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Intestinal parasites in a population of stone marten (*Martes foina*) in central Spain

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Abstract

*Intestinal parasites in a population of stone marten (*Martes foina*) in central Spain.* Twenty–one fresh fecal samples from stone marten (*Martes foina*) (Erxleben, 1777) collected in the Natural Park of Hoces del Río Riaza (Segovia) were processed using a modified version of Ritchie's concentration method. We identified 18 genera: four coccidian; two trematodes; three cestodes; eight nematodes; and one acanthocephalan. The prevalence was low for all genera except *Isospora* sp. (Schneider, 1875), *Eimeria* sp. (Schneider, 1875) and *Toxocara* sp. (Stiles, 1905) that showed values above 20 %. The richness of parasites was lower than that detected in other populations, and it is likely that the stone martens help disseminate these parasites as their external appearance was good.

Key words: Central Spain, Coccidia, Gastrointestinal parasites, Helminths, *Martes foina*, Mustelids, Prevalence

Resumen

Estudio de los parásitos intestinales en una población de garduñas (Martes foina) *del centro de España.* Se han analizado 21 muestras coprológicas frescas de garduña (*Martes foina*) (Erxleben, 1777) recogidas en el Parque Natural de las Hoces del río Riaza (Segovia) que han sido procesadas utilizando el método de Ritchie modificado como técnica de concentración. Se han detectado cuatro géneros de coccidios, destacando por su abundancia *Isospora* y *Eimeria*, dos de trematodos, tres de cestodos, ocho de nematodos y un único acantocéfalo. Las prevalencias de la mayoría de los parásitos son bajas en todos los géneros detectados excepto *Isospora* sp. (Schneider, 1875), *Eimeria* sp. (Schneider, 1875) y *Toxocara* sp. (Stiles, 1905), que presentan valores superiores al 20 %. La riqueza de parásitos es menor a la detectada en otras poblaciones y es probable que estos mustélidos actúen como dispersadores de los parásitos debido a que el aspecto externo de los ejemplares observados es bueno.

Palabras clave: Coccidios, España central, Helmintos, *Martes foina,* Mustélidos, Parásitos gastrointestinales, Prevalencia

Resum

*Estudi dels paràsits intestinals en una població de fagines (*Martes foina*) del centre d'Espanya*. S'han analitzat 21 mostres coprològiques fresques de fagina (*Martes foina*) (Erxleben, 1777) recollides al Parc Natural de les Gorges del riu Riaza (Segòvia) que han estat processades mitjançant el mètode de Ritchie modificat com a tècnica de concentració. S'hi han detectat quatre gèneres de coccidis, entre els quals destaquen per l'abundància *Isospora* i *Eimeria*, dos de trematodes, tres de cestodes, vuit de nematodes i un d'acantocèfal. Les prevalences de la majoria de paràsits són baixes en tots els gèneres detectats excepte *Isospora* sp. (Schneider, 1875), *Eimeria* sp. (Schneider, 1875) i *Toxocara* sp. (Stiles, 1905), que presenten valors superiors al 20 %. La riquesa de paràsits és inferior a la detectada en altres poblacions i és probable que aquests mustèlids actuïn com a dispersadors dels paràsits atès que l'aspecte extern dels exemplars observats és bo.

Paraules clau: Coccidis, Espanya central, Helmints, *Martes foina,* Mustèlids, Paràsits gastrointestinals, Prevalença

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Introduction

The stone marten (*Martes foina*) is classified as a generalist and highly adaptable carnivore. It is one of the mustelids with the widest distribution in the Eurasian region, found from Central and Southern Europe to eastern Mongolia, Afghanistan and Tibet (Genovesi et al., 1996). It lives in deciduous forests and woodland margins (Macdonald, 1993) and is often seen around cities and towns (Rasmussen and Madsen, 1985; Lucherini and Crema, 1993; Tóth, 1998; Lanszki, 2003). In the centre of the Iberian peninsula, the stone marten can be found from forest areas with a low human density to the low mountain areas where oak and Mediterranean scrub are predominant and population density is high. (Virgós and Casanovas, 1998; Virgós et al., 2000; Mangas et al., 2007).

Studies on endoparasites in Iberian wild carnivores are scarce and mainly based on the analysis of a small number of hosts (Simón–Vicente, 1975; Martínez et al., 1978; Sanmartín et al., 1989; Rosalino et al., 2011). Most studies have been performed in marten (Martes martes Linnaeus, 1758) (Segovia et al., 2007) and badgers (*Meles meles;* Linnaeus, 1758) (Rocamora et al., 1978; Jones et al., 1980). Furthermore, these studies have generally focused on the identification of the helminth fauna (Casanova, 1993; Miquel, 1993; Miquel et al., 1994b; Torres et al, 2001; Millán et al., 2004; Miterpáková et al., 2013) and data regarding intestinal parasites is precarious in mustelids.

Such studies are more plentiful at a European level (Soltys, 1962; Shakhmatova, 1966; Libois and Waechter, 1991) but they are generally limited to specific species (Segovia et al., 2007). Several publications have recently appeared from in Central Europe (Koubek et al., 2004a, 2004b; Kornás et al., 2013) and Italy (Millán and Ferroglio, 2001; Ribas et al., 2004), complementing those made in recent decades in Spain –mainly in Catalonia (Miquel, 1993; Miquel et al., 1994a, 1994b) and collected in Aubry et al. (2012). Despite the impact that environmental conditions have on the richness and diversity of species (Rosalino et al., 2011), most of these studies have focused on the European Mediterranean basin where

environmental conditions are more favourable but studies of the parasitological fauna in continental climates in current populations appear to be increasing (Massey et al., 2009; Nugaraité et al., 2014; Figuereido et al., 2018).

The aim of this study was to analyze the intestinal parasite burden in the stone marten (*Martes foina*) in a protected area in the centre of the Iberian Peninsula and to identify possible differences with species in other areas of the Iberian peninsula and Europe. We also aimed to analyze the prevalence of these diverse parasites.

Material and methods

Study area

The study was carried out in the Natural Park of Hoces del Río Riaza (UTM 30TVL59) in the NE of Segovia province, close to the border with the province of Burgos and with an approximate area of 6,740 has (fig. 1; https://patrimonionatural.org/espacios–naturales/parque–natural/parque–natural–hoces–del–rio–riaza)

Geographically, the study area is nestled in the Mediterranean region, in the lower supra–Mediterranean zone that has almost nine months a year of probable frosts and four months of drought (Rivas Martínez, 2007). The varied topography of the area, its limestone formation and the region's economic exploitation are the determinant factors of the presence of this diverse vegetation formed by juniper, holm oak and Portuguese oak trees. The rest of the vegetation in the area consists of riparian forest at the bottom of the valley with communities of scrubland, grassland and aquatic species, besides the existence of a major area of rain fed crops, and to a lesser extent, of irrigation in the meadow of the river Riaza.

Material and methods

We obtained 21 fecal samples from stone marten (*Martes foina*) collected along a fixed itinerary from Valdevacas to the Riaza river within the Natural Park of Hoces del Río Riaza (Segovia) using photo–trapping with DLC–Covert 2 cameras in 58 previously established catchment areas. We considered the habitat requirements of the species as well as the distance to water points, vegetation cover and type of land management (Park area vs authorized area) using the existing government layers in GIS (www.esri.com). The cameras were installed in November 2013 and were active at each point for 5 days. Each capture zone was baited with cans of sardines and the cameras were programmed to emit bursts of three photos at 20–second intervals. These capture areas were separated from each other by at least 1 km. We only selected the faeces identified by experienced observers as fresh samples of stone marten in the areas where presence of the species was confirmed through camera traps and where no other species with similar faeces were present (Davison et al., 2002). Fecal samples were placed in plastic bags, labelled and sealed for shipment and storage in the laboratory where they remained refrigerated for a maximum of 24 hours at 4 °C to prevent the growth of fungi. We then maintained the samples in potassium dichromate (3.5 %) for seven days to enhance the sporulation of oocysts. Next, we took 3 g of each sample for later concentration and the remaining samples were placed in 10 % formalin to fixation for re–use if necessary.

For later microscopic analysis and to better localize the parasites in the faeces, we performed Ritchie's modified biphasic concentration method (Allen and Ridley, 1970; García, 2007) with the 3 g of each of the previously selected fecal samples. This technique allows the effective treatment of the faeces and facilitates the identification of elements or parasites whose distribution is irregular in fecal mass (Kaufmann, 1996; Cöplü et al., 2007; Hendrix and Robinson, 2011; Saez et al., 2011).

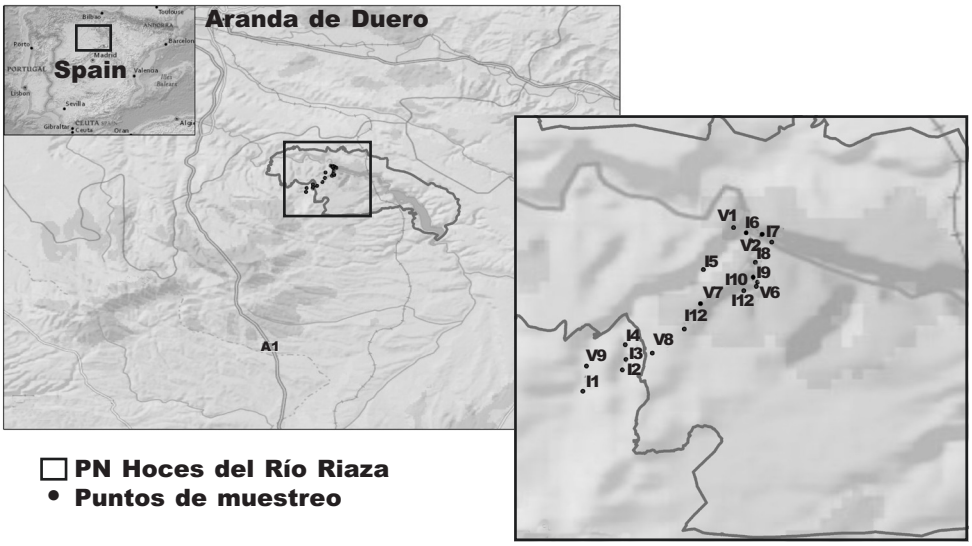

Fig. 1. Map showing the location of the study area, highlighting the Natural Park of Hoces del Río Riaza and points where cameras were placed.
Fig. 1. Mapa que muestra la localización del área de estudio en el que se destacan el Parque Natural de las Hoces del río Riaza y los puntos elegidos para el emplazamiento de las cámaras.

To improve the visualization of the morphological details we added iodine solution (García, 2007). Parasites were identified using an ocular micrometer to facilitate the calculation of dimensions and the keys suggested by Kaufmann (1996), Thienpont et al. (1979), Soulsby (1982), Cordero del Campillo and Rojo Vázquez, (2000) and Gibbons et al. (2009) were used.

Results

We found parasites in 16 of the 21 analyzed samples (80.95 %), and a total of 18 different genera of parasites, between Protozoa and Helminths: four genera of coccidia [*Eimeria* sp. (Schneider 1975), *Cyclospora* (Schneider 1881)*, Isospora* sp. (Schneider 1881) and *Sarcocystis* sp. (Lankester 1882)]; eight nematodes (*Eucoleus* sp. Dujardin [1844], *Paersonema* sp. [Freitas and Mendonca 1960], *Aonchotheca* sp. [López–Neyra 1947]*, Strongyloides* sp. [Grassi 1879], *Uncinaria* sp. [Froelich 1789], *Crenosoma* sp. [Molin 1861], *Toxocara* sp. [Stiles 1905] and *Baylisascaris* sp. [Sprent 1968]); three cestodes (*Taenia* sp. [Linnaeus 1758], *Hymenolepis* sp. [Weinland 1858] and *Oochoristica* sp. [Luehe 1898]); two trematodes (*Troglotrema* sp. [Odhner 1914] and *Heterophyes* sp. [Cobbold, 1866]); and one acanthocephalan (*Centrorhyncus* sp. [Lühe 1911]).

Due to the morphological similarity of some eggs, we were unable to clearly distinguish between them using only the diagnostic methods applied in this study so we also compared them with the collection of parasites of the Department of Biodiversity, Ecology and Evolution of the Faculty of Biological Sciences of the Universidad Complutense de Madrid. Regarding prevalence, coccidia and nematodes were found in 61.9 % of the samples, cestodes in 23.81 %, trematodes in 19.05 %, and acanthocephalan in 4.76 %. (table 1).

Isospora sp. and *Eimeria* sp. showed the highest prevalence within the coccidia (table 1). Among the helminths, nematodes were the most abundant taxon, and within these, *Toxocara* sp. Among cestodes, the most abundant taxon were *Oochoristica* sp. and *Taenia* sp. with a prevalence of 15 to 20 %, and the prevalence of *Troglotrema* sp. was 14.29 % among trematodes. Acanthocephala was the only *Centrorhyncus sp*. found (table 1).

Only five of the analyzed samples were free of parasites (23.81 %). Taking the samples with parasites into account, 10 of the samples had more than one parasite, and sample I10 had 12 different parasites (table 2).

Discussion

Analysis of fecal samples limits the parasites obtained to particular groups (related with the digestive tract) and to certain stages of those parasites (cysts, eggs, larvae). It does not allow specific determination without further genetic studies. These analyses are usually a standard procedure in the study of parasites because sampling is much simpler (it is not necessary to capture the specimen), the variety of parasitic species is sufficiently high, and relative specificity is low, and identification to genus is relatively simple (Painer et al., 2011; Pfukenyi et al., 2007). The species of coccidia are identified after sporulation (Levine, 1985).

Although the stone marten is common in the Iberian peninsula, studies concerning its intestinal parasite fauna are few. At a European level, 30 different species of parasites have been related to this mustelid (Aubry et al., 2012). In this study, we confirmed the presence of 18 genera previously reported in the literature.

Table 1. List of genera detected indicating the group and the prevalence found in the Natural Park of Hoces del Río Riaza.
Tabla 1. Lista de géneros detectados con indicación de su grupo y prevalencia en el Parque Natural de las Hoces del río Riaza.

Group		Group	
Genus	**Prevalence (%)**	**Genus**	**Prevalence (%)**
Coccidia		Nematodes	
Eimeria sp.	47.62	*Eucoleus* sp.	19.05
Cyclospora sp.	4.76	*Personema* sp.	4.76
Isospora sp.	47.62	*Aonchoteca* sp.	4.76
Sarcocystis sp.	19.05	*Strongyloides* sp.	14.29
Total	61.9	*Uncinaria* sp.	14.29
Trematodes		*Crenosoma* sp.	19.05
Heterophyes sp.	4.76	*Toxocara* sp.	23.81
Troglotrema sp.	14.29	*Baylisascaris* sp.	4.29
Total	19.05	Total	61.9
Cestodes		Acanthocephala	
Taenia sp.	19.05	*Centrorhynchus* sp.	4.76
Oochoristica sp.	14.29	Total	4.76
Hymenolepis sp.	9.52		
Total	23.81		

Table 2. Parasitic load obtained in the analyzed samples: ogf, number of oocyst per gram of faeces; egf, number of egg per gram of faeces.
Tabla 2. Carga parasitaria registrada en las muestras analizadas: ogf, número de oocistos por gramo de heces; egf, número de huevos por gramo de heces.

	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	V1	V2	V3	V4	V5	V6	V7	V8	V9
Coccidia (ogf)	2,66	7,98	0,00	0,00	0,00	0,00	2,66	21,30	10,60	10,60	7,98	10,60	5,32	10,60	0,00	0,00	0,00	0,00	2,66	29,30	7,98
Eimeria sp.	0.00	2,66	0,00	0,00	0,00	0,00	2,66	13,30	7,98	0,00	0,00	7,98	2,66	5,32	0,00	0,00	0,00	0,00	2,66	2,66	2,66
Cyclospora sp.	0.00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,66	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Isospora sp.	2,66	2,66	0,00	0,00	0,00	0,00	0,00	7,98	0,00	5,32	7,98	2,66	2,66	5,32	0,00	0,00	0,00	0,00	0,00	26,60	2,66
Sarcocystis sp.	0.00	2,66	0,00	0,00	0,00	0,00	0,00	0,00	2,66	2,66	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,66
Nematoda (egf)	0,00	23,94	15,92	0,00	0,00	0,00	0,00	21,28	5,32	164,96	2,66	61,18	29,26	0,00	0,00	2,66	0,00	0,00	0,00	0,00	0,00
Eucoleus sp.	0.00	0,00	10,60	0,00	0,00	0,00	0,00	0,00	5,32	5,32	0,00	53,20	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Personema sp.	0.00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	5,32	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Aonchoteca sp.	0.00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	5,32	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Strongyloideis sp.	0.00	5,32	0,00	0,00	0,00	0,00	0,00	2,66	0,00	47,90	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Uncinaria sp.	0.00	0,00	0,00	0,00	0,00	0,00	0,00	5,32	0,00	21,30	2,66	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Crenosoma sp.	0.00	0,00	0,00	0,00	0,00	0,00	0,00	7,98	0,00	66,50	0,00	2,66	2,66	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Toxocara sp.	0.00	13,30	5,32	0,00	0,00	0,00	0,00	5,32	0,00	13,30	0,00	5,32	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Baylisascaris sp.	0.00	5,32	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	26,60	0,00	0,00	2,66	0,00	0,00	0,00	0,00	0,00
Cestoda (egf)	16,00	0,00	0,00	0,00	0,00	10,60	0,00	0,00	0,00	5.32	34,60	0,00	0,00	0,00	0,00	0,00	0,00	0,00	34.6	0,00	0,00
Taenia sp.	16,00	0.00	0,00	0,00	0,00	10,60	0,00	0,00	0,00	0,00	2.66	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2.66	0,00	0,00
Oochoristica sp.	0.00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	5,32	13.3	0,00	0,00	0,00	0,00	0,00	0,00	0,00	26.6	0,00	0,00
Hymenolepis sp.	0.00	0.00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	18.6	0,00	0,00	0,00	0,00	0,00	0,00	0,00	5.32	0,00	0,00
Trematoda (egf)	0,00	0.00	0,00	0,00	0,00	0,00	0,00	2,66	0,00	0,00	2,66	13,30	0,00	0,00	0,00	0,00	0,00	0,00	2.66	0,00	0,00
Heterophyes sp.	0.00	0.00	0,00	0,00	0,00	0,00	0,00	2,66	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Troglotrema sp.	0.00	0.00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,66	13,30	0,00	0,00	0,00	0,00	0,00	0,00	2.66	0,00	0,00
Acantocephala (egf)	0,00	0.00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2.66	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Centrorhynchus sp.	0.00	0.00	0,00	0,00	0.00	0.00	0.00	0.00	0.00	2.66	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Comparison of results with those from studies in the literature showed several differences. The prevalence of coccidia was higher in our study (61.9 %), but comparison is difficult in view of the few references found and the low number of samples in the study of Rodríguez and Carbonell (1998) (n = 4). Focusing on the helminth fauna, the most studied group to date, and comparing our data with those from Miquel (1993) and Miquel et al. (1994a, 1994b), we found that prevalences were lower in our studies for all analyzed genera, except for *Uncinaria* sp. and *Baylisascaris* sp. (in both cases with values of 14.29 % compared to their 1.98 % or the 25 % of Di Cerbo et al. (2008) in *Uncinaria* sp.). Findings from other studies were also higher for the genera *Eucoleus* sp. (34 compared to our 19.05 %) and *Personema* sp. (22 % vs our 4.76 %). When comparing the values of *Taenia* sp. of Di Cerbo et al. (2008) we find a lower percentage (12.5 % compared to our 19.05 %) but the prevalence of *Aonchoteca* sp. was higher (22.2 % vs our 4.76 %).

The explanation for these differences may be the type of the sample and lower number of samples in our study. Neither can it be ruled out that the harsh environmental conditions in this area compared to the Mediterranean areas may play a role (Miquel et al., 1994b; Rosalino et al., 2011). External environmental conditions have been found to be indispensable for the survival of helminths (Kates, 1965) and protozoa (Schustera and Visvesvara, 2004), especially when frosts occur (Robertson et al., 1992), a frequent circumstance in the study area.

All the parasites found have a low host specificity and are common in several families of wild carnivores, probably due to their similar diet composition (Miquel et al., 1994b; Ribas et al., 2004). The lower parasite richness detected in comparison with other populations and the good external appearance of the observed specimens may indicate that the stone martens help to disseminate these parasites in wild carnivores.

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