Multiple cysts in a dog liver

Contributors: Jelena Palić, Stefan Unterer, Vera Geisen, Kathrin Busch
Clinic for Small Animal Medicine, Centre for Clinical Veterinary Medicine, Faculty of Veterinary Medicine, Ludwig-Maximilians-Universität München, Germany

Signalment: 3-year-old female neutered Labrador named Pippa

Specimen: Computed tomographic (CT) image of the cranial abdomen, FNA of the fluid from one of the cysts

History and clinical findings:

Pippa was a 3-year-old female castrated Labrador that was referred to the Clinic for Small Animal Medicine, Ludwig Maximilian University Munich. Pippa's current medication included metacam twice daily due to her existing orthopedic (meniscus) problems. On her initial presentation a week earlier, she had markedly enlarged abdomen and decreased general condition. CBC and biochemistry analysis (performed on a Fuji dry-chemistry analyzer FDI 4000i) by a referring veterinarian were unremarkable, except for a mild increase in alkaline phosphatase activity (90 U/l, reference interval 13-83 U/l), and a mild increase in total protein (7.8 g/dl, reference interval 5-7.2 g/dl). Albumin was 3.2 g/dl (reference interval 2.6-4 g/dl), revealing increased globulins of 4.6 g/dl.

Pippa was referred to imaging center for CT of the abdomen. Multiple non-vascularized, fluid-filled cysts have been detected (Fig. 1). In addition, focal areas of partial mineralization were noted in the right liver lobe. Hepatic lymph nodes were moderately enlarged, and other abdominal structures were unremarkable. Differential diagnosis included echinococcosis, abscess, and hepatic necrosis (e.g. secondary to hepatic neoplasia, but considered unlikely in this case). In order to further investigate cysts detected on CT, patient has been referred for an ultrasound-guided biopsy.

Clinical examination performed in Clinic for Small Animal Medicine at LMU was unremarkable, except for mild dehydration (less than 5%), and a bulging, non-painful abdomen that could not be palpated completely. Contrast-enhanced ultrasonographic imaging has been performed. The liver was significantly enlarged. Only small parts on the right side showed a normal liver architecture. Most of the liver parenchyma appeared very inhomogeneous with large cavitary lesions of up to 20 cm diameter. Cystic structures with irregular wall and hypoechoic content were detected in several liver lobes. No increased perfusion of the cystic wall and no perfusion of the cysts could be seen on color doppler examination. Ultrasound-guided fine-needle aspirate (FNA) samples of the fluid from one of the cysts have been obtained for cytological examination. Analysis of the fluid revealed total nucleated cell count of 11.77 x 10^9/L, and refractometric protein concentration of 4.3 g/dl.

Figure 1. Transverse computed tomographic image of the cranial abdomen of a 3-year old Labrador showing one of the liver cysts.

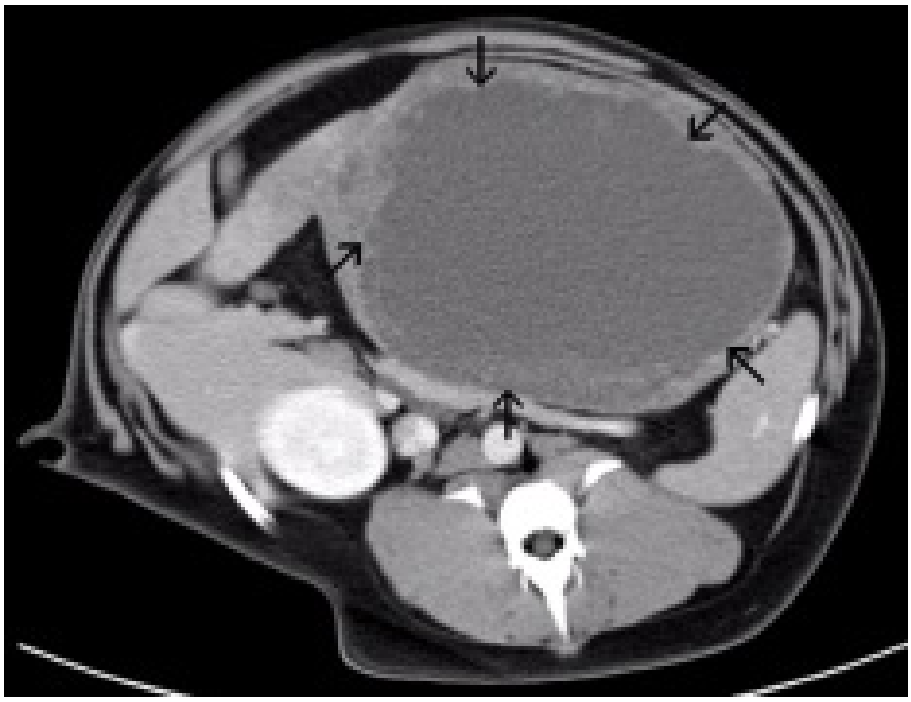


Figure 2. Fine-needle aspirate of a liver cyst from a 3-year old Labrador. Note the numerous large membrane-like structures. Modified Wright's stain. Scale bar = 500 µm.

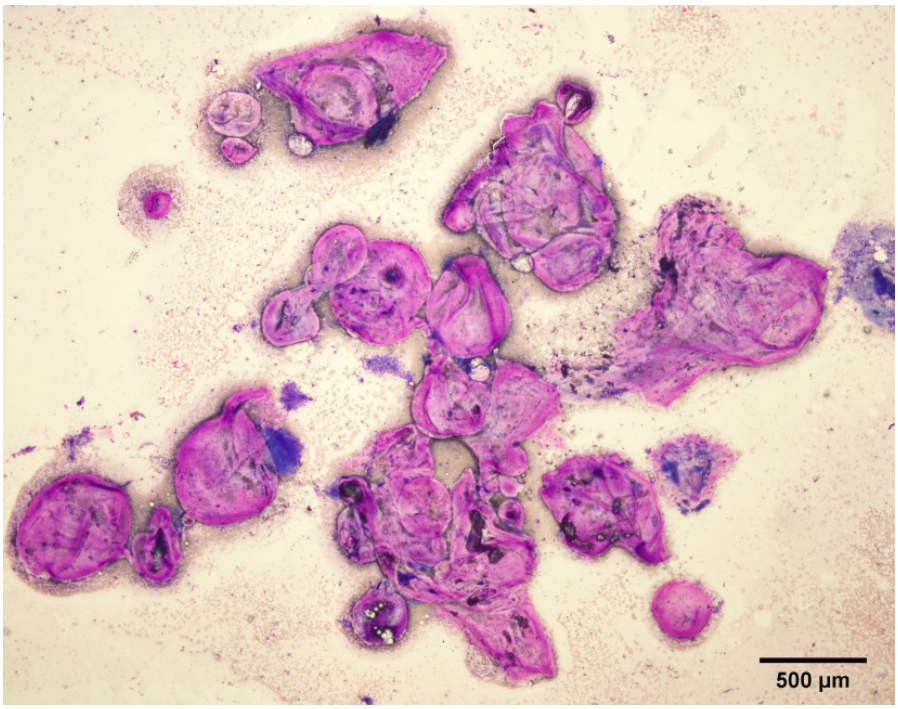


Figure 3. Fine-needle aspirate of a liver cyst from a Labrador. Note the presence of protoscolex attached to a membrane-like structure below. Modified Wright's stain. Scale bar = 100 µm.

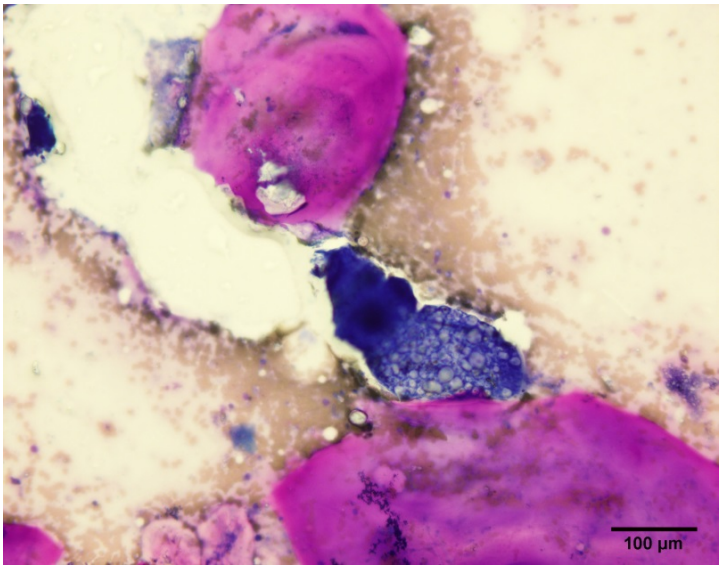
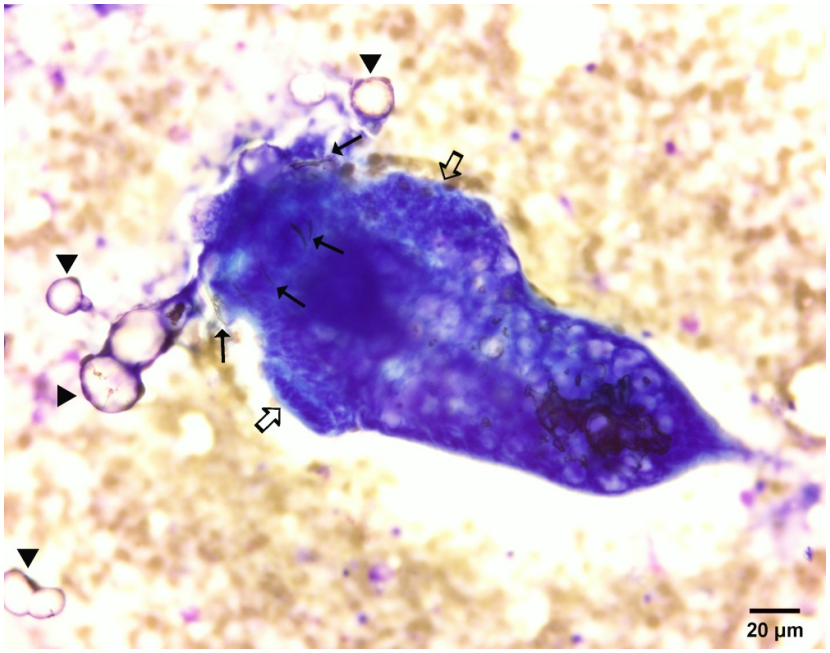


Figure 4. Fine-needle aspirate of a liver cyst from a Labrador. On a close-up view of the protoscolex, a circular row of hooks on the rostellum (arrows), and the presence of suckers (open arrows) can be detected. Several crystalline non-staining structures (calcareous corpuscles) are present in the background (arrowheads). Modified Wright's stain. Scale bar = 20 µm.



Questions:

1. Which additional test can be performed?
2. Does this dog pose a zoonotic risk to the owner?

Cytology interpretation: Tapeworm cysts and protoscolices consistent with larval stage of *Echinococcus multilocularis*, moderate lymphocytic and plasmacytic inflammation, mild cholestasis

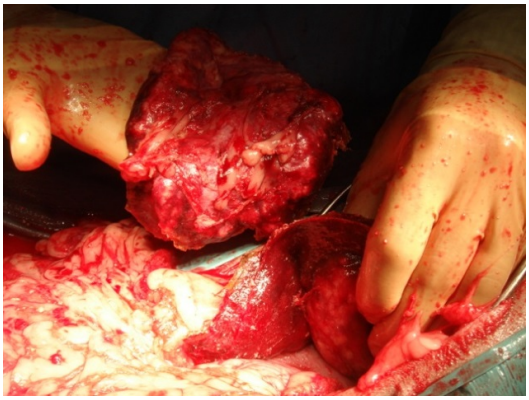
Cytology description and comments: FNA preparations of the liver were of high cellularity with moderate numbers of RBC in the background. Numerous large membrane-like structures (approximately 50-500 µm in size) were detected (Fig. 2). In addition, moderate numbers of protoscolices were seen (Fig. 3). Protoscolices were dark basophilic in color, had a circular row of hooks on the rostellum, and the suckers on the anterior end (Fig. 4). Non-staining crystalline structures (approximately 5-10 µm in diameter) consistent with calcareous corpuscles were also noted (Fig. 4). Small lymphocytes and plasma cells were moderately increased in numbers. Clusters of hepatocytes that were present did not show significant atypia. Mild cholestasis was also seen, indicated by the presence of bile-filled cannalicular plugs.

Additional tests: PCR testing of the fluid aspirated from one of the cysts has been performed in Institute for Parasitology at the University of Zurich, and confirmed the presence *of E. multilocularis* DNA.

Final diagnosis: Alveolar echinococcosis caused by the infection with metacestode (larval) stage of *Echinococcus multilocularis*

Surgery and follow-up: Debulking surgery has been performed. A large cyst (approximatelly 20 cm in diameter) located in the right medial liver lobe has been excised (Fig.5) and the remaining omentalized. Prior to excision, 1.5 liters of the yellowish fluid has been 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. Pippa recovered uneventfully, and was placed on daily albendazol 10 mg/ kg SID. Two months after the surgery, a recheck has been done. On clinical examination Pippa appeared fit, and the abdomen was soft and palpable. CBC, biochemistry, and liver function tests (ammonia and bile acids) have also been performed. All analytes were within reference intervals. Ultrasonographic examination revealed structurally altered left medial liver lobe with patchy hyperechogenic areas; a cyst of approximately 3.5 cm in diameter was present. The remainder of the right medial liver lobe showed normal structure.

Figure 5. A large cyst located on the right medial liver lobe has been drained and surgically removed.



Discussion:

Echinococcus multilocularis is a zoonotic tapeworm found in most of the European countries, large parts of Eurasia, and parts of North America[1]. The main definitive host in Europe is the red fox, with the Arctic fox, the raccoon dog, the domestic dog and to a much lesser extent the domestic cat as the other potential definitive hosts[2]. The natural intermediate hosts of *E. multilocularis* are small rodents such as voles. Adult parasites are found in the small intestine of the definitive hosts; eggs released in the feces from the gravid proglotid are morphologically indistinguishable from *Taenia*-type eggs. Importantly, eggs 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, therefore behaving as an invasive tumor[3]. The disease caused by the hydatid cyst growth results in extensive damage to the liver and in spread to other organs, and is termed alveolar echinococcosis (AE). When a definitive host ingests an infected intermediate host, protoscolices evaginate, attach to the intestinal mucosa and develop into adult parasites in 4-5 weeks[4].

Accidental hosts include many species of monkeys, pigs, dogs and humans which are infected by oral ingestion of the eggs. In dogs, the source of infection with *E. multilocularis* eggs is either the contaminated environment shared with wildlife such as red foxes, or autoinfection in association with the presence of adult tapeworms in the small intestine[3]. Ingestion of eggs by people also results in AE, a potentially severe, fatal disease that is currently an emerging issue in parts of central Europe[5]. The estimated number of new AE cases in Western and Central Europe is in the range of 170–200 per year, with the highest numbers in France, Germany, Switzerland, Lithuania and Poland)[2].This data correlates with a meta-analysis study that revealed the highest prevalence (> 10 %) of *E. multilocularis* in red foxes in Czech Republic, Estonia, France, Germany, Latvia, Lithuania, Poland, Slovakia, Liechtenstein and Switzerland[6]. Studies from Finland, Ireland, the United Kingdom and Norway reported the absence of *E. multilocularis* in red foxes. However, *E. multilocularis* was detected in Arctic foxes from the Arctic Archipelago of Svalbard in Norway[6]. 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 be exposed to a common source of infectious eggs in the environment. Additionally, dogs with AE can also harbor adult tapeworms in the intestine. Therefore, fecal examination is recommended for dogs diagnosed with AE.

The most commonly reported clinical finding in dogs with AE is progressive abdominal enlargement over several weeks[7], similar as in the case presented here. Hematologic and biochemistry results reported in literature are also nonspecific, and include mild-to-moderate hypoalbuminemia with concurrent hyperproteinemia[7].Some of the less frequently reported abnormalities are increased activities of ALT and/or AST and/or AP, leukocytosis with left shift and lymphocytosis[7]. Dog in our report had hyperproteinemia with hyperglobulinemia and albumin within the reference interval. In addition, our patient had mild increase in alkaline phosphatase activity, and the presence of bile-filled cannalicular plugs on cytology.

Characteristic findings on radiographs, ultrasonographic examination and CT in dogs include detection of multiple abdominal masses with or without wall mineralization. The masses appear cavitary and cystic to vesicular (alveolar) or granular; vascularization is absent on ultrasonographic examination and on CT[7].

Cytology is an excellent diagnostic tool for AE. Cytological examination of fluid aspirated from alveolar cysts, or from abdominal fluid consistently revealed the presence of large membrane-like structures and calcareous corpuscles (round refractile concretions composed of Ca, Mg, P, CO2, and organic components)[8,9]. However, the presence and detailed morphology of protoscolices found on cytology in dog AE cases has not yet been reported. Even on histopathology, in most cases, metacestodes contained no or few protoscolices and were thus sterile[10]. As a possible explanation, it has been hypothesized that as aberrant intermediate hosts, dogs appear to be less able to support the production of protoscolices than natural intermediate hosts[10]. Moreover, it has been demonstrated on histopathology that alveolar cysts in dogs are surrounded by fibrous tissue intermixed with inflammatory cells comprising variable numbers of macrophages,T and B lymphocytes, plasma cells, neutrophils and/or eosinophils, resembling lesions observed in man and in contrast to those observed in the natural intermediate hosts, which had less fibrosis and more protoscolices[10]. In our case, moderate numbers of inflammatory cells (lymphocytes and plasma cells) have been observed, together with moderate numbers of protoscolices.

Serological tests using affinity-purified Em2 antigen are available in dogs, however, such tests were not able to differentiate between intestinal *E. multilocularis* infections and AE[2]. Moreover, rise or fall in follow-up antibody titers did not correlate with progression or regression of the AE lesion based on ultrasonographic examination[11]. In humans, serological follow-up is routinely used since an antibody response to the recEm18-antigen proved to correlate with clinical evolution[12]; the antigen investigated in dogs was the Em2-antigen[11].

In order to diagnose *E. multilocularis* metacestode (larval) infection, macroscopic, cytological, histological and/or immunohistological (HE- and PAS-stain) findings, and molecular analysis results should be evaluated.The method of choice for identifying *E. multilocularis* from small non-fertile or calcified lesions is PCR[2]. Recently, a classification system has been proposed by which a case is considered as possible AE with only a positive serology, probable AE with both a positive serology and imaging findings consistent with AE, and confirmed AE with a positive microscopic or PCR identification[11].

The recommended treatment both in humans and in dogs is a radical resection combined with medical management[11]. Lifelong medical treatment with albendazole at a daily dose of 10 mg/kg is indicated in all dog cases in which a therapeutic approach is regarded appropriate[11]. In endemic areas, any lesion in a dog suspicious for AE should be sampled for cytological and PCR analysis to confirm diagnosis[2].

In summary, this is the description of alveolar echinococcosis caused by the infection with metacestode (larval) stage of *Echinococcus multilocularis* in a dog from an endemic area in southern Germany, with a particular emphasis on cytological detection and description of protoscolices.

References:

1. Eckert J. and Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev.* 2004;17(1):107-35.
2. Conraths FJ, Deplazes P. Echinococcus multilocularis: Epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. *Vet Parasitol.* 2015;213(3-4):149-61.
3. Peregrine AS. Alveolar echinococcosis in dogs: an emerging issue? *Vet Rec.* 2015;177(22):567-8.
4. Deplazes P, van Knapen F, Schweiger A, Overgaauw PAM. Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis. *Vet Parasitol.* 2011;182(1):41-53.
5. Gottstein B, Stojkovic M, Vuitton DA, Millon L, Marcinkute A, Deplazes P. Threat of alveolar echinococcosis to public health - a challenge for Europe. *Trends Parasitol.* 2015;31(9):407–12.
6. Oksanen A, Siles-Lucas M, Karamon J, Possenti A, Conraths FJ, Romig T, Wysocki P, Mannocci A, Mipatrini D, La Torre G, Boufana B, Casulli A. The geographical distribution and prevalence of *Echinococcus multilocularis* in animals in the European Union and adjacent countries: a systematic review and meta-analysis. *Parasit Vectors*. 2016;9(1):519-42.
7. Scharf G, Deplazes P, Kaser-Hotz B, Borer L, Hasler A, Haller M, Flückiger M. Radiographic, ultrasonographic, and computed tomographic appearance of alveolar echinococcosis in dogs. *Vet Radiol Ultrasound.* 2004;45(5):411-8.
8. Oscos-Snowball A, Tan E, Peregrine AS, Foster R, Bronsoiler J, Gottstein B, Jenkins E, Gesy Ka, Bienzle D. What is your diagnosis? Fluid aspirated from an abdominal mass in a dog. *Vet Clin Pathol.* 2015;44(1):167–8.
9. Brosinski K, Gutbrod A, Venzin C, Grest P. What is your diagnosis? Peritoneal fluid from a dog with abdominal pain. *Vet Clin Pathol.* 2012;41(2):297-8.
10. Weiss AT, Bauer C, Köhler K. Canine alveolar echinococcosis: morphology and inflammatory response. *Comp Pathol.* 2010;143(4):233-8.
11. Corsini M, Geissbühler U, Howard J, Gottstein B, Spreng D, Frey CF. Clinical presentation, diagnosis, therapy and outcome of alveolar echinococcosis in dogs. *Vet Rec.* 2015;177(22):569-75.
12. Ammann RW, Renner EC, Gottstein B, Grimm F, Eckert J,Renner EL, Swiss Echinococcosis Study Group. Immunosurveillance of alveolar echinococcosis by specific humoral and cellularimmune tests: long-term analysis of the Swiss chemotherapy trial (1976–2001). *J Hepatol.* 2004;41(4):551–59.