Pathology in Practice
In collaboration with the American College of Veterinary Pathologists

**History**

A 5-month-old spayed female Border Collie mixed-breed dog was evaluated because of a 1-week history of progressive lethargy, inappetence, weight loss, and diarrhea. The dog had no prior medical or travel history and was evaluated in Iowa.

**Clinical and Clinicopathologic Findings**

Initial evaluation of the puppy revealed generalized muscle wasting, dull mentation, diffuse icterus, and palpable hepatomegaly. A CBC, serum biochemical analyses, urinalysis, coagulation profile, pre- and postprandial bile acid measurements, radiography, and abdominal ultrasonography were performed. Results of the CBC indicated normocytic, normochromic anemia, moderate thrombocytopenia, and an inflammatory leukogram with a left shift and toxic changes suggestive of an infectious process. Hepatic dysfunction was evident based on high concentrations of total bilirubin (2.22 mg/dL; reference range, <0.6 mg/dL) and preprandial bile acids and prolonged prothrombin time (25 seconds; reference range, 5.5 to 7.9 seconds) and partial thromboplastin time (30 seconds; reference range, 10.4 to 19.3 seconds). Serum biochemical analyses also revealed hypoalbuminemia (1.5 g/dL; reference range, 2.7 to 4.0 g/dL), hypoglycemia (60 mg/dL; reference range, 68 to 115 mg/dL), hypercalcemia (13.2 mg/dL; reference range, 9.7 to 11.3 mg/dL), hyperphosphatemia (7.3 mg/dL; reference range, 3.2 to 6.0 mg/dL), and high alkaline phosphatase activity (349 U/L; reference range, 20 to 150 U/L). Results of thoracic radiography were unremarkable, whereas abdominal radiography revealed generalized hepatomegaly, with homogenous echotexture evident on ultrasonography. Shortly after diagnostic procedures were performed, the puppy had cardiac arrest and died.

On necropsy, mild bicavitary fluid was present. The liver was enlarged and had generalized pallor. Associated hepatic lymph nodes were also enlarged. The brain had a focus of encephalomalacia and hemorrhage present in the right cerebral hemisphere and a tan tissue mass dorsal to the midbrain.

Formulate differential diagnoses, then continue reading.

**Histopathologic Findings**

Microscopic examination of the lungs, liver, hepatic lymph nodes, spleen, small intestine, colon, adrenal gland, and thyroid glands revealed that the tissues were effaced by macrophages that often contained basophilic yeast, approximately 3 μm in size (Figure 1). Fewer numbers of lymphocytes, plasma cells, and neutrophils were also present. The kidneys had multifocal areas of inflammatory cells consisting primarily of macrophages with occasional lymphocytes and plasma cells, but no yeast was noted. Multifocal mineralization of medullary tubules was present. The right cerebral cortex had an area of locally extensive necrosis and hemorrhage, with aggregates of mononuclear cells and degenerate neutrophils at the periphery. The mass dorsal to the midbrain was identified as rarefied neural tissue with hemorrhage. No yeast was noted within the neural tissue. There were no notable lesions in tissue samples of the heart, stomach, bone marrow, pancreas, or eye.

**Diagnosis and Case Summary**

Cytologic and morphologic interpretation of small intestines, liver, lymph nodes, spleen, lung, and adrenal glands: granulomatous inflammation with basophilic intracellular yeast.

Case summary: disseminated histoplasmosis in a dog.

**Comments**

Histoplasmosis is a systemic fungal disease caused by *Histoplasma capsulatum*, which is a soil-borne dimorphic fungus known to be found in the gastrointestinal tracts and guano of bats, the primary reservoir. It has a worldwide distribution with most infections in the United States originating from the Ohio, Missouri, Tennessee, and Mississippi river...
valleys. Infections in dogs occur primarily via inhalation of microconidia found in the environment. In the body, the conidia transform into a yeast phase that is phagocytosed by macrophages. The organism has the ability to resist macrophage-killing mechanisms and replicate intracellularly causing disease in the respiratory tract.\textsuperscript{2,3} \textit{Histoplasma} is able to parasitize macrophages due to its ability to interact with phagocytic receptors while minimizing immune cell signaling recognition.\textsuperscript{4} The infected macrophages allow for hematogenous and lymphatic dissemination causing life-threatening multisystemic disease. In dogs, disease is often disseminated and most commonly found in the lungs, gastrointestinal tract, liver, spleen, and bone marrow.\textsuperscript{5}

Histoplasmosis has been reported in dogs of all ages but tends to affect young adults.\textsuperscript{5–7} Sporting and working dogs are overrepresented due to increased risk of exposure.\textsuperscript{7} The most common clinical signs are small or large bowel diarrhea and wasting.\textsuperscript{5} Other signs can vary widely dependent on organ systems involved including intermittent lameness, dyspnea, cough, dermal lesions, icterus, and neurologic signs such as changes in mentation, seizures, or nystagmus.\textsuperscript{5,7}

Bloodwork changes are often nonspecific and reflect chronic inflammation. Results of a CBC typically indicate normocytic, normochromic anemia secondary to inflammation.\textsuperscript{7} The WBC counts can be increased, normal, or decreased, but dogs most commonly have an inflammatory leukogram characterized by neutrophilia and monocytosis.\textsuperscript{7} In severe disease, thrombocytopenia, band neutrophils, and toxic changes can be present. On the serum biochemical analyses, hypoalbuminemia is present in most affected dogs.\textsuperscript{7} Other changes can be present such as azotemia, hyperglobulinemia, hyperbilirubinemia, and elevations in alkaline phosphatase, alanine aminotransferase, and \( \gamma \)-glutamyl transferase.\textsuperscript{7}

Definitive diagnosis of histoplasmosis can be made on cytology or histopathology and must be differentiated from other conditions with similar clinical signs. Samples of affected tissues are commonly obtained by fine-needle aspirate, impression smear, or rectal scrape. Various stains are successful in identifying the organism including modified Romanowsky stain or Wright stain.\textsuperscript{8} The organisms are found primarily within macrophages.\textsuperscript{8} They often appear as 1 or more oval to round, 2 to 4 \( \mu \)m in size intrahistiocytic yeast with a basophilic body and clear halo (Figure 1).\textsuperscript{8} The cytology also typically contains evidence of pyogranulomatous inflammation. Cytologic differentiation from other systemic fungal infections, including those caused by \textit{Blastomyces}, \textit{Cryptococcus}, or \textit{Coccidioides}, is often made based on size, budding, and evidence of endosporulation.
Histopathologic samples will yield similar results of intracellular yeast surrounded by pyogranulomatous inflammation. Fungal culture can have varied results but is not recommended due to the health hazard to laboratory personnel and long incubation times of 2 to 4 weeks. Serologic antibody testing is available for dogs as a noninvasive diagnostic tool but is generally unreliable and can yield false positives and negatives. Antigen testing via ELISA assays is preferred as urine antigen testing has been shown to be highly sensitive but poorly specific due to cross-reactivity with Blastomyces. Polymerase chain reaction testing can also be utilized to extract DNA from paraffin-embedded tissue samples.

Prognosis for histoplasmosis is dependent on the organ systems involved, with dogs with gastrointestinal signs and dissemination having a poor prognosis. Histoplasmosis can affect all organ systems and should be maintained on a differential diagnosis list for patients with hepatomegaly.

References