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

Ana Figueiredoa, Lucia Oliveiraa, Luís Madeira de Carvalhob, Carlos Fonsecaa, Rita Tinoco Torresa,*,

a Department of Biology & CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal
b Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

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.

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 (Alvares, 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 (McCullum 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
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 km². It is a mountainous region with altitudes ranging from 150 to 781 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 detect 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 (Dominguez & 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; Popiolek 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, Silva 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 (Mizgajksa et al., 2000; Saeed et al., 2006; Magi et al., 2009; Al-Sahi 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 39.2%. 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%, T. 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, allowing 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 (Silvio 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 andhumanized ecosystems.
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 helmith 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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References

Aguirre, A.A., 2009. Wild canids as sentinels of ecological health: a conservation medicine perspective. Parasit. Vectors 2 (Suppl. 1). 57.
Aguirre, A.A., Tabor, G.M., 2008. Global factors driving emerging infectious diseases. Annu. N. Y. Acad. Sci. 1149 (1), 1–3.
Al-Sabi, M., Kapel, C., 2013. First report of Eucoleus boehmi in red foxes (Vulpis vulpis) in Denmark, based on coprological examination. Acta Parasitol. 58 (4), 570–576.
Álvares, F., 2011. Ecologia e conservação do lobo (Canis lupus, L,) no noroeste de Portugal. PhD thesis in Conservation Biology. Faculdade de Ciências da Universidade de Lisboa.
Balnori, A., Rico, M., Naves, J., Llamazares, E., 2000. Contribución al estudio de los endoparásitos del lobo en la Península Ibérica: una investigación coprológica. Galenýms 12, 13–26.
Bradley, C.A., Altizer, S., 2007. Urbanization and the ecology of wildlife diseases. Trends Ecol. Evol. 22 (2), 95–102.
Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 575–583.
Cabral, M.J., Almeida, J., Almeida, P.R., Dellinger, T., Ferrand de Almeida, N., Oliveira, M.E., Santos-Reis, M., 2005. Livro vermelho dos vertebrados de Portugal. Instituto do Conservação da Natureza.
Carvalho, G.L.X.D., Moreira, L.E., Pena, J.L., Marinho, C.C., Bahia, M.T., Machado-Coelho, G.L.L., 2012. A comparative study of the TF-Test2, Kato-Katz, Hoffman-Pons-Janer, Willis and Baermann-Moraes coprologic methods for the detection of human parasitosis. Memorias do Inst. Oswaldo Cruz 107 (1), 80–84.
Carvalho-Varela, M., Marcos, M.V.M., 1993. A helmintofauna da Raposa (Canis lupus C.19) en el norte de Burgos. Galeries em Bol. SECEM 14 (2), 49–58.
Eira, C., Vingada, J., Torres, J., Miquiel, J., 2006. The helminth community of the red fox, Vulpes vulpes, in Dinas de Mira (Portugal) and its effect on host condition. Wildl. Biol. Pract. 2 (1), 25–36.
Eleni, C., Grifoni, G., Di Egidio, A., Meoli, R., De Liberato, C., 2014. Pathological findings of Angiostrongylus vasorum infection in red foxes (Vulpes vulpes) from Central Italy, with the first report of a disseminated infection in this host species. Parasitol. Res. 113 (3), 1247–1250.
Guerra, D.R.A., 2012. The Sylviace and Synanthropic Cycles of Echinococcus SPP: Taenia SPP. And Toxocara SPP. in Portugal: Coprologic and Molecular Diagnosis in Canids. Master dissertation. Universidade Técnica de Lisboa. Faculdade de Medicina Veterinária.
Guerra, D., Armua-Fernandez, M.T., Silva, M., Bravo, I., Santos, N., Deplazes, P., de Carvalho, L.M.M., 2013. Taenid species of the Iberian wolf (Canis lupus signatus) in Portugal with special focus on Echinococcus spp. Int. J. Parasitol. Parasites Wildl. 2, 50–53.
Kloch, A., Bednarska, M., Bajer, A., 2005. Intestinal macro-and microparasites of wolves (Canis lupus L.) from north-eastern Poland recovered by coprological study. Ann. Agric. Environ. Med. 12 (2), 237.
Mackenstedt, U., Jenkins, D., Romig, T., 2015. The role of wildlife in the transmission of parasitic zoonoses in peri-urban and urban areas. Int. J. Parasitol. Parasites Wildl. 4 (1), 71–79.
Macpherson, C.M., 2013. The epidemiology and public health importance of (UID/AMB/50017) through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement. CIISA-FMV-Ulisboa, under project UID/CVT/00276/2013 (FCT), provided additional funding.

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

| Species                          | Parasite found | N° positive | Prevalence (%) | Confidence interval (95%) |
|----------------------------------|----------------|-------------|----------------|--------------------------|
| Red fox (n = 28)                 | Crenosoma vulpis | 11          | 39.29          | (3.70–54.66)            |
|                                  | Toxocara canis   | 6           | 21.43          | NA                       |
|                                  | Ancylostomatidae | 3           | 10.71          | NA                       |
|                                  | Toxascaris leonina | 3        | 10.71          | (4.76–348.76)           |
|                                  | Angiostrongylus   | 2           | 7.14           | NA                       |
|                                  | vasorum vulpis   | 2           | 7.14           | NA                       |
|                                  | Balantidium coli | 1           | 3.57           | NA                       |
|                                  | Ancylostomatidae | 2           | 18.18          | NA                       |
| Iberian wolf (n = 11)            | Crenosoma vulpis | 1           | 9.09           | NA                       |
|                                  | Toxocara canis   | 1           | 9.09           | NA                       |
|                                  | Toxascaris leonina | 1       | 9.09           | NA                       |

a NA — not applicable.
b NC — not calculated.
toxocariasis: a zoonosis of global importance. Int. J. Parasitol. 43 (12), 995–1008.
Magi, M., Macchioni, F., Dell’Omodarme, M., Prati, M.C., Calderini, P., Gabrielli, S., Cancrini, G., 2009. Endoparasites of red fox (Vulpes vulpes) in central Italy. J. Wildl. Dis. 45 (3), 881–885.
Mathews, F., 2009. Zoonoses in wildlife: integrating ecology into management. Adv. Parasitol. 68, 185–209.
McCallum, H., Dobson, A., 1995. Detecting disease and parasite threats to endangered species and ecosystems. Trends Ecol. Evol. 10 (5), 190–194.
Mizgajski, H., Luty, T., Andrzejewska, I., 2000. Prevalence of Toxocara canis and other intestinal parasites in red foxes (Vulpes vulpes) in Poznan, Poland region. In: 9th International Symposium on Veterinary Epidemiology and Economics, 2000.
Otranto, D., Cantacessi, C., Dantas-Torres, F., Brianti, E., Genchi, C., Capelli, G., Deplazes, P., 2015. The role of wild canids and felids in spreading parasites to dogs and cats in Europe. Part II Helminths arthropods. Vet. Parasitol. 2015. http://dx.doi.org/10.1016/j.vetpar.2015.04.020.
Paradies, P., Schnyder, M., Capogna, A., Lia, R.P., Sasanelli, M., 2013. Canine angiostrongylosis in naturally infected dogs: clinical approach and monitoring of infection after treatment. Sci. World J. 2013.
Pawłowski, Z., 2001. Toxocariasis in humans: clinical expression and treatment dilemma. J. Helminthol. 75 (04), 299–305.
Popiołek, M., Szczepesia, I., Nowak, S., Myslajek, R.W., 2007. Helminth infections in faecal samples of wolves Canis lupus L. from the western Beskidy Mountains in southern Poland. J. Helminthol. 81 (04), 339–344.
Saeed, I., Maddox-Hyttel, C., Monrad, J., Kapel, C.M., 2006. Helminths of red foxes (Vulpes vulpes) in Denmark. Veterinary Parasitol. 139 (1), 168–179.
Santos-Reis, M., da Luz Mathias, M., 1996. The historical and recent distribution and status of mammals in Portugal. Hystrix, Italian J. Mammal. 8, 1–2.
Shimalov, V.V., Shimalov, V.T., 2000. Helminth fauna of the wolf (Canis lupus Linnaeus, 1758) in Belarusian Polesie. Parasitol. Res. 86 (2), 163–164.
Silva, M., Ferreira, L.B., Guerra, D., Deplazes, P., Rio-Maior, H., Nakamura, M., Álvares, F., Santos, N., Madeira de Carvalho, L.M., 2012. Rastreio de parasitas gastrointestinalis, pulmonares e musculares em canídeos domésticos e silvestres no Norte de Portugal. III Cong. Ibérico Lobo, Lugo, Espanha, 23–25 Nov. 2012, 57.
Slifko, T.R., Smith, H.V., Rose, J.B., 2000. Emerging parasite zoonoses associated with water and food. Int. J. Parasitol. 30 (12), 1379–1393.
Smith, K.F., Sax, D.F., Lafferty, K.D., 2006. Evidence for the role of infectious disease in species extinction and endangerment. Conserv. Biol. 20 (5), 1349–1357.
Thienpont, D., Rochette, F., Vanparijs, O.F.J., 1986. Diagnosing Helminthiasis through Coprological Examination, second ed. Janssen Research Foundation, Beerse, Belgium, p. 205.
Thompson, R.C.A., Lymbery, A.J., Smith, A., 2010. Parasites, emerging disease and wildlife conservation. Int. J. Parasitol. 40 (10), 1163–1170.
Torres, R.T., Fonseca, C., 2016. Perspectives on the Iberian wolf in Portugal: population trends and conservation threats. Biodivers. Conserv. 25 (3), 411–425.
Torres, J., Perez, M.J., Segovia, J.M., Miquel, J., 2001. Utilidad de la coprología parasitaria en la detección de helminthes parasitos en los cánidos silvestres ibéricos. Galemys 13, 75–83.
Torres, R.T., Silva, N., Brocas, G., Fonseca, C., 2015. To eat or not to eat? the diet of the endangered iberian wolf (Canis lupus signatus) in a human-dominated landscape in Central Portugal. PloS one 10 (6), e0129379.
Wang, C.R., Xiao, J.Y., Chen, A.H., Chen, J., Wang, Y., Gao, J.F., Zhu, X.Q., 2010. Prevalence of coccidial infection in sheep and goats in northeastern China. Veterinary Parasitol. 174 (3), 213–217.