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CASE REPORT

Cystic echinococcosis due to *Echinococcus equinus* in a Swiss donkey

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Summary

A 22-year-old donkey (*Equus asinus*) mare was presented for investigation of a 3-day history of lethargy and anorexia. Serum biochemistry profile revealed severe increased liver-derived enzymes. Abdominal ultrasound demonstrated several large round cysts in the liver. Broad-spectrum antimicrobials and anti-inflammatory therapy were started. However, exacerbation of the clinical signs occurred, and the donkey was euthanised. Necropsy and histopathological evaluation showed multiple hydatid cysts with protoscoleces from *Echinococcus* spp. in the liver with replacement of parenchyma by fibrosis accompanied by liver atrophy, severe ascites and thorax effusion. The present report describes the first case of a molecularly confirmed *Echinococcus equinus* infection in a donkey in northern Switzerland.

KEYWORDS

horse, cystic echinococcosis, donkey, *Echinococcus equinus*, hepatic cysts, Switzerland

INTRODUCTION

Cystic echinococcosis (CE) is a helminth zoonotic disease of global importance and distribution (Casulli et al., 2022). It is caused by the larval stages (metacestodes, hydatid cysts) of tape-worm parasites that belong to the *Echinococcus granulosus* sensu lato complex. The life cycles of these parasites are indirect and involve two mammalian hosts: an intermediate and definitive host, which are connected in the trophic chain (D'Alessandro & Rausch, 2008). Infections with metacestodes may be found in a variety of animal species, including sheep, goats, cattle, pigs, buffalo, equids and camels (Boufana et al., 2015). Canids, mainly dogs are definitive hosts of these parasites and carry adult stages in their small intestines. Molecular data show that *E. granulosus* is a species complex that includes several species or genotypes: *E. granulosus* sensu stricto, *E. canadensis*, *E. intermedius*, *E. borealis*, *E. ortleppi*, *E. equinus* and *E. felidis* (Lymbery, 2017). *E. equinus* was long believed to be non-zoonotic (Thompson & McManus, 2001). However, two cases of human infections have been reported recently in Turkey and Uzbekistan (Kim et al., 2020; Macin et al., 2021). In Europe, CE is generally a rare finding in horses and donkeys and is usually diagnosed incidentally at slaughter or post-mortem examination. The liver, lungs or both are mostly affected by the metacestodes or hydatid cysts. (Edwards, 1981; Goto et al., 2010; Haridy et al., 2008; Rezabek et al., 1993; Varcasia et al., 2008). To the authors' best knowledge, in equids, only one case report has described functional impairment of the affected organ because of cyst growth (Blutke et al., 2010). The present report describes a case of cystic echinococcosis in the liver of a 22-year-old donkey presented with lethargy and anorexia.

CASE HISTORY

A 22-year-old donkey mare was presented with a 3-day history of lethargy and anorexia to the Clinic for Equine Internal Medicine,

Vetsuisse Faculty, University of Zurich. On the day of the presentation, the donkey showed mild abdominal pain and had been treated with scopolamini butylbromidum and, metamizolum natricum (5 mL/100 kg bwt i.v., Buscpoan compositum® ad us. vet., Boehringer Ingelheim, Schweiz GmbH) by the referring veterinarian.

CLINICAL FINDINGS

Upon presentation, the donkey was lethargic, with a heart rate of 60 beats per minute, respiration rate of 12 breaths per minute, mucous membranes were pink with a capillary refill time of 3 s, and the rectal temperature was 37.3°C. Intestinal motility was decreased on auscultation in all four quadrants of the abdomen. She weighed 114 kg with a body condition score of 5/9. The remainder of the physical examination was unremarkable.

Laboratory assessment included complete blood count, which was within normal limits with the exception of a mild neutrophilia (11.75×10^9/L; reference range [RR]: $1.78–6.74 \times 10^9$/L) and lymphopenia (1.58×10^9/L; RR: $2.37–9.46 \times 10^9$/L). The serum biochemistry profile showed mild hyperglycaemia (7.9 mmol/L; RR: 3.8–6.0 mmol/L), hyperlipidaemia (4.7 mmol/L; RR: 0.3–2.3 mmol/L), increased activity of alkaline phosphatase ([AP], 1164 U/L; RR: 95–313 U/L), aspartate aminotransferase ([AST], 540 U/L; RR: 234–512 U/L), gamma-glutamyltransferase ([GGT], 2394 U/L; RR: 15–72 U/L), sorbitol dehydrogenase ([SDH], 16.9 U/L; RR: 0.9–5.8 U/L), glutamate dehydrogenase ([GDH], 118.5 U/L; RR: 1.4–19.1 U/L), lactate dehydrogenase ([LDH], 586 U/L; RR: 172–563 U/L) and a moderate increase in serum bile acid concentration (49.3 µmol/L; RR: 1.4–9.8 µmol/L), while total bilirubin was within normal limits. Plasma fibrinogen was within normal limits and serum amyloid A mildly increased (94.3 mg/L; RR: 0.5–1.2 mg/L). Elevated enzyme activities of blood urea nitrogen ([BUN], 13.2 mmol/L; RR: 2.8–7.7 mmol/L), and creatinine (181 µmol/L; RR: 57–121 µmol/L), were also present. To further assess liver function, prothrombin time, partial thromboplastin time and ammonia were determined and were within normal limits.

A complete ultrasonographic examination of the abdomen was performed using a broad-spectrum convex transducer, which showed that the liver was surrounded by anechogenic free fluid. The liver on the left was slightly heterogeneous with one small hypo- to anechogenic round structure of less than 1 cm. The liver on the right side was interspersed with several large well-defined cystic lesion measuring about 10 cm. One of the cysts showed a detached, irregular membrane inside, which appeared to float within the contents of the cyst. Little normal liver tissue remained between the cystoid structures (Figure 1).

WORKING DIAGNOSIS

Based on the serum biochemical profile and the multiple cystoid structures in the liver, the differential diagnosis included parasitic infection such as echinococcosis, congenital biliary cysts, a chronic inflammatory process, obstructive cholangiohepatitis, or neoplasms such as cystadenoma or cystadenocarcinoma. Further diagnostic procedures, such as ultrasound-guided aspiration of the cyst fluid and a liver biopsy were not performed due to the risk of potential cyst rupture and spread of the parasites. For prognostic purposes, to assess the extent of the cysts in relation to other organs and vital structures, to identify the presence of multi-organ involvement and to increase the likelihood of a correct diagnosis, a full-body computed tomography scan was discussed with the owner. However, due to financial constraints they decided to only pursue supportive therapy and monitor the disease progression.

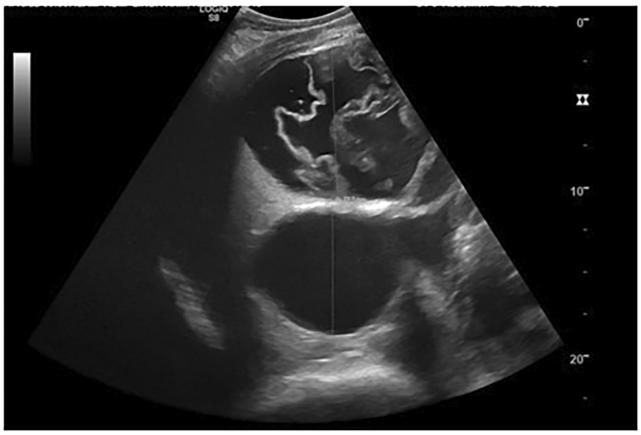

FIGURE 1 Sonogram obtained via transabdominal ultrasonography of the right liver. The diameter of the cysts are 9.5 and 7.7 cm, respectively.

TREATMENT

A short-stay 16-gauge intravenous catheter was placed in the left jugular vein. The donkey received a continuous infusion of lactated Ringer's solution (4 mL/kg bwt/h, Bichsel AG). Glucose continuous rate infusion (1 mg/kg/min, Energiedex ad us. vet., Vetoquinol AG) was administered to treat hyperlipidaemia. Trimethoprim-Sulfadimidin (30 mg/kg bwt per os q. 12 h, Rota TS Oraldoser ad us. vet., Vetoquinol AG) was selected to provide broad-spectrum antimicrobial coverage as bacterial cholangiohepatitis could not be excluded at this stage. In addition, anti-inflammatory treatment with meloxicam (0.6 mg/kg bwt i.v. q. 24 h, Inflacam® 20 mg/mL ad us. vet. Virbac (Switzerland) AG) was started. As the elimination half-life in donkeys is shorter than in horses, more frequent administration of meloxicam is usually recommended to provide adequate analgesia (Mahmood & Ashraf, 2011). However, as the donkey had a history of mild abdominal pain, meloxicam was given only once a day to avoid masking clinical signs associated with the gastro-intestinal tract. Antioxidative treatment with d-alpha-tocopherol (10 IU/kg bwt per po q. 24 h; Elevate® W.S.), was also provided. Although widely discussed in the literature, heparin sodium (50 IU/kg bwt q. 12 h, Heparin Natrium, Braun Medical AG) was administered to stimulate

lipoprotein lipase activity to improve triglyceride clearance from the circulation, as there were no abnormalities in the coagulation profile or platelet count (McKenzie, 2011). The donkey was offered a variety of diets on a voluntary basis for correction of the negative energy balance. These included carrots, apples, fresh grass, soaked beet pulp with molasses and corn syrup (Karo Light Corn Syrup) mixed with concentrates.

OUTCOME

The donkey ate small quantities of the food offered. Plasma triglycerides were repeated after 24 h of treatment and returned almost to normal (2.4 mmol/L; RR: 0.3–2.3 mmol/L). Liver-derived enzymes were repeated after 3 days of treatment. Gamma-glutamyltransferase (2644 U/L; RR: 15–72 U/L), SDH (19.1 U/L; RR: 0.9–5.8 U/L), GDH (193 U/L; RR: 1.4–19.1 U/L), and serum bile acid concentration (54.4 μmol/L; RR: 1.4–9.8 μmol/L) continued to rise, indicating progressive liver damage. Due to the poor prognosis, the owner elected for euthanasia. Post-mortem examination including histopathological evaluation was carried out with the owner's consent.

POST-MORTEM FINDINGS

The Institute's policy is to take the following actions if a zoonotic disease is suspected. The veterinary pathologists wore FFP2 masks, glasses and two pairs of gloves during necropsy. At necropsy, the euthanised animal was found to be in moderate body condition. The main gross findings were multiple large cysts in the liver, reaching from dorsal to ventral, up to 20 cm in diameter. To prevent cystic fluid to contaminate the section hall and to get in contact with any person, the liver was removed in toto and immediately put into an aluminium bowl (Figure 2) under a Microbiological Safety Cabinet Type BDK-SB (Class II, according to EN 12469:2000, with HEPA filter), where further examination of the tissue and sample collection took place. The cysts were filled with clear and partly reddish-clear fluid or beige soft to friable material (Figure 2). The walls of the cysts were white with a slightly irregular luminal surface. In the left lobe of the liver there were multiple white nodules varying in size up to 2 cm in diameter, which were hardly cuttable. Other macroscopic findings included severe ascites (approximately 15 L) and moderate thoracic effusion (2 L). Samples from all major organs were collected, fixed in 10% buffered formalin and routinely processed for histopathological examination.

HISTOPATHOLOGICAL FINDINGS

Histologically, multiple hydatid cysts in the liver could be detected. A few cross-sections of metacestode protoscoleces, which have a parenchymatous body and rostellar hooks (Figure 3) were detected in the described cysts in the liver and in multiple dilated biliary ducts. The hydatid cysts were surrounded by a capsule of connective tissue and were lined by an acellular laminated layer which had a narrow, single-layered, nucleated cell layer towards the lumen which often contained calcareous corpuscles. The macroscopically beige material in some cysts presented histologically as cellular debris mixed with remnants of laminated layers, few intact and degenerating neutrophils and macrophages, and numerous necrotic protoscoleces. Between the cysts, only scattered islets of hepatocytes were visible, mostly replaced and surrounded by wide bands of fibrous tissue connecting the portal fields (bridging fibrosis). The little remaining hepatic parenchyma consisted of few intact hepatocytes, regenerative nodules, and bile duct hyperplasia with biliary stasis.

PARASITOLOGY

Liver cyst material was analysed for the presence of *E. granulosus* sensu lato DNA. Amplification and sequencing of a part of the mitochondrial gene for the small subunit of ribosomal RNA identified the parasite as *E. equinus* (Trachsel et al., 2006). Sequence homology

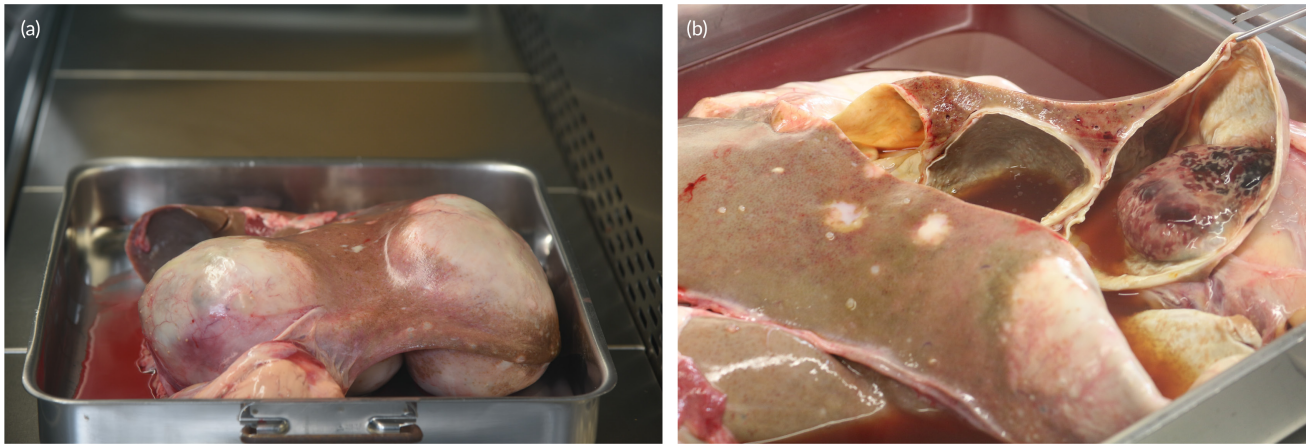

FIGURE 2 Multiple large cysts in the liver (a) filled with clear and partly reddish-clear fluid or beige soft to friable material (b).

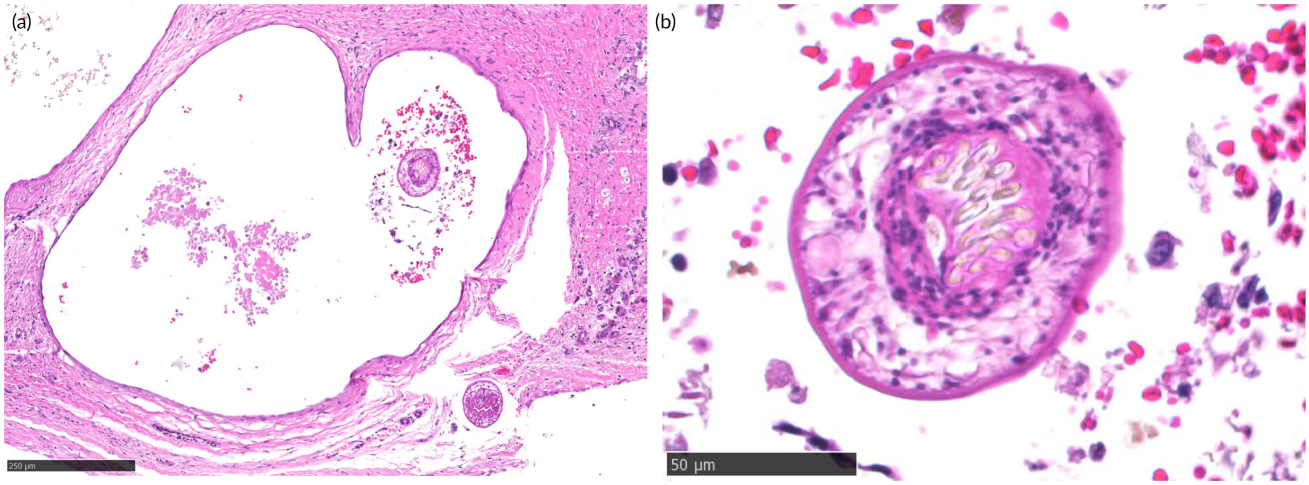

FIGURE 3 Typical parasitic material found inside the hydatid cyst surrounded by a capsule (a) and a cross-section of a metacestode protoscoleces (b).

with corresponding *E. equinus* sequences published in GenBank was >99% (190/191 bp; e.g. KY766905).

DISCUSSION

The present report describes a case of CE of the liver due to *Echinococcus equinus* of a 22-year-old donkey from Switzerland who presented with lethargy and anorexia. The diagnosis was made post-mortem. In humans, imaging studies, especially ultrasonography and chest radiography are the main diagnostic tests for CE while computer tomography, and magnetic resonance imaging, can also prove useful (Mihmanli et al., 2016; Moro & Schantz, 2009; Stojkovic et al., 2012). Percutaneous fine needle aspiration (FNA) under ultrasound guidance is used in suspected cases with equivocal radiological and serological test results (Agudelo Higuita et al., 2016). However, percutaneous procedure requires meticulous care due to the associated risk of anaphylaxis, cystic rupture, and metastatic spread (Junghanss et al., 2008). Therefore, in order to avoid the aforementioned complications, it was decided not to aspirate the cystic fluid in this donkey. Important to note is that transmission to humans (accidental intermediate hosts) occurs by the uptake of parasite eggs in contaminated food, water or soil, or by direct contact with infected definitive hosts (carnivores; Macin et al., 2021). For humans, therefore, contact with hydatid cysts (metacestodes) is not a route of infection (Agudelo Higuita et al., 2016).

Post-mortem examination revealed multiple large cysts causing massive loss of liver parenchyma with sequential fibrosis and regenerative nodules. This led to a severe loss of liver function, indicating that the cysts were not just an incidental finding, but were in fact pathological.

Echinococcus equinus is assumed to be the species that is most closely adapted to donkeys and other equidae as intermediate hosts, achieving fertility rates of up to 68% (Mulinge et al., 2023). Data on prevalence of CE in donkeys are few worldwide, e.g. from Tunisia 8.5% (Lahmar et al., 2014), eastern Africa 5.7% (Mulinge et al., 2023), Morocco 4.2% (Pandey, 1980) and, Egypt 6.9–15.5% (Aboelhadid et al., 2013; Ahmed et al., 2018; Barghash et al., 2017; Desouky et al., 2017; Haridy et al., 2008; Mahdy et al., 2014). However, only a few studies have identified *E. equinus* as causative species (Aboelhadid et al., 2013; Boufana et al., 2015; Desouky et al., 2017; Lahmar et al., 2014; Mousa et al., 2020). In Europe, *E. equinus* appears to be endemic in the United Kingdom (Boufana et al., 2015), Ireland (Torgerson & Budke, 2003), Spain (Carmena et al., 2008), and Italy (Varcasia et al., 2008). However, CE cases have also been reported in horses from geographical areas where *E. equinus* is not endemic. In these cases, the affected animals had either been imported from an endemic area or had been raised in close proximity to dogs imported from such an area and carrying adult *E. equinus* stages (Blutke et al., 2010; Hoberg et al., 1994; Merz et al., 2017).

Regarding the present case, the donkey was born in Switzerland and lived for 13 years on the same property in northern Switzerland. The donkey was kept on a pasture all year round and no stays outside Switzerland were known. Next to the premises was a dairy farm with a free-running farm dog. Since the infection must have originated from the uptake of *E. equinus* eggs from the faeces of an infected final host, this dog is one of the possible sources. However, there was no information available on the dog's breed, origin, stays abroad or diet. Although it seems very unlikely in view of the regulations on the disposal of slaughterhouse waste in Switzerland, dogs or wild carnivores (e.g. foxes) of local origin with access to infectious horse/donkey waste must also be considered as potential sources of *E. equinus* eggs. Under Swiss law, dead Equidae and slaughterhouse waste showing signs of diseases transmissible to humans or animals must be disposed in accredited rendering plants, and there are strict legal requirements for the treatment of slaughterhouse waste and its use as pet food (Schweizer Bundesrat, 2018). Therefore, the route of the donkey's infection remains purely speculative. This underlines the importance of better assessing the epidemiological situation and the prevalence of *E. equinus* in its definitive hosts, with particular attention to wild carnivores. So far, the potential role of foxes in the *E. equinus* life cycle remains uncertain (Eckert & Thompson, 1988).

The present case may be epidemiologically significant and should warrant investigation of possible transmission patterns of *E. equinus* among equine hosts in Switzerland. It is possible that the equine hydatid parasite will spread into areas where equine hydatidosis is not yet endemic. Research on *Echinococcus* transmission in Switzerland is also not without relevance to humans, as *E. equinus*, long thought to be of no zoonotic importance, has recently been reported as the causative agent of human CE (Kim et al., 2020; Macin et al., 2021). In Switzerland, echinococcosis is an animal disease which is under surveillance and must therefore be report.

CONCLUSION

This case report emphasises that any suspected case of equine cystic hydatidosis detected post-mortem or at slaughter, or, as in the present case, considered as a differential diagnosis due to cystic lesions within the liver, should be thoroughly investigated including molecular identification of the cysts.

AUTHOR CONTRIBUTIONS

Isabelle L. Piotrowski: Writing – original draft; conceptualization; investigation. **Rosalie Fabian:** Visualization; writing – original draft. **Stefanie M. Ohlerth:** Visualization; writing – review and editing. **Felix Grimm:** Writing – review and editing. **Meret E. Wehrli Eser:** Writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

ETHICS STATEMENT

No ethical review required – retrospective case report of clinical case.

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