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# *Sarcocystis* spp. infection in roe deer *(Capreolus capreolus)* from the north-west of Spain

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## 1 Introduction

Roe deer (*Capreolus capreolus* L.), the smallest of Spanish cervids, are widespread in north-western Spain. They have an important value as a sport hunting species, as well as a source of meat. Roe deer from north-western Spain are characterized by the presence of two winter-white spots on the neck and are unofficially assigned sub-specific status as *C. c. decorus*.

*Sarcocystis* spp. (Apicomplexa: Sarcocystidae) are protozoan parasites common in the musculature of wild and domestic animals. Completion of the *Sarcocystis* cycle requires two host species: an intermediate (or prey) and a definitive (or predator) host. Intermediate hosts can harbour more than one species of *Sarcocystis*, and according to Cornaglia et al. (1998) the same species of *Sarcocystis* can infect different wild and domestic ruminants. In most animals, infections are not considered to have any serious pathogenic significance, but heavy infections in intermediate hosts can result in loss of weight, anaemia, abortion, and even death (Dubey et al., 1989).

The number of *Sarcocystis* species in roe deer is not clear and a confused nomenclature has been used for a long time. Dubey et al. (1989) described four species: *S. gracilis, S. capreoli, S. sibirica* and the unnamed species *Sarcocystis* sp. More recently, Sedlaczek and Wesemeier (1995) reconsidered and corrected the existing descriptions of *Sarcocystis* species in the European roe deer, establishing four species: *S. gracilis, S. capreolicanis, S. hofmanni* and *Sarcocystis* sp.

The purpose of this study was to determine the prevalence and density of infection by *Sarcocystis* spp. in roe deer, and to study by light (LM) and transmission electron microscopy (TEM) the morphology and ultrastructure of sarcocysts in order to determine the species infecting roe deer in north-western Spain.

## 2 Materials and Methods

### 2.1 Animals and study area

Between 1993 and 1995 a total of 132 free-ranging roe deer, hunted in game reserves from Lugo province (north-western Spain), were examined for *Sarcocystis* infection. The animals, 126 males and 6 females, were shot principally in summer and autumn and their age ranged between 2 and 7 years. Thirty-seven of the 132 roe deer were captured at 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). This reserve is managed by the Galician government and is an important area for protection of the autochthonous flora (*Ilex aquifolium, Erica cillaris, Quercus* sp., etc.) and fauna (*Ursus arctos, Tetrao urogallus*, etc.), where hunting is only allowed under special conditions. In this area, people inhabiting the small villages principally practise traditional agriculture. The remaining 95 samples were obtained

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from small hunting preserves distributed throughout the province. According to MARTÍNEZ (1997) Ancares preserve has the highest density of roe deer in Spain (26.5/100 ha), whereas in the other preserves included in this study, the population is much lower because of the closeness to big human settlements that modify considerably the deers' habitat.

### 2.2 Samples

After killing the animals and separating their heads, that hunters keep as trophy, the deer were maintained in refrigerated condition until carried to the laboratory for necropsy.

Samples of diaphragm, oesophagus and heart were examined for the presence of sarcocysts by the compression method. Thirty samples of about 0.5 g (1–1.5 cm long × 1–2 mm thick) from each muscle were cut, pressed between trichinoscope plates and examined under the dissecting microscope at 45× magnification. Because of the thickness of the myocardium, in this muscle three parts (inner, middle and outer part) were differentiated, taking 10 samples from each one. Fresh samples were stained with a drop of methylene blue-eosin to enhance the visibility of the microcysts.

In order to study the shape and size of the cysts by light microscopy, samples from positive animals were fixed in buffered 10% formalin, embedded in paraffin, sectioned and stained with haematoxylin-eosin (H&E).

For the ultrastructural study, positive samples were fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, post-fixed with 1% osmium tetroxide and embedded in Epon-812 resin. Ultra thin sections were made by a RMC MT6000 XL (RMC, Tucson AZ), stained with uranyl acetate and lead citrate, and observed using a Jeol JM 100 SX (Jeol Co. LTD, Tokyo).

### 2.3 Statistical analysis

Analysis of data included the chi-square test to compare prevalences in the studied areas, and the McNemar's test to establish differences in the percentage 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

### 3.1 Prevalence and density of infection

The overall prevalence of infection by *Sarcocystis* was 85.6% (113 out of 132 roe deer). The mean density of infection was 15 ± 16.8 cysts/g.

The overall prevalence varied among locations (97.3% in Ancares *vs.* 81.1% in the small reserves as a whole), with significantly more animals from Ancares being infected ($\chi^2 = 5.701$; $P = 0.017$). The density of infection was also higher in Ancares (32 ± 45.42 *vs.* 9.22 ± 11.16), but the differences were not significant ($F = 2.305$; $P = 0.087$).

The prevalence and intensity of infection in the examined muscles are shown in Table 1. The myocardium was the most commonly infected muscle, followed by the diaphragm and the oesophagus. Significant differences occurred in the density of infection as indicated by the FRIEDMAN test ($\chi^2 = 44.981$; d.f. = 2; $P < 0.001$), and the Wilcoxon test found that the

*Table 1.* **Percentage and mean intensity of infection (cysts/g) by *Sarcocystis* spp. in different muscles of roe deer from Lugo (north-western Spain).**

| | Diaphragm | Oesophagus | Myocardium | | | |
|---|---|---|---|---|---|---|
| | | | Total | Inner part | Middle part | Outer part |
| Prevalence (%) | 75.2 | 73.1 | 77.3 | 75.2 | 78.1 | 76.3 |
| Intensity ± SD | 6.6 ± 10.62 | 9 ± 16.2 | 28.8 ± 48.28 | 35.8 ± 57.48 | 31.2 ± 50.98 | 23.2 ± 43.68 |

heart was more often infected than the oesophagus ($Z = -6.182$; $P < 0.001$) 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 was lower in the outer part than in the middle ($Z = -5.740$; $P < 0.001$) and the inner part ($Z = -6.028$; $P < 0.001$), and in the middle it was also lower than in the inner part ($Z = -2.036$; $P < 0.05$).

### 3.2 Morphology and ultrastructure

By light microscopy all cysts were fusiform-shaped and compartmentalized in their interior. Size differences were observed in the sarcocysts from each of the three examined organs (Table 2). Cysts localized in the myocardium were smaller than those found in the diaphragm.

*Table 2.* **Size (in µm, mean ± SD) of cysts of *Sarcocystis* in heart, oesophagus and diaphragm of roe deer from the north-west of Spain.**

| | Myocardium | Oesophagus | Diaphragm |
|---|---|---|---|
| Length | 299 ± 81.8 (150–575) | 1143.1 ± 307.2 (225–2500) | 801.16 ± 322.9 (230–1875) |
| Width | 79.5 ± 20.7 (50–125) | 126.3 ± 53.2 (25–250) | 70.5 ± 27.4 (25–175) |

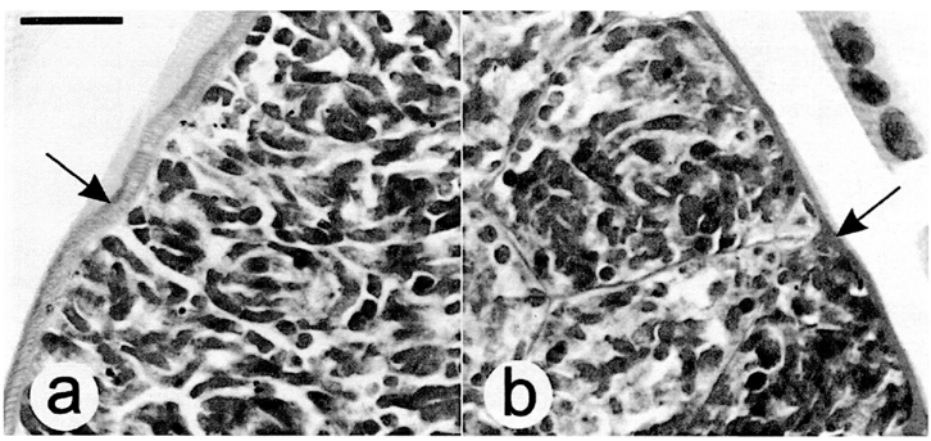


*Fig. 1.* (a) Thick-walled cyst of *Sarcocystis* in roe deer with palisade shaped projections (H&E, bar = 15 µm). (b) Thin-walled cyst of *Sarcocystis* in roe deer (H&E, bar = 15 µm).

In contrast, a wide range of length and width characterized oesophageal cysts. Regarding the arrangement of the cysts in the muscle, in the myocardium and diaphragm all of them ran in the same direction (parallel with muscle fibres), whereas those in the oesophagus were arranged perpendicularly.

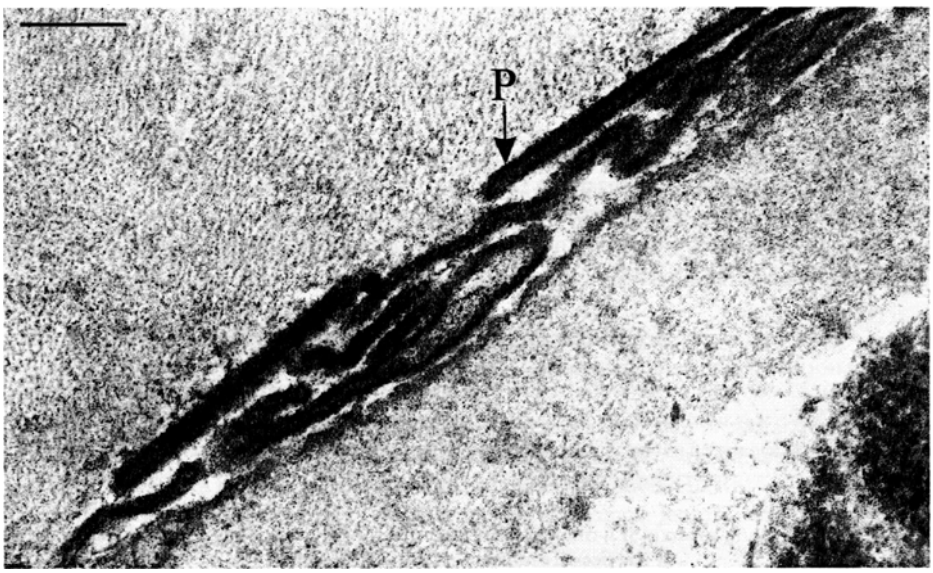


*Fig. 2.* TEM microphotograph of the primary sarcocyst wall with folded hair-like protrusions running in parallel with the surface of the cyst (bar = 0.25 µm).

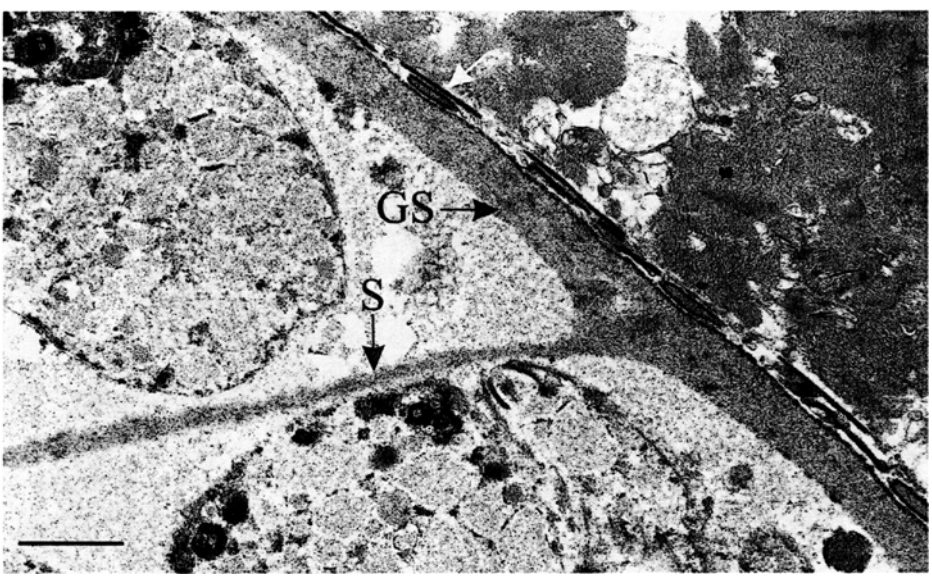


*Fig. 3.* TEM microphotograph of the primary sarcocyst wall. Ground substance (GS) and septa (S) separating compartments (bar = 1 µm).

Two morphologically distinct sarcocysts were observed in histological sections: Thin-walled cysts (< 1 µm) were observed in all of the parasitized animals (Fig. 1a). One thick-walled sarcocyst found in the oesophagus of a single roe deer, associated with thin-walled cysts; this sarcocyst was 370 µm long and 105 µm wide and its wall was 2 µm thick with palisade shaped projections (Fig. 1b).

The cyst wall appeared thin under the TEM and only one type of cyst wall could be differentiated on the basis of the size and shape of protrusions of the primary wall. This wall formed numerous folded villi-like protrusions without microtubules, which were 0.28 µm wide in their thicker part and 0.05 µm in their thinner part, and up to 0.94 µm long. These protrusions were running parallel to the surface of the cyst and were arranged in several closely spaced layers compressed against the cyst (Fig. 2). A 0.48 µm wide granular layer or ground substance was immediately beneath the primary sarcocyst wall. 0.26-µm wide septa, which arise from the granular layer traversed the sarcocyst separating compartments (Fig. 3).

## 4 Discussion

The results presented herein indicate a very high proportion of roe deer infected by *Sarcocystis*, which is in agreement with DUBEY et al. (1989), who consider this parasite as one of the most prevalent in wild animals and especially in cervids. However, prevalences were lower than the 100% reported by SANTINI et al. (1997) in Italy and by DÍEZ-BAÑOS et al. (1997) in the neighboring province of León (north-western Spain), although the values were much higher than the 16.6% found by NAVARRETE et al. (1990) in Caceres province (south-western Spain).

The high prevalence has epizootiological significance, as Lugo province has the highest density of population of roe deer in Spain (BRAZA et al., 1989), being considered as an important point of expansion of this ungulate, and its parasites, throughout the north-west of Spain. The high prevalence, especially in the Ancares preserve, could be explained by the abundance of roe deer in this preserve that increases the infection pressure. The prevalence of *Sarcocystis* infection increases with host age (ORYAN et al., 1996; HUONG, 1999), and in this study all of the animals were older than 2 years. Despite the high prevalence, the mean density of infection can be considered as moderate, being lower than the 27 cyst/g reported by DÍEZ-BAÑOS et al. (1997) in roe deer from León.

As shown by light microscopy, cysts can vary in size and shape depending on the species of the parasite, their localization, the age of the sarcocyst and the method used for study. It can be concluded that cyst size is not useful to differentiate *Sarcocystis* species. In this work, sarcocyst morphology varied from very long and narrow in the diaphragm to short and wide in the myocardium. SAVINI et al. (1996) attributed variations in shape and size of cysts in different muscles to the structure of the muscle itself and the pressure on the cyst depending on their location. Our results support this hypothesis since the myocardium showed the smallest cysts. In our opinion, this phenomenon could be attributed to the continuous contraction of this muscle or, according to WHEATER et al. (1987), to the smaller diameter of type I fibers that are very abundant in this organ.

By LM at 100× magnification all the cysts were thin-walled, except for one found in the oesophagus. This thick-walled cyst, that showed closely arranged finger-like protrusions, could be considered as *S. hofmanni*, in agreement with the microscopical description of SEDLACZECK and WESEMEIER (1995). Due to its low prevalence in our study, this species could not be studied by TEM. However, like SEDLACZECK and WESEMEIER (1995) we consider that cysts from this species can be easily distinguished by light microscopy from other *Sarcocystis* species that can infect roe deer.

Most *Sarcocystis* species produce a sarcocyst wall which shows unique structural features by LM, and therefore it fails to distinguish different species within the same intermediate host. All the sarcocysts observed by TEM had the primary wall folded to form hair-like protrusions that ran in parallel with the surface of the cyst. Ultrastructural morphology was consistent with the unnamed species of *Sarcocystis*, characerised as thin-walled cysts (SCHRAMLOVÁ and BLAŠEK, 1978) and cyst wall type IV (ENTZEROTH, 1982).

According to these results, 99.2% of infected roe deer showed a monoinfection with *Sarcocystis* sp., whereas 0.8% had mixed infection with *Sarcocystis* sp. and *S. hofmanni.* SANTINI et al. (1997) also found single infections of *Sarcocystis* sp. in roe deer from Italy. However, NAVARRETE et al. (1990) in a similar study in south-western Spain identified the species infecting roe deer as *S. capreoli* (syn. *S. capreolicanis*). SEDLACZECK and WESEMEIER (1995) detected three *Sarcocystis* species in the European roe deer from different areas in Germany and Poland, which corresponded to *S. gracilis, S. capreolicanis* and *S. hofmanni.*

Wild cervids may serve as important reservoirs of parasites for domestic ruminants. In Lugo province, especially in mountainous areas, cattle and sheep are maintained on pasture in an extensive or semi-extensive system that favors a mixed grazing with wild ungulates, multiplying the opportunities of accidental parasite transmission. However, this point remains unclear in the case of *Sarcocystis*. Some authors as ODENING et al. (1994) and CORNAGLIA et al. (1998) found that the same species can infect different intermediate hosts, whereas others, like ENTZEROTH (1982), affirmed that *Sarcocystis* is generally more host specific for intermediate than for definitive hosts. Taking into account that the species identified in this study are specific for the roe deer, this ungulate cannot be considered as an important reservoir of *Sarcocystis* for domestic ruminants in north-western Spain. However, further studies are needed to identify their definitive hosts.

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## Summary

Samples of heart, oesophagus and diaphragm muscle from 132 roe deer *(Capreolus capreolus)* hunted in game reserves from Lugo province (north-western Spain) were examined to determine the prevalence of *Sarcocystis* infection. Sarcocysts were examined by light (LM) and transmission electron microscopy (TEM). The overall prevalence of infection was 85.6%, with a density of 15 ± 16.8 cysts/g of muscle. The highest prevalence was observed in the large preserve in Ancares (97.3%) compared to that of the smaller reserves (81.1%). Size and shape differences were observed in the sarcocysts from the different muscular organs. The highest density of infection was in the heart, particularly to the inner part of the myocardium. LM distinguished two types of sarcocysts: thin-walled cysts present in all the positive organs and a thick-walled cyst, identified as *S. hofmanni,* found in the oesophagus of one animal. By TEM only one type of cyst wall, having a thin primary wall that formed folded hair-like protrusions, could be differentiated. Ultrastructural features of the cyst wall demonstrated that this species corresponds with the unnamed species *Sarcocystis* sp.

*Key words: Capreolus capreolus*, roe deer, *Sarcocystis* spp., ultrastructure, north-western Spain

## Zusammenfassung

*Infektion mit Sarcocystis spp. bei Rehen (Capreolus capreolus) aus Nordwest-Spanien*

Zur Bestimmung der Prävalenz von Sarcocystis-Infektionen wurden Herz-, Ösophagus- und Zwerchfellmuskel-Proben von 132 Rehen *(Capreolus capreolus)* aus Wildschutz-Gebieten in der Provinz Lugo (Nordwest-Spanien) untersucht. Die gefundenen Zysten wurden mittels Lichtmikroskopie und Transmissions-Elektronenmikroskopie untersucht. Die allgemeine Sarkosporidiose-Prävalenz betrug 85,6 %, die mittlere Befallsdichte lag bei 15 ± 16,8 Zysten/g Muskelgewebe. Die höchste Prävalenz wurde mit 97,3 % in einem großen Gehege in Ancarez beobachtet, während sie in kleineren Gehegen 81,1 % betrug. Es bestanden Unterschiede in Größe und Form zwischen den Zysten aus den Muskeln verschiedener Organe. Die höchste Befallsdichte wies der Herzmuskel, speziell das innere Myokard auf. Lichtmikroskopisch ließen sich zwei Zystentypen unterscheiden. Dünnwandige Zysten waren in allen positiven Organen vorhanden. Ferner fand sich eine einzelne dickwandige, als *Sarcocystis hofmanni* identifizierte Zyste im Oesophagus eines Rehes. Elektronenmikroskopisch ließ sich nur ein einziger Zystenwand-Typ differenzieren, der durch eine dünne Primärwand mit gefalteten, haarartigen Protrusionen charakterisiert war. Ultrastrukturelle Merkmale der Zystenwand zeigten, dass diese Art der unbenannten Spezies *Sarcocystis* sp. entspricht.


*Schlüsselwörter: Capreolus capreolus*, Reh, *Sarcocystis* spp., Ultrastruktur, Nordwest-Spanien


## Résumé

*Infection de Sarcocystis spp chez le Chevreuil (Capreolus capreolus) du Nord-Ouest de l'Espagne*

Des échantillons de muscles de cœur, d'œsophage et de diaphragme de 132 chevreuils *(Capreolus capreolus)* prélevés sur des animaux tirés dans les réserves de la province de Lugo (Nord-Ouest de l'Espagne) ont été examinés afin de déterminer la prévalence d'une infection de *Sarcocystis*. Les sarcocystes ont été analysés par microscopie optique (LM) et par microscopie électronique de transmission (TEM). La prévalence globale de l'infection était de 85,6 %, avec une densité de 15 ± 16,8 cystes par g de muscle. La prévalence la plus élevée fut observée dans la grande réserve d'Ancares (97,3 %), comparée à celle de réserves plus petites (81.1 %). Les différences de taille et de forme étaient observées dans les sarcocystes provenant de différents organes musculaires. La densité la plus élevée d'infection se situait dans le cœur, en particulier dans la partie interne du myocarde. Par LM on a pu distinguer deux types de sarcocystes: des cystes à paroi mince présents dans tous les organes positifs et un cyste à paroi large, identifié comme étant *S. hofmanni*, trouvé dans l'œsophage d'un animal. Par TEM un seul type de paroi de cyste, présentant une paroi primaire mince formant des protrusions filiformes, put être différencié. Des formations ultrastructurales de la paroi du cyste démontraient que cette espèce correspond à l'espèce innomée de *Sarcocystis* sp.


*Mots clefs: Capreolus capreolus*, Chevreuil, *Sarcocystis* spp., ultrastructure, Nord-Ouest de l'Espagne

Trad.: S. A. DE CROMBRUGGHE

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