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Short communication

Fatal pulmonary cysticercosis caused by *Cysticercus longicollis* in a captive ring-tailed lemur (*Lemur catta*)

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ABSTRACT

Here we describe fatal pulmonary cysticercosis caused by *Cysticercus longicollis*, the larval stage of *Taenia crassiceps* in a 15-year-old female ring-tailed lemur (*Lemur catta*) from Sarajevo Zoo. After sudden death, the lemur was subjected to necropsy and large multicystic structure, subdivided with fibrous septa and filled with numerous translucent, oval to ellipsoid bladder-like cysts (cysticerci), almost completely replacing right lung lobe was observed. In addition, numerous free and encysted cysticerci were found in the thoracic cavity. Histopathology revealed connective tissue outlined cavities that compress lung parenchyma. Each cavity contained several thin walled cysticerci with single inverted protoscolex, one or more suckers and rostelum with two rows of hooks. In many of the cysticerci one or several exogenous buds of daughter cysticerci were observed. Based on morphology and microscopic appearance the parasite was identified as *C. longicollis*. Subsequent molecular analysis and sequencing confirmed presumptive diagnosis. To our knowledge, this case represents the first report of *T. crassiceps* and cysticercosis caused by *C. longicollis* in Bosnia and Herzegovina.

1. Introduction

Metacestodes of various taeniid species have been documented in humans and non-human primates (Heldwein et al., 2006; Tappe et al., 2007; Luzon et al., 2010; Boufana et al., 2012; Ntoukas et al., 2013; De Liberato et al., 2014; Flammer-Anikpeh et al., 2014; Denk et al., 2016). In ring-tailed lemur (*Lemur catta*) fatal infections with larval forms of *Echinococcus granulosus, E. multilocularis, Taenia martis* and *T. crassiceps* have been described in several isolated cases (Luzon et al., 2010; Umhang et al., 2013; De Liberato et al., 2014; Denk et al., 2016). Furthermore, fatal cysticercosis caused by *T. crassiceps* was documented in two other species of lemurs, namely black lemur (*Eulemur macaco macaco*) (Dyer and Greve, 1998) and red ruffed lemur (*Varecia variegata rubra*) (Young et al., 2000).

Taenia crassiceps (Zeder, 1800) is a cestode species that inhabits the small intestine of domestic and wild carnivores in Northern hemisphere. In Europe, infection has been reported in different wild carnivores (Bagrade et al., 2009; Bružinskaitė-Schmidhalter et al., 2012; Takács et al., 2014), but red fox (*Vulpes vulpes*) is considered as the most common definitive host (Shimalov and Shimalov, 2003; Bružinskaitė-Schmidhalter et al., 2012; Vergles-Rataj et al., 2013). Although numerous species could serve as intermediate hosts, the common vole (*Microtus arvalis*) is regarded as the most important in Old Continent (Bröjer et al., 2002). In intermediate hosts, following the ingestion of oncospheres, the larval stage *Cysticercus longicollis* mostly resides in subcutis and body cavities (Freeman, 1962). Unique feature of *T. crassiceps* i.e. the ability of cysticerci to proliferate indefinitely by exogenous and endogenous budding, results in massive infections making the parasite very invasive. Definitive hosts ingest the cysticercus with intermediate hosts and adult stage develops in the intestine (Freeman, 1962; Ballweber, 2009). Rarely, larval form of the parasite develops in the immunocompromised definitive host (Hoberg et al., 1999; Wünschmann et al., 2003; Ballweber, 2009; Konjević et al., 2016; Whipp et al., 2017). Moreover, infection with *T. crassiceps* has been shown to induce a feminization process in male mice due to significant changes in sex hormone levels (Larralde et al., 1995).

In Bosnia and Herzegovina there is no data on the presence of this taeniid species, thus herein we describe the case of severe fatal pulmonary cysticercosis caused by *C. longicollis* in ring-tailed lemur from Sarajevo Zoo.

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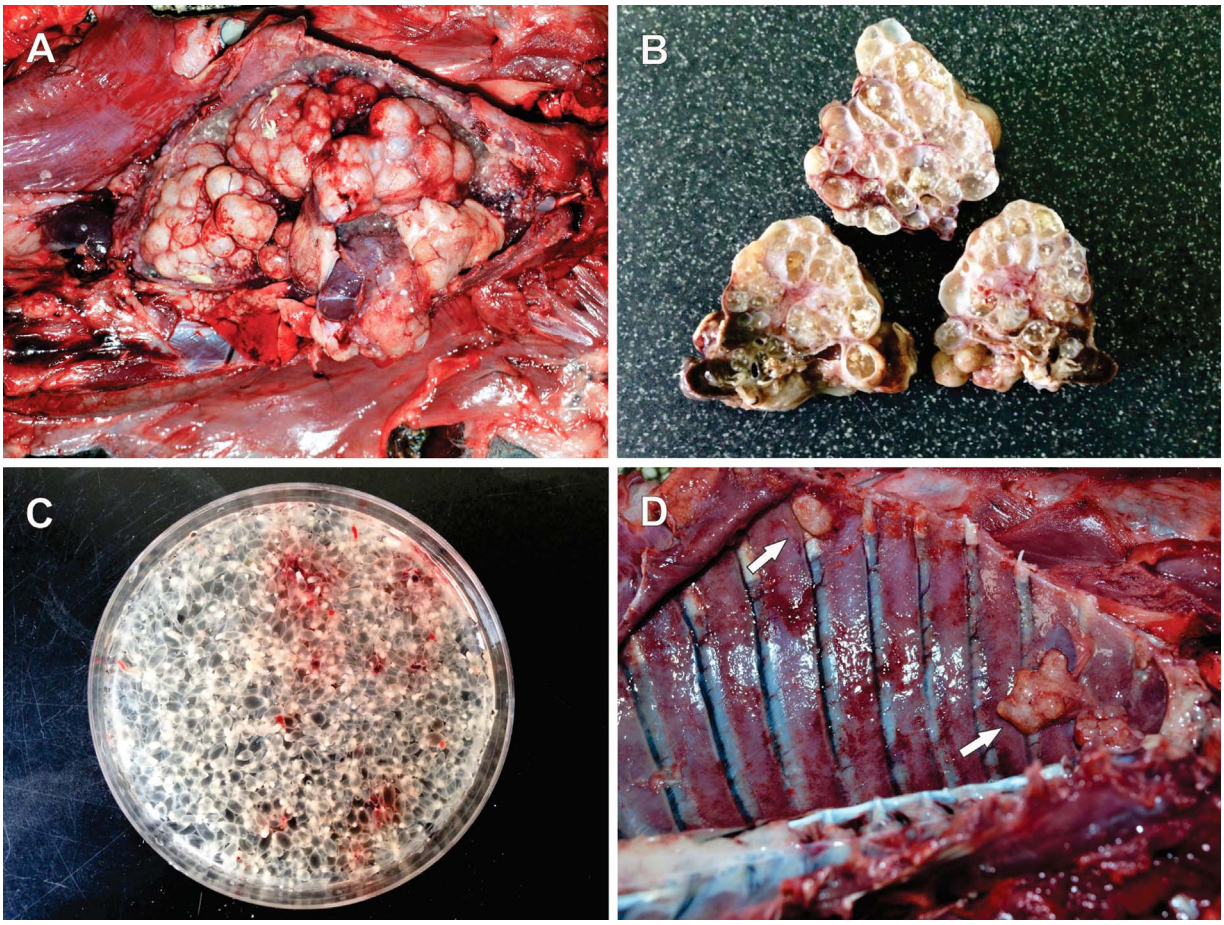

Fig. 1. *Cysticercus longicollis* cysticercosis in a captive ring-tailed lemur (*Lemur catta*). Multicystic structure filled with numerous cysticerci almost completely replaces the right lung lobe of a ring-tailed lemur (A). Cross section of cysts. Note numerous cysticerci in connective tissue subdivided cysts (B). Numerous free cysticerci collected from thoracic cavity. Note unipolar, opaque white 1 mm spot (protoscolex) in each cysticercus (C). Multiple cysts (arrows) surrounded with thin fibrous capsule attached to the inner thoracic wall (D).

2. Case report

In September 2016, a 15-year-old female ring-tailed lemur was submitted for necropsy at the Department of Pathology of the Faculty of Veterinary Medicine in Sarajevo. The female was in the breeding collection of four adults at the Sarajevo Zoo. These animals were donated from the Zagreb Zoo (Croatia) in October 2014. Lemurs were kept in two pairs in separated outdoor-indoor enclosure. Outdoor part was isolated with glass walls to prevent contact with other animals or possible intruder wild animals such as rats or birds. Animals were fed with fresh vegetables, eggs and vitamin and mineral supplements. Prior to sudden death the animal was in perfect body condition and no signs of illness or changes in behavior were observed by the veterinarians or animal keepers. Also, the male mate and the second pair of lemurs were in good body condition and show no signs of illness.

At necropsy, the right lung lobe was almost completely (90%) replaced with large multicystic structure subdivided with connective tissue septa and surrounded with fibrous capsule (Fig. 1A and B). Cystic cavities contained numerous up to 1 cm long and 1–5 mm in diameter, oval to ellipsoid thin walled transparent cysts, and each contained unipolar, opaque white 1 mm spot (Fig. 1C). Multiple cysts were also observed in thickened pericardium. Mediastinum was expanded with numerous similar free or grouped and fibrous capsule contained cysts submerged in gelatinous translucent material. Some of the cysts surrounded with thin fibrous capsule were also attached to the inner thoracic wall (Fig. 1D). No cysts were observed elsewhere in the body and no other significant gross lesions were observed. Multiple samples of lung, mediastinum, pericardium, heart, intestine, liver, spleen, kidney and brain were collected and processed routinely for histopathology. Moreover, numerous cysticerci were collected and used for molecular analysis. Genomic DNA was extracted with DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) and amplified by PCR with primers F/COI and R/COI (Nakao et al., 2000). Resulting amplicon was sequenced by a commercial company (Microsynth, Austria) in both directions employing the same primer set as used in the PCR.

Histopathology revealed multiple variously sized and shaped, connective tissue outlined, cavities that replaced and compressed the lung parenchyma. Each cavity contained several thin walled cysticerci. In the cyticerci single inverted protoscolex, one or more acetabula and rostelum with two rows of hooks, and numerous calcareous bodies were visible (Fig. 2). In many of the cysticerci single or multiple exogenous buds of daughter cysticerci were spotted (Fig. 3). In the remaining parenchyma, narrow stretches of adjacent compressed parenchyma were infiltrated with moderate numbers of various combinations of lymphocytes, plasma cells, neutrophils, macrophages and eosinophils. Infiltrates were extending in to the layers of connective tissue capsule around the parasitic cysts where multifocal lymphoid follicles were also present. Occasionally, multinuclear giant cells were seen. In vicinity of some parasitic cysts, expanded alveoli filled with moderate aggregates of alveolar macrophages, neutrophils, and eosinophilic and granular tissue debris interchanged with moderate proliferation of variously sized and shaped bronchioles lined with single layer of low cuboidal cells. Moderate proliferation of peribronchal and peribronchiolar follicular lymphoid tissue was also seen. Furthermore, severe hyperemia and multifocal hemorrhages were observed in the intestines, liver, kidney and brain. Diffusely, variously sized clear vacuoles were present in hepatocytes, and no other significant lesions were noticed.

Finally, molecular analysis and subsequent sequencing of an