Prevalence of Sarcocystis in free ranging roe deer (Capreolus capreolus) from Lugo (NW, Spain) and its distribution in different muscles

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Received: 22.04.01 Accepted: 31.05.01

Abstract: The esophagus, diaphragm and heart muscle of 154 roe deer (Capreolus capreolus) hunter-killed in Lugo province (Northwestern Spain) were examined, by the compression method, for the presence of sarcocysts of Sarcocystis spp. The overall prevalence of infection was 80.5%, with a density of infection of 15 ± 16.8 cysts/g. The highest density of infection corresponded to the heart, and in particular to the inner part of the myocardium. Considering the different studied areas, higher prevalence was observed in Ancares reserve (97.3%) than in other areas (81.1%).

Key words: Wildlife, roe deer, Capreolus capreolus, Sarcocystis spp, Lugo.

Resumen: Se ha analizado, mediante el método del compresorio, el esófago, diafragma y corazón de 154 corzos (Capreolus capreolus) capturados en la provincia de Lugo (Noroeste de España). La prevalencia de Sarcocystis spp fue del 80%, con una densidad de parasitación de 15 ± 16,8 quistes/g, siendo el corazón y, más concretamente, la porción interna del miocardio la más afectada. Los valores de prevalencia, en relación con las áreas ecológicas estudiadas, son superiores en la reserva de Ancares (97,3%) que en otras zonas (81,1%).

Palabras clave: Fauna silvestre, corzo, Capreolus capreolus, Sarcocystis spp, Lugo.

1. Introduction
Roe deer (Capreolus capreolus, L.), the smallest of Spanish cervids, are widespread in the Northwest of Spain. In this area, roe deer are characterized by the presence of two winter-white spots on the neck and are unofficially assigned sub-specific status as Capreolus capreolus decorus. In Europe roe deer are of considerable economic value as a source of meat. In addition, they are a sport hunting species, which also generates revenue. Approximately 2.5 million are shot in Western Europe each year (Linnell and Andersen, 1998).

Sarcocystis is a protozoan parasite common in the muscles of domestic and wild animals. In most animals, infections are not considered to be of any serious pathogenic significance. However, heavy infections can result in loss of weight, anemia, abortion, and even death (Dubey et al., 1989). The life cycle of Sarcocystis involves two hosts, a predator and a prey. In Northwestern Spain, principally wolves and stray dogs assume the predator role, whereas domestic and wild herbivorous act as intermediate hosts. So that, a roe deer eating the grass contaminated by infected faeces becomes the intermediate host, in which the cysts survive for years before eventually degenerating and becoming reabsorbed.

The work described herein is part of a larger study examining the parasite fauna of roe deer in Lugo province. Its purpose was to estimate the prevalence and density of infection by Sarcocystis spp in roe deer, as well as the influence of different factors on the incidence of this parasite.

2. Materials and Methods
2.1) Animals and study area
Between 1993 and 1995, 154 roe deer, hunter-killed in Lugo province (Northwestern Spain), were
examined for *Sarcocystis* spp infection. The animals were shot principally in summer and autumn and their age ranged from 2 to 7 years.

Collections of animals were from hunting reserves throughout the province. Forty-one of the roe deer were obtained from Ancares, a large national hunting reserve (12,667 ha) located in the western region of the Cantabrian mountain range (42°49'N-6°52'W). The remaining samples were obtained from small reserves distributed throughout the province that were grouped in four different areas (Figure 1).

### 2.2) Samples

Samples were taken from the diaphragm, esophagus and heart, and examined for the presence of sarcocysts by the compression method. Thirty samples of about 0.5 g (1-1.5 cm long x 1-2 mm thick) from each muscle were cut, pressed between trichinoscope plates and examined under the dissecting microscope at 4.5x magnification. Because of the thickness of the myocardium, in this muscle we differentiated an inner, middle and outer part; and we took 10 samples from each one. Samples were stained with a drop of metilen blue-eosin to make easier the visualization of the microcysts. Density of infection is given as number of sarcocysts/gram ± standard deviation.

### 2.3) Statistical analysis

Analysis of data included the chi-square test to compare prevalences in different geographic areas; furthermore, McNemar’s test was used to establish differences in the level of infection among the different muscular organs.

Analysis of variance (ANOVA) was used to compare the density of infection in the studied areas. The Friedman test for dependent samples was employed to determine if there were statistical differences between the densities of infection in the examined muscles, and if so, the range Wilcoxon test was applied to localize these differences. All tests were performed by the statistical package SPSS, version 6.1.3 (SPSS Inc. 1995).

### 3. Results

*Sarcocystis* spp was found in 124 of 154 roe deer (80.5%), in at least one of the examined muscular organs. The mean density of infection was 15 ± 16.8 sarcocysts/g. No macroscopic sarcocysts were found in any of the muscular samples.

The percentage and density of infection in the examined muscles are shown in Table 1. The frequency of sarcocysts in the heart, esophagus and diaphragm was very similar and no statistical differences were found. Nevertheless, taking into account the density of infection the Friedman test showed significant differences ($A^2 = 44.981$; df. = 2; $p < 0.001$); the Wilcoxon test found that the heart was more infected than the esophagus ($Z = -6.182$; $p < 0.001$) and the diaphragm ($Z = -5.675$; $p < 0.001$). With regard to the part of the myocardium where the cysts were found, the density of infection in the outer part was lower than in the intermediate ($Z = -5.740$; $p < 0.001$) and the inner part ($Z = -6.028$; $p < 0.001$), and in the same way that of the intermediate it was lower than in the internal part ($Z = -2.036$; $p < 0.042$).

Data referring the origin of the animals are showed in Table 2. The overall prevalence varied among locations,
with significantly more animals from Ancares being infected ($A^2 = 5.701; p = 0.017$) in comparison to those of smaller reserves as a whole. The density of infection was also higher in Ancares, but the differences were not significant ($F = 2.305; p = 0.087$). In the minor game reserves, we found that in the mountainous area, the prevalence was very high and statistically superior to the Coastal ($A^2 = 6.660; p = 0.020$), Central ($A^2 = 8.552; p = 0.005$), and Southern ($A^2 = 11.921; p = 0.001$) areas of the province.

### 4. Discussion

Sarcocystis is one of the most prevalent parasites of wild animals and especially in cervids (Dubey et al., 1989). The current study showed that in Northwestern Spain, roe deer are frequently infected by Sarcocystis spp.

However, our prevalence was lower than the 100% reported by Santini et al. (1997) in Italy and Diez-Baños et al. (1997) in the neighboring province of León, but much higher than the 16.66% found by Navarrete et al. (1990) in Cáceres province. Despite the high prevalence, the density of infection can be considered as moderate, being inferior to the 27 pointed by Diez-Baños et al. (1997) in roe deer from León.

The tendency for prevalence to increase with host age seems to be a common pattern with Sarcocystis. This should be interpreted as a function of enhanced infection opportunities with the passage of time. In the present study all of the animals were adults, which may explain the high prevalence rate.

Although there was no difference in the frequency of infection among the examined organs, the myocardium was the most intensely infected muscle, which is in agreement with another studies in fallow (Poli et al., 1988) and roe deer (Diez-Baños et al., 1997). The finding of relatively large numbers of cysts in the myocardium could indicate a preference of the species implicated for aerobic and well vascularized muscular fibers, very abundant in the heart (Wheater et al., 1987).

Sarcocysts can be present all over the myocardium (Dubey and Kistner, 1985). The particular distribution of the sarcocysts throughout the myocardium in this study could indicate that the invasion by the merozoites of muscular fibers starts by the inner part, followed by the middle and the outer part.

Roe deer from mountainous areas presented higher prevalences and densities of infection that could be explained by the abundance of definitive and intermediate hosts that increase the infection pressure.

However, further studies are needed to identify the species of Sarcocystis implicated and their definitive hosts.

### 5. Acknowledgments

The authors thank the “Delegation Provincial de Medio Ambiente” of Lugo for the opportunity and facilities to carry out this study. We are also in debt with Dra Natividad Díez Baños (Faculty of Veterinary of León) for her valuable advices and with the gamekeepers of the Hunting National Reserve of Ancares for their collaboration in the fieldwork.

### 6. References


### Table 2. Percentage and density of infection (cysts/g) by Sarcocystis spp in roe deer from different game reserves in Lugo (Northwestern Spain).

<table>
<thead>
<tr>
<th>Area</th>
<th>Percentage (%)</th>
<th>Density of infection ± SD</th>
</tr>
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<tbody>
<tr>
<td>Ancares</td>
<td>97.3</td>
<td>32 ± 45.42</td>
</tr>
<tr>
<td>Another reserves</td>
<td>81.1</td>
<td>9.22 ± 11.16</td>
</tr>
<tr>
<td>Coast</td>
<td>78.9</td>
<td>16.08 ± 17.66</td>
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<tr>
<td>Center</td>
<td>73.9</td>
<td>13.78 ± 18.62</td>
</tr>
<tr>
<td>Mountain</td>
<td>100</td>
<td>46.12 ± 75.80</td>
</tr>
<tr>
<td>South</td>
<td>65.2</td>
<td>4.36 ± 3.24</td>
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