Not so Fun- Guy

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Introduction

Gastrointestinal diseases are a common disease concern for most canine owners. Most gastrointestinal diseases start acutely and can be medically managed until resolution is achieved, or clinical signs are manageable[12]. Occasionally, disease processes that affect the gastrointestinal tract can be considered life threatening unless treatment is instituted rapidly. One such life-threatening disease is caused by an oomycete called *Pythium insidiosum,* which is an aquatic pathogen that causes cutaneous and gastrointestinal disease following colonization of damaged mucosa[1]. Pythiosis, or an infection caused by *Pythium*, differs from fungi since its cell wall and membrane have different components than fungi. *Pythium* is typically associated with coastal areas but is considered ubiquitous and arid climates such as Arizona and California have diagnosed cases of *Pythium*[1,6]. *Pythium* more commonly affects horses and dogs, but it has been reported in other species including: cats, cattle, and humans. Treatment for *Pythium* is typically aggressive and needs to be instituted following diagnosis due to rapid progression of disease and the poor prognosis for chronic cases, which is labeled as infections greater than 2 months[2,4,5]. Currently, treatment for Pythium is difficult due to its reduced susceptibility to antifungal drugs and its aggressive progression[4].

History and Presentation (both typical and atypical)

Pythium tends to be diagnosed in young (less than 3 years old), immunocompetent, large breed male dogs who work outdoors or have exposure to wetlands[1,2,14]. Canine pythiosis is associated with gastrointestinal disease or cutaneous lesions with the gastrointestinal form presenting more frequently, but these forms rarely coexist in the same patient[2,9]. A typical presentation of the gastrointestinal form includes weight loss, vomiting, diarrhea, and hematochezia being present[1]. However, these patients may also present with an emaciated body frame due to the weight loss

and with a palpable abdominal mass. Severe thickening of the stomach, small intestines, colon, rectum, esophagus or pharynx occurs with the gastrointestinal form, but only segmental sections are usually involved. The palpable abdominal mass is typically a segmental thickening of the gastrointestinal tract. The disease may spread to the pancreas, mesenteric lymph nodes and the bile ducts, but this is not a typical presentation for this disease[2]. Mesenteric lymphadenopathy is present in most cases; however, it is usually reactive hyperplasia instead of infection[9]. Systemic illness is not usually seen unless an intestinal obstruction, infarction or perforation occurs following *Pythium* hyphae extending into the lumen, mesenteric vessels or undermining the integrity of the gastrointestinal tract[1,4].

Pathophysiology (include anatomical considerations)

Pythium insidiosum is an aquatic oomycete, which differs from a true fungus by producing flagellate zoospores and the makeup of the cell wall and cell membrane[1]. The flagellated zoospores are the infective stage that is released into warm environments before it encysts in damaged skin, gastrointestinal mucosa or decaying plants[1,2]. The zoospores will adhere to the cut edges of the skin and encyst but does not adhere to undamaged tissue[2]. A glycoprotein is secreted by the encysted zoospores allow it to adhere to the injured tissues. The body temperature of the host stimulates the encysted zoospore to form a germ tube to enter the tissues of the host.

Once in the tissues, the hyphae exude exoantigens that stimulates a Th2 (T helper 2) immune response, which leads to recruitment of eosinophils and mast cells[3]. An Hoeppli-Splendore phenomenon from the recruited mast cells and degranulated eosinophils is responsible for the extensive tissue damage which occurs with pythiosis. The viable hyphae located within the extensive tissue damage excretes proteases and exhibit mechanical force to allow progression of

the hyphae[2,3]. It is shown that protease activity and tissue damage is needed to weaken the tissues prior to hyphae extending through them. Once *Pythium* is established within the body, it will progress rapidly by extending hyphae throughout the tissue and stimulating a Th2 reaction to create the tissue damage needed to help with its proliferation within the tissues[3].

The commonly affected regions of the gastrointestinal tract are the gastric outflow area, proximal duodenum, and ileocolic junction[9]. From these colonized areas, the hyphae radiate out and colonize additional tissue; however, it is not uncommon to find multiple segmented lesions within the same patient. Mesenteric lymphadenopathy is common with *Pythium,* but it is usually reactive hyperplasia instead of hyphae infiltration. If the disease extends into the mesenteric root, it can cause severe mesenteric lymphadenopathy with the lymph nodes coalescing until one large firm mass.

Differential Diagnoses

The clinical signs of pythiosis are weight loss, vomiting, diarrhea, palpable abdominal mass, and hematochezia[1]. Several different diseases can mimic the clinical signs that pythiosis causes within the patient, which can complicate the diagnosis. Abdominal neoplasia can mimic clinical signs of the gastrointestinal illness along with the palpable abdominal mass[9]. Zygomycosis can mimic the clinical signs and the disease process of pythiosis since it is another oomycete which behaves in a similar manner as Pythium. Pyloric outflow obstruction due to hypertrophy and intussception can mimic the gastrointestinal signs of pythiosis including vomiting and diarrhea[12]. Other disease processes which can mimic some of the clinical signs of pythiosis are histoplasmosis, eosinophilic gastroenteritis, and chronic intestinal foreign bodies.

Diagnostic Approach/Considerations

The typical presentation of worsening gastrointestinal signs indicates further investigation including abdominal radiographs, abdominal ultrasound, complete blood count, and serum chemistry to identify an origin of the disease[12]. On a complete blood count, eosinophilia and nonregenerative anemia may be a response to the hematochezia and the hyphae. Hyperglobinemia and occasional hypercalcemia can be noted on serum chemistry with the hypercalcemia being a paraneoplastic response to the pythiosis[2,6,12]. Poor abdominal details along with an abdominal mass may be present on abdominal radiographs[9]. In rare cases, small bowel obstruction can be seen on abdominal radiographs following occlusion of the lumen of hollow organs[4,9]. Abdominal ultrasound is considered the best choice for imaging to diagnose gastrointestinal pythiosis[9]. Abdominal ultrasonography can allow better visualization of the abdominal contents due to the superimposition of soft tissue structures that occurs with abdominal radiographs, which can hide subtle disease changes. Abdominal ultrasound can aid in the decision for surgical resection and fine needle aspiration of suspicious areas seen on abdominal ultrasound. Severe segmental thickening of the gastrointestinal tract and mesenteric lymphadenopathy can be seen on abdominal ultrasonography. Invasion into the mesenteric vessels by pythiosis which can be evaluated by the doppler function of an ultrasound impacts prognosis and surgical resection. Loss of layer distinction and thickening of the walls within the gastrointestinal tract is noted with gastrointestinal pythiosis; however, it is not considered pathognomonic for pythiosis since gastrointestinal neoplasia and other disease processes can also display these characteristics[11]. Ultrasound guided fine needle aspiration can be used to perform cytology with a Gomori methenamine silver (GMS) to identify the hyphae associated with pythiosis to gain a more certain diagnosis[4,9].

Transport of organic materials containing *Pythium* to an ancillary lab should be packaged correctly so that an accurate diagnosis can be made quickly[2]. If transport to an ancillary laboratory will take longer than 2 days, transport the specimen with a couple drops of antibiotics in the fluid to limit bacterial colonization. For transport, wash the specimen in distilled water and transport at room temperature in saline or water. Biopsy specimens of pythiosis can be refrigerated up to 5 days but shipping on ice has inhibited growth in about 20% of samples[2]. A culture and wet mount examination can be performed on suspected *Pythium* tissues particularly on cutaneous form of the disease to allow for identification or grow enough organisms so that an PCR or rRNA gene sequencing can be performed to identify the infection[2,9]. A wet mount examination is performed using freshly taken samples by examining with 10% KOH and looking for sparsely septate hyphae[2]. Small pieces of fresh, nonmacerated tissue are placed on a vegetable extract agar with an antibiotic selective media with mycelial growth expected within 12 to 24 hours in room temperature. *Pythium insidiosum* are adapted to grow well at temperatures from room temperature to typical mammalian body temperature with optimal growth occurring between 34-36 degrees Celsius and 40-45 degrees Celsius is the maximal temperature in which growth can occur. Diagnosis can be made following sporulation of Pythium within the cultures; however, the zoospores alone cannot be sufficient for positive diagnosis alone[2]. It is also rare to see sexual reproductive structures that are needed doing a culture which typically means another identification method needs to be performed to identify *pythium*[9].

Pythiosis can also be detected by serological assays, immunofluorescence, and DNA sequencing which require sending out tests to ancillary labs that perform these tests[2]. Immunodiffusion was the first method developed to detect anti- Pythium antibodies. However, while the specificity

was very good the sensitivity was low so other diagnostic tests were developed to increase the sensitivity and specificity. Enzyme linked immune-sorbent assay (ELISA), Western blot and immunochromatographic assay were later developed for *Pythium insidiosum* to increase the specificity and sensitivity compared to the immunodiffusion test[2]. Louisiana State University has developed an ELISA for pythiosis, which can also be used to detect a decrease in antibodies during treatment and following surgical resection to evaluate progress in treatment[9]. The Louisiana State University IgG ELISA is reported to have an 100% sensitivity and specificity for *Pythium insidiosum*[5]. The results are expressed as a percentage of positivity compared to a strong control with a greater that 40% positivity being correlated to an 100% sensitivity and specificity. *Pythium insidiosum* infected fixed tissues can have immunofluorescence and immunoperoxidase staining techniques performed to detect *Pythium insidiosum* antibodies[9]. Detection of the DNA of *Pythium insidiosum* can be identified within tissue by PCR and DNA sequencing eliminating the need for culturing[2]. Molecular techniques may be limited due to normal bacterial contamination of the lesions which may interfere with the testing. A species-specific DNA probe has been developed which uses the 530 bp fragment of a ribosomal intergenic spacer (IGS) to help identify pythiosis infections. Another molecular technique uses the internal transcribed spacer (ITS) of the rRNA locus of *Pythium insidiosum* to identify markers of pythiosis. However, results should be interpreted with caution due to the contamination with normal flora microbes may interfere with the results.

Cytological examination for *Pythium insidiosum* can be a less invasive method to diagnosis pythiosis through obtaining samples through fine needle aspirations using ultrasonography[9]. *Pythium insidiosum* does not stain well in a hematoxylin and eosin (H&E stain) and periodic acid- Schiff (PAS), so Gomori methenamine silver (GMS) staining is preferred to visualize

Pythium hyphae. In H&E and PAS stains, negative white space within the slide can be attributed to hyphae from *Pythium* with a band of eosinophils surrounding the white space. PAS stains hyphae from most fungal agents; however, *Pythium* does not contain chitin in their cell walls unlike true fungi[10]. The H&E stain is used to identify the morphology, localization and extent of the lesions and the categorize the inflammatory response seen. Sirius red (SR) and GMS stain can be combined to demonstrate the intralesional hyphae of pythiosis and eosinophils since SR can be used to demonstrate eosinophil granules. Sirius red can also identify the patterns and eosinophils associated with the Splendore- Hoeppli reaction, which occurs in a Th1 (T helper 1) immune response. On GMS staining, the hyphae are described as having dark brown, rarely septate hyphae with non-parallel cell walls[4,9]. On histopathologic examination, two patterns of inflammatory response can be seen but when they converge it can be described as pyogranulomatous[10]. The two main inflammatory patterns are necro-eosinophilic and granulomatous. Necro-eosinophilic zones of inflammation are described as having numerous, strongly positive GMS staining hyphae that are morphologically normal. With an H&E stain, the pattern is characterized with broad zones of eosinophilic necrosis, cellular debris and eosinophils. Occasionally, negative profile staining of hyphae can be seen in an H&E stain. Granulomatous inflammation is the second form of inflammation with pythiosis that consists with macrophages and giant cells that is surround with thin capsules of connective tissue with occasionally necrotic cores and surrounding lymphocytes and plasma cells. Lesions will be noticed primarily within the submucosa and muscularis; however, one to all layers of the gastrointestinal tract lining can be affected[10].

Treatment and Management Options

Medical management is unrewarding in most cases due to the main target for antifungal drugs, ergosterol, is not a main component of its cell membrane[1]. Antifungal drugs were originally used to treat pythiosis due to the historical belief that *Pythium* was a fungal agent[2]. The cell wall has both cellulose and beta-glucan but is missing the chitin which is present in fungal organisms[1,2]. Ergosterol is typically a target for fungal drugs, but this is not an important component of the cell membrane which complicates treatment[1]. Due to the decreased importance of ergosterol in their cell membrane, they are suspected not to be susceptible to azoles, terbinafine, and amphotericin B since these drugs affects the biosynthesis of ergosterol[2]. However, some of these antifungal drugs have been noted to cause remission to a complete cure of *Pythium insidiosum*[1,2,4]. The efficacy of terbinafine and itraconazole was reported to be less than 20% in cases with pythiosis in a paper by Schmiedt et al[5]. Synergism between the antifungal agents is suspected when used in pythiosis since the effect of the antifungal drugs in combination and separately in rabbits showed that combination therapy caused a decrease in lesion size[4]. Synergistic fungicidal levels for *Pythium* insidiosum was noted in 17% of samples between the azoles (voriconazole and itraconazole) and terbinafine in an in vitro study evaluating the effects of the drugs on pythiosis[8]. The drug combinations studied showed that the effects are indifferent or synergistic but are not antagonist in respect to pythiosis treatment. The synergistic effect of the azoles and terbinafine is thought that one drug may increase the cell permeability to the other drug; however, Pythium does not contain ergosterol within their cell membrane, so it should not be effective. Terbinafine was noted to be the most effective drug within this study with a lower dosage needed to reach fungicidal levels after 24 hours of incubation[8]. One case report published by Dr. Grooters et al. incorporated a commercial fungicide called mefenoxam into a treatment regimen following side effects from itraconazole, which led to a curative result along with administration of

terbinafine[13]. Mefenoxam is not evaluated by the FDA for use in dogs for the treatment of fungal diseases, but the EPA required that the drug be evaluated on dogs for safety purposes. The EPA required that dogs were administered it for 6 months with no harm, so the case report utilized the same dosage of 1 mg/kg BID to treat the *Pythium* infection alongside other treatments used. It has been noted that 90% inhibition of Pythium has been attributed to mefenoxam in an in vitro study at the concentration of 1 microgram per ml. However, it is not recommended to currently administer mefenoxam to a patient since a thorough drug evaluation has not been completed through the FDA to indicate that it is safe to use in patients[13]. Treatment with glucocorticoids have also been used to reduce clinical signs and decrease vomiting in patients with pythiosis to increase the quality of life during treatment or until humane euthanasia[9]. On the rare occasion, a small subsegment of dogs on prednisone alone at the dosage of 1 mg/kg SID have been cured of Pythium per Dr. Grooters[9].

Aggressive surgical resection of the gastrointestinal tract segments affected by *Pythium* is the treatment of choice since a complete cure can be achieved if complete excision is done[1]. The goal is 5 cm margins at each area of segmental thickening to attempt to remove all tissue with pythiosis along with taking biopsies of regional lymph nodes since it can be a prognostic indicator[1,9]. If non resectable lymph nodes are noted on exploratory laparotomy near an affected area of bowel, it should not dissuade a surgeon from removing the affected segment of intestine[9]. Enlarged lymph nodes of the mesentery seen during surgery may not contain Pythium hyphae but in most cases will be reactive lymphadenopathy[1]. Post-operative reoccurrence can occur following aggressive surgical resection so treatment with antifungals is recommended for 2 months or until margins can be verified[1,4,9]. Dr. Grooters recommends treating with itraconazole at 10 mg/kg SID and terbinafine at 5 to 10 mg/kg SID for at least 2 to 3 months following

surgical resection[9]. If incomplete margins are taken, the lesions may progress despite medical therapy and clinical signs will reoccur, so a repeat surgery may be indicated[9,13]. Reoccurrence of the disease can be monitored through serial ultrasonographic studies to evaluate thickness of the gastrointestinal tract and ELISA serology performed before and serially following surgery[9,13]. If the ELISA percentage from Louisiana State University drops by 50% or more within 3 months of surgery, complete surgical remission was achieved, and medical therapy can be discontinued at that portion[1,9].

A newer component to treatment of *Pythium insidiosum* is immunotherapy or vaccines which are created from the soluble mycelial antigens and secreted exoantigens[1,5]. The addition of the cytoplasmic antigens along with the exoantigens in the vaccine appears to improve the efficacy of the vaccine particularly in equine cases[5]. However, a lower cure rate for canine patients has been noted when compared to equine patients with 72% of equine cases including chronic cases and 33% of dog cases obtaining a cure[1,3]. However, another paper mentioned that equine cure rates can vary between 50-83.3% with the same lymphozied immunotherapeutic vaccine, which has also been used on canine patients as well[4]. Acute disease, which is noted to be infections of less than 2 months, tends to show the maximum benefit to immunotherapy in canine patients[5]. One paper assessed that canine patients that had an injection site reaction following vaccination tended to have a better response in response to treatment[4]. Immunotherapy alone has not been associated with cure unless it is used in combination with other treatment options including antifungal drugs or surgical resection[5].

Several papers evaluated how immunotherapy modulates the immune response and how it may provide a benefit to the patient. A change from Th2 to Th1 immunity was noted in the papers by Mendoza et al. and Bach et al. through evaluating cytokines, cell types and enzymes associated

with the immune response[2,7]. NTPDase was evaluated to understand the change from Th2 to Th1 immune response that was purposed by Mendoza following immunotherapy[7]. ATP was measured to correlate the NTPDase activity in the study by Bach et al. since NTPDase is present in lymphocytes and hydrolyzes ATP into AMP. Decreased ATP levels were associated with increased NTPDase activity following exposure to *Pythium* but returned to normal levels following 4 doses of immunotherapy. Lymphocytes have a receptor (P2Y) that binds extracellular ATP when in low concentrations, which stimulates the Th2 response. But when ATP hydrolysis returns to normal levels following immunotherapy it can be assumed that NTPDase activity has decreased. The decrease in NTPDase activity occurs with increased extracellular ATP, which stimulates ATP binding to P2X7 receptors to cause activation of a Th1 response. This leads to stimulation of cytotoxic T lymphocytes and macrophages which would destroy the hyphae and decrease the infection[7]. Following immunotherapy, prevention of reinfection has been seen in equine patients due to the production of IgG classes for 18 months[3]. The proposed injection schedule for immunotherapy is 6 injections spaced out by 15 days with a Benadryl premedication to limit hypersensitivity and injection site reaction following vaccination[4,14].

Combination treatment involving immunotherapy, surgery and antifungal drug has been argued as the ideal treatment since it provides a multimodal approach towards treatment[4]. Dietary concerns in pythiosis patients is important due to decreased absorption of food over infected areas and impacts digestive function[5]. Ideally, a low fat and highly digestible diet should be given following diagnosis due to the disruption of intestinal function.

Expected Outcome and Prognosis

Treatment for *Pythium insidiosum* should be started early to have the best success at clearance of the disease[2]. The recovery rate for patients diagnosed with Pythium is low, but it is hard to identify the exact percentages since the location of the disease and surgical resection causes a variability in treatment. Immunotherapy with the combination of other treatments including antifungal medications and surgery may have a cure rate between 33% to 72% depending on what the vaccine was made from. The main treatment option for pythiosis is extensive surgery to remove the *Pythium* infected tissues from the body; however, clean margins may not be achieved even with aggressive surgical intervention. However, adjunctive therapies should be considered even if margins are not achieved since even those patients with marginal excisions have been cured of *Pythium*[4,5]. Treatment is extremely difficult and the disease itself confers a poor prognosis[4]. One case report of 10 dogs demonstrated a mean survival time of 26.5 days with each dog eventually dying of the disease[6]. Unsuccessful treatment is associated with location and extent of the lesion, the patient's immune response to the disease, and Pythium's inherent resistance to most antifungal drugs.

Conclusion

Pythium insidiosum has been diagnosed in arid climates that do not fit with the typical distribution leading some to suspect that both flooding and global warming are contributing to the spread of Pythium outside of its historical range[2]. While canine and equine patients are considered the most commonly infected species, other species including humans have been diagnosed with pythiosis resulting in similar treatment outcomes. Due to *Pythium* causing similar disease in varying species, it is important to evaluate data from other species to improve treatment options and results[4]. While there is no established treatment protocol yet, extensive surgery is still considered the treatment of choice to reduce the disease burden, but it may not be successful in complete removal and death

can still occur[2]. Antifungal treatment can be attempted and has been associated with cures, but cellular makeup of *Pythium insidiosum* limit the benefit that antifungals can achieve. Lastly, immunotherapy has relatively successful outcomes, but marked difference in responses between species and patients show that the response may be unpredictable. Therefore, it is important to understand that with these limitations that treatment is extremely difficult, and that diagnosis alone confers a poor prognosis[4].

References

1. Greene, Craig E. Pythiosis. In: *Infectious Diseases of the Dog and Cat*. 4th ed., Elsevier Saunders, 2006; 677-681

2. Gaastra, Wim, et al. Pythium insidiosum: An overview. *Vet Microbiol* 2010;146:(1-2):1-16 Available at: https://www.sciencedirect.com/science/article/pii/S0378113510003548?via%3Dihub . Accessed July 5, 2018.

3. Mendoza, Leonel, et al. "An Improved Pythium Insidiosum-Vaccine Formulation with Enhanced Immunotherapeutic Properties in Horses and Dogs with Pythiosis." *Vaccine*, 2003;21(21-22):2797-2804. Available at: *https://www.sciencedirect.com/science/article/pii/S0264410X03002251* . Accessed July 5, 2018.

4. Schmiedt, Chad W., et al. "Treatment of Intestinal Pythiosis in a Dog with a Combination of Marginal Excision, Chemotherapy, and Immunotherapy." *J Am Vet Med Assoc* 2012; 241(3):358–363.Available at: https://avmajournals-avma-org.proxy4.athensams.net/doi/pdf/10.2460/javma.241.3.358 . Accessed July 5, 2018.

5. Aeffner, Famke, et al. "Pathology in Practice." *J Am Vet Med Assoc* 2015; 246(5):511–513., Available at: https://avmajournals-avma-org.proxy4.athensams.net/doi/pdf/10.2460/javma.246.5.511. Accessed July 5, 2018.

6. Berryessa, N.a., et al. "Gastrointestinal Pythiosis in 10 Dogs from California." *J Vet Int Med*, 2008;22(4):1065–1069., Available at: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1939-1676.2008.0123.x . Accessed July 5, 2018.

7. Bach, Barbara Charlotte, et al. “Immunotherapy for Pythiosis: Effect on NTPDase Activity in Lymphocytes of an Experimental Model.” *Biomed Pharmacother*, 2010: 64(10): 718–722. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0753332210001666 . Accessed July 5, 2018.

8. Argenta, J. S., et al. In Vitro Activities of Voriconazole, Itraconazole, and Terbinafine Alone or in Combination against Pythium Insidiosum Isolates from Brazil. *Antimicrob Agents Chemother*,2008;52(2):767–769., Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2224725/ . Accessed July 5, 2018.

9. Grooters, Amy E. Chapter 69: Pythiosis, Lagenidiosis, and Zygomycosis. *Canine and Feline Infectious Diseases*. Elsevier/Saunders, 2014:668-678. Available at: www.sciencedirect.com/science/article/pii/B9781437707953000697. Accessed July 5, 2018.

10. Martins, T.B., et al. “A Comparative Study of the Histopathology and Immunohistochemistry of Pythiosis in Horses, Dogs and Cattle.” *J Comp Pathol*, 2012;146(2-3):122–131. Available at: https://www.sciencedirect.com/science/article/pii/S002199751100096X . Accessed July 5, 2018.

11. Graham, John P., et al. “Ultrasonographic Features of Canine Gastrointestinal Pythiosis.” *Vet Radiol Ultrasound*, 2000;41(3):273–277. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1740-8261.2000.tb01490.x. Accessed July 5, 2018.

12. Côté, Etienne. Pythiosis and Lagenidiosis. In: *Clinical Veterinary Advisor: Dogs and Cats*. 3rd ed. Elsevier Mosby, 2007: 937-938.

13. Hummel, James, et al. "Successful Management of Gastrointestinal Pythiosis in a Dog Using Itraconazole, Terbinafine, and Mefenoxam." *Med Mycol*, 2011;49(5):539–542., Available at: https://academic.oup.com/mmy/article/49/5/539/973624 Accessed July 5, 2018.

14. Pereira, Daniela I.B. "Canine Gastrointestinal pythiosis treatment by combined antifungal and immunotherapy and review of published studies". *Mycopathologia*. 2013;176: 309-315.