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Echinococcus and *Taenia* spp. from captive mammals in the United Kingdom

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ABSTRACT

Taeniid tapeworms which include *Echinococcus* and *Taenia* spp. are obligatory parasites of mammals with pathogenicity usually related to the larval stages of the life cycle. Two species (or genotypes) of *Echinococcus*, *E. granulosus sensu stricto* and *E. equinus*, as well as several *Taenia* spp. are endemic in the UK. Here we report on the occurrence of larval cystic stages of *Echinococcus* and *Taenia* spp. in captive mammals in the UK. Using molecular techniques we have identified *E. granulosus* (G1 genotype) in a guenon monkey and a Philippine spotted deer; *E. equinus* in a zebra and a lemur; *E. ortleppi* in a Philippine spotted deer; *E. multilocularis* in a macaque monkey and *Taenia polyacantha* in jumping rats. To the best of our knowledge this is the first report of *E. multilocularis* in a captive primate translocated to the UK. As far as we know these are the first reports of *E. equinus* in a primate (lemur) and in a zebra; as well as *E. granulosus* (G1 genotype) and *E. ortleppi* in a cervid translocated to the UK. These infections and implications of the potential establishment of exotic species of cestodes are discussed.

1. Introduction

Tapeworm species in the family Taeniidae and genera *Echinococcus* and *Taenia* are obligatory parasites of mammals transmitted within predator–prey interactions. Adult tapeworms occur in carnivore definitive hosts while the larval cystic (metacestode) infections are found in herbivorous intermediate hosts. Herbivores are infected through the accidental ingestion of taeniid eggs passed in definitive host faeces, which then develop over months or years into fluid-filled cysts, usually causing cysticercosis or echinococcosis/hydatidosis in organs, tissues or body cavities (Jones and Pybus, 2001). Some taeniid species are also important zoonoses for example *T.*

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solium and most *Echinococcus* spp. (Flisser and Craig, 2005). In addition, several species infect domestic livestock and may facilitate transmission in synanthropic cycles. Cysticercosis or echinococcosis in domestic animals is usually benign and often asymptomatic. In contrast, taeniid larval cestode infections in wild or captive herbivores may be highly pathogenic for example, hydatidosis caused by *E. granulosus* in wild wallabies (Barnes et al., 2011) and *E. multilocularis* in captive great apes (Rehmann et al., 2003).

In the UK, several *Taenia* spp. are known to be transmitted in mammalian wildlife cycles including *T. pisiformis* and *T. serialis* (between foxes and rabbits/hares), in perisylvatic cycles including *T. taeniaeformis* (between cats and rodents), or within domestic cycles e.g. *T. hydatigena* (between dogs and sheep). Classically, two of the 10 genotypes or strains of *E. granulosus* occur in the UK, the G1 genotype (sheep strain) and the G4 (horse strain) genotype (McManus and Thompson, 2003). The latter genotype has now been elevated to species status as *E. equinus*, on the basis of morphological, molecular and epidemiological considerations (Thompson and McManus, 2002; Thompson, 2008). *E. granulosus sensu stricto* (G1 genotype or sheep strain) is cosmopolitan in distribution (Eckert et al., 2000) and in the UK, is particularly endemic in mid/south Wales (Howells and Taylor, 1980; Stallbaumer et al., 1986; Palmer and Biffin, 1987). Maintenance of transmission of *E. granulosus* (genotype 1) in Wales appears to result from farm dogs acquiring infected sheep offal (liver and lungs) through either home-slaughter (with deliberate feeding), and/or through unleashed dogs scavenging on sheep carcasses that have died on high ground and moorland (Williams, 1976; Walters and Clarkson, 1980; Buishi et al., 2005). Human cystic echinococcosis (CE) contracted in the UK is assumed to be due to the sheep G1 strain of *E. granulosus* however, to date no molecular analysis has been conducted on locally acquired human hydatid isolates to identify the zoonotic genotypes and haplotypes of *E. granulosus*.

E. equinus is similarly endemic in the British Isles, occurring in dogs and horses but is assumed on epidemiological grounds to be non-zoonotic (Thompson and McManus, 2002). *E. multilocularis* on the other hand, is endemic to several continental European countries (Eckert et al., 2001) where its life cycle is largely sylvatic, involving the red fox (*Vulpes vulpes*) and microtine rodents (*Microtus* spp. and *Arvicola* spp.) serving as definitive and intermediate hosts respectively. In highly endemic areas of Europe, human alveolar echinococcosis (AE) has been estimated to have an annual incidence of 0.02–1.4 cases per 100,000 (Eckert and Deplazes, 1999). There is however, no evidence for the transmission of *E. multilocularis* in the British Isles (Smith et al., 2003; Torgerson and Craig, 2009).

Here we summarise the clinical history and present molecular genotypic analysis for 5 larval cestode infections detected at post-mortem or following surgical biopsy in captive mammals notified from wildlife veterinary services, zoos and/or safari parks in the UK. In doing so, we describe new records for *Echinococcus* spp. in the UK.

2. Materials and methods

2.1. Hosts and parasites

Parasite material originating from captive mammals was used in this report. Material was submitted for analysis to Cestode Diagnostics (http://www.star.salford.ac.uk/page/Cestode_Diagnostics), a service laboratory specialising in the molecular identification of cestode infections at the University of Salford. Samples were received from zoos, safari parks and veterinary clinics across the United Kingdom during the period from 2006 to 2011. Details of the animal's sex, age, country of origin, as well as type of cyst/larval material recovered from seven parasite samples including metacestode tissue from 2 deers, 1 equid, 3 primates and 1 rodent are shown in Table 1.

2.2. DNA extraction

Genomic DNA was extracted from 95% ethanol-fixed cysts/larval tissue (Table 1) using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Cyst fluid samples were centrifuged at 3600 × *g* for 2–3 min and the pellets retrieved were used for DNA extraction. Paraffin-embedded parasite tissue from a Barbary macaque (*Macaca sylvanus*) was sectioned using a standard microtome at 5 μm. Genomic DNA was extracted from the resultant paraffin ribbons using the Qiagen DNA FFPE tissue kit as recommended by the manufacturer (Qiagen, Hilden, Germany).

2.3. DNA amplification

Total DNA extracted from the various larval parasites was used as template in PCR assays to amplify fragments within three mitochondrial genes, namely, cytochrome *c* oxidase 1 (*cox1*), NADH dehydrogenase subunit 1 (*nad1*) and the small ribosomal RNA gene (12S rRNA) as previously described (Table 1). Amplified products were separated by electrophoresis on a 1.5% TAE agarose gel, stained with gel red DNA dye (Cambridge Biosciences, UK) and visualised using UV illumination (Syngene G:Box gel documentation and analysis system). Genomic DNA extracted from Libyan *E. granulosus* protoscoleces (G1 genotype), from adult Chinese *E. multilocularis* tapeworms and from an Austrian isolate of *E. multilocularis* (rodent origin) as well as from adults of *T. hydatigena* were used as positive controls in the relevant PCR assays. Negative controls (molecular grade water) were also included. Bands of amplified products were cut out under UV light and gel purified using PurLink™ quick gel purification Kit (Invitrogen, Paisley, UK). Purified PCR products were commercially sequenced (Beckman Coulter, Essex, UK).

2.4. Sequence analysis

Nucleotide sequences were analysed using FinchTV software package (Geospiza, Seattle, WA) and compared with those deposited in international databases through the use of BLAST (Basic Local