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Urinary capillariosis in a free-ranging Marsican brown bear (*Ursus arctos marsicanus*)

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ABSTRACT

Extraintestinal nematodes have been seldom investigated in the brown bear (*Ursus arctos*). In this study, a case of urinary capillariosis and bladder associated lesions is reported in a deceased free-ranging Marsican brown bear (*Ursus arctos marsicanus*) from Central Italy. Gross lesions in the urinary bladder consisted of scattered foci of mucosal hyperemia, while at histological examination mild cystitis was observed. At microscopic examination of urine and bladder lavage fluid, capillariid adult female nematodes and eggs were found, suspected of belonging to the genus *Pearsonema* based on their location in the urinary bladder. This is the first report of *Pearsonema* infection and associated bladder lesions in a brown bear.

1. Introduction

The Marsican brown bear population, also known as the Apennine brown bear and ascribed to the subspecies *Ursus arctos marsicanus* by Altobello (1921), survives as an isolated nucleus of 40–50 individuals in Central Italy, mainly concentrated in the Abruzzo, Lazio and Molise National Park (Ciucci et al., 2015). Since this population is classified as critically endangered by the IUCN (McLellan et al., 2016), cases of mortality are fully investigated through forensic necropsies and appropriate laboratory testing. Main goals of forensic examination are to assess the overall health conditions of deceased bears, to ascertain the cause of death and to rule out any signs of unlawful killing, since poaching with firearms, traps or poison are the major causes for species mortality (Gervasi and Ciucci, 2018).

In Europe, previous studies on endoparasite infections of the brown bear (*Ursus arctos*) are scarce (Rogers and Rogers, 1976; Borka-Vitális et al., 2017; Paoletti et al., 2017; Papadopoulos et al., 2017). Moreover, previous parasitological surveys on free-ranging brown bears from Italy only included examination of faecal samples, while urinary parasite infection were not investigated (Stancampiano et al., 2008; Paoletti et al., 2017).

Capillariid nematodes of the genus *Pearsonema* infect the urinary tract of domestic and wild carnivorous and omnivorous mammals (Moravec, 1982; Basso et al., 2013). *Pearsonema* spp. infections have been mainly reported in canids, felids and mustelids (Butterworth and Beverley-Burton, 1980; Fernández-Aguilar et al., 2010; Moravec et al., 1987). Although more rarely, the infection has been described also in other definitive hosts belonging to other families, such as small Indian mongoose *Herpestes auropunctatus*, raccoon dog *Nyctereutes procyonides*, raccoon *Procyon lotor* and masked shrew *Sorex cinereus* (Huizinga et al., 1976; Butterworth and Beverley-Burton, 1980; 1981; Bourque, 2011; Bružinskaitė-Schmidhalter et al., 2011). In the present study, a case of urinary capillariosis and associated lesions in a deceased free-ranging Marsican brown bear (*Ursus arctos marsicanus*), is reported.

2. Materials and methods

In June 2018 the carcass of an adult male Marsican brown bear found dead in the Abruzzo, Lazio and Molise National Park (Italy), was submitted to the National Reference Center for Veterinary Forensic Medicine (Grosseto, Italy) for post-mortem examination by local authorities. A forensic necropsy was undertaken, including complete skinning of the carcass, skull opening and photographic documentation with metric reference. In order to assess the possible presence of urinary parasites, urine was collected from the urinary bladder through centesis and fixed with 70% ethanol. The urinary bladder was opened and microscopically examined for gross lesions and parasites. Then, the urinary bladder was pressure-washed with 70% ethanol. Under an optical microscope, the urine sample collected by cystocentesis and urinary bladder lavage fluid were examined for capillariid adult nematodes and

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eggs according to previously published methods (Maurelli et al., 2014; Mariacher et al., 2015). Samples from the urinary bladder were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 4 µm, stained with haematoxylin-eosin and examined for histopathological lesions. A 10-grams muscle sample was collected from the cranial tibial muscle and submitted to research of *Trichinella* larvae with the Trichomatic 35 automated digestion method (Dupouy-Camet and Murrell, 2007).

3. Results and discussion

The examined male Marsican brown bear was in fair body condition with a mass of 142 kg, and the carcass showed moderate putrefactive changes. Necropsy revealed that the bear had suffered a severe trauma (fall from a height) following intra-specific aggression. Cause of death was septic peritonitis from traumatic gastric rupture. Multiple foci of hyperemia were observed in the bladder mucosa, sometimes associated with the presence of thread-like and rolled up nematodes, visible to the naked eye.

At microscopic examination, urine and lavage fluid were positive for the presence of capillariid eggs and large fragments of adults. A large number of mature capillariid eggs measuring 59.8-65 X 26–28.6 µm and showing a thick wall, protruding bipolar plugs and a rough egg-shell surface, was observed both in mature females and at microscopical analysis of the urinary sediment after flotation test (Fig. 1). Immature eggs measuring 52-54.6 X 23.4–26 µm were also observed in the uterus of adult females. Six adult female worms were counted, while males were not found. Adult females showed a thread-like appearance, a subterminal anus, a vulva with a funnel-like appendage (Fig. 1) and their width was about 54.6 µm at the vulvar level. Due to the extreme

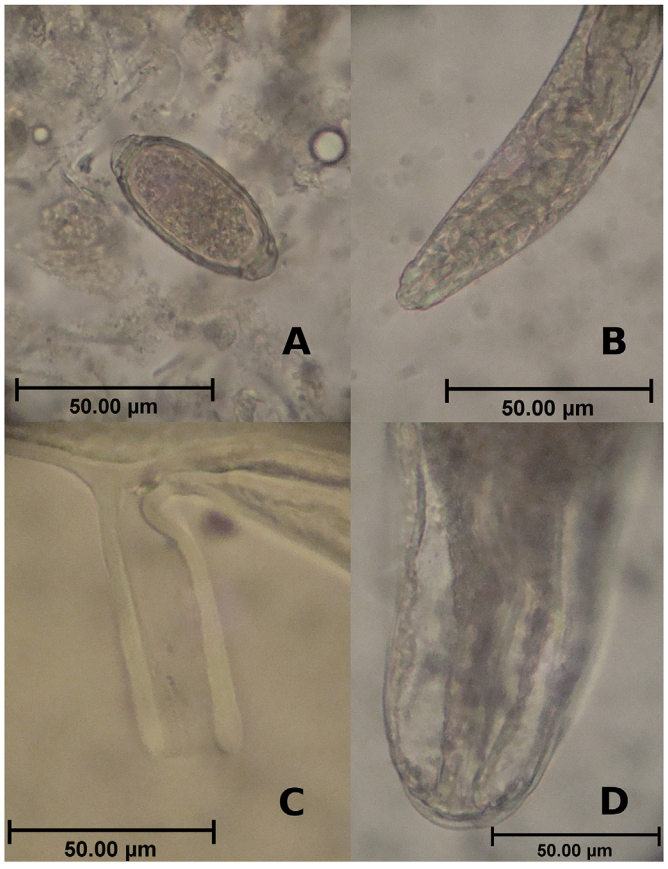

Fig. 1. Urinary parasites, light microscopy. A) Mature capillariid egg, 40X, B) Adult female, vulvar appendix, 40X, C) Adult female, anterior extremity, 40X, D) Adult female, posterior extremity, 40X.

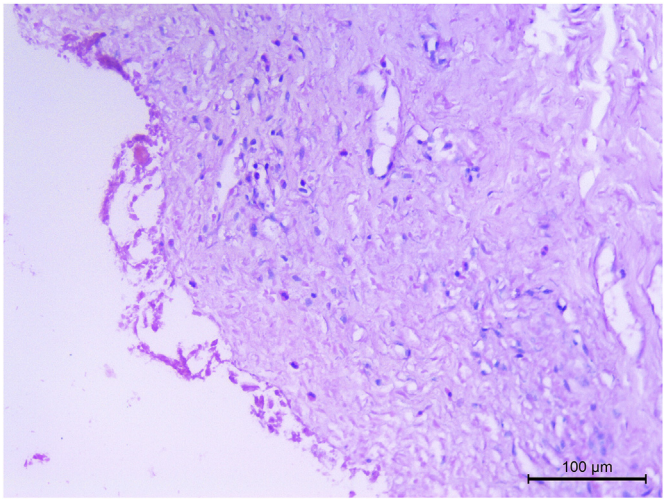

Fig. 2. Urinary bladder, histology. Scattered eosinophils in the submucosa. Hematoxylin-eosin, 10X.

fragility and fragmentation of the adult parasites, nematode length was not possible to assess. Based on their location in the urinary bladder, these capillariid adult female nematodes and eggs were suspected of belonging to the genus *Pearsonema*, the only capillariid genus known to occur in this location and of which there are at least four species known to infect carnivorous mammals (Moravec, 1982). At histological examination, despite mild autolytic changes, a very mild cystitis (Fig. 2) with scattered eosinophils and few lymphocytes, and multifocal small haemorrhages in the submucosa, was observed.

Negative results were obtained from the search of *Trichinella* larvae.

Among extraintestinal nematodes of the brown bear (*Ursus arctos*), only *Crenosoma* sp. (Borka-Vitális et al., 2017), *Eucoleus aerophilus* (Paoletti et al., 2017), *Dirofilaria immitis* (Papadopoulos et al., 2017) and *Trichinella* spp. (Borka-Vitális et al., 2017) have been reported in Europe. In the brown bear, *Pearsonema* nematodes have been previously reported only once in a captive animal and identified with the species *Pearsonema plica* (Rukhlyadev and Rukhlyadeva, 1953), but pathological aspects were not investigated.

Pearsonema nematodes are generally considered to have a low pathogenic impact on infected animals. Nevertheless, especially in case of *P. plica* heavy parasite burdens, the infection has been accounted for urinary clinical signs and bladder lesions, both in domestic carnivores (Senior et al., 1980; Callegari et al., 2010; Rossi et al., 2011) and in wild canids. Lesions associated with *P. plica* infection generally consist of eosinophilic or lymphocytic infiltration of urinary bladder, kidney and ureters in foxes (Fernandez-Aguilar et al., 2010; Bork-Mimm and Rinder, 2011; Alić et al., 2015), while follicular chronic cystitis has been reported in wolves (Mariacher et al., 2015). In the bear of the present case, only scattered hyperemic foci in the bladder mucosa and mild cystitis were observed.

In Europe, foxes (*Vulpes vulpes*) and wolves (*Canis lupus*) are regarded as the reservoir hosts of *P. plica* (Bagrade et al., 2009; Bork-Mimm and Rinder, 2011; Magi et al., 2014; Mariacher et al., 2015). Likely, the populations of red fox and wolf that live in the same area of the bear (Di Sabatino et al., 2014) may have contributed to environmental contamination with *P. plica* eggs.

The life cycle of most *Pearsonema* species is indirect with earthworms as intermediate hosts (Butterworth and Beverley-Burton, 1981; Moravec et al., 1987). As for his dietary habits, the brown bear is considered a highly opportunistic omnivore (Bojarska and Selva, 2012). Therefore, the bear examined in this study could have fed on both earthworm intermediate hosts and, more probably, on putative paratenic hosts, such as other invertebrates, small mammals or amphibia (Seville and Addison, 1995; Rossi et al., 2011).

Biomolecular diagnostic methods for Trichuridae have been scarcely investigated (Guardone et al., 2013). However, the development of species-specific genetic markers could be a fundamental aid in the diagnosis of this infection, especially in unusual host species, in low parasite burdens or in poorly preserved carcasses (as is often the case for free-ranging animals discovered only a few days after death), considering that the fragility of the adult worms may limit parasite identification.

4. Conclusions

This report provides the first description of *Pearsonema* infection and associated cystitis in a brown bear. Further parasitological studies on a wider number of free-ranging brown bears would be needed to assess the actual prevalence and impact of urinary capillariosis in the brown bear in Europe. Nevertheless, to this aim new and more sensitive molecular diagnostic tools should be implemented.

Conflict of interest

Authors declare no conflict of interest.

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