CYSTIC FLUID FROM A LIVER LESION IN A HORSE

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Signalment:

15-year-old Cob mare

Clinical history:

One-year history of muscle and weight loss despite a normal appetite and no evidence of dental disease. Vaccinations and deworming were up to date. The horse was foaled and raised in England and it had never been abroad.

Clinical findings:

Temperature, pulse and respiratory rate were within normal limits. Thoracic and abdominal auscultations revealed no abnormalities and increased digital pulses or warm feet were not noted on physical examination.

Diagnostic procedures:

Initial clinicopathological data (Table 1 - April) revealed hyperproteinemia, hyperglobulinemia and a low albumin:globulins ratio. Hyperfibrinogenemia (heat precipitation method) and mildly increased CK activity were also detected. Endogenous ACTH was within its reference interval for the time of the year.

	April*	**May****	**December***	**Units**
WBC	9.6 (6.0-12.0)	11.8 (6.00-12.00)	11.2 (6.0-12.0)	$x10^9/l$
Neutrophils	6.0 (2.7-6.0)	**8.6 (3.00-7.00)**	**6.4 (2.7-6.0)**	$x10^9/l$
Lymphocytes	2.6 (1.5-5.0)	2.0 (1.50-5.00)	4.0 (1.5-5.0)	$x10^9/l$
Monocytes	0.4 (0.0-0.7)	0.7 (0.20-1.00)	0.3 (0.0-0.7)	$x10^9/l$
Eosinophils	0.6 (0.0-0.9)	0.5 (0.00-1.00)	0.4 (0.0-0.9)	$x10^9/l$
RBC	7.21 (5.50-9.50)	5.87 (5.50-9.50)	6.06 (5.50-9.50)	$x10^{12}/l$
Haemoglobin	12.5 (8.0-14.0)	10.9 (8.00-14.00)	10.5 (8.0-14.0)	g/dl
Hct	34 (24.0-44.0)	29.8 (24.00-44.00)	29.0 (24.0-44.0)	%

MCV	47.2 (39.0-52.0)	50.8 (39.00-52.0)	47.9 (39.0-52.0)	fl
MCH	17.3 (15.2-18.6)	18.6 (15.20-19.00)	17.3 (15.2-18.6)	pg
MCHC	**36.8 (30.0-35.0)**	36.7 (30.00-37.00)	**36.2 (30.0-35.0)**	g/dl
Platelets	210 (90-400)	184 (90.00-400.00)	252 (90-400)	$x10^9$/l
Total protein	**91.2 (55.0-80.0)**	**88 (52.00-80.00)**	**88.6 (55.0-80.0)**	g/l
Albumin	28.3 (25.0-38.0)	28.0 (28.00-39.00)	**23.2 (25.0-38.0)**	g/l
Globulin	**62.9 (20.0-43.0)**	**60.0 (13.00-52.00)**	**65.4 (20.0-43.0)**	g/l
Albumin:globulin ratio	**0.4 (0.5-2.4)**	0.5	**0.4 (0.5-2.4)**	
Fibrinogen	**4.9 (1.0-4.0)**		**5.7 (1.0-4.0)**	g/l
Serum Amyloid A	0.1 (0.0-10.0)		0.0 (0.0-10.0)	mg/l
Urea	5.4 (2.5-8.3)	5.8 (3.57-8.93)	6.9 (2.5-8.3)	mmol/l
Creatinine	76 (50-155)	**54 (62.00-159.00)**	75 (50-155)	umol/l
Bile acids	4.2 (0.0-20.0)		6.5 (0.0-20.0)	umol/l
Total bilirubin	13.4 (10.0-40.0)	14.0 (0.00-43.00)	12.9 (10.0-40.0)	umol/l
GGT	23.0 (0.0-34.0)	40.0 (10.00-55.00)	24.0 (0.0-34.0)	u/l
AST	388 (10-594)		458 (10-594)	u/l
ALP	240 (1-250)		**272 (1-250)**	u/l
CK	**560 (0-540)**		**654 (0-540)**	u/l
GLDH	4.6 (0.0-12.0)		9.3 (0.0-12.0)	u/l
Calcium	3.11 (2.60-3.50)	3.24 (2.75-3.55)	3.12 (2.60-3.50)	mmol/l
Phosphate	0.81 (0.80-1.80)		0.99 (0.80-1.80)	mmol/l
Potassium	4.24 (2.80-5.30)	3.8 (3.20-5.50)	3.88 (2.80-5.30)	mmol/l
Sodium	135 (134-145)	139 (130.00-142.00)	134 (134-145)	mmol/l
Chloride	99 (98-102)	100 (95.00-108.00)	98 (98-102)	mmol/l
Triglycerides	0.10 (0.01-0.87)		0.15 (0.01-0.87)	mmol/l
ACTH	11.80 (<30)		20.90 (<30)	pg/ml
Glucose		6.2 (4.44-6.61)		mmol/l

*Sysmex XT-2000i, Milton Keynes, UK and Beckman Coulter, High Wycombe, UK

**Boule Medical AB, Boule Diagnostics AB, Spånga, Sweden and Fuji Film Dri-Chem NX500i, St Martins Way, UK

Table 1 – Haematology, biochemistry and endocrinology laboratory test results

(reference intervals between brackets)

Ultrasonographic examination of the abdomen revealed a mildly increased wall thickness of the large colon on the right side of the abdomen and an approximately 47 mm in diameter, hypoechoic, cystic structure in the liver (Figure 1). Doppler mode showed this structure not to be vascularised.

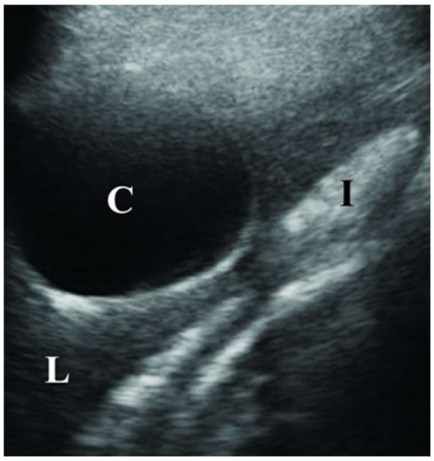


Figure 1 – Abdominal ultrasound (L - liver; C – cyst; I – intestine)

Clinical differential diagnoses for the liver lesion included a neoplasm, abscess and parasitic cyst.

Treatment and follow-up:

The horse was treated with albendazole (25 mg/kg; 400 mg/tablet, PO, BID for 30 days), doramectin for feather mites ([10mg/ml], 3 ml/100Kg, 50 ml IM, 2 weeks apart) and suxibuzone (3g/day, 1.5 g sachets, PO, BID). Clinical re-assessment revealed loud gut sounds on both sides and short-term, self-resolving diarrhoea. In-house general clinicopathological data (Table 1 – May) showed a mild, mature neutrophilia, mild hyperproteinemia with moderate hyperglobulinemia and low creatinine. Electrolytes were within their respective reference intervals.

Faecal parasitology and bacteriology (*Clostridium perfringens* and *Clostridium difficile* toxins, *Salmonella* spp and *Campylobacter* spp) were negative.

Six months later, a repeat ACTH measurement (Table 1 – December) was also within its reference interval. At this stage, self-resolving diarrhoea recurred. A third haematology and biochemical profile revealed mild, mature neutrophilia, hyperproteinemia, hypoalbuminemia, hyperglobulinemia, hyperfibrinogenemia, high ALP activity and high CK activity. Dexamethasone (20 ml, 0.2% w/v) IM injections were given once a day for approximately 1.5 months. After 13 days, the dose was reduced by 1 to 2 ml every week as there was a good clinical response.

Euthanasia was elected approximately 1 month later due to further weight loss and the owner's perception of poor welfare of the horse.

A full necropsy was carried out. In addition to poor condition, gross examination revealed one white liver cyst on the caudo-dorsal aspect of the right liver lobe (Figure 2) and a thickened area of the small intestine.

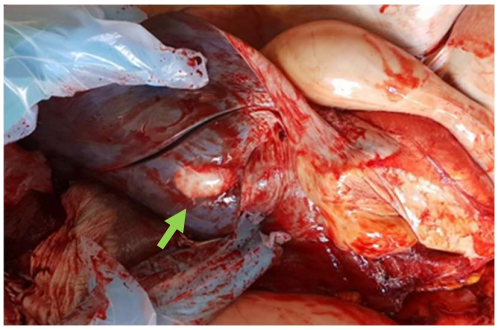


Figure 2 – Liver cyst (green arrow) noted during necropsy

When cut open, the liver cyst was unilocular and it had a 1 to 2 mm thick, firm, white wall with many low, irregular folds protruding from the wall into the cavity. The cyst was filled with haemorrhagic fluid containing numerous floating, white, approximately 1 mm, round flecks (hydatid sand) (Figure 3).

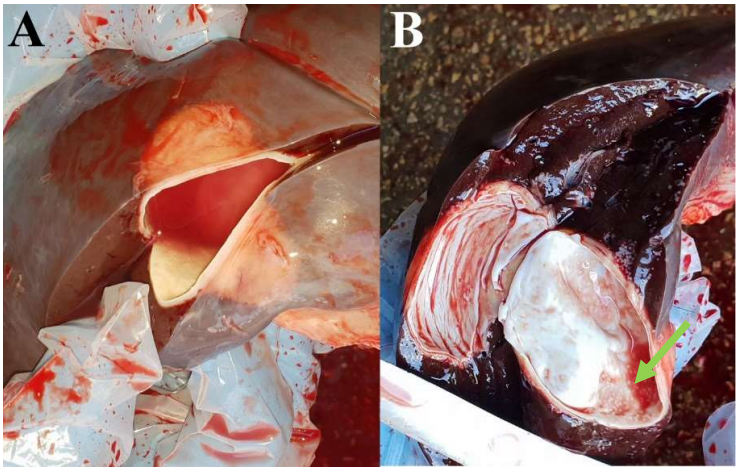
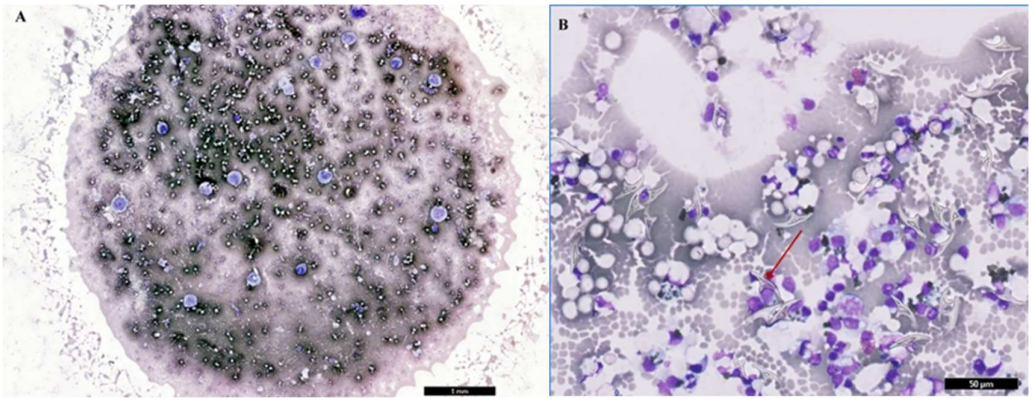


Figure 3 – Unilocular liver cyst filled with haemorrhagic fluid (A) and aspect of the inner wall with multiple folds and hydatid sand (green arrow) (B).

Fluid from the liver cyst was collected in an EDTA tube and submitted for cytological examination.



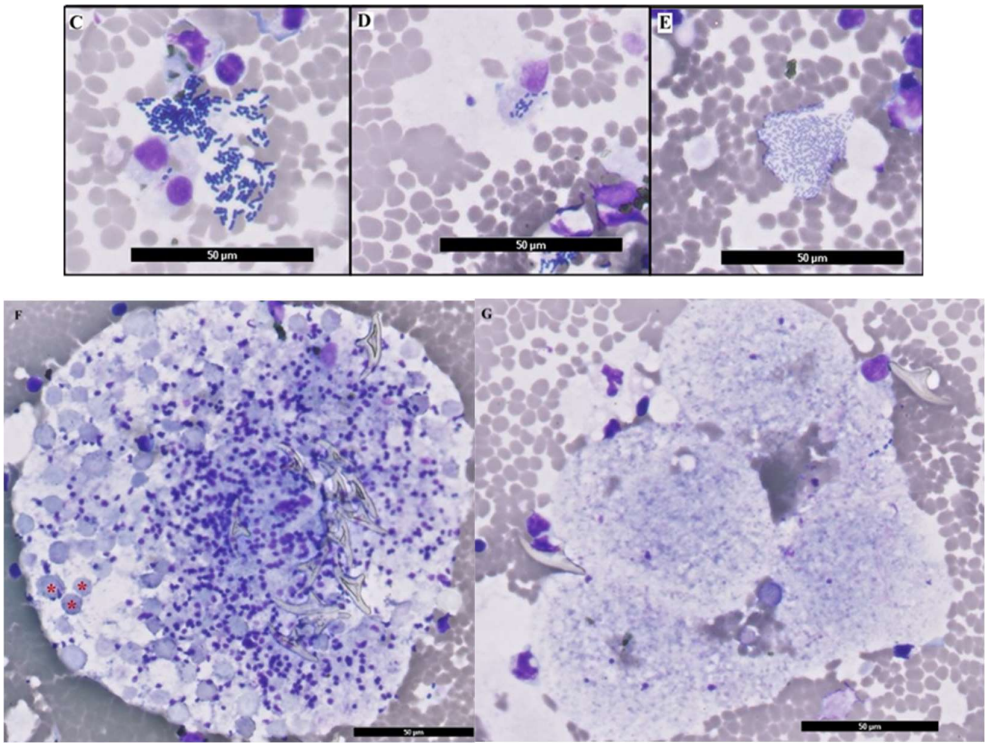


Figure 4 – Cytocentrifuge preparation of cystic fluid from a liver lesion (Wright-Giemsa stain)

Cytological examination of a cytocentrifuge preparation of the cystic fluid revealed a moderately cellular specimen on a moderate to heavy background of red blood cells (Figure 4A). Cellular preservation was moderate. A 500-nucleated cell differential count yielded 44% macrophages, 37% small lymphocytes and 19% neutrophils. Eosinophils and plasma cells were rare (Figure 4B). Bacteria (cocci and bacilli) were present in small numbers free in the background and rarely within the cytoplasm of degenerate macrophages/neutrophils (Figure 4, C-E). The cytoplasm of the macrophages often contained variably sized, coarse, dark-green staining material. Moderate numbers of palely to moderately basophilic, round to oval membrane-like structures measuring up to 100 µm in diameter were scattered throughout the cytological preparation (Figure 4, F and G). These structures were coarsely granular with none (Figure 4G) to many, approximately 2 µm in diameter, basophilic, round structures (calcareous corpuscles) (Figure 4F, red asterisks). Protoscoleces were occasionally noted and these were associated with variable number of hooks (21 µm in length and 10 µm in thickness at the widest part). Free hooks were also seen in small numbers in the background (Figure 4B, red arrow). Distinct suckers were not detected.

Automated total nucleated cell count and protein concentration in the cystic fluid were not determined due to insufficient sample.

PCR for *Echinococcus* spp was carried out on DNA extracted from the cytology slides of cystic fluid. Part of the mitochondrial cytochrome oxidase subunit 1 (cox1) gene was amplified by PCR. Sequences were identical (313/313 bp) to published sequences of *Echinococcus equinus* (i.e. GenBank KY766905).

Samples of the liver cyst and a thickened area of the small intestine were submitted in 10% buffered formalin for histopathological assessment.

The liver cyst wall was composed of an outer, thick, host-derived, adventitial layer of fibrous and fibroblastic tissue (Figure 5), the inner layers of which were necrotic with scattered macrophages, lymphocytes and plasma cells within the viable areas of the periphery (Figure 6). The adventitial layer was lined by a thin laminated membrane of palely eosinophilic to amphophilic material followed by a narrow, frequently separated, parasite-derived germinal membrane (Figure 5). Two, approximately 33 x 29 µm protoscoleces with an eosinophilic outer wall and many small,

basophilic, rounded bodies (calcareous corpuscles) together with a rostellum were noted in close association with the germinal membrane (Figure 7, inset).

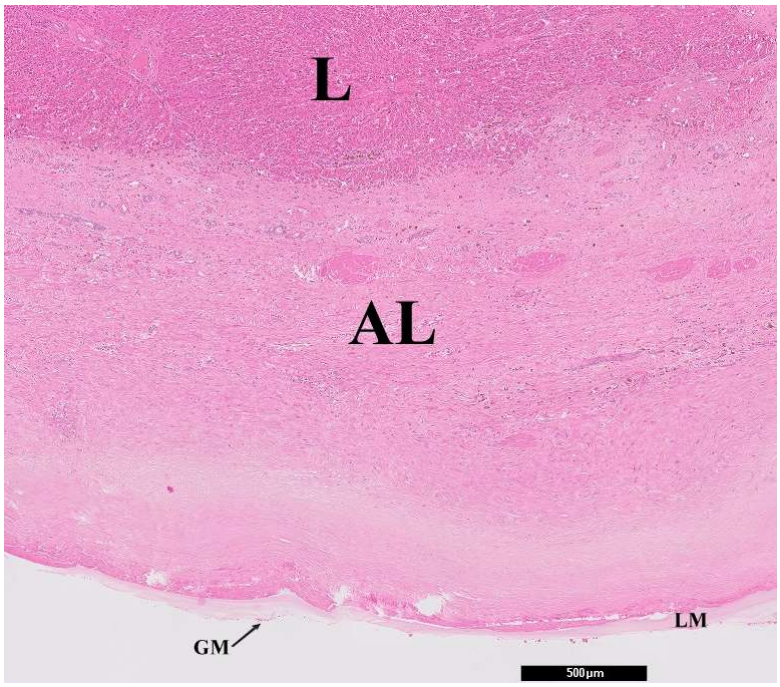


Figure 5 - Detail of the liver cyst wall, H&E stain.

(L – liver parenchyma; AL – adventitial layer; LM – laminated membrane;

GM – germinal membrane [arrow]).

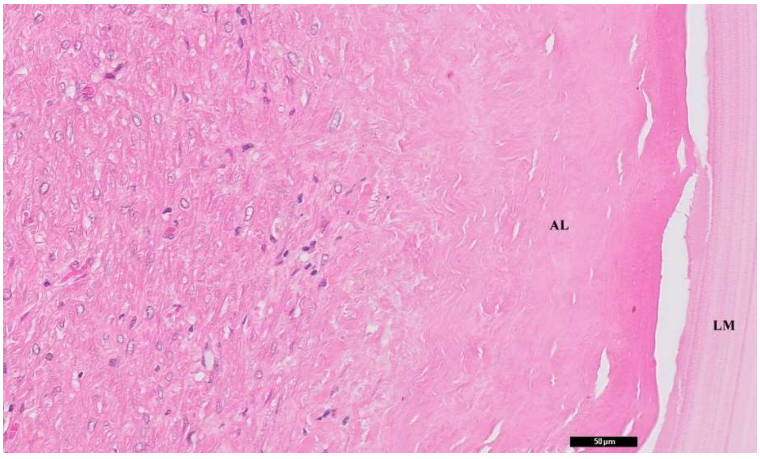


Figure 6 - Detail of the host-derived adventitial layer (AL). Note infiltration by inflammatory cells (macrophages, lymphocytes and plasma cells) on the left hand side and the necrotic appearance of the inner aspect (right hand side of the adventitial layer). LM – laminated membrane. H&E stain.

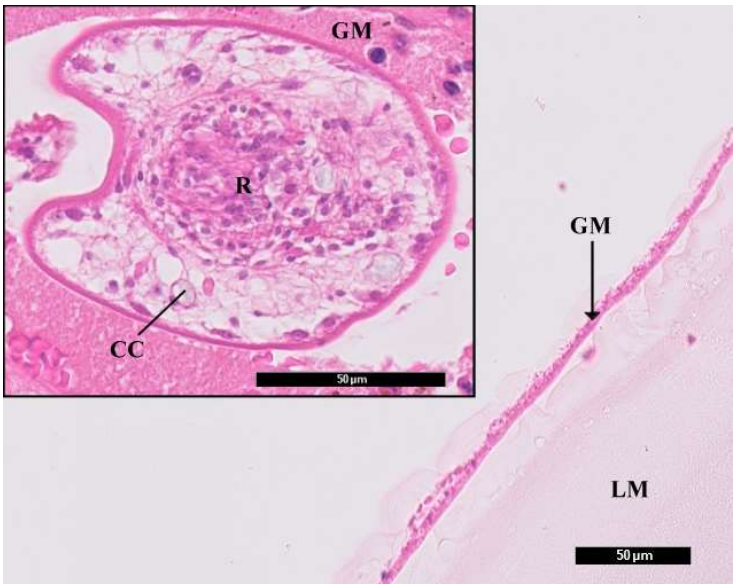


Figure 7 - Detail of the parasite-derived germinal membrane (GM - black arrow in main image).

LM – laminated membrane.

Inset: detail of a protoscolice (R – rostellum; CC – calcareous corpuscles). H&E stain.

The hepatic parenchyma adjacent to the cyst was compressed and portal areas occasionally appeared to be expanded by fibrous tissue with an occasional impression of portal-portal bridging and scattered, individualised or clustered haemosiderophages. Hepatocytes had granular, eosinophilic cytoplasm and frequently contained brown, granular pigment (suspected to be haemosiderin).

A section of small intestine was moderately affected by autolysis with loss of much of the surface epithelium and the tips of most villi. Within the lamina propria there was a multifocal, mild increase in the number of plasma cells and lymphocytes. This finding was interpreted as mild lymphoplasmacytic enteritis.

Discussion

To the authors' knowledge, the present case is only the second clinical report of molecularly confirmed, cystic echinococcosis caused by *E equinus* in a horse from the UK. Cytological examination of hydatid sand was included in the reports of *E granulosus* infection in the liver of two imported horses in the USA and *E equinus* echinococcosis in the lungs of a horse from Germany.[1,2]

The UK is currently considered to be free of *Echinococcus multilocularis*, but *E equinus* and *E granulosus* ss are known to be endemic, maintained primarily in horse/foxhound and sheep/dog transmission cycles, respectively.[3,4,5] Farm dogs may acquire *E equinus* infection if they have access to hydatic cysts from equids, for example, from unregulated slaughter and foxhounds are expected to have a high probability of exposure to *E equinus* as they are still frequently fed horse offal in some hunt kennels.[5] Some authors have hypothesised that a decline in the frequency of infection with *E equinus* in horses may follow the prohibition of fox hunting in the UK.[6] The last epidemiological study on equine

hydatidosis in the UK dates from the 1960-70s and it found a prevalence ranging from 10% to 50% throughout England, Wales and Ireland.[7] A small study on the molecular epidemiology of *E equinus* and *E granulosus* ss in the UK found that all 31 horse isolates (hydatid cysts collected at abattoirs) had 100% sequence identity with *E equinus*.[5]

E equinus is also endemic in Ireland, Spain and Italy. It is sporadically reported in Belgium and Switzerland and rarely in Germany.[2] Molecularly confirmed *E equinus* hydatidosis in equids has also been reported in Egypt, Tunisia, Namibia, Turkey and Kyrgyzstan.[2,5,8]

The taxonomy of the genus Echinococcus has undergone major changes in recent years.[9,10,11] At the beginning of the 1980s, there were four undisputed species (*E granulosus*, *E multilocularis*, *E oligarthra* and *E vogeli*) in this genus and taxonomic uncertainty was largely due to the limitations of morphological descriptions and lack of evidence for geographical or ecological segregation.[6,12] From the early 1990s onwards, phylogenetic analyses of Echinococcus populations based on sequence data from the mitochondrial cytochrome c oxidase subunit 1 (cox1), NADH dehydrogenase 1 (nad1), adenosine triphosphate 6 (ATP6) and small ribosomal RNA (12S rRNA) genes and the nuclear rDNA internal transcribed spacer 1 (ITS-1) revealed a series of largely host-adapted species and genotypes that are maintained in distinct cycles of transmission, now representing distinct species.[3,6,10] The agents of cystic echinococcosis (*E granulosus* sensu lato) have been subdivided into *E granulosus* sensu stricto (ss) (encompassing the former "sheep strain", genotype G1; "Tasmanian sheep strain", G2; and "buffalo strain", G3), *E felidis* (former "lion strain"), *E equinus* ("horse strain", G4), *E ortleppi* ("cattle strain", G5) and *E canadensis* ("camel strain", G6; "pig strain", G7; G9, probably a variant of the pig strain; and cervid strains, G8 and G10). Recently, a wildlife cycle of *E equinus* was discovered in the Etosha National Park of Namibia between lions and black-backed jackals as definitive hosts and plain zebras as intermediate hosts.[10] *E multilocularis*, *E oligarthra*, *E vogeli* and *E shiquicus* are the causative agents of alveolar and polycystic echinococcosis.[6,9,10]

The liver is the most common site of hydatid cysts in equids. Other less commonly reported locations include the lungs, cranium, brain, pericardium, pleura, spleen, kidneys, muscle, uterus and ocular structures.[1] One extradural spinal hydatid cyst causing hindlimb ataxia in a horse has been reported.[13] Hydatid cysts have a longevity of several years (at least 16 years) in equids and they are generally an incidental finding at slaughter and post- mortem examination with no ante-mortem clinical signs.[1,2,8] Equine cystic echinococcosis can be caused by various Echinococcus taxa, but only *E equinus* is known to produce fertile cysts.[2] Molecular confirmation is therefore required to identify the Echinococcus species involved.[10] Other differential diagnoses for the metacestode structures (e.g. hooks, calcareous corpuscles) were considered in this case, but there are no other known genera of cestodes (e.g. Taenia and Anoplocephala) which cause similar cysts in horses.

E equinus seems to be a highly specific parasite of Equidae (horses, donkeys and zebras) as intermediate hosts and it is assumed to be apathogenic to humans with no reported cases.[1,2,8,11] However, fatal hydatidosis by *E equinus* was recently reported in two captive lemurs born and raised in the UK (one red ruffed lemur [*Varecia variegata rubra*] and one ring-tailed lemur [*Lemur catta*]).[3,4] Additionally, similar cases have since been diagnosed in another lemur (black ruffed lemur [*Varecia variegata*]) and lar gibbon (*Hylobates lar*) (Dr Daniela Denk, personal communication). The natural infection of primates with *E equinus* suggests that the possibility of zoonotic susceptibility of humans should not be completely excluded, reinforcing the need for continued good hygiene, enhanced surveillance efforts and awareness of this disease.[4]

In the present case, the clinical signs of continued weight loss were attributed to intestinal disease with consequent malabsorption and protein loss whilst infection by *E equinus* was considered to be sub-clinical. A thickened area of large intestine was noted on ultrasound examination ante-mortem, but a rectal biopsy was not carried out due to owner's cost concerns. The clinically noted loud gut sounds and diarrhoea with a good clinical response to dexamethasone could support an underlying inflammatory enteropathy. Histopathological assessment of a section of thickened small intestine collected at necropsy revealed mild enteritis only, but it is possible that the intestinal sample

was not representative of the full degree of intestinal inflammation or that the absence of more marked inflammation was secondary to long-term treatment with dexamethasone. The consistent findings of hyperproteinemia, hyperglobulinemia and hyperfibrinogenemia were compatible with inflammation. The development of peripheral neutrophilia further supported inflammation and hypoalbuminemia may have been caused by protein losing enteropathy, inflammation or both. At one point, low creatinine was noted in-house and this analyte returned to its reference interval on the following measurement at a commercial laboratory. This was surprising as muscle mass loss would explain low creatinine, but its return to its reference interval, in the absence of evidence of dehydration (e.g. increased urea or hypernatremia), can only be explained by variability between the two analysers used. Increased CK activity in serum indicated skeletal muscle damage. The increased activity of ALP in serum detected in the last set of laboratories results for this horse could be due to gastrointestinal disease and, to a lesser extent, neutrophilia, as this enzyme is present not only in the liver, but also bone, intestine, kidney, placenta and leucocytes.[14] The increased MCHC in the absence of anaemia was most likely due to *in vitro* haemolysis.

Currently, there are no guidelines for the treatment of hydatid disease in horses. In human medicine, continuous administration of albendazole at an average dosage of 15 mg/kg/day is recommended in some cases[9] and this protocol was used as a template for the treatment of the horse in this case. At necropsy, the liver cyst in this horse was approximately the same size as estimated on abdominal ultrasonography and this was interpreted as evidence of no response to anthelmintic treatment.

The contents of cysts associated with hydatid disease are usually sterile. In the present case, the inflammation and presence of bacteria noted cytologically could represent post-mortem changes. Contamination of the cystic fluid by peripheral blood was unlikely as, if this was the case, predominance of neutrophils would be expected as a reflection of peripheral neutrophilia, but instead macrophages and small lymphocytes outnumbered neutrophils on cytology.

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