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Helminth parasites in faecal samples from the endangered Iberian lynx (*Lynx pardinus*)

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ABSTRACT

The Iberian lynx is the most endangered felid in the world. Enteropathogens may threaten its survival, and therefore we analysed faecal samples from 66 different individuals (37 males and 29 females), the largest population representation studied to date. The samples were obtained from November 2005 to October 2008 in the two areas where the Iberian lynx survives: Sierra Morena and Doñana (Andalusia, southern Spain). A total of 56.1% samples were parasitized with at least 6 species of helminths, including two cestodes (*Hymenolepis* spp. and *Taenia* spp.) and four Nematodes (*Ancylostoma* spp., *Toxocara* spp., *Toxascaris leonina*, and *Capillaria* sp.). In this work, the presence of *Hymenolepis* is reported for the first time in *Lynx pardinus*. The relevance of our findings is discussed focussed on the conservation of this endangered felid.

1. Introduction

According to the World Conservation Union, the Iberian lynx – *Lynx pardinus* (Temminck, 1827) – is the most endangered felid species in the world (IUCN, 2010). The current population is estimated at no more than 200 individuals, distributed in two isolated metapopulations, both in Andalusia, southern Spain: Doñana area (DA), with only about 50 lynxes, and Sierra Morena (SM) with about 150 lynxes (López-Bao et al., 2010).

Endangered populations are markedly sensitive to diseases as a result of a variety of extrinsic and genetic factors (Munson et al., 2005). Among these diseases, helminthoses may play an important role in mortality and morbidity in the population of their hosts, in this case the Iberian lynx (Vicente et al., 2004). Nevertheless, there is limited published information concerning the helminth fauna of *L. pardinus*, due to the difficulty in obtaining samples. In fact, only 13 carcasses (5 from Montes de Toledo, 2 from DA and 6 from SM) of Iberian lynx individuals confiscated from poachers or road kills have been analysed to date (Torres et al., 1998; Millán and Casanova, 2007). According to these authors, the helminths recovered from *L. pardinus* in the Iberian Peninsula include: *Brachylaima* sp. (Yamaguti, 1971) (Trematoda); *Taenia pisiformis* (Bloch, 1780), *T. polyacantha* (Leuckart, 1856), *T. teaniaeformis* (Bloch, 1780), *Mesocestoides litteratus* (Batsch, 1786), *Mesocestoides* sp. and *Joyeuxiella pasqualei* (Diamare, 1893) (Cestoda); and *Eucoleus aerophilus* (Creplin, 1839), *Ancylostoma tubaeforme* (Zeder, 1800), *Toxocara cati* (Schrank, 1788), *Toxascaris leonina* (Von Linstow, 1902), *Vigisospirura potekhina potekhina* (Petrow et Potekhina, 1953), *Mastophorus muris* (Gmelin, 1790), and *Physaloptera praeputialis* (Von Linstow, 1889).

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When carcasses are difficult to obtain, coproparasitological surveys may be a useful alternative in spite of their inconveniences related to the difficulty in making certain specific adscriptions of the materials or when ecological studies are needed. In this sense, only one scientific paper reported data on egg excretion by nine individuals of lynxes originating from SM (Rodriguez and Carbonell, 1998), namely *T. pisiformis* and *Taenia* spp. (Cestoda); and *Capillaria aerophila*, *Ancylostoma* spp., *T. cati*, *T. canis* (Werner, 1782), *T. leonina* and *Strongyloides stercoralis* (Bavay, 1876) (Nematoda). More recently, Vicente et al. (2004) reported a high prevalence and abundance of *A. tubaeforme* eggs in lynx faeces in Doñana Natural Park.

The aim of the present study is to determine the prevalence of the helminth fauna in the Iberian lynx, considering samples from the two metapopulations and taking into account a significant number of individuals from each geographical area, through a study of eggs and/or larval stages found in faecal samples.

2. Materials and methods

2.1. Study area

The study was carried out in the areas currently inhabited by the two last populations of *L. pardinus*. The Doñana area (37°0′N, 6°30′W) covers 2000 km^2 of land, mostly within Doñana National Park and Doñana Natural Park in the provinces of Cadiz and Huelva, with altitudes ranging from 0 to 47 m above sea level. Sierra Morena (38°13′N, 4°10′W) includes 1125 km^2 in two bordering protected natural parks and several private hunting estates at 500–1300 m above sea level. The climate of both areas is sub-humid Mediterranean with marked seasons.

2.2. Sampling and animals

From November 2005 to October 2008, faecal samples of 66 different individuals of *L. pardinus* were collected, 32 (48%) from Doñana Area and 34 (52%) from Sierra Morena. 37 (56.1%) were males and 29 (43.9%) females, aged 0–12 years (mean 3.17; DT: 2.14 and V: 4.57), 32 juveniles (<2 years) and 34 adults (>2 years).

All the samples studies originated from the Iberian lynx Biological Resource Bank (Leon-Quinto et al., 2009), and the collection was performed under agreement with the Environmental Council of the Regional Government of Andalusia.

The samples were taken from dead as well as live animals. In the latter case, taking advantage of animal manipulation for other reasons (radio-tracking, health check-ups, and ectoparasite fauna studies) (Millán et al., 2007, 2009; Leon-Quinto et al., 2009). Several biological parameters such as age (juveniles < 2 years old; and adults > 2 years old), sex, and geographical origin were considered.

2.3. Methods and techniques

All samples obtained from different lynx individuals were processed at the laboratory within 24 h, and three aliquots were made of each faecal sample: one was preserved in 10% formalin solution (1:3; v:v) at room temperature, one at −80 °C, and one in absolute ethanol (1:1; v:v) at 4 °C. Parasitological studies were carried out at Laboratorio de Parasitologia of Universidad Miguel Hernandez. Samples fixed in 10% formalin were processed using the diphasic technique of formol-ether (Knight et al., 1976). Helminth eggs and larvae were identified by size and morphological characteristics following Khalil et al. (1994) for cestodes and Burrows (1962), Sprent (1968), Warren (1970) and Moravec (1982) for nematodes. Microscopic slides and materials from the parasite collection of the Department of Parasitology at University of Valencia were used for reference checks when needed.

2.4. Statistical analysis

Statistical analysis was carried out using SPSS 16.0 software (SPSS Institute, Chicago, IL). Correlations among variables host origin, sex, categorized age and parasitisation, were calculated by X^2 test and Fisher Exact statistical when necessary. A p value below 0.05 was considered statistically significant.

3. Results

The coprological study revealed that 56.1% of Iberian lynxes were infected, without statistical differences between the two areas (50% DA *vs* 61.8% SM). At least six helminth species were found in the faecal samples (Table 1). Cestodes belonging to the genera *Taenia* and *Hymenolepis* were detected. The first were found solely in SM, while the latter were encountered in either area. Total prevalences observed were lowest among helminths.

However, the greatest diversity was detected among nematodes, covering an identical spectrum in the two areas. At the same time, this group showed the highest parasitation prevalences, ranging from 12.1% to 24.2%. The existence of statistical differences in the prevalence of certain species between both areas, i.e. *Ancylostoma* spp. (43.7% in DA *vs* 5.9% in SM; p 0.000), *Toxocara* spp. (9.4% *vs* 29.4%; $p = 0.041$) and *T. leonina* (3.1% *vs* 29.4%; $p = 0.004$), was remarkable.

Up to three different helminth species were found in the same host, concretely in four individuals of the Iberian lynx. No significant statistical differences between host sex (Table 2) and age group (Table 3) were encountered in prevalence of infection.

4. Discussion

The best way to shed light on the helminth fauna of a host species is by means of a complete analysis of a carcass. Nonetheless, when this proves difficult, as in this particular case since dealing with the world's most endangered felid species, the analysis of faecal samples may be useful to close the knowledge gap concerning its helminth fauna without involving the death of the host species.

The structure of the helminth fauna in the Iberian lynx detected in this study is in agreement with the data so far available on its helminth fauna although based on the

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Table 1
Number of individuals studied (n) and infected (n_i) of the Iberian lynx and helminth species detected with their respective prevalences (%) according to the area and in the total of study.

	n_i (%) Doñana area $n=32$	Sierra Morena $n=34$	Total study $n=66$	p-Value
Cestoda				
Taenia spp.	–	4 (11.8)	4 (6.1)	0.114
Hymenolepis spp	1 (3.1)	1 (2.9)	2 (3.0)	1.000
Nematoda				
Capillariinae gen. sp.	4 (12.5)	4 (11.8)	8 (12.1)	1.000
Ancylostoma spp.	14 (43.7)	2 (5.9)	16 (24.2)	0.000*
Toxocara spp.	3 (9.4)	10 (29.4)	13 (19.7)	0.041*
Toxascaris leonina	1 (3.1)	10 (29.4)	11 (16.7)	0.004*
Total	16 (50.0)	21 (61.8)	37 (56.1)	0.336

* Statistically significant.

Table 2
Number of individuals studied (n) and infected (n_i) of the Iberian lynx and helminth species detected with their respective prevalences (%) by sex according to the area and in the total of study.

	Doñana area			Sierra Morena			Total study	
	n_i (%)		p-Value	n_i (%)		p-Value	n_i (%)	
	Male $n=16$	Female $n=16$		Male $n=21$	Female $n=13$		Male $n=37$	Female $n=29$
Cestoda								
Taenia spp.	–	–		2 (9.5)	2 (15.4)	0.63	2 (5.4)	2 (6.8)
Hymenolepis spp.	1 (6.2)	–	1	1 (4.8)	–	1	2 (5.4)	–
Nematoda								
Capillariinae gen. sp.	3 (18.7)	1 (6.2)	0.60	2 (9.5)	2 (15.4)	0.63	5 (13.5)	3 (10.3)
Ancylostoma spp.	9 (56.2)	5 (31.2)	0.154	1 (4.8)	1 (7.7)	1	10 (27.0)	6 (20.7)
Toxocara spp.	2 (12.5)	1 (6.2)	1	5 (23.8)	5 (38.5)	0.45	7 (18.9)	6 (20.7)
Toxascaris leonina	–	1 (6.2)	1	5 (23.8)	5 (38.5)	0.45	5 (13.5)	6 (20.7)
Total	9 (56.2)	7 (43.7)		11 (52.3)	10 (76.9)		20 (54.0)	17 (58.6)

examination of a very small number of carcasses, i.e. only 13 individuals (Torres et al., 1998; Millán and Casanova, 2007).

Digenetic trematodes, with an aquatic life cycle including vertebrates containing metacercariae, were not found in this study.

Taenia eggs were not identified at species level since, until now, three different species have been reported to parasitize the Iberian lynx, namely *T. pisiformis*, *T. polyacantha* and *T. taeniaeformis*. The first is the least frequently taeniid detected by Torres et al. (1998), although lagomorphs are considered the major intermediary host for this species. However, *T. polyacantha* and *T. taeniaeformis* use several small mammals as their intermediate hosts (Abuladze, 1970).

Table 3
Number of individuals studied (n) and infected (n_i) of the Iberian lynx and helminth species detected with their respective prevalences (%) by age group according to the area and in the total of study.

	Doñana area			Sierra Morena			Total study	
	n_i (%) Juvenile $n=14$	Adult $n=18$	p-Value	n_i (%) Juvenile $n=18$	Adult $n=16$	p-Value	n_i (%) Juvenile $n=32$	Adult $n=34$
Cestoda								
Taenia spp.	–	–		2 (11.1)	2 (12.5)	1	2 (6.2)	2 (5.9)
Hymenolepis spp.	1 (7.1)	–	0.44	1 (5.5)	–	1	2 (6.2)	–
Nematoda								
Capillariinae gen. sp.	2 (14.3)	2 (11.1)	1	2 (11.1)	2 (12.5)	1	4 (12.5)	4 (11.8)
Ancylostoma spp.	8 (57.1)	6 (33.3)	0.12	–	2 (12.5)	0.21	8 (25.0)	8 (23.5)
Toxocara spp.	2 (14.3)	1 (5.5)	0.57	5 (27.8)	5 (31.2)	1	7 (21.8)	6 (17.6)
Toxascaris leonina	–	1 (5.5)	1	6 (33.3)	4 (25.0)	0.71	6 (18.7)	5 (14.7)
Total	8 (57.1)	8 (44.4)		10 (55.5)	11 (68.7)		18 (56.2)	19 (55.9)

Juvenile, <2-year old; adult, >2-year old.

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Large eggs (75–80 μm diameter) with an oncosphere surrounded by a membrane with two characteristic polar knobs and polar filaments were found in the faeces of two juvenile male lynxes from both areas. These *Hymenolepis* eggs are frequently detected in Spanish rodents (Feliu et al., 1997), but this finding constitutes the first report in the Iberian lynx. Molecular studies may be convenient to determine whether this is a species for which the Iberian lynx acts as a definitive host or if, on the other hand, these eggs were ingested by the lynx through a prey, other than the rabbit, which acts as its definitive host.

Mesocestoides sp. and *M. litteratus* turned out to be the most prevalent cestodes in the Iberian lynx (40% and 37.5%, respectively) reported to date, both in SM only (Torres et al., 1998; Millán and Casanova, 2007). The non-detection of *Mesocestoides* eggs in this study is noteworthy and almost certainly related to the biological nature of this genus. In this sense, information on the biology of these cestodes is rather limited, although their infective form is known to occur in amphibians, reptiles and small mammals. Moreover, other factors related to possible fluctuations in the reproductive status of cestodes could have had their implications on the day of the sampling and consequently in their total absence in this study.

The detection of *J. pasqualei* in only one Iberian lynx of SM (Millán and Casanova, 2007), a species known to use lizards as its intermediate host, may explain its absence in this study.

Nematodes are in any case a heterogeneous group. The capillarinid egg detected in the faecal sample might turn out to be *Capillaria aerophila* (=*Eucoleus aerophilus*) since it is the only trichurid species detected up to date in the Iberian lynx, in post-mortem studies (Torres et al., 1998), as well as in coprological surveys (Rodriguez and Carbonell, 1998). It is a parasite of the lungs causing infection by direct transmission or via an earthworm (Anderson, 1992).

A male nematode identified as *A. tubaeforme* on the basis of the length of the spicules obtained from an adult lynx may confirm the identification of the eggs of *Ancylostoma* observed in faecal samples as *A. tubaeforme*. Moreover, this species is the only hookworm previously reported in the Iberian lynx from DA (Torres et al., 1998; Vicente et al., 2004; Millán and Casanova, 2007), which agrees with the higher prevalence obtained (43.7%) in the present study. The epidemiology of this species in the endangered Iberian lynx in Doñana National Park has previously been studied by Vicente et al. (2004). These authors demonstrated that juvenile lynxes showed a statistically higher prevalence of *Ancylostoma* than adults, which agrees with the results obtained here in this area. However, the absence of this nematode in juvenile lynxes and their presence in adults (12.5%) in SM is rather surprising, because this nematode had so far not been reported in this area. The life cycle of this species is probably similar to that of other *Ancylostoma* species, such as *A. caninum*, thus several modes of transmission, including through the skin, prenatally, transmammary, or through insects or rodents as paratenic hosts (Anderson, 1992), can be considered. Moreover, the low prevalence of SM may be related with the not suitable environmental conditions for development and survival of free-living infective larvae.

Among the nematodes infecting the Iberian lynx, ascarids (*Toxocara* spp. and *T. leonina*) are present in both areas, but are significantly more prevalent in SM (in both species 29.4%). These two species are cosmopolitan, usually found in *Lynx* species, but particularly in the Iberian lynx (Rodriguez and Carbonell, 1998; Torres et al., 1998; Millán and Casanova, 2007), and have two main paths of infection, either by direct transmission or indirect involving a paratenic host, usually rodents or earthworms (Anderson, 1992).

The sporadic presence of *M. muris*, *P. praeputialis* and *V. p. potekhina* in only one Iberian lynx until now (Torres et al., 1998; Millán and Casanova, 2007) is likely to be related to the biological nature of these species, always linked to their requirement to use an insect as intermediate host. This fact makes it possible to understand their total absence in the faecal samples analysed.

Small populations are particularly susceptible to stochastic processes, including outbreaks of diseases that can even cause local extinctions. Little is known about the diseases affecting lynx. However, helminthoses may play an important role in the mortality and morbidity of the Iberian lynx (Vicente et al., 2004). These health considerations must be taken into account in breeding programs such as reintroductions or translocations. Further studies must be conducted in order to assess the impact of the intestinal helminths on the Iberian lynx health, by comparing the presence of the parasites with clinical, biochemical and hematological parameters.

5. Conclusion

The helminth fauna detected in this study can be considered for the first time, and on the basis of the number of specimens analysed, representative of both Iberian lynx populations. This carnivore does not forage opportunistically on the ground and grass, and insects are usually absent in its faeces. Consequently, most infections with monoxenic nematodes may occur through direct transmission between mother and kittens, or through rabbits as paratenic hosts, while the rest of helminth species might be related to the dominance of a single or a reduced number of prey species in the diet of the Iberian lynx. Control measures to prevent helminthoses could have a positive impact on the Iberian lynx conservation.

Conflict of interest statement

The authors declare that they have no competing interests.

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