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Prevalence of zoonotic parasites in an endangered Iberian wolf (*Canis lupus signatus*) population in Portugal

Ana M. Figueiredo[1], Tânia Barros[1], Ana M. Valente[1,2], Carlos Fonseca[1], Luís Madeira de Carvalho[3], Rita Tinoco Torres[1*]

[1]Department of Biology & CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

[2]Instituto de Investigación en Recursos Cinegéticos (UCLM-CSIC-JCCM), Ronda de Toledo 12, 13071 Ciudad Real, Spain

[3]CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisbon, Portugal

*Corresponding author

E-mail: rita.torres@ua.pt

Department of Biology & CESAM,

University of Aveiro, Campus de Santiago,

3810-193 Aveiro, Portugal

Telephone: +351 234 370975

As a top predator, the Iberian wolf (*Canis lupus signatus*) plays a major role shaping interactions within food webs. Due to its conservation status in Portugal, *i.e.* endangered, it is important to understand the role of parasites in this population, since they can be a limiting factor for the population fitness and trophic interactions and, ultimately, their survival. From November 2017 to August 2018, 33 fresh faecal samples were collected in several transects distributed throughout Montesinho Natural Park. Samples were analysed by means of four coprological techniques. A total of three helminth parasites (*Ancylostoma* spp., *Uncinaria* spp. and *Eucoleus aerophilus*) were identified based on size and morphology. The overall prevalence was low (15.5 %), being

Ancylostoma spp. the most prevalent parasite. The three parasites found are of major concern, once they are pathogenic to humans and other wild and domestic animals. We suggest surveillance programs that include both parasite and wildlife monitoring. To our knowledge, this is the first coprological study performed with this Iberian wolf population.

Keywords Parasites, Zoonoses, Iberian wolf, Public health, Portugal.

As key components of biological communities, parasites have an important impact on ecosystem functioning, altering connectivity and nesting of food webs, which lead to different patterns of parasite transmission (Hudson *et al.*, 2006; Lafferty *et al.*, 2008; Thompson *et al.*, 2010). Increasing interactions between wildlife, domestic animals and even humans, enables parasite spillover, which not only can represent a health threat to humans and domestic animals - since wildlife can act as reservoirs and spreaders of diseases - but also a spillback to wildlife (Thompson *et al.*, 2009), especially when concerning endangered species (Hudson *et al.*, 2006).

As a top predator, the Iberian wolf (*Canis lupus signatus*) plays a major role in modelling food web interactions (Estes *et al.*, 2011; Lesniak *et al.*, 2018). In Portugal, due to wolf population decline throughout the 20th century, where its numbers and distribution have sharply reduced, it is listed as "Endangered" in the Portuguese Red Data Book of Vertebrates (Cabral *et al.*, 2005) and protected by law since 1988 (Torres and Fonseca, 2016). Once parasites can reduce population fitness, alter trophic interactions and competition (Hudson *et al.*, 2006) and even affect the trophic pyramid stability (Lafferty *et al.*, 2008), it is essential to understand the role of such parasites in the endangered Iberian wolf population in Portugal.

Few studies have evaluated parasite diversity and prevalence of the Iberian wolf populations in Portugal (Guerra, 2012; Silva *et al.*, 2012; Guerra *et al.*, 2013; Figueiredo *et al.*, 2016; Silva, 2018). But, to our knowledge, none have focused on estimating the prevalence and diversity of parasites in the area where the Iberian wolf populations reach the highest densities (Pimenta *et al.*, 2005), being this population of special

epidemiological interest because it is connected with other wolf populations located both in Spain and in Portugal. Hitherto, this is the first assessment of parasites in Iberian wolf inhabiting Montesinho Natural Park, located on the northeast of Portugal. This cross-border wolf population can potentially share a wide range of parasite species with other neighbouring populations of wolves, as well as other domestic and wild canid species, representing a health risk. Additionally, the close contact with the rural communities, either to humans and domestic animals, can enhance the transmission of parasites (Santos, 2015), especially with a faecal-oral transmission cycle (Beiromvand *et al.*, 2013) and zoonotic importance. This study aimed to investigate, by means of coprological analyses, the parasite prevalence of this Iberian wolf population.

Our research was conducted in Montesinho Natural Park (MNP) (6º30'-7º12'W, 41º43' to 41º59'N), comprised as one of European Union's Natura 2000 Network sites (Fig. 1). The total prospected area was 35,000 ha, and the landscape is characterized by the presence of mountain formations, with elevation ranging from 438 to 1,481 m. The climate is mainly Mediterranean, with an annual average temperature ranging between 3ºC in the coldest month and 21ºC in the warmest, and precipitation between 600 and 1,500 mm (Castro *et al.*, 2010). The area exhibits a mosaic of deciduous and coniferous forest, characterized by oaks (*Quercus pyrenaica*, *Q. rotundifolia*, *Q. suber*), sweet chestnut (*Castanea sativa*) and maritime pine (*Pinus pinaster*). The shrub vegetation is dominated by heather (*Erica* spp.), gum rockrose (*Cistus ladanifer*) and furzes (*Ulex europaeus* and *U. minor*) and fragmented by small cultivated fields (Valente *et al.*, 2014; Torres *et al.*, 2015a). According to the last wolf census, conducted in 2002/2003, 20 wolf packs were confirmed for this region (Pimenta et al., 2005), however it is important to stress that our study area is smaller than the whole region.

Between November 2017 and August 2018, experienced and field-trained personnel randomly collected 33 fresh faeces from Iberian wolf throughout our study area (Fig. 1). Collected samples were stored at 4ºC, in order to avoid degradation or evolution of the stages of development of parasitic forms until further examination in the laboratory (Zajac and Conboy, 2012). Scats were submitted to genetic analysis to confirm as being from *Canis lupus signatus*. DNA extraction was performed using QIAamp® DNA Stool

Mini Kit (QIAGEN Hilden, Germany) following manufacturer instructions. A fragment of 350bp from the control region (mitochondrial region) (Saccone *et al.*, 1987), was amplified using the universal primers Thr-L 15926 5'-CAATTCCCCGGTCTTGTAAACC-3′ and DL-H 16340 5′-CCTGAAGTAGGAACCAGATG-3′ (Vilà *et al.*, 1999). Reaction mixtures were initially denatured at 94ºC for 3 min, followed by 42 amplification cycles (94ºC for 1 min; annealing for 2 min at 50º and extension for 1.5 min at 72ºC) and a final extension step at 72ºC for 10min, adapted from Vilà *et al.* (1999) and Barros *et al.* (2016). Samples were visualized by electrophoresis on 1.4 % agarose gel. Mitochondrial fragments were purified using ExoSap-IT® (USB Corporation) and sent to sequence in both directions using sequencers ABIPRISM® 3730-XL DNA Analyser from Applied BiosystemsTM.

Sequences were then manually aligned using MEGA version 6.0 (Tamura *et al.*, 2013) and compared with previously published *Canis lupus signatus* sequences (Vilà *et al.*, 1999).

Egg/larvae parasite prevalence and mean intensity were evaluated by means of four different quantitative and qualitative techniques: 1) modified McMaster test and 2) Willis flotation technique were performed with sugar saturated solution, in order to isolate gastrointestinal nematode/cestode eggs and coccidian oocysts (Thienpont *et al.*, 1986; Zajac and Conboy, 2012); 3) sedimentation technique, using a methylene blue dye to select the trematode eggs (Domínguez and De La Torre, 2002); and 4) modified Baermann technique, to detect first-stage larvae of cardiopulmonary nematodes (Zajac and Conboy, 2012). Ancylostomatidae eggs were differentiated according to Thienpont et al. (1986); *Uncinaria* eggs (*e.g. Uncinaria stenocephala*) measure between 63-80 µm in length and 32-50 µm in width, bigger than *Ancylostoma* eggs (*e.g. Ancylostoma caninum*), which measure between 56-65 µm in length and 37-43µm in width. *Uncinaria* eggs have dissimilar poles with both sides more parallels, while *Ancylostoma* eggs have more similar poles and barrel side walls.

Parasite prevalence was calculated based on Bush *et al*. (1997), using Rstudio® software (R Core Team, 2014). Confidence limits were established with 95 % confidence intervals (CI) using the same Rstudio® software.

From a total of 33 faecal samples, five (15.15 %) were infected with at least one of the three helminth parasites that were found in this Iberian wolf population (Table 1). All helminths found were from phylum Nematoda: two from the digestive tract, *Ancylostoma* spp. and *Uncinaria* spp. / one respiratory, *Eucoleus aerophilus*. Three faecal samples (3/5, 60 %) showed a co-infection with two different endoparasites and two samples (2/5, 40 %) were monospecific (Table 2).

All the parasites found in this study have been previously reported in Iberian wolf, both in Portugal (Silva *et al.*, 2012; Figueiredo *et al.*, 2016; Silva, 2018) and Spain (Balmori *et al.*, 2000; Nunes, 2017; Muñoz *et al.*, 2018), but also on other wolf populations in Europe (*e.g.* Popiołek *et al.*, 2007; Hermosilla *et al.*, 2017; Al-Sabi *et al.*, 2018) (Table 3). In Portugal, Silva *et al.* (2012) found higher prevalence of *Ancylostoma/Uncinaria* (45.7 %), but lower of *Eucoleus aerophilus* (4.3 %). Ancylostomatidae eggs were found in higher prevalence in Figueiredo *et al.* (2016) (18.18 %) and also in Silva (2018) (15.2 %); nevertheless, *Eucoleus aerophilus* was reported in lower prevalence (6.5 %) (Silva, 2018). In Spain, Balmori *et al.* (2000), Nunes (2017) and Muñoz *et al.* (2018) found a prevalence of Ancylostomatidae of 100 %, 21.60 % and 19,35 %, respectively; *Capillaria* sp., was present in higher prevalence in Balmori *et al.* (2000) (20 %) and Muñoz *et al.* (2018) (50.4 %), however in lower prevalence in Nunes (2017) (5.53 %). In Poland, Popiołek *et al.* (2007) study showed the same prevalence for *Ancylostoma caninum* (12.3 %), but higher for *Uncinaria stenocephala* (37 %) and *Eucoleus aerophilus* (14.6 %). In Croatia, Hermosilla *et al.* (2017) found a prevalence of 13.1 % for *Ancylostoma/Uncinaria* spp. and 16 % for *Capillaria* spp. In Sweden, Al-Sabi *et al.* (2018) reported a 90 % prevalence for *Uncinaria stenocephala*, however, they used carcasses of hunted wolves in their study instead of faecal samples, increasing the possibility of finding a greater parasite diversity, with higher prevalence.

As a cross-border population, we would expect higher parasite diversity and prevalence, due to the close contact with the other Spanish and Portuguese wolf populations. Since this is the first study performed in this area, we were not able to compare to previous studies. Muñoz *et al.* (2018) analysed the parasite prevalence of the

Sierra de la Culebra wolf population, which represents the Spanish neighbouring population, and also found both Ancylostomatidae gen sp. and *Eucoleus aerophilus*. The total parasite prevalence (66.67 %) and richness (7 different species) found in Muñoz *et al.* (2018) study was higher than ours (15.15 %, and only 3 species identified). Likewise, previous studies performed with other European wolves populations reported higher prevalence rates and several parasites species, which we did not find in the present study, including *Toxocara canis, Toxascaris leonina, Trichuris vulpis, Crenosoma vulpis, Taenia hydatigena, Taenia krabbei, Dipylidium caninum, Alaria alata,* and other nematodes, cestodes, trematodes and protozoa species (Table 3). The low parasite prevalence associated with low parasite richness that we found can be associated to events of parasitic fauna extinction, due to the absence of the required intermediate host (Torchin *et al.*, 2003), seasonal variation effects, like moisture and temperature (affecting both abundance and activity of intermediate hosts and parasite development) (Jones, 2001), which can lead to non-predation of key intermediate species (Al-Sabi *et al.*, 2018). Nonetheless, several factors can be associated with parasite prevalence and richness on host species; these can include body mass and life-history traits, social contact (parasites can spread from one host to another by direct transmission), but also biogeography, which may constitute an aspect that dictates parasite prevalence and diversity, in a way that it may even influence patterns of parasite species richness (Lindenfors *et al.*, 2007). Thus, host species with larger geographical ranges may have higher parasite richness, since they can use a wider range of habitats and consequently contact with a larger number of different definitive hosts, but also more intermediate and paratenic host species (Lindenfors *et al.*, 2007).

Considering now a previous study carried out in an area where Iberian wolf densities are low, using the same coprological techniques, the results showed a prevalence close to 40 % (Figueiredo *et al.*, 2016), being significantly higher than the one found in this study. *Toxocara canis*, *Toxascaris leonina,* both with zoonotic importance (Okulewicz *et al.*, 2012), and *Crenosoma vulpis*, were the nematodes species found in this isolated and fragmented population, but these were not found in the northeast population, as stated before. Higher rates of parasitism infection in this southern population may be associated with higher abundance of other sympatric species that can help in the spread of zoonotic agents, like the red fox or feral dogs (Beiromvand *et al.*, 2013; Figueiredo *et al.*, 2016). Additionally, the southern area has higher human density, together with higher densities of livestock and domestic carnivores, using an extensive

and traditional grazing system (Torres *et al.*, 2015b), which may promote the transmission and spread of a higher number of zoonotic diseases, explaining the higher parasite richness and prevalence found. Although 70 % of the emerging zoonoses have a wildlife origin (Jones *et al.*, 2013), their spread and prevalence are driven by the continuous expansion and urbanization of the human population, together with travel and trade, land-use change, but also to the proliferation of both domestic and wild host populations (Hassell *et al.*, 2017). Wildlife migration and colonization of rural areas increase the transmission of zoonotic agents, not only to livestock and other domestic animals, but also to humans. This is relevant, since both *Ancylostoma/Uncinaria (*Prociv and Croese, 1996) and *Eucoleus aerophilus* (Lalošević *et al.,* 2008) found have zoonotic importance, and because they can use wild canids, domestic cats and dogs as definitive hosts, representing a potential risk for public health. Moreover, these parasites have direct life cycles, requiring only one host for its development, which means that its transmission to humans, domestic or other wild animals can easily occur through accidental ingestion of the eggs (*Eucoleus aerophilus*) or percutaneous contact (*Ancylostoma* spp./*Uncinaria* spp.) in contaminated soil, water, faeces or food (Böhm *et al.*, 2007; Al-Sabi *et al.*, 2018). Moreover, *E. aerophilus* infection can also be acquired through the consumption of earthworms, which act as transport hosts (Anderson, 2000).

We are aware that because we used a non-invasive sampling technique, based on the collection of fresh faeces in the environment, our study has some constraints. The coprological results we obtained only allowed us to detect the parasites, without knowing the true impact that intestinal and pulmonary nematodes have on the wolf. However, this methodology is suitable for studies on wildlife and crucial for the ones with endangered populations, such as ours. Therefore, a broader understanding of how parasites interact with the different organisms in the ecosystem is needed in order to provide additional information about the ecosystem health status and even help conservation programs of threatened species. Future studies should focus both on parasite and endangered species conservation, highlighting that wildlife conservation and public health concerns cannot be translated into parasite jeopardize.

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Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Table 1 – Number of positive samples, prevalence and confidence intervals (CI, 95 %) of parasites in Iberian wolf (*Canis lupus signatus*) faeces (N=33) collected in Montesinho Natural Park.

Parasites	Nº positive	Prevalence (%)	Confidence Interval (95 %)
Ancylostoma spp.	4	12.12	(3.40-28.20)
Eucoleus aerophilus	3	9.09	(1.90-24.33)
Uncinaria spp.	1	3.03	(0.07-15.6)
Total positives	5	15.15	NA*

*NA – Not applicable

Table 2 – Sample identification (ID) related with the presence (+) and absence (-) of each type of nematode egg found in faeces (N=33) of Iberian wolf (*Canis lupus signatus*) collected in Montesinho Natural Park.

Sample ID	*Ancylostoma* spp.	*Uncinaria* spp.	*Eucoleus aerophilus*
L6	-	-	+
L9	+	-	+
L12	+	+	-
L32	+	-	-
L33	+	-	+

Table 3 –Literature review of parasite prevalence in grey wolf (*Canis lupus*) in Europe. Prevalence rates are organized in intervals, where the lowest value corresponds to the lowest prevalence and the highest value to the highest prevalence found. Parasite species found in the studies [14], [16], [18], [19], [22], [24], [26], [27], [30], [32], [33], [37] were obtained from post mortem examination, while the others were from faecal examination. Reference list: [1]Popiołek *et al.*, 2007; [2]Guerra, 2012; [3]Silva *et al.*, 2012; [4]Muñoz *et al.*, 2018; [5]Silva, 2018; [6]Balmori *et al.*, 2000; [7]Torres *et al.*, 2000; [8]Kloch *et al.*, 2005; [9]Borecka *et al.*, 2013; [10]Pereira, 2015; [11]Figueiredo *et al.*, 2016; [12]Hermosilla *et al.*, 2017; [13]Paoletti *et al.*, 2017; [14]Shimalov & Shimalov, 2000; [15]Torres *et al.*, 2001; [16]Segovia *et al.*, 2001; [17]Dominguez & De La Torre, 2002; [18]Bagrade *et al.*, 2009; [19]Varodi *et al.*, 2017; [20]Bindke *et al.*, 2019; [21]Papadopoulos *et al.*, 1997; [22]Moks *et al.*, 2006; [23]Szczęsna-Staśkiewicz, 2009; [24]Fiocchi *et al.*, 2016; [25]Lesniak *et al.*, 2017; [26]Ali-Sabi *et al.*, 2018; [27]Ćirović *et al.*, 2015; [28]Szczęsna & Popiołek, 2007; [29]Szafrańska *et al.*, 2010; [30]De Liberato *et al.*, 2017; [31]Penezić *et al.*, 2014; [32]Ionică *et al.*, 2017; [33]Kornyushin *et al.*, 2011; [34]Poglayen *et al.*, 2017; [35]Guerra *et al.*, 2013; [36]Gori *et al.*, 2015; [37]Korol *et al.*, 2016.

Nematodes	Portugal[a]	Spain[a]	Italy	Germany	Poland	Sweden	Croatia	Greece	Serbia	Romania	Ukraine	Belarus	Latvia	Estonia	Literature
Strongyloides spp.	1.5-21.3	27.0	-	-	1.1	-	-	-	-	-	-	-	-	-	[1][2][3][4] [5]
Ancylostomatidae	6.5-57.7	16.6-100.0	4.34	-	6.9-30.8	-	13.1	-	-	-	-	-	-	-	[3][4][5][6][7][8][9][10][11][12][13]
Ancylostoma caninum	-	8.5-16.6	-	20.29	12.3	-	-	-	-	-	6.2	13.5	2.9	-	[1][14][15][16][17][18][19][20]
Uncinaria stenocephala	-	11.1-90.0	66.6	11.0-20.29	37.0-72.0	90.0	-	50.0	-	-	65.6	15.4	41.2	77.0	[1][14][15][16][17][18][19][20][21] [22][23][24][25][26]
Toxocara spp.	11.8-38.5	5.0-37.0	-	-	-	-	-	-	-	-	-	-	-	-	[2][7][10]
Toxocara canis	7.3-9.09	5.5-42.9	4.34-33.3	11.0-13.04	5.6-13.5	-	2.8	16.6	3.9	-	15.6	21.2	5.8	8.0	[1][3][4][6][8][9][11][12][13][14][16] [17][18][19][20][21][22][23][24][25][27]
Toxascaris leonina	1.9-9.09	2.15-20.0	-	4.0-5.80	1.1-6.0	-	0.5	-	-	-	15.6	13.5	-	8.0	[1][2][3][4][5][6][8][11][12][14][16][17] [19][20][22][23][25]
Trichuris spp.	1.5-11.5	3.7-28.1	-	-	-	-	-	-	-	-	-	-	-	-	[2][3][5][7][10][15]
Trichuris vulpis	5.9	9.68-42.9	-	5.80-12.3	13.9-38.5	-	1.5	-	-	-	18.8	3.9	-	-	[2][4][6][8][9][12][13][14][16][17][19][20]
Trichinella spp.	-	12.8	-	4.0	33.0	-	-	-	-	-	-	19.2	69.7	50.0	[14][16][18][22][23][25]
Rictularia sp.								16.6							[21]
Pterigodermatites affinis	-	-	33.3	-	-	-	-	-	-	-	3.1	-	-	-	[19][24]
Spirocerca lupi	-	-	-	-	2.32-11.5	-	-	-	-	-	-	7.7	-	-	[14][28][29]
Macracanthorhynchus catulinus	-	-	-	-	-	-	-	-	-	-	-	3.9	-	-	[14]
Capillaria spp.	-	-	-	-	-	-	16.0	-	-	-	-	-	-	12.0	[12][22]
Eucoleus aerophilus	0.9-4.3	14.3-50.54	21.74	15.0-31.88	14.6-17.0	-	-	-	-	-	9.4	7.7	-	-	[1][3][4][5][6][10][13][14][19][20][23][25][29]
Capillaria plica	-	-	-	25.0	-	-	-	-	-	-	9.4	13.5	41.4	-	[14][18][19][25]
Capillaria vulpis				25.0	17.0										[23][25]
Crenosoma vulpis	9.09	20.0	-	-	-	-	4.6	-	-	-	6.2	7.7	9.1	-	[6][11][12][14][18][19]
Angiostrongylus vasorum	-	2.1	28.0	-	-	-	3.1	-	-	-	-	-	-	-	[12][16][30]
Dirofilaria immitis	-	2.1	-	-	-	-	-	-	1.43	-	-	-	-	-	[16][31]
Dirofilaria repens	-	-	-	-	-	-	-	-	-	7.14	-	-	-	-	[32]
Cestodes															
Taenia spp.	13.1-15.4	-	11.1-100.0	21.74	1.4-11.2	-	1.5	50.0	-	-	3.13	-	8.8	19.0	[1][4][5][6][7][9][10][12][15][18][20][21][22] [24][26][33][34]
Taenia hydatigena	11.8	9.68-95.0	19.6-40.7	13.33-15.0	56.0	40.0	-	16.6	9.8	-	37.5	26.9	41.2	12.0	[14][16][18][20][21][22][23][25][26][27][33] [34][35][36]
Taenia multiceps	-	44.7	-	-	-	25.0	-	-	3.9	-	9.38	-	47.1	27.0	[16][18][22][27][33]
Taenia serialis	5.9	29.8	-	-	-	-	-	-		-	9.38	-	-	-	[16][27][33][35]
Taenia pisiformis	2.9	2.1							1.0		3.13	7.7	20.6	8.0	[14][18][22][27][33][35]
Taenia polyacantha	1.5	-	1.8	-	-	-	-	-	2.9	-	6.25	5.8	11.8		[14][18][27][33][34][35]
Taenia krabbei	-	-	4.5-22.2	13.33-77.0	-	25.0	-	-	-	-	-	7.7	8.8	-	[14][18][20][25][26][34][36]
Taenia taeniaeformis	-	-	0.6	-	-	-	-	-	2.0	-	-	-	-	-	[27][36]

Taenia crassiceps	-	-	0.6	-	-	-	-	-	-	-	6.25	5.8	8.8	-	[14][18][33][36]
Taenia ovis	-	-	2.2	-	-	-	-	-	-	-	-	-	-	15.0	[22][36]
Echinococcus multilocularis	-	-	-	2.0	-	-	-	-	-	-	-	-	5.9	-	[18][25]
Echinococcus granulosus	1.5[b]	-	5.5-56.0	-	-	-	-	-	-	-	6.25	11.5	2.9	4.0	[14][18][22][33][34][35][36]
Dipylidium caninum	-	5.5-6.4	-	-	3.8	5.0	-	50.0	-	-	3.13	15.4	-	-	[8][21][14][16][17][26][33]
Mesocestoides sp.	-	-	-	-	-	-	-	33.3	-	-	-	-	-	-	[21]
Mesocestoides lineatus	-	-	-	-	-	-	-	-	-	-	28.13	7.7	5.9	12.0	[14][18][22][33]
Mesocestoides litteratus	-	4.3	-	9.0	-	-	0.3	-	1.0	-	-	-	-	-	[12][16][25][27]
Spirometra erinacei	-	-	-	-	-	-	-	-	-	-	9.38	15.4	-	-	[14][33]
Trematodes															
Alaria alata	3.8	2.1-60.0	5.3	15.94-53.0	2..2-80.1	15.0	0.3	-	1.0	-	-	-	-	-	[1][6][7][12][14][15][16][18][20][22][23][24][25][26][27][29][37]
Brachylaima spp.	-	-	5.3	-	-	-	-	-	-	-	-	-	-	-	[24]
Isthmiophora melis	-	-	-	-	-	-	-	-	-	-	31.5	17.3	85.3	89.0	[14]
Opisthorchis felineus	-	-	-	-	-	-	-	-	-	-	-	5.8	-	-	[14]
Protozoa															
Sarcocystis spp.	-	55.5	-	-	-	-	19.1	-	-	-	-	3.9	-	-	[12][17]
Sarcocystis canis	7.9	-	-	-	-	-	-	-	-	-	-	-	-	-	[3]
Sarcocystis felis	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	[3]
Cystoisospora spp.	0.9-3.7	20.0	-	-	-	-	-	-	-	-	-	-	-	-	[3][5][6]
Cystoisospora canis	-	-	-	-	-	-	1.8	-	-	-	-	-	-	-	[12]
Cystoisospora ohioensis	-	-	-	-	-	-	2.1	-	-	-	-	-	-	-	[12]
Cryptosporidium spp.	13.5	-	-	-	54.9	-	1.8	-	-	-	-	-	-	-	[3][8][12]
Coccidiae	-	20.0	-	-	-	-	-	-	-	-	-	-	-	-	[6]
Giardia spp.	-	-	-	-	-	-	2.1	-	-	-	-	-	-	-	[8][12]
Neospora spp.	-	-	-	-	-	-	2.6	-	-	-	-	-	-	-	[12]

[a]*Canis lupus signatus*

[b]G7 strain of *Echinococcus granulosus*

STUDY AREA AND SAMPLE LOCATIONS

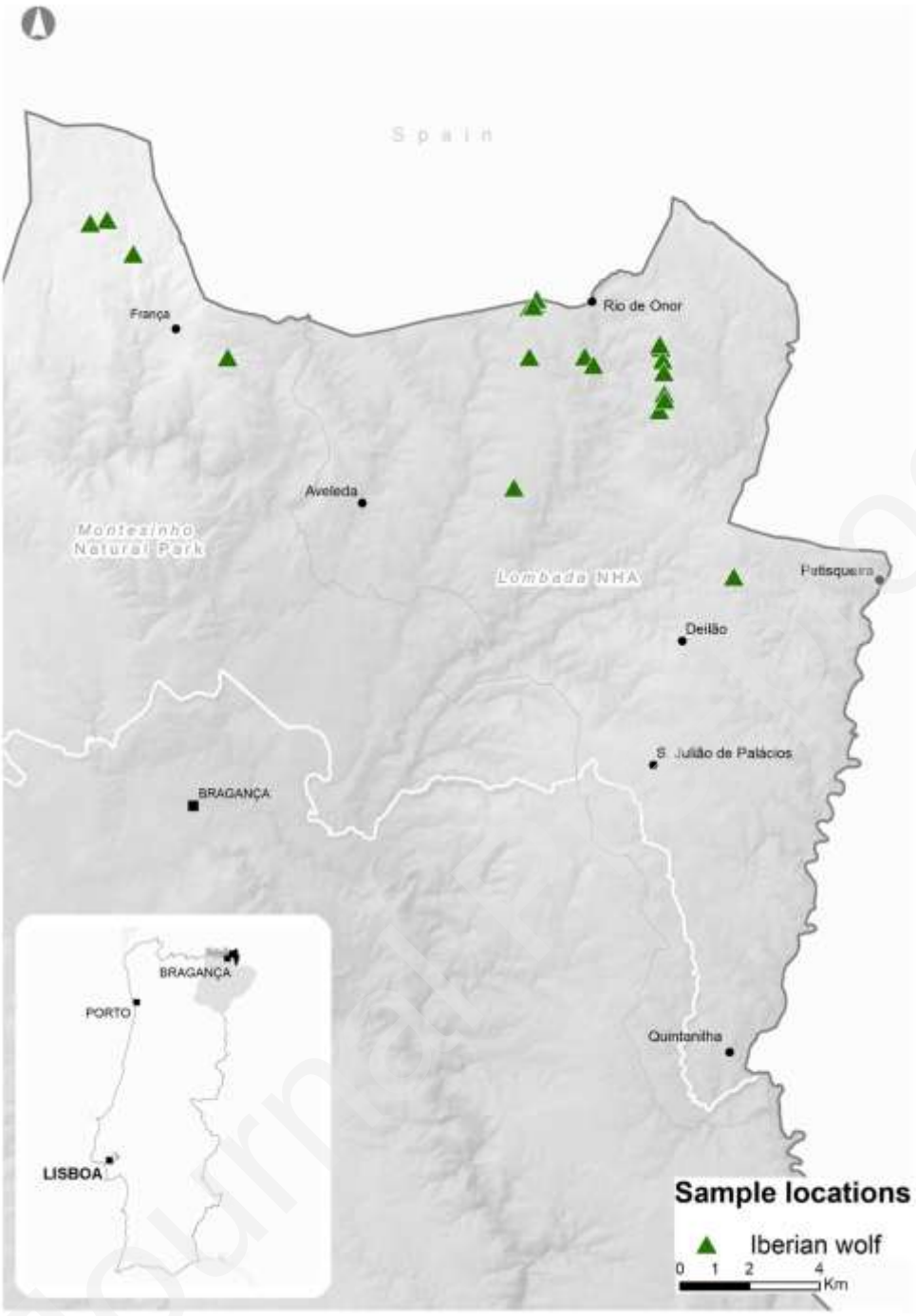

Fig. 1 – Location of the study area in Portugal, where the green triangles represent the sites of Iberian wolf faeces collected for coprological analysis at Montesinho Natural Park.