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Research paper

# Fatal echinococcosis in three lemurs in the United Kingdom—A case series

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ABSTRACT

Tapeworms of the genus *Echinococcus* reside in the small intestine of a number of carnivorous species, predominantly canids. In enzootic areas, hydatidosis caused by taeniid metacestodes can present a significant problem in accidental intermediate hosts, including humans. Whereas the United Kingdom is currently considered free of *Echinococcus multilocularis*, *Echinococcus granulosus* sensu stricto (s.s.) and *Echinococcus equinus* are endemic in the UK and have been reported in a variety of captive mammals. The presentation of echinoccocosis in non-human primates widely parallels disease in humans, and public health concerns are related to the four genera, *E. granulosus*, *E. multilocularis*, *Echinococcus* vogeli and *Echinococcus oligarthrus*. In contrast, sporadic outbreaks and individual hydatid disease cases in non-human primates have been associated with several *Echinococcus* and *Taenia* species. Here we describe three fatal cases of cystic echinococcosis in two captive ring-tailed lemurs (*Lemur catta*) and one captive red-ruffed lemur (*Varecia variegata rubra*) and provide molecular tapeworm characterisation. To the best of the authors' knowledge, this includes the first report of *Echinococcus ortleppi* in a UK born ring-tailed lemur and provides the first in depth case reports of echinococcosis due to *E. equinus* in UK born ring-tailed and red ruffed lemurs with detailed clinical and pathological findings. The cestode life cycle and implications for zoo collections are discussed.



## 1. Introduction

Taeniid tapeworms are obligatory parasites of mammals. Their pathogenicity usually relates to the distinctive metacestode stage, or hydatid cyst. Four species are known in the genus *Echinococcus*. Taeniid infections in humans and non-human primates results in cystic echinococcosis (CE) caused by *Echinococcus granulosus*, alveolar echinococcosis (AE) caused by *E. multilocularis*, and polycystic forms either caused by *Echinococcus vogeli* or *Echinococcus oligarthrus*. *E. granulosus* is most prevalent in countries of the temperate zones and infection has re-emerged in certain areas where it was once believed to be controlled (Moro and Schantz, 2009). The typical life cycle involves dogs and other canids including the red fox (*Vulpes vulpes*) as definitive hosts and a range of domestic and wild ungulates as intermediate hosts for the larval stages (Eckert et al., 2001). Herbivorous intermediate hosts are infected through uptake of taeniid eggs passed in the faeces of definitive hosts. Following ingestion, eggs hatch in the small intestine and release an oncosphere, which penetrates the bowel wall and migrates into the viscera, where it develops over months or years into fluid-filled metacestode cysts containing protoscolices. Definitive hosts in turn become infected by ingestion of infected tissues.

To date, studies have identified 10 distinct genetic types (G1—10) amongst the *E. granulosus* genus demonstrating geographic distinction, different host affinities and varying development rates, sensitivity to chemotherapeutic agents, and pathology (Thompson et al., 2001; Nakao et al., 2007). *E. granulosus* sensu lato (s.l.) species complex is the causative agent of cystic echinococcosis which includes *E. granulosus* sensu stricto (s.s.) (G1–G3 genotypes), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5) and *E. canadensis* (G6–G10) (Nakao et al., 2007; Thompson, 2008). In the UK, *E. granulosus* (s.s) and *E. equinus* circulate endemically (Thompson and McManus, 2002; McManus and Thompson, 2003), whereas the G5 strain (*E. ortleppi*) has to date only been reported in a zoo ungulate imported from France (Boufana et al., 2012). *E. granulosus* (s.s.) is the causative agent of human CE contracted in the UK,

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and recent case reports confirm human infections with *E. granulosus* (s.s.) (Nakamura et al., 2011; Craig et al., 2012; Boufana et al., 2015). To date, *E. equinus* is not known to be zoonotic (Thompson and McManus, 2002).

Whereas echinococcosis in domestic animals is often benign and not associated with clinical symptoms, the mortality rate is high in untreated primates and wild or captive herbivores (Eckert and Deplazes, 2004; Moro and Schantz, 2009; Boufana et al., 2012). Infections with metacestodes of a variety of taeniid species have been described in non-human primates with limited to severe clinical consequences (Shahar et al., 1995; Tappe et al., 2007; Luzon et al., 2010; Boufana et al., 2012; De Liberato et al., 2014). Individual fatal tapeworm infections in Lemuroidea could be attributed to *Taenia martis* metacestodes in a ring-tailed lemur (*Lemur catta*) living in an Italian zoological garden (De Liberato et al., 2014), *Taenia crassiceps* cysticercosis in a ring-tailed lemur from Madrid Zoo (Luzon et al., 2010), a black lemur (*Eulemur macaco macaco*) and red ruffed lemur in the United States (Dyer and Greve, 1998; Young et al., 2000), *E. multilocularis* in ring-tailed lemurs in a Japanese and French zoo, respectively (Kondo et al., 1996; Umhang et al., 2013) and *E. granulosus* in a ring-tailed lemur in a zoological collection in Israel (Shahar et al., 1995). Two of the echinococcosis cases in lemurs included in this case series were previously described in brief in a survey of UK tapeworm cases in exotic species (Boufana et al., 2012, 2015). In the following report we present detailed clinical and pathological findings and summarise molecular genotypic data for three cases of larval cestode infection detected at post-mortem examination in captive UK lemurs including the first case of *E. ortleppi* (G5 genotype) infection in a UK born and raised ring-tailed lemur.

## 2. Material and methods/results

### 2.1. Case 1

A six year old adult female red ruffed lemur (*Varecia variegata rubra*) was found dead in its enclosure in June 2011. The animal had been captive born in 2004 in Suffolk, United Kingdom, and was moved to Essex in 2005. It lived in a naturalistic outdoor exhibit and had shown no prior signs of illness. On post-mortem examination, large numbers of cystic larval cestode parasites distended the abdominal cavity, both free floating, embedded within mesenteric and omental connective tissues, and on the surfaces of liver and spleen. Cysts ranged in size from approximately 2 to 40 mm diameter, and from clear to opaque in colour. Serosal roughening and hyperaemia were consistent with diffuse polyserositis. No significant abnormalities were detected in the thoracic cavity.

A set of tissues was routinely fixed in formalin and processed for histology. Histological examination confirmed the presence of significant fibrinosuppurative peritonitis accompanying abundant parasitic cysts, both intact and degenerating (accumulations of fibrin, granulocytes including eosinophils and macrophages around folded capsular material). Numerous protoscolices, both within cysts and free floating were observed (Fig. 1). Mild haemosiderosis of liver and spleen, considered within normal limits, was the only additional finding. Parasitic cysts were preserved in 95% ethanol, and genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A 396 bp fragment within the cytochrome *c* oxidase subunit 1 mitochondrial gene (*cox 1*) (Bowles et al., 1992; Bart et al., 2006) amplified using the extracted DNA exhibited 99% homology to *E. equinus* (Accession no. EF143834) (Boufana et al., 2012).

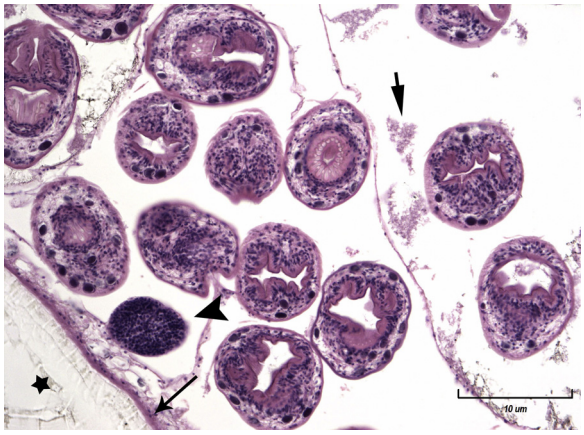


**Fig. 1.** Case 1. Hydatid cyst of the diaphragm showing the thick, acellular, adventitial layer (star) and the thin germinal layer (long arrow) lining the brood capsule which is filled with numerous *E. equinus* protoscolices. Invaginated scolices (tip and arms of arrow head) arise as buds from the inner surface of the brood capsule which also comprises hydatid "sand" (short arrow). H&E, ×20.

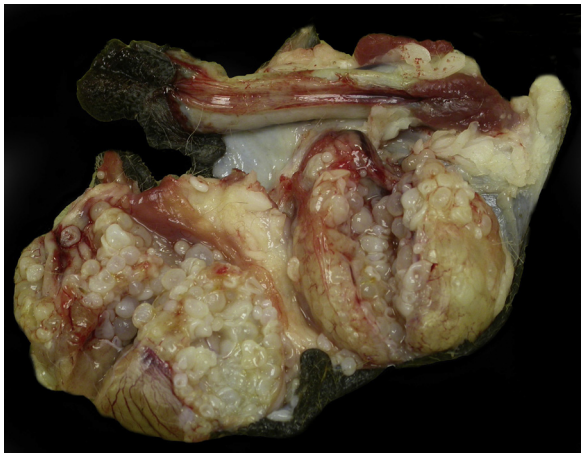

**Fig. 2.** Case 2. Enlarged scrotum and penis with opened urethra. On incision the vaginal tunics are packed with multiple free-floating and adherent larval cestode cysts, ranging from approximately 1–6 mm diameter. The testes are compressed by the abundant parasites, and parasitic cysts are tightly attached to the testicular capsule.

### 2.2. Case 2

A five year old male ring-tailed lemur (*Lemur catta*), which was born in Essex in 2007 into the same lemur troop as animal 1 and remained in the collection, presented in April 2012 with persistent penile prolapse. Clinical examination under gaseous anaesthesia revealed paraphimosis. The testes appeared hard on palpation and the bladder was distended. The animal had difficulties urinating which continued despite catheter placement and supportive care including antibiotic treatment. The caudal abdomen became distended and an exploratory laparotomy revealed numerous cystic masses in the abdomen. Due to the poor prognosis the animal was euthanased. At necropsy, numerous individual free-floating larval cestode cysts ranging from 1 to 6 mm in diameter were present within the abdominal cavity. Typically, these were translucent to cream coloured, fluid-filled or collapsed, and distributed at random over serosal surfaces. An extensive mass of adherent, densely packed metacestode cysts was present dorsal to the bladder, surrounding the colon and rectum and filling much of the volume of the pelvis. The bladder wall was thickened, mucosal surfaces roughened with multifocal petechial haemorrhages, and the lumen contained mucopurulent turbid yellow urine/exudate. Upon inci-