Diagnostic Exercise

From The Davis-Thompson Foundation*

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Answer Sheet

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Clinical history: An 18-month-old, intact female Australian Shepherd mixed breed dog with a several-week history of appetite loss and anorexia was submitted for post-mortem examination.

Necropsy Findings: The abdomen was moderately distended by 500 ml of reddish brown opaque fluid. A 14.0 x 5.6 x 4.0 cm, 1.0 kg, dark gray to red, soft mass with irregular smooth serosal surface expanded the mesentery and firmly attached to a 15.0 cm long segment of the proximal jejunum (Figures 1A-B). On cut surface of the mass, there were multiple irregular cavities filled with red to dark red viscous opaque fluid. The wall of the affected segment of jejunum had variable thickness, with red to gray roughened mucosal surface (Figure 1C). Elsewhere in the mesentery there were multiple 1.0 x 1.5 x 0.6 cm, round, black, soft masses (mesenteric lymph nodes) (Figure 1C).

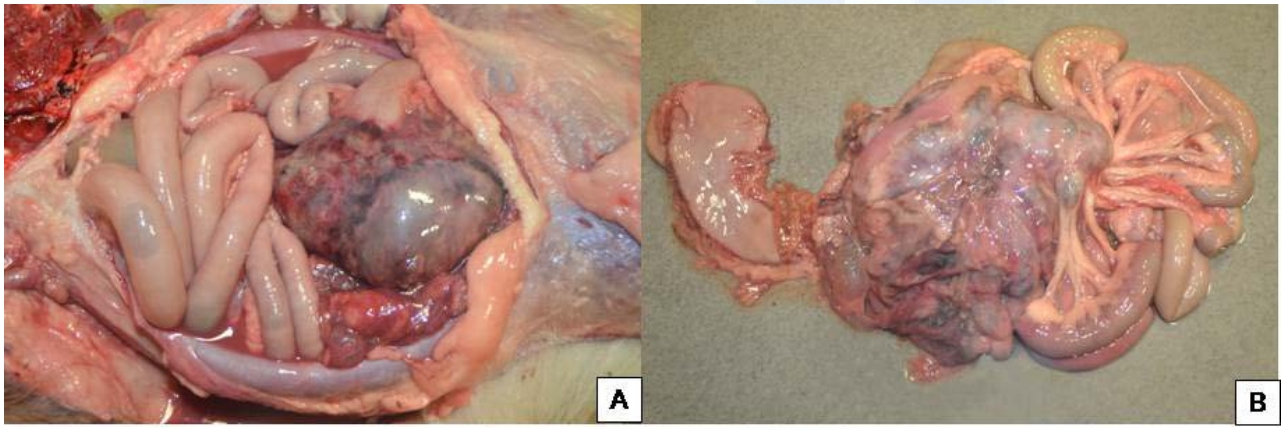

Figure 1 - A and B

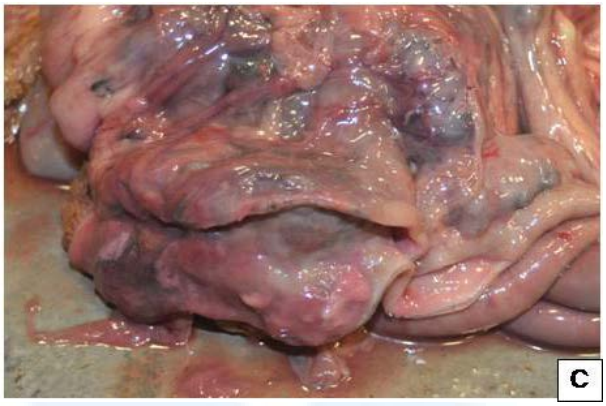

Figure 1 - C

Microscopic findings: Jejunum and Mesenteric lymph nodes: Effacing transmurally a segment of the proximal jejunum were multifocal to coalescing granulomas with a core of necrotic debris and variable numbers of degenerate neutrophils surrounded by epithelioid macrophages, multinucleate (primarily Langhans-type) giant cells, lymphocytes, and plasma cells (Figure 2A). Abundant mature fibrosis surrounded and separated the multiple coalescing inflammatory foci. Alterations were much more extensive throughout the outer layer of the intestine and the adjacent mesentery, where they formed the large mass noted at necropsy. The grossly noted round, black, soft masses throughout the remaining mesentery were histologically confirmed to be lymph nodes of which the normal architecture was extensively (up to 90% in the sections examined) replaced by coalescing granulomatous inflammation similar to that seen in the jejunum and adjacent mesentery (Figure 2B). Abundant poorly discernible, Gomori's methenamine silver (GMS)-positive hyphae were present within these inflammatory foci, both extracellularly among the central necrotic debris and intracellularly in macrophages and giant cells (Figures 2B-C). Hyphae were 4-10 µm wide and had non-parallel walls, rare septations, and non-dichotomous branching (Figure 2D).

Morphologic Diagnoses: Abdominal cavity: Peritoneal effusion, moderate; Jejunum: Jejunitis, granulomatous, segmental, transmural, chronic, marked, with intralesional hyphae; Mesentery and Mesenteric lymph nodes: Lymphadenitis, granulomatous, multifocal to coalescing, chronic, marked to severe, with intralesional hyphae.

Diagnosis: Intestinal and Mesenteric Pythiosis.

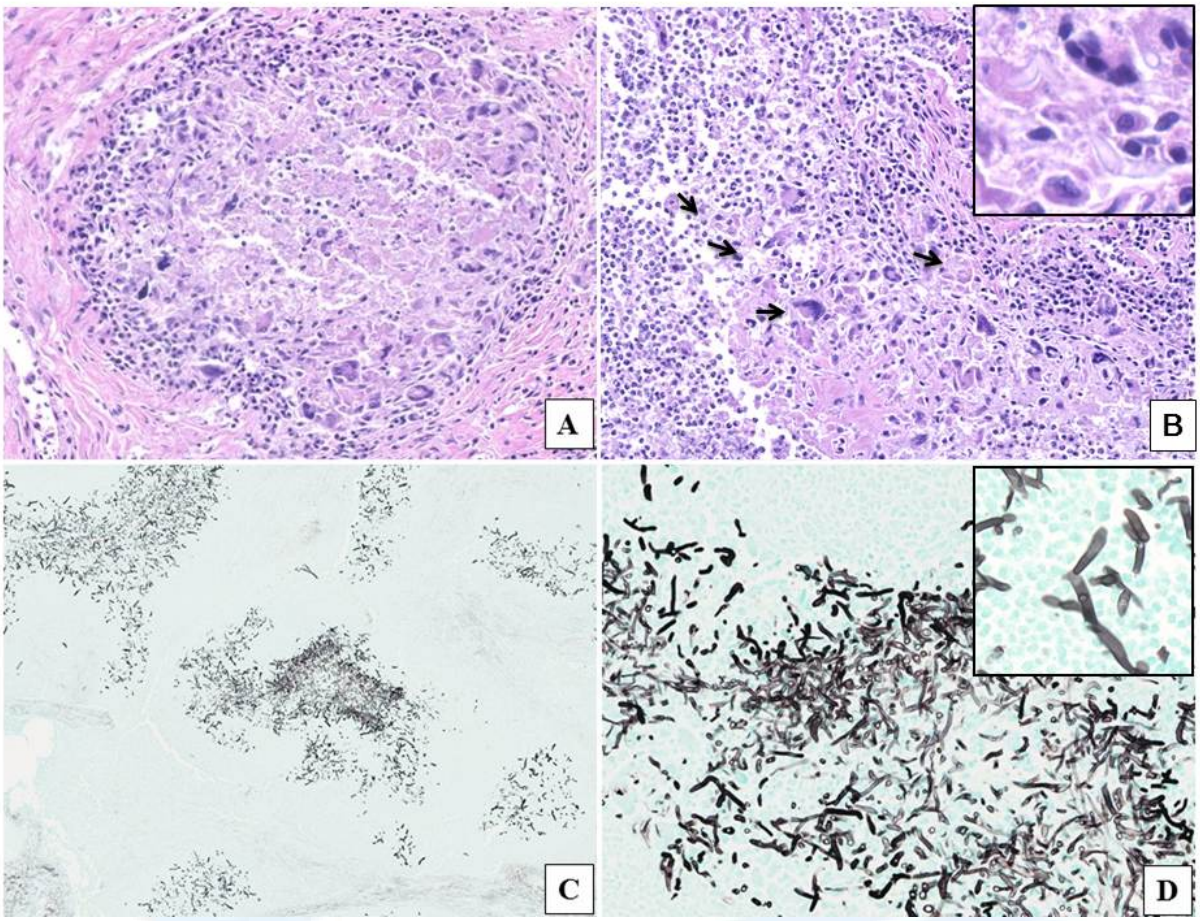

Figure 2. Dog, Intestinal and Mesenteric Pythiosis. (A) Jejunum. A granuloma focally disrupts the normal intestinal architecture, characterized by a core of necrosis rimmed by epithelioid macrophages, multinucleate giant cells, lymphocytes, and plasma cells. H&E, 20x. (B) Mesenteric lymph node. A similar granuloma effaces the nodal parenchyma containing multiple poorly discernible hyphae-like structures (arrows). H&E, 20x; Inset, 40x. (C) Mesenteric lymph node. Numerous hyphae are present within the coalescing granulomas. Gomori's methenamine silver stain (GMS), 10x. (D) Higher magnification of (C) highlighting the intralesional 4-10 µm wide hyphae with non-parallel walls, rare septations, and non-dichotomous branching. GMS, 20x; Inset, 40x.

Discussion: Pythiosis is a chronic granulomatous to pyogranulomatous non-transmissible disease often reported in wetlands and swampy areas of tropical, subtropical, and temperate regions.[9,11,14] Confirmed cases of pythiosis occur most commonly in North America in the Gulf Coast region but have also been reported as far north as New Jersey, Virginia, Kentucky, southern Indiana and southern Illinois, and as far west as Arizona and California.[2,5,11] *Pythium insidiosum*, an aquatic pathogen (class Oomycetes; kingdom Stramenopila), is now recognized as the etiologic agent of pythiosis in mammals, affecting horses, dogs, cattle, cats, sheep, camels, humans, and other species.[7,10,13,17] Two

forms of pythiosis have been described: cutaneous and gastrointestinal.[15] The cutaneous form is preponderant in horses whereas the gastrointestinal type occurs more frequently in dogs.[11]

Canine pythiosis is most often seen in young, large-breed, male, outdoor working dogs, with Labrador Retrievers and German Shepherds possibly predisposed to the disease.[11] Epidemiologic records indicate that the affected dogs are referred to a veterinarian mostly in the fall, winter, and early spring.[2] *P. insidiosum* shows high preference towards damaged skin and gastrointestinal mucosa. The encysted zoospores in the aquatic environment, where they use a plant substrate for the asexual phase of the life cycle, will develop a germ tube and later hyphae that are invasive, stimulating the pathogen to spread. Outbreaks of pythiosis often occur after heavy rains or floods, which expand the areas contaminated with *P. insidiosum.*[2,4] Exposure of damaged skin to or ingestion of water containing *P. insidiosum* should therefore be avoided to decrease the risk of infection.[2]

The clinical signs of the gastrointestinal form include vomiting, intermittent diarrhea, progressive weight loss, hematochezia, abdominal pain, and abdominal masses.[3,5] At necropsy, severe segmental thickening is seen most commonly in the stomach, duodenum, and ileocolic junction.[7] *P. insidiosum* infection of the gastrointestinal tract can be transmural but lesions are mainly located in the submucosa and muscularis propria, extending into the serosal surface. The infection may also extend to adjacent tissues, such as pancreas and mesenteric lymph nodes, leading to peritonitis, omental adhesions and mesenteric lymph node enlargement. Frequently there is mucosal ulceration as well.[4]

The cutaneous form of canine pythiosis involves the extremities, tail, head, ventral neck, perineum, or medial thigh.[7] The lesions are characterized by non-healing wounds, ulcerated nodules and draining tracts in the skin, affecting primarily the dermis and subcutis. The diagnosis of pythiosis might therefore fail if deeper tissues are not adequately represented in biopsies.[7]

Cytologic aspirates reveal pyogranulomatous, suppurative or eosinophilic inflammation.[10] Histopathologic findings are characterized by similar inflammation occasionally accompanied by fibrosis. Inflammatory foci consist of central necrosis surrounded by epithelioid macrophages, multinucleate giant cells, neutrophils, and eosinophils.[10] Intralesional hyphae do not stain well with Romanovsky and Hematoxylin & Eosin (H&E) stains and are therefore often identified as clear spaces ('hyphal ghosts') surrounded by a narrow band of eosinophilic material within the center of the granulomatous inflammation. Special stains such as Gomori's methenamine silver (GMS) elevate the contrast and highlight the morphology of the hyphae.[11] *P. insidiosum* pathogens have slender hyphae, measuring 2-7 µm in width, with rare septation, occasional branching, and non-parallel walls.[11]

Because the definitive diagnosis of pythiosis can be a challenge due to similarities with lagenidiosis and zygomycosis and possible unsuccessful culture of the organism, additional diagnostic methods have been developed to circumvent these issues.[5] A soluble mycelial antigen-based enzyme-linked immunosorbent assay (ELISA) for detection of anti-*P. insidiosum* antibodies, *P. insidiosum*-specific PCR assays applicable to cultured isolates, properly preserved infected tissue samples or paraffin-embedded tissue samples, and immunohistochemical techniques using polyclonal antibodies for detecting *P. insidiosum* antigen in tissues have proven to have high sensitivity and specificity toward the diagnosis of pythiosis.[5-7,9,10]

In dogs with the gastrointestinal form, segmental lesions should be resected. Similarly, in dogs with the cutaneous form, the recommendation is of complete excision of the infected tissue or, if a limb is affected, amputation.[3] Because *P. insidiosum* lacks ergosterol in the cytoplasmic membrane, antifungal drugs interfering with ergosterol biosynthesis, such as azoles, terbinafine and amphotericine B, have reduced efficacy.[1,4] Nonetheless, combination of traditional antifungal drugs with mefonaxam or immunotherapy has shown some success in affected dogs.[8,16] A vaccine formulation based on soluble mycelial antigens and secreted exoantigens of *P. insidiosum* has been successfully used as treatment in horses with cutaneous pythiosis but their efficacy in dogs appears less than that reported in horses.[12] Long-term prognosis in dogs infected with *P. insidiosum* therefore depends on chronicity of infection (poor in infections <2 months), location of lesion and treatment modalities used.[12]

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