COMPANION OR PET ANIMALS

Lethal cysticercosis in a pet rabbit

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SUMMARY
A one-year-old neutered female crossbreed rabbit died unexpectedly after initially responding to symptomatic treatment over a three-month period for recurrent gut stasis, inappetence and lethargy. Postmortem examination revealed numerous fibrous tracks within the liver from which flatish ovoid parasites could be extruded. Parasites were also found in large numbers throughout the peritoneal cavity. Histopathology confirmed verminous hepatitis with numerous parasitic granulomas within the parenchyma of the liver containing intact and degenerate parasites. The severity of the parasitic burden and associated liver damage was the presumed cause of death. Intact parasites showed morphological features consistent with Taenia pisiformis at 6–15 days postinfection. Species identification was confirmed by PCR sequence analysis. The rabbit was fed on hay sourced from a local farm, commercially available nuggets and washed vegetables. It did not graze outside. Hay contaminated with dog or fox faeces was the presumed source of infection.

BACKGROUND
Cestodes are of major importance to animal and public health. The family Taeniidae (Taenia and Echinococcus species) are of particular note, since both intermediate and definitive host roles may be filled by companion animal and livestock species.1 Several Taeniidae are also notable for their zoonotic potential.

Definitive hosts, typically carnivorous species, harbour adult tapeworms which reside in the gastrointestinal tract. Infection occurs through the consumption of an infective metacestode larva contained within the body of an infected intermediate host, commonly livestock and other domestic and wild herbivorous species, which in turn become infected through the consumption of eggs passed in the faeces of the definitive host. Depending on the species the larval metacestode may undertake a range of morphological changes and migratory paths to become mature and reach their predilection site, resulting in a range of clinical presentations. Important factors with respect to clinical manifestation include intensity of infection, organs/tissues affected and type of metacestode (eg, cysticercus, hydatid cyst and so on). In many instances infection is apathogenic.2

In the definitive host, species identification through morphology is relatively straightforward.3 Morphological species identification of metacestodes, however, is more challenging due to within-species variation, particularly at different stages of migration and maturity.3–5 Furthermore, early migratory patterns and morphological features of different species can be very similar.6 7 In instances of aberrant larval migration and/or unusual clinical presentations, species identification is further complicated since predilection site and associated pathological changes cannot be used to differentiate one species from another.

Rabbits and other lagomorphs are intermediate hosts for several Taeniid species, most commonly Taenia pisiformis and T serialis.1 Additionally, rabbits may act as intermediate hosts of zoonotic cestodes including Echinococcus granulosus and, under experimental conditions, T hydatigena and T ovis, although infection with these latter two species does not produce infective metacestodes.11

T pisiformis is known to infect domestic pet rabbits through faeco-oral transmission from a canine definitive host, in the UK usually a dog or fox. Following the ingestion of food contaminated with eggs, hatched oncospheres penetrate the epithelium of the duodenum and jejunum and travel via the hepatic portal vein to the liver.8 In the liver oncospheres develop into cysticerci that migrate through the parenchyma before entering the abdominal cavity where they attach to the serosal surfaces.12 A mature cysticercus measures up to 18 mm, with a fluid-filled, transparent spherical or ellipsoid appearance, possessing a single invaginated scolex formed from the body wall.8 13 Presence of mature cysticerci within the abdominal cavity is the most common clinical presentation. These may be found during exploratory laparotomy or postmortem examination and are considered to be incidental findings unless infection is heavy.14–17

Relatively little is known of the prevalence and species diversity of cestode infections in rabbits because of their low pathogenicity and the limited opportunities available to diagnose infection. This article documents a case of atypical, pathogenic infection caused by larval T pisiformis (cysticercosis).

CASE PRESENTATION
Over a three-month period, a one-year-old neutered female crossbreed rabbit (2.4 kg) was presented three times for veterinary treatment of loss of appetite, lethargy and abdominal pain. The rabbit was one of three that lived in a house without access to the garden. The household contained no other domestic species. All three rabbits were fed on a diet of commercially available nuggets, fresh vegetables from the supermarket, dandelions from the owner’s enclosed garden and hay sourced from a local farm.


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The owner meticulously washed and rinsed all vegetables and dandelions before they were offered as feed.

On each of the three occasions the rabbit was presented for examination, it was hospitalised and treated symptomatically for gut stasis (see below for specifics). Hospitalisation was predominantly to facilitate treatment with medications the owner was unable to administer at home. However, during these periods abdominal radiographs and blood samples were taken at regular intervals for glucose, haematocrit and total protein using a portable glucometer, microhaematocrit and spectrometer, respectively. Blood results were consistently unremarkable for the duration of treatment apart from slightly raised glucose values, presumably due to stress.

On the first occasion, treatment consisted of syringe feeding followed by domperidone (0.5 mg/kg orally twice daily, Motilium Oral Suspension; Winthrop Pharmaceuticals), ranitidine (5 mg/kg orally twice daily, Zantac Syrup; GlaxoSmithKline) and meloxicam (0.15 mg/kg orally twice daily, Metacam 1.5 mg/ml oral suspension; Boehringer Ingelheim), subcutaneous fluids with Hartmann’s solution (10–20 ml/kg, Aquapharm No 11; Animalcare), tramadol (5 mg/kg subcutaneously twice daily, Zamadol; Meda Pharmaceuticals) and metoclopramide (0.5 mg/kg subcutaneously twice daily, Emeprid; Ceva Animal Health).

Abdominal radiographs revealed some mild non-specific changes, including increased radiodensity around the area of the uterine stump and displacement of the descending colon and rectum. Hence, due to a suspicion of bacterial involvement (eg, uterine stump abscess), antibiotics were administered in the form of trimethoprim/sulpha, with an initial dose by subcutaneous injection (48 mg/kg, Trimacare 24%; Animalcare) followed by oral drops (40 mg/kg orally twice daily, Sulfatrim Oral Drops; Virbac). The rabbit responded to treatment and was discharged after five days.

On the second occasion, symptomatic treatment (minus metoclopramide) and antibiotic therapy were continued. The rabbit again responded positively to treatment and was discharged after 14 days.

On the third occasion, nine days after the previous discharge date, the rabbit again presented with a recurrence of clinical signs. Hospitalisation and symptomatic treatment were continued as previously described. On this occasion antibiotics were given in the form of procaine benzylpenicillin (40 mg/kg subcutaneously every four days, Ultrapen; Norbrook).

Two days postadmission a rabbit-specific biochemistry and haematology profile (performed by Carmichael Torrance Diagnostics, Garforth) was unremarkable apart from a raised phosphate level of 2.1 mmol/l (reference range 1.1–1.6 mmol/l), a raised alanine aminotransferase level of 121 U/l (reference range 20–80 U/l) and eosinophilia of 4 per cent. The profile did not include bilirubin, aspartate aminotransferase or γ-glutamyltransferase. Serology for Encephalitozoon cuniculi was negative, with IgG and IgM antibody levels less than 1:40.

Unlike previous radiographs, a lateral view of the abdomen taken seven days postadmission revealed hepatomegaly (figure 1). Further diagnostic procedures, such as ultrasonography and possible exploratory laparotomy, had been scheduled for the following day; however, the rabbit died unexpectedly overnight. On the evening before death, the rabbit had been quiet, alert and responsive with a mildly elevated respiratory rate and a slightly raised blood glucose of 9.6 mmol/l. It had passed normal urine and faeces overnight and there were no signs of disturbance.

**INVESTIGATIONS**

**Postmortem examination**

At postmortem examination the rabbit was in good body condition, although the abdomen was distended with fluid. Investigation of the abdominal cavity revealed a large volume of fluid containing large numbers of unidentified small semitransparent parasites measuring 4–8 mm in length. The abdominal fluid was clear-yellow-tinged and proteinaceous, frothing readily when shaken. Small fibrinous strands were also present within the abdomen, and the abdominal fluid was observed to solidify when collected and left to stand. No adhesions were observed between any digestive structures. A mild lymphadenopathy was observed. The stomach and intestines appeared pale. The stomach contained food from syringe feeding the night before. Extensive sublumbar fat was present.

The liver was enlarged, thickened and firm with a generalised yellow-white discoloration of the surface (figure 2A). Multiple incisions into the parenchyma revealed numerous fibrotic tracks throughout the tissue from which parasites could be extruded (figure 2B). The parasites were not identifiable. A tentative diagnosis of liver fluke was made from the gross pathological changes and the presence of large numbers of parasites within fibrous tracks in the liver.
Apart from some mild agonal changes and pallor, no abnormalities were detected grossly in the spleen, intestines, reproductive tract, bladder, diaphragm, heart and lungs. The thoracic cavity contained some pleural fluid of a similar consistency to the abdominal fluid, but contained no parasites. The liver, heart, lungs, spleen, kidney, pyloric antrum, and jejunal, caecal and rectal sections were submitted along with a faecal sample to Abbey Veterinary Services for further pathological assessment. Twelve parasite specimens were collected from the abdominal fluid and submitted to Liverpool Veterinary Parasitology Diagnostics (LVPD) in either industrial methylated spirit to be transferred to a liver lobe showing numerous parasitic granulomas. The parasites are encompassed by a proliferative inflammatory reaction with fibrosis. (B) Higher magnification view of granulomas containing cysticerci. The parasites have an eosinophilic tegument encompassing a solid body without a digestive tract. A scolex is evident within one of the parasites in this view (arrow). The granulomas are present in the parenchyma of the liver. The triad of hepatic portal vein, hepatic artery and bile duct are unaffected (arrowhead). Scale bars denote relative magnifications and dimensions. Image credits: Abbey Veterinary Services.

Histopathology
Examination of several sections of the liver showed numerous discrete to coalescing granulomas containing substantial intact and degenerate parasites (Figure 3A). The parasites had an eosinophilic tegument enclosing a solid loosely arranged parenchymatous body without a cavity or digestive tract. An occasional scolex was evident (Figure 3B). The granulomas were composed of an inner core of macrophages, including foreign body-type giant cells and more peripherally located lymphocytes, plasma cells and heterophils, accompanied by varying degrees of fibrosis. Although large numbers of parasites were present in the parenchyma, none could be demonstrated within the bile ducts.

Small intestine and caecal sections showed well-differentiated enterocytes, while the propria displayed a moderate to heavy population of lymphocytes and plasma cells. Lung sections showed mild congestion and atelectasis with intracapillary heterophilia. No remarkable lesions were noted in the intestine, lungs, myocardium, kidney and spleen.

A diagnosis of verminous hepatitis, with numerous parasitic granulomas, was made from the histopathological examination. The lack of internal organs led to a provisional diagnosis of cestodes rather than trematodes.

Parasite species identification
Species identification was performed on specimens submitted to LVPD through a combination of morphological and molecular approaches.

Morphological identification
Grossly, specimens measured 4.8–8 mm x 0.75–2 mm and appeared as small, transparent, fluid-filled cysts with a broad anterior and narrow, tail-like posterior (Figure 4A). Specimens were prepared for further investigation by light microscopy using one of two separate staining techniques:

Modified Baxby staining,19 that is, no fixation, staining with steaming hot 1 per cent safranin for two minutes, then 5 per cent malachite green in 10 per cent ethanol for 30 seconds. These specimens were mounted in lactophenol.

Haematoxylin staining, that is, acid formal alcohol immersion overnight, manual flattening of specimens between two slides and staining with neat haematoxylin for 2.5 hours, decolourising in 1 per cent HCl in 70 per cent alcohol, dehydration in ascending grades of alcohol, then xylene and mounting in distyrene plasticizer xylene (DPX).

Examination by light microscopy following modified Baxby staining revealed features consistent with described morphologies of T. pisiformis cysticerci,3–6 that is, corrugated tegument, apical tegument invagination and invagination canal (Figure 4B). In addition, scolex and rostellar annule were revealed following haematoxylin staining (Figure 4C).

Collectively, observable morphological features in the specimens varied, indicating a spectrum of development and postinfection period of 6–15 days.

PCR sequence analysis
Specimens in industrial methylated spirit were transferred to molecular grade 70 per cent ethanol ahead of DNA extraction using QIAGEN DNeasy Blood and Tissue Kit following manufacturer’s recommendations (www.qiagen.com). Purified DNA was quantified using a NanoDrop (Thermo Fisher Scientific, Waltham, USA) before amplification by PCR using a previously published generic primer pair designed to amplify the Taeniidae mitochondrial 16 s ribosomal RNA gene (rrnL-F: 5’-TTATTTG-CCTTTTGCATCA-3’; rrnL-R: 5’-AAAAAGATCCTAGGGTC TTTCCG-3’). Sequence analysis of the PCR product revealed a 600 bp product. Subsequent purification of PCR product using QIAGEN QIAquick PCR Purification Kit was performed following manufacturer’s recommendations (www.qiagen.com) and samples were sent for sequence analysis (TabeSeq Service, Eurofins Genomics, Germany). Forward/reverse read consensus alignment using BioEdit (software) yielded a 558-bp sequence. A BLAST search of the nucleotide database using Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/blast.cgi), a 99 per cent alignment (2-bp difference) was found with a previously published mitochondrial genome of T. pisiformis (GenBank accession no MH005823).

DISCUSSION
T. pisiformis is historically a common parasite of domestic rabbits when reared in outdoor enclosures.20 Early accounts of the parasite describe the effects of light infection as apparent, and heavy infections as causing abdominal distension.
and discomfort. In rabbits farmed as food-producing animals, infection with *T. pisiformis* cysticerci has been linked to poor body condition, reduced weight gain and economic losses. A recent study demonstrated that *T. pisiformis* cysticercosis in rabbits has a profound impact on the endocrinology of female rabbits, resulting in significant reductions in both litter size and live birth weights.

While *T. pisiformis* cysticercosis is listed as a cause of liver damage by some authors, this stage of infection is generally not considered as a differential diagnosis for liver disease in rabbits. The fatal outcome in this case is therefore a notable exception. The severity of the liver damage was not evident from the vague clinical signs, while hepatomegaly was only seen on abdominal radiography on the day before the rabbit died. Similarly, blood sample results did not reflect the degree of liver damage, although the biochemistry profile used did not consider all liver parameters. Raised liver enzymes and low total protein values are reported to be associated with *T. pisiformis*. Ultrasonography would have demonstrated liver pathology. The rabbit had been scheduled for this on the day it died.

Should hepatic cysticercosis have been diagnosed while the animal was alive, treatment would not have been straightforward. First, the severity of liver pathology means a complete recovery would have been unlikely even with curative therapy. Secondly, while oral albendazole (20 mg/kg once a day for 15 days) has been shown to be both preventive and curative against *T. pisiformis* cysticercosis in rabbits, there would have been little chance of recovery even with curative therapy. The rabbit remained clinically healthy for the duration of the afflicted animal’s treatment and diagnostic work-up. Hay was sourced from a local farm with a dog that roamed freely over fields that were also frequented by foxes. Faecal contamination of this hay therefore seems to be the only potential source of infection. Given the high parasitic burden and the estimated age of all parasites examined, it is likely that infection in this case was acquired from the local farm with a dog that roamed freely over fields that were also frequented by foxes.

Under experimental conditions rabbits develop resistance to *T. pisiformis* infection from around eight weeks of age, while a primary exposure has been shown to protect against reinfection for up to 12 months. It is possible that some of the discrepancies relating to the stage of parasite development and duration of clinical signs in this case may therefore be explained by immunity resulting from a previous exposure: A study examining over 17,000 livers from eight-week to 10-week-old rabbits reared outdoors for meat detected hepatic cysticercosis in 9 per cent of livers examined. Most infected livers contained only a few fibrous tracks but, occasionally, granulomatous inflammatory responses were found associated with heavy infections. The authors postulated these marked inflammatory responses represent a protective immune response which effectively traps and kills the parasite within the hepatic parenchyma. The descriptions of the histological changes detailed in these instances closely match those present in the liver of the rabbit in this case report. Delayed migration of oncospheres and cysticerci through the liver may explain some of the disparity between the estimated stage of development of the cysticerci and the observed duration of illness.

The source of infection and heavy parasitic burden are another interesting aspect of this case. The rabbit belonged to a meticulous owner who washed all vegetables and dandelions offered in addition to nuggets and hay. The rabbit did not graze in the garden. The owner’s other two rabbits remained clinically healthy for the duration of the afflicted animal’s treatment and diagnostic work-up. Hay was sourced from a local farm with a dog that roamed freely over fields that were also frequented by foxes. Faecal contamination of this hay therefore seems to be the only potential source of infection. Given the high parasitic burden and the estimated age of all parasites examined, it is likely that infection in this case was acquired from the local farm with a dog that roamed freely over fields that were also frequented by foxes.
instance occurred as ingestion of a single very large infective dose of eggs, potentially an intact or recently degenerated gravid proglottid was responsible. Hay was also believed to be the source of cysticercosis in two laboratory rabbits that came from a specific disease-free colony.31

Presumably, many rabbits could be exposed to T.pisiformis eggs through consumption of contaminated hay, yet the severe pathology observed in this case is extremely uncommon. This case report is therefore intended to document and raise awareness of this unusual presentation and the options available to clinicians when approaching similar cases in the future.

While a presumptive diagnosis of T.pisiformis could theoretically be made based on morphological features and distribution of the parasites within the liver and peritoneum, the generic PCR sequence analysis added greater certainty to the species diagnosis. This was important in this instance, since the atypical nature of the case led the authors to consider the possibility of aberrant migrations and/or infection with other cestode species not typically associated with rabbits such as T. hydatigena. The 2-bp difference between the authors’ current PCR product sequence and the reference mitochondrial genome of T. pisiformis is likely the result of within-species genetic variation. This sequence has been submitted to the National Center for Biotechnology Information GenBank database (accession no MH005823).

In conclusion, this case report describes an unusual presentation of cysticercosis caused by T. pisiformis in a pet rabbit with severe liver pathology and an extremely high infectious burden. A combination of pathological, parasitological and molecular-based techniques was employed to reach the diagnosis. Such cases could represent an unusual, but noteworthy, differential diagnosis in rabbits presenting with similar clinical signs. Consideration should also be given to preventing transmission by identifying sources of infection to rabbits, administration of routine tapeworm treatments to dogs and avoidance of feeding them rabbit offal. This case also highlights the difficulty in ensuring that hay is free from contamination by dog or fox faeces.

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REFERENCES
