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Current opinion

Parasite species of the endangered Iberian wolf (*Canis lupus signatus*) and a sympatric widespread carnivore

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ABSTRACT

Parasites have a profound impact on wildlife population dynamics. However, until some years ago, studies on the occurrence and prevalence of wildlife parasites were neglected comparatively with the studies on humans and domestic animals. In this study, we determined the parasite prevalence of two sympatric wild canids: the endangered Iberian wolf (*Canis lupus signatus*) and the widespread red fox (*Vulpes vulpes*), in central Portugal. From November 2014 to July 2015, fresh fecal samples from both species were collected monthly in several transects distributed throughout the study area. All samples were submitted to several coprological techniques. In total, 6 helminth parasites (*Crenosoma vulpis*, *Angiostrongylus vasorum*, *Toxocara canis*, *Trichuris vulpis*, Ancylostomatidae, *Toxascaris leonina*), and a protozoa (*Balantidium coli*) were identified based on size and morphology. The red fox was infected by seven different parasites while the Iberian wolf was infected by four. All parasites present in wolf were also present in the red fox. *C. vulpis* had the higher prevalence in red fox, while Ancylostomatidae were the most prevalent parasites in wolf. To our knowledge, this is the first study in this isolated subpopulation of the Iberian wolf. Our results show that both carnivores carry parasites that are of concern as they are pathogenic to humans and other wild and domestic animals. We suggest that surveillance programs must also include monitoring protocols of wildlife; particularly endangered species.
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1. Introduction

Parasites have a profound impact on wildlife population dynamics, by having a broader effect in the ecosystem health and function and can cause temporary or permanent declines on local populations (Daszak et al., 2000; Smith et al., 2006; Thompson et al., 2010). Although the prevalence of parasites have been widely studied in humans and domestic animals (both pets and livestock), only recently has the prevalence of parasites begun to be studied on wildlife species (Thompson et al., 2010), particularly those with relevant conservation status (Aguirre and Tabor, 2008).

The Iberian wolf (*Canis lupus signatus*) is an endemic subspecies of the Iberian Peninsula. In Portugal, this top predator is protected by law since 1988, being listed as “Endangered” in the Portuguese Red Data Book (Cabral et al., 2005). The Iberian wolf population declined throughout the 20th century, mostly in Portugal, where its numbers have plummeted and its range has massively contracted (Torres and Fonseca, 2016). Despite its high cultural value (Álvares, 2011) and the role it plays as a keystone species, very few studies evaluated the prevalence of parasites in this species in Portugal (Guerra, 2012; Silva et al., 2012; Guerra et al., 2013). Given that parasites may impact host populations by causing temporary or permanent declines in abundance, they can potentially be a major cause of species extinctions. Following this line, endangered populations can be more prone to extinction as their population size is small and they can serve as reservoir hosts (McCallum and Dobson, 1995; Smith et al., 2006). Therefore, a better understanding of Iberian wolf parasitism is required for optimal conservation and management plans of this endangered species. This is particularly relevant in a humanized environment where zoonosis can occur in the interface human-domestic-wildlife animals (Mathews, 2009).

Iberian wolf populations occur at low density in central Portugal making it difficult to implement an adequate parasite sampling protocol. Therefore, it is vital to use another widespread and abundant canid species that spatially overlap and can reflect the

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presence of parasites of the target population (i.e. Iberian wolf) (Aguirre, 2009). The Iberian wolf coexists with the abundant and widespread red fox (*Vulpes vulpes*). The red fox is a synanthropic carnivore, providing the link between urban and natural habitats, favoring parasites transmission (Bradley and Altizer, 2007). Sympatric carnivores, such as the red fox, can act as reservoir hosts of helminths. However, there is no information about the parasites in the red fox in this area.

The aim of this study was to investigate, by means of a coprological survey, the occurrence and prevalence of parasites among the endangered Iberian wolf, a highly isolated subpopulation, and a sympatric widespread population of red fox, in the south of the Douro River, Portugal.

2. Materials and methods

Our study area was located in central-west Portugal and includes the range occupied by an isolated and fragmented wolf pack located on the south of the Douro River (for details see Torres and Fonseca, 2016), occupying two sites of the Natura 2000 network (Freita-Arada and Montemuro mountain range), with an area of 750 km^2. It is a mountainous region with altitudes ranging from 800 to 1,381 m and steep slopes. The climate is mainly Mediterranean, with strong oceanic influence. The study area is composed mainly by forests (46%), scrubland (26%), agricultural land (20%) and urban area (8%). Tree species like *Quercus robur*, *Q. pyrenaica*, *Castanea sativa* and *Pinus pinaster* are common in this area, and also different types of scrublands, like *Erica* spp., *Ulex* spp. and *Pterospartum tridentatum* (Torres et al., 2015).

Between November 2014 and July 2015, fresh faeces from Iberian wolf (n = 11) and red fox (n = 28) were randomly collected in 43 transects that were monthly prospected, distributed throughout the study area, by experienced and field-trained personnel. Morphology, size, color, smell, contents and spatial position were, in combination, diagnostic attributes of wolf scats (Torres et al., 2015). Collected samples were stored at 4 °C until examined in the laboratory (Wang et al., 2010).

The prevalence of eggs/larvae parasites were evaluated using three techniques: 1) modified Baermann technique with 24 h-readings (Paradies et al., 2013) was used to detected L1 lungworms nematodes; 2) flotation technique, Willis technique, was performed with a sugar saturated solution to isolate nematode/cestode eggs and coccidia oocysts (Thienpont et al., 1986) by filling up a test tube, so that a meniscus could be formed on the surface (Carvalho et al., 2012) in order to make the eggs float and 3) sedimentation technique with methylene blue dye to select the trematode eggs (Domínguez & De La Torre, 2002). All the eggs and larvae were examined under different magnifications on a light microscope to identify the species based on its size, color, shape and structure (Balmori et al., 2000). In both carnivore species, the prevalence of the parasites was calculated according to Bush et al. (1997). Confidence limits were established with 95% confidence intervals (CI). Data was analyzed using Microsoft Excel 2013®.

3. Results

From a total of 39 collected samples, 21 samples (Iberian wolf = 4; red fox = 17) were infected by six different helminth parasites and one protozoa. The red fox was infected by 7 different parasites, while the Iberian wolf was infected by 4 (Table 1). Ten (58.8%) red fox faeces were infected with only one endoparasite, four (23.5%) with two endoparasites and three (17.6%) with three species of endoparasites. Three (75.0%) Iberian wolf faeces were infected with one endoparasite, and only one (25.0%) with three endoparasites. The parasites found and their prevalence is shown in Table 1.

4. Discussion

All the parasites found in the Iberian wolf had already been described in previous studies in Spain (Balmori et al., 2000; Torres et al., 2001) and in other wolf populations in Portugal (Guerra, 2012; Silva et al., 2012) and elsewhere in Europe (Kloch et al., 2005; Popiołek et al., 2007). The only exception was *Crenosoma vulpis*, which, to our knowledge, had only been described in Belarus (Shimalov and Shimalov, 2000). Balmori et al. (2000) found higher prevalence of *Toxocara canis* (16.7–42.9%), *Toxascaris leonina* (14.3–20%) and Ancylostomatidae (20–42.9%) comparatively to ours (*T. canis* and *T. leonina*: 9.09%; Ancylostomatidae: 18.18%) in the Iberian wolf in Spain. In Portugal, both Guerra (2012) (*T. canis* - 7.3–11.8%) and Silva et al. (2012) (*T. canis* and *T. leonina*: 7.3%) found prevalence levels similar to ours. In the European context, Shimalov and Shimalov (2000), found higher prevalence of *Toxocara canis* (21.2%), *Toxascaris leonina* (13.5%), but lower prevalence of *C. vulpis* (7.7%). Although comparisons are difficult due to differences in techniques, sampling locations, and subspecies; the number of positive samples in the present study is rather low compared with other studies undertaken in Spain and in Europe. This could be attributed to the scarcity of Iberian wolves in the study area and its endangered status (Torres and Fonseca, 2016). Likewise, the parasites found in the red fox had been previously described in Portugal (Carvalho-Varela and Marcos, 1993; Eira et al., 2006; Guerra, 2012; Silva et al., 2012) and in other European countries (Mizgajska et al., 2000; Saeed et al., 2006; Magi et al., 2009; Al-Sabi and Kapel. 2013; Eleni et al., 2014). In Portugal, Eira et al. (2006), Guerra (2012) and Silva et al. (2012) reported a higher prevalence of *T. canis*, than ours (37.1%, 15- <40% and 24.7% respectively). Similarly, the prevalence was also higher regarding *Angiostrongylus vasorum* (16.1%) and *Trichuris vulpis* (8.6%) (Eira et al., 2006) but lower regarding *C. vulpis* (3.23%) while we reported a prevalence of 39.29%. On the other hand, Carvalho-Varela and Marcos (1993) only found one parasite (*T. leonina*) with a higher prevalence than ours (*T. leonina*: 11.4%) and all the remaining prevalence was lower (*T. canis*: 11.1%, *Trichuris vulpis*: 2.0%, *C. vulpis*: 1.3% and *A. vasorum*: 0.3%).

In this study, we used a non-invasive sampling technique, based on collection of fresh faeces in the environment, which is suitable for studies on wildlife and particularly essential for studies with endangered populations, such as ours. Due to financial constraints, we did not use molecular analyses for individual identification of the animal. Therefore, we cannot exclude that some fresh faeces samples are from the same animal however we find it very unlikely. Consequently, we used the term *prevalence* to describe the proportion of infected faeces in the wolf and red fox population investigated.

Toxocara canis, *Toxascaris leonina* and nematodes of the Ancylostomatidae family were found in both carnivores. *Toxocara canis* and *Toxascaris leonina* are significant species because they are widespread public health zoonotic parasitic infections that humans share with dogs, cats and wild canids, particularly foxes, therefore, representing a potential risk to public health (*e.g.*, Pawlowski, 2001; Macpherson, 2013). In the last decades, there have been dramatic changes in natural/rural landscapes due to urbanization, which allowed a closer contact between humans, livestock/domestic animals, and wildlife (Mackenstedt et al., 2015). Consequently, the risk for diseases of wildlife to spread to domestic carnivores and the other way around; and for zoonotic agents to emerge in human populations increased, and there is an urgent need to obtain more-recent parasite data especially in natural/rural settings (Slifko et al., 2000; Otranto et al., 2015). This has the potential to be more problematic in respect to our country as the red fox is a widespread and abundant carnivore in Portugal (Santos-Reis and Luz, 1996), providing a link between more natural and humanized ecosystems.

Table 1
Number of positive samples and the prevalence of parasites found in fresh feces collected in central Portugal.

Species	Parasite found	Nº positive	Prevalence (%)	Confidence interval (95%)
Red fox (n = 28)	*Crenosoma vulpis*	11	39.29	(3.70–54.66)
	Toxocara canis	6	21.43	NA[a]
	Ancylostomatidae	3	10.71	NC[a,b]
	Toxascaris leonina	3	10.71	(4.76–348.76)
	Angiostrongylus vasorum	2	7.14	NA
	Trichuris vulpis	2	7.14	NC
	Balantidium coli	1	3.57	NC
	Ancylostomatidae	2	18.18	NA
Iberian wolf (n = 11)	*Crenosoma vulpis*	1	9.09	NC
	Toxocara canis	1	9.09	NC
	Toxascaris leonina	1	9.09	NC

[a] *NA* – not applicable.
[b] *NC* – not calculated.

Additionally, our results stress that monitoring the prevalence of parasites should be a priority in the conservation of the endangered population of the Iberian wolf population, due to the potential detrimental effects of parasites for the persistence of small populations. None of the other species found in both canids are known to infect livestock, even though they are known to infect canine and feline species. All the helminth parasites found in the Iberian wolf were also present in the red fox, which means that a route of transmission between these two carnivores could exist, namely because most have direct life-cycles, enhancing an easy and fast transmission between both carnivores by contact or environmental transmission.

Hitherto, as far as we know, no other study has focused on the parasite community in this isolated subpopulation of the Iberian wolf. Even though we acknowledge that our sample size was rather small, this only highlights the difficulty of collecting enough samples to perform a study of this kind in an endangered species, such as the one in this study. Nevertheless, we suggest that further studies evaluate the role of the red fox as a sentinel species, as there are parasites species common to both canids, it is possible to consider that, given the degree of infection present in the collected samples of red fox, that communities living in the same area can also be found infected in the case there is a form of transmission between species, including the Iberian wolf.

The prevalence of several potentially zoonotic parasites found in the present study indicates a need to prevent public health risks. Monitoring the presence of parasites in these two carnivore species should be a continuous task due to the risk of zoonotic infections and the potential impact on public health. In the particular case of the endangered Iberian wolf, a better understanding of the role that these agents have on the host, and it is required precise data on the diversity and abundance of parasitic communities at local level is required. A sampling protocol is vital to implement, so that it could be possible to determine the parasitic communities present in the area which could jeopardize the wolf population, but also a focus on the factors that influence the presence of these parasites in these two canids and their transmission routes. We must not forget the importance to acknowledge the dynamic between wild species, humans and domestic animals, being particularly important their own host-parasite relationship.

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