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 are reported in a deceased free-ranging Marsican brown bear (*Ursus arctos marsicanus*) from Central Italy. Gross lesions observed in the urinary bladder consisted of scattered foci of mucosal hyperemia, while at histological examination mild cystitis was found. At microscopic examination of urine and bladder lavage fluids, 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.

Key words: cystitis; Marsican brown bear; *Pearsonema*; urinary capillariosis

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; Borka-Vitális 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, 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 then 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 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. Moreover, 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 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 evidenced.

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). Therefore, the abundant populations of red fox and wolf that live in the same area of the bear (**bisogna assolutamente aggiungere dei riferimenti bibliografici!!!!**) may have likely contributed to high 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). In fact, although few studies are available on the diet of the Marsican brown bear, a recent paper (Ciucci et al., 2014) reported small mammals and various insects being present at scat analysis of this animal, but earthworms remains were not observed. Nevertheless, laboratory processing of scat samples could lead to overlook some minor diet components because only few sub-samples are microscopically analysed from the whole scat specimen collected on the field (Di Domenico et al., 2012). Moreover, scat analysis could be less adequate to demonstrate earthworm ingestion rather than direct observation of feeding bears (Mattson et al., 2001).

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 burden or in poorly preserved carcasses, especially of free-ranging animals deceased from some days, considering that the fragility of the adult worms may limit parasite identification. Aggiungere un commento più approfondito in risposta al commento 1 del revisore 2.

Figure 1. Urinary parasites. Immature eggs in the uterus of an adult female, 40X.

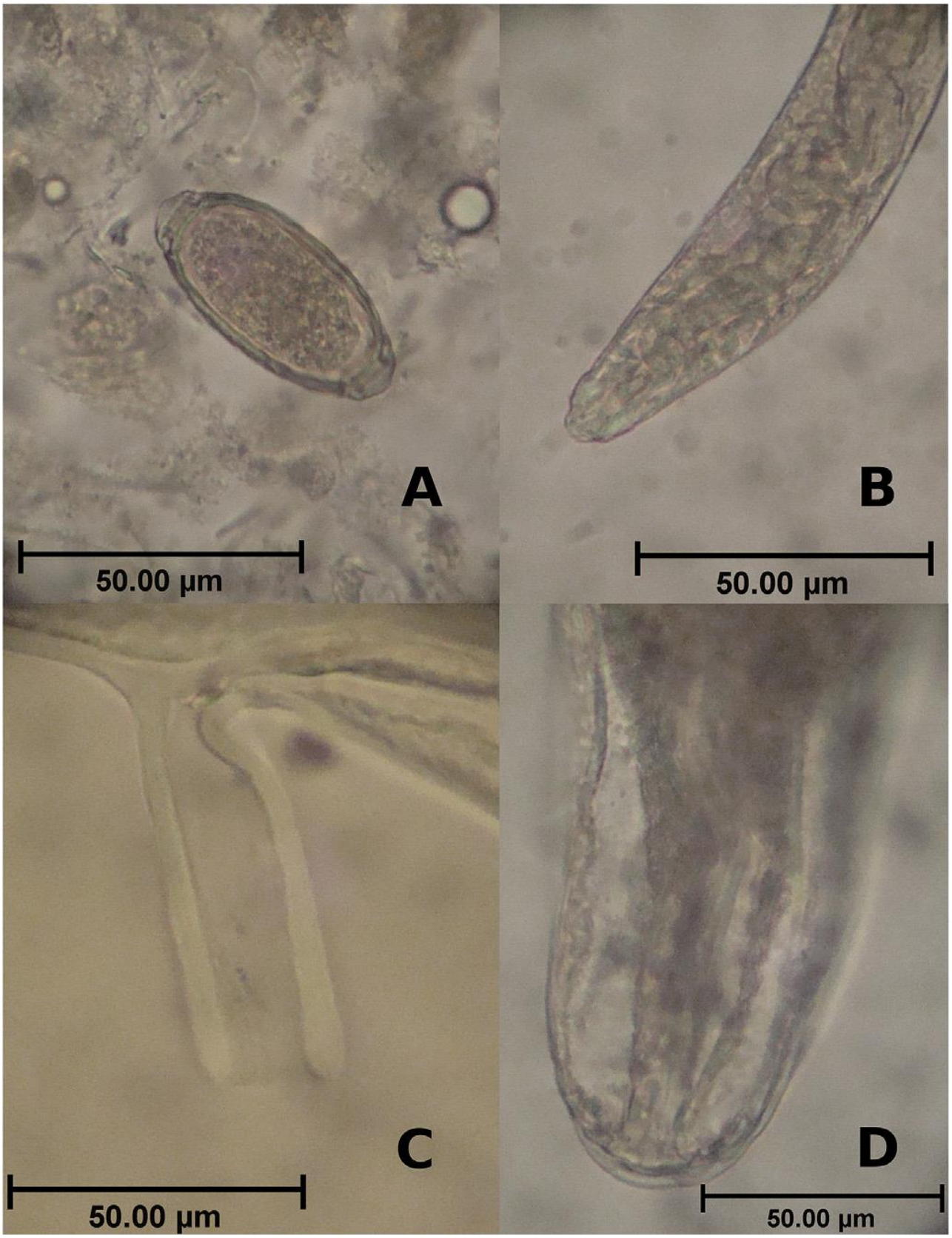
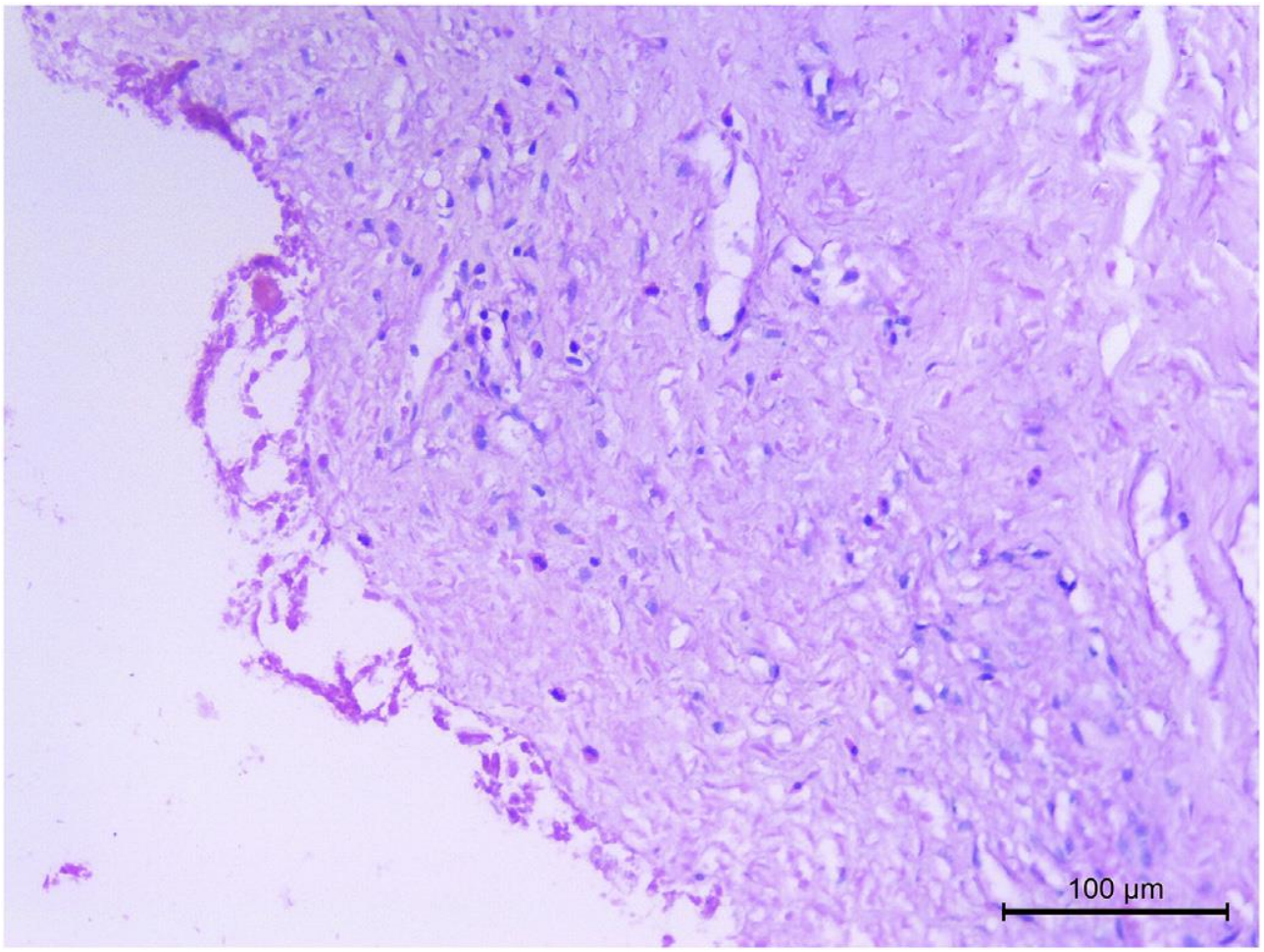


Figure 2. Urinary bladder, histology. Scattered eosinophils in the submucosa. Hematoxylin-eosin, 10X.



3.1 Conclusions

This report provides the first description of *Pearsonema* infection and associated cystitis in a Marsican brown bear and in the brown bear more in general. Further parasitological studies on a wide number of free-ranging brown bears are 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 are also needed.

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REFERENCES

Altobello, G. 1921. Fauna dell'Abruzzo e del Molise. Vertebrati, Mammiferi. IV. I Carnivori (Carnivora). Colitti, Campobasso, Italy.

Alić, A., Hodxić, A., Kadrić, M., Besirović, H., Prasović, S., 2015. *Pearsonema plica* (*Capillaria plica*) infection and associated urinary bladder pathology in red foxes (*Vulpes vulpes*) from Bosnia and Herzegovina. Parasitol. Res. doi: 10.1007/s00436-015-4382-6.

Bagrade, G., Kirjušina, M., Vismanis, K., Ozoliņš, J., 2009. Helminth parasites of the wolf *Canis lupus* from Latvia. J. Helminthol. 83, 63-68. doi: 10.1017/S0022149X08123860.

Basso, W., Spänhauer, Z., Arnold, S., Deplazes, P., 2013. *Capillaria plica* (syn. *Pearsonema plica*) infection in a dog with chronic pollakiuria: challenges in the diagnosis and treatment. Parasitol. Int. 63, 140-142. doi: 10.1016/j.parint.2013.09.002.

Bork-Mimm, S., Rinder, H., 2011. High prevalence of *Capillaria plica* infections in red foxes (*Vulpes vulpes*) in Southern Germany. Parasitol. Res. 108, 1063-1067. doi: 10.1007/s00436-010-2196-0.

Borka-Vitális, L., Domokos, C., Földvári, G., Majoros, G., 2017. Endoparasites of brown bears in Eastern Transylvania, Romania. Ursus. 28 (1), 20-30. doi: 10.2192/ursu-d-16-00015.1.

167 Bojarska, K., Selva, N., 2012. Spatial patterns in brown bear Ursus arctos diet: the role of
168 geographical and environmental factors. Mammal Rev. 42, 120-143. doi: 10.1111/j.1365-
169 2907.2011.00192.x.
170 Bourque, M., 2011. The masked shrew (*Sorex cinereus*), a new host for *Capillaria plica*. Can. J.
171 Zool. 59 (12), 2393-2394. doi: 10.1139/z81-320.
172 Bružinskaitė-Schmidhalter, R., Šarkūnas, M., Malakauskas, A., Mathis, A., Torgerson, P.R.,
173 Deplazes, P., 2011. Helminths of red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes*
174 *procyonoides*) in Lithuania. Parasitol. 139 (1), 120-127. doi: 10.1017/S0031182011001715.
175 Butterworth, E.W., Beverley-Burton, M., 1980. The Taxonomy of *Capillaria* spp. (Nematoda:
176 Trichuroidea) in Carnivorous Mammals from Ontario, Canada. Syst. Parasitol. 1(3/4), 211-
177 236.
178 Butterworth, E.W., Beverley-Burton, M., 1981. Observations on the Prevalence and Intensity of
179 *Capillaria* spp. (Nematoda: Trichuroidea) in Wild Carnivora from Ontario, Canada. Proc.
180 Helminthol. Soc. Wash. 48(1), 24-37.
181 Callegari, D., Kramer, L., Cantoni, A.M., Di Lecce, R., Dodi, P.L., Grandi, G., 2010. Canine
182 bladderworm (*Capillaria plica*) infection associated with glomerular amyloidosis. Vet.
183 Parasitol. 168, 338-341. doi: 10.1016/j.vetpar.2009.11.008.
184 Ciucci, P., Tosoni, E., Di Domenico, G., Quattrociocchi, F., Boitani, L., 2014. Seasonal and annual
185 variation in the food habits of Apennine brown bears, central Italy. J. Mammal. 95(3), 572-
186 586. doi: 10.1644/13-MAMM-A-218.
187 Ciucci, P., Gervasi, V., Boitani, L., Boulanger, J., Paetkau, D., Prive, R., Tosoni, E., 2015.
188 Estimating abundance of the remnant Apennine brown bear population using multiple non-
189 invasive genetic data sources. J. Mammal. 96, 206-220. doi: 10.1093/jmammal/gyu029.
190 Di Domenico, G., Tosoni, E., Boitani, L., Ciucci, P., 2012. Efficiency of scat-analysis lab
191 procedures for bear dietary studies: the case of the Apennine brown bear. Mamm. Biol. 77,
192 190-195. doi: 10.1016/j.mambio.2012.01.002.

193 Dupouy-Camet, J., Murrell, K.D., 2007. FAO/WHO/OIE Guidelines for the surveillance,
194 management, prevention and control of Trichinellosis. WHO, OIE. ISBN 9290447044, 122
195 pp.
196 Fernández-Aguilar, X., Mattsson, R., Meijer, T., Osterman-Lind, E., Gavier-Widén, D., 2010.
197 *Pearsonema* (*syn Capillaria*) *plica* associated cystitis in a Fennoscandian arctic fox (*Vulpes*
198 *lagopus*): a case report. Acta Vet. Scand. 52 (39). doi: 10.1186/1751-0147-52-39.
199 Gervasi, V., Ciucci, P., 2018. Demographic projections of the Apennine brown bear population
200 *Ursus arctos marsicanus* (Mammalia: Ursidae) under alternative management scenarios. Eur.
201 Zool. J. 85 (1), 243-253. doi: 10.1080/24750263.2018.1478003.
202 Guardone, L., Deplazes, P., Macchioni, F., Magi, M., Mathis, A., 2013. Ribosomal and
203 mitochondrial DNA analysis of Trichuridae nematodes of carnivores and small mammals.
204 Vet. Parasitol. 197, 364-369. doi: 10.1016/j.vetpar.2013.06.022.
205 Huizinga, H.W., Cosgrove, G.E., Sturrock, R.F., 1976. Renal capillariasis in the small Indian
206 mongoose, *Herpestes auropunctatus*. J. Wildlife Dis. 12 (1), 93-96.
207 Levine, N.D., 1968. *Nematode parasites of domestic animals and of man*. Burgess Publishing
208 Company, Minneapolis, USA.
209 Magi, M., Guardone, L., Prati, M. C., Mignone, W., Macchioni, F., 2014. Extraintestinal nematodes
210 of the red fox (*Vulpes vulpes*) in north-west Italy. J. Helminthol. 11, 1-6. doi:
211 10.1017/S0022149X1400025X.
212 Mariacher, A., Eleni, C., Fico, R., Ciarrocca, E., Perrucci, S., 2015. *Pearsonema plica* and *Eucoleus*
213 *böhmi* infections and associated lesions in wolves (*Canis lupus*) from Italy. Helminthologia.
214 52 (4), 364-369. doi: 10.1515/helmin-2015-0058.
215 Mattson D.J., French, M. G., French, S. P., 2001. Consumption of earthworms by Yellowstone
216 grizzly bears. Ursus 13, 105-110.

Maurelli, M.P., Rinaldi, L., Rubino, G., Lia, R., Musella, V., Cingoli, G., 2014. FLOTAC and Mini-FLOTAC for uro-microscopic diagnosis of *Capillaria plica* (syn. *Pearsonema plica*) in dogs. BMC Res. Notes. 7, 591. doi: 10.1186/1756-0500-7-591.

McLellan, B.N., Proctor, M.F., Huber, D., Michel, S., 2016. Brown bear (*Ursus arctos*) isolated populations (supplementary material to *Ursus arctos* Redlisting account). The IUCN Red List of Threatened Species 2016.

Moravec, F., 1982. Proposal of a new systematic arrangement of nematodes of the family Capillariidae. Folia Parasitol. 29 (2), 119-132.

Moravec F., Prokopic J., Shlikas A.V., 1987. The Biology of Nematodes of the Family Capillariidae Neveu-Lemaire 1936. Folia Parasitol. 34(1), 39-56.

Paoletti, B., Iorio, R., Traversa, D., Di Francesco, C.E., Gentile, L., Angelucci, S., Amicucci, C., Bartolini, R., Marangi, M., Di Cesare, A., 2017. Helminth infections in faecal samples of Apennine wolf (*Canis lupus italicus*) and Marsican brown bear (*Ursus arctos marsicanus*) in two protected national parks of central Italy. Ann. Parasitol. 63 (3), 205-212. doi: 10.17420/ap6303.107.

Papadopoulos, E., Komnenou, A., Putachides, T., Heikkinen, P., Oksanen, A., Karamanlidis, AA., 2017. Detection of *Dirofilaria immitis* in a brown bear (*Ursus arctos*) in Greece. Helminthologia. 54 (3), 257-261. doi: 10.1515/helm-2017-0033.

Rogers, L.L., Rogers, S.M., 1976. Parasites of Bears: A Review. Bears: Their Biology and Management 3. doi: 10.2307/3872791.

Rossi, M., Messina, N., Ariti, G., Riggio, F., Perrucci, S., 2011. Symptomatic *Capillaria plica* infection in a young European cat. J. Feline Med. Surg. 13, 793-795. doi: 10.1016/j.jfms.2011.07.006.

Rukhlyadev, D.P., Rukhlyadeva, M.N., 1953. Studies on the helminthofauna of the brown (caucasian) bear. In: Papers of helminthology presented to Academician K.I. Skrjabin on his 75th Birthday. Moscow: Izdatel'stvo Nauk SSSR, pp.598-602. Cited by Butterworth EW,

Beverley-Burton M (1980) The Taxonomy of *Capillaria* spp. (Nematoda: Trichuroidea) in Carnivorous Mammals from Ontario, Canada. Systematic Parasitology 1(3/4):211-236.

Senior, D.F., Solomon, G.B., Goldschmidt, M.H., Joyce, T., Bovee, K.C., 1980. *Capillaria plica* infection in dogs. J. Am. Vet. Med. Assoc. 176, 901-905.

Seville, R.S., Addison, E.M., 1995. Non-gastrointestinal helminths in Marten (*Martes americana*) from Ontario, Canada. J. Wildlife Dis. 31 (4), 529-533. doi: 10.7589/0090-3558-31.4.529.

Stancampiano, L., Poglayen, G., Marchesi, B., Barbieri, N., Gentile, L., 2008. Apennine brown bear (*Ursus arctos marsicanus*): does host population structure influence intestinal parasite community? In: Prigioni, C., Meriggi, A., Merli, E. (eds), 2008. VI Congr. It. Teriologia, Hystrix It. J. Mamm. (n.s.) Supp. 2008, 31.