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Diagnostic Exercise
From the Latin Comparative Pathology Group and the Davis-Thompson Foundation:

Gastric pythiosis in a dog

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Clinical History:

A 1-year-old, female intact, mixed breed dog had eaten a live duck prior to the onset of chronic vomiting and hyporexia, which progressed to severe weight loss. Vomiting improved with the administration of antacids, gastrointestinal protectants, anti-emetics, antibiotics, and appetite stimulants. These clinical signs returned once medical management was stopped. Endoscopic gastrointestinal biopsies showed a lymphoplasmacytic gastritis and enteritis. As the dog continued to decline, an exploratory laparotomy was done, revealing a severely enlarged, firm stomach that bled when touched. No gastrointestinal foreign body was found. The patient was humanely euthanatized.

Gross Findings:

The dog was in poor body condition. A 13 cm surgical scar from the laparotomy was over ventral midline. The greater omentum was adhered to multiple sections of the gastrointestinal tract. The stomach was markedly enlarged, thick, firm, and discolored red, brown, to gray (Fig. 1). On the cut section, the gastric walls were thickened up to 2.75 cm. The thick and discolored areas encompassed parts of the cardia, the entire fundus, and pyloric antrum. Within the lumen was a red to brown fluid that contained mucoid material. Samples of stomach were submitted for histopathology.

Follow-up questions:

- *Histological description?*
- *Additional histochemical stains?*
- *Morphologic diagnosis?*
- *Differential diagnoses?*

**The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation (DTF). These exercises are contributed by members and non-members from any country of residence. Consider submitting an exercise! A final document containing this material with answers and a brief discussion will be posted on the DTF website (https://davist- hompsonfoundation.org/diagnostic-exercise/).*

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ANSWERS

Histological Description:

Transmurally expanding and markedly effacing the mucosa, submucosa, muscularis, and serosa of the gastric wall are large numbers of eosinophils, macrophages, neutrophils, lymphocytes, plasma cells, and rare multinucleated giant cells. The inflammatory infiltrate is multifocally nodular, forming granulomas (Fig. 2) encircled by reactive fibroblasts and abundant collagen (fibrosis), and filled with degranulated material, karyorrhectic debris, and numerous 3-5 µm diameter, irregular fungal-like hyphae with non-parallel walls and occasional branching (Fig. 3; confirmed via GMS, Fig. 4). Multifocally throughout the gastric wall are large areas of edema, hemorrhage, necrosis, fibrosis, and karyorrhectic debris. Inflammatory cells are infiltrating vessels (Fig 5.). Vessels are hypertrophied and have increased eosinophilic material within necrotic tunica media (fibrinoid necrosis). There are multifocal thrombosed vessels. The gastric lamina propria has pale, eosinophilic staining with minimal cells (necrosis) and fibrosis and is infiltrated by lymphocytes, plasma cells, erythrocytes, macrophages filled with yellow to brown intracytoplasmic granules (hemosiderin), and clumps of basophilic rods (bacteria). Gastric glands are multifocally effaced and replaced with hemorrhage and inflammatory cells. The gastric mucosa is denuded, pale, eosinophilic, and minimally cellular (autolysis).

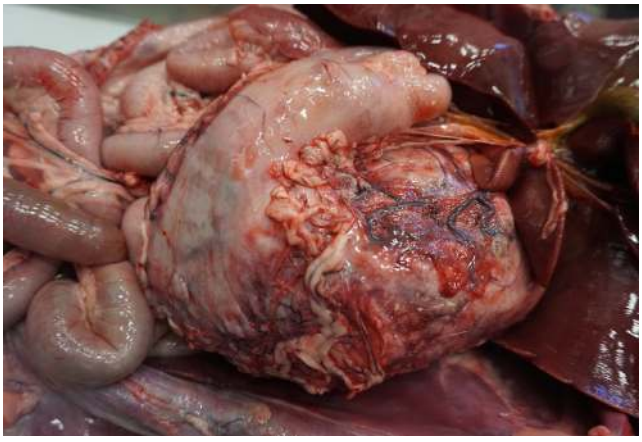

Figure 1. Approximately 75% of the stomach is enlarged and discolored red, brown, to gray throughout parts of the cardia, the entire fundus, pyloric antrum, with the greater omentum adhered to the ventral surface of the gastric body. The gastric vessels traveling down the lesser curvature to the ventral aspect of the fundus are dilated, torturous, and congested.

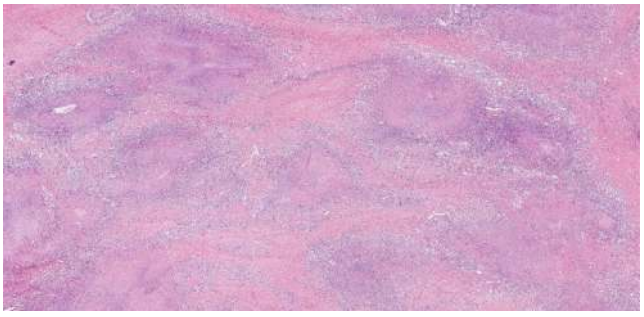

Figure 2. Multifocal to coalescing eosinophilic granulomas and fibrosis expand the gastric wall.

Morphologic Diagnosis:

Stomach. Severe, chronic, transmural, pyogranulomatous and eosinophilic gastritis with vasculitis and intralesional fungal-like hyphae

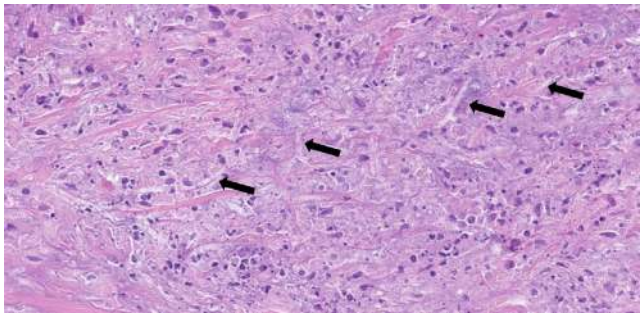

Figure 3. Poorly demarcated, wide, clear, occasionally branching hyphae (arrows) are centered in an area of necrosis.

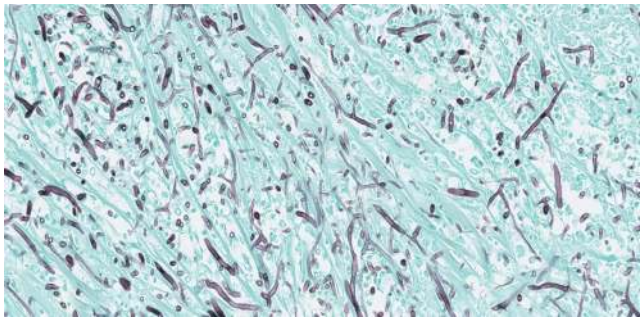

Figure 4. Broad, irregular, black-staining filaments with occasional branching congregate within granulomas and areas of necrosis. Gomori's methenamine silver (GMS) stain.

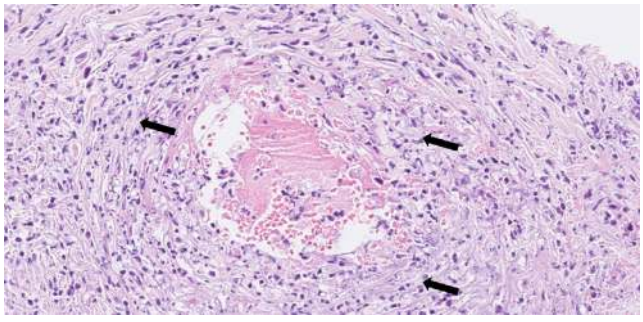

Figure 5. The hypertrophied gastric vessel is surrounded by reactive fibroblasts, collagen, and clear, poorly demarcated, irregular hyphae (arrows) with rare septation. The tunica media is expanded by a pale eosinophilic, fibrillar to amorphous material and karyorrhectic debris (fibrinoid necrosis).

Cause:

Pythium insidiosum

Pathogenesis:

Oospores on aquatic plants produce sporangia —> asexual reproduction results in motile zoospores —> differentiation into infective biflagellate secondary type zoospores —> infective zoospores released in warm/wet weather—> zoospores swim directed by chemotaxis to damaged skin and/or mucosa and encyst—> encysted zoospores secrete sticky glycoproteins to increase adherence of additional zoospores to damaged tissue —> germ tube (hypha) formation stimulated by host body temperature —> entry into tissue and blood vessels—> proteases secreted and hyphae elongate to perpetuate host tissue invasion —> granulomatous reaction

Comments:

Pythium insidiosum is an aquatic oomycete of the kingdom Stramenopila, class Oomycetes, order Pythiales, and family Pythiaceae (5). As opposed to fungi, *Pythium* spp. do not contain chitin within their cell walls or utilize ergosterol in their cell membranes, making *Pythium* more closely related to algae than fungi (4, 5). This organism is typically found in fresh bodies of water and soil in warm, wet conditions, and infections usually develop after a heavy rain or flooding event (4, 8). In this case, a history of eating a live duck suggests that the patient was around pond water or other conditions favorable for *P. insidiosum* transmission.

The zoospores of *P. insidiosum* show chemotaxis towards plant and animal tissues; however, transmission from one infected animal to another has yet to be documented (4, 8). Strong chemotaxis is exhibited towards damaged skin and mucosa, where adhesion and infiltration can occur even within immunocompetent animals (4, 8). Transmission usually occurs when an animal is in contact with water containing *P. insidiosum* zoospores or has a wound that contacts hyphae (4). While not explicitly documented, there is a concern of insects transmitting this organism, as *P. insidiosum* has been isolated from a Southern house mosquito (*Culex quinquefasciatus*) in India (9).

Pythium spp. have been documented to infect a wide variety of species, and the clinical signs vary based on the species infected and where lesions develop (Table 1). The highest incidence of infection is seen in young, large breed dogs and horses, which do not have an age, sex, or breed predilection, and the most common lesion sites are cutaneous and gastrointestinal (4, 5). One case of disseminated pythiosis has been reported in a dog, and it still remains rare for both cutaneous and gastrointestinal pythiosis to develop concurrently (5). Cutaneous

Table 1. Documented distributions of species and lesions associated with *Pythium* spp. (1, 4, 6, 7).

Species	Lesion Site
Horses	Cutaneous, mammary glands, nasal cavity
Dogs	Alimentary, cutaneous, respiratory
Cats	Alimentary, cutaneous, nasal cavity, retrobulbar
Donkeys	Cutaneous
Mules	Cutaneous
Donkeys	Cutaneous
Cattle	Cutaneous
Goats	Cutaneous
Sheep	Nasal cavity, cutaneous, alimentary
Camels (*Camelus dromedarius*) (*Camelus bactrianus*)	 Cutaneous, muscle, lymph node, salivary gland Alimentary
Jaguar (*Panthera onca*)	Pulmonary
Tiger (*Panthera tigris tigris*)	Alimentary
Spectacled bears (*Tremarctos oronatus*)	Cutaneous, preputial glands, alimentary
Ostrich	Alimentary
White-faced ibis (*Plegadis chihi*)	Cutaneous
Porpoise (*Phocoena phocoena*)	Cutaneous
Humans	Cutaneous, vascular, ocular, disseminated
Rabbits	Experimentally induced only

pythiosis manifests as non-healing wounds or tumor-like lesions that develop at the site of infection, which are usually the legs, tail, and face, and may cause pruritus in the affected areas (4, 5). Like equine habronemiasis, cutaneous pythiosis in horses frequently present with "kunkers," which are small, stoney masses that develop due to eosinophil degranulation, and are not found in most other infected species (4).

Animals that develop gastrointestinal pythiosis, which is mostly seen in dogs, may have a palpable abdominal mass, vomiting, weight loss, and diarrhea (4, 5). These are non-specific signs that were seen in this patient but can also be seen with various other gastrointestinal diseases. Prior to necropsy, additional differential diagnoses considered by the submitting clinician based on laparotomy findings included neoplasia, fungal infection, or gastric leiomyositis. Common gross findings in dogs with gastrointestinal pythiosis include segmental, transmural thickening of affected gastrointestinal tissues, and due to their proximity, infiltration of the pancreas, mesenteric lymph nodes, bile duct, uterus, and prostate have been documented (5). Sequelae to gastrointestinal invasion of pythiosis seen grossly include gastric or intestinal lumen diameter reduction, obstruction, bowel ischemia, infarction, perforation, and hemoabdomen (5).

Histologically, pythiosis is commonly identified by tissue samples containing eosinophilic granulomatous to pyogranulomatous inflammation with broad, clear, occasionally branching hyphae within granulomas, multinucleated giant cells, or areas of necrosis when stained with hematoxylin and eosin (H&E) (3, 5). To better visualize the hyphae, tissue sections can be stained with Gomori's methenamine silver (GMS), where the hyphae branches stain black on a green background (5). Histopathologic findings of pythiosis, lagenidiosis, and zygomycosis have a high degree of resemblance, so histology alone cannot be used to definitively diagnose these conditions (5). The histological findings need to be interpreted alongside the gross findings because knowledge regarding predilection sites of these organisms can help in prioritizing differentials. In canines, pythiosis commonly occurs in the pyloric antrum, proximal duodenum, and ileocolic junction (5). Incidences of lagenidiosis have been reported in dogs; however, none of these reports include dogs with gastrointestinal lesions (5). Dogs with zygomycosis typically have lesions within the nasopharyngeal region, and gastrointestinal lesions have only been noted in cats (5). Additional diagnostics may be warranted to better classify a patient's prognosis, especially with cutaneous cases, since zygomycosis may respond better to treatment than lagenidiosis or pythiosis (5).

Other mechanisms of diagnosing pythiosis include wet mount examination, culture, serological assays, and PCR (4, 5). For all diagnostic methods, adequate tissue sampling is required for the detection and visualization of *P. insidiosum*, for this organism tends to be located deep within affected tissues (5). While additional diagnoses going beyond histology were not attempted for this case, the diagnosis of *P. insidiosum* is based off the morphology of the hyphae, the presence of eosinophilic and granulomatous inflammation, the host species, and the gastric localization.

Early diagnosis is imperative to providing curative treatment, whereas chronically affected individuals have a poor prognosis (4, 8). Surgical debulking and removal of masses caused by pythiosis can be unrewarding due to its high reoccurrence rate, and the use of anti-mycotic agents are often unsuccessful due to the lack of ergosterol in the cell membrane of *P. insidiosum*, which is the common target for most anti-mycotic drugs (4). The use of immunotherapy in addition to anti-mycotic drugs and surgical removal may be the most currently effective treatment for this condition (4).

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