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Original papers

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

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ABSTRACT. This article reports the results of a copromicroscopic and molecular investigation carried out on faecal samples of wolves (n=37) and brown bears (n=80) collected in two protected national parks of central Italy (Abruzzo Region). Twenty-three (62.2%) samples from wolves were positive for parasite eggs. Eight (34.78%) samples scored positive for single infections, i.e. *E. aerophilus* (21.74%), *Ancylostoma/Uncinaria* (4.34%), *Trichuris vulpis* (4.34%), *T. canis* (4.34%). Polyspecific infections were found in 15 samples (65.21%), these being the most frequent association: *E. aerophilus* and *Ancylostoma/Uncinaria*. Thirty-seven (46.25%) out of the 80 faecal samples from bears were positive for parasite eggs. Fourteen (37.83%) samples were positive for *B. transfuga*, and six (16.21%) of them also contained *Ancylostoma/Uncinaria*, one (2.7%) *E. aerophilus* and one (2.7%) both *E. aerophilus* and *Ancylostoma/Uncinaria*. Of the other samples, 19 (51.35%) were positive for *Ancylostoma/Uncinaria*, two (5.4%) for *E. aerophilus* and two (5.4%) for both. Molecular analysis found the roundworm and capillariid eggs found in wolves and bear samples to be *Toxocara canis*, *Baylisascaris transfuga* and *Eucoleus aerophilus* (syn. *Capillaria aerophila*). Considering the high prevalence of zoonotic intestinal helminths detected in this study, it is important to improve the knowledge and awareness of the general public and park operators regarding the potential health risk associated with infections in wildlife.

Key words: intestinal helminths, wolves, bears, faeces, zoonosis

Introduction

Wild animals play an important role in the epidemiology of several diseases of veterinary and zoonotic concern, as they may act as reservoirs and spreaders of pathogens capable of infecting animals and human beings. Various diseases are increasingly associated with human-modified ecological transition zones [1], and urbanization is an ongoing global phenomenon that has a significant impact on ecosystems and host-parasite interactions [2]. Additionally, several parasites pose a risk for the health and welfare of wildlife, as disease epidemics can severely reduce and isolate animal populations within protected areas [3] where pathogen transmission from domestic species is believed to have affected wild species [4]. In fact, the threat of disease transmission from domestic animals to wildlife has become recognized as an increasing concern within the conservation community in recent years [5].

In Europe, wolves are hosts and frequent spreaders of various helminths of zoonotic concern and/or veterinary importance, e.g. taeniid tapeworms [6–8], roundworms [9], hookworms [10,11], whipworms [12], *Trichinella* spp. [13] and extraintestinal nematodes [14–18]. Roundworms and hookworms are the most frequent intestinal helminths affecting wolves. For instance, *Toxocara canis* has been found in wolves from Poland, Hungary, Latvia, Spain and Italy with prevalence rates up to ~20% [10,19–22]. Similarly, ancylostomatid hookworms, e.g. *Ancylostoma caninum* and *Uncinaria stenocephala*¸ have been recorded in wolves in eastern Europe, the Iberian peninsula and Italy, with a prevalence of up to ~30% [13,19,23].

These nematodes are able to cause subclinical diseases in wolves but some of them, e.g. *T. canis,* can also infect humans and domestic animals [23]. For instance, when humans accidentally ingest larvated eggs from the environment, migrating *Toxocara* larvae can cause severe damage (i.e. *larva migrans* syndromes) [24]. Also, zoonotic hookworms may infect humans and cause skin, enteric and pulmonary diseases, such as cutaneous lesions and eosinophilic enteritis [25]. While the role of *A. caninum* and *U. stenocephala* as a cause of cutaneous *larva migrans* is still unclear [26], in humans this parasite has been associated with folliculitis, ephemeral and papular/pustular eruptions [27] and also with the penetration of muscle fibres and lung infiltrates [28]. As wolves mark their territories by scats and urine [29] and sporadically prey on livestock [30], the possibility of parasite transmission between wolves and humans or domestic animals may become a risk factor for public health due to increased contact with human settings.

Wild bears may harbour different zoonotic helminths e.g. tapeworms, flukes, roundworms and hookworms [31]. Nonetheless, the most important endoparasite affecting all species of bears worldwide is the roundworm *Baylisascaris transfuga* [32], which has been widely described in North America [33,34] and in some areas of Europe [35–37]. Roundworms of the *Baylisascaris* genus have been implicated in clinical and subclinical diseases in a range of natural hosts including bears; importantly, they are also known to play a role in life-threatening *larva migrans* syndrome in a number of domestic animals and humans [38–41]. There is no unequivocal evidence of natural infections by *B. transfuga* in non-ursid animals or humans [42], although migrating larvae have been reported to experimentally invade the central nervous system causing visceral, neural, and ocular disease in some animals, including mice [40,43–45].

The mountainous Abruzzo Region of central Italy encompasses four protected regional or national parks, and numerous others natural reserves and protected areas. These territories host large wild endangered predators, such as the Apennine wolf (*Canis lupus italicus*) and the Marsican brown bear (*Ursus arctos marsicanus*) [46]. The wolf population inhabiting some parks of the Abruzzo Region is currently stable, comprising about seven reproductive packs with a total number of about 30 wolves in Abruzzo, Lazio and Molise Park [47] and 10 reproductive packs with a total amount of about 70-80 individuals in Majella Park [48].

Importantly, the Marsican bear population comprises an isolated population of a subspecies [49] of the European brown bear (*Ursus arctos*, Linnaeus, 1758). The population is small, with an estimated number of about 40 individuals, and threatened (95% CI: 37–52) [50].

At present, scattered information is available on parasites of wolves in Italy, although intestinal helminths have been reported in some surveys from limited areas [8,19,51,52]. For instance, studies carried out on illegally killed wolves in the Apennine mountainous chain have found them to harbour various parasites, the most frequent being *T. canis*, *Trichuris vulpis* and *Echinococcus granulosus* [8].

Little information is available in Italy on the occurrence of parasites in free-ranging bears living in natural reserves. One study conducted in the Abruzzo, Lazio and Molise National Park and from its adjacent buffer zone found that *B. transfuga* was identified in a relatively large number of faecal samples (14.8%) followed by *Trichuris* spp. (1.8%) and *Strongyloides* (1.4%) [53]. Elsewhere, *Baylisascaris* spp. and *Trichinella* spp. were recorded in captive bears in Italy [37,54].

Therefore, given the importance of parasitosis of veterinary and zoonotic concern for endangered wildlife of Italian national parks, the aim of the present study was to obtain novel information on the distribution of intestinal helminths in wolves and bears living in two national parks of the Abruzzo mountainous territory of the country.

Materials and Methods

From May 2013 to October 2014, faecal samples from wolves (n=37) were collected from different areas of two national protected parks of central

Italy: 11 from the "Abruzzo, Lazio and Molise Park" (41°48'N, 13°47'E), and 26 from the "Majella Park" (42°10'4"N, 14°1'48"E). Eighty samples were collected from brown bears in an area within the "Abruzzo, Lazio and Molise Park".

The two study areas are located at similar latitudes, share a mountainous backcountry, this being a mosaic of forested and open habitats, and are exposed to similar seasonal climatic variations. Wolf and bear scats were discriminated from each other and from those of other animal species by size, shape, smell, composition and location. In particular, wolf adult droppings are 10–15 cm long and 3–5.5 cm thick, cylindrical, with sub-divisions, and are tapered at one of the extremities The secretion produced by the anal gland, which is atrophied in most dog breeds, adheres to the faeces during defecation and gives it an acrid and characteristic smell. Moreover, wolves usually use faeces in territorial marking. In the wild, faeces are more common along trails and roads, particularly at junctions, and are placed on conspicuous objects. In addition, undigested prey remains (i.e. fur, snails and parts of bones), especially from large wild ungulates such as wild boar and deer, were taken into account [8]. Samples were stored at 5°C in labelled plastic bags and put in a cooler for transportation. Wolf samples were then frozen at –80°C for four days for safety precautions [55], and then placed at refrigeration temperature. All samples were also included in a monitoring program aimed at identifying the major enteropathogenic viruses in wildlife. Molecular screening against Canine parvovirus type 2 (CPV-2), Canine adenovirus type 1 and 2 (CAV-1 and CAV-2) and Canine distemper virus (CDV) was negative for all samples (unpublished data).

For parasitological exams, faecal samples were divided into aliquots for copromicroscopic and molecular analysis, respectively. Copromicroscopic examination was made with a classical flotation procedure using a saturated $NaNO_3$ solution (specific gravity 1.300) [56]. Parasite elements were identified according to their morphological features and micrometric measurements at 40× and 100× magnifications [38,57].

The identity of roundworm eggs and capillariids found in bear and wolf faeces was genetically confirmed as follows. Genomic DNA was extracted from an aliquot of each positive sample using the commercial NORGEN Stool DNA Isolation Kit (Norgen Biotek Corp. Canada). Roundworm eggs from wolves were identified with primers specific for the ribosomal internal transcribed spacer (ITS-2) of *Toxocara* [58], while a primer set internal to the mitochondrial gene encoding for the cytochrome c

Table 1. Percentage of intestinal parasites recorded in wolves (*Canis lupus italicus*) and bears (*Ursus arctos marsicanus*) from two protected national parks in Italy based on copromicroscopic and molecular analysis of samples

Animal	Total positive samples	Monospecific infection	Two species	Three species
Wolves (N=37)	23 (62.2%)	8 34.78% on positive samples: – 5 (21.74 %) *E. aerophilus* – 1 (4.34%) *Ancylostoma/Uncinaria* – 1 (4.34%) *T. vulpis* – 1 (4.34%) *T. canis*	12 52.17% on positive samples: – 7 (30.43%) *E. aerophilus* + *Ancylostoma/Uncinaria* – 5 (21.74%) *T.vulpis* + *T.canis*	3 13.04% on positive samples: – 2 (8.7%) *E. aerophilus* + *Ancylostoma/Uncinaria* + *T. canis* – 1 (4.34%) *Ancylostoma /Uncinaria* + *T. vulpis* + *T.canis*
Bears (N=80)	37 (46.25%)	28 75.66% on positive samples: – 7 (18.91%) *B. transfuga* – 19 (51.35%) *Ancylostoma/Uncinaria* – 2 (5.4%) *E. aerophilus*	8 21.61% on positive samples: – 6 (16.21%) *B. transfuga* + *Ancylostoma/Uncinaria* – 2 (5.4%) *E. aerophilus* + *Ancylostoma/Uncinaria*	1 2.7% on positive samples: – 1 (2.7%) *B. transfuga* + *E. aerophilus* + *Ancylostoma/Uncinaria*

oxidase subunit I (*cox*1) of the *Baylisascaris* genus was chosen for the bear faeces [59]. Capillariid eggs were genetically identified with a semi-nested PCR amplifying diagnostic regions within the *cox*1 gene [60]. The amplicons obtained were purified and sequenced, and the sequences were aligned using Data Analysis in Molecular Biology and Evolution version 4.5.55 (DAMBE); the results were compared to sequences available in the GenBankTM database using the Nucleotide-Nucleotide "Basic Local Alignment Search Tool" (BLAST).

Results

Wolves

Twenty-three (62.2%) out of the 37 faecal samples from wolves were positive for parasite eggs (Table 1). Roundworms, capillariids, *Ancylostoma/ Uncinaria* and *Trichuris vulpis* eggs were identified. Roundworm and capillariid eggs were molecularly confirmed to be *T. canis* and *Eucoleus aerophilus (*syn. *Capillaria aerophila)*, i.e. 100% homology with sequence AB110034 and KC341988 deposited in the Genbank^{TM} database, respectively. Monospecific infections were found in eight (34.78%) samples: *E. aerophilus* (21.74%) being the most frequent, followed by *Ancylostoma/ Uncinaria*, *T. vulpis* and *T. canis*, all with a proportion of 4.34%. Polyspecific infections were found in 15 samples (65.21%), 12 of which scored positive for two parasites and three for three parasites at the same time. The most frequent association was represented by *Ancylostoma/ Uncinaria* and *E. aerophilus* (39%) (Table 1).

Bears

Thirty-seven (46.25%) out of the 80 faecal samples were positive for parasite eggs (Table 1). Roundworm, capillariid and *Ancylostoma/ Uncinaria* eggs were identified. Roundworm and capillariid eggs were molecularly confirmed to be *Baylisascaris transfuga* and *E. aerophilus (*syn. *Capillaria aerophila)*, i.e. 100% homology with sequence AB125694 and 100% homology with KC341988 deposited in the Genbank^{TM} Database respectively.

Of the 37 positive samples, 14 (37.8%) were positive for *B. transfuga* eggs; of these, six (16.21%) were positive also for *Ancylostoma/ Uncinaria*, one for *E. aerophilus* and one (2.7%) for both. Of the other positive samples, nineteen (51.35%) were positive for *Ancylostoma/ Uncinaria*, two (5.4%) for *E. aerophilus* and two (5.4%) for both *E. aerophilus* and *Ancylostoma/ Uncinaria* (Table 1).

Discussion

The present results show that wolves and bears living in the national parks of central Italy are infected with different nematodes, some of which have zoonotic potential. As parasites found in examined wolves and bears have a direct life cycle, the study of parasitic communities in wildlife can provide valuable information on the health status, dietary habits, and other ecological aspects of these animals, such as their home range and population dispersion: the high number of parasites being an indication of a large home range and wide dispersion in the habitat.

The nematodofauna recorded from the wolves in the Abruzzo Region is similar to that described in other countries from Eastern Europe, such as Latvia [13]; in addition, the rate of infection by the most frequent parasites, i.e. *E. aerophilus* and hook-worms, are in accordance with another study performed in Poland [22]. This is the first report of *E. aerophilus* in wolves from Italy, this parasite being previously recorded in wolves from Poland [22] and Latvia [13]. Importantly, this nematode also infects the lungs of a range of domestic and wild animals and, sometimes, people [61]. In the present study, *T. canis* and *T. vulpis* eggs were found with a lower percentage in comparison with rates recorded for hookworms, as reported previously [19]. While the prevalence of *T. canis* agrees with data from other European countries, i.e. Greece [62], Belarus [63], Poland [21] and Spain [23], the rates recorded for *T. vulpis* are lower than those recorded in eastern countries, e.g. Poland (38.5%) [21].

Infection of wolves with the nematode species reported here could result from direct transmission from other wild or domestic canids (e.g. *T. vulpis*, *E. aerophilus*) [64] or, in the case of *Toxocara*, through predation of infected small animals acting as paratenic hosts [65]. No taeniid eggs were found in the wolf faeces; however, this cannot suggest a true negativity to these parasites. In fact, previous studies based on necroscopic or genetic examinations have shown that the Italian wolf may act as a definitive host of many taeniids, i.e. *Taenia* spp. and *Echinococcus granulosus* [8,19,66]. The absence of tapeworm eggs in the samples can be attributed to the fact that cestode occurrence is often underestimates by conventional microscopy in comparison with other more sensitive methods. Therefore, infection by tapeworms in wolves from the studied areas should not be ruled out, especially considering that the examined geographic areas are endemic for *E. granulosus* [8], a tapeworm that can infect various canids, including wolves, and can cause potentially fatal infections in humans.

Few reports are available on the distribution of parasites of brown bears in wild territories of Europe. The present results confirm the frequent occurrence of *B. transfuga* in brown bears, as recorded in previous surveys in Italy [53] and Croatia [67]. While it is possible that samples from the same animal were examined, resulting in an overestimation of the infection rate, the values could also be underestimated due to possible false negative results at the copromicroscopy. The prevalence of ascarids based on faecal examinations is, in general, significantly lower than the real prevalence at necropsy [22]; in fact, this technique has some limitations, including variable sensitivity depending on parasite species [68], the inability to identify eggs morphologically beyond family or genus level [69], and is beset by potential temporal sampling mismatches, i.e. the samples might be collected during the prepatent period [70] or outside egg excretion peaks, when the number of eggs excreted is markedly reduced [71].

Infected bears often eliminate a large number of eggs in the environment [44]; therefore, the high number of samples that scored positive for *B. transfuga* indicate that bears may highly contaminate the area where they live and may potentially represent a threat for human health. In fact, until the role of *B. transfuga* as a zoonotic agent is ultimately clarified, this parasite should be considered as a potential threat for people. The presence of *Ancylostoma/Uncinaria* and *E. aerophilus* is also not surprising as these parasites have already been found in species of bears in Europe and other parts of world [31,34,72].

In the present study, wolves and bears were positive to *T. canis*, *B. transfuga* and *E. aerophilus*, i.e. nematodes able to cause intestinal and respiratory diseases in animals and with zoonotic potential. Humans become infected by ingesting larvated ascarid eggs from the environment, the eggs hatch in the intestine and the migrating larvae cause a number of clinical syndromes, e.g. *ocular* and *visceral larva migrans*, especially in toddlers and children [73–75]. In addition, when swallowing infective eggs of *E. aerophilus*, humans may display an infection characterized by fever, bronchitis, cough, haemoptysis and dyspnoea, and which can mimic bronchial carcinoma [76].

The present survey provides an update on the occurrence of parasites in free-ranging wolves and bears living in two national parks in central Italy. The identification of parasites with a zoonotic potential indicates the risk of transmission, for tourists, especially children, and local workers in different National Parks. Educational programs for the prevention of zoonotic parasitoses should be implemented to protect visitors in the National Park and continuous monitoring of areas potentially contaminated with wolf and bear faeces should ne initiated. Also, veterinary control programs should be implemented to reduce the parasitic burden on wildlife to achieve improvements in animal health and welfare.

References

[1] Despommier D., Ellis B.R., Wilcox B.A. 2006. The role of ecotones in emerging infectious diseases. *EcoHealth* 3: 281-289. doi:10.1007/s10393-006-0063-3

[2] Mackensted U., Jenkins D., Romig T. 2015. The role of wildlife in the transmission of parasitic zoonoses in peri-urban and urban areas. *International Journal for Parasitology: Parasites and Wildlife* 4: 71-79. doi:10.1016/j.ijppaw.2015.01.006

[3] León-Vizcaíno L., Ruíz de Ybáñez M.R., Cubero M.J., Ortíz J.M., Espinosa J., Pérez L., Simón M.A., Alonso F. 1999. Sarcoptic mange in Spanish ibex from Spain. *Journal of Wildlife Diseases* 35: 647-659. doi:10.7589/0090-3558-35.4.647

[4] Foreyt W.J., Jessup D.A. 1982. Fatal pneumonia of bighorn sheep following association with domestic sheep. *Journal of Wildlife Diseases* 18: 163-168. doi:10.7589/0090-3558-18.2.163

[5] Aguirre A.A. 2009. Wild canids as sentinels of ecological health: a conservation medicine perspective. *Parasitic and Vectors* 2 (Suppl. 1): S7. doi:10.1186/1756-3305-2-S1-S7

[6] Hirvelä-Koski V., Haukisalmi V., Kilpelä S.-S., Nylund M., Koski P. 2003. *Echinococcus granulosus* in Finland. *Veterinary Parasitology* 111: 175-192. doi:10.1016/s0304-4017(02)00381-3

[7] Guerra D., Armua-Fernandez M.T., Silva M., Bravo I., Santos N., Deplazes P., Carvalho L.M. 2012. Taeniid species of the Iberian wolf (*Canis lupus signatus*) in Portugal with special focus on *Echinococcus* spp. *International Journal for*

Parasitology: Parasites and Wildlife 2: 50-53. doi:10.1016/j.ijppaw.2012.11.007

[8] Gori F., Armua-Fernandez M.T., Milanesi P., Serafini M., Magi M., Deplazes P., Macchioni F. 2015. The occurrence of taeniids of wolves in Liguria (northern Italy). *International Journal for Parasitology: Parasites and Wildlife* 4: 252-255. doi:10.1016/j.ijppaw.2015.04.005

[9] Craig H.L, Craig P.S. 2005. Helminth parasites of wolves (*Canis lupus*): a species list and an analysis of published prevalence studies in Nearctic and Palaearctic populations. *Journal of Helminthology* 79: 95-103. doi:10.1079/joh2005828

[10] Borecka A., Gawor J., Zięba F. 2013. A survey of intestinal helminths in wild carnivores from the Tatra National Park, southern Poland. *Annals of Parasitology* 59: 169-172.

[11] Takács A., Szabó L., Juhász L., Takács A., Lanszki J., Takács P., Heltai M. 2014. Data on the parasitological status of golden jackal (*Canis aureus* L., 1758) in Hungary. *Acta Veterinaria Hungarica* 62: 33-41. doi:10.1556/AVet.2013.058

[12] Szafrańska E., Wasielewski O., Bereszyński A. 2010. A faecal analysis of helminth infections in wild and captive wolves, *Canis lupus* L., in Poland. *Journal of Helminthology* 84: 415-419. doi:10.1017/s0022149x10000106

[13] Bagrade G., Kirjušina M., Vismanis K., Ozoliņš J. 2009. Helminth parasites of the wolf *Canis lupus* from Latvia. *Journal of Helminthology* 83: 63-68. doi:10.1017/s0022149x08123860

[14] Otranto D., Cantacessi C., Mallia E., Lia R.P. 2007. First report of *Thelazia callipaeda* (Spirurida, Thelaziidae) in wolves in Italy. *Journal of Wildlife Diseases* 43: 508-511. doi:10.7589/0090-3558-43.3.508

[15] Otranto D., Dantas-Torres F., Mallia E., DiGeronimo P.M., Brianti E, Testini G., Traversa D, Lia R.P. 2009. *Thelazia callipaeda* (Spirurida, Thelaziidae) in wild animals: report of new host species and ecological implications. *Veterinary Parasitology* 166: 262-267. doi:10.1016/j.vetpar.2009.08.027

[16] Cirovi D., Penezić A., Pavlović I., Kulišić Z., Cosić N., Burazerović J., Maletić V. 2014. First records of *Dirofilaria repens* in wild canids from the region of Central Balkan. *Acta Veterinaria Hungarica* 62: 481-488. doi:10.1556/avet.2014.021

[17] Eleni C., De Liberato C., Azam D., Morgan E.R., Traversa D. 2014. *Angiostrongylus vasorum* in wolves in Italy. *International Journal for Parasitology: Parasites and Wildlife* 3: 12-14. doi:10.1016/j.ijppaw.2013.10.003

[18] Mariacher A., Eleni C., Fico R., Ciarrocca C., Perrucci S. 2015. *Pearsonema plica* and *Eucoleus böhmi* infections and associated lesions in wolves (*Canis lupus*) from Italy. *Helminthologia* 52: 364-369. doi:10.1515/helmin-2015-0058

[19] Guberti V., Stancampiano L., Francisci F., 1993. Intestinal helminth parasite community in wolves (*Canis lupus*) in Italy. *Parassitologia* 35: 59-65.

[20] Segovia J.M., Guerrero R., Torres J., Miquel J., Feliu C. 2003. Ecological analyses of the intestinal helminth communities of the wolf, *Canis lupus*, in Spain. *Folia Parasitologica* 50: 231-236. doi:10.14411/fp.2003.041

[21] Kloch A., Bednarska M., Bajer A. 2005. Intestinal macro- and microparasites of wolves (*Canis lupus L.*) from north-eastern Poland recovered by coprological study. *Annals of Agricultural and Environmental Medicine* 12: 237-245.

[22] Popiołek M., Szczęsna J., Nowak S., Mysłajek R.W. 2007. Helminth infections in faecal samples of wolves *Canis lupus* L. from the western Beskidy Mountains in southern Poland. *Journal of Helminthology* 81: 339-344. doi:10.1017/s0022149x07821286

[23] Segovia J.M., Torres J., Miquel J., Llaneza L., Feliu C. 2001. Helminths in the wolf, *Canis lupus*, from north-western Spain. *Journal of Helminthology* 75: 183-192. doi:10.1079/JOH200152

[24] Rubinsky-Elefant G., Hirata C.E., Yamamoto J.H., Ferreira M.U. 2010. Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. *Annals of Tropical Medicine and Parasitology* 104: 3-23. doi:10.1179/136485910x12607012373957

[25] Bowman D.D., Montgomery S.P., Zajac A.M., Eberhard M.L., Kazacos K.R. 2010. Hookworms of dogs and cats as agents of cutaneous larva migrans. *Trends in Parasitology* 26: 162-167. doi:10.1016/j.pt.2010.01.005

[26] Lee A.C.Y., Schantz P.M., Kazacos K.R., Montgomery S.P., Bowman D.D. 2010. Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. *Trends in Parasitology* 26: 155-161. doi:10.1016/j.pt.2010.01.002

[27] Rivera-Roig V., Sánchez J.L., Hillyer G.V. 2008. Hookworm folliculitis. *International Journal of Dermatology* 47: 246-248. doi:10.1111/j.1365-4632.2008.03469.x

[28] Little M.D., Halsey N.A., Cline B.L., Katz S.P. 1983. *Ancylostoma* larva in a muscle fiber of man following cutaneous larva migrans. *The American Journal of Tropical Medicine and Hygiene* 32: 1285-1288. doi:10.4269/ajtmh.1983.32.1285

[29] Zub K., Theuerkauf J., Jędrzejewski W., Jędrzejewska B., Schmidt K., Kowalczyk R. 2003. Wolf pack territory marking in the Bialowieza Primeval Forest (Poland). *Behaviour* 140: 635-648. doi:10.1163/156853903322149478

[30] Jedrzejewski W., Nowak S., Schmidt K., Jedrzejewska B. 2002. The wolf and the lynx in Poland – results of the census conducted in 2001. *Kosmos* 51: 491-499 (in Polish with summary in

English).

[31] Choquette L.P.E., Gibson G.G., Pearson A.M. 1969. Helminths of the grizzly bear, *Ursus arctos* L., in northern Canada. *Canadian Journal of Zoology* 47: 167-170. doi:10.1139/z69-038

[32] Schaul J.C. 2006. *Baylisascaris transfuga* in captive and free-ranging populations of bears (Family: Ursidae). Dissertation for the Degree Doctoral of Philosophy in the Graduate School of The Ohio State University, The Ohio State University, Ohio, USA.

[33] Duffy M.S., Greaves T.A., Burt M.D.B. 1994. Helminths of the black bear, *Ursus americanus*, in New Brunswick. *Journal of Parasitology* 80: 478-480. doi:10.2307/3283422

[34] Foster G.W., Cunningham M.W., Kinsella J.M., Forrester D.J. 2004. Parasitic helminths of black bear cubs (*Ursus americanus*) from Florida. *Journal of Parasitology* 90: 173-175. doi:10.1645/ge-127r

[35] Huber D., Stahan Z. 1983. Helmintofauna nekih vrsta divljači Samoborskog gorja: ekološki odnosi. *Veterinarska Stanica* 14: 23-27 (in Croatian).

[36] De Ambrogi M., Aghazadeh M., Hermosilla C., Huber D., Majnaric D., Reljic S., Elson-Riggins J. 2011. Occurrence of *Baylisascaris transfuga* in wild populations of European brown bears (*Ursus arctos*) as identified by a new PCR method. *Veterinary Parasitology* 179: 272-276. doi:10.1016/j.vetpar.2011.02.025

[37] Testini G., Papini R., Lia R.P., Parisi A., Dantas-Torres F., Traversa D., Otranto D. 2011. New insights into the morphology, molecular characterization and identification of *Baylisascaris transfuga* (Ascaridida, Ascarididae). *Veterinary Parasitology* 175: 97-102. doi:10.1016/j.vetpar.2010.09.017

[38] Sprent J.F.A. 1968. Notes on *Ascaris* and *Toxascaris*, with a definition of *Baylisascaris* gen. nov. *Parasitology* 58: 185-198. doi:10.1017/s0031182000073534

[39] Papini R., Renzoni G., Lo Piccolo S., Casarosa L. 1996. Ocular larva migrans and histopathological lesions in mice experimentally infected with *Baylisascaris transfuga* embryonated eggs. *Veterinary Parasitology* 61: 315-320. doi:10.1016/0304-4017(95)00825-x

[40] Sato H., Matsuo K., Osanai A., Kamiya H., Akao N., Owaki S., Furuoka H. 2004. Larva migrans by *Balisascaris transfuga*: fatal neurological diseases in Mongolian jirds, but not in mice. *Journal of Parasitology* 90: 774-781. doi:10.1645/GE-3330

[41] Cho S., Egami M., Ohnuki H., Saito Y., Chinone S., Shichinohe K., Suganuma M., Akao N. 2007. Migration behaviour and pathogenesis of five ascarid nematode species in the Mongolian gerbil *Meriones unguiculatus*. *Journal of Helminthology* 81: 43-47. doi:10.1017/s0022149x07212118

[42] Bauer C. 2013. Baylisascariosis – infections of animals and humans with 'unusual' roundworms. *Veterinary Parasitology* 193: 404-412. doi:10.1016/j.vetpar.2012.12.036

[43] Papini R., Casarosa L. 1994. Observations on the infectivity of *Baylisascaris transfuga* eggs for mice. *Veterinary Parasitology* 51: 283-288. doi:10.1016/0304-4017(94)90166-x

[44] Papini R., Renzoni G., Malloggi M., Casarosa L. 1994. Visceral larva migrans in mice experimentally infected with *Baylisascaris transfuga* (Ascarididae: Nematoda). *Parassitologia* 36: 321-329.

[45] Papini R., Demi S., Della Croce G. 1996. Observations on the migratory behaviour of *Baylisascaris transfuga* larvae in rabbits. *Revue de Médicine Vétérinaire* 147: 893-896.

[46] Altobello G. 1921. Fauna dell'Abruzzo e del Molise. Mammiferi IV. I Carnivori (Carnivora). Casa Tipografico-Editrice, Cav. Uff. Giov. Colitti e figlio, Campobasso, Italy (in Italian).

[47] Grottoli L., Ciucci P., Boitani L., Gentile L., latini R. 2012. Consistenza e assetto della popolazione di lupo nel parco Nazionale D'Abruzzo, Lazio e Molise, 2006-2009. Atti VIII Convegnonazionale ATIt, 9-11maggio2012, Piacenza (in Italian).

[48] Majella National Park. 2013. Apennine wolf. http://www.parcomajella.it/en/natura/fauna-del-parco/mammiferi/lupo-appenninico

[49] Randi E., Gentile L., Boscagli G., Huber D., Roth H.U. 1994. Mitochondrial DNA sequence divergence among some west European brown bear (*Ursus arctos L.*) populations. Lessons for conservation. *Heredity* 73: 480-489. doi:10.1038/hdy.1994.146

[50] Gervasi V., Ciucci P., Boulanger J., Randi E., Boitani L. 2012. A multiple data source approach to improve abundance estimates of small populations: the brown bear in the Apennines, Italy. *Biological Conservation* 152: 10-20. doi:10.1016/j.biocon.2012.04.005

[51] Arru E., Garippa G., Fico R. 1986. Sulla presenza di *Echinococcus granulosus* nella volpe (*Vulpes vulpes*) e nel lupo (*Canis lupus*). *Atti della Società Italiana di Scienze Veterinarie* 42: 927-929 (in Italian).

[52] Guberti V., Francisci F. 1991. Cause di mortalitŕ di 60 Lupi raccolti in Italia dal 1984. *Ricerche di Biologia della Selvaggina* 19: 599-603 (in Italian).

[53] 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? *"Hystrix" The Italian Journal of Mammalogy* 19: 31.

[54] Pozio E. 2015. *Trichinella* spp. imported with live animals and meat. *Veterinary Parasitology* 213: 46-55. doi:10.1016/j.vetpar.2015.02.017

[55] Eckert J., Gottstein B., Heath D., Liu F.-J. 2001. Prevention of echinococcosis in humans and safety precaution. In: *WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern*. (Eds. J. Eckert, M.A. Gemmel, F.X. Meslin, Z.S. Pawlowski). World Organization for

Animal Health, Paris, France: 238-246. http://apps.who.int/iris/handle/10665/42427

[56] Euzeby J. 1981. Diagnostic expérimental des helminthoses animales: travaux pratiques d'helminthologie vétérinaire. Informations Techniques des Services Vétérinaries, Paris, France.

[57] Sloss M.W., Kemp R.L., Zajac A.M. 1994. Veterinary clinical parasitology. 6th Edition, Wiley-Blackwell, London.

[58] Fogt-Wyrwas R., Jarosz W., Mizgajska-Wiktor H. 2007. Utilizing a polymerase chain reaction method for the detection of *Toxocara canis* and *T. cati* eggs in soil. *Journal of Helminthology* 81: 75-78. doi:10.1017/s0022149x07241872

[59] Sato H., Une Y., Kawakami S., Saito E., Kamiya H., Akao N., Furuoka H. 2005. Fatal *Baylisascaris* larva migrans in a colony of Japanese macaques kept by a safari-style zoo in Japan. *Journal of Parasitology* 91: 716-719. doi:10.1645/ge-3374rn

[60] Di Cesare A., Castagna G., Otranto D., Meloni S., Milillo P., Latrofa M.S., Paoletti B., Bartolini R., Traversa D. 2012. Molecular detection of *Capillaria aerophila*, an agent of canine and feline pulmonary capillariosis. *Journal of Clinical Microbiology* 50: 1958-1963. doi: 10.1128/jcm.00103-12

[61] Traversa D., Di Cesare A. 2013. Feline lungworms: what a dilemma. *Trends in Parasitology* 29: 423-430. doi:10.1016/j.pt.2013.07.004

[62] Papadopoulos H., Himonas C., Papazahariadou M., Antoniadou-Sotiriadou K. 1997. Helminths of foxes and other wild carnivores from rural areas in Greece. *Journal of Helminthology* 71: 227-232. doi:10.1017/s0022149x00015960

[63] Shimalov V.V., Shimalov V.T. 2000. Helminth fauna of the wolf (*Canis lupus* Linnaeus, 1758) in Belorussian Polesie. *Parasitology Research* 86: 163-164. doi:10.1007/s004360050026

[64] Urquhart G.M., Armour J., Duncan J.L., Dunn A.M., Jennings F.W. 1996. Veterinary parasitology. Blackwell Science, Glasgow, Scotland.

[65] Ceruti R., Sonzogni O., Origgi F., Vezzoli F., Cammarata S., Giusti A.M., Scanziani E. 2001. *Capillaria hepatica* infection in wild brown rats (*Rattus norvegicus*) from the urban area of Milan, Italy. *Journal of Veterinary Medicine Series B* 48: 235-240. doi:10.1046/j.1439-0450.2001.00436.x

[66] Guberti V., Bolognini M., Lanfranchi P., Battelli G. 2004. *Echinococcus granulosus* in the wolf in Italy. *Parassitologia* 46: 425-427.

[67] Aghazadeh M., Elson-Riggins J., Reljić S., De Ambrogi M., Huber D., Majnarić D., Hermosilla C. 2015. Gastrointestinal parasites and the first report of *Giardia* spp. in a wild population of European brown bears (*Ursus arctos*) in Croatia. *Veterinarski Archives* 85: 201-210.

[68] Dryden M.W., Payne P.A., Ridley R., Smith V. 2005. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. *Veterinary Therapeutics* 6: 15-28.

[69] Taylor M.A., Coop R.L., Wall R.L. 2007. Veterinary Parasitology. 3rd Edition. Blackwell Publishing Ltd., Ames, Iowa, USA.

[70] Snyder D.E., Fitzgerald P.R. 1987. Contaminative potential, egg prevalence, and intensity of *Baylisascaris procyonis* infected raccoons (*Procyon lotor*) from Illinois, with a comparison to worm intensity. *Proceedings of the Helminthological Society of Washington* 54: 141-145.

[71] Kapel C.M.O., Torgerson P.R., Thompson R.C.A., Deplazes P. 2006. Reproductive potential of *Echinococcus multilocularis* in experimentally infected foxes, dogs, raccoon dogs and cats. *International Journal for Parasitology* 36: 79-86. doi:10.1016/j.ijpara.2005.08.012

[72] Crum J.M., Nettles V.F., Davidson W.R. 1978. Studies on endoparasites of the black bear (*Ursus americanus*) in the southeastern United States. *Journal of Wildlife Diseases* 14: 178-186. doi:10.7589/0090-3558-14.2.178

[73] Despommier D. 2003. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clinical Microbiology Reviews* 16: 265-272. doi:10.1128/cmr.16.2.265-272.2003

[74] Roddie G., Stafford P., Holland C., Wolfe A. 2008. Contamination of dog hair with eggs of *Toxocara canis*. *Veterinary Parasitology* 152: 85-93. doi:10.1016/j.vetpar.2007.12.008

[75] Overgaauw P.A.M., van Zutphen L., Hoek D., Yaya F.O., Roelfsema J., Pinelli E., van Knapen F., Kortbeek L.M. 2009. Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. *Veterinary Parasitology* 163: 115-122. doi:10.1016/j.vetpar.2009.03.044

[76] Lalošević D., Lalošević V., Klem I., Stanojev-Jovanović D., Pozio E. 2008. Pulmonary capillariasis miming bronchial carcinoma. *The American Journal of Tropical Medicine and Hygiene* 78: 14-16. doi:10.4269/ajtmh.2008.78.14

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