Unusual cases of *Taenia crassiceps* cysticercosis in naturally infected animals in the Czech Republic

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ABSTRACT: The tapeworm *Taenia crassiceps* has an indirect life cycle. Occasionally, metacestode stages have been reported from aberrant hosts as dogs, cats, lemurs and humans. This study describes an unusual series of serious cysticercosis cases: an 18-month-old male Yorkshire terrier dog with pleural cysticercosis accompanied by a cough, a 10-year-old male Shih Tzu dog with subcutaneous cysticercosis as well as a Cape ground squirrel and a Senegal bushbaby, both with generalised cysticercosis. Surgery was successful only in the Shih Tzu. The Yorkshire terrier died a few hours after surgery, while the Cape ground squirrel was euthanised and the Senegal bushbaby died before surgery. Cysticerci from the four cases were identified morphologically and using molecular methods. Fragments of genes coding for cytochrome oxidase subunit 1 were sequenced for each of the four isolates. Their affiliation to *T. crassiceps* was confirmed by comparison with the sequence data of other isolates available in the GenBank database. In general, the comparison of sequences of all isolates showed low variability in nucleotide composition (at most five positions). The cases from captive zoo animals represent the first findings of *T. crassiceps* in the Cape ground squirrel and Senegal bushbaby. The optimal treatment of cysticercosis caused by *T. crassiceps* remains unclear. Successful attempts usually include extensive surgical interventions and prolonged anthelmintic treatment. Chemotherapeutic options are limited. Although regular deworming targeting intestinal helminths of dogs is not effective against *T. crassiceps* cysticerci, it may help to prevent contamination of the environment by tapeworm eggs contained in dog faeces and reduce the risk of infection for susceptible animals and humans.

Keywords: tapeworm; metacestode; dog; *Xerus inauris*; *Galago senegalensis*

The tapeworm *Taenia crassiceps* Zeder, 1800 (Eucestoda, Cyclophyllidea, Taeniidae) is widely distributed across Europe, North America and Asia. This parasite has an indirect life cycle. Rodents and insectivores are common intermediate hosts; metacestodes of the cysticercus type usually develop in their body cavities and subcutis upon infection which is acquired by ingestion of eggs containing the larval stage (oncosphere). These further multiply asexually by exogenous budding (Loos-Frank 2000). Carnivores (mainly foxes, but also other canids) serve as definitive hosts and harbour adult tapeworms in their small intestines. Dogs with predatory inclinations and capabilities to hunt rodents can be infected by cysticerci which develop into adult tapeworms releasing gravid proglottids containing eggs. Another risk for dogs, cats and also humans can be the infection by eggs from a contaminated environment, which leads to formation of cysticerci (Wunschmann et al. 2003; Ballweber 2009; Goesseringer et al. 2011). In human populations, the most vulnerable individuals

are HIV-positive and immunocompromised patients (Arocker-Mettinger et al. 1992; Francois et al. 1998). Metacestodes of *T. crassiceps* have rarely been diagnosed in immunocompetent patients (Ntoukas et al. 2013).

Natural infections of dogs with metacestodes are most commonly described in elderly individuals and castrates, which has been explained by the lack of sex hormones (Beugnet and Chermette 1999), and in immunosuppressed individuals (Hoberg et al. 1999). The most common form is the subcutaneous cysticercosis (Beugnet et al. 1996; Bauer et al. 1998; Ballweber 2009). Cerebral cysticercosis is a fatal form of the infection with symptoms very similar to those of rabies (Suja et al. 2003). Neurocysticercosis has also been described in cats (Wunschmann et al. 2003). Other forms of *T. crassiceps* cysticercosis (intraperitoneal, generalised) were described in isolated cases in herbivorous mammals (Luzon et al. 2010; Basso et al. 2014).

MATERIAL AND METHODS

The material originated from different cases of cysticercosis in two pet dogs and two species of captive mammals from a zoo in the Czech Republic.

The first dog patient was an 18-month-old male Yorkshire terrier previously treated with antibiotics for a cough accompanied by febrile illness five times over the course of 2011 with no success. The dog was examined using X-rays and sonography, followed by thoracocentesis. Soon after the patient perished, and necropsy was performed immediately (Hypska et al. 2013).

The second dog patient was a ten-year-old male Shih Tzu, which had undergone several surgeries in the past and castration at the age of eight years. The owner observed painless swelling of the skin in the region of the right hip bump about two months previously. The swelling did not grow significantly, but progressive soreness was evident at the time of clinical examination. Immediate surgery was performed in the affected area (Coufal et al. 2015).

The examined Cape ground squirrel (*Xerus inauris* Zimmermann, 1780) came from a group of animals kept in an enclosure in the Pilsen zoological garden (zoo). In March 2014, the squirrel was hospitalised because of swelling of the mammary glands and the apparent presence of a tumour or post-traumatic lesion on the hind leg. The squir-

rel was euthanised after unsuccessful symptomatic therapy, and inspection at necropsy was performed. Nine months after this finding, similar symptoms (swelling of the hind leg) appeared in an adult Senegal bushbaby (*Galago senegalensis* Geoffroy, 1796) kept in the same zoo. This animal died and was also dissected.

In all cases, the material containing cysts (cysticerci) obtained from body cavities and tissues was microscopically examined to determine morphological features (Loos-Frank 2000). Morphological species determination was followed by molecular identification. Material fixed in pure ethanol was used for the isolation of DNA. Total DNA was extracted from 6–10 cysticerci of each isolate using the DNeasy Blood and Tissue Kit (Qiagen) according to the kit manual. The concentration of isolated gDNA was adjusted to 50 ng/µl in all samples. Sequences of primers suitable for PCR amplification and sequencing of a fragment of the cytochrome oxidase 1 gene (*COX1*) were adopted from Bowles et al. (1992): forward 5'-TTTTTTGGGCATCCTGAGGTTTAT-3' and reverse 5'-TAAAGAAAGAACATAATGAAAATG-3'. The reaction mixture contained 1 µl DNA sample, 1 µl of each of the primers (10 µmol), 12.5 µl EmeraldAmp GT PCR Master Mix (Takara) and 9.5 µl H_2O. PCR conditions were as follows: 94 °C for 4 min, 30 × (94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s) and 72 °C for 10 min. Resulting products were electrophoresed in agarose gels, corresponding bands were excised and DNA was extracted using Zymoclean^{TM} Gel DNA Recovery kit (Zymo Research). The products were ligated into the pGem$^®$ vector (Promega) and transformed into competent *E. coli* according to the manufacturer's manual. Bidirectional Sanger sequencing of the isolated plasmids containing inserts was performed using the same primers in the Sequencing Laboratory of the Section of Biology, Faculty of Science, Charles University. Consensus sequences were compared to the NCBI Nucleotide Collection database using the blastn tool (https://blast.ncbi.nlm.nih.gov) and aligned with a complete sequence of the *T. crassiceps COX1* gene extracted from the NCBI GenBank mitochondrial genome sequence NC_002547.1, and with additional partial sequences from various *T. crassiceps* isolates (AB033411.1, EU544549.1, EU544547.1, KF751222.1) using BioEdit software (Hall 1999). Final alignment of the sequences trimmed to the length of our isolates (where applicable) was performed in EMBL-EBI Kalign (www.

ebi.ac.uk/Tools/msa/kalign/) employing the default setup, and a sequence percent identity/difference count matrix was drawn up. Nucleotide identities at different positions were identified and highlighted in BoxShade (www.ch.embnet.org/software/BOX_form.html).

RESULTS

In the Yorkshire terrier, the X-ray examination of the thorax revealed pleural effusion and the sonography examination showed an atypical appearance of the pleural effusion formed by multiple hyperechoic particles with diameters of 5–10 mm. Only a minimal amount of aspirated fluid (about 0.2 ml) was obtained by thoracocentesis; however, after repeated aspiration, thin-walled cysts (1–5 mm in size) were captured. Only a small number of cysts (around 30) were aspirated in total. All the cysts were identical in appearance, and each of them contained a whitish spot (Figure 1A). Microscopic examination revealed scolices with hooks inside the cysts (Figure 1B), typical for cysticercus. The dog died 18 hours after surgery. The autopsy revealed massive occurrence of parasitic cysts in the thoracic cavity (Figure 1C). The total volume of the haemorrhagic exudate with cysticerci was ca. 90 ml. Lung surface was smooth with whitish deposits, fibrin fusions occurred on the pleura. The diagnosis was pleural cavity cysticercosis.

The clinical examination of the Shih Tzu revealed a visible, slightly painful bulge in the subcutaneous tissue of the hip, 10 × 12 cm in size, without significant tempering, but fluctuating and elastic. Surgery revealed hundreds of cysticerci in bloody fluid (Figure 1D). The affected tissue was surgically removed. The diagnosis was subcutaneous cysticercosis. The dog had no problems and the affected area was without filling or other alterations two months after the operation.

The Cape ground squirrel from the zoo exhibited no reaction to symptomatic therapy; the oedema grew larger and distended to the caudal part of the mammary glands as a tumour, which, however, could not be confirmed by cytological examination. Surgery revealed numerous small cysts (1–5 mm) in the connective tissue. The cysts were microscopically confirmed as larval stages of *Taenia* sp. (cysticerci). The attending veterinarian decided to perform euthanasia of the affected animal because of the massive infection. Necropsy revealed generalised cysticercosis. The cysticerci were not only in the connective tissue of the thigh, but also in the abdominal cavity (Figures 2A and 2B). The other squirrels from the same enclosure did not display any clinical signs of the illness.

The Senegal bushbaby was less mobile with swellings on hind limbs and the infection quickly lead to death. Necropsy revealed numerous cysticerci (1–5 mm) in the connective tissue and muscles of its right hind leg (Figures 2C and 2D).
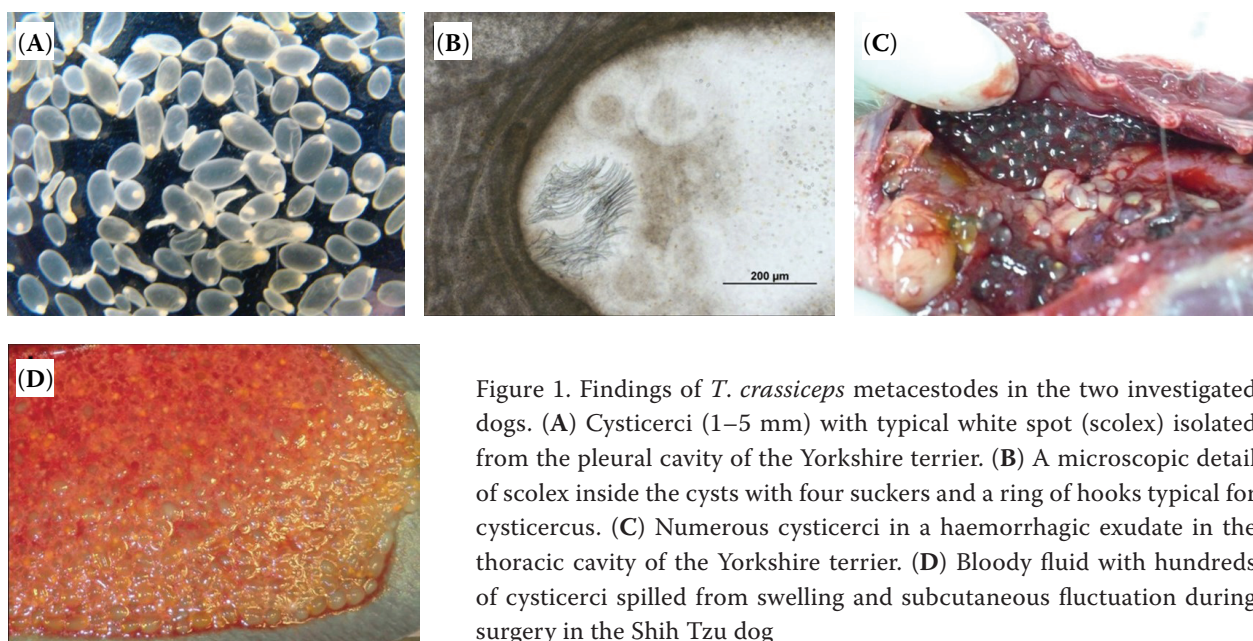

Figure 1. Findings of *T. crassiceps* metacestodes in the two investigated dogs. (**A**) Cysticerci (1–5 mm) with typical white spot (scolex) isolated from the pleural cavity of the Yorkshire terrier. (**B**) A microscopic detail of scolex inside the cysts with four suckers and a ring of hooks typical for cysticercus. (**C**) Numerous cysticerci in a haemorrhagic exudate in the thoracic cavity of the Yorkshire terrier. (**D**) Bloody fluid with hundreds of cysticerci spilled from swelling and subcutaneous fluctuation during surgery in the Shih Tzu dog
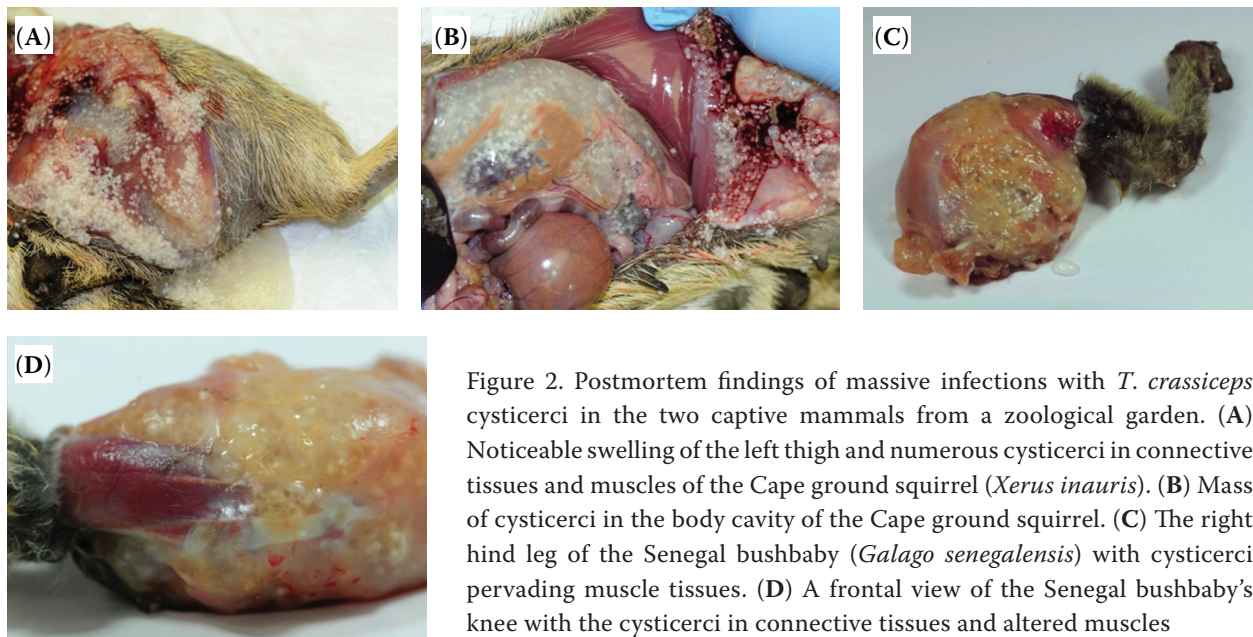
Figure 2. Postmortem findings of massive infections with *T. crassiceps* cysticerci in the two captive mammals from a zoological garden. (**A**) Noticeable swelling of the left thigh and numerous cysticerci in connective tissues and muscles of the Cape ground squirrel (*Xerus inauris*). (**B**) Mass of cysticerci in the body cavity of the Cape ground squirrel. (**C**) The right hind leg of the Senegal bushbaby (*Galago senegalensis*) with cysticerci pervading muscle tissues. (**D**) A frontal view of the Senegal bushbaby's knee with the cysticerci in connective tissues and altered muscles

In all cases, the microscopic examination of obtained cysticerci led to preliminary determination of the species as *T. crassiceps* based on general morphology and the number, shape and size of hooks (Hoberg et al. 1999).

Fragments of *COX1* genes were sequenced for each parasite isolate and the sequences (all 444 bp in length) were deposited in the NCBI GenBank database under the accession numbers indicated in parentheses: dog 1 (Yorkshire terrier, KY321318), dog 2 (Shih Tzu, KY321319), Cape ground squirrel (KY321320) and Senegal bushbaby (KY321321). Blasting of the sequences against the NCBI nucleo-tide collection confirmed their similarity with other *T. crassiceps* isolates. The alignment and sequence identity/difference count matrix of our isolates and the corresponding regions of the *T. crassiceps COX1* sequences from the NCBI GenBank database are shown in Table 1 and Figure 3, respectively. The alignment of translated amino acid sequences of our isolates and the reference sequences from GenBank indicated that in the case of the Yorkshire terrier there were missense mutations within the partial *COX1* DNA sequence causing amino acid changes at positions 127 (conservative mutation Val→Ala) and 137 (non-conservative mutation

Table 1. Sequence percent identity/difference count matrix for partial *COX1* genes of *Taenia crassiceps* isolates compared with selected sequences from the NCBI Gen Bank database

	Yorkshire	Shih Tzu	*Xerus*	*Galago*	NC_002547.1	AB033411.1	EU544549.1	EU544547.1	KF751222.1
Yorkshire	ID	5	3	2	7	2	3	6	2
Shih Tzu	98.8	ID	4	3	8	0	1	4	0
Xerus	99.3	99.0	ID	1	6	1	2	5	1
Galago	99.5	99.3	99.7	ID	5	0	1	4	0
NC_002547.1	98.4	98.1	98.6	98.8	ID	0	1	4	0
AB033411.1	99.4	100	99.7	100	100	ID	1	4	0
EU544549.1	99.2	99.7	99.4	99.7	99.7	99.7	ID	3	1
EU544547.1	98.4	98.9	98.7	98.9	98.9	98.9	99.2	ID	4
KF751222.1	99.4	100	99.7	100	100	100	99.7	98.9	ID

The lower part of the table shows per cent identity between the sequences, the upper part shows the number of different nucleotides between the sequences; the comparison was made between the maximum overlapping parts of particular sequence pairs based on the alignment in Figure 3
ID = identical sequences

Figure 3. The sequence alignment of corresponding parts of the *COX1* genes of *Taenia crassiceps* isolates. GenBank sequence accession numbers: Yorkshire terrier dog – KY321318, Shih Tzu dog – KY321319, *Xerus inauris* Cape ground squirrel – KY321320, *Galago senegalensis* Senegal bushbaby – KY321321. Accession numbers of reference sequences of *COX1* genes are indicated in the Table 1. Numbers in particular sequences indicate the true positions within the non-trimmed partial sequences from our isolates and within the complete/partial sequences extracted from NCBI Gen Bank

Phe→Ser); missense mutations were observed also in the case of the Shih Tzu, resulting in an amino acid change at position 4 (non-conservative mutation His→Ala) (data not shown).

DISCUSSION

Cysticercosis is a rare condition in dogs and the occurrence of two cases of larval *T. crassiceps* cysticercosis within two years is disturbing. The first patient (Yorkshire terrier) was probably infected in the first months of his life, and the undeveloped immunity of the puppy might have allowed the fatal infection. The second patient (Shih Tzu) was an aged male castrate, conditions which are generally thought to increase susceptibility to infection by metacestode stages of *T. crassiceps*. For this case, an analogy could be found in experimental infections of mice, where the cysticerci stimulate a shift towards a Th2 T-helper cell immune response. Increased production of certain cytokines mediates the elevated conversion of testosterone to estradiol. Simultaneously, the pathway leading to the production of dihydrotestosterone is blocked and changes in the expression of receptors for particular steroids occur. All this results in the inhibition of an effective Th1 immune response, feminisation of males and massive multiplication of metacestodes (for a review see Morales-Montor and Larralde 2005).

Cysticercosis of the thoracic cavity has been reported only relatively sporadically. A case of pericardial effusion caused by cysticerci of *T. crassiceps* was reported in a dog by Mascher and Hoffman (2005). Infections of the pleural cavity are also reported only rarely (Hoberg et al. 1999). On the other hand, subcutaneous cysticercosis in dogs has been globally regarded as the most common form (Chermette et al. 1993; Beugnet et al. 1996; Bauer et al. 1998; Ballweber 2009). Besides the usual way of the infection by ingestion of the parasite eggs from the contaminated environment when the infection is inflicted by the larval stages (oncospheres), experiments in mice have shown that intraperitoneal cysticercosis by *T. crassiceps* can also develop after ingestion of metacestodes (cysticerci), which are able to penetrate the intestinal wall and reach the abdominal cavity within 24 hours of infection (Kroeze and Freeman 1982). Although the second possibility was not experimentally verified in dogs, it cannot be completely ruled out, especially in cases of individuals with an incomplete immune response. Therefore, small rodents might potentially serve as another source of cysticercosis in dogs with strong prey drives.

Other cases of *T. crassiceps* cysticercosis in small mammals from the zoo followed in the same year. First, necropsy findings which were macroscopically similar to the dog cases were noticed by the zoo veterinarian in a thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*), but material from this animal was not preserved for subsequent analyses; therefore, it was not included in this study. The next two cases of cysticercosis from the same zoo in the Cape ground squirrel and the Senegal bushbaby followed in a few months. These animals were probably infected by fodder or bedding (hay, straw) contaminated with canid faeces containing eggs of *T. crassiceps*.

Sequencing of the *COX1* genes of our isolates confirmed the diagnosis made on the basis of microscopic examination. Although just partial sequences were obtained and no far-reaching conclusions on the genetic relationship of the isolates can be drawn, it is obvious that the two isolates from the zoo in western Bohemia are almost identical, suggesting a common source of infection. The two dog cases were from different geographical regions (central and north-eastern Bohemia), and in this case the difference was the greatest among our isolates (five nucleotide positions). To our knowledge, the infections described in this study represent the first findings of *T. crassiceps* in *Xerus inauris* and *Galago senegalensis*. However, this is not surprising as the spectrum of mammalian intermediate hosts can be assumed to be very broad.

Not only dogs, but also foxes, wolves and jackals have been described as sources of *T. crassiceps* eggs in Europe (Takacs et al. 2014; Gori et al. 2015; Hauser et al. 2015). Speculation regarding a significant contamination of the environment in Europe with *T. crassiceps* eggs can be supported by a rising number of cases described recently from various mammalian species (not only dogs or zoo animals), and from humans, who represent aberrant intermediate hosts. Among animals, fatal cases of *T. crassiceps* cysticercosis were described in, e.g., a ring-tailed lemur (*Lemur catta*) from Madrid Zoo in Spain (Luzon et al. 2010), in a chinchilla (*Chinchilla laniger*) from Switzerland (Basso et al. 2014), in a wild red fox (*Vulpes vulpes*) in Croatia (Konjevic et al. 2016) and the subcutaneous form

in a dog in Germany (Nolte et al. 2016). As for recent human infections, e.g., two cases in immunocompetent patients in Germany were described (Ntoukas et al. 2013; Roesel et al. 2014); further, cysticercosis was described in a HIV-positive woman from Switzerland who worked as an employee in a zoo (Anikpeh et al. 2014).

The treatment of *T. crassiceps* cysticercosis is demanding, mainly because chemotherapeutic options are limited. Successful strategies include extensive surgical interventions and protracted anthelminthic drug therapy. Experiments in mice showed a significant reduction of cysticerci after prolonged 20- and 25-day administration of albendazole (Zurabian et al. 2013). In human medicine, the synergistic action of praziquantel and albendazole was employed to successfully treat infection (Garcia et al. 2011; Fraga et al. 2012; Anikpeh et al. 2014). As for regular deworming of dogs, it is not effective against *T. crassiceps* cysticercosis, but may help to prevent contamination of the environment by tapeworm eggs contained in faeces of dogs parasitised by adult tapeworms in the intestine. However, the spread of the parasite through the faeces of infected foxes likely represents a more serious problem.

In conclusion, four cases of cysticercosis caused by *Taenia crassiceps* were recorded in the Czech Republic in domestic and captive animals within a relatively short period. The number of such findings in animals and humans in Europe is growing, probably as a result of environmental contamination by parasite eggs distributed through the dense population of (also urban) red foxes. *T. crassiceps* cysticercosis represents a serious disease with possibly lethal consequences; the treatment is difficult. Regular deworming targeting intestinal tapeworms of the relevant categories of dogs is strongly recommended as it can help to reduce the risk of infection for susceptible hosts such as humans and domestic mammals.

Acknowledgements

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