A 3-year-old 24.8-kg castrated male Bluetick Coonhound was referred to the Veterinary Hospital at North Carolina State University because of a history of weight loss (3.4 kg over approx 1 month) and recurrent episodes of tenesmus and hematochezia that were refractory to empirical treatment with prednisone (0.4 mg/kg, PO, q 12 h for 8 days), metronidazole (19 mg/kg, PO, q 12 h for 13 days), fenbendazole (unknown dosage), and gabapentin (11.5 mg/kg, PO, q 24 h) over an approximately 1.5-month period. The dog frequently strained to defecate (>15 times/d), producing either large volumes of soft to watery feces with frank blood and mucus, or small amounts of hemorrhagic diarrhea. Fecal flotation was performed, and no parasite ova or larvae were observed. Six other dogs in the household were not affected.

Clinical and Gross Findings

On physical examination, digital rectal examination revealed a firm, circumferential mass along the rectum and extending approximately 3 cm cranially from the anus. CT revealed a severely thickened descending colon and rectum, with mild thickening of the transverse colon, ascending colon, and cecum. Moderate medial and lateral iliac, sacral, and left colic lymphadenomegaly were also noted. Abdominal ultrasonographic examination revealed persistent colonic wall thickening and lymphadenomegaly. A CBC and serum biochemical profile were also performed (Table 1). After 2 months of treatment, a second abdominal CT scan was performed, and findings were consistent with complete colonic obstruction. Euthanasia was elected.

Gross postmortem examination revealed marked asymmetrical thickening of the rectum and descending colon (up to 4 cm thick), causing partial luminal obstruction, and resulting in severe dilation of the transverse and ascending colon (Figure 1). A few scattered, depressed, pale-tan mucosal ulcerations (up to 0.6 cm in diameter) were in the descending colonic mucosa. Starting at the anus and extending...
along the rectum about 8 cm orad were multifocal to coalescing cavitations and ulcerations spanning approximately 70% of the mucosal surface. On the cut surface, the wall of the descending colon was firm with mottled dark-green to yellow discoloration and effacement of normal wall layering. In addition, lymph nodes associated with the descending colon and rectum were moderately enlarged, measuring up to 2.5 X 1.5 X 1 cm.

Formulate differential diagnoses, then continue reading.

Cytologic, Histopathologic, Immunohistochemical, and Laboratory Findings

Cytologic examination of an antemortem fine-needle aspirate sample of the rectal mass (taken 1 month after onset of clinical signs) and a postmortem impression smear of the descending colon revealed multiple, nonstaining, branching, sparsely septate hyphae with abundant mixed mononuclear cells, variable numbers of neutrophils, and necrotic debris (Figure 2).

Multiple tissue samples from the gastrointestinal tract, mesenteric lymph nodes, lungs, heart, liver, kidneys, and spleen were collected; fixed in neutral-buffered 10% formalin; and routinely processed for histologic examination. Histologically, the submucosa and muscularis of the descending colon and rectum were markedly expanded by multifocal to coalescing, poorly organized pyogranulomas composed of central areas of lytic necrosis with myriad, nonstaining, 7- to 12-µm-diameter, sparsely septate hyphae with nearly parallel walls and nondichotomous branching.

The hyphal morphology was highlighted by Grocott-Gomori methenamine silver stain. Immunohistochemical staining revealed that the hyphae were strongly immunoreactive for anti-\textit{Pythium} antibody (rabbit polyclonal antibody performed by the Auburn University Pathobiology Diagnostic Services). These hyphae were surrounded by variable numbers of neutrophils, moderate numbers of epithelioid macrophages, and occasional Langhans-type multinucleated giant cells rimmed by abundant fibrosis, with moderate numbers of intermixed lymphocytes and plasma cells. Granulomatous inflammation occasionally extended through the serosa into the adjacent mesentery. The submucosa of the rectum within the most affected areas also had multiple, extensive lytic tracts with abundant intermixed cocci and bacilli. Occasional blood vessel walls along the serosa and adjacent adipose tissue were hyalinized, fragmented, and infiltrated by abundant degenerating inflammatory cells with intraluminal fibrin thrombi (fibrinoid vascular necrosis and vasculitis, likely secondary to extensive local necrosis). Colonic lymph nodes were moderately enlarged with expansion of the cortex and paracortex by lymphocytes and plasma cells (lymphoid hyperplasia). There was no histologic evidence of extension of infection to other portions of the gastrointestinal tract, lymph nodes, or other major organ systems.

At the initial presentation, blood was collected from the dog for assessment of serum anti-\textit{Pythium insidiosum} antibodies via ELISA performed by the Auburn University Pathobiology Diagnostic Services. Results of the ELISA indicated that the dog tested positive (100% positive at 1:1,000 dilution) for anti-\textit{P. insidiosum} antibodies. After 1 month of treatment,
a repeat ELISA revealed a slight reduction in percent positivity compared to the positive control (91% positive at 1:1,000 dilution). Based on these results, treatment was initiated with itraconazole (10 mg/kg, PO, q 24 h), terbinafine (10 mg/kg, PO, q 24 h), and a tapering dose of prednisone (1 mg/kg, PO, q 24 h initially). After 1 month of treatment, the dog had gained approximately 2.2 kg, with continued diarrhea but with less straining.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis: marked segmental pyogranulomatous colitis and proctitis with myriad intrallesional hyphae (consistent with *P. insidiosum*), lytic tracts, and fibrosis.

Case summary: colorectal pythiosis in a dog.

Figure 2—Postmortem photomicrographs of an impression smear from the wall of the descending colon (A), a tissue section at the level of transition between the rectum (arrow) and descending colon (arrowhead; B), and the descending colon (C and D) of the dog described in Figure 1. A—Multiple, nonstaining, occasionally branching, sparsely septate hyphae are surrounded by abundant degenerating mixed mononuclear cells and necrotic debris. Modified Romanowsky stain; bar = 20 µm. B—The colonic mucosa, submucosa, and muscularis are markedly expanded and replaced by coalescing granulomas (asterisk). H&E stain; bar = 0.4 cm. C—The centers of granulomas consist of abundant degenerating neutrophils and eosinophilic debris with interspersed nonstaining hyphae of about 7 to 12 µm in diameter with nearly parallel walls (arrowheads). Granulomas are rimmed by occasional Langhans multinucleated giant cells and a layer of lymphocytes and plasma cells (upper left). H&E stain; bar = 20 µm. D—Hyphae are stained black (positive) and have nondichotomous branching and occasional septation. Grocott-Gomori methenamine silver stain; bar = 20 µm. Inset—Hyphae exhibit positive immunoreactivity (brown staining). Immunohistochemical staining with antibodies against *Pythium insidiosum*; bar = 20 µm.

Comments

The history, clinical, serologic, cytologic, histologic, and immunohistochemical findings in this case confirmed a diagnosis of canine colorectal pythiosis. Although relatively uncommon, pythiosis is a disease of growing importance, as cases are being documented in a wider range of areas, including Wisconsin, California, and arid regions such as Arizona.1,2 In this case, the dog had access to a fenced-in backyard with 2 lined koi ponds and had a history of swimming in a lake in Southern Pines, NC, earlier the same year (approx 8 months before the onset of clinical signs). In addition, a history of travel and swimming in Texas within the year prior to presentation was also reported.

Pythiosis is a condition caused by *P. insidiosum* (class Oomycota), an aquatic oomycete. This organism most frequently causes gastrointestinal,
cutaneous, or subcutaneous disease in dogs, horses, calves, and sheep, but vascular, ocular, and, rarely, systemic involvement have also been described. The gastrointestinal form is commonly reported in dogs, whereas cutaneous or subcutaneous disease is more common in horses. Various disease states have also been documented in humans, cats, and some nondomestic species in captivity, such as spectacled bears, a jaguar cub, and a dromedary camel.

**Pythium insidiosum** is present in the aquatic environment in tropical, subtropical, and temperate climates. Cases have been noted in a wider range of climates, suggesting that changing environmental conditions may create more habitats conducive to *P insidiosum* zoosporation. Although it has been demonstrated that *P insidiosum* is nearly ubiquitous and genetically diverse in ponds and lakes in North Central Florida, no further studies have investigated the prevalence of environmental *P insidiosum* in other parts of the United States. The ubiquity of *P insidiosum* in bodies of water in Florida compared to the prevalence of clinical pythiosis demonstrates that specific conditions must be met for an exposure to become an infection. *Pythium insidiosum* does not appear to be capable of infiltrating through healthy, intact tissue. Instead, tissue trauma or secretion of tissue-dissolving enzymes by the invading organisms is required for mammals to be infected, whether it be through the skin or the intestinal mucosa. A higher prevalence of infection has also been noted in medium- and large-breed dogs ≤ 3 years old.

Pythiosis presents as a chronic granulomatous disease that most commonly affects the skin or gastrointestinal tract. Clinical signs of gastrointestinal pythiosis are often similar to those in this case—weight loss, hematochezia, and diarrhea—and may include vomiting as well. Physical examinations can be unremarkable besides a poor body condition and an eventually palpable abdominal mass, depending on the affected site. Signs of systemic illness, such as hyporexia and lethargy, generally are not present unless intestinal infarction, perforation, or obstruction occurs. Cutaneous pythiosis in small animals is often characterized by nonhealing wounds and invasive masses with ulceration and draining tracts.

Common clinicopathologic abnormalities associated with gastrointestinal pythiosis include eosinophilia, anemia, hyperglobulinemia, and hypealbuminemia. In this case, the CBC demonstrated a mild leukocytosis characterized by a mild, mature neutrophilia, most consistent with mild or chronic inflammation. There was also a mild nonregenerative normocytic, normochromic anemia, most consistent with anemia of chronic inflammation. The serum biochemical profile revealed minimal hypealbuminemia with a low albumin-to-globulin ratio, mild hypocholesterolemia, and mild hypomagnesemia, which may have been a result of loss through the compromised intestinal wall. The total protein concentration was still within the reference interval, given that the globulin concentration was near the upper end of the reference interval, which is typical of chronic inflammation in animals with pythiosis.

Ultrasonographic signs of gastrointestinal pythiosis include thickening and obliteration of the normal layered appearance of the intestinal wall, along with regional lymph node enlargement, as seen in this case. Similar changes can also be seen with gastrointestinal neoplasms (eg, gastrointestinal adenocarcinoma, lymphoma, and leiomyosarcoma), and they should be included as clinical differential diagnoses. Therefore, further diagnostic tests are required for differentiation, including examination of tissue aspirates or biopsies.

Definitive diagnosis of pythiosis is based on the results of culture performed on samples of affected tissue that yield growth of *P insidiosum*, which can be identified by its characteristic sporulation. Culture can be challenging, and sporulation is not always achieved; therefore, other ancillary methods must be used. In the absence of a full-thickness biopsy of the affected gastrointestinal segment or skin, ELISA is generally used for noninvasive ante-mortem diagnosis of pythiosis because it only requires a serum sample. ELISA has a reported sensitivity and specificity of 100%. A sample is considered positive if it has more than 40% positivity compared to a strongly positive control run on the same plate with 3 different dilutions being applied. ELISA can also be used to track treatment progress, as a decreasing percent positivity correlates with improvement of lesions. In addition, dogs with gastrointestinal or cutaneous pythiosis that were treated successfully with surgery had results of ELISA percent positivity within reference limits 2 to 19 months after treatment. Thus, ELISA seems to correlate fairly well with active infection. Rarely, ante-mortem diagnosis via microscopic examination of fine-needle aspirate samples can be used, but this is reliant on aspiration of samples with hyphae present, as noted in this case. Immunohistochemical staining can be performed as an ancillary ante- or postmortem diagnostic tool in the evaluation of either fine-needle aspirate samples or biopsy specimens with hyphae, as was the case in this dog. However, because the antibody used is polyclonal, cross reactivity with *Lagenidium* sp may occur; therefore, the final diagnosis cannot be based solely on results of immunohistochemical analysis. In addition, immunohistochemical analysis is less commonly performed, compared with ELISA, because the method for collecting the samples is more invasive than simply collecting the serum.

Histologically, 2 distinct patterns can be seen in canine cutaneous and gastrointestinal pythiosis: either a necro-eosinophilic pattern, characterized by zones of eosinophilic necrosis, collagenolysis, cellular debris, and variable numbers of eosinophils; or a pyogramulomatous to granulomatous pattern (seen in this case), characterized by epithelioid macrophages and Langhans giant cells mixed in varying proportions. These patterns can coexist in the same patient.
Lesions can efface 1 to all layers of the gastrointestinal tract, but the submucosa and muscularis are often primarily affected. In cutaneous pythiosis, lesions can extend from the usually ulcerated epidermis to the subcutis. Hyphal morphology is characteristically sparsely septate with nondichotomous, near-right-angle branching, and hyphae are best highlighted with silver stains such as Grocott-Gomori methenamine silver stain. There is weak to no staining with periodic acid-Schiff stain, because *Pythium* does not produce chitin.

Treatment for pythiosis is often unsuccessful, and overall prognosis is poor. A multifaceted approach to treatment appears to have the most success. The best outcomes are generally associated with surgical excision of the lesion with margins of a minimum of 3 to 4 cm. In this case, granulomatous inflammation (observed clinically as intestinal wall thickening) extended to the internal anal sphincter, so surgical excision was not a viable option because successful margins could not be obtained. A slightly better treatment response rate (approx 20%) has been reported with the administration of a combination of itraconazole and terbinafine, rather than itraconazole or amphotericin B alone. Most antifungals have variable success in treating pythiosis because of the lack of ergosterol in the cell walls of oomycetes. There have been reports of successful long-term treatment with a combination of antifungals, surgical excision, and immunotherapy. A recent case report also documented the resolution of gastrointestinal pythiosis lesions in a dog treated with a combination of itraconazole, terbinafine, and mafenoxam (an agricultural fungicide).

Immunotherapy has shown variable success, ranging from being part of successful treatment in some cases to having no discernible effect in others. This treatment method appears most effective in acute cases diagnosed within 2 months of infection because it purportedly works by displaying injected *P. insidiosum* cytoplasmic antigens to the host’s immune system.

Medical management alone with a combination of itraconazole, terbinafine, and prednisone is reported to have some success. An anti-inflammatory dosage of prednisone combined with the traditional itraconazole and terbinafine protocol was a successful long-term treatment in 3 dogs. The addition of prednisone is thought to help to reduce the overactive immune response that leads to debilitating granulomas, allowing the animal to survive while antifungals treat the infection. Unfortunately, this protocol was unsuccessful for our patient.

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References