Pathology in Practice

In collaboration with the American College of Veterinary Pathologists

History

A 3-year-old 38.5-kg spayed female Labrador Retriever was evaluated because of a markedly enlarged abdomen and decreased alertness. Results of a CBC and biochemical analyses were unremarkable, except for mildly increased alkaline phosphatase activity (90 U/L; reference interval, 13 to 83 U/L) and total protein concentration (7.8 g/dL; reference interval 5 to 7.2 g/dL). Albumin concentration was 3.2 g/dL (reference interval, 2.6 to 4 g/dL), revealing a mildly elevated globulin fraction of 4.6 g/dL. The patient was referred for abdominal CT.

Clinical, Gross, and Cytologic Findings

Multiple nonvascularized, fluid-filled cysts associated with the liver were detected on CT. Focal areas of partial mineralization were noted in the right liver lobe. Hepatic lymph nodes were moderately enlarged. The patient was referred for abdominal ultrasonography and collection of ultrasound-guided fine-needle aspirate (FNA) samples of the cysts detected on CT.

A week later, the dog was presented for ultrasonography. On clinical examination, the dog had mild dehydration and a bulging abdomen, the contents of which could not be palpated completely. Abdominal palpation did not elicit signs of pain. On ultrasonographic examination, the liver appeared markedly enlarged. Only small parts on the right side showed normal architecture. Most of the liver parenchyma was inhomogeneous, with large cavitary lesions of up to 20 cm in diameter. Cystic structures with irregular walls and hypoechoic contents were detected in several liver lobes. To differentiate between benign and malignant lesions, contrast-enhanced ultrasonographic imaging (CEUS) was performed to assess in more detail the perfusion pattern of cyst walls and abnormal liver structures. Focal perfusion changes were not observed in the inhomogeneous areas of the liver. Cystic walls appeared very thin, rim sign could not be demonstrated, and perfusion of the cysts was not seen on color flow Doppler ultrasonography or CEUS. Ultrasound-guided FNAs of fluid from 1 of the cysts were obtained, and analysis of the fluid revealed a refractometric protein concentration of 4.3 g/dL, high cellularity (total nucleated cell count, 11.77 X 10⁹/L), and numerous large (approx 50 to 500 µm in size) membrane-like structures that had moderate numbers of large elongated dark basophilic forms attached (Figure 1).

Formulate differential diagnoses, then continue reading.

Figure 1—Photomicrographs of lower (A) and higher (B) magnifications of ultrasound-guided fine-needle aspirate samples from a cystic liver mass in a 3-year-old 38.5-kg spayed female Labrador Retriever with marked abdominal enlargement and decreased alertness. Numerous large, membrane-like structures (arrowheads) and a large, elongated, dark basophilic form (arrow) are present. A—Modified Wright stain; bar = 500 µm. B—Modified Wright stain; bar = 100 µm.
Additional Cytologic, Clinicopathologic, and Histopathologic Findings

On closer inspection, large forms attached to membrane-like structures were identified as protoscolices, which had a circular row of hooks on the rostellum and the suckers on the anterior end (Figure 2). Nonstaining crystalline structures (5 to 10 µm in diameter) consistent with calcareous corpuscles were also observed. Moderate eosinophilic and lymphoplasmacytic and mild neutrophilic inflammation was noted. Mild cholestasis was also present, indicated by bile-filled canalicular plugs.

Results of PCR assay performed on an FNA sample from one of the cysts confirmed the presence of *Echinococcus multilocularis* DNA.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis: tapeworm cysts and protoscolices consistent with larval stage of *E multilocularis*, moderate eosinophilic and lymphoplasmacytic and mild neutrophilic inflammation, and mild cholestasis.

Case summary: alveolar echinococcosis (AE) caused by the infection with metacestode (larval) stage of *E multilocularis* in a dog.

Comments

*Echinococcus multilocularis* is a zoonotic tapeworm found in most of the European countries, large parts of Eurasia, and parts of North America. The main definitive host in Europe is the red fox. In North America, main definitive hosts are coyotes, red foxes, wolves and Arctic foxes, with prevalence varying depending on the geographic location. The natural intermediate hosts of *E multilocularis* are small rodents. Adult parasites are found in the small intestine of the definitive hosts; eggs released in the feces from the gravid proglottid are immediately infective for intermediate hosts. When ingested, the oncosphere hatches, migrates to the liver, and develops into metacestode (larval) stage called hydatid (alveolar) cyst containing numerous protoscolices of the parasite. Growth of the hydatid cyst in the liver remains indefinitely in the proliferative stage and is in the form of exogenous budding. Alveolar echinococcosis is the disease caused by the hydatid cyst growth, with resulting extensive damage to the liver and spread to other organs. When a definitive host ingests an infected intermediate host, protoscolices evaginate, attach to the intestinal mucosa, and develop into adult parasites.

Accidental hosts (such as dogs and humans) are infected by oral ingestion of the eggs. In dogs, the source of infection with *E multilocularis* eggs is a contaminated environment shared with wildlife or autoinfection in association with the presence of adult tapeworms in the small intestine. The endemic areas for *E multilocularis* in Canada and the United States included Alaska, the Northern Tundra region of Canada, the north central region of the United States, and 3 Canadian Prairie provinces. In the past several years, geographic range expanded into parts of British Columbia and Ontario, at least partially due to large numbers of foxes and coyotes establishing in urban areas. Also, a case of AE was reported recently in a dog in northern Virginia and a case of intestinal *E multilocularis* infection was reported in Missouri. Increased numbers of the reported cases of AE in dogs suggest that *E multilocularis* infection is on the rise both in North America and Europe. Even though alveolar cysts in the abdomen of dogs are not zoonotic, it is important to keep in mind that the owner and the dog could have been exposed to a common source of infectious eggs in the environment. If owners think they may have been infected, serologic testing is recommended for screening. Dogs with AE can also harbor adult tapeworms in the intestine. Fecal examination is recommended for dogs diagnosed with AE. Because taeniid eggs (*Taenia* and *Echinococcus* spp eggs...
are morphologically indistinguishable, the preferred identification method in the feces is multiplex PCR assay.\textsuperscript{13} Commercial real-time PCR assay testing of fecal material, tissue aspirates, fresh refrigerated tissue specimens, or formalin-fixed paraffin-embedded tissue is available in North America and Europe.\textsuperscript{14} A common clinical finding in dogs with AE is progressive abdominal distension,\textsuperscript{15,16} similar as in the dog of the present report. Other clinical signs described were lethargy, anorexia, vomiting, diarrhea, and weight loss.\textsuperscript{16} Findings on CBC may include mild nonregenerative anemia,\textsuperscript{3,17} neutropenia,\textsuperscript{4} lymphopenia,\textsuperscript{7} neutrophilia,\textsuperscript{17} leukocytosis with left shift,\textsuperscript{15} or lymphocytosis,\textsuperscript{15} some likely due to inflammatory response. Biochemical results are nonspecific and included mild to moderate hypoalbuminemia,\textsuperscript{15,17} hyperglobulinemia,\textsuperscript{5,15} hyperphosphatemia and hypocalcemia,\textsuperscript{3} or mildly increased liver enzyme activities,\textsuperscript{3,15,17} alone or in combination. Besides hepatic involvement, additional spread to omentum, peritoneum, diaphragm, lung, spleen, vertebral bodies, brain,\textsuperscript{16,18} and prostate\textsuperscript{19} has been reported. The dog of the present report had mild hyperproteinemia with mild hyperglobulinemia and albumin fraction within the reference interval, mildly increased alkaline phosphatase activity, and bile-filled canicular plugs (likely due to intrahepatic cholestasis). Our initial differential diagnoses included echinococcosis, nonneoplastic\textsuperscript{20} (adult-type polycystic liver disease, possibly including Von Meyenburg complexes) and neoplastic\textsuperscript{21} (cholangiocellular adenoma and cholangiocellular carcinoma) cystic hepatic lesions, abscess, and hepatic necrosis (eg, secondary to hepatic neoplasia, but considered unlikely).

Cytology is an excellent diagnostic tool for AE. Cytologic examination of fluid aspirated from alveolar cysts or from abdominal fluid of affected patients consistently reveal the presence of large membrane-like structures and calcareous corpuscles (round refractile concretions composed of Ca, Mg, P, CO\textsubscript{3}, and organic components).\textsuperscript{8,17} In dogs, protoscolices are not always detected on cytology and are rarely reported,\textsuperscript{22} whereas natural intermediate hosts can harbor more than 200,000 protoscolices.\textsuperscript{3} As a possible explanation, it was hypothesized that as aberrant intermediate hosts, dogs appear to be less able to support the production of protoscolices than natural intermediate hosts.\textsuperscript{23}

To diagnose \textit{E multilocularis} metacestode (larval) infection, macroscopic, cytologic, and histologic (H&E stain and Periodic acid–Schiff stain) findings and molecular analysis results should be evaluated. The method of choice for identifying \textit{E multilocularis} from small nonfertile or calcified lesions is PCR assay.\textsuperscript{2} Serologic tests using affinity-purified Em2 antigen were investigated in dogs; however, such tests were not able to differentiate between intestinal \textit{E multilocularis} infections and AE.\textsuperscript{2}

The recommended treatment both in humans and in dogs is a radical resection combined with medical management.\textsuperscript{16} Lifelong medical treatment with albendazole at a daily dose of 10 mg/kg is indicated in all affected dogs for which a therapeutic approach is regarded appropriate.\textsuperscript{16} The affected patient and all other dogs and cats in the household should be treated with praziquantel (5 mg/kg, PO, q 24 h for 2 days) to eliminate adult tapeworms that might be concurrently present in the small intestine.\textsuperscript{3} For the dog of the present report, debulking surgery was performed. A large cyst (20 cm in diameter) in the right medial liver lobe was excised and the remaining omentum. Prior to excision, 1.5 L of the yellowish fluid was removed from the cyst to prevent leakage in the abdominal cavity. The remaining liver cysts were integrated into the liver tissue and could not be surgically removed. The dog recovered uneventfully and was treated with albendazole (10 mg/kg, PO, q 24 h). Two months later, the dog’s abdomen was soft, less distended, and more palpable, and results of a CBC, biochemical analyses, and liver function tests (ammonia and bile acids) were within reference limits. Two years after diagnosis and surgery, the dog underwent a second surgery for cyst removal. Repeated analysis of abdominal fluid obtained by abdominocenteses revealed modified transudate without detectable parasitic structures, and 1 year after the second surgery, the dog was euthanized due to weight loss and ascites.

In summary, AE caused by the infection with metacestode (larval) stage of \textit{E multilocularis} is an important differential diagnosis in dogs from endemic areas with cavitory masses in the liver. Cytology is a valuable tool for establishing the final diagnosis.

References