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Artículo Original

The occurrence of endoparasites of roe deer (*Capreolus capreolus*) in two different areas from nw Spain

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ABSTRACT

From April to October 2007 individual faecal samples from the rectum of 185 roe deers were collected. The alimentary tracts were dissected out of the animals killed during hunting in two different areas from NW Spain. Samples were collected from animals living in a mountainous area and in another one closer to farmland. The presence of parasitic forms was assessed by means of coprological techniques. Eighty-eight percent (95% CI 84, 92) of the faecal samples had parasitic forms, and a prevalence of 35% (28, 42) coccidia, 3% (1, 5) cestoda, 66% (59, 73) gastrointestinal and 54% (47, 61) bronchopulmonary nematoda was achieved. Trematoda-eggs were not detected. Seven species of *Eimeria* were identified, being the most prevalent *E. patavina, E. capreoli* and *E. catubrina*, and *E. rotunda, E. ponderosa, E. panda* and *E. superba* the lowest ones. We observed eggs from Trichostrongylids (65%), *Trichuris* spp. (5%) *Nematodirus* spp. (3%) and *Moniezia* spp. (3%). By means of the migration technique, larvae from *Varestrongylus capreoli* (45%) and *Dictyocaulus capreolus* (26%) were recovered. A significantly higher risk for gastrointestinal nematode infection in the animals from the areas closer to farmland was shown. Our findings support the interest for studies about the role of wild animals as natural reservoirs for parasite infections in domestic livestock. Further research is in progress to gain more data on this subject.

Key words: Roe deer, endoparasites, oceanic climate, coprology, mountain habitat.

RESUMEN

De abril a octubre de 2007, se recogieron 185 muestras de heces de corzo directamente del recto. Durante la temporada de caza, se recogió el tracto digestivo de los corzos abatidos en dos zonas diferentes del noroeste de España, una era una zona montañosa y la otra era más llana y próxima a zonas de cultivos agrícolas. La presencia de formas parasitarias se evaluó mediante diferentes técnicas coprológicas. En el

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88% (95% IC 84, 92) de las muestras de heces se observaron formas parasitarias, siendo la prevalencia de ooquistes de coccidios del 35% (28, 42), de huevos de cestodos del 3% (1, 5), de huevos de nematodos gastrointestinales del 66% (59, 73) y de larvas de nematodos broncopulmonares del 54% (47, 61) y no se detectaron huevos de trematodos. Se identificaron siete especies de *Eimeria*, siendo las más frecuentes *E. patavina*, *E. capreoli* y *E. catubrina*, mientras que *E. rotonda*, *E. ponderosa*, *E. superba* y *E. panda* fueron las que en presentaron prevalencias más bajas. Además, se identificaron huevos de Tricostrongílidos (65%), *Trichuris spp.* (5%), *Nematodirus spp.* (3%) y *Moniezia spp.* (3%) y larvas de *Varestrongylus capreoli* (45%) y *Dictyocaulus capreolus* (26%). Se detectó un riesgo significativamente mayor de infección por nematodos gastrointestinales en los animales de las zonas más cercanas a las tierras agrícolas. Nuestros resultados muestran la importancia de los estudios sobre el papel que los animales silvestres pueden desempeñar como reservorios naturales de infecciones parasitarias para el ganado doméstico; no obstante, es necesario continuar las investigaciones sobre este tema.

Palabras clave: Corzo, endoparásitos, coprología, habitat de montañas, España.

INTRODUCTION

It has been demonstrated that digestive infections by parasites cause production reduction in domestic animals worldwide (Irvine et al, 2006). The main effects are often sub clinical, consisting of reduction of appetite, growth, reproduction and lactation (Forbes et al, 2000).

Although most parasitological studies have been focussed on domestic animals, it has been recently established that parasitic infections are just as common and important in wild animals, which can serve as potential reservoirs (Samuel et al, 2001; Simpson 2002; Shimalov and Shimalov 2003; Böhm et al, 2007).

The region of NW Spain has an oceanic climate (Kottek et al, 2006), which coincides with the weather found along the west coasts at the middle latitudes (40°-60°N) of all the world continents and in southeastern Australia. Similar climates are also found at coastal tropical highlands and tropical coasts on the leeward sides of mountain ranges. These conditions allow that domestic ruminants are kept under field conditions in semi-extensive management throughout the year (Díaz et al, 2007).

The application of the Common Agricultural Policy (CAP) has initially led to considerable agricultural intensification in EU member states (Matson et al, 1997). The main consequences were the increment in the arable surface and the reduction in the forested areas, responsible for the fall in the number of wild animals also. This stimulated the appearance of reserve areas for preserving these animals.

The EU Rural Development Regulation of the Agenda 2000 has lessened to develop some measures (agro-environmental, early retirement schemes, forestation of cultivated land, and payments to assist farmers in Less Favoured Areas, LFA) and other measures, such as farm investment, the installation of young farmers, training, investment aid for processing and marketing facilities, additional assistance for forestry, promoting adaptation and development of rural areas (Stoate et al. 2001).

Because of this new policy, the colonisation of natural vegetation over the arable landscape has been favoured, and an increase in the roe deer population in that areas during the last decade has been proved (Acevedo et al, 2005; Gamarra et al, 2008). Some estimates indicated that the roe deer population is over than 22,500 animals in the region of study.

The main goal of the current study was to get more knowledge about the endoparasites affecting roe deer in Northwest Spain. For this purpose, faecal and digestive samples from 185 animals were collected in a National Game Reserve and in several small hunting areas. The prevalence and intensity of gastrointestinal and pulmonary parasitic infections in roe deer was established by means of coprological techniques and analyzed according to the age of the animals and their origin.

There is a lack of knowledge about the endoparasites infecting this host species, which could reduce their health status and their resistance to

other diseases, diminishing thus their possibilities for survival. For this reason, a study to analyze the presence of endoparasites in NW Spain was conducted.

MATERIALS AND METHODS

Animals and study area: This study was carried out throughout the hunting season of 2007, between April and October, in Galicia (NW Spain) (42º20' - 43º45' N, 6º49' - 8º00' W). The climate in this region is warm temperate, with mild and dry summers and rainy winters with moderate temperatures (Díaz et al, 2008). In order to analyze the effect of different climatic factors such as mean annual temperature and precipitation, data recorded from different weather stations next to the study areas between the years 1999 and 2004 were used (Meteogalicia, Xunta de Galicia). We used the program Autocad 2004 and 1:5,000 topographical maps also.

A total of 185 faecal and digestive samples were collected from two different zones (Table 1). Sixty eight samples were collected from zone I, corresponding to the Ancares National Game Reserve (402,084.2 ha) (42°49′ N - 6°52′ W). This is a mountainous area with an altitude ranging from 650 to 1,500 m and a mean slope higher than 25%, where the mean annual temperature is below than 11ºC and the mean annual rainfall is higher 1500 mm. Nowadays this reserve is under the control of the Galician regional government and constitutes an important area for protection of autochthonous flora (*Ilex aquifolium*, *Erica cillaris*, *Quercus* sp., etc.) and fauna (*Ursus arctos*, *Tetrao urogallus*, etc). Hunting is only allowed under special conditions (Panadero et al, 2001; López et al, 2003). In this area, people inhabits in small villages dedicated principally to traditional agricultural practices, in especially to the production of meat cattle by extensive system. According to Martínez (1997), the mean density of roe deer is over 6.3/km^2.

The remaining roe deer (117) were from zone II, composed by some small game reserves in NW Spain, where the population density of this cervid is lower (5.2/km^2) because of the closeness to human settlements. In these reserves the altitude varies from sea level to 650 m, the mean slope from 13 to 25%, the mean annual temperature between 12 and 14ºC, and the mean annual rainfall from 1,300 to 1,500 mm.

By considering the age of roe deer, two groups were considered, one with animals less than 3 years (28 animals) and another one with deer between 3 and 10 years (157 animals).

Samples: Faecal samples were collected directly from the intestine of harvested animals. The coprological analyses (flotation, sedimentation and migration) were made in duplicate (MAFF, 1986). The results were expressed as the number of oocysts (opg), eggs (epg) and larvae per gram of faeces (lpg).

The identification of *Eimeria* species was done according to Pellérdy (1974). According to the morphological characteristics of gastrointestinal nematode eggs were identified as Trichostrongylids, *Nematodirus* and *Trichuris*. Larvae of bronchopulmonary nematodes were identified by considering previous investigations (Panadero et al, 2001, Carreño et al, 2009).

Statistical analysis: The median (Me) was calculated as a tendency marker and the interquartile range (IQR) as a dispersion measure. Version 12 of the SPSS software package (SPSS Inc., Chicago, IL) was used to conduct analyses of median (by using the non-parametric Kolmogorov-Smirnov test), and the prevalences (by means of the χ^2 test). A P-value ≤ 0.05 was considered indicative of a statistically significant difference. The possible relationship between the different factors was assessed by calculating the crude odds ratio (OR).

The prevalences were expressed as the value and the 95% Confidence Inteval (CI).

RESULTS

a) Parasitological findings: The presence of endoparasites was detected in 88% (95% CI 84, 92) of the faecal samples. As drawn in Table 2, we observed oocysts of *Eimeria*, eggs of cestoda, gastrointestinal nematodes or larvae of bronchopulmonary nematodes. Nevertheless, the samples were negative to trematode eggs.

b) Prevalence of endoparasites: The overall prevalence for *Eimeria* was 35% (28, 42). We found the most prevalent were *E. patavina* (57%; 50, 64), *E. capreoli* (21%; 15, 27) and *E. catubrina* (12%; 8, 16), and the lowest *E. rotunda, E. ponderosa,*

E. panda and *E. superba* (minor than 1%). The presence of gastrointestinal nematodes eggs was observed in 66% (59, 73) of the samples analysed.

The prevalence of infection by bronchopulmonary nematodes was elevated (54%; 47, 61). Larvae of *Dictyocaulus capreolus* and *Varestongylus capreoli* were identified in the faeces by means of the migration technique. Finally, a prevalence of 3% by cestodes was achieved.

Taking into account the age of the animals (Table 3), the highest percentages of infection by *Eimeria*, Trichostrongylids, *Trichuris*, *Moniezia* were detected in the youngest animals (less than 3 years). On the contrary, the greatest percentages of infection by lungworm nematodes were observed in the adult animals.

According to the origin of the samples (Table 4), the highest percentages of infection and odds ratio values by lungworm nematodes were detected in the zone I, while the highest percentages of infection by Trichostrongylids and *Trichuris* were observed in the zone II.

c) Intensity of infection by endoparasites: The level of infection by the different parasites is drawn in Table 3. Elevated values for *Eimeria* were obtained, whereas the egg-output of gastrointestinal nematodes was moderate, and that of cestodes and bronchopulmonary nematodes low.

Regarding age of the animals (Table 3), the youngest animals passed the highest values of *Eimeria*-oocysts. The greatest values of cestodes egg-output were obtained in the roe deer with more than 3 years. A similar value for gastrointestinal egg-output was obtained in respect to the age of the roe deer. The presence of lung nematode larvae was higher in the youngest animals. The Kolmogorov-Smirnov test showed no differences related to the age of the animals.

Table 4 summarizes the influence of the origin of the samples on the intensity of elimination. The greatest values for coccidian egg-output were obtained in the zone I roe deer, although those for cestodes and nematodes were achiveved in the samples belonging to zone II. Significant differences were proved only in the number of nematode eggs-passed by faeces.

d) Risk analysis (OR): The values for OR are represented in Tables 3 and 4. Although significant differences were not proved by the Kolmogorov-Smirnov test, the highest values for coccidian, cestodes and gastrointestinal nematodes were achieved in the youngest animals (Table 3). On the contrary, the animals older than 3 yr showed the greatest OR values for bronchopulmonary nematodes.

The relation between the zone where the animals were living and the occurrence of endoparasites is drawn in Table 4. A significantly higher OR value for gastrointestinal nematoda (Trichostrongylids) in the animals from zone II was observed. We proved also a higher risk infection by lung nematodes in the zone I, but significant differences were not achieved ($P > 0.05$).

Table 1. Distribution of the faecal samples obtained from hunted roe deer in NW Spain

Age	Area		Total
	Zone I	Zone II	
< 3 yr	5	23	28
3-10 yr	63	94	157
Total	68	117	185

Table 2. Endoparasites identified in the faeces of roe deer by coprological methods

Parasitic group	Genera	Species identified
Protozoa	*Eimeria spp*	*E. patavina* *E. capreoli* *E. catubrina* *E. rotunda* *E. ponderosa* *E. panda* *E. superba*
Cestoda	*Moniezia* spp	
Nematoda	Gastrointestinal	Trichostrongylids *Nematodirus* *Trichuris*
	Lung	*Varestrongylus capreoli* *Dictyocaulus capreolus*

DISCUSSION

We observed oocysts of *Eimeria*, eggs of cestodes, gastrointestinal nematodes and larvae of bronchopulmonary nematodes. These results are in

Table 3. Analysis of the endoparasites found in the faeces of hunted roe deer by considering the age of the animals

Parasitic group	Genera	Group age (years)	Prevalence (95% CI)		χ^2 analysis		OR opg / epg / lpg			K-S analysis	
					χ^2	P	Median	IQR		F	P
Protists	*Eimeria* spp	<3	43% (36, 50)	0.949	0.390	1.5	600	988		1.061	0.210
		3-10	33% (27, 40)			0.7	250	638			
Cestodes	*Moniezia* spp	<3	4% (1, 6)	0.010	1	1.1	100	0			
		3-10	3% (1, 6)			0.9	400	425			
Gastro-intestinal Nematodes	Total	<3	79% (73, 84)	2.224	0.192	2.1	100	150			
		3-10	64% (57, 71)			0.5	100	100			
	Trichos-trongylids	<3	75% (69, 81)	1.541	0.284	1.8	100	150		0.396	0.998
		3-10	63% (56, 70)			0.6	100	100			
	Nematodirus spp	<3		0.923	0.108						
		3-10	3% (1, 6)				50	75			
	Trichuris	<3	7% (3, 11)	0.360	0.628	1.6	50	0		0.000	1
		3-10	4% (2, 7)			0.6	50	50			
Lung Nematodes	Total	<3	46% (39, 53)	0.723	0.417	0.7	4	13			
		3-10	55% (48, 72)			1.4	1	4			
	Varestrongylus capreoli	<3	43% (36, 50)	0.039	1	0.9	1	3		0.655	0.784
		3-10	45% (38, 52)			1.1	1	3			
	Dictyocaulus capreolus	<3	18% (12, 23)	1.026	0.357	0.6	16	24		0.986	0.285
		3-10	27% (21, 33)			1.7	2	7			

agreement with that reported from different European areas (Hidalgo et al. 1996; Rossi et al. 1997; Panadero et al. 2001; Pérez et al. 2003; Pilarczyk et al, 2005). We did not observe trematode eggs in the faeces. Samuel et al, (2001) proved that wild cervids are not significant reservoirs for *F. hepatica* and the limited infections may be explained by an inherent resistance to infection of these hosts.

The coccidian prevalence was higher in the youngest animals. The prevalence of infection by gastrointestinal nematodes in this study was lower that that obtained by necropsy (100%) by Díez-Baños et al, (1995) and (100%) Pilarczyk et al, (2005).

Most of the studies about wild animals have been carried out on that living in a mountain habitat (Rossi et al, 1997; Pérez et al, 2003). Roe deer usually live in mountain areas where the climate conditions do not favour survival of parasitic transmission stages, thus the potential sources of infection are relatively few.

Nevertheless, the population of roe deer living in areas close to human settlements, including gardens and pastures is increasing (Hamnes et al, 2006). This might expose them to more faecal contamination from various sources and thus they might have increased possibility of ingesting resistant parasitic forms. In the current investigation, faecal samples were analyzed from roe deer hunted in two different areas, one mountainous and the other near to domestic animal farms. We obtained a significantly higher OR value for gastrointestinal nematodes (Trichostrongylids) in the animals from farmland localizations. This could be attributable to that those zones with smaller altitude and slopes, and moderate temperatures are favourable for the development and survival of free gastrointestinal nematode stages and their persistence in the grass (Waruiru et al, 2001). Besides this, it seems reasonable to believe that feeding close to the ground would bring them in closer contact with faecal deposits from other animals, thus increasing

Table 4. Analysis of the endoparasites found in the faeces of hunted roe deer by considering the habitat of the animals

Parasite group	Genera	Area	Prevalence (95% CI)	χ^2 analysis χ^2	OR	opg / epg / lpg P	Median	IQR	K-S analysis F	P
Protists	*Eimeria* spp	Zone I	34% (28, 41)	0.010	1	1.0	250	600	0.985	0.227
		Zone II	35% (28, 42)			1.0	450	700		
Cestodes	*Moniezia* spp	Zone I		3.552	0.088					
		Zone II	5% (2, 8)				300	413		
Gastro-intestinal Nematodes	Total	Zone I	60% (53, 67)	2.056	0.195	0.6	150	150	1.464	0.028
		Zone II	70% (64, 77)			1.6	100	100		
	Trichos-trongylids	Zone I	58% (51, 65)	1.928	0.165	0.6	150	150	1.416	0.036
		Zone II	68% (62, 75)			1.6	100	100		
	Nemato-dirus spp	Zone I	6% (3, 9)	4.217	0.060	7.4	50	113		
		Zone II	1% (0, 2)			0.1	50	0		
	Trichuris	Zone I	3% (1, 5)	0.823	0.364	0.5	50	0	0.356	1
		Zone II	6% (3, 9)			2.1	50	50		
Lung Nematodes	Total	Zone I	63% (56, 70)	3.347	0.091	1.8	1	4	0.703	0.707
		Zone II	49% (42, 56)			0.6	2	10		
	Varestron-gylus capreoli	Zone I	49% (42, 56)	0.938	0.358	1.3	1	3	0.615	0.844
		Zone II	42% (35, 49)			0.7	1	3		
	Dictyo-caulus capreolus	Zone I	27% (21, 33)	0.097	0.861	1.1	3	8	0.568	0.811
		Zone II	25% (19, 31)			0.9	2	17		

the possibility of ingesting nematode larvae. However, the high prevalence of *V. capreoli* and *D. capreolus* in roe deer from the mountain zone could be explained by a greater abundance of intermediate hosts in this area.

This study demonstrates that endoparasites are widespread among the roe deer population. A rapid development of shrub caused for an aabandonment of agriculture results in a food supply increase and more defense of roe deer, and this may increase the number of that wild ruminants. Large wild animals may provide a significant reservoir of infection because of the substantial quantity of droppings, which they produce. As free roaming animals, roe deer can contaminate not only pastures but surface waters and recreational areas also, and thus the occurrence of parasites needs further investigation.

Our findings support the interest for studies about the role of wild animals as natural reservoirs for parasite infections in domestic livestock. Further research is in progress to gain more data on this subject.

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