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Case Report

Preliminary findings on the gastrointestinal parasites of the brown bear (*Ursus arctos*) in the Cantabrian mountains, Spain

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ABSTRACT

No study is currently available on the parasitofauna of the population of brown bears (*Ursus arctos*) inhabiting the Cantabrian Mountains in Spain. The aim of the present study was to obtain novel information on diversity and prevalence of gastrointestinal parasites in these individuals. During August 2016 and from May to July 2017, 14 fecal samples were collected from the western Cantabrian bear subpopulation, in the Somiedo Natural Park, in the Spanish province of Asturias. The prevalence of parasites detected was 71% and two genera were identified: *Dicrocoelium* sp. and *Trichuris* sp. Since the impact that pathogens such as endoparasites can have on the health of bears, together with other stressors, is still poorly understood, research efforts that include disease surveillance are critical to the successful protection of this emblematic species. Our preliminary findings require further investigations, with a wider sampling effort, and bring awareness for the need of carrying further studies on this area as a part of a proactive species management plan.

1. Introduction

The brown bear (*Ursus arctos*), once a widespread distributed mammal in Europe, is now restricted to regions where human impact remains low, such as the Cantabrian Mountains, in Spain. The Cantabrian brown bear has almost faced extinction in the past, due to hunting and habitat degradation (Martínez Cano et al., 2016), which led to the population being fragmented into two separated subpopulations (Pérez et al., 2010). Fortunately, following the implementation of conservation measures, these sub-populations are now showing an increase in numbers, in range and in connectivity between each other (Gonzalez et al., 2016).

While bears generally avoid humans and their settlements, as their numbers increase, it is expected to also occur an increase of interactions with human populations, leading to an increment of conflicts, such as crop and apiary damages and livestock predations (Dorresteijn et al., 2014; Naves et al., 2018). The subsequent increase in coexistence between humans, domestic animals and these large carnivores may also lead to greater risk of transmission of zoonotic diseases and maintenance of a sylvatic-domestic parasite transmission cycle, since bears play an important role as reservoirs and spreaders of several parasitic diseases of veterinary and zoonotic concern (Paoletti et al., 2017; Di Salvo and Chomel, 2020).

At the moment, no information is available regarding the parasitofauna of the Cantabrian brown bear population. Throughout Europe, there are reports that brown bears may harbour different helminths, and the most commonly reported include species such as *Baylisascaris transfuga*, *Uncinaria* sp., *Diphyllobothrium latum*, *Dicrocoelium* sp. and *Trichuris* sp. (De Ambrogi et al., 2011; Orosová et al., 2016; Borka-Vitális et al., 2017; Paoletti et al., 2017; Mariacher et al., 2018; Štrkolcová et al., 2018; Molnár et al., 2020). Combined with the already big list of threats that this species already faces, some with immunosuppressive potential, such as human persecution, poaching and exposure to contaminants (Zedrosser et al., 2011; Haugen, 2020), high parasitic infections may contribute to individual poor health and eventually lead to population declines.

Thus, given the impact that parasitic infections can have on the health of brown bears, other animals and humans, and considering that no information is available regarding the parasitofauna of the Cantabrian brown bear population in Spain, the aim of the present study was

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to obtain novel information on diversity and prevalence of gastrointestinal parasites in these endangered animals.

2. Material and methods

2.1. Study area

This study was conducted in the Somiedo Natural Park in the western region of the Cantabrian Mountains, located along the Atlantic coast of north-western Spain, in the province of Asturias (Fig. 1). The Cantabrian mountains area is characterized by a climate with mild temperatures and high humidity, displays a complex mountainous topography and a forest coverage on 25% of its territory (García et al., 2007). Although the human population density in the area is low, human activities have converted large patches of natural cover into pastures and agricultural lands, with the main economic activity being the extensive breeding of livestock (Fernández-Gil et al., 2006).

The Cantabrian brown bears are distributed in two connected subpopulations (Gonzalez et al., 2016), occupying an area around 7000 km^2, with above 200 individuals in the western subpopulation (CI95% = 168–260 individuals) and 20 in the eastern subpopulation (CI95% = 12–40 individuals) (Pérez et al., 2014).

2.2. Sample collection

During August 2016 and from May to July 2017, brown bear fecal samples were collected opportunistically ($N = 8$ and $N = 6$, respectively) from the western population of Cantabrian bears, during camera trapping setting up events in Somiedo Natural Park. The samples were recognized by their distinctive size, shape and smell by experienced field technicians from Fondo para la Protección de los Animales Salvajes (FAPAS). The samples were obtained from the central portion of fresh-looking scats. Each sample was stored in a plastic container at 4 °C and subsequently frozen until further processing.

2.3. Coprology

The frozen samples were thawed 24 h prior to coprological examination. The samples were submitted to standardized flotation and sedimentation techniques (Thienpont et al., 1986). About 4 g of feces were mixed with 50 ml of a saturated sodium chloride (NaCl) solution. The mixture was strained through cheesecloth and left to stand for 1 h. The surface film was then mounted on three glass slides. Three hours later, the supernatant was discarded and the sediment was transferred to three other glass slides and stained the methylene blue. All the slides were examined under a light microscope Olympus BX 50, using the facilities of the Laboratory of Parasitology and Parasitic Diseases, at the Faculty of Veterinary Medicine, University of Lisbon, Portugal. Identification of parasite eggs was based on available information on their morphology and morphometry ()(Hendrix and Robinson, 2012b,a)

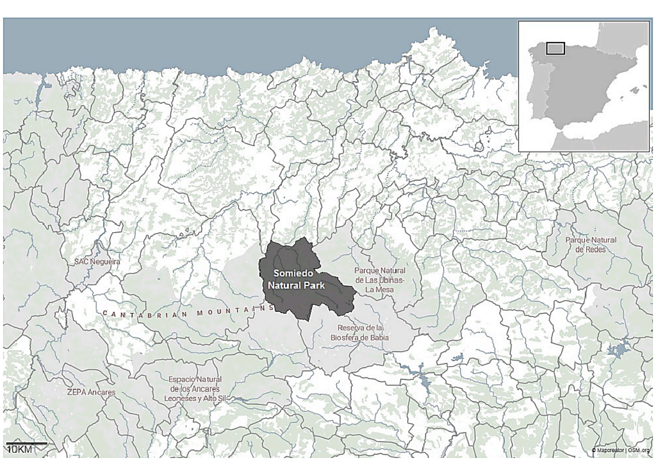

Fig. 1. Map of the study area, with Somiedo Natural Park highlighted.

3. Results

During our study period we were able to collect 14 brown bear fecal samples from the western subpopulation. We detected parasites in 10 out of the 14 samples (71%). Trematode eggs were found in 10 samples (71%), all of them structurally corresponding to typical representatives of the genus *Dicrocoelium*. Additionally, in 2 of those 10 samples there were also eggs compatible with and identified as *Trichuris* sp. (14% of the total samples).

Furthermore, in one of the samples it was detected a nematode egg with a perforated membrane, being infected by nematophagous fungi, of unknown species.

4. Discussion

Few surveys of parasitofauna in brown bears have been conducted in Europe. In Central Slovakia, of 17 scat samples collected, 76% were positive for the presence of parasites. *Baylisascaris transfuga* and *Ancylostoma* spp. were the dominant species (Orosová et al., 2016). In Romania, endoparasites were found in 38% of 211 scats samples, including *Baylisascaris transfuga*, *Crenosoma* sp., *Trichuris* sp. and *Dicrocoelium dendriticum* (Borka-Vitális et al., 2017). In Poland, no parasite eggs were found in the 12 brown bear fecal samples collected (Borecka et al., 2013). In Croatia, gastrointestinal parasites were detected in 33% of 94 brown bear fecal samples, including *Baylisascaris transfuga* and *Syngamus* sp. (Aghazadeh et al., 2015). In our study, we detected endoparasites in 71% of the samples collected, belonging to the genera *Dicrocoelium* and *Trichuris*.

D. dendriticum infects the bile ducts of the liver and gall bladder of its definitive hosts— herbivorous and carnivorous mammals, and its life cycle involves two intermediate hosts - terrestrial gastropod mollusks and ants ()(Hendrix and Robinson, 2012b). Ants represent an important food for bears (Purroy, 2017; Keis et al., 2019), so the consumption of infected secondary intermediate hosts might potentially lead to the transmission of *D. dendriticum*. Alternatively, this can also be a case of pseudo-parasitism, following the consumption of an infected definitive host's liver by the bear, namely domestic and wild ungulates/lagomorphs, being this alternative for egg shedding a very rare one.

Trichuris sp. has been previously reported in European brown bears (Borka-Vitális et al., 2017); *Trichuris vulpis* has been reported in northern Spain in wolves (*Canis lupus*) (Segovia et al., 2003), and in several European countries in wolves, red foxes (*Vulpes vulpes*), dogs and pine martens (*Martes martes*) (Borecka et al., 2013; Kirkova et al., 2006), all species that share the habitat with the Cantabrian bear. The presence of *Trichuris* sp. can also be a case of pseudo-parasitism, since *Trichuris suis* can be found in domestic pigs and wild boar (*Sus scrofa ferus*) and *T. ovis* in domestic and wild ruminants)(Hendrix and Robinson, 2012a), which can be occasionally ingested by bears, being, however, a rare alternative for a real patent infection.

One important aspect to consider is the fact that bears, due to hibernation, have a specific parasitic seasonal dynamic. The highest prevalence of endoparasites in bears has been recorded in autumn and the lowest in spring (Gau et al., 1999; Orosová et al., 2016), presumably because they empty their bowels before hibernation and start to get reinfected later in spring (Gau et al., 1999). Specifically, a study on the pattern of *B. transfuga* prevalence and excretion in European brown bears concluded that the intensity of excretion of the parasite eggs had its peak in autumn (Molnár et al., 2020). The fact that the samples of our study were mainly collected in late spring and summer, added to the small sample size, can explain the low diversity of gastrointestinal parasite species detected and the absence of nematode eggs of the widespread bear specialist *B. transfuga*. In another study, *B. transfuga* eggs were not detected in the scats of bears in which the nematode infection was confirmed both by positive Polymerase Chain Reaction (PCR) and dissection of the intestine (Aghazadeh et al., 2015). Underestimated prevalence levels yielded by coprological studies may be the consequence of the low sensitivity of flotation techniques (Paoletti et al., 2017). Nevertheless, flotation/sedimentation tests were used in the present study, since they are cost-effective and easy to perform, as a first approach for preliminary parasitological studies. In the other hand, *Dicrocoelium* sp. is a parasite normally found in the liver and gall bladder, which may explain the high prevalence of this parasite, regardless of the bears' hibernation dynamics. Further, there is also the possibility that the scats collected do not all belong to different bears and, thus, some of the samples might represent the infection of the same individual over different time-points.

Interestingly, in one of the samples it was detected nematophagous fungi infecting a nematode egg. Nematophagous fungi are ubiquitous and generally regarded as soil organisms, with the capacity to attack and infect nematodes at all stages (Lopez-Llorca et al., 2008). Considering that they are able to reduce the number and viability of gastrointestinal parasites' eggs in distinct animal species and in the environment (Canhão-Dias et al., 2020), they may play an important role in the dynamics of disease transmission in the wild and it may partially explain the absence of nematodes in the samples of this study.

5. Conclusion

Despite the positive news of the Cantabrian brown bear population growth and range expansion, it is expected that this will lead to increasingly more individuals getting displaced towards humanized areas and to an increase in interactions with human populations and domestic animals. Since the impact that pathogens such as endoparasites can have on the health of bears, together with other stressors, is still poorly understood, research efforts that include disease surveillance are critical to the successful protection of this emblematic species. For future research, a wider sampling is suggested, throughout different seasons, with coprology techniques complemented with molecular methods. Nevertheless, our preliminary results bring awareness for the need of carrying further studies on this area as a part of a proactive species management plan.

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Animal welfare

Not applicable.

Ethical statement

Not applicable.

Declaration of Competing Interest

None.

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