

Short communication. *Sarcocystis* infection: a major cause of carcass condemnation in adult sheep in Spain

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Abstract

The frequency, distribution and impact of small ruminant *Sarcocystis* infection in the European Union is largely unknown; this study reports the prevalence of macroscopic *Sarcocystis* sp. cysts and associated carcass condemnation, in 6,065 adult, cull, small ruminants from 145 farms in Spain. Macrocysts were detected in 12% of sheep from 60% flocks, and in none of the 345 goats examined. Most affected sheep had cysts in more than one body part and as a result, 79% of sheep carcasses with cysts were totally condemned. Consequently, it is estimated that *Sarcocystis* spp. infection could be costing the Spanish sheep industry € 20 million yr⁻¹. Three types of cysts were identified according to size, shape and location: narrow, filament-shaped measuring 2-10 × ≤ 1 mm, present striated muscles only, and two wider types measuring 2-20 × 2-6 mm, including oval-shaped oesophageal cysts and more elongated cysts in striated muscles. Narrow and wide macrocysts were found in the same sheep and are compatible with *Sarcocystis gigantea* and *Sarcocystis medusiformis*, respectively, as described in New Zealand in the 1970s. However, cyst size and morphology varies with age and location. Moreover, *S. medusiformis* has not been reported in Europe and species-specific diagnosis is necessary to ascertain the ethiology of macrocysts in this study.

Additional key words: financial impact; goats; macroscopic cysts; muscle.

Resumen

Comunicación corta. Infección por *Sarcocystis*: una causa importante de decomiso de canales en ovino adulto en España

Apenas existe información sobre la frecuencia, distribución e impacto actual de la sarcocistiosis en la Unión Europea; este trabajo describe la prevalencia de quistes macroscópicos de *Sarcocystis* spp. y el porcentaje de decomisos asociados a los quistes, en 6.065 pequeños rumiantes adultos de desvieje, de 145 granjas de España. Se observaron quistes en 12% de ovejas de 60% de rebaños, y en ninguna de las 345 cabras examinadas. La mayoría de las ovejas afectadas presentó quistes en varias zonas corporales decomisándose el 79% de las canales afectadas. Según esto, las pérdidas por la infección por *Sarcocystis* spp. para la industria ovina española se estimaron en 20 millones de euros año⁻¹. Se observaron tres tipos de quistes según el tamaño, forma y localización: estrechos, filiformes, de 2-10 × ≤ 1 mm, en músculo estriado solamente y dos tipos de quistes anchos, de 2-20 × 2-6 mm, ovales en esófago y los mas elongados en musculatura estriada, que podrían corresponderse con las especies *Sarcocystis gigantea* y *Sarcocystis medusiformis*, descritas en Nueva Zelanda en la década de 1970. Sin embargo, sería necesario un diagnóstico específico para confirmar la etiología de los quistes de este estudio, ya que el tamaño y morfología de éstos varía según la edad y la localización, y *S. medusiformis* no está descrita en Europa.

Palabras clave adicionales: cabras; impacto económico; músculo; quistes macroscópicos.

Sarcocystis sp. are apicomplexan coccidial protozoans with a two host prey-predator life cycle, including more than one hundred and twenty host-specific species infecting mammals, birds, reptiles and fish (Levine, 1986). Oocysts and free sporozoites shed in the faeces by the carnivore definite host are ingested by the intermediate (prey) host, released sporozoites undergo schizogony generations in vascular endothelial cells and other cell types, before developing muscular cysts (sarcocyst) containing infectious bradyzoites. *Sarcocystis* sp. speciation remains a challenge; the traditional method based on cyst size and morphology lacks specificity and although DNA sequence analysis is considered the most accurate method, specific probes are only available for some species (Joachim *et al.*, 1996; Heckerroth & Tenter, 1999). Sheep are the intermediate host for four species: *S. gigantea* (syn.= *S. ovifelis*) and *S. medusiformis* that form macroscopic cysts and cats are the definite host, and *S. tenella* (Syn.= *S. oivicanis*) and *S. arieticanis* that form microscopic cysts and canines are the definite hosts. Among the recognised caprine species, *S. hircifelis* (syn. *S. moulei*) develops macroscopic cysts and cats are the definite host and *S. capracanis* y *S. hircicanis* produce microscopic cysts and canines are the definite hosts (Tenter, 1995). Ruminants are considered among the most sensitive hosts, particularly to dog-transmitted *Sarcocystis* sp; acute infections can cause non-specific symptoms that can lead to abortion and death, and chronic *Sarcocystis* spp. infection results in reduced production and macroscopic cysts are a major cause of carcass condemnation in the abattoir (Tenter, 1995). *Sarcocystis* spp. infection is not a communicable disease in the European Union and recording of associated lesions is not compulsory (Dorny *et al.*, 2009) hence, there is no up-to-date information on the prevalence and impact of the disease in small ruminants in Europe.

The present study was carried out between October 2009 and October 2010 to estimate the extent and impact of small ruminant *Sarcocystis* spp. infection in Spain. It involved 5720 sheep and 345 goats over 12 months of age, from 145 farms, including 96 sheep flocks, 7 goat herds and 42 mixed sheep and goat herds. They included every adult cull sheep and goat slaughtered in this abattoir during this period, whose origin could be unequivocally traced back to its farm of origin. Table 1 shows that animals were mostly from Castilla-La Mancha, 97% were females, 67% were meat-producing and sheep breeds were predominantly

Manchega. Postmortem inspection of slaughtered animals was carried out following the standard CE 854/2004 European Union regulation (OJ, 2004) by two veterinarians working as official meat inspectors at the abattoir, who systematically examined the external surfaces of the carcass, oesophagus, heart, diaphragm, pleura and peritoneum to detect macroscopic *Sarcocystis* sp. cysts. Complying with the above legislation, carcasses with one or more cysts in one body area were partially condemned and the affected area was trimmed off, instead, carcasses were totally condemned when cysts were found in two or more body areas. Given that small ruminant sarcocysts are not considered zoonotic, carcass condemnation is justified on the basis of the negative visual impact that cysts may have on the consumer. The length, width and body location of cysts conforming to two distinct morphologies were investigated on carcasses from thirty two randomly selected infected sheep. Differences in prevalence of sheep with macroscopic cysts according to study independent variables including production type (dairy or meat), breed and origin (autonomous community) were assessed using Yates-corrected chi-squared test in EpiInfo 2002 (CDC Atlanta). Random effects logistic regression in SAS 9.0 (SAS Institute) was then employed to evaluate the independent contribution of flock (random variable) and production type and origin (fixed variables) to cyst prevalence among Manchega sheep. Model estimation was based on the maximum likelihood, *p*-values were calculated using the likelihood-ratio chi-squared test and alpha was set at 5% for a double test.

Macroscopic *Sarcocystis* sp. cysts were detected in 12% (712/5720) of sheep from 60% (83/138) of flocks and in none of the 345 goats examined (Table 1). Median and interquartile range flock prevalence was 17% and 6-33%, respectively. Prevalence in sheep from mixed sheep and goat flocks was 7.5% (127/1699). The percentage of partial and total carcass condemnations were 3% (148/5720) and 10% (564/5720), respectively. Hence, 79% (564/712) of condemnations involved the whole carcass. Three different types of macroscopic cysts, were detected according to their size, shape and location: narrow filiform cysts in striated muscles and two wider types including cysts with almost parallel sides and bluntly rounded ends present in the oesophagus only and other more elongated cysts in striated muscle. The size of the 141 cysts detected in the 32 sheep selected for cyst characterisation, were 2-20 × 2-6 mm for wider cysts (n = 68) and 2-10 × ≤1 mm

for the narrow filiform cysts ($n = 73$). Wide cyst location (frequency) included the oesophagus (40%), internal side of the abdominal muscles (28%), limbs (12%), intercostal muscles (10%), diaphragm (9%) and neck (<2%). In contrast, narrow filiform cysts were only found in the internal side of the abdominal muscles (66%), back limb (27%) and diaphragm (7%). The mean (range) age of sheep harbouring wide macrocysts only ($n = 4$), narrow macrocysts only ($n = 9$) and both types ($n = 18$) were similar: 7.0(6-9), 7.3 (5-9) and 7.9 (5-9) years old, respectively ($p > 0.05$). Bivariate chi-square comparisons indicated that macrocyst prevalence in sheep differed significantly between breeds, it was greater in meat producing compared to dairy animals, including for the Manchega breed, and among autonomous communities with more than one flock sampled, in Castilla-La Mancha than in Valencia ($p < 0.001$) (Table 1). However, logistic regression analysis of prevalence in Manchega sheep, indicated that prevalence did not vary significantly according to production type (milk or meat) or flock origin (Valencia or Castilla-La Mancha) ($p > 0.05$) and confirmed significant differences between flocks ($p < 0.05$).

Given that macroscopic cysts represent a small proportion of cysts present in small ruminants (Tenter,

1995), results suggest that *Sarcocystis* spp. infection remains widespread in Spanish sheep. No goats were found infected and the reasons for this are unclear. To the authors' knowledge the prevalence of goat *Sarcocystis* spp. infection in Spain has not been previously investigated however, other studies elsewhere have similarly reported null prevalence of *Sarcocystis* in goats (Shastri, 1990; Kudi *et al.*, 1991; Dafedar *et al.*, 2008). Differences in management may account for prevalence differences between species, however, most goats in this study came from mixed sheep and goat flocks and the prevalence of macrocysts in sheep from these flocks was 7.5%. A minimum number of animals may be required for the parasite to be maintained in the flock. Although the goat census in mixed flocks was unknown it is likely that it was smaller than the sheep census because comparatively few goats were slaughtered and commonly, mixed flocks in Spain have only enough goats to suckle lambs from ewes with insufficient milk. Further studies should be carried out for a more accurate estimation of the prevalence of macroscopic cysts in this species.

Macroscopic cysts are typical of certain species and the three cyst types identified in this study based on size, shape and location are like those described by

Table 1. Macroscopic *Sarcocystis* sp. cysts in muscle from small ruminants examined post-mortem in an abattoir in Spain ($n = 6,065$)

	Animals		Flocks	
	No.	% with cysts ¹	No.	% with cysts ¹
<i>Species</i>				
Ovine	5,720	12	138	60
Caprine	345	0	49	0
<i>Autonomous community²</i>				
Castilla-La Mancha	5,107	11	128	61
Andalucía	305	41	1	100
Valencia	260	2	8	37
Aragón	48	40	1	100
<i>Sheep breeds</i>				
Manchega	4,472	10	115	61
Segureña	950	25	17	71
Lacaune	250	0	5	0
Rasa Aragonesa	48	40	1	100
<i>Sheep production</i>				
Dairy	1,844	7	37	49
Meat	3,876	15	101	64
<i>Sheep breed and production</i>				
Manchega-dairy	1,594	8	32	56
Manchega-meat	2,878	11	83	63

¹ macroscopic cysts, ² for sheep only.

Collins et al. (1976, 1979) in New Zealand. Based on macrocyst wall structure the latter authors grouped the wide cysts into a single species namely *S. gigantea* and named the narrow cysts as *S. medusifformis*, (Collins et al., 1976, 1979). Later experimental transmission studies with the two species indicate *S. gigantea* is found in oesophageal and striated muscle instead *S. medusifformis* was found in laryngeal, abdominal and diaphragmatic musculature only (Munday & Obendorf 1984a,b; Obendorf & Munday, 1987). The size and shape of cysts for a particular species may change depending on the age and location (striated vs smooth muscle) and as mentioned earlier, *S. medusifformis* is endemic to Australasia and has not been described in sheep carcasses in Europe (Tenter, 1995). Further studies would be necessary to confirm the presence of both species in Spanish sheep. To the authors' knowledge, DNA-based speciation methods have been described for *S. gigantea* but not for *S. medusifformis* (Heckerth & Tenter, 1999).

Since the recent enforcement of EU legislation (OJ, 2009), livestock keepers in Spain are obliged to have a container in the farm premises to store dead animals and raw animal by-products not intended for human consumption, which is periodically disposed off by specialist contractors. Compliance with such legislation should clearly facilitate the control of *Sarcocystis* infection and other major parasitic infections of small ruminants with typical predator-prey life cycles such as cisticercosis, hidatidosis and toxoplasmosis.

In contrast to bovine sarcocystiosis by *S. hominis* and porcine sarcocystiosis by *S. suis*, small ruminant sarcocystiosis are not zoonotic (Fayer, 2004) nonetheless, *Sarcocystis* sp. possess the powerful sarcocystin neurotoxin, but no studies have investigated its potential toxicity to humans following the consumption of cyst-containing meat. Results from this and other recent studies (Borji & Parandeh, 2010) reinforce the importance of *Sarcocystis* spp. infection as a cause of condemnation of adult sheep meat. The financial losses due to the condemnations were not calculated however, an uninformed calculation of the cost of *Sarcocystis* spp. infection to the Spanish sheep industry could be made as follows: given an adult sheep population in Spain of 20 million and an average 4 million sheep culled every year, at € 50 sheep⁻¹ (25 kg carcass weight and € 2 kg⁻¹ sale cost for the abattoir), *Sarcocystis* spp. infection losses would amount to € 20 million yr⁻¹.

In conclusion, the study confirms previous reports that sheep *Sarcocystis* spp. infection is widespread in Spain and is an important cause of carcass condemnation (Perez & González, 1970; Diez, 1978; Sánchez et al., 1983; Martínez-Moreno, 1989). The distribution of the infection elsewhere in Europe requires investigation and may be similar where comparable sheep husbandry practices are used. The presence of two distinct macroscopic cysts prompts the need for species-specific diagnosis to ascertain whether both *S. gigantea* and *S. medusifformis* are endemic in Spain or both cysts are morphological variations of the first species.

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