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CASE REPORT

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# Colorectal basidiobolomycosis in a dog

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## Abstract

A 7-year-old castrated male French Bulldog was examined for chronic large intestinal enteropathy. A colonic mass and thickened rectal mucosa were identified, and histopathologic examination of endoscopic biopsy specimens disclosed eosinophilic proctitis with large (5-20 μm), irregularly shaped, pauciseptate hyphae that were Gomori methenamine silver and periodic acid-Schiff positive. Amplification and sequencing of ribosomal DNA extracted from paraffin-embedded tissues yielded a sequence with 97% identity to GenBank sequences for *Basidiobolus ranarum*. After itraconazole, terbinafine, and prednisone administration, clinical signs resolved rapidly, and sonographic lesions were largely absent after 6 weeks. Treatment was discontinued by the owner 15 weeks after diagnosis. Three weeks later, the dog collapsed acutely and was euthanized. Necropsy identified metastatic islet cell carcinoma and grossly unremarkable colorectal tissues. However, histopathology of the rectum disclosed multifocal submucosal granulomas with intralesional hyphae morphologically similar to those previously observed. This report is the first to describe medical treatment of gastrointestinal basidiobolomycosis in a dog.

**KEYWORDS**

*Basidiobolus ranarum*, entomophthoromycosis, itraconazole, pythiosis, zygomycosis

## 1 | INTRODUCTION

Basidiobolomycosis is a rare fungal infection that most often manifests as gastrointestinal (GI), cutaneous, or subcutaneous disease in people.[1-3] The causative agent, *Basidiobolus ranarum*, is classified in the order Entomophthorales, which also contains *Conidiobolus coronatus*, a cause of rhinofacial, retrobulbar, and respiratory disease in mammals.[3] The older species designations *Basidiobolus haptosporus*, *Basidiobolus meristosporus*, and *Basidiobolus heterosporus* are synonymous with *B ranarum*.[4] Previously, these fungi were classified in the phylum Zygomycota, and although recent reclassification based on molecular phylogeny now places them in the phylum Zoopagomycota,[5] it is likely that the term "zygomycosis" will continue to be used colloquially to refer to infections caused by these fungi. *Basidiobolus* species are commonly found in soil and decaying vegetation, and have been isolated from insects as well as from the intestinal tracts of amphibians and reptiles.[6] It is hypothesized that infection in mammals occurs after percutaneous inoculation of spores by traumatic injury or insect bites, or by ingestion.[3]

In people, GI basidiobolomycosis causes focal or multifocal segmental thickening that may be mass-like. It typically affects the colon

**Abbreviations:** FFPE, formalin-fixed paraffin-embedded; GI, gastrointestinal; GMS, Gomori methenamine silver; PAS, periodic acid-Schiff.

and rectum, but also can involve small intestine, stomach, and liver.[1,2] Histologically, GI basidiobolomycosis resembles infection caused by the pseudofungal oomycete *Pythium insidiosum*.[7] Historically, most cases of basidiobolomycosis have occurred in tropical or subtropical climates, including Africa, Asia, and South America.[3] More recently, however, GI basidiobolomycosis has been identified in a number of human patients from Arizona, Saudi Arabia, and Iran, indicating that the disease can occur in arid climates as well.[1,2,8,9]

Reports of basidiobolomycosis in dogs have been limited to a dog with multifocal ulcerative, draining skin lesions,[10] 1 with fungal pneumonia,[10] 1 with a colonic mass,[11] and 1 with disseminated disease involving the stomach, small intestine, pleura, mediastinum, diaphragm, mesenteric root, liver, and spleen.[12,13] In an additional case, *Basidiobolus microsporus* was cultured from a dog's feces, but there was no evidence of tissue infection.[14] Information available about treatment from these reports is sparse. Recently, we have had success treating GI pythiosis using a combination of prednisone, itraconazole, and terbinafine, based on the hypothesis that prednisone may contribute to resolution of lesions by decreasing the severe inflammation typically associated with the disease.[15] This report describes the clinicopathologic findings as well as response to treatment using this same antifungal and prednisone combination in a dog with colorectal basidiobolomycosis.

## 2 | CASE REPORT

A 7-year-old castrated male French Bulldog was evaluated for large intestinal diarrhea, tenesmus, hematochezia, and decreased activity of 1 month's duration. The dog had been adopted 1 year earlier and had lived in central Louisiana since that time. A caudal abdominal mass and circumferential thickening of the rectum with mucosal irregularity were noted on physical examination. A CBC identified eosinophilia (5300/μL; reference interval, 100-1200/μL) and thrombocytosis (753 000/μL; reference interval, 220 000-600 000/μL). Serum biochemistry results were normal with the exception of moderate hypoglycemia (65 mg/dL; reference' interval, 80-115 mg/dL) and mild hypokalemia (3.5 mmol/L; reference interval, 3.8-5.5 mmol/L). Thoracic radiographs were normal. Abdominal ultrasound examination identified severe (up to 8.8 mm) circumferential thickening of the colonic wall with loss of wall layering extending from the mid-descending colon to the pelvic inlet. Medial iliac lymph nodes were mildly rounded (up to 6.9 mm) and hypoechoic. Ultrasound-guided needle aspirates of the distal colonic wall yielded poor cellularity, precluding cytologic interpretation. Colonoscopy identified hyperemic and irregular colonic and rectal mucosa, with multifocal ulcerative mucosal lesions in the rectum. Anti-*P insidiosum* antibody serology was strongly positive (94% positivity at 1:1000 dilution, Auburn University). Based on a presumptive diagnosis of colonic pythiosis, medical treatment was initiated with prednisone (1 mg/kg PO q12h for 5 days, then 1 mg/kg PO q24h), itraconazole (10 mg/kg PO q24h), and terbinafine (10 mg/kg PO q24h).

Histopathologic examination of endoscopic rectal biopsy specimens showed mixed eosinophilic proctitis with intralesional hyphae that measured 5 to 20 μm in diameter (mean ± SD; 11 ± 4 μm), were irregularly shaped with thin, nonparallel walls and rare septae, and were surrounded by a narrow sleeve of eosinophilic material (Figure 1). They were positive with Gomori methenamine silver (GMS) stain and the periodic acid-Schiff (PAS) reaction. Because the size and morphology of the hyphae were thought to be incompatible with *P insidiosum* and instead suggestive of basidiobolomycosis, formalin-fixed paraffin-embedded (FFPE) rectal biopsy tissues were sent to the Texas A&M Dermatopathology Specialty Service for pan-fungal PCR and sequencing. After DNA extraction from the FFPE tissues, conventional PCR was performed targeting the internal transcribed spacer 2 (ITS2) region of the ribosomal RNA gene using the following primers: ITS3-F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4-R (5′-TCCTCCGCTTATTGATATGC-3′). Electrophoresis yielded a single band of approximately 450 base pairs. Sequencing of this product produced a 266 base pair sequence that, when queried using the NCBI BLAST tool, matched *B ranarum* with up to 97.4% identity. The sequence was deposited in the GenBank database under accession number MT154079.

At reevaluation after 6 weeks of treatment, all clinical signs had resolved. A repeat CBC indicated resolution of the previously noted eosinophilia and thrombocytosis. Serum biochemistry results included a moderate increase in ALT activity (248 U/L; reference interval, 0-60 U/L), a mild increase in ALP activity (110 U/L; reference interval, 0-100 U/L), mild hypercholesterolemia (271 mg/dL; reference interval, 150-240 mg/dL), mild hypokalemia (3.5 mmol/L; reference interval, 3.8-5.5 mmol/L), and normoglycemia (85 mg/dL; reference interval, 80-115 mg/dL). Abdominal ultrasound examination identified a substantial decrease in the thickness of the colonic wall, measuring 1.1 mm with normal wall layering at the proximal descending colon, and 2.8 mm with indistinct wall layering at the distal descending colon. Medial iliac lymphadenopathy

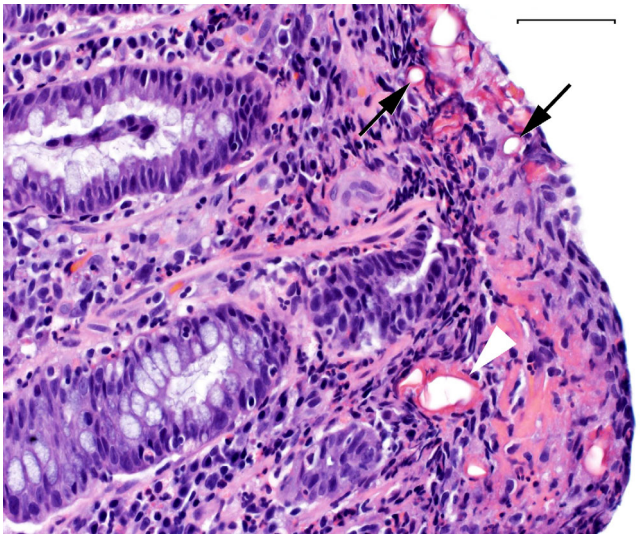

**FIGURE 1** Photomicrograph of an endoscopically obtained rectal pinch biopsy specimen showing mixed eosinophilic proctitis with large, irregularly shaped hyphae (black arrows) with thin, nonparallel walls and rare septae. The hyphae are surrounded by a thin sleeve of eosinophilic material (white arrowhead). Hematoxylin and eosin stain; scale bar = 50 μm

had resolved. Medical treatment was continued as described above until 9 weeks later (after 15 total weeks of treatment), when the owner discontinued all treatments and reevaluations for financial reasons. Three weeks later (4.5 months after diagnosis), the dog collapsed twice and became acutely lethargic. No GI signs were reported. The owner declined additional diagnostic testing and elected to have the dog euthanized. Necropsy was performed the same day at the Louisiana Animal Disease Diagnostic Laboratory.

At necropsy, no macroscopic lesions were observed in the GI tract. The right lobe of the pancreas had a single, 1-cm-diameter, well-demarcated, round, white nodule. The pancreaticoduodenal lymph node was twice normal size. The liver contained scattered 1-mm-diameter white nodules. Five filarial nematodes were present within an intrapulmonary branch of the pulmonary artery. Nine sections of the brain were examined and found to be normal. Despite the lack of macroscopic lesions in the rectum, histopathologic examination of rectal tissues identified multifocal to coalescing granulomas in the submucosa and tunica muscularis containing intralesional hyphae that were morphologically consistent with those previously identified in colonoscopic biopsy specimens (Figure 2A). Walls of these hyphae were positive on GMS and PAS staining (Figure 2B,C). In addition, histopathology and immunohistochemistry (the latter performed at the Michigan State University Veterinary Diagnostic Laboratory) identified an insulin-secreting pancreatic islet cell carcinoma with liver and pancreaticoduodenal lymph node metastases. This finding along with the initial moderate hypoglycemia and mild hypokalemia suggest that some of the dog's initial lethargy may have resulted from hypoglycemia that improved during prednisone administration, but worsened and caused collapse when it was discontinued. Frozen tissue and FFPE sections of rectum were submitted for pan-fungal PCR and sequencing. Unfortunately, despite multiple attempts, no sequences of diagnostic quality were obtained.

## 3 | DISCUSSION

Gastrointestinal basidiobolomycosis is a rare disease in dogs, having been previously described only twice. In 1984, a dog with gastric and small intestinal basidiobolomycosis that died before surgery could be attempted was described, and necropsy identified disseminated disease that also involved the pleura, mediastinum, diaphragm, mesenteric root, liver, and spleen.[12] This same case also was included in a large retrospective analysis of pythiosis and entomophthoromycosis.[13] More recently, a dog with a colonic mass was reported that died shortly after radical excision was attempted.[11] As in our patient, the 2 previously described cases had clinical findings similar to those associated with GI pythiosis, characterized by signs of chronic enteropathy with or without vomiting, severe segmental wall thickening or mass-like lesions in the GI tract, and peripheral eosinophilia.[7] In addition, sonographic findings in our patient were identical to those previously observed in dogs with GI pythiosis, including severe, hypoechoic, segmental thickening of the GI tract associated with loss of wall layering and abdominal lymphadenopathy.[7,15] The histologic characteristics of GI basidiobolomycosis also are similar to those associated with pythiosis, and include pyogranulomatous and eosinophilic inflammation associated with wide, pauciseptate hyphae with nonparallel walls and occasional right-angle branching.[7]

Given the many clinicopathologic similarities between GI basidiobolomycosis and GI pythiosis, differentiating between these diseases is challenging. Assessment of anti-*P insidiosum* antibodies often is used to support the diagnosis of pythiosis in dogs and to differentiate it from other causes of segmental GI wall thickening.[7] Unfortunately, in the case described here, measurement of anti-*P insidiosum* antibodies produced a strong false-positive result, indicating that this assay may not be as specific as was previously thought. An anti-*P insidiosum* assay using a whole cell extract-derived soluble mycelial antigen previously developed by 1 of the authors (A. M. G.) and since discontinued was found to be 100% specific when evaluated using sera from dogs with

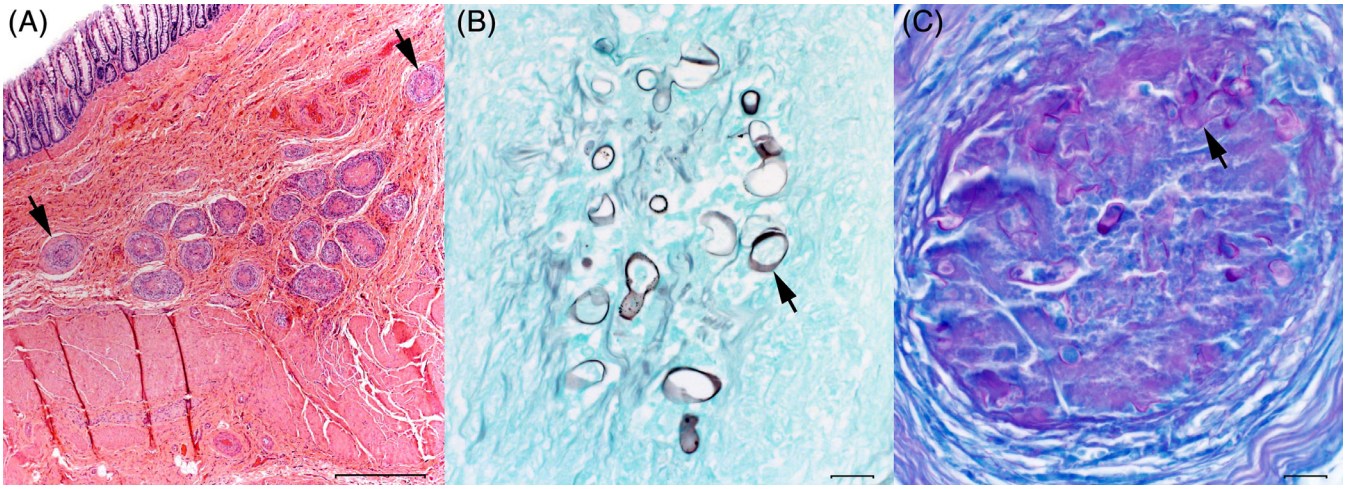


**FIGURE 2** Photomicrograph of rectal tissue taken at necropsy showing multifocal to coalescing granulomas (arrows) in the submucosa (A); hematoxylin and eosin stain; scale bar = 500 μm. Large, thin-walled, irregularly shaped hyphae (arrows) within granulomas were GMS (B) and PAS (C) positive; scale bar = 20 μm

other types of oomycotic and fungal infections.[16] However, the dogs with basidiobolomycosis that were evaluated in that study had focal cutaneous disease rather than GI or disseminated disease (Grooters AM, unpublished data), and it is possible that sera from dogs with more extensive lesions would have produced a false-positive result in that assay as well. Another potential explanation for the false-positive result observed in our patient is that the antigen used in the assay offered by Auburn University includes secreted proteins in addition to a whole-cell extract,[15] which may decrease the specificity of the assay. Data regarding the sensitivity and specificity of that assay have not been published.

Despite their similarities, some histologic characteristics may assist in differentiating basidiobolomycosis from pythiosis. One feature that was crucial to making a diagnosis in our patient is the size of the hyphae, with *B ranarum* hyphae being much larger than *P insidiosum* hyphae. Hyphal diameter in the 2 previously described cases of GI basidiobolomycosis in dogs ranged from 5 to 30 μm,[11,12] and in the case described here was 5 to 20 μm, with a mean of 11 μm. In contrast, the diameter of *P insidiosum* hyphae has been reported to be 2 to 9 μm, with a mean of 5 μm.[13] In addition, hyphae of the Entomophthorales often are surrounded in tissue by a radiating sleeve of eosinophilic material (Splendore-Höeppli phenomenon),[2,8,12] which is typically more narrow or absent around *P insidiosum* hyphae.[13] Finally, histochemical staining characteristics may help differentiate *B ranarum* from *P insidiosum*. Periodic acid-Schiff, which reacts strongly with polysaccharides that are polymers of glucose (glycogen, cellulose) or N-acetylglucosamine (chitin),[17] typically stains the hyphal walls of the Entomophthorales strongly.[2,11] However, PAS reactivity of *P insidiosum* hyphae is more variable, less intense, and often negative.[15,18] This is likely because of differences in the carbohydrate composition of the oomycete as compared to the fungal cell wall; oomycetes such as *Pythium* and *Phytophthora* spp generally are devoid of chitin and other N-acetylglucosamine-based polysaccharides.[19]

Although the histopathologic characteristics described above may raise the index of suspicion for basidiobolomycosis, definitive diagnosis can be achieved only by culture or molecular methods. Culture can be successful if appropriate tissue samples are obtained at the time of biopsy, but this is not always possible. Recently, a technique for extraction, amplification, and sequencing of fungal DNA from FFPE tissues has become routinely available.[20] This method allows a diagnosis to be made when culture is not performed or is unsuccessful. Because the pan-fungal primers used in this assay amplify DNA from pathogenic oomycetes as well as from fungi, the assay also can be used to confirm a diagnosis of pythiosis.[20] One limitation of this approach is that it should only be performed when organisms are visualized histologically. Because pythiosis and basidiobolomycosis tend to affect deeper GI tissues (muscularis mucosae, submucosa, muscularis propria) most severely,[1,13] endoscopic biopsy specimens that do not extend into the submucosa may fail to include hyphae.

Information about treatment of basidiobolomycosis in dogs is very limited. One previously described case with cutaneous disease failed to respond to itraconazole, amphotericin, and potassium iodide.[10] Another dog with fungal pneumonia initially responded to 6 months of itraconazole treatment, but the disease recurred after the treatment was discontinued.[10] Of the 2 previously described dogs with GI basidiobolomycosis, 1 died before surgery could be attempted[12] and the other died immediately after resection of the colon and ileum.[11] In humans, most cases of GI basidiobolomycosis have been managed using a combination of surgical resection and medical treatment, and it has been recommended that surgery be followed with 6 months of itraconazole treatment to prevent recurrence.[2] However, response to medical treatment alone also has been described, with itraconazole being the drug used most commonly in the past.[1] Results of in vitro susceptibility testing for isolates of *Basidiobolus* spp have been highly variable, but have suggested resistance to amphotericin for some isolates.[1,21,22] This finding, along with reports of amphotericin treatment failure,[23,24] suggest that amphotericin may not be a good choice for the treatment of GI basidiobolomycosis.[3] Recently, several reports have described successful treatment of GI basidiobolomycosis in human patients using voriconazole[9,25-28] and 1 report using posaconazole,[29] suggesting that 1 of these newer triazoles may be the current treatment of choice.

In our patient, a combination of itraconazole, terbinafine, and prednisone initially was chosen based on a presumptive diagnosis of pythiosis. Once the diagnosis of basidiobolomycosis was made, the dog's clinical signs had dramatically improved, and the decision was made to continue that treatment. This decision was based in part on the fact that the inflammatory pattern associated with basidiobolomycosis is very similar to that associated with pythiosis, suggesting that the use of prednisone to decrease the severe inflammatory response (especially the eosinophilic component) would be similarly beneficial. During the 15 weeks that treatment was administered to our patient, the response was identical to what we have previously observed and what has been reported in dogs with nonresectable GI pythiosis (rapid resolution of clinical signs and near resolution of sonographic lesions).[15] Unfortunately, discontinuation of treatment after 15 weeks and subsequent euthanasia because of signs associated with metastatic insulinoma precluded determination of response to long-term medical management in this case. Despite the lack of macroscopic lesions in the GI tract at necropsy, microscopic examination of the rectum identified submucosal granulomas containing hyphae. This finding emphasizes the need to continue antifungal treatment for a long period of time after resolution of clinical signs and sonographic lesions. We recommend treatment of entomophthoromycosis for a minimum of 12 months, and a similar approach has been used for successful treatment of human patients.[9,26,27,29]

In summary, GI basidiobolomycosis is a rare infection in dogs that shares many clinicopathologic similarities with pythiosis. Large hyphal diameter, presence of a substantial eosinophilic sleeve, and positive PAS reactivity should alert diagnosticians to the possibility that a lesion is caused by basidiobolomycosis rather than pythiosis. When lesions are not readily resectable, long-term medical treatment with itraconazole, voriconazole, or posaconazole should be considered, and combining antifungal treatment with corticosteroids for the first 3 to 4 months may lead to rapid resolution of clinical signs.

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## CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

## OFF-LABEL ANTIMICROBIAL DECLARATION

Itraconazole and terbinafine are not approved for use in dogs in the United States.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

## HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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