Escola Universitária Vasco da Gama

VETERINARY MEDICINE MASTER´S DEGREE

Study of Gastrointestinal Parasites in the Iberian Wolf (*Canis lupus signatus*)

Ana Luísa Pereira

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Author

Ana Luísa Pereira

Scientific Supervisors

Sofia Cancela Duarte, DVM PhD

Teresa Letra Mateus, DVM MSc

(Escola Universitária Vasco da Gama, Coimbra, Portugal)

Dr. Brett Gardner, DVM

(Johannesburg Zoo, Johannesburg, South Africa)

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Abstract

Iberian wolf (*Canis lupus signatus*), is the largest carnivore in Iberian Peninsula and is threatened with extinction.

The aim of this study was to contribute to the knowledge of helminthic and protozoa fauna, in the wolf populations from the North of Spain, with the perspective to evaluate the health and surveillance of the diseases of these packs.

One hundred and seventy seven samples of faeces of *Canis lupus signatus*, were collected from the environment in the North of Spain, between 2013 and 2014 and submitted to coprological methods. Helminth eggs were detected on 101 (57.0 %), and simple infections were more frequent with a prevalence of 53.4 % comparing with multiple infections. In the overall samples, eggs from *Capillaria* spp. (41.5 %) were the most frequently detected, followed by *Toxocara* spp. (40.5 %), Ancylostomatidae (29.7 %), Taeniidae (26.7 %) and *Trichuris* spp. (25.7 %). Results are discussed and compared with earlier published studies, especially to check the present status of helminthic infections in the wolf populations in that region.

Additionally a subsample was analysed using a immunofluorescent assay (IFA) to identify the presence of *Giardia* spp. and *Cryptosporidium* spp. Fifty faecal samples were analysed, of which ten (20 %) were positive. Simple infections were more frequent, with seven (14 %) samples positive for *Giardia* spp. and two (4 %) positive for *Cryptosporidium* spp. Multiple infections with both protozoan species were found in just one (2 %) sample. Faecal samples, even frozen, revealed to be a valuable tool, to detect and identify helminthic and protozoa infections from wolves in the scope of surveillance programs.

Keywords: *Cryptosporidium*; *Giardia*; Helminths; Parasites; Spain; Wolf; Zoonoses

Resumo

O Lobo Ibérico (Canis lupus signatus) é o maior carnívoro da Península Ibérica e encontra-se ameaçado de extinção.

O presente estudo, tem como objetivo, contribuir para o estudo e conhecimento da fauna helmíntica e protozoária (nomeadamente *Giardia* spp. e *Criptosporidium* spp.) nas populações de lobos do Norte de Espanha, com a perspetiva de avaliar o estado sanitário destas populações.

Cento e setenta e sete amostras fecais de *Canis lupus signatus* foram recolhidas do meio ambiente entre os anos de 2013 e 2014 e foram analisadas usando métodos de coprologia. Foi detetada a presença de ovos de helmintes em 101 (57,0 %), e foi mais frequente a presença de infeções simples (53,4 %) comparativamente com infeções múltiplas. No total de animais infetados, incluindo infeções múltiplas, os ovos de *Capillaria* spp. (41,5 %) foram os mais frequentes, seguidos por *Toxocara* spp. (40,5 %), Ancylostomatidae (29,7 %), Taeniidae (26,7 %) e *Trichuris* spp. (25, 7%). Os resultados foram comparados e discutidos com estudos anteriores. Adicionalmente uma sub-amostra foi analisada para determinar a presença de *Giardia* spp. e *Cryptosporidium* spp., usando um teste comercial de imunofluorescência (IFA). Cinquenta amostras foram analisadas e dez (20 %) foram positivas ao teste. Foi mais frequente a presença de infeções simples causadas por *Giardia* spp., sete (10 %) e duas (4 %) positivas para *Cryptosporidium* spp. e infeções múltiplas em apenas umas das amostras (2 %). A utilização de amostras fecais congeladas, revelou-se uma ferramenta útil, para deteção e identificação de infeções por helmintes gastrointestinais e protozoários, em lobos selvagens "*in- vivo*" e em risco de extinção, no âmbito da vigilância epidemiológica.

Palavras-Chave: *Cryptosporidium*; Espanha; *Giardia*; Helmintes; Lobo; Parasitas; Zoonoses

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" Una persona puede cambiarlo todo: de cara, de casa, de familia, de novio, de religión… de Dios…Pero hay una cosa que no se puede cambiar… La Pasión…"

" El SECRETO DE SUS OJOS"

By Juan José Campanella

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List of abbreviations

d. 1.2- density of 1.2

HCl- hydrochloric acid

IFA- immunofluorescent assay

NaOH- sodium hydroxide

PBS- phosphate-buffered saline

RR2- probed-antibody

RMG- mounting medium

1. Introduction

More than ever, the relation between humans/ livestock and wildlife is increasing and leading to the so called “spill-over” and “spill-back” events, with implications in veterinary public health (Thompson, 2013; Carmona and Cardona, 2014) and also in nature conservation (Young *et al.*, 2014). Infectious agents can affect conservation strategies, given the threat that represent for the endangered wildlife species, by causing population declines, and menacing reintroduction programs (Almberg *et al.*, 2012). Parasites on sentinel animals can also be effective indicators of ecosystem integrity and human interference (Aguirre, 2009).

Many aetiological agents can infect multiple host species, being one of the major causes of emerging diseases if environmental changes occur (Woolhouse, 2002), and these possibilities are just unknown (Gortázar *et al*. 2007). In that context, human helminthic and protozoan parasites, especially of zoonotic nature, represent very important costs, namely because the morbidity, mortality and negative effects on the economy that embody (Chomel *et al.*, 2007; Thompson *et al.*, 2010; Gazzinelli *et al.*, 2012; Parsons *et al.*, 2014).

Zoonoses represent three quarters of the human emerging diseases (Magwedere *et al.*, 2012), being wildlife parasites the major source of zoonotic emerging pathogens among humans (Taylor *et al.*, 2001). Parasites have further impact on livestock, food security and economic costs through their deleterious effects (Chomel *et al.*, 2007).

Based on the “One Health” approach various studies have been carried out, around the world, in wildlife, demonstrating the increasing importance of research in all perspectives, as follows: 1) The importance of maintaining the biodiversity; 2) “Spill-over” and “Spill-back” of diseases; 3) “One Health” as a conservation target; 4) Health of humans and non-humans.

The Iberian Wolf (*Canis lupus signatus*), one of the major wildlife predator in the Iberian Peninsula, is a currently endangered species, carrying a cultural and historical stigma that contributed to its decline. In human-dominated landscapes the occurrence of wolves is the result of a complex interaction among several environmental and human factors (Llaneza *et al.*, 2011). It is recognized that wolves can be a reservoir of many zoonotic diseases (Torres *et al.*, 2000). On the other hand, humans and/ or livestock can be a risk for wolves, as the previously described human transmission of *Mycobacterium tuberculosis* to suricats and elephants (Chomel *et al.*, 2007), and *Giardia* spp. to wolves, beavers and gorilas (Thompson, 2013). Wolves have a great capacity to adapt to new and different environments, talking full advantage of all available food, depending of the prey population (wild and/ or domestic animals) (Dominguez and De La Torre, 2002).

Like in humans and other species (Degiorgis-Ryser, 2012) the presence of parasitic infections in wolves, can cause high levels of morbidity, mortality and impacts on reproduction, leading to dramatic impacts in small populations (Almberg *et al.*, 2012).

Wildlife parasitic infection control begins with surveillance schemes to identify the occurrence of parasites/ diseases (Gotázar *et al.*, 2007). Disease surveillance is an important element of recovery plans for rare and endangered species (Thompson *et al.*, 2010). In a reintroduction program of wolves

in the Yellowstone Park it was observed a marked vulnerability of the introduced animals when in contact with endemic aetiological agents, probably with origin in local coyotes (*Canis latrans*). Wildlife disease surveillance is also a valuable tool to monitor the health status of the population (Domingez and De La Torre, 2002) and to detect, at an early stage, entries of new agents on the environment (Thompson *et al.*, 2010; Thompson, 2013).

The aim of this study was to evaluate the occurrence and diversity of gastrointestinal parasites namely helminths and protozoa in faecal samples of Iberian Wolf collected in the North of Spain, as part of a large-scale surveillance and protection project. The obtained results were discussed and compared with previous studies. To the authors' knowledge, this is the first report of occurrence of *Giardia* spp. and *Cryptosporidium* spp. in the Iberian Wolf.

2. Material & Methods

2.1. Sampling

The faecal samples were collected in the North of Spain, in the different transects of wolves packs, as part of a large-scale surveillance and protection project.

Given the nature of the sampling procedure no information was available regarding the sex or age of the animals. Differentiation of the wolf´s faeces from, namely dog faeces, was achieved by indirect signs such as the composition/ morphology of faeces (shape, size, and contents like hair and pieces of bones with a characteristic odour), typical localization, and ground scratch marks (Harris and Ream, 1983, *cit*. by Llaneza *et al.*, 2011).

Samples were collected in the summer (May, June, July and August) and winter (September and October) seasons of two consecutive years (2013 and 2014). The 177 faecal samples collected were frozen at -20 ºC, and maintained in this condition until analysis.

2.2. Conventional coprological analysis

For qualitative coprological analysis, flotation (Benchop) and natural sedimentation qualitative methods were used in each sample, as previously described (Zajac and Conboy, 2012).

Briefly, in the floatation method saturated sodium chloride solution (d. 1.2) was mixed with the faeces and both were passed through a sieve. The tubes were filled until formation of a meniscus and a coverslip was placed on the top of the tube. After 10-15 minutes, the coverslip was transferred to a glass slide and examined by conventional microscopy (Zajac and Conboy, 2012).

For the natural sedimentation method (Zajac and Conboy, 2012), the faecal sample was mixed with tap water in a container and left to rest for approximately one hour after which the supernatant was decanted. These procedures were repeated until the supernatant appeared clean. One drop of sediment was taken and placed on a microscope slide along with a drop of 0.1 % methylene blue (Panreac ®, Barcelona, Spain), to stain the eggs with a yellowish brown colour.

In the coprological assays, the eggs were identified based on morphological characteristics (shape and structure), as described by Zajac and Conboy (2012). A sample was considered contaminated by the presence of a parasitic form, regardless the method used.

2.3. Immunofluorescence assay

Of the 177 samples analysed, 50 faecal specimens were randomly selected to determine the presence of *Cryptosporidium* oocysts and *Giardia* cysts. With that purpose a commercial direct immunofluorescence assay (Cellabs® Pty Ltd, Brookvale, Australia) was used. Faeces (50 µL or 5 mm diameter) were diluted (1:10) with phosphate-buffered saline (PBS). PBS was prepared by diluting 0.2 g potassium chloride, 0.2 g potassium dihydrogenphosphate, 1.2 g anhydrous disodium hydrogen phosphate and 8 g sodium chloride in 1 L of distilled water, with pH adjusted to 7.4 through the use of 1 M HCl and 0.1 M NaOH. In a microscope slide, 20 µL of the faecal specimen was placed and allowed to completely air dry. The slides were fixed for five minutes in acetone and allowed to air dry. The probed-antibody (RR2; 25 µL) was added to the fixed specimen and positive control, covering all area. The slides were incubated at 37 ºC in a humid chamber for 30 minutes, and then rinsed gently in a bath of PBS for one minute. The slide was drained and the excess moisture around well was removed with tissue. A drop of mounting fluid (RMG) was added to the slide well, and a coverslip was placed on top of the drop and the air bubbles were removed. The entire specimen was immediately scanned using a fluorescence microscope.

Cryptosporidium oocysts (2-6 µm in size) appeared with a round or oval shape with bright green fluorescence. A fold or suture could be seen on the surface. *Giardia* cysts appeared elliptical in shape, with bright green fluorescence. The test was considered positive if one or more oocysts and cysts were present.

3. Results

From the 177 samples analysed, 101 (57.0 %) were found to be positive while in the remaining 76 (42.9 %) no helminth eggs were found with the coprological methods employed.

Through the Benchtop flotation technique, 57 (32.2 %) of the faecal samples were considered positive while, through natural sedimentation technique, 89 (50.2 %) of the samples were determined positive. Positive results in both techniques were only observed in 34 (33.6 %) of the analysed samples.

Considering both simple and multiple infections, *Capillaria* spp. was the most frequently observed helminth (41.5 %), followed by *Toxocara* spp. (40.5 %), Ancylostomatidae (29.7 %), Taeniidae (26.7 %) and *Trichuris* spp. (25.7 %).

Regarding the pattern of infection, simple infections (Table 1) were more prevalent (53.4 %) when compared with multiple infections, whether with two- (33.6 %), three- (8.9 %) or more than three-helminth (3.9 %) detected.

Table 1- Prevalence (%) results of simple infections (n=54) in each of the coprological methods used.

	Flotation Positive Sedimentation Positive n (%)	Flotation Positive Sedimentation Negative n (%)	Flotation Negative Sedimentation Positive n (%)	**Total n (%)**
Ancylostomatidae	1 (1.8 %)	3 (6 %)	5 (9.2 %)	**9 (16.6 %)**
Toxocara spp.	6 (11.1 %)	2 (3.7 %)	12 (22.2 %)	**20 (37 %)**
Taeniidae	2 (3.7 %)	2 (3.7 %)	4 (7.4 %)	**8 (14.8 %)**
Capillaria spp.	6 (11.1 %)	1 (1.8 %)	8 (14.8 %)	**15 (27.7 %)**
Trichuris spp.	0	0	2 (3.7 %)	**2 (3.7 %)**
Total	**15 (27.7 %)**	**8 (14.8 %)**	**31 (57.4 %)**	**54 (100 %)**

Similarly to helminth parasites, protozoan simple infections were more frequent (90 %), with seven (14 %) samples positive for *Giardia* spp., and two (4 %) samples positive for *Cryptosporidium* spp. Of the fifty samples analysed, only ten (20 %) were positive for both *Giardia* spp. and *Cryptosporidium* spp.

4. Discussion

The present study had some limitations, firstly because the dataset was a convenience sample. Secondly, it was not possible to determine if the samples were fresh, given that they were collected from the soil. Samples were collected following transects and snowtracks of the packs and then the faeces were put on bags and frozen until analysis. Recent studies showed that samples with low egg counts prior to freezing were especially prone to false negative results after thawing. A possible explanation is the rupture or damage of large numbers of eggs which compromise its identification, and thus decrease the diagnostic value of the sample (Schurer *et al.*, 2014). Nevertheless coprology of field collected faecal samples with freezing procedures is the best alternative when working with wild and endangered species, difficult to find and from remote places (Schurer *et al.*, 2014). Furthermore, the collection is safer, easier and can also provide dynamic information in short- and medium-term surveys. Finally, specimen freezing is a logistically easier alternative to preserve a large number of samples (Torres *et al.*, 2000; Abdybekova and Torgerson, 2012; Schurer *et al.*, 2014).

Another limitation concerning to the present study, is the unavailability of georeferencing data of the faecal specimens to ascertain the impact on the public health/ livestock and environment in that region.

Coprology is a non-invasive procedure, however it can compromise the quantification and identification of parasite forms. Furthermore, low levels of infection as well as patent infections or irregular elimination of eggs that may not be detected through coprology can also compromise the sensibility of the method (Torres *et al.*, 2001). Finally, coprology allows the detection of only respiratory and gastrointestinal parasites (Balmori *et al.*, 2000). Necropsy can overcome some limitations of the coprology technique, as reviewed by Torres *et al.* (2001). Through necropsy it is possible to visualise the host, i.e. knowledge of the age, gender, body condition among others is

available (Segovia *et al.*, 2001). Although necropsy renders the possibility of both identify, in more detail, and quantify, it is difficult to get carcasses and furthermore to preserve them until analysis (Torres *et al.*, 2001). Accordingly, in the literature review of Craig and Craig (2005) roughly 93 % of helminthic identifications in published studies of wolves were made by necropsy, proving to be a more sensitive method comparing with coprology. Studies on the occurrence of wolf parasites through coprology are thus scarce. The occurrence and diversity of gastrointestinal parasites in wolves reported, through coprological techniques and necropsy are presented in Table 2 and Table 3, respectively, regarding only the parasites found in the present study.

The results of the present study suggest that the coprological methods employed (Flotation and Sedimentation) are complementary, despite sedimentation retrieved more positive results. Already previously (Szczesna and Popiolek, 2007) sedimentation technique was described as more sensitive.

Comparing with previous studies, all of which with a smaller number of samples, it is of notice that the present study featured lower overall prevalence (57.0 %), for instance in comparison with Spain (72 %-100 %; Dominguez and De La Torre, 2000; Torres *et al.*, 2000; Segovia *et al.*, 2001), Belarus (80 %; Shimalov and Shimalov, 2001), Poland (27.8 %-78.6%; Popiolek *et al.* 2007; Szafranska *et al.*, 2010; Borecka *et al.*, 2013), and Ethiopia (70,2 %; van Kesteren *et al.*, 2014).

It is generally accepted, that surveys carried out through coprological methods in wild canids, provide acceptable results as most of the wolf´s endoparasites are detectable with coprological methods (Popiolek *et al.*, 2007).

According to the results of the present study*, Capillaria* spp. was the most prevalent parasite among the analysed wolf samples with a relatively high frequency (41.5 %). These parasites have only been reported in Spain by Balmori *et al.* (2000). At least four species of *Capillaria* can infect canids: *C. plica* (bladder), *C. hepatica* (liver), *C. boehmi* (nasal cavities and sinuses) and *C. aerophila* (respiratory tract). Given their corresponding location, none of the eggs of this genus are visible in faeces (van Kesteren *et al*., 2014), except from *C. aerophila* in highly infected animals (Taylor *et al*., 2007). Thus a possible explanation for the presence of *Capillaria* spp. eggs in the faeces of wolf is the hunted preys (van Kesteren *et al.*, 2014), as *Capillaria* spp. is resistant and thus can cross the intestinal tract intact.

Toxocara spp. it is a zoonotic parasite, and can cause ocular and visceral larva *migran*s in humans (Jenkins *et al.,* 2011).The high prevalence of *Toxocara* spp. can compromise the growth of the young cubs. These parasites have a vertical transmission (placenta or milk), which increase the number of infected animals in a population. They also can get infected, by the ingestion of eggs present in the environment (Baños *et al*., 2002), or in the hunted preys (e.g. dogs). The presence of *Toxocara* spp. is suggestive of the presence of juveniles in the pack (Torres *et al.*, 2000). Comparing with other studies, coprological methods can offer results of *Toxocara* spp. occurrence very close to the real infection (Torres *et al.,* 2001), although it was observed that freezing procedures caused damage in the ascarid eggs (Schurer *et al.,* 2014).

Table 2- Prevalence (%) and diversity of gastrointestinal helminthic parasites in wolves through coprology.

		Portugal	**Spain**	**Poland**	**Poland**	**Poland**	**Portugal**	**Ethiopia**	**Spain**
Cestodes	Taeniidae	15.4 %	9.4 %	11.2 %	----------	1.4 %	23.5 %	4.3 %	26.7 %
Nematodes	Ancylostomatidae	57.7 %	29.7 %	*A.caninum*:12.3 % *U. stenocephala*: 37 %	*A. caninum*: 35.9 %	5.6 %	----------	22.3 %	29.7 %
	Toxocaridae	38.5 %	9.4 %	5.6 %	3.9 %	6.9 %	----------	14.9 %	40.5 %
	Capillaridae		----------	14.6 %	23.1 %	----------	----------	52.1 %	41.5 %
	Trichuridae	11.5 %	28.1 %	----------	15.5 %	13.9 %	----------	22.3 %	25.7 %
Animal species (n)		Wolves (26)	Wolves (19) Fox (45)	Wolves (89)	Wolves (103)	Wolves (72)	Wolves (68)	Wolves (94)	Wolves (177)
Reference		Torres *et al.* (2000)	Torres *et al.* (2001)	Popiolek *et al.* (2007)	Szafranska *et al.* (2010)	Borecka *et al.* (2013)	Guerra *et al.* (2013)	van Kesteren *et al.* (2014)	Present study

Table 3- Prevalence (%) and diversity of gastrointestinal helminthic parasites in wolves through necropsy.

	Country	**Belarus**	**Spain**	**Spain**	**Spain**	**Kazakhstan**
Cestodes	Taeniidae	[5-26.9 %]	95 %	*T. hydatigena*: 44.7 % *T. multiceps*: 29.8 % *T. serialis*: 2.1 %	46.8 %	*E. granulosus*: 36 % *Taenia* spp.: 31.7 %
Nematodes	Ancylostomatidae	*A. caninum*: 13.5 % *U. stenocephala*: 15.4 %	55 %	*A. caninum*: 8.5 % *U. stenocephala*: 51.1 %	62.5 %	
	Toxocaridae	21.2 %	5 %	6.4 %	42.2 %	39 %
	Capillaridae	13.5 %				
	Trichuridae	3.9 %	10 %	10.6 %	31.2 %	22 %
Animal species (n)		Wolves (52)	Wolves (20)	Wolves (47)	Wolves (19) Fox (45)	Wolves (41)
Reference		Shimalov and Shimalov. (2000)	Torres *et al.* (2000)	Segovia *et al.* (2001)	Torres *et al.* (2001)	Abdybekova and Togerson (2012)

The family Ancylostomatidae has two species of veterinary concern: *A. caninum* and *U. stenocephala*. The results were given at the family level given the difficulty to distinguish some related helminth species just through the egg morphology (Balmori *et al.*, 2000; Torres *et al.*, 2000). The herein determined prevalence of *Ancylostoma* spp. was very similar to the one described by Torres *et al.* (2001). *Ancylostoma caninum* is a zoonotic parasite and can cause cutaneous larva *migrans* (Baños *et al*., 2002), although only occasionally humans can be infected and become the final host (Taylor *et al.*, 2007). *A. caninum* is more frequently transmitted by milk (Segovia *et al*., 2001), although horizontal transmission can occur (e.g. percutaneous or oral transmission by ingestion of third-stage larvae from the environment; Taylor *et al.,* 2007). Both species (*A. caninum* and *U. stenocephala*) could be fatal for the young cubs. The lower prevalence determined in the present study, comparatively to the one reported by Torres *et al.* (2001), can be explained by the high sensitivity of the eggs to the freezing stage, which could result in false negatives (Schurer *et al*., 2014).

By coprology is not possible to make the distinction between different species of *Taenia* neither to distinguish the later from *Echinococcus* spp. (Balmori *et al.*, 2000; Torres *et al.*, 2001). Furthermore it is difficult to detect Taeniidae eggs and predict the real flow on the packs. The possibility of underestimation cannot be excluded because these parasites have irregular elimination of pregnant proglotides to the environment. In addition, the possibility to be shed intact to the environment it is high and that makes impossible the detection of eggs. The freezing procedure for Taeniidae eggs was not a problem as showed previously by Schurer *et al.* (2014), according to whom, Taeniidae eggs were relatively unaffected by freezing.

Although coprological methods are suitable to detect *Trichuris* eggs, identification at the species level is hindered by the resemblance between *Trichuris* eggs from same genera but with origin on hunted preys (Torres *et al.*, 2001). Infection can occur in wolves by ingestion of eggs from the soil and water, because these eggs have a high resistance to environmental conditions and can remain infective for many years in temperate climates (Baños et al., 2002). *Trichuris* infection in humans, are rare (Zajac and Conboy, 2011).

Besides helminths, the analysed faecal specimens were contaminated with protozoan of *Giardia* spp. and *Cryptosporidium* spp. These protozoan parasites have already been reported in wolves in Poland by Kloch *et al.* (2005) with a high prevalence (45.5 % for *Giardia* spp. and 54 % for *Cryptosporidium* spp.) and by Paziewska *et al.* (2007) regarding *Cryptosporidium* spp. only (37.5 %). In Canada (Stronen *et al.,* 2011) the protozoan were also detected with a prevalence of a 29.5 % for *Giardia* spp. and 1.2 % for *Cryptosporidium* spp.

To the authors' knowledge, no studies were carried out to detect the presence of *Cryptosporidium* oocysts and *Giardia* cysts in the wolf packs of Spain. For that reason, discussion and comparison were hindered. It is of notice that these agents could belong to zoonotic species and can cause mortality in some parts of the world, among humans and non-humans. Cysts of *Giardia* spp. and oocysts of *Cryptosporidium* spp. (infective stages) can feature a high faecal excretion to the environment and can remain on the ground surface or soil even after the faeces were decomposed.

As such accidental infection of humans, livestock or companion animals can ensue, especially *Giardia duodenalis* which has been reported in many mammals, including humans (Ryan and Cacció, 2013). On the other hand, *Cryptosporidium* spp. has a negative impact in immunocompromised population, being humans infected by many species of *Cryptosporidium* (Plutzer and Karanis, 2009). *Giardia* spp. in canids can cause chronic diarrhoea, weight loss, lethargy and growth retardation (Taylor *et al*., 2007). *Cryptosporidium* spp. is normally asymptomatic in canids, but in immunosuppressed animals can be a cause of chronic diarrhoea (Taylor *et al*., 2007).

5. Conclusions

The non-invasive collection and analysis of frozen faeces samples from living animals can provide, even with simple techniques, valuable information. We can conclude with the present study, that the packs are infected and taking in account that the majority of parasites identified have zoonotic potential (e.g. *Toxocara* spp., Ancylostomatidae., Taeniidae, *Giardia* spp and *Cryptosporidium* spp.) further studies are needed as well as the development of molecular techniques to identify the species of the infectious agents. On the other hand, one of the most prevalent helminthic parasites found, was *Toxocara* spp. which can put in risk the growth of the packs, and for such reasons requires concern. In these landscapes shared with humans, wolves can act like a reservoir for many zoonotic diseases, but are wolves being the victims of humans and livestock?

Vigilance and routine surveillance of wildlife ultimately aiming at the concept of “One health” are needed.

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