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Diagnosis of systemic cryptococcosis by fecal cytology in a dog.

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Companion Notes
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Article Abstract
A 3-year-old Boxer was presented with progressive diarrhea, vomiting, and lethargy of 5-months duration. The dog had watery black feces, a mature neutrophilia, and microcytic anemia. Cytologic evaluation of a direct fecal smear stained with Wright's-Giemsa revealed numerous encapsulated, narrow-based, budding organisms consistent with Cryptococcus sp. Pyogranulomatous inflammation and Cryptococcus organisms also were observed in ultrasound-guided fine-needle aspirates of the small intestine and mesenteric lymph nodes, and in histologic sections of colonic biopsies obtained by endoscopy. Multifocal chorioretinitis by fundic examination was consistent with systemic mycosis, and the reciprocal antigen titer (1600) on a cryptococcal antigen latex agglutination test for Cryptococcus neoformans was markedly increased. Using immunohistochemistry, the organism was identified further as C neoformans var. grubii (C neoformans var. neoformans serotype A). After 3 weeks of antifungal treatment, ultrasound examination revealed urinary bladder wall thickening, and Cryptococcus organisms were found in a urine sediment preparation. After 4 months of treatment, the dog was clinically normal and had no abnormal findings on CBC, serum biochemistry, urinalysis, or fecal cytology; however, the antigen titer remained unchanged, mesenteric lymphadenomegaly and jejunal wall thickening were still evident, and cytologic evaluation of fine-needles aspirates of the jejunal wall revealed budding Cryptococcus organisms. Intestinal involvement in dogs with cryptococcosis is rare, and diagnosis by fecal cytology has not been documented previously.

Keywords
cytology, disseminated disease, feces, gastrointestinal tract

Full Text of Article

A 3-year-old, 22.8-kg (50.2-lb), castrated male Boxer was referred to the University of Illinois Veterinary Teaching Hospital for evaluation of progressive diarrhea, vomiting, and lethargy of 5-months duration. Previous treatment had included metronidazole, sucralfate, prednisone, and dietary modification, with no improvement in clinical signs. Trichuris vulpis infection and heartworm disease had been diagnosed and treated in the 2 months before presentation. On physical examination, the dog was lethargic and had a dry, coarse hair coat. The dog's feces were watery and black, and there was fecal staining on the perineum. Rectal temperature, heart rate, and respiratory rate were normal. No other abnormalities were noted on initial physical examination.

A CBC revealed leukocytosis (25,200 cells/µL, reference interval 6000–17,000 cells/µL) with a mature neutrophilia (24,800 cells/µL, reference interval 3000–11,500 cells/µL). The lymphocyte concentration was low (326 cells/µL, reference interval 1000–4800 cells/µL). Microcytic (55.2 fL, reference interval 60.0–77.0 fL), normochromic (35.7 g/dL, reference interval 32–36 g/dL) anemia (HCT 31.9%, reference interval 35–52) also was identified. Nucleated RBCs (2/100 WBCs), a few target cells, and rare microfilaria were observed on blood smears. Serum biochemical results included high alkaline phosphatase activity (185 U/L, reference interval 12–110 U/L), high alanine aminotransferase activity (140 U/L, reference interval 17–87 U/L), and high total carbon dioxide (CO2) concentration (30.2 mmol/L, reference interval 15.0–27.0 mmol/L). Urinalysis of a sample obtained by cystocentesis revealed mild (30 mg/dL) proteinuria.

A fecal flotation was performed and was negative for intestinal parasite ova. Cytologic evaluation of a direct smear of feces stained with Wright's-Giemsa revealed numerous encapsulated, narrow-based, budding organisms consistent with Cryptococcus sp (Figure 1). Clostridial overgrowth (>5 spores per ×100 oil immersion field) was also diagnosed. Fundic examination revealed multifocal areas of chorioretinitis, consistent with systemic mycosis. Results of thoracic radiography were normal. Abdominal ultrasound revealed severe mesenteric lymphadenomegaly, diffuse intestinal wall thickening, a slightly small liver, and a small amount of free abdominal fluid. Ultrasound-guided fine-needle aspiration of the mesenteric lymph nodes and colonic wall was performed. Cytologic findings in both specimens were indicative of pyogranulomatous inflammation with Cryptococcus sp organisms (Figure 2).

On proctoscopic examination, the rectal and colonic mucosa were nodular, erythematous, and edematous. Colonic biopsy samples were submitted for histopathologic evaluation. Histologically, the colonic mucosa and submucosa were partially effaced by large aggregates of Cryptococcus organisms, with severe granulomatous inflammation in the surrounding tissue (Figure 3). A latex cryptococcal antigen agglutination test (LCAT) (Meridian Bioscience Inc, Cincinnati, OH, USA) performed at the University of Georgia, Department of Medical Microbiology, revealed a markedly increased reciprocal antigen titer (1600) for Cryptococcus neoformans. A previously validated immunohistochemical method1 was used to identify the organism as C neoformans var. grubii (C neoformans var. neoformans serotype A) (Figure 4). Because gastrointestinal C neoformans infection is not common in the dog, we suspected underlying immunosuppression. Serum was submitted for measurement of immunoglobulin (IgG) concentrations. The serum IgG concentration was only slightly low (715 mg/dL, reference

interval 1000-2000 mg/dL) and was not consistent with immunodeficiency. No other immune function tests were done.

Initial treatment of the dog included itraconazole (5 mg/kg, PO, q12h), metronidazole (10 mg/kg, PO, q12h), famotidine (0.5 mg/kg, PO, q12h), and sucralfate (50 mg/kg dissolved in water, PO, q8h). The dog was released from the hospital but returned 10 days later with development of aggressive behavior, third-eyelid prolapse, and thoracic spinal pain, which may have reflected central nervous system infection. Bilateral mucopurulent nasal discharge, polyuria, polydipsia, and submandibular lymphadenomegaly also had developed, while the diarrhea was unchanged. Because of its better penetration into the central nervous system,2 fluconazole was substituted for itraconazole, and intravenous amphotericin B was given (cumulative dose of 240 mg) because of the possibility of poor intestinal absorption of the drugs.

The dog remained hospitalized for 23 days, during which time the dog developed regenerative anemia, with a HCT of 20% and 188,000 reticulocytes/μL (reference interval 0–60,000/μL). The anemia may have been caused by gastrointestinal bleeding, but a fecal occult blood test was not done. On day 13 after the initial presentation the dog developed hypoxemia associated with right caudal lobar pneumonia and syncopal episodes associated with electrocardiocraphic findings of an accelerated idioventricular rhythm with periods of ventricular premature contractions. To investigate myocardial and endocardial disease, echocardiography was performed and revealed mild subaortic stenosis. The arrhythmia resolved following packed RBC transfusions. Subsequent radiographs revealed mild caudal thoracic spondylosis and disk space narrowing between the 12th and 13th thoracic vertebrae. Follow-up abdominal ultrasound examination revealed unchanged mesenteric lymphadenomegaly and intestinal wall thickening, and a new finding of urinary bladder wall thickening. Cryptococcus sp organisms were detected microscopically on unstained wet mount specimens of the urine sediment. The dog's appetite and attitude eventually improved, and the diarrhea resolved on day 32. The dog was released from the hospital on long-term fluconazole treatment (100 mg BID). Four months after initial presentation the dog was clinically normal, with no abnormal findings on CBC, serum biochemistry, urinalysis, or fecal cytology. The pneumonia had resolved, but mesenteric lymphadenomegaly and jejunal wall thickening were still evident on abdominal ultrasonography. Cytology of fine-needles aspirates of the jejunal wall revealed budding Cryptococcus sp organisms, and the LCAT titer was unchanged.

Discussion

To our knowledge, this is the first report in either animals or human beings in which cryptococcal organisms were identified by cytologic evaluation of feces. In this case, the initial diagnosis was based on the results of fecal cytology, demonstrating its value in the investigation of diarrhea in dogs.

Cryptococcosis of the gastrointestinal tract has been reported infrequently in dogs, either as an isolated finding or in disseminated disease.3 In comparison with the pulmonary and central nervous systems, involvement of the gastrointestinal tract and other abdominal organs is rare in dogs infected with C neoformans.4 In this case, cryptococcal organisms were identified in aspirates of the colon wall and mesenteric lymph nodes and in urine sediment. Two case reports4,5 previously documented gastrointestinal infection with C neoformans in 3 dogs. In 1

report,4 a 3-year-old male Doberman Pinscher was presented for vomiting. Gastroscopy demonstrated severe gastric lesions suspected to be gastric carcinoma. Euthanasia was performed and a postmortem diagnosis of cryptococcosis was made from histopathologic evaluation of gastric biopsy samples collected during necropsy. In the second report,5 a 14-month-old male Border Collie was presented for lethargy and vomiting and a 2-year-old spayed female Giant Schnauzer was presented for neurologic signs and vomiting. In both cases, C neoformans infection was diagnosed from histopathologic evaluation of tissue biopsy samples (mesenteric lymph nodes, small intestinal masses) collected during exploratory laparotomy. Fecal cytology was either not performed or not reported in any of these cases but based on the the findings in the present case, may have been useful in making a diagnosis.

The dog in this report developed polyuria and polydipsia during hospitalization, and the urine contained Cryptococcus sp organisms. However, renal abnormalities were not seen by ultrasonography, and renal biopsies were not performed, so renal infection was not confirmed. In a recent report,6 cryptococcal pyelonephritis was diagnosed in a 5-year-old castrated male Golden Retriever. Yeast organisms were seen in that dog's urine sediment, but the organisms were initially considered to be contaminants. In that report, a definitive diagnosis of cryptococcal infection was made on histopathologic examination of the kidneys. One other report7 of fungal pyelonephritis in a dog suggested that analysis of urine may provide a diagnosis via culture, cytologic evaluation of sediment, or capsular antigen test for Cryptococcus sp. Overall, cryptococcal infection should be considered in the differential diagnosis of dogs with gastrointestinal tract lesions, mesenteric lymphadenomegaly, and renal disease.4,8

Although the clinical signs in the dog in this case had completely resolved, cytologic and serologic evidence of infection remained. A 2- to 4-fold decline in the LCAT titer per month during initial therapy (which was not observed in this dog) corresponds to an adequate clinical response.9 Additional amphotericin B therapy could have been used, but the owners elected to continue fluconazole therapy, and at the time of writing the dog was clinically normal.

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