

**“And So It Grows”**

**A case of colonic pythosis in a golden retriever**

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## **Introduction:**

The etiologic agent for pythiosis is *Pythium insidiosum*, a water mold from the class Oomycetes.<sup>5</sup> This disease presents similarly to those caused by *Lagenidium*, another oomycete, and *Zygomycetes*, a true fungus.<sup>1,5,10</sup> Pythiosis most often affects horses, dogs, humans, and cattle, although other animals can become infected.<sup>3,7,10,11</sup> Traditionally, all species can present with the cutaneous form of pythiosis; however, dogs are the only species that commonly presents with the gastrointestinal form of this disease.<sup>7,10,11</sup> In dogs, young medium to large breed dogs are most commonly infected.<sup>4,13</sup> Pythiosis is progressively fatal and is often associated with very poor outcomes, especially in dogs.<sup>5,10,14</sup>

In the United States pythiosis occurs most frequently in the southeastern states, although cases have been documented as far north as Wisconsin and as far west as California.<sup>1,5,11</sup> Infection with *P. insidiosum* is often associated with contact with stagnant or standing water, such as lakes and rice fields.<sup>9,5,14</sup> Treatment for pythiosis may be unrewarding, especially in late stage disease, and includes aggressive surgical resection and antifungals; however, other drugs may have some use in treating pythiosis.<sup>1,5,7,11,15</sup>

## **History and Presentation:**

Miller was an approximately 3-year-old male intact Golden Retriever who was presented to MSU-CVM Small Animal Surgery on 10/28/19 for a consultation for his gastrointestinal (GI) Pythium. About 4 weeks prior, Miller began to vomit and strain to defecate. After going to his primary veterinarian, he was prescribed Provable (a probiotic), maropitant, and Hill's Prescription Diet i/d. He did well for about a week and then began to have watery diarrhea and became anorexic. Miller was then brought back to his primary veterinarian where abdominal radiographs were performed which showed a soft tissue mass caudal to the stomach in the region

of the spleen, pancreas and transverse colon. At this time, Miller was referred to AERC. Bloodwork was within normal limits. An ultrasound was performed, and a thickened large intestinal wall and enlarged lymph nodes were found and aspirated. The lymph node aspirate was consistent with a reactive lymph node; however, the results of the intestinal wall aspirate were inconclusive as the spleen was accidentally aspirated instead. Pythiosis was suspected due to the patient's age, history, and thickened bowel loops seen on ultrasound and serum titers were sent out to Auburn University. They returned as 100% for pythiosis. Terbinafine, mefenoxam, and itraconazole were added to Miller's medications. His owner stated upon presentation that while Miller had solid stool previously, the mefenoxam caused him to have diarrhea. He was reluctant to eat at home and only wanted to eat boiled chicken and rice. Miller was up to date on his vaccinations and received Proheart and Simparica for flea/tick/heartworm prevention.

On presentation Miller was bright, alert, and responsive. He had a body condition score of 2/9 with his ribs being clearly visible. He weighed 26.70 kg. His heart rate was 96 bpm and he had strong synchronous pulses. His temperature was 102.4° F. He was panting, but had no evidence of distress. His mucous membranes were pink and moist with a capillary refill time of less than two seconds. His teeth were clean and appeared in good condition. No ocular, nasal or aural discharge was appreciated. No crackles or wheezes could be heard on lung auscultation. No arrhythmias or murmurs could be heard on cardiac auscultation. He had an erythematous, shaved, lesion on his right forelimb approximately 2 inches in length, which was from a previous catheter site. Upon rectal exam his prostate was smooth and symmetrical, but his rectum was full of soft orange feces. No masses, organomegaly, or thickened bowel palpated on abdominal palpation. His GI tract felt empty. His cranial nerves were intact, and no gait abnormalities could be detected.

### **Diagnostic Approach/Consideration:**

Miller was previously diagnosed via a fine needle aspirate and Pythium titers from Auburn University, which returned as 100% for pythiosis at a 1:1000 dilution. Pythium titers are read as a percent positivity in relation to a strong positive control. Titers above 40% were considered positive. Pythiosis can be diagnosed by immunodiffusion (ID), enzyme-linked immunosorbent assay (ELISA), hemagglutination (HA), or immunochromatographic test (ICT).<sup>7</sup> Each test has its advantages and disadvantages. ELISA is the most popular method of diagnosing pythiosis via detection of anti-pythium antibodies.<sup>1,7</sup> New research into a protein A/G ELISA. A/G is a protein that can bind the immunoglobulins from various animal species.<sup>7</sup> This new ELISA appears to be 100% sensitive and 98% specific across 3-4 standard deviations for multiple species.<sup>7</sup> Most ELISAs currently in use are species specific.<sup>7</sup> It is important to note that ELISAs measure anti-pythium antibodies, not antigens from *P. insidiosum* itself; however, decreasing titers are associated with a positive response to treatment.<sup>1,11</sup>

A CBC and serum chemistry were taken on the day of presentation. Miller's serum chemistry was largely within normal limits, except for a mild hyperglobulinemia, mild hypokalemia, and mild hyperchloremia. The hyperglobulinemia is consistent with immune stimulation by pythiosis, and the hypokalemia and hyperchloremia can be explained by his diarrhea. His CBC showed a mild to moderate eosinophilia at 1641/ul (normal range 120 – 1300) which is consistent with pythiosis.<sup>5,11,14</sup>

A contrast enhanced CT was performed for surgical planning. The wall of the proximal to middle portion of the descending colon was diffusely thickened with irregular contrast enhancement. The mesocolon in that region was also diffusely thickened and contrast enhancing.

Abdominal lymphadenopathy was noted, including several jejunal and left colic lymph nodes that measured up to 51x23x24mm.

### **Pathophysiology:**

Zoospores are the infective stage of *P. insidiosum*.<sup>3,5</sup> The zoospores are flagellated and motile.<sup>5,13</sup> They are chemoattracted to decaying plant matter, injured and necrotic animal tissue, and human and horse hair.<sup>9</sup> *Pythium* colonizes intestinal tissue through deficits made by intestinal parasites or mechanical damage, additionally, the zoospores themselves may be capable of direct invasion via their germ tube.<sup>9</sup> Within the GI tract, *Pythium* especially likes to colonize the gastric outflow tract and the ileocolic junction.<sup>14</sup> Non-resectable GI pythiosis has been reported to have a mean survival time of 26.5 days.<sup>14</sup> Both *Pythium sp.* and *Lagenidium sp.* are more closely related to red algae and *Prototheca sp.* than they are true fungi.<sup>5, 15,16</sup> Pythiosis, lagenidiosis, and zygomycosis have similar clinical signs, and cannot be differentiated histologically from one another without use of immunohistochemistry.<sup>5</sup> Oomycetes lack ergosterol in their cell walls.<sup>4,5,6</sup> Rather, the cell wall of *P. insidiosum* contains cellulose and  $\beta$ -glucan.<sup>15</sup> Pythiosis is characterized by poorly staining, sparsely septate hyphae with nonparallel walls and tapered ends and eosinophilic inflammation on histopathology.<sup>3,5,10</sup> Despite looking like a fungus on histopathology, the lack of ergosterol can make treatment difficult, as most antifungals target ergosterol synthesis.<sup>4, 16</sup>

Despite the lack of ergosterol in the cell walls, antifungals such as itraconazole, amphotericin B, or terbinafine may occasionally have an effect, although this is estimated to be an under 20% response to antifungals.<sup>6</sup> Due to this, these drugs are often used as adjunct therapy, especially itraconazole combined with terbinafine. While it is not FDA-approved for use in animals, mefenoxam 22% (Subdue MAXX, SyngentaCrop Protection Inc, Greensboro, NC) is an

EPA controlled agricultural fungicide used to control oomycetes in plants. It has been shown to inhibit *in vitro* growth of *P. insidiosum* and seems to be successful in controlling clinical pythiosis.<sup>5</sup> 1 µg/ml mefenoxam can completely inhibit up to 90% of *P. insidiosum* laboratory isolates, although it should be noted many laboratory isolates are Brazilian *P. insidiosum* strains.<sup>16</sup> Mefenoxam inhibits ribosomal RNA polymerases.<sup>5</sup> It can be dosed as frequently as 4 mg/kg PO every 12 hours with minimal side effects.<sup>5</sup> A study by the manufacturer showed no observable adverse effect level in dogs dosed 8 mg/kg/day for 6 months.<sup>5</sup> There is only one reported case of the use of this therapeutic compound; however, multiple later articles allude to other unreported cases. Should Mefenoxam be chosen as a treatment, informed consent of the owners must be obtained, considering it is not an FDA approved drug and is being used off label from its EPA directions.<sup>6</sup> Other drugs may have some efficacy in the treatment of pythiosis, particularly in cases that are not surgically resectable or have local recurrence.

Other drugs that may have some efficacy as adjuvant treatments are antimicrobials such as minocycline, which had a 17% cure rate in a rabbit model, and azithromycin, which had an 83% cure rate in the rabbit model.<sup>16</sup> Minocycline and azithromycin have also been shown to have synergistic anti-*P. insidiosum* activity when used in combination. It should be noted that rabbits, which are the most common animal model for pythiosis, develop subcutaneous pythiosis, and do not accurately represent the clinical presentation seen in dogs, humans, and other animals.<sup>16</sup>

One other drug that may have efficacy against *P. insidiosum* is prednisone. There is a case report of 3 dogs with nonresectable GI pythiosis who were managed with terbinafine, itraconazole, and anti-inflammatory doses of prednisone who did very well.<sup>14</sup> Each of the dogs treated with this combination showed improved clinical signs, negative pythium titers in 3-4

months, and all were alive with a good quality of life over 6 months post treatment start.<sup>14</sup> The improvement of clinical signs is at least in part attributed to the corticosteroid effect on eosinophils – it reduces adherence and inhibits chemotaxis and apoptosis.

It is important for clinicians to remember pythiosis as a potential for dogs with chronic colitis, particularly if they are losing weight. This is especially true considering the spread of pythiosis outside of its traditional range over the last 20 years.<sup>1</sup> Detecting the disease early is critical for more positive outcomes. Other differentials include: neoplasia, zygomycosis, lagenidiosis, chronic foreign bodies, and eosinophilic gastroenteritis.<sup>5</sup> Lagenidiosis is primarily a dermatologic infection and very rarely disseminates to other organs.<sup>5</sup>

### **Treatment and Management:**

Recurrence of pythiosis is a common complication associated with resection of affected body parts, especially with inadequate margins. The CT scan revealed that as a minimum, the majority of Miller's colon would need to be removed, as a minimum of 5 cm margins are recommended for the resection of GI pythiosis to prevent regrowth.<sup>5, 14</sup> There is at least one report of successful treatment of GI pythiosis of a dog with lesser surgical margins; however, it was also treated concurrently with immunotherapy and antifungals.<sup>15</sup> Getting appropriate margins on the aboral end was the largest concern for Miller's surgery. It was likely a subtotal colectomy would need to be performed in order to get appropriate margins.<sup>5,15</sup>

Dogs are thought to be poor doers with subtotal colectomies; however, this information is largely anecdotal.<sup>15</sup> In addition to the usual short-term complications of dehiscence, hemorrhage, and infection associated with GI surgery, dogs frequently suffer from medium to long term postoperative diarrhea.<sup>2</sup> There is also a risk for bacterial overgrowth if the ileocolic junction is not preserved.<sup>12</sup> In cats, subtotal colectomy is often a salvage treatment for megacolon; cats tend

to do well, but generally use the litter box more frequently for the rest of their lives due to decreased ability to store feces.<sup>2</sup> This is a post-surgical side effect also seen in dogs, and both dogs and cats may have soft feces for the remainder of their lives.<sup>2, 8, 12, 15</sup> This is something that must be discussed with owners prior to surgery. Fecal consistency should improve post-surgery as it is believed that villous height increases in the ileum to improve absorption.<sup>2</sup> A 2002 study showed that the lengthening of the villi occurred most significantly in dogs during the 8<sup>th</sup> to 12<sup>th</sup> week post operation.<sup>8</sup> Depending on the amount of intestine removed, some dogs may not regain the ability to form even soft stool and may never regain the ability to store feces more than 3 hours, even six months post operation.<sup>15</sup> One study showed that dogs that underwent a subtotal colectomy with the ileocolic junction preserved returned to normal defecation (defined feces of normal or near normal consistency passed 2-3 times a day) within 5 to 10 weeks of the subtotal colectomy.<sup>12</sup> Removal of the distal end of the ileum may also require patients to be supplemented with cobalamin.<sup>15</sup> Finally, post-op dietary management may be implemented by feeding dogs diets that are highly digestible to reduce fecal volume and high in fiber to slow gastric emptying to increase absorption time.<sup>15</sup> Overall, dogs can do very well with subtotal colectomies, as long as the owners understand the long-term post-operative management.

Miller was taken to surgery on October 29, 2019 for a subtotal colectomy and gastrostomy tube (G-tube) placement. He was given a sanitary clip of the back of his hind legs, his perianal region, and the base of his tail prior to surgery. An abdominal explore was performed. The diaphragm, liver, kidneys, pancreas, adrenal glands, bladder, stomach, duodenum, illeum, and cecum were within normal limits. The omentum was red, thickened, with a cobblestone appearance, and multiple mesenteric lymph nodes were enlarged. The transverse and descending colon was markedly thickened from about 6 cm aborad to the cecum to about 14

cm orad to the pelvic brim and had multiple omental adhesions to it. The adhered omentum was transected using the Precise Ligasure handpiece (Medtronic, Minneapolis, MN) to isolate the affected segment of colon. Initially resection margins were approximately 10 cm aborad and 6 cm orad to preserve the cecum; however, the two free ends were unable to be opposed due to excessive tension. The cecum was carefully dissected from its mesenteric attachments using the Ligasure and was removed. The ileum and colon were anastomosed in a simple interrupted pattern using 3-0 PDS. The abdominal cavity was lavaged with sterile saline, a mesenteric lymph node biopsy was taken, and a g-tube was placed to assist with feeding. Recovery was without complications. The resected portions of intestine and lymph node biopsy were submitted for histopathology.

Miller's biopsies returned as follows: the cecum showed mildly increased eosinophils within the basal lamina propria, increased lymphatic dilation, and peyers patch collapse, but no signs of pythiosis. The colon had a poorly defined transmural and multifocally circumferential mass causing expansion of the intestinal wall from the lamina propria to the serosa. The mass was composed of fine fibrovascular stroma interspersed with many reactive fibroblasts and neovascularization (granulation tissue), numerous macrophages, numerous eosinophils, and fewer neutrophils. Throughout the lesion there were multifocal, discrete to coalescing, inflammatory aggregates of coagulative necrosis and intralesional nonstaining hyphal-like structures. Upon the use of Grocott's methenamine silver stain (GMS) the hyphal-like structures became visible and demonstrated the extent of *P. insidiosum's* growth. The margins were shown to be complete. The lymph node was reactive but showed no signs of pythium invasion. After surgery, Miller was continued on his regimen of itraconazole, terbinafine, and mefenoxam.

### **Case Outcome:**

The biggest hurdle to Miller's recovery was controlling his post-operative diarrhea. His owners elected for him to stay at MSU-CVM as they both worked, and Miller was initially passing liquid diarrhea nearly every hour. He was fed Hills Prescription Diet i/d and given Fortiflora once a day. By 3 days post op, his fecal consistency increased slightly, but he continued to have severe frequent diarrhea and developed perineal irritation. Metronidazole was added to help control his diarrhea by decreasing intestinal inflammation and metoclopramide was added to address what was perceived to be gastric stasis caused by his surgery and subsequent fentanyl CRI. By 4 days post op he needed to be sedated with 0.1 mg/kg hydromorphone to clean his rear due to the pain from the irritation. On day 5, loperamide was added to his treatments to slow gut motility and hopefully decrease the frequency of his diarrhea. 6 days post-op Miller was able to store feces for up to a maximum of 3 hours, although he continued to have diarrhea and painful perineal irritation. On day 7, *Psyllium* fiber was added to his treatments to bulk up his stool. On this day, Miller's perineal irritation increased to the point the superficial skin of his scrotum sloughed off. On his 9<sup>th</sup> day post op, the most cranial edge of his incision became red and inflamed. The incision in this area developed a serous to purulent discharge. Miller was placed on amoxicillin/clavulanic acid to control potential infection, and it was determined that this damage was due to the pressure point over his xyphoid created by his low body condition. Thicker padding was added to his kennel. By his discharge 10 days post op, Miller was able to store feces a maximum of 7 hours if he was asleep, but he still needed to go more frequently if he was awake.

Miller was discharged on 11/8/19. He was sent home on itraconazole, terbinafine, mefenoxam, loperamide, psyllium powder, amoxicillin/clavulanic acid, trazadone, and Resinol

(2% resorcinol) ointment. His owners were instructed on how to use his G-tube, care for the G-tube stoma site, and how to clean and care for the inflammation on his perineum and scrotum. He was sent home with a sample of Hills Prescription Diet i/d and it was requested of his owners to maintain a defecation log, so modifications to his diet could be made as needed. His owners planned on having a doggy door installed in their home once his G-tube was removed. His one-month recheck was delayed until January 2020, due to the holiday season. During his recheck, it was found that his *Pythium* titers had decreased from 100% to 60%. He had gained 3 kg in weight and his stool was persistently a “cow-pie” consistency. His GI panel results for TLI, PLI, cobalamin, and folate were within normal limits, so no supplementation was needed at that time. His antifungal regimen of mefenoxam, itraconazole, and terbinafine were continued, as well as his *Psyllium* fiber, and loperamide. It was recommended that Miller’s diet be changed to Purina EN Fiber Balance to help Miller continue to gain weight and improve his stool consistency. Unfortunately, further information on this patient was lost to follow up.

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