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Systematic Review

Gastrointestinal Parasites in Iberian Wolf (*Canis lupus signatus*) from the Iberian Peninsula

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Abstract: The Iberian Peninsula is one of the most humanized areas in Europe, yet humans may cohabit with large predators, such as the Iberian wolf (*Canis lupus signatus*), at the expense of many contributions to its conservation. The limited wolves' territory leads to a close relationship between this wild species, humans, and other animals, which may promote the spillover of pathogens, such as gastrointestinal parasites. This review intends to provide an update concerning gastrointestinal parasite findings performed using coprological methods on fecal samples from Iberian wolves. Studies conducted in Portugal and Spain through coprology presented a prevalence of gastrointestinal parasites of 57.0–100% in Spain and 21.5–68.3% in Portugal. Parasites belonging to Protozoa, Trematoda, Cestoda, and Nematoda were specified, alongside thirteen genera and twenty species of gastrointestinal parasites. In this study, 76.9% (10/13) of genera and 65.0% (13/20) of species of gastrointestinal parasites were identified as having zoonotic potential. These results highlight that further studies are needed to better understand the parasitic agents circulating in the wild in humanized areas, such as the Iberian Peninsula.

Keywords: *Canis lupus signatus*; gastrointestinal parasites; helminths; Iberian wolf; Portugal; protozoans; Spain

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1. Introduction

The Iberian Peninsula is one of the most humanized landscapes in Europe, where humans, livestock, and wildlife cohabit in close contact [1]. Therefore, pathogens (viruses, bacteria, and parasites) can infect multiple hosts in these systems and are thus responsible for emerging diseases if environmental changes occur [2]. These possibilities are unpredictable [3] for wild carnivores, which are excellent sentinels for assessing the health status of their natural prey (wild boar, roe deer, or red deer) and ecosystems. Furthermore, evaluating their health allows us to assess risks to their own sustainability.

Wild animals can act both as reservoirs of agents and sources of transmission to domestic animals, which in turn have close contact with humans. Conversely, domestic animals or even humans can introduce agents into the environment, which can endanger wild animals [4]. Several anthropogenic factors have intensified animal-human interfaces [5].

These relations can promote events such as spillover and/or spillback of infectious and parasitic diseases between humans, livestock, and wildlife, exposing different interfaces and potential sources of emerging zoonotic diseases (EZDs). Parasitic infections are responsible for high economic losses, morbidity, or even mortality. A better understanding of the host and parasite's natural history and the possible mechanisms underlying changes in disease dynamics might improve our knowledge of diseases affecting wild animals [4].

In human-dominated landscapes, the occurrence of wolves results from complex interactions among several environmental and human factors [5].

The conservation of large carnivores is a challenge for biodiversity conservation efforts in territories fragmented by solid human pressure, as is the case for wolves in almost all of Europe. In the past, wolves were stigmatized due to their negative impacts on humans [6]. However, it has been reported [7] that in one-third of Europe, the population of at least one species of large predator is either stable or growing [8]. This equilibrium is due to significant investments in conservation, education, and public support, as well as protective legislation and implementation that have contributed to a possible coexistence. The European scenario reveals that large carnivores and people share the same landscape, such as the example of the increasing number of wolves all over Europe [7].

Studying the parasitic fauna present in wolves allows us to predict valuable information, obtain reliable data, and, in a non-invasive way, provides crucial evidence that is challenging to access in vivo. For example, if the populations are growing (prevalence of *Toxocara* spp. in relation to the presence of juveniles in the packs), determining seasonal or inter-annual changes could be an essential aspect of population dynamics in this highly social species because it is recognized that wolves can act as a reservoir and spreader of some zoonotic diseases [9,10]. Knowing the circulating agents in wild canid populations can help us understand their health status and the environment where they live.

According to Craig and Craig [11], most parasitic agent findings should be detected by necropsy. Nevertheless, when working with a protected species, this is a considerable limitation.

The fox (*Vulpes vulpes*) is the most abundant mesocarnivore on the Iberian Peninsula (IP) and possibly has closer contact with humans, especially in rural areas sharing the environment with both wild carnivores (such as wolves) and domestic animals (such as dogs). Monitoring wolf cohabitants, such as dogs or foxes, that may host certain agents and thus introduce them into the environment, should be essential [12].

Coprological techniques provide a good alternative when working with endangered and elusive species in remote areas, mainly because they allow us to access information about free-living animals without interfering with their life cycle and behavior. It was suggested by Torres et al. [10] that coprology can offer parasitic evidence, except perhaps for cestode eggs due to the intermittent excretion of ovigerous proglottids into the environment, thus yielding false negative results [13]. Additionally, coprology can provide information about the diversity of parasites that circulate in prey, such as *Trichuris* spp., which have high resistance to the gastrointestinal tract [14]. Thus, coprology can help determine the health status of prey. This raises the question about what role wild carnivores play, as reservoirs, since most parasitic eggs have high resistance to the environment or must develop in the environment [15,16].

This review aimed to collect information about parasitic agents in Iberian wolf populations in Portugal and Spain found using coprological methods, with a particular focus on agents with zoonotic potential and the potential risk for infection to domestic animals. We compare the prevalence of potential zoonotic agents detected in Iberian wolves with that reported in foxes and dogs in the same region as the Iberian wolf using coprological techniques.

2. Results

Overall, five master's dissertations, five articles, and two conference abstracts were analyzed. From Portugal, three master's dissertations were found and analyzed from the Faculty of Veterinary Medicine of the University of Lisbon [17–19] and two articles were found [20,21]. From Spain, two masters' dissertations were found and analyzed from Vasco da Gama University School, Coimbra [22,23], as well as three articles [24–26] and three congress abstracts [24,25,27].

Based on the literature review, a global range of prevalence was established from 21.5% to 100% for gastrointestinal parasites in Iberian wolves, with an average global prevalence of 61.0%. Regarding each country, the prevalence of gastrointestinal parasites (GI) ranged between 21.5% and 68.3% [17–20] in Portugal and from 57.0% to 100% in Spain [22–29].

Parasites of the phyla Protozoa, Platyhelminthes (classes Trematoda and Cestoda), and Nematoda were reported via coprological methods. Moreover, thirteen genera and twenty species of gastrointestinal parasites were identified. Among them, 76.9% (10/13) of genera and 65.0% (13/20) of species had zoonotic potential. Table 1 summarizes the GI parasites reported in Portugal and Spain, found through coprology [17–29].

Table 1. Gastrointestinal parasites reported in wolves from Portugal and Spain.

	Agents	Portugal	Spain
Nematoda	Ancylostomatidae	+	+
	Ancylostoma caninum	+	+
	Unicinaria stenocephala	+	+
	Toxocara spp.	+	+
	Toxascaris leonina.	+	+
	Crenosoma vulpis	+	–
	Trichuris spp.	+	+
	Trichuris vulpis	+	+
	Spirocerca lupi	–	+
	Ascaris suum *	–	+
	Nematodirus spp.*	+	+
	Eucoleus spp.	+	+
	Eucoleus aerophilus	+	++
	Strongyloides spp.	+	+
Cestoda	Taeniidae	+	+
	Taenia hydatigena	+	–
	Taenia polyacanta	+	–
	Taenia pisiformis	+	–
	Taenia serialis	+	–
	Moniezia expansa *	+	+
	Dipylidium caninum	–	+
	Hymenolepis diminuta *	–	+
Trematoda	*Dicrocoelium dendriticum*	–	+
Protozoa	*Giardia* spp.	–	+
	Cryptosporidium spp.	–	+
	Sarcocystis spp.	–	+
	Sarcocystis canis	+	+
	Cystoisospora spp.	+	+
	Eimeria sp.*	+	+

* Likely a pseudo parasite rather than a patent infection.

The occurrence and diversity of gastrointestinal parasites reported in wolves through coprological techniques in Portugal and Spain are presented in Tables 2 and 3, respectively.

Table 2. Prevalence (%) of parasitic agents reported in wolves in Portugal [17–21].

	Agents	Zoonotic Potential	% (n)	Technique	References
Nematoda	Ancylostomatidae	Yes	45.7 (75/164)	Coprology	[17]
			6.5 (7/107)		[19]
	Toxocara spp.	Yes	11.7 (16/68)	Coprology	[18]
	Toxocara canis	Yes	7.30 (12/164)	Coprology	[17]
			9.0 (1/11)		[21]
	Toxascaris leonina	No	7.3 (12/164)	Coprology	[17]
			7.4 (5/68)		[18]
			9.0 (1/11)		[21]
			1.9 (2/107)		[19]
	Crenosoma vulpis	No	9.0 (1/11)	Coprology	[20]
	Trichuris spp.	Yes	3.7 (5/164)	Coprology	[17]
			1.5 (1/68)		[18]
			2.8 (3/107)		[19]
	Trichuris vulpis	Yes	5.9 (4/68)	Coprology	[18]
			0.9 (1/107)		[19]
	Eucoleus aerophilus	No	4.3 (7/164)	Coprology	[17]
	Nematodirus spp. *	No	0.6 (1/164)	Coprology	[17]
	Strongyloides spp.	Yes	21.3 (35/164)	Coprology	[17]
			1.5 (1/68)		[18]
			1.9 (2/107)		[19]
Cestoda	Taeniidae	Yes	4.3 (7/164)	Coprology	[17]
			22.1 (15/68)		[20]
			13.1 (14/107)		[19]
	Taenia hydatigena	Yes	11.8 (8/68)	PCR-Multiplex and Sequencing	[20]
	Taenia polyacantha	No	1.5 (1/68)	PCR-Multiplex and Sequencing	[20]
	Taenia pisiformis	No	2.9 (2/68)	PCR-Multiplex and Sequencing	[20]
	Taenia serialis	Yes	5.9 (4/68)	PCR-Multiplex and Sequencing	[20]
	Echinococcus granulosus	Yes	1.5 (1/68)	PCR-Multiplex and Sequencing	[20]
	Moniezia spp. *	No	0.6 (1/164)	Coprology	[17]
Protozoa	*Eimeria* spp. *	No	4.9 (8/164)	Coprology	[17]
			0.9 (1/107)		[19]
	Sarcocystis canis	No	7.9 (13/164)	Coprology	[17]
	Cystoisospora spp.	No	3.7 (6/164)	Coprology	[17]
			0.9 (1/107)		[19]
	Cryptosporidium sp.	Yes	13.5 (22/164)	Coprology	[17]

* Likely a pseudo parasite rather than a patent infection.

Table 3. Prevalence (%) of parasitic agents reported in wolves in Spain [22–29].

	Agents	Zoonotic Potential	% (n)	Technique	References
Nematoda	Ancylostomatidae	Yes	21.6 (86/398)	Coprology	[23]
			19.3 (18/93)		[25]
			16.2 (122/752)		[26]
			17.0 (17/100) 30.0 (30/100)		[27]
			1.9 (2/101)		[28]
	Ancylostoma caninum	Yes	16.6 (3/18)	Coprology	[24]
	Uncinaria stenocephala	No	11.1 (2/18)	Coprology	[24]
	Toxocara spp.	Yes	40.0 (71/177)	Coprology	[22]
			7.5 (30/398)		[23]
			6.5 (49/752)		[26]
			5.0 (5/100)		
			7.0 (7/100)		[27]
			4.9 (5/101)		[28]
	Toxocara canis	Yes	5.5 (1/18)	Coprology	[24]
			10.7 (10/93)		[25]
	Trichuris spp.	Yes	25.5 (45/177)	Coprology	[22]
			43.9 (174/398)		[23]
			8.1 (61/752)		[26]
	Trichuris vulpis	Yes	11.1 (2/18)	Coprology	[24]
			9.6 (9/93)		[25]
	Spirocerca lupi	Yes	16.6 (3/18)	Coprology	[24]
			1.5 (6/398)		[23]
			0.9 (1/101)		[28]
	Ascaris suum *	Yes	0.5 (2/398)	Coprology	[23]
	Toxascaris leonina	No	5.5 (1/18)	Coprology	[24]
			0.2 (1/398)		[23]
			2.1 (2/93)		[25]
			1.0 (1/100)		[27]
			0.9 (1/101)		[28]
	Nematodirus spp. *	No	0.20 (1/398)	Coprology	[23]
	Eucoleus spp.	No	5.5 (22/398)	Coprology	[23]
			17.1 (129/752)		[26]
			13.8 (14/101)		[28]
	Eucoleus aerophilus	No	50.5 (47/93)	Coprology	[25]
	Strongyloides spp.	Yes	27.0 (25/93)	Coprology	[25]
Cestoda	Taeniidae	Yes	26.7 (47/177)	Coprology	[22]
			8.0 (8/100)		[27]
			4.0 (4/100)		
			7.5 (30/398)		[23]
			9.6 (9/93)		[25]

Table 3. *Cont.*

	Agents	Zoonotic Potential	% (n)	Technique	References
			10.7 (81/752)		[26]
			5.9 (6/101)		[28]
	Moniezia expansa *	No	0.5 (2/398)	Coprology	[23]
	Dipylidium caninum	Yes	5.5 (1/18)	Coprology	[24]
	Hymenolepis diminuta *	Yes	0.5 (2/398)	Coprology	[23]
Trematoda	*Dicrocoelium dendriticum*	Yes	3.0 (12/398)	Coprology	[23]
			1.0 (1/100)		[27]
			3.0 (3/100)		[27]
Protozoa	*Giardia* sp.	Yes	14.0 (7/50)	Coprology/IFD **	[29]
	Cryptosporidium spp.	Yes	4.0 (2/50)	Coprology/IFD **	[29]
	Sarcocystis spp.	Yes	44.4 (8/18)	Coprology	[24]
	Cystoisospora spp.	No	1.0 (4/398)	Coprology	[23]
	Eimeria spp. *	No	11.1 (2/18)	Coprology	[24]

* Likely a pseudo parasite rather than a patent infection. ** Direct Immunofluorescence.

Data associated with domestic dogs (*C. familiaris*) and foxes (*V. vulpes*) in Portugal [17–19,30–32] and Spain [33–36], using the same methodology, are compiled on Tables 4 and 5, with a focus on zoonotic agents.

Table 4. Prevalence (%) of parasitic agents with zoonotic potential reported in domestic dogs (*Canis familiaris*) and red foxes (*Vulpes vulpes*) in Portugal [17–19,30–32].

		Domestic Dog *(C. familiaris)* % (n)	Red Fox *(V. vulpes)* % (n)	References
Nematoda	Ancylostomatidae	53.8 (21/39)	64.2 (52/81)	[17]
		19.5 (57/296)	–	[30]
		14.8 (29/195)	–	[30]
		20.7 (21/101)	–	[30]
		22.0 (38/173)	15.2 (32/211)	[19]
		–	24.4 (20/82)	[31]
	Ancylostoma caninum	33.0 (21/63)	–	[32]
	Uncinaria stenocephala	–	–	–
	Toxocara spp.	–	12.1 (4/33)	[18]
		0.6 (1/173)	2.8 (6/211)	[19]
		–	34.1 (28/82)	[31]
	Toxacara canis	10.3 (4/39)	24.7 (20/81)	[17]
		29.0 (18/63)	–	[32]
	Trichuris spp.	7.7 (3/39)	2.5 (2/81)	[17]
		13.3 (23/173)	8.1 (17/211)	[19]
	Trichuris vulpis	1.6 (1/63)	–	[32]
	Strongyloides spp.	25.6 (10/39)	42.0 (34/81)	[17]
		1.7 (3/173)	1.9 (4/211)	[19]

Table 4. *Cont.*

		Domestic Dog *(C. familiaris)* % (n)	Red Fox *(V. vulpes)* % (n)	References
Cestoda	Taeniidae	2.6 (1/39)	–	[17]
		–	6.1 (2/33)	[18]
		4.6 (8/173)	4.3 (9/211)	[19]
	Taenia hydatigena	–	–	–
	Taenia serialis	–	3.0 (1/33)	[18]
	Taenia multiceps	–	–	–
	Echinococcus granulosus	–	–	–
	Echinococcus multilocularis	–	–	–
	Diphylidium caninum	6.0 (2/63)	–	[32]
	Hymenolpis spp.	–	0.5 (1/211)	[19]
	Hymenolepsis diminuta	–	–	–
Protozoa	*Cryptosporidium* spp.	–	–	–
	Giardia sp.	–	–	–
	Sarcocystis spp.	–	–	–
	Sarcocystis canis	2.6 (1/39)	1.2 (39)	[17]

Table 5. Prevalence (%) of parasitic agents with zoonotic potential reported in domestic dogs (*Canis familiaris*) and red foxes (*Vulpes vulpes*) in Spain [33–36].

		Domestic Dog *(C. familiaris)* % (n)	Red Fox *(V. vulpes)* % (n)	References
Nematoda	Ancylostomatidae	31.2 (114/365)	–	[33]
	Ancylostoma caninum	1.1 (11/1040)	–	[34]
	Uncinaria stenocephala	28.4 (295/1040)	–	[34]
	Toxocara spp.	27.7 (101/365)	–	[33]
		–	2.0 (1/49)	[35]
	Toxocara canis	5.6 (58/1040)	–	[34]
		–	27.0 (69/257)	[36]
	Trichuris spp.	26.6 (97/365)	–	[33]
	Trichuris vulpis	1.7 (17/1040)	–	[34]
		–	12.0 (30/257)	[36]
	Spirocerca lupi	1.1 (4/365)	–	[33]
	Strongyloides spp.	–	–	–
Cestoda	Taeniidae	4.0 (42/1040)	–	[34]
		–	6.1 (3/49)	[35]
		1.9 (7/365)	–	[33]
	Taenia hydatigena	1.1 (11/1040)	–	[34]
		–	0.4 (1/257)	[36]

Table 5. *Cont.*

		Domestic Dog *(C. familiaris)* % (n)	Red Fox *(V. vulpes)* % (n)	References
	Taenia serialis	–	–	-
	Taenia multiceps	0.1 (1/1040)	–	[34]
	Echinococcus granulosus	0.5 (5/1040)	–	[34]
	Echinococcus multilocularis	–	–	–
	Diphylidium caninum	23.1 (240/1040)		[34]
		–	2.0 (5/257)	[36]
	Hymenolepsis diminuta	–	–	–
Protozoa	*Cryptosporidium* spp.	1.9 (7/365)	–	[33]
	Giardia spp.	27.1 (99/365)	–	[33]
	Giardia duodenalis	27.1 (99/365)	–	[33]
	Sarcocystis spp.	5.5 (20/365)	–	[33]
	Sarcocystis canis	2.6 (1/39)	–	[33]

The prevalence of agents with zoonotic potential reported in wolves in the Iberian Peninsula was compared with the prevalence reported in other studies in wolves in Europe (Table 6), regardless of the technique (coprology or necropsy) [37–51].

Table 6. Prevalence (%) of parasitic agents with zoonotic potential reported in wolves in Europe [37–51].

	Agents	% (n)	Country	References
Nematoda	Ancylostomatidae	6.9 (5/72)	Poland	[37]
		20.2 (14/69)	Germany	[38]
		18.4 (7/38)	Italy	[39]
	Ancylostoma caninum	12.3 (11/89)	Poland	[40]
		*	France	[41]
		2.9 (1/34)	Latvia	[42]
		2.7 (4/147)	Greece	[43]
		6.2 (2/32)	Ukraine	[44]
		7.1 (3/42)	Italy	[45]
	Uncinaria stenocephala	77.0 (20/26)	Estonia	[46]
	Toxocara spp.	–	–	–
	Toxocara canis	8.0 (2/26)	Estonia	[46]
		3.5 (2/58)	Poland	[47]
		5.6 (5/89)	Poland	[40]
		*	France	[41]
		5.8 (2/34)	Latvia	[42]
		5.9 (5/72)	Poland	[37]
		3.9 (1/102)	Serbia	[48]

Table 6. *Cont.*

	Agents	% (n)	Country	References
		9.5 (4/49)	Italy	[45]
		13.0 (9/69)	Germany	[38]
		5.2 (2/38)	Italy	[39]
	Trichuris vulpis	1.7 (1/58)	Poland	[47]
		13.9 (10/72)	Poland	[37]
		6.8 (10/147)	Greece	[43]
		18.8 (6/32)	Ukraine	[44]
		5.8 (4/69)	Germany	[38]
	Spirocerca lupi	4.7 (7/147)	Greece	[43]
	Strongyloides spp.	1.1 (1/89)	Poland	[40]
Cestoda	*Taenia* spp.	19.0 (5/26)	Estonia	[46]
		11.2 (10/89)	Poland	[40]
		8.6 (5/58)	Poland	[47]
		8.8 (3/34)	Latvia	[42]
		1.4 (1/72)	Poland	[37]
		7.4 (10/147)	Greece	[43]
		45.0 (8/18)	Sweden	[49]
		21.7 (15/69)	Germany	[38]
		34.1 (13/38)	Italy	[39]
	Taenia hydatigena	12.0 (3/26)	Estonia	[46]
		41.2 (14/34)	Latvia	[42]
		9.8 (10/102)	Serbia	[48]
		19.6 (35/179)	Italy	[50]
		22.2 (29/130)	Italy	[51]
	Taenia serialis	1.0 (1/102)	Serbia	[48]
		10.5 (4/38)	Italy	[39]
	Taenia multiceps	27.0 (7/26)	Estonia	[46]
		47.1 (16/34)	Latvia	[42]
		3.9 (4/102)	Serbia	[48]
		76.2 (32/42)	Italy	[45]
	Echinococcus granulosus	4.0 ((1/26)	Estonia	[46]
		2.9 (1/34)	Latvia	[42]
		5.6 (10/179)	Italy	[50]
		5.5 (7/130)	Italy	[51]
		26.3 (10/38)	Italy	[39]
	Echinococcus multilocularis	8.6 (2/23)	Slovakia	[52]
		5.9 (2/34)	Latvia	[42]
	Diphylidium caninum	4.8 (2/42)	Italy	[45]
	Hymenolepsis diminuta	–	–	–

Table 6. *Cont.*

	Agents	% (n)	Country	References
Protozoa	*Cryptosporidium* spp.	25.8 (37/147)	Greece	[43]
	Giardia spp.	–	–	–
	Giardia duodenalis	–	–	–
	Sarcocystis spp.	46.9 (68/147)	Greece	[43]
	Sarcocystis canis	–	–	–

* Study only reported presence/absence of agents.

3. Discussion

Our review estimated an average global prevalence of 61.0% for gastrointestinal parasites in Iberian wolves in the IP ranging from 21.5 to 100%. More than 50% of the found genera/species were of zoonotic concern. These results were mostly based on coprological methodologies, addressing their usefulness for wildlife studies with such an inconspicuous species as the Iberian wolf (*Canis lupus signatus*).

Non-invasive techniques based on coprology have proven advantageous, can be adapted to any species, and are valuable when working with wild, rare, and/or remote species. It has been widely recognized that coprological methods can provide acceptable results for wild canids. Remarkably, most wolf endoparasites are detectable with these methods [53]. However, coprological methods have limitations, such as the impossibility of seeing the host (assessment of body condition) or evaluating gender and age, among other available parameters [54]. Low levels of infection, patent infections, or irregular elimination of eggs (cestodes) may not be detected through coprology and can compromise its sensitivity [40]. Coprology also has the advantage of being able to complement other techniques important to study species such as Taeniidae [20].

Some studies may aim to perform coprological techniques and isolate Taeniid eggs using the combined floatation method in zinc chloride solution (density 1.45 g/mL) before sieving [55], and then performing observations using conventional microscopy. For DNA extraction, commercial kits were used, such as the Qiamp DNA mini kit (Qiagen, Hilden, Germany) and a kit from Bio-Rad Laboratories (Hercules, CA, USA), according to the manufacturers' instructions [56]. As justified, other methods can be used when sensitivity is necessary. For instance, to determine the presence of *Giardia* cysts and *Cryptosporidium* oocysts in fecal samples, a commercial direct immunofluorescence assay was applied [29].

Although parasitic identification is achieved through morphological analysis, in some cases it is only possible to identify parasites at family or genus levels. Other potential limitations of these studies include the difficulty of estimating the prevalence in different regions/countries using different techniques and disparity in the number of samples analyzed among the studies.

Portugal and Spain have common parasitic agents circulating in the environment or in their domestic and/or wild populations. The presence of Nematoda, Cestoda, and Protozoa was confirmed. In Portugal, *C. vulpis* in the phylum Nematoda, *T. hydatigena*, *T. polyacantha*, *T. pisiformis,* and *T. serialis* in the phylum Cestoda, and *S. canis*, in the phylum Protozoa were detected. In Spain, *A. caninum, U. stenocephala, S. lupi, A. suum* in the phylum Nematoda, *D. caninum,* and *H. diminuta* in the phylum Cestoda, *Giardia* spp. in the phylum Protozoa, and *D. dendriticum* in the phylum Trematoda were found (Tables 2 and 3).

Foxes are a hunting species; therefore, it is relatively easy in both countries to obtain animals for necropsy, while samples for coprology are not so frequently found. Necropsy studies in foxes suggest that these animals are not parasite-free, quite the opposite [11].

Despite the disparity among techniques and number of samples used for detecting gastrointestinal parasites in wolves across Europe, the results should not be underestimated. Belarus reported an overall prevalence of gastrointestinal parasites of 80% [57]. Poland reported a prevalence of gastrointestinal parasites of 27.8–78.6% [37,40,47]; Sweden

reported a prevalence of 90% [49]; Germany reported a prevalence of 60.8% [38]; Serbia reported a prevalence of 16.7% [48]; Slovakia reported a prevalence of 66% [52,58]; Greece reported a prevalence of 83.0% [43]; Italy reported a prevalence of 74.3–85.7% [45,59]; Spain reported a prevalence of 57.0–100% [22–29]; and Portugal reported a prevalence of 21.5–68.3% [17–19,30–32]. Potentially zoonotic parasites were reported in all of these studies. Some of these parasites can cause ocular and visceral larva migrans *(Toxocara* spp.) and cutaneous larva migrans *(A. caninum).*

Among the reported nematodes, the family Ancylostomatidae has two species of veterinary concern: *A. caninum* and *U. stenocephala.* Both were reported in Portugal [17,19] and Spain [23–26], with a prevalence ranging between 6.0% and 45.7% and between 16.2% and 30.0%, respectively. This family was also identified in dogs with a prevalence between 14.0 and 53.8% [17,19,30,31] and in 64.2% of Portuguese foxes. In Portugal, this family was reported with a higher prevalence in dogs and foxes than in wolves, and in Spain, the prevalence was similar among the different species. The species *A. caninum* was identified in dogs [32] and foxes [19,31] in Portugal. In Spain, it was identified in wolves [24] and dogs [34]. This family was already reported in wolves in Poland [37], Germany [38], and Italy [39] (Table 6) and *T. canis* was reported in France [41], Italy [45], and Poland [40], although with a lower prevalence than in the IP. However, *A. caninum* had a higher prevalence in the IP than in other European countries, such as France [41], Italy [45], Greece [43], Poland [40], Ukraine [44], and Latvia [42]. *A. caninum* is more frequently transmitted by milk from females to cubs [14], although horizontal transmission can occur via percutaneous or oral transmission of third-stage larvae from the environment and ingestion of paratenic hosts, respectively, whereas transmission of *U. stenocephala* more frequently occurs by ingestion. Both species, *A. caninum* and *U. stenocephala,* can have a direct life cycle and their microbiotope is the small intestine [13,14].

A. caninum is more pathogenic due to it is hematophagous characteristics, which can cause severe anemia and therefore cause mortality in young cubs. *A. caninum* is a zoonotic parasite that can cause cutaneous larva migrans in humans [60,61], although occasionally humans can be infected and become the final host [14]. The family Ancylostomatidae was the most prevalent in Portugal and Spain.

Other relevant reported nematodes belonged to *Toxocara* spp. with *T. canis* being the species with zoonotic potential. This genus was reported in wolves in Portugal [18] and in Spain [22,23,26–28], with a similar prevalence. In Portugal, this genus had a similar prevalence among the three hosts, whereas wolves and dogs had a similar prevalence and foxes had a lower prevalence in Spain. This family was also reported in dogs [17] and foxes [31]. The species *T. canis* was identified in wolves [17,21], dogs [17,32], and foxes [17], with a higher prevalence in dogs than in wolves or foxes in Portugal. Spain had a higher prevalence of *T. canis* in wolves and foxes than in dogs. Wolves in the IP had a similar prevalence of this agent as that reported in wolves in Germany [38] and Italy [45], compared with other countries where the species was also identified, but with a lower prevalence, such as Poland [37,40,47], Latvia [42], Estonia [46], and Serbia [48]. The animals can be infected by ingesting eggs present in the environment [13,14], or cubs can be infected by vertical transmission (via placenta or milk), thus establishing a direct cycle inside the pack. The presence of this agent in cubs can cause morbidity and eventually mortality with high levels of infection. Additionally, it can cause loss of body condition, pneumonia accompanied by pulmonary edema, and partial or complete bowel occlusion, causing a risk of peritonitis [13,14]. The presence of this agent may therefore be a risk to sustainable pack growth. In addition, *T. canis* is a parasite with zoonotic potential, with children being more predisposed to infection and causing ocular and visceral larva migrans in humans [13,14,61].

The presence of eggs from *Strongyloides* spp. was reported in Portugal [17–19] and Spain [25], with a similar prevalence. This genus was also reported in dogs and foxes in Portugal, but the presence of this agent was not reported in dogs and foxes in Spain. It was only reported in wolves in Poland [40], with a low prevalence. Primary infection of

the host usually occurs through skin penetration. Nevertheless, trans mammary infection may also occur if the host has been infected during lactation. Heavy infections can produce respiratory signs from migrating larvae or enteritis associated with the presence of adults. *S. stercoralis* is an example of a species of this genus that can lead to severe or even fatal infections in immunocompromised humans. Canine strains infecting humans are little known, but due to the seriousness of some reported human cases, it is considered a zoonotic agent [13].

The presence of *A. suum* was reported in wolves in Spain [23]. This nematode infects mainly pigs (wild and/or domestic), which become infected by ingesting eggs from the environment. Larval migration through the liver and lungs can lead to a predisposition to bacterial or viral pneumonia. The adults develop in the small intestine, which can cause poor growth. The larval stages can migrate to other species, such as humans [13]. Nevertheless, this nematode was reported with a low prevalence and may have been a case of pseudo parasitism since there were no known patent infections in wolves.

Other nematodes, causing respiratory but not gastrointestinal infections, are not transmissible to humans but can cause morbidities in canids with high levels of infection, such as *Eucoleus* spp. The presence of *E. aerophilus* was described in Portugal [17] and the genus *Eucoleus* was reported in Spain [25], but with a higher prevalence [23,26,28]. These nematodes have been described as cosmopolitan. Adult forms are found in the respiratory system (trachea, bronchi, and bronchioles) of canids (wild and/or domestic). *E. aerophilus* is a vital pathogen that causes bronchopneumonia and chronic cough [13].

The presence of *C. vulpis* was reported in wolves only in Portugal [20]. The host is infected by ingesting a terrestrial snail containing third-stage larvae, which have tropism for the respiratory system. The adult forms are coughed up, swallowed, and the eggs are passed from the host to the environment in feces [13]. High infections can produce chronic respiratory disease in canids. It is not reported in humans.

Trichuris was identified as *T. vulpis* [18] in Portugal [17–19] and was identified at the genus level [22,23,26] and as the species *T. vulpis* in Spain [24,25]. The genus *Trichuris* had the highest prevalence in dogs in Portugal (Table 4). In Spain, dogs and wolves had a similar and higher prevalence of *Trichuris* than foxes. *T. vulpis* is an agent of importance in veterinary and human medicine. Although infection is rare in humans [14], it has been described in several studies, especially in children, [62] but also in adult humans as a cause of visceral larva migrans [63]. Canids become infected by ingesting eggs in the environment. The adult worms have tropism to the caecum and large intestine and shed eggs through feces, where it develops into the infective stage [13].

Eggs of *Nematodirus* spp. were reported in Portugal [17] and Spain [23]. These parasites are present in ruminants' small intestine, and most of these species do not cause clinical disease. Ruminants become infected when they ingest infective larvae, but their detection in wolves may be considered a pseudo parasitic phenomenon [14]. This nematode was reported with a low prevalence in both countries.

S. lupi was reported in wolves [23,24,28] and dogs [33] only in Spain. This species was only reported in wolves in Greece [43], with a higher prevalence than in Spain. The adult forms are found on the wall of the esophagus, stomach, and eventually the aorta. Canids become infected when ingesting insects (dung beetle) or paratenic hosts (rodents, other mammals). The infections were considered subclinical. However, dysphagia, regurgitation, esophageal rupture, or obstruction, can occur. Occasionally, it can also infect humans, although very rarely [14].

The class Cestoda, mainly of the family Taeniidae, has species of high importance in terms of public health, such as *Echinococcus* spp. or *T. multiceps* [14]. The Taeniidae family was reported in Portugal [17,19,20] and in Spain [22,23,25–28] with a high but similar prevalence. This family was found in dogs in Portugal [17] and Spain [34] and in foxes in Portugal [35]. The prevalence in both countries was higher in wolves than in other canid species. This family has been reported a little all over Europe, with a prevalence in Sweden [49] and Italy [39] similar to that in the IP. Taeniidae were also identified in

Estonia [46], Poland [37,40,47], Latvia [42], Greece [43], and Germany [38]. It is impossible to identify the species solely by the morphology of the eggs, even to differentiate between *Taenia* spp. and *Echinococcus* spp. or any species of this family. In Portugal, molecular techniques were applied to identify the species of Taeniidae eggs [20]. It was detected in the presence of *T. hydatigena* (ungulates act as intermediate hosts (IHs), *T. polyacantha* (with rodents as IHs), and *T. pisiformis* and *T. serialis* (both with rabbits as IHs) in Portugal. This last species can eventually infect humans [14]. *T. hydatigena* was not reported in dogs or foxes in Portugal but was detected in Spain through necropsy in wolves and dogs [34,36]. After necropsy, this species was also reported in Estonia [46] and Serbia [48], with a prevalence similar to that in Portugal. This species was reported across Europe, with high prevalence in Latvia [42] and Italy [50,51]. *T. serialis* was reported in wolves and foxes in Portugal [20], but no findings were reported in Spain. Despite the low prevalence of this species, it was reported in Serbia [48] and Italy [39]. *T. multiceps* was not identified in any of these canids in Portugal but was reported in one dog in Spain [34]. It was reported with a high prevalence in Italy [45] and Latvia [42], but with a more negligible prevalence in Estonia [46] and Serbia [48]. *Echinococcus* spp. were reported in wolves in Portugal through molecular techniques [20] and in wolves and dogs in Spain through necropsy [34,64]. Other countries in Europe revealed that *Echinococcus* spp. were mainly detected by necropsy because they are countries with large populations of wolves that are hunted. These species were reported with a high prevalence in Italy [45,50] compared to that in other countries, such as Estonia [46] and Latvia [42]. The Taeniidae family has the particularity of always being dependent on a predation cycle to complete their cycle. Wild or domestic ungulates, or even rodents or lagomorphs, can act as the IH and carnivores as the definitive host (by ingesting the immature metacestode stages on prey tissues), where they complete the life cycle and excrete their eggs through feces [13,14].

The presence of *Moniezia* spp. cestodes was also reported in Portugal [17] and Spain [23]. These cestodes are present in the gastrointestinal tract of ruminants. The animals become infected when eggs are shed in their feces to the environment, where they develop as cysticercoid larvae inside oribatid mites living in the fecal pat and pasture environments and are ingested during grazing. It is reported that a high level of infection may lead to a delay in the growth of young ruminants. Nevertheless, their presence in wolves may be considered a pseudo parasitism situation and can also highlight the sort of prey ingested by these carnivores [14]. The prevalence of this agent was low and similar in both countries.

D. caninum was detected in dogs [17] and foxes [19] in Portugal and was reported in wolves [24], dogs with a high prevalence [34], and foxes [36] in Spain. The presence of this agent was reported in wolves only in Italy [45]. Animals get infected by ingesting the arthropod intermediate host (fleas) with larval cysticercoids. Infections with this cestode can cause anal pruritis with the passage of segments. However, this cestode has zoonotic potential, especially in children [14]. This agent was found with a low prevalence.

H. diminuta was detected only in foxes in Portugal [19] and only in wolves in Spain [23]. The presence of this agent in wolves in the rest of Europe has not been reported. This agent is present in the small intestine of rodents, and eventually in humans. The eggs are shed in feces and ingested by intermediate beetle hosts. Infection occurs when these beetles are eaten or by the ingestion of eggs by the definitive host. It can also be considered a pseudo parasite [14].

D. dendriticum was reported only in wolves in Spain [23,27]. This trematode has a tropism for bile ducts and is present in several species (domestic and wild ungulates, such as ruminants or pigs). This trematode needs two intermediate hosts to complete their cycle: embryonated eggs in the environment are ingested by terrestrial snails (*Zebrina detrita*) in which long tailed cercariae develop inside the daughter sporocysts. Cercariae leave the snail as sporocysts through mucus and are ingested by ants of the genus *Formica* (*Formica fusca*), in which the cercariae encyst as metacercariae. Several infections can lead to

extensive cirrhosis in the liver, leading to anemia and poor body condition [14]. This agent was found with a low prevalence.

Coccidia were detected in both countries as *Eimeria* spp. in Portugal [17,19] and Spain [24]. There are many host-specific species of *Eimeria*, but they generally infect the intestinal tract of ruminants and are already reported in canids. Infection occurs when fecal oocysts sporulate in the environment and are ingested in the pasture. Coccidia infect young ruminants, lagomorphs, and birds, leading to diarrhea and consequently, poor body condition. Nevertheless, the level of pathogenicity is variable, depending on the coccidian species [14]. This agent was detected with a low prevalence in both countries.

Cystoisospora spp., formerly known as *Isospora* spp., were reported in Portugal [17,19] and Spain [23]. Many species of this protozoan have been described as infecting the intestinal system of canids. Intermediate hosts become infected by ingesting sporulated oocysts (ruminants, rodents, or birds), followed by development in the intestine of the final host, and once again, shed through feces to the environment. Clinical cystoisosporosis is more frequent in young animals and can be exacerbated by high stress levels, causing diarrhea and abdominal pain [14]. This agent was found with a low prevalence.

The presence of other protozoan parasites, namely members of the genus *Sarcocystis*, was detected in Portugal, namely the species *S. canis* [17], while only the genus *Sarcocystis* was identified in Spain [24]. A wide range of species can infect canids, each with a specific intermediate host (ungulates, pigs, and rodents), but all of them are present in the small intestine of canids. The animals become infected by ingesting intermediate host tissue contaminated with *Sarcocystis* cysts. It is reported that *Sarcocystis* spp. do not cause illness in the definitive host, but some species can cause severe disease on the IH [14]. This genus was found in Spain with a higher prevalence than in Portugal.

The presence of *Cryptosporidium* spp. was reported in wolves in Portugal [17] and in wolves [29] and dogs [33] in Spain. These protozoans were reported in wolves in Greece [43], with a higher prevalence than in the IP. These protozoans have tropism in the small intestine and a biological cycle. Canids become infected by ingesting oocysts, which multiplicate in the intestine and are shed in feces to the environment. However, the role of canids in transmission of this species to man remains unknown, compared with *C. parvum*, which is an intestinal parasite in ruminants. *C. parvum* can cause subclinical or severe diarrhea in young animals and has zoonotic potential, especially in children [14].

Giardia spp. cysts were reported in wolves in Spain [29] and with a higher prevalence in dogs [33]. Their presence was not reported in wolves across Europe. These flagellates are present in the small intestine of canids and other animals. Very common in canids, animals get infected by ingesting cysts in the environment. Many infections are asymptomatic but can cause mild to severe diarrhea and poor body condition, especially in young animals. Although the role of canids in transmitting this parasite is controversial, *Giardia* spp. always have zoonotic potential [14].

Our study highlights the importance of surveillance and monitoring of sylvatic and domestic species, especially in humanized territories.

4. Materials and Methods

We analyzed scientific articles in PubMed, ResearchGate, master's dissertations in university repositories, and congress abstracts reporting gastrointestinal parasites in Iberian wolves performed in the Iberian Peninsula (Portugal and Spain) using coprological methods from January 2000 to May 2022.

We searched studies reporting gastrointestinal parasites in Iberian wolves beginning in the early 2000s. However, the number of studies available detailing the prevalence and distribution of gastrointestinal parasites in this species is still being determined, and limited information was available. Most of these publications were the result of single point studies, without continuous monitoring in space and/or time, as a way of assessing the health status of the species since carnivores are not subject to any regular veterinary monitoring regarding gastrointestinal parasites.

For this paper, we used the following keywords: "*Canis lupus signatus*," "coprology," "gastrointestinal parasites," "helminths," "Iberian wolf," "Portugal," "protozoans," and "Spain".

A survey of the published data on gastrointestinal parasites in domestic dogs (*C. familiaris*) and foxes (*V. vulpes*) was also carried out using data obtained through coprology and/or necropsy in the same geographical area as that of the Iberian wolf in Portugal and Spain (Tables 4 and 5, respectively).

With agents with zoonotic potential reported in the IP in mind, we searched for other studies with domestic dogs and red foxes in the same territory as thate of the Iberian wolf.

Regardless of the methodology used (coprology and/or necropsy), we compared the prevalence of the zoonotic agents reported in wolves with the prevalence of these agents registered in Europe (Table 6).

5. Conclusions

Although coprology has lower sensitivity than necropsy, the results obtained from the non-invasive technique suggest it can still be an interesting, alternative diagnostic tool that provides important results. The method allows access to samples of wild animals, qualitative and quantitative estimates of parasitic levels, and indirectly provides information about the health status of the animal(s), as well as the agents circulating in the ecosystem, especially those with zoonotic potential.

Although the number of the studies was low, the number of available samples was variable, the use of coprological methods in these studies provided precious information, revealing the utility of this kind of technique for wild carnivores and the importance of carrying out these studies.

Half of the agents reported in wolves, through coprology, have zoonotic potential. Most of the reported parasitic agents circulating in wild cycles represent a potential risk of transmission for domestic animals and even humans, especially in human-modified landscapes. Considering the proximity of canids and humans, agents infecting wild canids can potentially infect domestic canids, and their closeness with humans puts human health at risk. We highlighted agents that were detected with zoonotic potential and with a high prevalence, such as Ancylostomatidae, *Sarcocystis* spp., *Cryptosporidium* spp., *Giardia* spp., and Taeniidae, which were reported in three canid host species (although with a lower prevalence) and require attention due to their severe consequences in terms of public health.

This report also shows the importance of monitoring parasitic diversity in wild carnivores, if possible, on a regular basis, because they contribute to the possible dissemination and/or maintenance of parasitic agents in circulation, especially in the case of land-sharing and highly fragmented ecosystems, such as the ones in the northern IP. Particularly relevant, this review highlights the prevalence of agents with zoonotic potential in domestic canids, sometimes higher than that in wild canids. These findings are in line with what has already been suggested by other authors, namely that we should increase awareness of animal care and welfare for domestic animals in rural areas, which are interface zones.

It is urgent to perform studies that establish the health status of wild carnivores in human-modified landscapes. To obtain a better epidemiological understanding of the sylvatic reservoirs under a One Health and Conservation Medicine approach, this knowledge is crucial to implement health measures when dealing with wild carnivores in humanized landscapes, as it is the case of the IP and its wolves and cohabitant carnivores.

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References

1. Alcaraz-Castaño, M.; Alcolea-González, J.J.; de Andrés-Herrero, M.; Castillo-Jiménez, S.; Cuartero, F.; Cuenca-Bescós, G.; Kehl, M.; López-Sáez, J.A.; Luque, L.; Pérez-Díaz, S.; et al. First modern human settlement recorded in the Iberian hinterland occurred during Heinrich Stadial 2 within harsh environmental conditions. *Sci. Rep.* **2021**, *11*, 15161. [CrossRef] [PubMed]
2. Woolhouse, J.E.M. Population biology of emerging and re-emerging pathogens. *Trends Microbiol.* **2002**, *10*, 3–5. [CrossRef] [PubMed]
3. Gortázar, C.; Ferroglio, E.; Höfle, U.; Frölich, K.; Vicente, J. Diseases shared between wildlife and livestock: A European perspective. *Eur. J. Wildl. Res.* **2007**, *53*, 241. [CrossRef]
4. Weinstein, S.B.; Lafferty, K.D. How do humans affect wildlife nematodes? *Trends Parasitol.* **2015**, *31*, 222–227. [CrossRef]
5. Llaneza, L.; López-Bao, J.; Sazatornil, V. Insights into wolf presence in human-dominate landscapes: The relative role of food availability, humans, and landscapes attributes. *Divers. Distrib.* **2011**, *18*, 459–469. [CrossRef]
6. Treves, A.; Karanth, K.U. Human-carnivore conflict and perspectives on carnivore management worldwide. *Conserv. Biol.* **2003**, *17*, 1491–1499. [CrossRef]
7. Chapron, G.; Kaczensky, P.; Linnell, J.D.C.; von Arx, M.; Huber, D.; Andrén, H.; López-Bao, J.V.; Adamec, M.; Álvares, F.; Anders, O.; et al. Recovery of large carnivores in Europe's modern human-dominated landscapes. *Science* **2014**, *346*, 1517–1519. [CrossRef]
8. Almberg, S.E.; Cross, C.P.; Dobson, P.A.; Smith, W.D.; Hudson, J.P. Parasite invasion following host reintroduction: A case study of Yellowstone's wolves. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **2012**, *367*, 2840–2851. [CrossRef]
9. Magouras, I.; Brookes, V.J.; Jori, F.; Martin, A.; Pfeiffer, D.U.; Dürr, S. Emerging Zoonotic Diseases: Should we rethink the Animal Interface? *Front. Vet. Sci.* **2020**, *7*, 582743. [CrossRef]
10. Torres, J.; Segovia, M.J.; Miquel, J.; Feliu, C.; Llaneza, L.; Fonseca-Petrucci, F. Helmintofauna del lobo Ibérico (*Canis lupus signatus* Cabrera, 1907). Aspectos potencialmente útiles en Mastozoologia. *Galemys* **2000**, *12*, 1–11.
11. Craig, L.H.; Craig, S.P. Helminth parasites of wolves (*Canis lupus*): A species list and an analyses of published prevalence studies in Neartic and Paleartic populations. *J. Helminthol.* **2005**, *79*, 95–103. [CrossRef] [PubMed]
12. Lledó, L.; Giménez-Pardo, C.; Saz, J.V.; Serrano, J.V. Wild red foxes (*Vulpes vulpes*) as sentinels of Parasitic Diseases in the Province of Soria, Northern of Spain. *Vector-Borne Zoonotic Dis.* **2015**, *15*, 743–749. [CrossRef] [PubMed]
13. Taylor, A.M.; Coop, L.R.; Wall, L.R. *Parasites of Dogs and Cats Veterinary Parasitology*, 4th ed.; Blackwell Publishing: Milton, NSW, Australia, 2016.
14. Zajac, M.; Conboy, A.G. *Veterinary Clinical Parasitology, 8th ed*; John Willey & Sons: Oxford, UK, 2012.
15. White, R.J.; Razgour, O. Emerging zoonotic diseases originating in mammals: A systematic review of effects of anthropogenic land-use change. *MammRev.* **2020**, *50*, 336–352. [CrossRef] [PubMed]
16. Carricondo-Sanchez, D.; Zimmermann, B.; Wabakken, P.; Eriksen, A.; Milleret, C.; Ordiz, A.; Sanz-Pérez, A.; Winkeros, C. Wolves at the door? Factors influencing the individual behavior of wolves in relation to anthropogenic features. *Biol. Conserv.* **2020**, *244*, 108514. [CrossRef]
17. Silva, M. Rastreio de Parasitas Gastrointestinais, Pulmonares, Cutâneos e Musculares em Canídeos Domésticos e Silvestres no Norte de Portugal. Master's Thesis, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, 2010.
18. Guerra, D. The Sylvatic and Synanthropic Cycles of *Echinococcus* spp., *Taenia* spp. and *Toxocara* spp. in Portugal: Coprologic and Molecular Diagnosis in Canids. Master's Thesis, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, 2012.
19. Silva, C. Rastreio de Parasitas Gastrointestinais e Pulmonares de Canídeos Domésticos e Silvestres no Distrito de Vila Real, Portugal. Master's Thesis, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, 2018.
20. Guerra, D.; Armua-Fernandez, M.T.; Silva, M.; Bravo, I.; Deplazes, P.; Madeira de Carvalho, M.L. Taeniid species of the Iberian Wolf (*Canis lupus signatus*) in Portugal with special focus on *Echinococcus* spp. *Int. J. Parasitol: Parasites Wildl.* **2013**, *2*, 50–53. [CrossRef]
21. Figueiredo, A.; Oliveira, L.; Madeira de Carvalho, L.; Fonseca, C.; Torres, R.T. Parasite species of the endangered Iberian wolf (*Canis lupus signatus*) and a sympatric widespread carnivore. *Int. J. Parasitol. Parasites Wildl.* **2016**, *5*, 164–167. [CrossRef]
22. Pereira, A. Study of Gastrointestinal Parasites in the Iberian Wolf. Master's Thesis, Vasco da Gama University School, Coimbra, Portugal, 2015.
23. Nunes, I. Assessing Gastrointestinal Parasites of Iberian Wolves in Northwestern Spain through Environmental Fecal Samples. Master's Thesis, Vasco da Gama University School, Coimbra, Portugal, 2017.

24. Domínguez, G.; De La Torre, A.J. Aportaciones al conocimiento de los endoparasitos del lobo Ibérico (*Canis lupus signatus* Cabrera, 1907) en el norte de Burgos. *Galemys.* **2002**, *14*.
25. Muñoz, S.; Ramos, P.L.; Carretón, E.; Diosdado, A.; González-Miguel, J.; Simón, F.; García, R.M. Intestinal Helminths in Iberian Wolf (from Northwest Spain). *Open Parasitol. J.* **2018**, *6*, 106–111. [CrossRef]
26. Pereira, A.L.; Mateus, T.L.; Llaneza, L.; Vieira-Pinto, M.M.; Madeira de Carvalho, L.M. Gastrointestinal helminths on the Iberian Wolf populations from northern Spain—A general view. In Proceedings of the 18th International Conference Life Sciences for Sustainable Development, Cluj-Napoca, Romania, 26 September 2019; p. 192.
27. Mateus, T.; Llaneza, L.; Ribeiro, J.N.; Vieira-Pinto, M.M. Diversidade e prevalencia de helmintes intestinais em fezes de lobo ibérico no Norte de Espanha-dados preliminares. In Proceedings of the III Iberian Wolf Congress, Lugo, Spain, 23–25 November 2012.
28. Vieira-Pinto, M.M.; Llaneza, L.; Neves, J.; Mateus, T.L. Diversity and intensity of environmental contamination with gastrointestinal parasites of wolves in Northern Spain. In Proceedings of the 11th Symposium on Wild Fauna, Viterbo, Italy, 25–28 September 2019.
29. Pereira, A.L.; Mateus, T.L.; Llaneza, L.; Duarte, S.C. *Giardia* sp. and *Crysptosporidium* sp. in Iberian Wolf. *J. Hellenic Vet. Med. Soc.* **2019**, *70*, 1579–1582. [CrossRef]
30. Mateus, T.L.; Castro, A.; Ribeiro, J.R.; Vieira-Pinto, M. Multiple Zoonotic Parasites Identified in Dog Feces Collected in Ponte de Lima, Portugal—A Potential Threat to Human Health. *Int. J. Environ. Res. Public Health* **2014**, *11*, 9050–9067. [CrossRef]
31. Montenegro, H.; Martins, A.I.; Melo, L.; Covas, A.; Carolino, N.; Cortes, H.; Brandão, R.; Santos, N.; Nakamura, M.; Rio-Maior, H.; et al. Diversidade de parasitas gastrointestinais em carnívoros silvestres de Portugal—O Caso de *Vulpes vulpes silacea*. In Proceedings of the XI Congreso Ibérico Sobre Recursos Genéticos Animales, SERGA—Sociedad Española para los Recursos Genéticos Animales, Murcia, Spain, 27–28 September 2018.
32. Silva, V.; Silva, J.; Gonçalves, M.; Brandão, C.; Vieira e Brito, N. Epidemiological survey on intestinal helminths of stray dogs in Guimarães, Portugal. *J. Parasit. Dis.* **2020**, *44*, 869–876. [CrossRef] [PubMed]
33. Remesar, S.; García-Dios, D.; Clabuig, N.; Prieto, A.; Díaz-Cao, J.M.; López-Lorenzo, G.; López, C.; Fernández, G.; Morrondo, P.; Panadero, R.; et al. Cardiorespiratory nematodes and co-infections with gastrointestinal parasites in new arrivals at dog and cat shelters in North-Western Spain. *Transbound. Emerg. Dis.* **2022**, *69*, e3141–e3153. [CrossRef] [PubMed]
34. Benito, A.; Carmena, D.; Postigo, I.; Estibalez, J.J.; Martinez, J.; Guisantes, J.A. Intestinal helminths in dogs in Alava, North of Spain. *Rev. Ibérica De Parasitol.* **2003**, *63*, 121–126.
35. Pereira, A.L.; Silva, P.A.; Rinaldi, L.; Mateus, T.L.; Vieira-Pinto, M.M.; Madeira de Carvalho, L. A Red Flag for free-range wild Iberian ugulates: Gastrointestinal parasites in red foxes (*Vulpes vulpes*) in a Cantabrian ecosystem. In Proceedings of the Book XII Reunião de Ungulados Silvestres Ibéricos, UTAD, Vila Real, Portugal, 1–2 October 2021.
36. Martínez-Rondán, F.J. Helminthfauna of American Mink (*Neovison vison*), the Iberian Wolf (*Canis lupus signatus*) and the Red Fox (*Vulpes vulpes*) in the Northwest of Iberian Peninsula. Ph.D. Thesis, Escuela Internacional de Doctorado, Universidad de Murcia, Murcia, Spain, 2019.
37. Borecka, A.; Gawor, J.; Zięba, F. A survey of intestinal helminths in wild carnivores from the Tatra National Park, southern Poland. *Ann. Parasitol.* **2013**, *59*, 169–172.
38. Bindke, J.D.; Springer, A.; Janecek-Erfurth, E.; Böer, M.; Strube, M. Helminth infections of wild European wolves (*Canis lupus* Linnaeus, 1758) in Lower Saxony, Germany, and comparison to captive wolves. *Parasitol. Res.* **2019**, *118*, 701–706. [CrossRef]
39. Macchioni, F.; Coppola, F.; Furzi, F.; Gabrielli, S.; Baldanti, S.; Boni, C.B.; Felicioli, A. Taeniid cestodes in a wolf pack living in a highly anthropic hilly agro-ecosystem. *Parasite* **2021**, *28*, 10. [CrossRef]
40. Popiolek, M.; Szczesna, J.; Nowak, S.; Myslajek, W.R. Helminth infections in faecal wolves *Canis lupus* L. from the western Beskidy Mountains in southern Poland. *J. Helminthol.* **2007**, *81*, 339–344. [CrossRef]
41. Laborde, E. Etude du Parasitisme Interne des Loups du Parc Alpha Dans le Mercantour. Ph.D. Thesis, Ecole Nationale Vétérinaire, Toulouse, France, 2008.
42. Bagrade, G.; Kirjušina, M.; Vismanis, K.; Ozolinš, J. Helminths parasites of the wolf *Canis lupus* from Latvia. *J. Helminthol.* **2009**, *83*, 63–68. [CrossRef]
43. Diakou, A.; Karaiosif, R.; Petridou, M.; Iliopoulos, Y. Endoparasites of the wolf (*Canis lupus*) n Central Greece. In Proceedings of the Conference EWDA 2014—11th European Wildlife Disease Association Conference, Edinburg, Scotland, 25–29 August 2014.
44. Varodi, E.I.; Malega, A.M.; Kuzmin, Y.I.; Kornyushin, V.V. Helminths of wild predatory Mammals of Ukraine. *Vestn. Zool.* **2017**, *51*, 187–202. [CrossRef]
45. De Macedo, M.R.P.; Zanet, S.; Bruno, S.; Tolosano, A.; Marucco, F.; Rossi, L.; Muller, G.; Ferroglio, E. Gastrointestinal helminths of wolves (*Canis lupus* Linnaeus, 1758) in Piedmont, north-western Italy. *J. Helminthol.* **2019**, *94*, 1–6.
46. Moks, E.; Jõgisalu, I.; Saarma, U.; Talvik, H.; Järvis, T.; Valdmann, H. Helminthologic survey of the wolf (*Canis lupus*) in Estonia, with an emphasis on *Echinococcus granulosus*. *J. Wildl. Dis.* **2006**, *42*, 359–365. [CrossRef] [PubMed]
47. Szczęsna, J.; Popiolek, M.; Wojciech, S. A study of the helminthfauna of wolves (*Canis lupus* L.) in the Bieszczady Mountains (south Poland)—Preliminary results. *Wiad Parazytol.* **2007**, *53*.
48. Ćirović, D.; Pavlović, I.; Penezić, A. Intestinal Helminth Parasites of the Grey Wolf (*Canis lupus* L.) in Serbia. *Acta Vet. Hung.* **2015**, *63*, 189–198. [CrossRef] [PubMed]

49. Al- Sabi, M.N.S.; Rääf, L.; Osterman-Lind, E.; Uhlhorn, H.; Kapel, C.M.O. Gastrointestinal helminths of gray wolves (*Canis lupus lupus*) from Sweden. *Parasitol Res.* **2018**, *117*, 1891–1898. [CrossRef] [PubMed]
50. Gori, F.; Armua-Fernandez, M.T.; Milanesi, P.; Serafini, M.; Magi, M.; Deplazes, P.; Macchioni, F. The occurrence of taeniids of wolves in Linguria (northern Italy). *Int. J. Parasitol. Parasites Wildl.* **2015**, *4*, 252–255. [CrossRef]
51. Poglayen, G.; Gori, F.; Morandi, B.; Galuppi, R.; Fabbri, E.; Caniglia, R.; Milanesi, P.; Galaverni, M.; Randi, E.; Marchesi, B.; et al. Italian wolves (*Canis lupus italicus* Altobello, 1921) and molecular detection of taeniids in the Foreste Casentinesi National Park, Northern Italian Apennines. *Int. J. Parasitol. Parasites Wildl.* **2017**, *6*, 1–7. [CrossRef]
52. Martínek, K.; Kolárová, L.; Hapl, E.; Literák, I.; Uhrin, M. *Echinococcus multilocularis* in European wolves (*Canis lupus*). *Parasitol Res.* **2001**, *87*, 838–839.
53. Segovia, M.J.; Torres, J.; Miquel, J.; Llaneza, L.; Feliu, C. Helminths in the wolf, *Canis lupus* from north-western Spain. *J. Helminthol.* **2001**, *75*, 183–192.
54. Torres, J.; Pérez, M.J.; Segovia, J.M.; Miquel, J. Utilidad de la coprologia parasitaria en la detección de helmintos parásitos en los cánidos silvestres ibéricos. *Galemys: Boletín Inf. De La Soc. Española Para La Conserv. Y Estud. De Los Mamíferos* **2001**, *13*, 75–83.
55. Mathis, A.; Deplazes, P.; Eckert, J. An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. *J. Helminthol* **1996**, *70*, 219–222. [CrossRef]
56. Štefanić, S.; Shaikenov, B.S.; Deplazes, P.; Dinkel, A.; Togerson, P.R.; Mathis, A. Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* ("sheep strain") in naturally infected dogs. *Parasitol. Res.* **2004**, *92*, 347–351. [CrossRef] [PubMed]
57. Shimalov, V.V.; Shimalov, V.T. Helminth fauna of the wolf (*Canis lupus* Linnaeus, 1758) in Bielorrussian Polesie. *Parasitol Res.* **2000**, *86*, 163–164. [CrossRef] [PubMed]
58. Čabanová, V.; Guimaraes, N.; Hurníková, Z.; Chovancová, G.; Urban, P.; Miterpáková, M. Endoparasites of the grey wolf (*Canis lupus*) in protected areas of Slovakia. *Ann. Parasitol.* **2017**, *63*, 283–289.
59. Ambrogi, C.; Ragagli, C.; Decaro, N.; Ferroglio, E.; Mencucci, M.; Apollonio, M.; Mannelli, A. Health survey on the wolf population in Tuscany, Italy. *Hystrix It. J. Mamm.* **2019**, *30*, 19–23.
60. Balmori, A.; Rico, M.; Naves, J.; Llamazares, E. Contribución al etudio de los endoparásitos del lobo en la Paenínsula Ibérica: Una investigación coprológica. *Galemys* **2000**, *12*, 13–26.
61. Baños, D.P.; Baños, D.P.; Pelayo, M. Nematodosis: Toxocarosis, Toxascariosis, Ancilostomatidosis, Tricuriosis, Estrongiloidosis, Espirocercosis y Olulanosis. In *Parasitología Veterinaria, 3rd ed*; McGraw-Hill-Interamericana: Madrid, Spain, 2002; pp. 615–651.
62. Traversa, D. Are we paying too much attention to cardio-pulmonary nematodes and neglecting old-fashioned worms like *Trichuris vulpis*. *Parasites Vectors* **2011**, *4*, 32. [CrossRef] [PubMed]
63. Dunn, J.J.; Columbus, S.T.; Aldeen, W.E.; Davis, M.; Carrol, K.C. *Trichuris vulpis* recovered from a patient with chronic diarrhea and five dogs. *J. Clin Microbiol.* **2002**, *40*, 2703–2704. [CrossRef]
64. Sobrino, R.; Gonzalez, L.M.; Vicente, J.; Fernández de Luco, D.; Garate, T.; Gostázar, C. *Echinococcus granulosus* (Cestoda, Taeniidae) in the Iberian Wolf. *Parasitol. Res.* **2006**, *99*, 753. [CrossRef]