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Epidemiology of *Ancylostoma* spp. in the endangered Iberian lynx (*Lynx pardinus*) in the Doñana National Park, south-west Spain

J. Vicente[1,2]*, F. Palomares[3], R. Ruiz de Ibañez[2] and J. Ortiz[2]

[1]Instituto de Investigación en Recursos Cinegéticos (CSIC/UCLM), PO Box 535, E-13080 Ciudad Real, Spain: [2]Parasitología y Enfermedades Parasitarias, Departamento Patología Animal, Facultad de Veterinaria de Murcia, Campus de Espinardo, 30100 Murcia, Spain: [3]Department of Applied Biology, Estación Biológica de Doñana (CSIC), Avda. María Luisa s/n, 41013 Sevilla, Spain

Abstract

The epidemiology of *Ancylostoma* spp. was studied in the endangered Iberian lynx (*Lynx pardinus*) in the Doñana National Park, south-west Spain. Faecal samples were collected throughout a complete annual cycle (August 1997 to September 1998). The overall egg prevalence of *Ancylostoma* spp. was 57.8%. The pattern of abundance of *Ancylostoma* spp. eggs in faeces was overdispersed. Juvenile lynx demonstrated a statistically higher prevalence and abundance of *Ancylostoma* spp. than in adults. These levels of egg output (maximum 21195 epg), as previously reported in free ranging large felid cubs, could be close to disease involvement. The potential pathogenicity of hookworms and the influence of individual and ecological factors on hookworm transmission in the Iberian lynx from the Doñana National Park population are discussed.

Introduction

The implications of natural diseases in large cats pose a general conservation problem because of their threatened status and can even lead to preventive or therapeutic management in free-living animals (Roelke *et al.*, 1985). The Iberian lynx (*Lynx pardinus*) is a medium sized carnivore species exclusive to the south-west of the Iberian Peninsula. According to the World Conservation Union (IUCN, Nowell & Jakson, 1996), *L. pardinus* is considered to be the most endangered feline species in the world, with an estimated number of not more than 300 individuals, distributed in several isolated populations (Delibes *et al.*, 2000). One of the two main populations, and the best known, is in the Doñana National Park, where ecological studies have been carried out for several years (Palomares *et al.*, 1996). This Iberian lynx population comprises about 40 individuals occupying a fragmented landscape and isolated from other lynx populations, which could make it theoretically non-viable (Gaona *et al.*, 1998). Postnatal mortality is known to be around 33%, the reasons for which are undetermined (F. Palomares *et al.*, unpublished data).

There is little available information about parasites and infectious diseases of the Iberian lynx (Delibes *et al.*, 2000). Faecal examination for parasite infective stages is a valuable alternative to obtaining host samples (Festa-Bianchet, 1991; Müller-Graf *et al.*, 1999), especially for rare and endangered animals where information from parasite studies is important for management and conservation purposes.

The ecology of helminth parasites, including hookworms, has been reported in several lynx species in the world, mainly in North American bobcats (*Lynx rufus*) and Canadian lynxes (*Lynx canadensis*) (Stone & Pence, 1978; Smith *et al.*, 1985; Mclaughlin *et al.*, 1993). *Ancylostoma tubaeforme*, a cosmopolitan and specific hookworm in Felidae, has previously been reported in the Iberian lynx (Torres *et al.*, 1998). *Ancylostoma*

*Fax: +34 926 225184
E-mail: jvicente@irec.uclm.es

tubaeforme has also been found in wild cats (*Felis silvestris*), wild cat–domestic cat hybrids (Miquel *et al.*, 1994) and stray domestic cats (Calvete *et al.*, 1998) in the Iberian Peninsula. Clinical signs in wild free-ranging felids have been associated with ancylostomosis by perinatal transmission in American large cats (Roelke *et al.*, 1985; Dunbar *et al.*, 1994).

The objectives of the present study were to evaluate: (i) the epidemiology of *Ancylostoma* spp. in the Iberian lynx population in the Doñana National Park through a study of faecal samples; and (ii) the effect of natural infections of hookworms on lynx in view of the disease implications of these parasites in comparable populations of endangered feline species. This investigation would provide information on a helminth pathogen which could potentially be involved in the neonatal survival of the Iberian lynx.

Materials and methods

Field site

The study was carried out in the Doñana National Park, a 550 km^2 reserve on the Atlantic coast in southeastern Spain, on the Guadalquivir river mouth (37°09′N, 06°26′W). The predominant habitats are marshes, mediterranean scrublands and sand dunes. Apart from stray cats, nine different carnivore species coexist in Doñana. The Iberian lynx is the heaviest, weighing 10–12 kg (Palomares *et al.*, 1996).

The study area is located in Coto del Rey, in the north of Doñana National Park and is characterized by scrubland patches of *Pistacia lentiscus* and *Halimium halimifolium* with scattered *Quercus ruber* trees and *Fraxinus angustifolius* flanking seasonal streams.

The climate is mediterranean with Atlantic influence. The dry season typically starts in May and lasts until September and is characterized by high temperatures (e.g. monthly maximum mean temperature of 32.7°C in August 1997). Most yearly rains are concentrated in the wet season, which normally begins by September and continues, with considerable variations, until April, with mild temperatures (e.g. monthly minimum mean temperature of 7.4°C in February 1998). These rainfall and temperature data were used to classify each season with precision, the dry season extending from 15 May to 15 September and the wet season for the remainder of the year.

Lynx populations

Three lynx foci were distinguished by Palomares *et al.* (1991) in the metapopulation of Doñana National Park. The lynx subpopulation from Coto del Rey contains the highest density in the Doñana area and is probably the highest worldwide. The lynx population of the study area consisted of three resident lynx couples, their offspring (three cubs as mean) and occasional wandering individuals. Faecal samples were identified as belonging to: (i) juvenile ($\leq$ 9 months) or adult lynx ($>$ 9 months); and (ii) dry or wet seasons (table 1).

Collection of faecal samples

Fresh faecal samples from Coto del Rey were collected within 24 h after their deposition. Sampling was carried out throughout the period from August 1997 to September 1998 in conjunction with an ecological study on lynx in the same area. The collection and precise assignation to juvenile (all juvenile samples but one were less than 6 months old according to birth date) or adult classes in the field were possible when animals were radiolocated or their latrines were visited. Immobilization of trapped lynx with a combination of ketamine hydrochloride and xylazine hydrochloride (Ferreras *et al.*, 1994) allowed the collection of faecal material from known individuals. Faeces were weighed and mixed with an equal volume of 10% buffered formalin until analysed in the laboratory.

Laboratory analysis

Analyses were performed using a flotation technique in Seather's sacharose solution with a specific gravity of 1.275 (Georgi & Georgi, 1990). Individual faecal egg counts were performed using McMaster cameras. Faeces were dehydrated in a stove at 100°C so that eggs per gram (epg) were always expressed in relation to dehydrated faeces. Helminth eggs were identified according to their morphology (Patton *et al.*, 1986; Georgi & Georgi, 1990; Patton & Rabinowitz, 1994; Müller-Graf, 1995) and linear lengths with a calibrated eyepiece micrometer.

The digestive tract of an adult road-killed female lynx from the Doñana population was examined for helminths. Hookworms were removed and preserved in 70% ethanol, cleared in lactophenol and morphologically examined under a light microscope. Hookworn specimens were deposited in the Laboratory of the Unidad Docente de Parasitología y Enfermedades Parasitarias del Departamento de Patología Animal de la Universidad de Murcia (ref. A. t. 01/1998).

Statistical analysis

The prevalence is defined as the number of faeces infected with eggs of *Ancylostoma* spp. related to all examined samples, and the mean abundance describes the mean number of eggs in a faecal sample related to both

Table 1. The prevalence (% ± S.E.) of *Ancylostoma* spp. eggs in faecal samples of Iberian lynx in the Doñana National Park, 1997/98.

			Dry season		Wet season	
	Adults	Juveniles	Adults	Juveniles*	Adults	Juveniles
No. of faecal samples examined	48	9	17		31	9
Prevalence (%)	50.0 ± 3.6	100.0	35.2 ± 5.5		58.0 ± 4.3	100.0

*No data available for juveniles in the dry season.

infected and uninfected samples (Margolis *et al.*, 1982). Standard errors for prevalences were estimated with the expression S.E. $(p) = p(1 - p)/n^{1/2}$ (Martin *et al.*, 1987).

The following statistical analyses are based on egg counts. Host age and seasonal status were treated as variables with two classes respectively: (i) juveniles ($\leq$ 9 months) or adults ($>$ 9 months); and (ii) collected during dry or wet season.

To test for significant differences in the faecal prevalence of eggs of *Ancylostoma* spp. between host age (in wet season) and season (in adults) classes, a Fisher two-tailed test was employed. To check if egg counts were normally distributed, they were contrasted with Kolmogorov-Smirnov test (K-S test). For faecal abundances, the significance of host age (in wet season) and season (in adults) were separately tested. For all comparisons the non parametric Mann-Whitney U-Test (M-W test) for two independent variables was used (Siegel, 1970). The level of significance was established at the 5%, using the SPSS 10.0.6 program (©SPSS Inc., 1999).

Results

Parasite identification

Thin-shelled and ellipsoidal-shaped eggs containing a morula in different segmented states were observed in coprological analyses. Eggs were initially classified as species of *Ancylostoma*, according to the morphology and linear measurements (n = 75) (egg size length: 57–63 μm, mean: 56.6 $\pm$ 3.2 S.D.; width: 37–41 μm, mean: 38.6 $\pm$ 3.0 S.D.).

The post-mortem examination of a lynx carcass revealed the presence of nine nematode specimens in the small intestine. They were identified as *A. tubaeforme* (Burrows, 1956). Eggs from faeces were found to be morphologically similar to a sample of eggs from mature and gravid *A. tubaeforme*.

Faecal infections

The distribution of *Ancylostoma* spp. egg outputs was not normal ($P < 0.05$). The mean prevalence value in the faeces was 58.8 $\pm$ 6.1 S.E., with a mean abundance of 1218.9 $\pm$ 3722.4 S.D. epg. The abundance of eggs in faecal samples showed an overdispersion, with variance/mean ratio significantly greater than unity.

Table 2. The range and mean number of eggs of *Ancylostoma* spp. eggs in faecal samples from lynx in the Doñana National Park, 1997/1998.

	Mean no. of eggs $\pm$SD	Range (min–max)
Adults	307.3 $\pm$ 790.2	0–4372.0
Juveniles	6081.0 $\pm$ 7857.9	36.9–21195.2
Dry season		
Adults	108.1 $\pm$ 185.9	0–577.3
Juveniles*		
Wet season		
Adults	416.5 $\pm$ 961.8	0–2900.9
Juveniles	6081.0 $\pm$ 7857.9	36.9–21195.2

*No data available for juveniles in the dry season.

Seasonal levels of infection

Hookworm eggs were widespread in the faeces of both adult (58.09%) and juvenile (100%) lynx during the wet season (table 1). Faecal samples from the juveniles showed statistically higher prevalences than those from the adults in the wet season ($P < 0.05$). With samples from adult lynx only, no significant difference in prevalence between the seasonal groups was found ($P > 0.05$).

Faecal abundances for season and host age groups are summarized in table 2. There were no statistical differences between the dry (mean: 108.1 $\pm$ 185.9 S.D.) and wet seasons (mean: 416.5 $\pm$ 961.8 S.D.) when only adults were considered ($P > 0.05$). Juveniles shed significantly more epg (mean: 6081 $\pm$ 7857.9 S.D.) than adults (mean: 416.5 $\pm$ 961.8 S.D.) ($P < 0.05$) during the wet season.

Discussion

Morphological similarities were found between the eggs of *Ancylostoma* spp. from faecal samples and those from gravid *A. tubaeforme* females removed from a necropsied adult lynx, hence confirming the identification as *A. tubaeforme*. This species is the only hookworm previously reported in the Iberian lynx, and found in an individual from the Doñana National Park (Torres *et al.*, 1998).

Ancylostoma tubaeforme is the most prevalent gastrointestinal parasite in faecal samples from our of study (J. Vicente, unpublished observations), while ascarids are the most prevalent parasites infecting the Iberian lynx in their current area of distribution (Torres *et al.*, 1998). Introduced felids can be a new source of hookworms for an indigenous population, including exotic and potentially pathogenic parasite species (Roelke *et al.*, 1985). Thus, from the point of view of Iberian lynx management, it is important to consider the special features concurring in the Doñana National Park, where Iberian lynx and wild cats coexist with stray cats, and the associated risks of parasite transmission and shared host-cycles.

Coprological results suggest that hookworms are widespread in this subset of Iberian lynx from the Doñana National Park. A mean faecal prevalence of 57.8% of *Ancylostoma* spp. is much higher than the coprological analysis (22.2%) of Iberian lynx in central Spain (Rodriguez & Carbonell, 1998). To our knowledge, only a higher prevalence (91.9%) of *Ancylostoma* spp. eggs in faecal samples of free ranging wild felids has been reported in *Lynx rufus* from southern Texas (Mitchell & Beasom, 1974).

The high density that the Iberian lynx reaches in the Doñana National Park, and especially in Coto del Rey, may favour infection and reinfection of direct cycle parasites. Parasitism by monoxenous parasites may be related to host density in wild mammal populations, including hookworms as potential indicators of densities in wild lynxes (Watson *et al.*, 1981). Large prevalences of hookworm infections in wild felids have been found in social species such as African lion *Panthera leo*, (Müller-Graf, 1995; Bjork *et al.*, 2000). However, the Iberian lynx does not demonstrate any social behavior (Ferreras *et al.*, 1997). The overall faecal prevalence of 67.5% in the wet

season in the Doñana National Park, being 58.0% for adults, is higher (Patton & Rabinowitz, 1994) or similar (Patton *et al.*, 1986) to that of hookworms in tropical wild felids. The climate in the area of the present study may influence the survival of free-living infective larvae, especially in dry and hot summer seasons.

The abundance data is difficult to interpret because of the scarcity of reference values in other wild felids. The abundance of eggs in the faecal samples showed an overdispersion. This agrees with an aggregated distribution of the parasite in the Iberian lynx from Coto del Rey (Shaw & Dobson, 1995). A very small proportion of high counts and a majority of low counts may indicate differences in exposure or/and immunity between individuals (Müller-Graf *et al.*, 1997).

Ancylostoma tubaeforme is widely spread among this subset of faecal samples in the Doñana National Park lynx population. The abundance of infection was significantly lower in adults than juveniles during the wet season. Also, there were no significant seasonal differences in abundance when only adult lynx were considered.

High faecal counts in juvenile lynx samples may reflect transient deficient immunity to hookworms and/or the effects of a larger exposure to infection. An early infection within the first days of life of the lynx, in the case of *Ancylostoma* spp., succeeds by the prenatal or transmammary route (Mclaughlin *et al.*, 1993). A relatively lightly infected female lynx can produce heavily infected young (Mitchell & Beasom, 1974). Moreover, an early exposure to *A. tubaeforme* infection may be related to the optimum microhabitat that natal dens could provide for maintaining infective larvae. Natal dens are repeatedly used by female Iberian lynx for breeding activity because they are a limited resource in Doñana (Fernández & Palomares, 2000). This finding may favour oral or percutaneous transmission to juvenile lynx (Knowlton, 1972). In adult lynx, which show a high prevalence of infection, the reactivation of tissue larvae and development to adult worms, especially in periparturient periods, could contribute to maintenance the parasite population in the host intestine (Schad & Page, 1982) as has been suggested in bobcats from southern Texas (Mitchell & Beasom, 1974).

Mean abundances have not been described for eggs of *Ancylostoma* spp. in faeces in wild felid populations, but there are reports of egg counts associated with juvenile clinical ancylostomosis in wild free-ranging felids (Dunbar *et al.*, 1994). Lower abundances of faecal outputs than we describe for juveniles Iberian lynx were found in a kitten of the Florida panther (*Felis concolor coryi*) with clinical parasitosis by *Ancylostoma pluridentatum*. In this clinical episode, egg shedding was monitored with a maximum of 936 epg of fresh faeces (Dunbar *et al.*, 1994) and a related increase of 4% to 24% of eosinophils was reported, decreasing to 6% after treatment. A juvenile recaptured lynx from the Doñana National Park has been reported to have high eosinophils levels (14%), returning to a value similar to those of adult animals (1%) when reaching adult age (Beltrán *et al.*, 1991), which could coincide with the life span of *Ancylostoma* spp., estimated to be from 6 to 18 months (Kalkofen, 1987).

There is no direct evidence of clinical events or mortality in juvenile Iberian lynx due to ancylostomosis. Nevertheless, the circumstances associated with this lynx population are similar to those of the endangered Florida panther. Ancylostomosis may play a role in neonatal morbidity and mortality in the population of Iberian lynx from the Doñana National Park, where individual losses are important. So, the study of parasite burdens and especially the possible clinical episodes relating to hookworms in cubs should be considered in future research for the purposes of management and conservation.

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