Case Report: Gastrointestinal Pythiosis in a Canine

Megen Danette Cummings

Class of 2017

Advisor: Jason Syrcle, DVM, DACVS

Mississippi State University

College of Veterinary Medicine

Clinicopathologic Conference

June 17, 2016

Introduction:

Pythium insidiosum is an aquatic pathogen belonging to the class Oomycete which belongs in the kingdom Stramenophila (Chromista). The life cycle begins with hyphal colonization that produces a zoosporagium with multiple, asexual biflagellate zoospores.[1,2,11] When the zoospores are released they adhere to other plants to complete reproduction. Additionally, the swimming zoospores are attracted possibly by chemotaxis to animal and human hair. Studies have shown that once the zoospores attach to the hair they accumulate at the follicular end of the hair shaft. This is when the disease process, which is known as pythiosis, begins.[3,11,12] Pythiosis causes gastrointestinal or ulcerative and pruritic cutaneous lesions in dogs, cats, horses, cattle and humans.[2,10,11] Dogs that are infected with this organism may have a history of being around stagnant, water sources. They either ingest the water or the organism enters through an open wound. The infective stage of *Pythium insidiosum* is a biflagellate aquatic zoospore.[4,5]

Pythium lives in an aquatic environment in temperate, tropical and subtropical climates around the world.[2] In The United States, the organism is most often found in the Gulf Coast states; however, it has been reported as far north as New Jersey and even in California.[2] *P. insidiosum* can also be found in the soil.[3] Oomycetes and all *Pythium species* are different from true fungi. Molecular studies demonstrate that Pythium is more closely related to *Prototheca spp*. (brown algae).[2,3] *Pythium* produces motile flagellate zoospores and their cells contain cellulose and beta-glucan, not chitin like other fungi. Another interesting component is that *Pythium* lacks ergosterol in the cell membrane.[5] Ergosterol is the main target for most anti-fungal pharmaceuticals. *Pythium* is known as a sterol auxotroph, which means the agent incorporates

sterols form the environment rather than producing them.[5] Pythiosis is over represented in young, large- working breed male dogs in the Southeastern United States.[3]

The gastrointestinal form of pythiosis, is a rapidly fatal disease with a poor to grave prognosis. There are only four documented cases of successful treatment of dogs with gastrointestinal pythiosis. All four cases were treated with itraconazole and terbinafine and three of the cases included radical surgical excisions with 3-4 cm margins and included procedures such as a partial gastrectomy, subtotal colectomy, and Billroth II.[2] This case report will describe a gastrointestinal form of *Pythiosis* at the duodenum-jejunum junction that was located around the cranial mesenteric artery and mesenteric root, which unfortunately could not be removed with wide margins.

History and Presentation:

Royal, a 12 month old intact female boxer which lived only in New Jersey presented to her primary veterinarian for a 1 month history of vomiting and anorexia. She had a progressive loss of appetite over that period of time. On physical exam, her temperature, heart rate, and respiratory rate were within normal limits. She weighed 20 kg and had a body condition score of 2/5. Fecal floatation and heartworm antigen tests were negative. There was a strong suspicion that she had pancreatitis, therefore a canine Pancreatic Lipase Immunoreactivity snap test was performed, which revealed abnormal levels of pancreatic lipase. Royal was treated with Omeprazole (20mg PO BID), Cerenia (24mg PO q24h), and a low-fat diet. After ten days of Cerenia and a month of Omeprazole she still was vomiting occasionally after meals and her appetite did not get better. She would also regurgitate periodically throughout the middle of the

night. At this time she was referred to the Mississippi State University College of Veterinary Medicine.

Pathophysiology:

Pythiosis is a disease caused by *P. Insidiosum* that causes cutaneous or gastrointestinal lesions. The gastrointestinal disease causes a pyogranulomatous inflammation of the affected tissue such as the stomach and small intestines. This can cause a partial or complete gastrointestinal obstruction. Areas most commonly affected are the proximal duodenum and the ileocolic junction.[3,7,9,15] When visualizing the area grossly, the submucosa is reddened and ulcerations maybe present. There is also commonly evidence of adhesions and peritonitis. Lymphadenopathy is also typically present and the lymph nodes are usually most palpable mid abdomen. The disease can metastasize to surrounding tissues through the vasculature which can cause acute hemoabdomen, infarction, perforation, and bowel ischemia.[9,11]

Diagnostic Approach/Considerations:

Upon presentation to MSU-CVM, Royal was alert, responsive but was very depressed. She was severely emaciated with a body condition score of 1/5 and a weight of 18.3 kg. Her heart and respiratory rates were within normal limits. She had a slight fever of 103.1°F/39.5 ˚C. Her capillary refill time was greater than 2 seconds and she was 5% dehydrated. Her heart and lungs auscultated normally. The remainder of her physical exam was normal. The complete blood count revealed mild thrombocytosis (698 K/ul; 160-650 K/ul), anemia/low HCT (28.1%; 34-60%), and low hemoglobin (9.2 g/dl;11-19g/dl). Serum biochemistry revealed hyponatremia (140 mmol/L; 143-153 mmol/L), low anion gap (9 mmol/L; 10-20 mmol/L), hypoalbuminemia

(1.6 g/dl; 2.5-3.9 g/dl), low alanine aminotransferase (4 U/L; 10-90 U/L) and low total bilirubin (0.1mg/dl; 0.2-0.6 mg/dl). The coagulation profile was all within normal limits.

A lack of serosal and retroperitoneal detail was seen on the abdominal radiographs, suggestive of emaciation. The axis of the stomach was shifted cranially, consistent with a small liver. The spleen was normal and the kidneys could not be evaluated. The small bowel contained a moderate amount of gas and fluid but did not display an obstructive pattern. The colon also contained mainly gas with some fecal material as seen in **Fig. 1**. The three thoracic views were normal besides an apparent opaque density in the region of the sternal lymph node only present in the left lateral view.

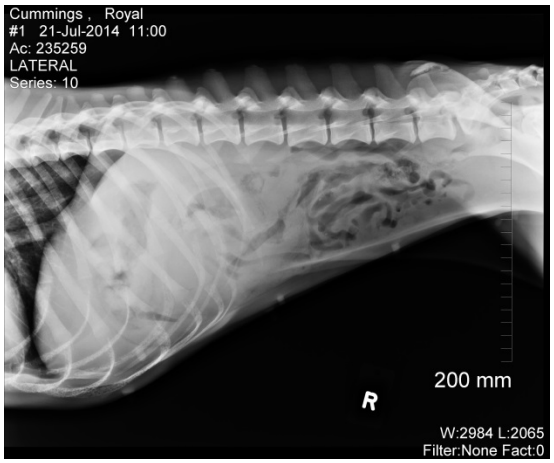

Fig. 1. Right lateral view showing the cranial shift in the axis of the stomach and the gas filled intestines. Also, this radiograph shows loss of serosal and retroperitoneal detail. This is due to her body condition score and peritoneal fluid.

A thickened gastric wall with loss of wall layering was seen on the abdominal ultrasound. The pyloric wall was hypoechoic. The normal thickness values of the stomach wall on ultrasound are 3-5mm. When thickness is greater than 1cm, it suggests malignancy.[12] Royal's stomach thickness measured greater than 1.44cm, seen in **Fig. 2.**

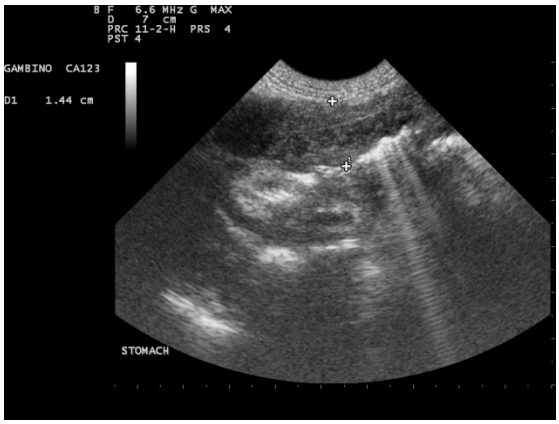

Fig. 2. This image shows the thickness of the stomach wall being 1.44cm wide.

Continuing the abdominal ultrasound, there was a pronounced thickness in the duodenum-jejunum junction and the mesenteric lymph node that measured 1.4 cm in width. Normal mesenteric lymph nodes should measure no more than 5 mm in width.[12] During the ultrasound, a fine needle aspirate of the thickened area and the mesenteric lymph node was obtained. Cytology of the mesenteric lymph node showed low cellularity, an admixture of nucleated cells that were necrotic, and fewer nucleated cells with a proteinaceous background. The cells that were intact and could be identified were almost exclusively degenerative neutrophils. The aspirate of the thickened area on the right side of the jejunum contained numerous amounts of red blood cells, platelet clumps, and non-degenerative neutrophils, admixed with fewer small lymphocytes, and occasional eosinophils.

With the diagnostics that were performed and with the aspirate slides results, the differential diagnostic list was limited to: intestinal lymphosarcoma (or other types of GI neoplasia), pythiosis, foreign body, inflammatory bowel disease, intussusception, or eosinophilic gastroenteritis. With the history and signalment the list was narrowed down to lymphosarcoma and pythiosis. A blood sample was sent to *Pan American Veterinary Laboratories* in Hutto, Texas to run an antigen-based enzyme linked immunosorbent assay (ELISA) for *Pythium*

insidiosum antibodies.[7,14] This test has been found to be highly sensitive and specific for the diagnosis of pythiosis.[5] The result of this test usually takes 5-7 days. As the results of this test were pending, Royal was not improving with medical management. Due to Royal's current clinical signs and her prognosis, an exploratory celiotomy was performed. There was a large intramural mass that extended from the distal duodenum to the proximal jejunum. It was ovoid and 10cm wide. It was firm, fibrous and was raised from the muscular tunics and markedly compressed the intestines at the duodenum-jejunum junction. The mass was located aboral to the cranial mesenteric artery and was connected to the mesenteric root. At this point the discussion was being made if excision with wide margins was even an option. The mesenteric lymph nodes were enlarged just as the ultrasound showed. The mass and mesenteric lymph node was aspirated with a 22-gauge needle and a STAT cytology was performed along with an impression smear of the mesenteric lymph node. The cytologic examination revealed severe pyogranulomatous inflammatory reaction. The impression smear of the lymph node revealed an increased number of medium to large lymphocytes compared to a normal lymph node. Also, there were scattered plasma cells and eosinophils present. During the exploratory, it was found that there were some other thickened intestines that could have been related. The extent of the mass and its anatomical location was not amenable to surgical excision, so humane euthanasia was performed.

At necropsy, the entire duodenum was dilated three times the normal width. There was also irregular firmness and thickening (up to 1cm) of the duodenum. Four 1-2cm wide irregular ulcers with mottled red/white beds were present in the mucosa of the gastroduodenal junction. The surrounding tissues were hyperplastic forming an umbilical rim. The remaining mucosa was rugose and mottled red/tan with abundant mucopurulent adherent material, seen in **Fig. 3.** The

duodenum-jejunum was markedly compressed by a firm/fibrous ovoid 10 cm mass that arose from the muscular tunics as seen in **Fig. 4 and 5.** When incised, many storiform fibromuscular bundles irregularly intersected one another. Throughout the intestines there were multifocally, poorly demarcated, brown-yellow, 2-10 mm wide caseous ovoid/round areas.

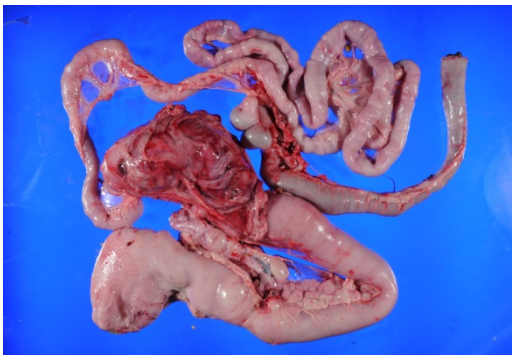

Fig.3 The gastrointestinal tract showing the ovoid 10cm long intramural mass.

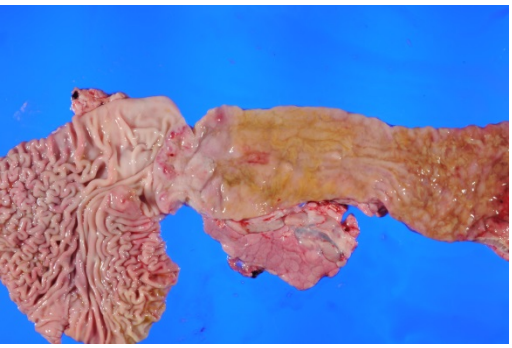

Fig.4 Ulcers with mottled red/white beds and thickened stomach wall and duodenum.

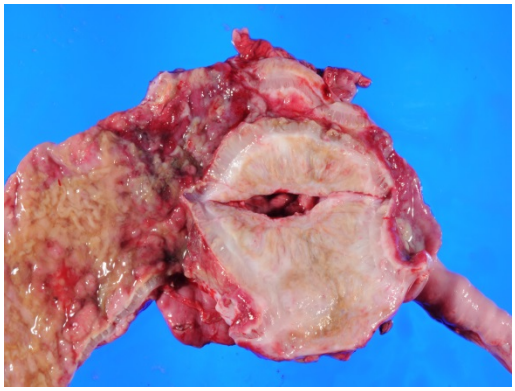

Fig.5 A cross section of the mass and the thickness of the muscular tunic layer of the duodenum.

Tissue samples were obtained and histopathology of the duodenum and proximal jejunum showed severe eosinophilic and pyogranulomatous inflammation and fibrosis that expanded the

muscular tunics and the submucosa of the gut. There were many coalescing nodules composed of multinucleated giant cells, eosinophils, large epithelioid macrophages, neutrophils that were surrounded by fibroblasts, lymphocytes, and plasmacytes. A Gormori's methenamine silver (GMS) stain showed occasional positive, irregular, 10-20 µm-wide hyphal-like structures, seen in **Fig 6.** This is suggestive of enteric pythiosis. There were surrounding fibrotic tissues that were composed of granulation tissue, ulceration, intense inflammation, epithelial regeneration, hemorrhage, and thrombosis. This also extended down to the mesentery. The liver was small and had diffuse plasmacytes, lymphocytes, and eosinophils in the perivenular stroma. All other organs were within normal histomorphological limits.

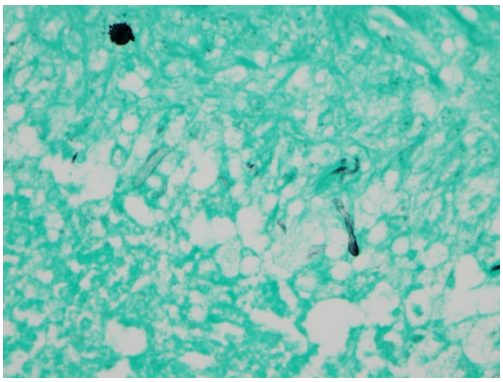

Fig.6. A section stained with Gomori methenamine silver showed irregular 10-20 µm-wide hyphal-like structures.

Treatment and Management:

As previously mentioned, *P. insidiosum* is an aquatic oomycete that is not a fungus. It is more closely related to brown algae. *P. insidiosum* lacks chitin in its cell wall and contains cellulose and beta glucan. Also, unlike fungi, *P. insidiosum* does not have ergosterol in its cell membrane, which is the target for anti-fungal pharmaceuticals.[4-6] Pythiosis can cause dermal or gastrointestinal lesions. Both forms must be aggressively treated, even though the prognosis is

still poor to grave.[1,2] There is a vaccine available that targets both exoantigens and the cytoplasmic antigens of *P. Insidiosum*. However, dogs do not have a strong clinical response to the vaccine. The reason for this poor response is unknown.[9] There are only four documented cases that show survival in dogs with the gastrointestinal form of pythiosis. These cases all included medical management, with or without radical surgical removal with 3-4 cm margins.[2] One of the patients was treated with a partial gastrectomy. The other patient was treated with a subtotal colectomy, along with itraconazole (11 mg/kg PO q24h), terbinafine (7 mg/kg PO q24h), and immunotherapy. The anti-*P insidiosum* vaccine is 3 doses. It should be injected immediately when diagnosed then every 14 days for the next 2 doses. The vaccine is 0.1 mL and should be administered intradermal.[10] The most recent surgical treatment was a Billroth II procedure. The fourth documented case was medical management only which included itraconazole, terbinafine and the agricultural fungicide mefenoxam. The mefenoxam was used at a dose of 4 mg/kg PO q12h of a 22% solution intended of agricultural applications. The combination of medical management is over a 6 to 18-month period. [2,6,15]

Case Outcome:

In this case report the cytology, histopathology, and blood work were diagnostic for pythiosis. Cytology revealed pyogranulomatous inflammation with a significant eosinophilic component. The Gomori's Methenamine Silver (GMS) stain showed the irregular hyphal-like structures. The histopathology showed a remarkable thickening of the muscular tunic layer in the duodenum. Furthermore, the patient was hypoproteinemic, anemic, and thrombocytopenic. To confirm the diagnosis three days after euthanasia, the ELISA test results were finalized and were positive for the detection of *P. insidiosum* antibodies.

The patient in this case is unique because of the history of the dog living in New Jersey and it still being winter when the clinical signs began. It has been reported that the infective stage, motile biflagellate zoospore, is not active during the winter months.[5,11] This is mainly due to low temperatures and the water and ground freezing. The northeastern states, especially New Jersey, are not common areas to find *Pythium insidiosum*. However, there is another case that was reported.[7-9] Unfortunately, the patient in this case report was not a candidate for wide surgical margins due to the distribution and progression of the disease.

The author would like to thank Wes Baumgartner, DVM, PhD, DACVP, for the cytology, histopathological, and necropsy images. Also, would like to thank Jennifer Gambino, DVM, DACVR for the diagnostic images.

References:

1. Austwick, P.K. and Copland, J.W., Swamp Cancer; *Nature,* 250, 84, 1974.
2. Dycus D., Fisher, C., Butler R., Surgical and Medical Treatment of Pyloric and Duodenal Pythiosis in a Dog. *Journal of American Animal Hospital Association:* Nov/Dec 2015. Vol.51, No.6, pp. 385-391.
3. Ettinger SJ and Feldman EC. *Textbook of Veterinary Internal Medicine*. 6th ed. Elsevier Saunders. 2005, 1219-1220,1246-1247
4. Gaastra W, Lipman LJA, De Cock Aw, et al. *Pythium insidiosum*: An overview. *Veterinary Microbiology* 2010; 140: 1-16.
5. Greene, Craig E. *Infectious Disease of the Dog and Cat.* 4the ed. Elsevier Saunders. St. Louis, MO: 2012 978-1-4160-6130-4, 677-681.
6. Grooters, A.M. Pythiosis, langenidiosis, zygomycosis in small animals. *Vet Clin North Am Small Animal Practice*. 2003; 33(4): 695-720
7. Grooters, A.M. Development and Evaluation of an Enzyme-Linked Immunosorbent Assay for the Serodianosis of Pythiosis in Dogs. *J Vet Intern Med.* 2022; 16: 142-146
8. Hensel, P et al. Immunotherapy for treatment of multicentric cutaneous pythiosis in a dog. *J Am Vet Med Assoc*. 2003; 223(2): 215-218
9. Jaeger G.H., Rotstein D.S. and Law J.M. Prostatic Pythiosis in a Dog. J Vet Intern Med. 2002; 16 (5): 598-602.
10. Mendoza L, Mandy W, Glass R. An improved *Pythium insidiosum*-vaccine formulations with enhanced immunotherapeutic properties in horses and dogs with pythiosos. Vaccine. 2003; 21:2791-2804

11. Mendoza, L., Hernandez, F, and Ajello, L, Life cycle of the human and animal oomycete pathogen *Pythium insidiosum, J. Clin. Microbiol*., 31, 2967, 1993.
12. Mendoza, L., Ajello, . and McGinnis, M.R., Infection caused by Oomycetous pathogen *Pythium insidiosum, J, Myco. Med.,* 6 151, 1996
13. Thrall, Donald. *Textbook of Veterinary Diagnostic Radiology*. 6e. St. Louis: Elsevier Saunders, 2013. print.
14. "Pythiosis Insidiosum." *Pan American Veterinary Laboratories*. Web. 4 Oct. 2015
15. Schmiedt CW, Stratton-Phelps M, Torres BT et al. Treatment of intestinal pythiosis in a dog with combination of marginal excision, chemotherapy, and immunotherapy. *J Am Vet Met Assoc* 2012;241: 358-363.