

The prevalence of ovine *Sarcocystis* species in Izmir province*

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Summary: This study was carried out to determine the prevalence of ovine *Sarcocystis* species in Izmir province. A total of 734 muscle samples of 200 sheep from four different age groups were surveyed by the digestion and histological examination methods for the presence of *Sarcocystis* species. *Sarcocystis ovifelis*, *Sarcocystis ovicanis*, and *Sarcocystis arieticanis* were detected as the responsible species for ovine sarcosporidiosis in Izmir province. Macrocysts of *S.ovifelis* were found in 24.5 % (49/200) of sheep and the highest prevalence was detected in the oesophagus in all age groups. Microcysts of *S.ovicanis* and *S.arieticanis* were present in 86.5 % (173/200) of sheep. The prevalence of *Sarcocystis* cysts increased significantly with the age. Although microcysts were detected in 46 % (23/50) of lambs up to 6 months of age, they were present in all muscle samples of sheep (150/150) over 6 months of age. Histologically, no inflammatory reaction was seen against sarcocysts within the infected muscles, unless they degenerated. There was no significant difference ($p>0.05$) statistically between sensitivities of two diagnostic methods (digestion and histology) in detecting *Sarcocystis* species. These results reveal the fact that the environment is heavily contaminated with sporocysts, and the ingestion of sporocysts by sheep begins from the young ages in Izmir province.

Key words: Izmir, prevalence, *Sarcocystis* spp, sheep.

İzmir İl'inde koyun *Sarcocystis* türlerinin yayılışı*

Özet: Bu çalışma, İzmir ilinde koyun *Sarcocystis* türlerinin yayılışını belirlemek amacıyla yapıldı. Bu amaçla dört farklı yaş grubundan 200 koyuna ait toplam 734 kas örneği, *Sarcocystis* türlerinin varlığı yönünden enzim tekniği ve histolojik muayene metodları ile araştırıldı. İzmir ilindeki koyunlarda sarcosporidiosis'den sorumlu türler, *S.ovifelis*, *S.ovicanis* ve *S.arieticanis* olarak tespit edildi. *S.ovifelis* makrokistleri, koyunların % 24,5 (49/200)'inde bulundu ve tüm yaş gruplarında en yüksek prevalans ösefagusta tespit edildi. *S.ovicanis* ve *S.arieticanis* mikrokistleri, koyunların % 86,5 (173/200)'inde mevcuttu. *Sarcocystis* kistlerinin prevalansı, yaş ile önemli derecede arttı. Mikrokistler, 6 aylığa kadar olan kuzuların % 46 (23/50)'sında bulunduğu halde, 6 aylıktan büyük koyunların tüm kas örneklerinde mevcuttu. Histolojik olarak, kistler dejenere olmadıkça, enfekte kas tellerindeki sarkokistlere karşı yangısal bir reaksiyon görülmedi. *Sarcocystis* türlerinin tespitinde, iki teşhis metodunun (enzim tekniği ve histoloji) duyarlılıkları arasında istatistiksel olarak önemli bir fark ($p>0,05$) bulunmadı. Bu sonuçlar, İzmir ilinde çevrenin sporokistler ile yoğun şekilde kontamine olduğu ve koyunlar tarafından sporokist alımının genç yaşlardan itibaren başladığı gerçeğini ortaya koymaktadır.

Anahtar sözcükler: İzmir, koyun, prevalans, *Sarcocystis* spp.

Introduction

Sarcocystis is one of the most prevalent protozoan parasites in the striated muscles of livestock slaughtered for food such as cattle, sheep and goat. Four species of *Sarcocystis* have so far been identified from domestic sheep. Of these, *S.ovicanis* (synonymous=*S.tenella*) and *S. arieticanis*, pathogenic species, form microscopic cysts and are transmitted via canids, the definitive hosts, whereas *S. ovifelis* (synonymous=*S. gigantea*) and *S. medusiformis*, non-pathogenic species, form macroscopic cysts and are transmitted via felids (5). Many reports (4, 6, 13, 22, 23) on the prevalence of ovine *Sarcocystis* species have revealed that sarcosporidiosis is still an

extremely common infection even in the most developed countries of the world. The prevalences of ovine *Sarcocystis* species detected in different provinces of Turkey have also ranged from 55 % to 100 % (16, 21, 26, 28, 29).

The main objective of the study reported here was to determine the prevalence of ovine *Sarcocystis* species in Izmir province by using the digestion and histological examination methods. In addition, the pathological lesions observed in the muscles with sarcocysts, and the influence of age and sex on the prevalence of *Sarcocystis* species were also investigated.

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Materials and Methods

Muscle samples were collected between October 1998 and October 2000 from 200 sheep both slaughtered at various abattoirs in Izmir province and necropsied in Bornova Veterinary Control and Research Institute under 6 months of age for the purpose of diagnosis. All sheep had been raised in different locations of Izmir province. A total of 734 muscle samples from the oesophagus, diaphragm, and heart of all sheep and also from the tongue and skeletal muscles (gluteus medius) in some of them were examined for the presence of *Sarcocystis* species by the digestion and histological techniques. Sheep were surveyed in four groups according to their ages: up to 6 months, over 6 months to 1 year, over 1 year to 2 years, and over 2 years. The muscle samples from a total of 50 sheep for each age group were taken. They were initially examined by the naked eye for the presence of macrocysts. The found cysts were counted and measured for their dimensions. Later, the samples were divided into two sets: one set for digestion procedures, the other set for histological examinations. For the digestion, ten grams of each muscle sample were homogenized in a blender and digested in a NaCl - buffered 0.25 % trypsin solution, as described by Erber (7). After a 10 to 15 min incubation at 20°-25° C, the digest was sieved through a nylon-meshed tea strainer and centrifuged within the tubes with conic bottom at 1500 rpm for 10 min (Hettich Universal 16). After discarding the supernatant fluid, one to two drops from the sediment at the bottom were examined microscopically for *Sarcocystis* microcysts.

For histological examinations, the muscle samples with or without macrocysts were fixed in 10 % neutral buffered formalin during the necropsy or within the latest two hours after slaughter, prepared the paraffin blocks, sectioned at 4 to 5 micron, stained with haematoxylin and eosin (H&E), and examined microscopically for sarcocysts. Selected tissue sections with macrocysts were also stained with periodic acid Schiff (PAS). The wall morphologies of sarcocysts in the muscle sections were examined under oil immersion.

Sarcocystis species present in the muscle samples were identified on the basis of the differences in the wall morphologies of sarcocysts, as described by Dubey et al. (5). The shortest and longest dimensions of each sarcocyst were also recorded. Both the sensitivities of two diagnostic methods (digestion and histology) and the differences in prevalences of microcysts according to the muscles of different organs and sex in sheep from four different age groups were evaluated statistically by using the z test (9). Values of $p < 0.001$ were considered significant.

Results

Sarcocystis macrocysts were found in 49 (24.5 %) of 200 sheep. The highest prevalence of macrocysts was detected in sheep over 2 years old and in the oesophagus in all age groups (Table 1).

Table 1. The prevalence of *sarcocystis* macrocysts in the muscles of different organs in 49 sheep with macrocysts from four different age groups.

Tablo1. Dört farklı yaş grubundan makrokistli 49 koyunda farklı organ kaslarında *sarcocystis* makrokistlerinin prevalansı.

Examined organ muscles	Numbers of sheep with macrocysts				Total number (%)
	1.group	2.group	3.group	4.group	
O	1	2	14	15	32 (16)
T	-	-	1	2	3 (1.5)
H	-	-	1	2	3 (1.5)
O + T	-	-	1	3	4 (2)
O + D	-	-	2	3	5 (2.5)
O + D + T	-	-	-	1	1 (0.5)
O + D + T + H	-	-	-	1	1 (0.5)
Total number (%)	1 (2)	2 (4)	19 (38)	27 (54)	49 (24.5)

O : Oesophagus; T : Tongue; H : Heart; D : Diaphragm

1.group: up to 6 months; 2.group: >6 months to 1 year; 3.group: >1 year to 2 years; 4.group: >2 years

Diaphragmatic macrocysts were spindle-shaped and elongated, and measured 8-14 x 3-6.5 mm, whereas those found in the oesophagus, tongue, and heart muscles were round-oval shaped, and measured 0.25-12 x 0.25-8 mm. In histological sections, macrocysts had the morphological features corresponding to the description of *S. ovifelis*. They were thin walled, surrounded by a secondary thick cyst wall which is stained positively with PAS (Figure 1). There was no evidence of any degeneration or inflammatory reaction in the muscle fibers surrounding macrocysts.

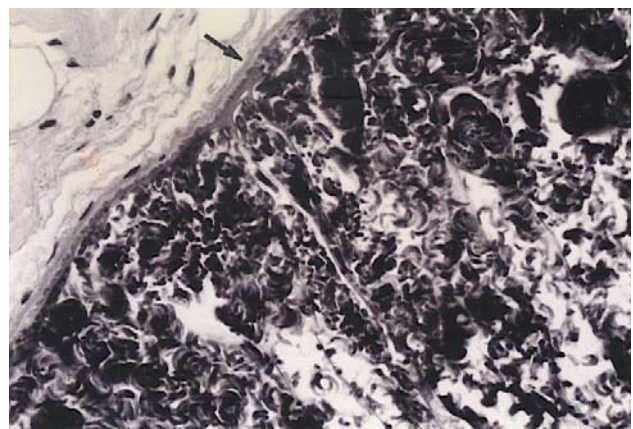


Figure 1. *Sarcocystis ovifelis* macrocyst in oesophagus. Secondary thick cyst wall (arrow). periodic acid Schiff x 400.

Şekil 1. Özefagusta *Sarcocystis ovifelis* makrokisti. Sekonder kalın kist duvarı (ok). periodic acid Schiff x 400.

Table 2. The prevalence of *sarcocystis* microcysts determined by the digestion method and histological examination in the muscles of different organs in 200 sheep from four different age groups.

Tablo 2. Dört farklı yaş grubundan 200 koyunun farklı organ kaslarında enzim tekniği ve histolojik muayene metodları ile tespit edilen *sarcocystis* mikrokistlerinin prevalansı.

Age groups	Examined organ muscles									
	<i>Oesophagus</i>		Diaphragm		Heart		Tongue		Skeletal muscles	
	D	H	D	H	D	H	D	H	D	H
1.group (%)	23/50* (46)	23/50* (46)	23/50 (46)	22/50 (44)	22/50 (44)	23/50 (46)	7/31 (22.6)	7/31 (22.6)	1/25 (4)	2/25 (8)
2.group (%)	50/50 (100)	50/50 (100)	50/50 (100)	50/50 (100)	50/50 (100)	50/50 (100)	15/15 (100)	15/15 (100)	3/3 (100)	3/3 (100)
3.group (%)	50/50 (100)	50/50 (100)	50/50 (100)	50/50 (100)	50/50 (100)	50/50 (100)	15/15 (100)	15/15 (100)	2/2 (100)	2/2 (100)
4.group (%)	50/50 (100)	50/50 (100)	49/50 (98)	50/50 (100)	50/50 (100)	50/50 (100)	37/38 (97.4)	38/38 (100)	5/5 (100)	5/5 (100)
Total (%)	173/200 (86.5)	173/200 (86.5)	172/200 (86)	172/200 (86)	172/200 (86)	173/200 (86.5)	74/99 (74.5)	75/99 (75.6)	11/35 (31.4)	12/35 (34.3)

D= Digestion method; H=Histological examination;

* x/n=The number of infected animal/The number of examined animal.

1.group: up to 6 months; 2.group: >6 months to 1 year; 3.group: >1 year to 2 years; 4.group: >2 years

The overall prevalence of *Sarcocystis* microcysts in all sheep with or without macrocysts was detected as 86.5 % (173 of 200) (Table 2). No significant difference ($p>0.05$) was statistically found between sensitivities of two diagnostic methods in detecting *Sarcocystis* species in all age groups. In histological sections, microcysts were observed from 2.5 months of age, and immature cysts (n:10) measured 7.5-15 x 10-25 μ m. Twentyseven sheep (54 %) aged up to 6 months had no microcyst (Table 2). *Sarcocystis* microcysts were present in all muscle samples of sheep over 6 months of age (Table 2). The prevalence of microcysts according to the sex was shown in Table 3.

Table 3. The prevalence of *sarcocystis* microcysts according to the sex in 200 sheep from four different age groups.

Tablo 3. Dört farklı yaş grubundan 200 koyunda cinsiyete göre *sarcocystis* mikrokistlerinin prevalansı.

	Age groups							
	1. group		2. group		3. group		4. group	
Sex	group	%	group	%	group	%	group	%
Female	18/35	51.4	19/19	100	39/39	100	46/46	100
Male	5/15	33.3	31/31	100	11/11	100	4/4	100
Total	23/50*	46	50/50	100	50/50	100	50/50	100

* x/n=The number of infected animal/The number of examined animal.

1.group: up to 6 months; 2.group: >6 months to 1 year; 3.group: >1 year to 2 years; 4.group: >2 years

Two types of microcysts were differentiated on the basis of their cyst wall morphologies. Sarcocysts, thin-walled with hair like projections, were identified as *S.arieticanis*, and sarcocysts, thick-walled with radial striations, as *S.ovicanis* (Figure 2). Of 173 sheep with microcysts, 63 (31.5 %) were only infected by *S.ovicanis* and 110 (55 %) had mixed infection. Whereas the numbers of *S.arieticanis* cysts in muscle samples in 95 (86.3 %) of 110 sheep with mixed infection were usually limited to a few sarcocysts, their numbers in the remainder (13.7 %) were more than or very close to those of *S.ovicanis* cysts.

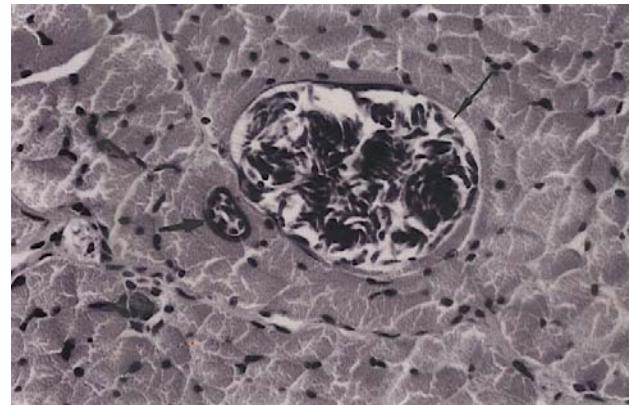


Figure 2. *Sarcocystis ovicanis* (thick arrow), thick-walled with radial striations, and *Sarcocystis arieticanis* (thin arrow), thin-walled with hair like projections, in the tongue. Haematoxylin and eosin x 400.

Şekil 2. Dilde radyal çizgili kalın duvarlı *Sarcocystis ovicanis* (kalın ok) ve saç benzeri uzantılara sahip ince duvarlı *Sarcocystis arieticanis* (ince ok) mikrokisti. H&E x 400.

Histologically, microscopic cysts from immature cysts with merozoites to mature cysts with bradyzoites were observed in varying intensity in muscle sections. In the heart muscles, sarcocysts were also seen in Purkinje fibres as well as in the muscle fibres of the myocardium. In all muscle samples examined, most of the muscle fibres containing seemingly intact sarcocysts showed usually no evidence of any pathological change or inflammatory reaction. However, there were intense mononuclear cell infiltrations with variable numbers of neutrophil leucocytes within and around the degenerated sarcocysts. In some of the muscle fibres with such degenerative cysts, sarcoplasm had also lost its striations and become amorphous. Most of the muscles with sarcocysts had focal to multifocal, mild interstitial mononuclear cell infiltrations, largely surrounding the vessels and rarely separating the muscle fibres, without associated with the intensity of sarcocysts. These cell infiltrations very rarely surrounded the muscle fibres with sarcocysts. Multifocal mild mononuclear cell infiltrations were also present in the subepicardial tissue of the hearts.

Discussion and Conclusion

Sarcocystis ovifelis, a macroscopic sarcocyst the most often identified from sheep, is of a worldwide distribution (1, 2, 4, 6, 13), whereas *S. medusiformis*, another ovine macrocyst, has so far been reported only from several countries (1, 3, 17). In Turkey, the prevalence rates of macrocysts in sheep have ranged from 6.1 % to 66 % in abattoir surveys performed in different provinces (8, 16, 26, 28, 29). In the present study, *S. ovifelis* sarcocysts were found in 24.5 % (49 of 200) of sheep. The highest prevalence (16 %) recorded in the oesophagus was similar to the observations of other some researchers (2, 11-13), except for that of Ozturk and Kucukerden (20) who detected the highest prevalence of cysts in the diaphragm. Macrocysts have been reported to be found mostly in adult sheep (1, 2, 26, 27, 29). In this study, the prevalence of *S. ovifelis* macrocysts increased with age and was significantly higher in sheep over 2 years old than in the younger sheep (Table 1). *S. ovifelis* was found in 3 sheep under 1 year old, supporting the finding of Svobodova and Nevole (27) who reported macrocysts in a young sheep. This finding suggests that macrocysts may develop in a time less than 1 year, unlike the previous observation that they need at least one year to develop (15).

Microscopic ovine *Sarcocystis* species have been observed to be more prevalent than macroscopic cyst-forming species in Turkey (14, 16, 21, 26, 29) and other many countries (1, 2, 4, 6, 13). The overall prevalence of

microcysts in 200 sheep surveyed in four different age group in the present study was also higher than that recorded for macrocysts. The differences in prevalence between the age groups were significant ($p < 0.001$) in sheep aged up to 6 months. Microcysts were found in 23 (46 %) lambs in this age group. While the prevalences of microcysts in the oesophagus, heart, and diaphragm were similar, the lowest prevalences were respectively in the skeletal muscles and tongue (Table 2). The prevalences of microcysts recorded in lambs in different countries have been reported as 22.6 % in those under 6 months of age in Austria (6), 58.33 % in those under 3 months of age and 79.05 % in those between 3 and 12 months of age in Spain (13), 35.67 % in Czechoslovakia (27), and 18.3 % in the United States (25). In some studies (20, 29) performed in Turkey, the prevalences of *Sarcocystis* microcysts have ranged from 34 % to 100 % in sheep under 1 year old. In this study, the prevalence of microcysts also significantly increased with age, as indicated by findings of the previous many studies (1, 6, 13, 20, 27). All sheep over 6 months of age had microcysts (Table 2).

The observations of some studies performed on ovine sarcosporidiosis have shown that no significant difference was found in prevalence between both sex of sheep (13, 25). While the prevalence of *Sarcocystis* species recorded in this study was higher in females than males of sheep aged up to 6 months ($p < 0.001$), the prevalence was 100 % in all female and male sheep over 6 months of age (Table 3). Therefore, it seems that the prevalence of microcysts increasing with age was not associated with sex.

The results of many surveys (10, 22-24, 26) on ovine *Sarcocystis* species have revealed that sheep are infected by either only one or both *Sarcocystis* species forming microcysts in the muscles, as in the present study. None of sheep was infected by only *S. arieticanis*, unlike the findings of some studies in Turkey (20, 26, 29). Additionally, the number of *S. arieticanis* microcysts present in the muscle samples in most of the mixed infections was also limited to a few sarcocysts, similar to the observations of Dubey et al. (4).

It has been reported that sarcocysts within the muscle fibres had usually no associated host reaction but some toxic substances may be released, causing a strong immune response, when they were ruptured (5). In experimentally infected lambs, O'Toole et al. (19) have also observed that an associated host response to cysts occurred largely within the infected muscle fibres, when sarcocysts degenerated. In his another study, O'Toole (18) has ultrastructurally detected that a mild or moderate myositis developed during both meront and cyst-forming

stages, and only a small proportion of muscle fibres with cysts attracted a local inflammatory reaction, consisting of macrophages and plasma cells. In this study, it was seen neither any degenerative change and necrosis within the infected muscle fibres, unless sarcocysts degenerated, nor haemorrhages even in the muscle samples with numerous cysts, in contrast to the observations of some studies (17, 20).

Various diagnostic methods are used for the detection of sarcosporidiosis (1, 4, 10, 11, 13). We used the digestion and histological examination methods and compared these two diagnostic methods. Both diagnostic methods had similar sensitivities ($p>0.05$), like the findings of Savini et al. (23) who compared the same methods in sheep. However, the high prevalence of ovine *Sarcocystis* species in Izmir province may have increased the our chance of detecting sarcocysts in the examined muscles.

The results of the present study have revealed that the prevalence of pathogenic ovine *Sarcocystis* species in Izmir province was 46 % in those less than 6 months of age, and 100 % in those over 6 months of age. In addition, these results points out the reality that the environment is heavily contaminated with sporocysts, and the ingestion of sporocysts by sheep begins from the young ages. Therefore, it has of great importance the farmers to be trained not to feed their dogs and cats with uncooked meat, and the abattoir remnants to be burned, in order to be effectively broken of infection cycle between the intermediate and the definitive hosts in Izmir province.

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