An abdominal mass in a young dog

Paola Cazzini, Kaori Sakamoto, Shawn Zimmerman, Bridget Garner
Department of Pathology, College of Veterinary Medicine,
University of Georgia, Athens, GA, USA

Signalment:

20-month-old, female spayed, mixed breed dog

History:

The dog presented at the UGA veterinary teaching hospital with a 2 week history of vomiting that progressively increased in frequency.

Clinical findings:

On presentation, the dog was quiet, alert, and responsive, and had a body condition score of 4/9. A firm mass was palpated in the mid- to caudal abdomen.

Diagnostic findings:

Hematologic analysis revealed leukocytosis, characterized by lymphocytosis, monocytosis, eosinophilia, and basophilia. No other significant changes were present.

Radiologic examination of the abdomen was performed. The stomach was moderately distended with gas and fluid. A lobulated, soft tissue opacity was seen in the mid-cranial abdomen and was suspected to be small intestinal in origin. A small volume of free abdominal fluid was also suspected due to mild, diffuse, decreased serosal detail.

Ultrasonographic examination of the abdomen was performed. A focal segment of jejunum was thickened and multiple mesenteric lymph nodes were enlarged. A scant volume of free abdominal fluid was also identified. Aspiration of the abdominal fluid (Table 1) and fine needle aspirates (FNA) of the intestinal mass (Figures 1-4) were performed.

Table 1

TEST	RESULT	UNITS
Color	beige	
Transparency	opaque	
Nucleated Cells	53.6	x 10^3/µl
Protein	3.9	g/dl
Differential cell count: • 95% non-degenerate neutrophils, • 5% eosinophils, small mature lymphocytes, and macrophages		

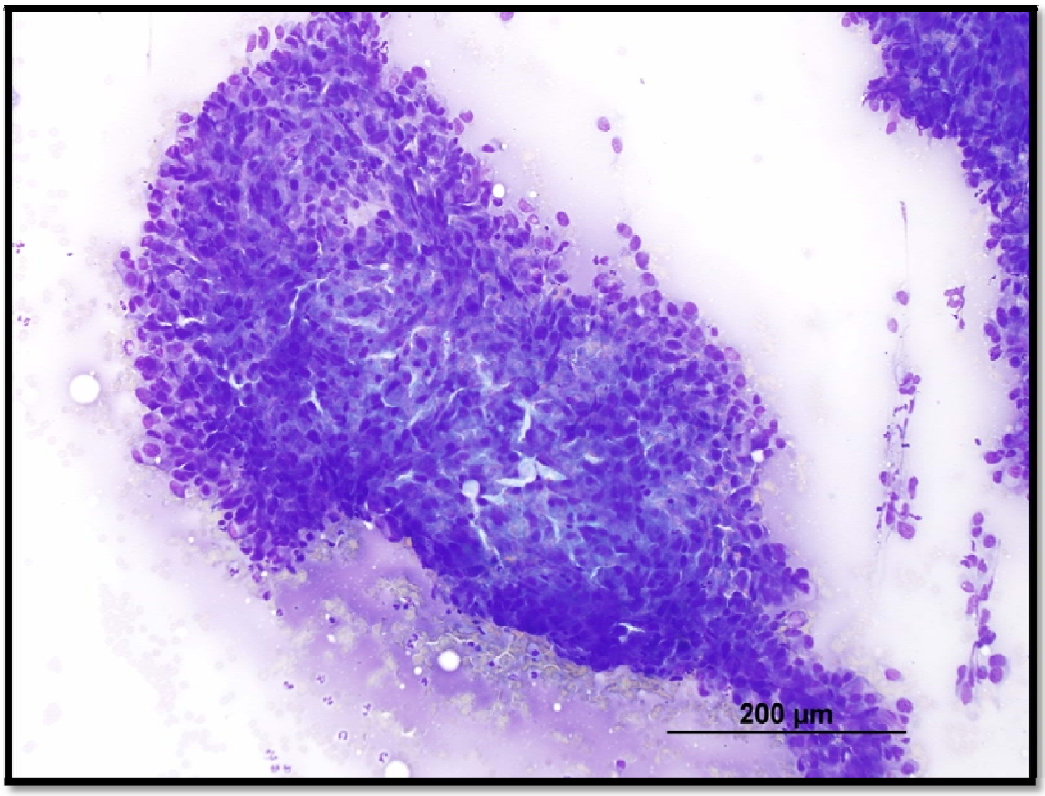

Figure 1. **FNA of the abdominal mass, Wright-Leishman-stain**

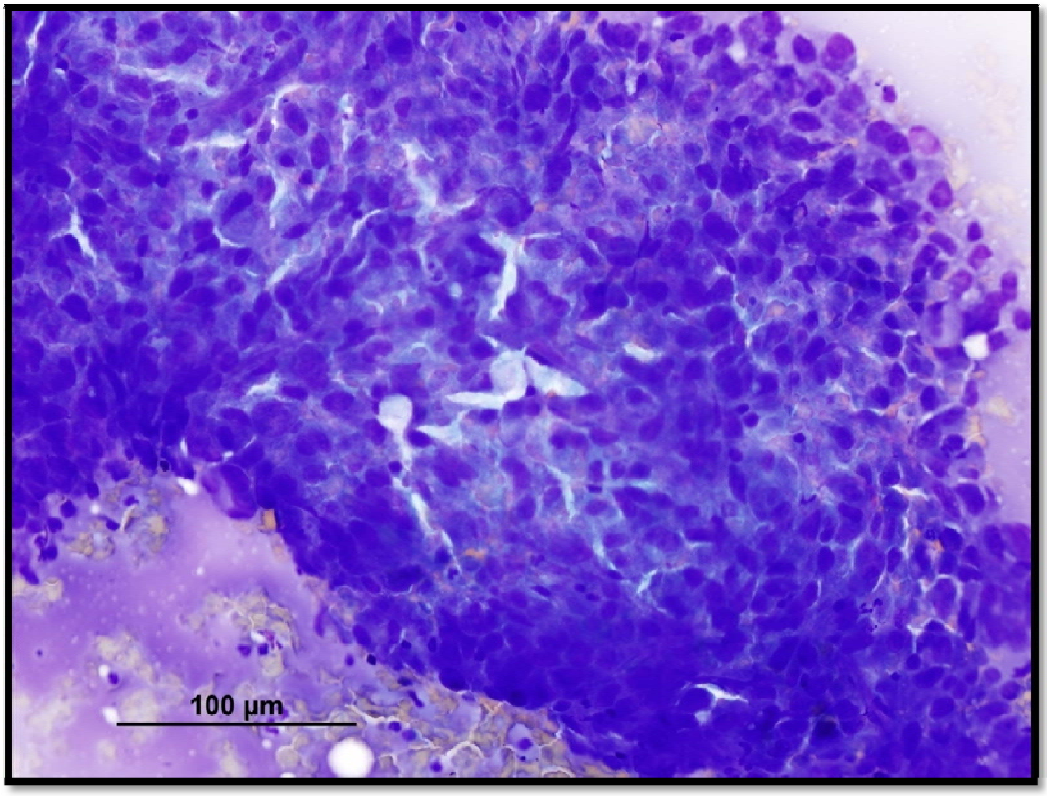

Figure 2. **Higher magnification of figure 1, Wright-Leishman-stain**

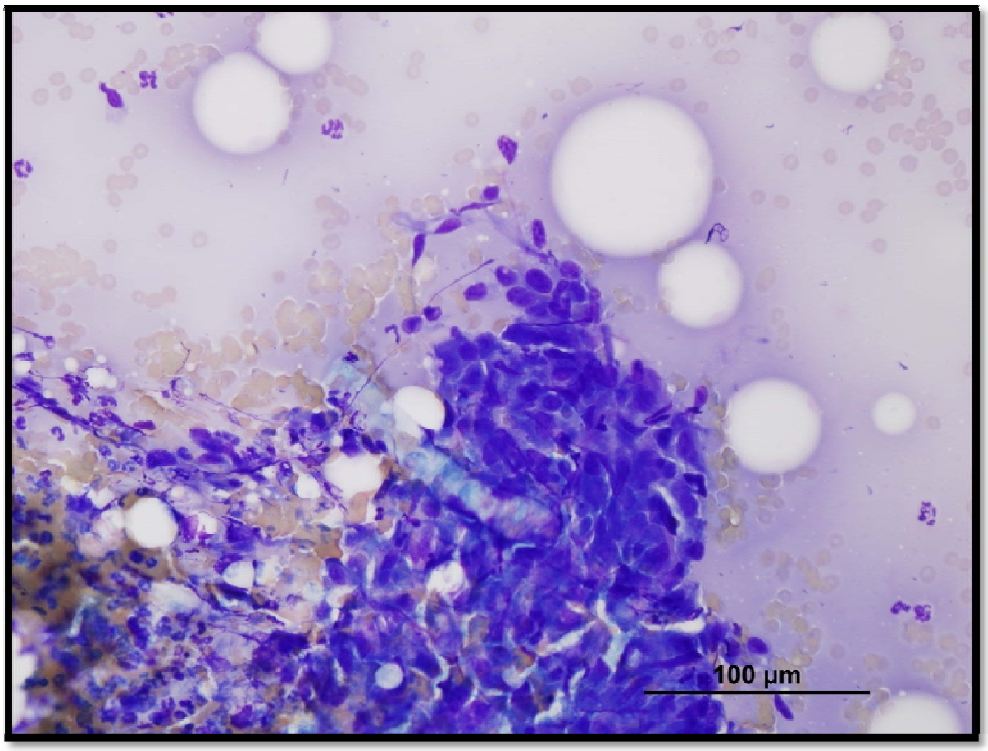

Figure 3. **FNA of the abdominal mass, Wright-Leishman-stain**

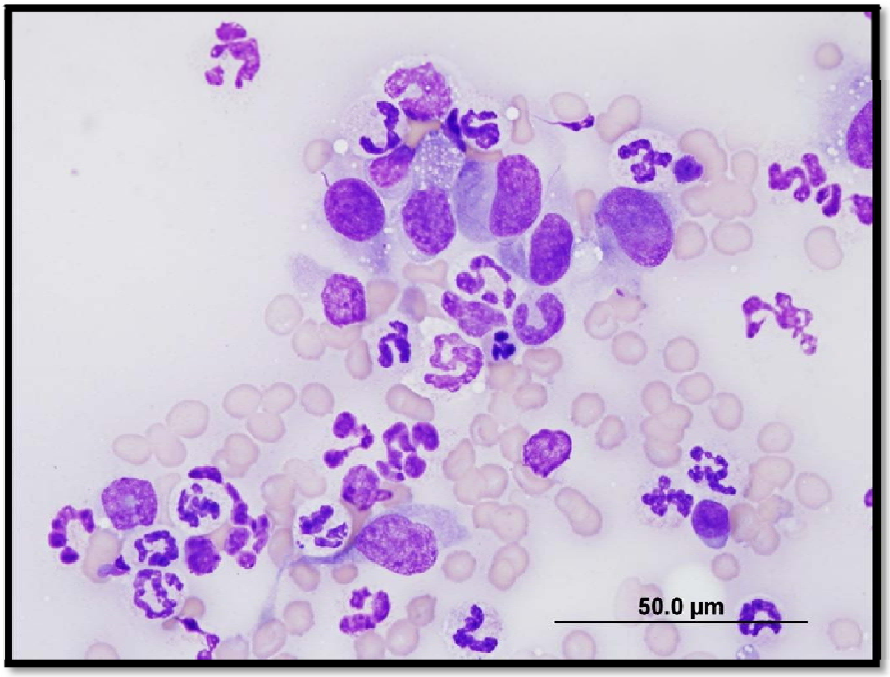

Figure 4. **FNA of the abdominal mass, Wright-Leishman-stain**

Questions:
What are your differential diagnoses?
What stains can you use to support your hypothesis?
What additional testing do you need so confirm your hypothesis?

Further investigations and discussion:

Cytology (Figures 1-4)**:**

The slides are highly cellular and a heterogeneous population of cells is present. Large numbers of mildly degenerate, segmented neutrophils, foamy macrophages, and lesser numbers of epithelioid macrophages, multinucleated giant cells, and eosinophils are present. These leukocytes are admixed with reactive fibroblasts. Within the thick cellular areas, there are low numbers of poorly to negatively staining, branching, poorly-septate hyphal elements with occasional bulbous ends.

Opinion: Pyogranulomatous inflammation with an eosinophilic component and intralesional hyphal elements, most likely *Pythium* sp. or *Lagenidium* sp.

Special stains:

Gomori-Grocott methenamine silver (GMS) stain was applied to an unstained cytological smear (Figure 5) and the hyphal elements were positively staining.

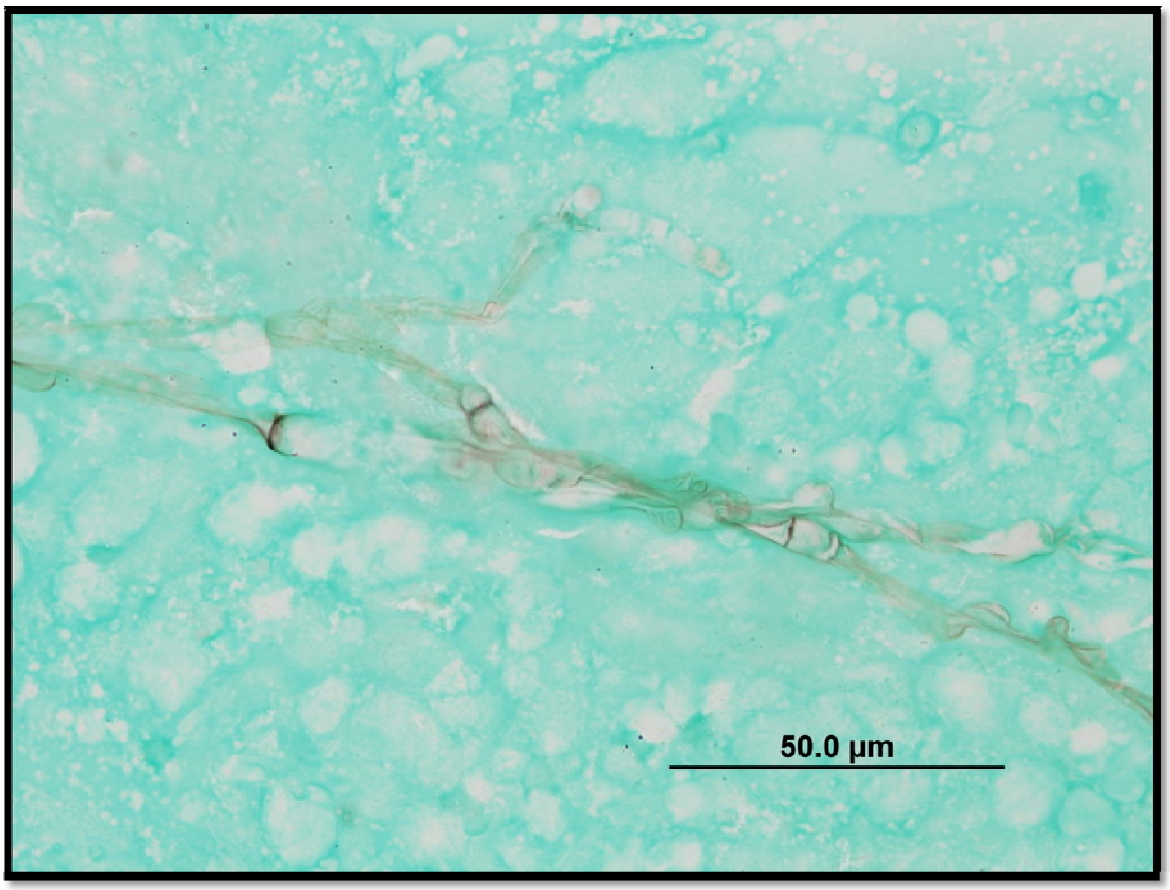

Figure 5. FNA of the abdominal mass, GMS stain

Additional diagnostics:

A serum sample from the dog was sent to Louisiana State University to perform ELISA serology for anti-*Pythium insidiosum* antibodies and the result was positive.
Aerobic bacterial culture from the intestinal mass was negative.

Case conclusion:

Surgical excision with wide margins of the affected intestinal tract and of 2 enlarged mesenteric lymph nodes was performed. The lumen of the affected intestinal segment was severely reduced and the intestinal walls were expanded by severe granulomatous inflammation and fibrosis with areas of necrosis. Intralesional hyphae similar to the ones described in the cytology specimen were observed. Reactive lymphoid hyperplasia with mild, focal, eosinophilic lymphadenitis was diagnosed in the mesenteric lymph nodes. The dog recovered uneventfully from surgery, and received three doses of a *Pythium insidiosum*-vaccine, used as an immunotherapy, and a 12-week therapy with terbinafine. At 18 months post-surgery, the dog had no signs of recurrence.

Discussion:

Pythium insidiosum (*P. insidiosum*) is an Oomycete. Although it is microscopically similar to fungi, this organism is more closely related to diatomeae and algae.[1] *P. insidiosum* can be found in stagnant waters, and more cases are seen after heavy rain or floods. This microorganism can develop zoospores, single nucleated cells without cellular walls that can swim with the help of flagella.[1] Zoospores are the infective propagules that show marked chemotaxis for damaged plants or injured animal tissues.[1] Upon contact and adhesion to injured parts of animals or plants, the zoospores encyst.[1] Stimulated by host body temperature, they develop a germ tube (hypha) that extends and infiltrates the surrounding tissues.[1] Most cases of pythiosis have been reported in humans, dogs, and horses, but sporadic cases in other animals have been reported.[1] Pythiosis is an infrequent, non-transmissible disease normally found in tropical, subtropical, and temperate regions and is considered an endemic disease in Thailand [1]. The location of the lesion is directly related to the parts of the body in direct contact with stagnant water. In horses, lesions are most commonly seen on the legs and ventral parts of the abdomen, while in dogs, both cutaneous and intestinal lesions, probably due to ingestion of infected water, can occur.[1,2] Among the cases of pythiosis in canines, there is a higher prevalence in young, large-breed dogs.[3,4] Common hematological and biochemical findings include anemia, eosinophilia, hypoalbuminemia, and hyperglobulinemia.[3] Hypocalcemia can be seen, normally attributed to the hypoalbuminemia, while hypercalcemia associated with *Pythium sp*. has also been reported.[4] The clinical symptoms associated with intestinal pythiosis include vomiting, weight loss, and intermittent diarrhea. Abdominal masses can also be palpated upon physical examination.[3,4]

<u>Diagnostics:</u>

In contrast with other similar microorganisms, *P. insidiosum* hyphae do not stain well in Hematoxyn and Eosin (H&E) or Romanovsky stains. Regardless, the presence of a granulomatous inflammation with an eosinophilic component should lead to the inclusion of pythiosis in the differential list.[1] Periodic acid-Schiff (PAS) and, preferably, Gomori-Grocott methenamine silver (GMS) staining, can be used to better identify this microorganism, although a longer exposure period of the specimen to the stain is recommended. Most *P. insidiosum* strains develop slender hyphae measuring 2.5 – 12 µm width, while some cryptic strains have

broader hyphae that can measure 8-20 µm or up to 30 µm with hyphal swelling [5]. Infections caused by other oomycetes, such as *Lagenidium* sp., and zygomycetes such as *Conidiobolus coronatus,* can be very similar to pythiosis.[4] Other diagnostic methods, such as culture, immunohistochemistry, serological tests, and PCR amplification of DNA extracted from infected tissues, are necessary to confirm the diagnosis.[2,4] In this case, the diagnosis was confirmed by serological testing.

Treatment:

Pythiosis can be difficult to treat and is often a fatal disease, especially if affecting the gastro-intestinal tract. Surgical excision with wide margins is recommended and remains the treatment of choice. *Pythium* sp. lacks ergosterol in its cytoplasmic membrane; for this reason antimycotic agents that specifically target ergosterol are poorly or not effective on *P. insidiosum*.[1] Immunotherapy for the treatment of pythiosis has been successfully developed in horses and dogs.[6,7] The most likely explanation for the mechanism by which this works is that a Th2 to Th1 switch in the immune response occurs.[1]

References:

1. Gaastra W, Lipman LJ, De Cock AW*, et al.* Pythium insidiosum: an overview. Vet Microbiol 2010;146:1-16.
2. Pereira DI, Schild AL, Motta MA, Fighera RA, Sallis ES, Marcolongo-Pereira C. Cutaneous and gastrointestinal pythiosis in a dog in Brazil. Vet Res Commun 2010;34:301-306.
3. Berryessa NA, Marks SL, Pesavento PA*, et al.* Gastrointestinal pythiosis in 10 dogs from California. J Vet Intern Med 2008;22:1065-1069.
4. LeBlanc CJ, Echandi RL, Moore RR, Souza C, Grooters AM. Hypercalcemia associated with gastric pythiosis in a dog. Vet Clin Pathol 2008;37:115-120.
5. Mendoza L, Vilela R. What is your diagnosis? Pythium insidiosum or Lagenidium sp.? Vet Clin Pathol 2009;38:273; author reply 273-274.
6. Hensel P, Greene CE, Medleau L, Latimer KS, Mendoza L. Immunotherapy for treatment of multicentric cutaneous pythiosis in a dog. J Am Vet Med Assoc 2003;223:215-218, 197.
7. Mendoza L, Mandy W, Glass R. An improved Pythium insidiosum-vaccine formulation with enhanced immunotherapeutic properties in horses and dogs with pythiosis. Vaccine 2003;21:2797-2804.