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Prevalence of ovine *Sarcocystosis* in Cordoba, Spain

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Summary

The presence of *Sarcocystis* spp. was surveyed in 97 sheep in the province of Cordoba. Three methods were used for the detection of the parasite in three different muscular location (heart, esophagus and diaphragmatic muscle). *Sarcocystis* bradyzoites were found in 85.6% of the sheep by trypsin digest. Microscopic *S. tenella* in all infected animals and *S. arieticanis* in the diaphragmatic muscle of one sheep, and macroscopic *S. gigantea* in the esophagus of 6 sheep were identified. The prevalence of the infection varied with the age of the animals.

Key Words: *Sarcocystis* spp., ovine, prevalence.

Resumen

Hemos investigado la presencia de *Sarcocystis* spp. en 97 ovinos de la provincia de Córdoba, empleando tres métodos de estudio distintos para la detección del parásito en los distintos grupos musculares estudiados (corazón, esófago y diafragma). Mediante digestión trípsica encontramos bradizoitos de *Sarcocystis* en el 85,6% de los animales. Se identificaron quistes microscópicos de *S. tenella* en todos los ovinos parasitados y *S. arieticanis* en la musculatura diafragmática de un animal y quistes macroscópicos de *S. gigantea* en los esófagos de 6 ovejas. La prevalencia de la infección mostró una clara variación según la edad de los hospedadores.

Palabras Clave: *Sarcocystis* spp., ovinos, prevalencia.

Introduction

Little is known of the prevalence of ovine sarcocystosis in Spain. There are some data of prevalence of the infection in three provinces (Granada, Leon and Zaragoza) obtained by parasitological methods (direct microscopical examinations for the presence of the parasite) and serological tests, but in no case are there any reference on the incidence of different *Sarcocystis* spp., only the existence of macro and microcyst is pointed out.

The present work is intended to bring some data on the epidemiology of ovine sarcocystosis in the province of Cordoba, in the south of Spain.

Materials and Methods

The presence of *Sarcocystis* spp. was surveyed in 97 sheep killed in two slaughterhouses of the city of Cordoba during 1986. They came from very different geographical points of the province.

Of these 97 animals 42 were males and 55 females, aged between one month and four years. The animals were distributed in three groups according to their age: under three months, 12 animals; between three and twelve months, 43 animals and more than twelve months old, 42 animals.

Three methods were used to detect *Sarcocystis*. Firstly, muscle was digested in a trypsin solution (Erber, 1977[4]) at

30°C for 15 minutes, and the digest was examined microscopically for bradyzoites. In the positive samples, direct microscopic observations in trichinoscope plates and histological examinations of tisular sections after staining with hematoxilin-eosin and periodic-acid Schiff (PAS) were performed in order to study the structure and the morfology of the cyst.

Results and Discussion

Sarcocystis bradyzoites were found in digests of 85.6% of sheep. Microscopic *Sarcocystis* that we identified as *S. tenella* were found in all infected animals. Macroscopic *S. gigantea* were found in the esophagus from 6 of these sheep. In one sheep we found a microscopic cyst with a thin wall (less than 1 μm) and hair-like protusions (approximately 10-12 μm long) all over the surface, that we identified as *S. arieticanis* Heydorn, 1985, the last described *Sarcocystis* species (Heydorn[5], Heydorn and Melhorn[6], Dubey *et al.*[3]).

We considered three locations in the muscular tissue of the animals: heart, esophagus and diaphragmatic muscle. 87.5% of the animals studied harboured the cysts in the three locations examined. 12.50% of the sheep had cysts located in two of the three muscular groups: 7.22% in the heart and esophagus and 4.28% in the heart and diaphragmatic muscle.

Prevalence varied with age. 100% of oldest animals were infected, followed by sheep between 3 and 12 months old (79.05%) and the lowest prevalence (58.33%) was in the youngest age group, younger than 3 months.

Prevalences were similar in males and females.

Results of this survey confirm the high prevalence of *Sarcocystis* infection in sheep in Spain. We determined a parasitation index of 85.67%, close to the data from other spanish provinces: Zaragoza, 62% (Otero[9]) and 98.18% (Sánchez-Acedo *et al.*[12]), León, 68.65% (Díez-Baños[2]) and 95% (Pereira and Bermejo[10]) and Granada, with a 96% (Pérez-Garro *et al.*[11]).

In additon, we differentiate between the prevalence of macroscopic and microscopic species of *Sarcocystis*. We identified the macrocyst of *S. gigantea* and the microcysts of *S. tenella* and *S. arieticanis*, in accordance with their morfology and microscopic structure. We did not find in our study *S. medusiformis* Collins *et al.*, 1979, the second macroscopic sarcocyst of the sheep. (Collins *et al.*[1], Moore[7], Obendorf and Munday[8]).

The life cycle and definitive host for ovine *Sarcocystis* species in Spain needs further studies. Only for *S. gigantea* the life-cycle between sheep and cat has been fulfilled (Sela-Pérez[13]).

Other results of the survey, as the location of the cysts and the influence of the age of the host in the prevalence of the infection, coincide with all the known data of ovine *Sarcocystis*.

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NOTA PARASITOLÓGICA

Enzymatic heterogeneity among strains of *Leishmania infantum* from human visceral and cutaneous leishmaniosis in Catalonia (Spain)

Identification of *Leishmania* strains by enzyme electrophoresis in the west mediterranean area displays the existence of a notable heterogeneity among isolates from human cutaneous (HCL) and visceral (HVL) leishmaniosis, not shared with those of canine origin (CL)[1-8].

During the last few years we have had occasion to study 16 *Leishmania* isolates from patients with HCL or HVL, acquired in Catalonia (Spain).

Isolation and culturing were carried out in N.N.N. medium. When growth was unsuccesful the liquid phase was supplemented with brain-heart broth or RPMI 1640 medium. Successful growth was not achieved on various occasions, mainly with inocula of cutaneous origin (five cases of HCL from the Island of Majorca and one HCL from Roda de Berà and one HVL from Bonastre, neighbouring towns in Tarragona province).

Enzymatic determination was performed in the Laboratoire d'Écologie Médicale et Pathologie Parasitaire (Montpellier) through fifteen enzymatic systems (Tables 1 and 2).

Table 1
Origin and enzymatic identification of *Leishmania infantum* strains from man in Catalonia

Strain	*Zymodeme*	*Clinical Manifestation*	*Patient's Age*	*Observations*
MHOM/ES/81/BCN-1	MON-29	C	Adult	
MHOM/ES/83/BCN-2	MON-28	V	1 year	
MHOM/ES/84/BCN-6	MON-1	V	1 year	
MHOM/ES/84/BCN-8	MON-1	V	1 year	
MHOM/ES/84/BCN-9	MON-1	V	1 year	
MHOM/ES/84/BCN-11	MON-29	C	Adult	
MHOM/ES/84/LEM-676	MON-1	V	Adult	
MHOM/ES/85/BCN-14	MON-1	V	5 months	
MHOM/ES/85/BCN-15	MON-80	V	4 months	
MHOM/ES/86/BCN-16	MON-1	C	10 years	
MHOM/ES/86/BCN-18	MON-33	C	8 years	
MHOM/ES/86/BCN-20	MON-1	V	8 months	
MHOM/ES/88/BCN-21	MON-1	V	Adult	Kidney transplant
MHOM/ES/88/BCN-22	MON-1	V	Adult	HIV +
MHOM/ES/88/BCN-24	MON-1	C	Adult	
MHOM/ES/89/BCN-32	MON-33	V	Adult	HIV +

Table 2
Profile of isoenzyme mobility of zymodemes from man in Catalonia

Enzyme	*Zymodemes*				
	1	*28*	*29*	*33*	*80*
MDH	100	104	104	104	104
ME	100	100	100	100	100
ICD	100	100	100	100	100
PGD	100	100	100	100	100
G6PD	100	102	105	105	100
GLUD	100	100	100	100	100
DIA	100	100	100	100	100
NP_1	100	140	140	100	130
NP_2	100	100	100	100	100
GOT_1	100	100	100	100	100
GOT_2	100	100	100	100	100
PGM	100	100	100	100	100
FH	100	100	100	100	100
MPI	100	100	100	100	100
GPI	100	100	100	100	100

Comments

Zymodeme MON-1 has been isolated in most cases of HVL and CL in west mediterranean area[1-6,8]. However it has been found in HCL repeatedly in France and Italy[3-6,7,9].

Other zymodemes from the complex *L. infantum* have been isolated less frequently and sometimes in a limited geographic area. MON-29 and MON-33, together with MON-11, are common cause of HCL in the north of Catalonia (Eastern Pyrenees-France)[4,5,7,8]. MON-80 is known through three strains of different origin: one HCL in Argel, one HVL in Greece and MHOM/ES/85/BCN15 from a HVL in Barcelona[5]. As far as we know, the only strain identified at the moment belonging to MON-28 is MHOM/ES/83/BCN-2 from a HVL.

From these results the following features should be emphasized:

- The heterogeneity of *Leishmania* strains causing human afection in Catalonia (five different zymodemes between 16 isolates).
- The heterogeneity among strains causing the same clinical manifestations (three zymodemes in 5 HCL and four zymodemes in 11 HVL).
- The different pathologies yielded by strains with the same enzymatic profile (HCL produced by strains belonging to zymodemes normally causing HVL –ex. MON-1–, and vice-versa –ex. MON-33–.

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