine. Terbinafine may become an acceptable alternative treatment of veterinary cryptococcal infections due to its efficacy and cost (as little as \$12/mo for the patient reported herein), but further investigation is needed. Although insufficient data are currently available to recommend terbinafine as a first-line agent, use in cases where primary therapy has failed to yield a response may prove beneficial.

## Conclusion

The patient described in this report had an unusual presentation of *C. neoformans*, leading to a protein-losing enteropathy. Novel therapy with terbinafine likely aided in the recovery of this patient. Additional studies investigating the efficacy of terbinafine for cryptococcosis of different organ systems are necessary. ■

The findings and conclusions of this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

## FOOTNOTES

- <sup>a</sup> Packed red blood cell transfusion; Iowa State University College of Veterinary Medicine Non-Resident Blood Donor Program, Ames, IA
- <sup>b</sup> Plasma transfusion; Iowa State University College of Veterinary Medicine Non-Resident Blood Donor Program, Ames, IA
- <sup>c</sup> 6% hetastarch in 0.9% sodium chloride injection; Hospira Inc., Lake Forrest, IL
- <sup>d</sup> Fluconazole 100 mg tablets; IVAX Pharmaceuticals Inc., Miami, FL
- <sup>e</sup> Abelcet 100 mg vial (amphotericin B lipid complex injection); Enzon Pharmaceuticals Inc., Bridgewater, NJ
- <sup>f</sup> Terbinafine 250 mg tablets; Northstar Rx LLC, Memphis, TN

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