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RESEARCH

Endoparasites of the Iberian wolf (*Canis lupus signatus*) and mesocarnivores in Central Portugal

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Abstract

At the end of the nineteenth century, massive population declines were observed in carnivores due to the emergence of infectious diseases. This study aims to investigate, by means of coprological analysis, the prevalence and intensity of the parasites that infect the endangered Iberian wolf *Canis lupus signatus* and two mesocarnivores (the red fox *Vulpes vulpes* and the stone marten *Martes foina*) in Central Portugal. In total, 67.2% of the samples screened were infected; *Toxascaris leonina* (40.6%) was the parasite with the highest prevalence, followed by Ancylostomatidae and *Eimeria* spp. (28.1%). *Eimeria* spp. was found in stone marten with the highest infection rate (37,800 OPG), followed by *T. leonina* (10,100 EPG) in a red fox sample. Moderate to high levels of parasitic infections were identified in 73.3% of red foxes from the western area. Our results highlight the possibility of cross-infection among these carnivore species and cross-contamination in the wildlife-livestock-human interface.

Keywords Iberian wolf · Mesocarnivores · Ancylostomatidae · *Toxascaris leonina* · *Eimeria* · Portugal

Introduction

By the end of the nineteenth century, the emergence of infectious diseases caused several worldwide massive wildlife populations to decline, particularly carnivores (Funk et al. 2001). Considering their ability to trigger sudden outbreaks and their fast evolutionary mechanisms, parasites and other pathogens became a major subject of studies on carnivore decline (Smith et al. 2006). Carnivores are hosts of ca. 43% of the human infection pathogens (Cleaveland et al. 2001; Otranto and Deplazes 2019), being susceptible to a wide array of pathogenic microparasites. Many of these microparasites are native or can be easily transmitted by domestic species, highlighting their zoonotic importance (Thompson et al. 2009; Otranto and Deplazes 2019). Because of the impact of parasites at individual and community levels, the study of parasitic and infectious diseases in wild carnivores is important, considering not only that several species are already endangered by the ongoing anthropogenic impact (Ripple et al. 2014) but also because there is a lack of information on this topic (Torres et al. 2006).

The Iberian wolf (*Canis lupus signatus*), an endemic top predator of the Iberian Peninsula, has seen its abundance and distribution decline, occupying now about 20% of its

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original range (Torres and Fonseca 2016). Their fragile conservation status sets the understanding of their health at the centre of conservation programmes; however, relative information is available (Figueiredo et al. 2016, 2019). This study aims to investigate the prevalence and intensity of parasites that infect the endangered Iberian wolf and other carnivore species, such as the stone marten (*Martes foina*) and the sympatric red fox (*Vulpes vulpes*), in Central Portugal.

Material and methods

Study area

The study was carried out in Central Portugal, south of the Douro River (Fig. 1), in two distinct but adjacent areas (western and eastern areas), covering a total area of ca. 8941 km^2 (Carvalho et al. 2020).

The western area has a total area of 750 km^2, comprising the Freita-Arada and Montemuro mountainous range and occupying two sites of the Natura 2000 network. The climate is mainly Mediterranean, with a strong Atlantic influence and high levels of rainfall (i.e. average annual rainfall of 2000 mm). It features a vast and rich flora with an abundant diversity of shrubs like *Ulex* spp., *Erica* spp. and *Pterospartum tridentatum* and trees species like *Quercus robur*, *Quercus pyrenaica* and *Pinus pinaster* (Figueiredo et al., 2016). The eastern area comprises the Douro International and Vale do Côa areas, also belonging to Natura 2000 network. The Mediterranean climate and geology enable a rich and diverse flora disposition, composed mainly of woodlands of *Quercus pyrenaica*, *Quercus rotundifolia* and *Quercus suber* and scrublands of *Cistus* spp. and *Juniperus* spp. (Carvalho et al. 2020). Both areas present a high diversity of wildlife species, like the Eurasian otter (*Lutra lutra*), the wild boar (*Sus scrofa*) and the roe deer (*Capreolus capreolus*).

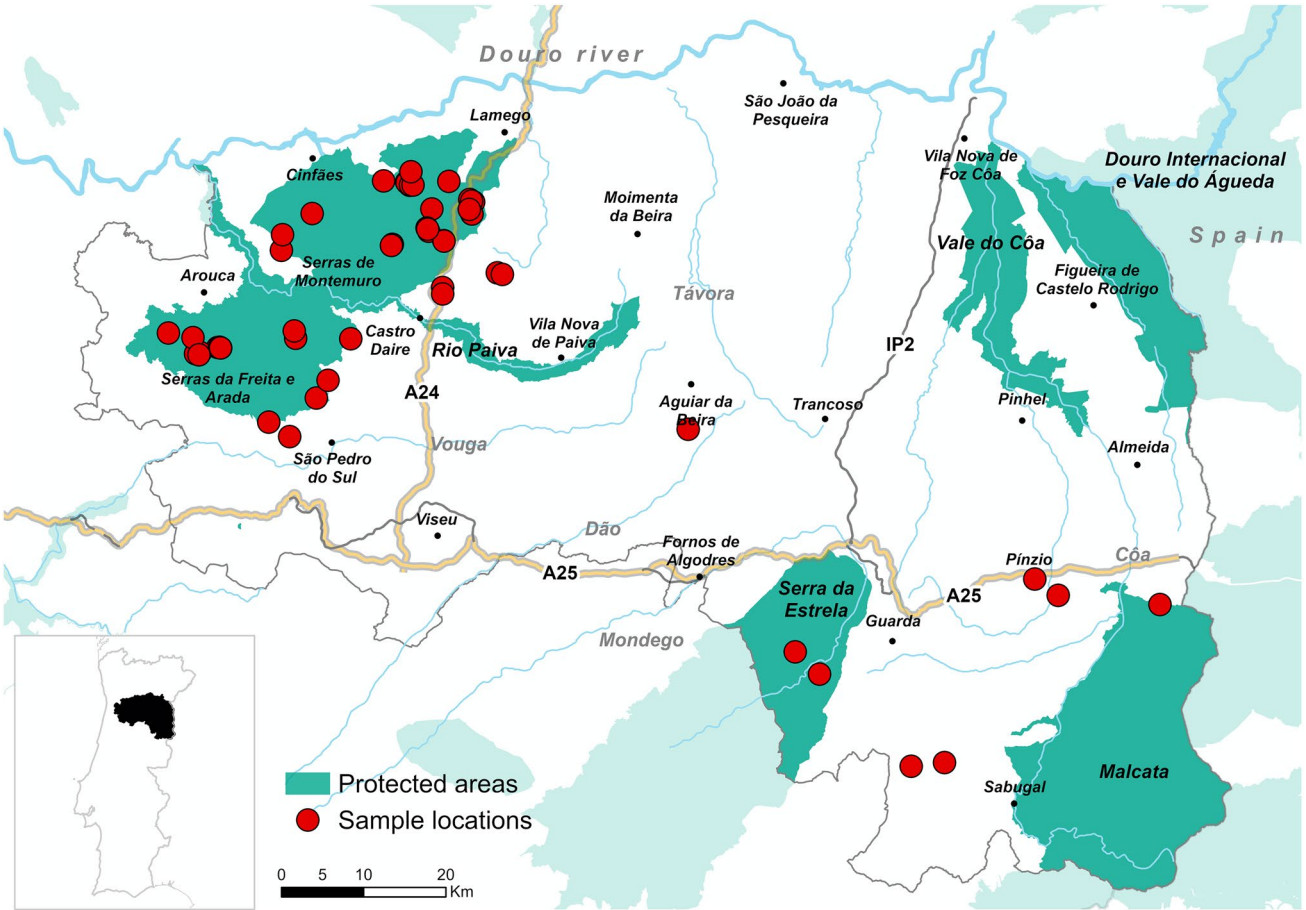

Fig. 1 Sample locations and intervention range of the Life WolFlux project, consisting of protected areas, represented in green (Natura 2000 network) and the unprotected areas between them. The western area contains the sites "Serras da Freita e Arada", "Serra de Montemuro" and "Rio Paiva". The eastern area comprises the sites "Vale do Côa", "Douro Internacional e Vale do Águeda", "Malcata" and "Serra da Estrela". Sample locations are outlined in red. Adapted from Carvalho et al. (2020)

Sample collection

Between October 2019 and July 2021, fresh faecal samples from red fox ($n=44$), stone marten ($n=12$) and Iberian wolf ($n=8$) were opportunistically collected in predefined transects distributed throughout the study area by experienced and field-trained personnel (Fig. 1). Sample collection was carried out according to morphological characteristics (i.e. size, shape, colour), smell, content and spatial position (Figueiredo et al. 2016). Collected samples were stored in airtight plastic bags at 4 °C until further examination in the laboratory. All Iberian wolf samples were submitted for molecular confirmation (see Figueiredo et al. 2019) as a regular procedure of the ongoing monitoring project of this endangered species.

Copromicroscopic and data analyses

Parasite intensity and prevalence were evaluated using one quantitative and three qualitative techniques for each sample. Egg/oocyst intensity was achieved by a modified McMaster technique (quantitative technique), combining a saturated sucrose solution with a McMaster chamber under a 10 × objective. The mean intensity of the infection was expressed in eggs per gram of faeces (EPG)/oocysts per gram of faeces (OPG), estimated as the mean number of parasite eggs/oocysts per infected hosts, with a sensitivity of 50 eggs per gram of faeces (Hansen and Perry 1990; Zajac and Conboy 2007). Willis's flotation and a sedimentation technique were performed with a saturated sucrose solution (3:1), using parasite buoyancy and liquid density as segregation factors. A modified Baermann technique was used to detect L1 lungworm nematodes after a 24-h larvae migration in lukewarm water (Zajac and Conboy 2007). Parasite species were identified based on shape, size, colour and structure using 10 × and 40 × objectives. Prevalence was estimated using a binomial test in R software (www.r-project.org), with confidence limits established with 95% intervals (CI). Individual and mean intensity were used to determine the infection level of the respective species, according to Hansen and Perry (1990) intensity classes, established as low (1–500 EPG/OPG), moderate to high (500–1500 EPG/OPG) and high (> 1500 EPG/OPG) level of infection (Mederos et al. 2010).

Results and discussion

From a total of 64 examined samples, 43 samples (67.2%) were infected with three helminths and two protozoa species (Table 1). In total, 81.8% (36/44) of red fox samples were positive for at least one parasite, 33.3% (4/12) for stone marten and 25.0% (2/8) for Iberian wolf. Nearly half of the infections were monospecific, and mixed infections were identified in the following combinations: Ancylostomatidae and *Eimeria* spp. 12.5% (8/64), *Toxascaris leonina* and *Eimeria* spp. 9.4% (6/64), Ancylostomatidae and *T. leonina* 4.7% (3/64), Ancylostomatidae, *T. leonina* and *Eimeria* spp. 3.1% (2/64) and *Trichuris* sp., *Cystoisospora* sp. and *Eimeria* spp. 1.6% (1/64). No L1 lungworm nematodes were detected

Table 1 Parasite prevalence and mean intensity in red fox (*Vulpes vulpes*) for both western and eastern areas and total parasite prevalence and mean intensity in Iberian wolf (*Canis lupus signatus*) and stone marten (*Martes foina*)

Host	Parasite		Study area	N° positive/total	Prevalence	Mean intensity
	Group	Species				
Red fox ($n=44$)	Nematode	*Toxascaris leonina*	Western	23/38	60.5% (43.4–76.0)	1339
			Eastern	2/6	33.3% (4.33–77.7)	925
			Total	25/44	56.8% (41.0–71.6)	1306
		Ancylostomatidae	Western	9/38	23.7% (11.4–40.2)	517
			Eastern	5/6	83.3% (35.9–99.6)	1350
			Total	14/44	31.8% (18.6–47.6)	773
	Coccidia	*Eimeria* spp.	Western	12/38	31.6% (17.5–48.6)	877
			Eastern	3/6	50.0% (11.8–88.2)	350
			Total	15/44	34.1% (20.5–49.9)	764
Iberian wolf ($n=8$)	Nematoda	Ancylostomatidae	Total	2/8	25.0% (3.2–65.1)	75
	Coccidia	*Eimeria* spp.	Total	2/8	25.0% (3.2–65.1)	50
Stone marten ($n=12$)	Nematoda	*Toxascaris leonina*	Total	1/12	8.33% (0.2–38.5)	150
		Ancylostomatidae	Total	2/12	16.7% (2.1–48.4)	150
		Trichuris sp.	Total	1/12	8.33% (0.2–38.5)	900
	Coccidia	*Eimeria* spp.	Total	2/12	16.7% (2.1–48.4)	19,600
		Cystoisospora sp.	Total	1/12	8.33% (0.2–38.5)	150

with the Baermann technique. The most common parasite in our study was the nematode *T. leonina*, present in nearly half of the samples (40.6%); however, it was only found in red foxes and stone marten.

When comparing the two study areas, the western area showed a higher prevalence and monospecificity for *T. leonina* in red fox: 60.5% (23/38) of red fox samples from the western area were parasitised by *T. leonina*, while 36.8% (14/38) were monospecific to this parasite (Table 1). In the eastern area, no monospecific infection was observed, and the most common parasite found across the three species was Ancylostomatidae, with a prevalence of 66.7% (8/12).

Due to the smaller sample size, parasite infection comparisons between areas were not conducted for the Iberian wolf and the stone marten (Table 1). The mean intensity of Ancylostomatidae was considerably higher in the eastern area, with a value of 1350 EPG. In the western area, *T. leonina* showed a higher mean intensity, with 1339 EPG. The mean intensity of *Eimeria* spp. was higher in the western area, with 877 OPG, compared with the eastern one (350 OPG). The parasite with the highest intensity of infection was *Eimeria* spp. (37,800 OPG), found on a stone marten individual, followed by *T. leonina* (10,100 EPG) on a red fox sample, both from the western area. According to Hansen and Perry (1990) intensity classes, in the western area, 22 foxes had moderate to high infection levels (73.3%); in the eastern area, only two foxes (40.0%) had moderate and high infection levels.

All parasites found in this study have been previously reported in the Iberian wolf (Figueiredo et al. 2016, 2019; Muñoz et al. 2018) and other wolf populations (Bindke et al. 2019), and in stone marten (Kirkova et al. 2011; Figueiredo et al. 2018; Olmedo et al. 2018) except for *Trichuris* sp., which, to our knowledge, was the first description for this species. Similarly to Figueiredo et al. (2018) study, *T. leonina* was found again in one stone marten sample in the western location with the exact prevalence (8.3%). This parasite was quantified with the McMaster technique at 150 EPG suggesting that it may not represent just a mechanical infection but an actual infection. For red foxes, all the parasites found were previously described in Portugal (Figueiredo et al. 2016) and elsewhere in Europe (Magi et al. 2009; Onac et al. 2015). No L1 lungworm nematodes were detected in the three carnivores, even though Figueiredo et al. (2016, 2018) were able to identify *Angiostrongylus vasorum* and *Angiostrongylus* sp. in red fox and stone marten, respectively, and *Crenosoma vulpis* in the same three species that cohabit in the western area.

Compared to other studies undertaken in Portugal, Spain and the European context, the present study revealed higher prevalence and intensity values, particularly for the ascarid *T. leonina* and the coccidian *Eimeria* spp. and in red foxes. *T. leonina* parasite exhibits a greater capacity to adapt to different climatic and environmental scenarios, representing an important source of environmental infection for both domestic and wild species (Okulewicz et al. 2012; Zajac and Conboy 2007). Additionally, according to Reperant et al. (2007), the prevalence of *T. leonina* in red foxes seems to be related to the level of habitat urbanisation, where higher prevalence levels can be linked to a decrease in habitat urbanisation levels (Okulewicz et al. 2012), i.e. rural environments. This statement is in concordance with our sampling site, which may explain the high prevalence values found. On the other hand, *Eimeria* spp. is a Coccidia that infects wild and domestic ungulates and can incur significant economic losses to the livestock industry. It has been suggested that reports in wild and domestic carnivores are usually related to mechanical infections and pseudoparasitims events due to predation or coprophagy of a definitive host (Zajac and Conboy 2007). Nevertheless, this species was detected in red fox samples with a high prevalence and high-intensity values, as high as 3150 OPG (and even 37,800 OPG in one stone marten sample), which suggests that it may be a real infection. Myšková et al. (2019) also reported *Eimeria* spp. in arctic foxes (*Vulpes lagopus*), using molecular biology in addition to coprology methods, identifying 3 *Eimeria* sequences. Two were coincident with predatory behaviour and pseudoparasitim (birds and rodents), and one infecting warm-blooded vertebrates, which could represent an actual parasite of foxes. A limitation of the present study was that only conventional microscopy-based methods and not molecular methods were used; therefore, it was not possible to determine if the *Eimeria* spp. parasite found in our samples was a similar species to the one Myšková et al. (2019) identified.

Other limitations include the following: (1) sampling effort was not balanced between areas, with sample collection duration being three times longer in the western area, and (2) the small sample size for the Iberian wolf and the stone marten, which may have overshadowed the true prevalence and intensity values of these populations. The low sample size can be attributed to (a) the fragmented and low densities of this Iberian wolf population (Figueiredo et al. 2016; Torres and Fonseca 2016) and (b) the requirement of using fresh faecal samples for parasite detection, a suitable noninvasive technique when dealing with endangered species which, invariably, decreases our sample size. Notwithstanding, based on the red fox data reported, it is possible to use the red fox as a sentinel species, acting as a proxy of the environment and reflecting the health status of the species that cohabit in its environment (Aguirre 2009; Figueiredo et al. 2016). Thus, we can expect that the different carnivore communities that cohabit in this area may find themselves infected with the same parasites when there is a transmission source/route available between species (e.g. contaminated water, soil, meat, faeces).

Our data highlights the possibility of cross-contamination between carnivore species since we discovered that the three species share the same parasites. Furthermore, the three nematode species (Ancylostomatidae, *Toxascaris leonina* and *Trichuris* sp.) and the Coccidia *Cystoisospora* sp. found in our study can be a source of infection to domestic canids, felines and even humans, which represents a public health concern considering that the parasite prevalence of wild animals with synanthropic habits is increasing (Moss et al. 2016). The identification of *Eimeria* spp. with high prevalence and intensity values emphasises the need to apply better measures to monitor and control free-roaming livestock species and wild ungulates, avoiding contact between herds, mainly because they share the same habitat with carnivore species, constituting a source of food for them, but also, inevitably, a reservoir of parasitic diseases. For this reason, more information on parasite prevalence, coupled with molecular identification and typing and their dynamics within the wildlife-livestock-human interface, are necessary to prevent the emergence and cross-transmission of parasites with zoonotic interest. The implementation of surveillance and monitoring programmes for parasitic outbreaks, along with other conservation measures, will ensure the long-term perseveration and thriving of the endangered Iberian wolf, with a special focus on the fragile and unstable populations located south of Douro River.

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Data availability Data supporting the conclusions of this article are included within the article and its supplementary table.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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