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SARCOCYSTIS INFECTION IN SHEEP FROM SOUTH-WESTERN NORWAY

By

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BRATBERG, B., O. HELLE and M. HILALI: *Sarcocystis infection in sheep from south-western Norway.* Acta vet. scand. 1982, *23*, 221—234. — The occurrence of Sarcocystis infection and pathological changes were recorded in samples of the heart, diaphragm, and oesophagus from 198 healthy sheep representing 3 different age groups, obtained from an abattoir.

The infection rate of S. gigantea (syn. S. tenella) was 18.2 %, and the distribution within groups was: ewes 30.0 %, yearlings 11.6 %, lambs nil. The infection rate of S. tenella (syn. S. ovicanis) was 65.1 %, and the corresponding distribution was: ewes 83.5 %, yearlings 74.4 %, and lambs 25.0 %. A third type of Sarcocystis sp. displaying thick wall was found in 3 samples.

Focal interstitial infiltrates of mononuclear cells were demonstrated in 47.9 % of the hearts, in 19.6 % of the diaphragms and in 31.3 % of the oesophagi. The occurrence of Sarcocystis and the focal interstitial mononuclear cell infiltrates were positively correlated ($P < 0.0001$). Morphologically identical sporocysts typical of S. tenella were produced by dogs and foxes fed naturally infected sheep tissues. A cat fed S. gigantea macrocysts produced sporocysts characteristic for the species.

Sarcocystis; pathology; life cycle; final hosts; sheep.

Sheep are reported to be intermediate hosts for 2 species of Sarcocystis: Sarcocystis tenella (syn. S. ovicanis) and Sarcocystis gigantea (syn. S. tenella) (*Markus* 1978, *Mehlhorn & Heydorn* 1978, *Fayer* 1980, *Levine & Tadros* 1980). Recently a third ovine sarcocyst species, S. medusiformis, was proposed (*Collins et al.* 1980). The pathogenic properties attributed to S. tenella are well demonstrated in experimental sheep by *Heydorn & Gestrich* (1976), *Leek et al.* (1977), and *Leek & Fayer* (1978). Spontaneous disease in sheep connected with S. tenella

infection has been described by *Landsverk et al.* (1978) and by *Landsverk & Bratberg* (1979). S. gigantea is considered to be nonpathogenic (*Dubey* 1976, *Markus* 1978)

Sarcocystis infection is supposed to be common in Norwegian sheep, but the significance of the infection is not known. The purpose of this investigation was to estimate the occurrence of S. gigantea and S. tenella by using the heart, oesophagus, and diaphragm as indicator organs, further to record the severity of Sarcocystis infection and pathological lesions in these organs, and to demonstrate the final hosts of the Sarcocystis species observed.

MATERIAL AND METHODS

Specimens of the heart, diaphragm, and oesophagus of apparently healthy sheep were collected at an abattoir (Forus, Sandnes) at the end of August 1979. On the basis of age estimates, the animals, individually labelled and totalling 198, were divided into 3 groups: 103 ewes (Group I), 43 yearlings (Group II), and 52 lambs 4—5 months old (Group III).

The following examinations were performed:

1. Macroscopic evaluation with respect to the presence of S. gigantea macrocysts and gross pathological changes.
2. Trichinoscopic method for detection of sarcocysts.
3. Homogenization technique according to *Erber* (1977) for detection of merozoites.
4. Histological examination: One specimen was selected from each ventricle of the heart, and 1 specimen each from the oesophagus and the diaphragm. The tissues were fixed in 10 % neutral buffered formalin, processed routinely, and stained with haematoxylin and eosin (HE), selected sections being stained with elastin van Gieson (EVG). Sarcocystis spp. were identified on cyst wall structure, and the frequency of sarcocysts was quantitated according to the average number of cysts observed in 5 fields of view in the same section, using a light microscope objective lens $\times$ 4. The size of the sarcocysts was measured with an ocular micrometer.
5. Experimental infection of final hosts: S. gigantea macrocysts of oesophageal origin were collected in physiological saline. Eighty S. gigantea cysts were fed to 1 coccidia-free, conventionally reared male cat. Two 2-months-old labrador retriever

dogs and 2 silver foxes, a colour mutation of the red fox Vulpes vulpes, approximately 4 months old, all coccidia-free and individually caged, were each fed 500 g of pooled samples of the heart, diaphragm, and oesophagus once. Two dogs and 2 silver foxes served as non-infected controls. Faeces from the cat, the dogs and the foxes were collected daily and examined for the presence of sporocysts. During the last part of the patent period, when sporocysts were passed intermittently, faecal samples were examined 3 times weekly. The size of the sporocysts was measured with an ocular micrometer.

RESULTS

The hearts, diaphragms, and oesophagi examined did not have any gross abnormalities, except for S. gigantea macrocysts in the oesophagus. The results of the examination as regards the presence of S. gigantea and S. tenella infections are presented in Table 1. Macrocysts of S. gigantea were found in the oesophageal muscles only. In Group I S. gigantea cysts were found on histology in 1 additional case negative for S. gigantea on gross examination, making a total of 31 positive samples (30.0 %). The overall infection rate for all 3 groups was 18.2 %.

S. tenella cysts were found in all the 3 organs investigated. In Groups I and II the samples positive for S. gigantea on gross examination were excluded when the trichinoscopy and homogenization methods were used. Mean S. tenella infection rates for all 3 groups were 87.1 % on using the homogenization

Table 1. Total prevalence of S. gigantea and S. tenella in the heart, diaphragm and the oesophagus in ewes (Group I), yearlings (Group II), and lambs (Group III).

Sarcocyst type and method of detection	Group I		Group II		Group III	
	Number of samples	Number positive (and %)	Number of samples	Number positive (and %)	Number of samples	Number positive (and %)
S. gigantea, macroscopic examination	103	30 (29.1)	43	5 (11.6)	52	0
S. tenella and/or S. gigantea, homogenization	73	62 (84.9)	38	32 (84.2)	52	48 (92.3)
S. tenella, trichinoscopy	73	39 (53.4)	38	21 (55.2)	52	26 (50.0)
S. tenella, histology	103	86 (83.5)	43	32 (74.4)	52	13 (25.0)
S. gigantea, histology	103	5 (4.8)	43	2 (4.6)	52	0

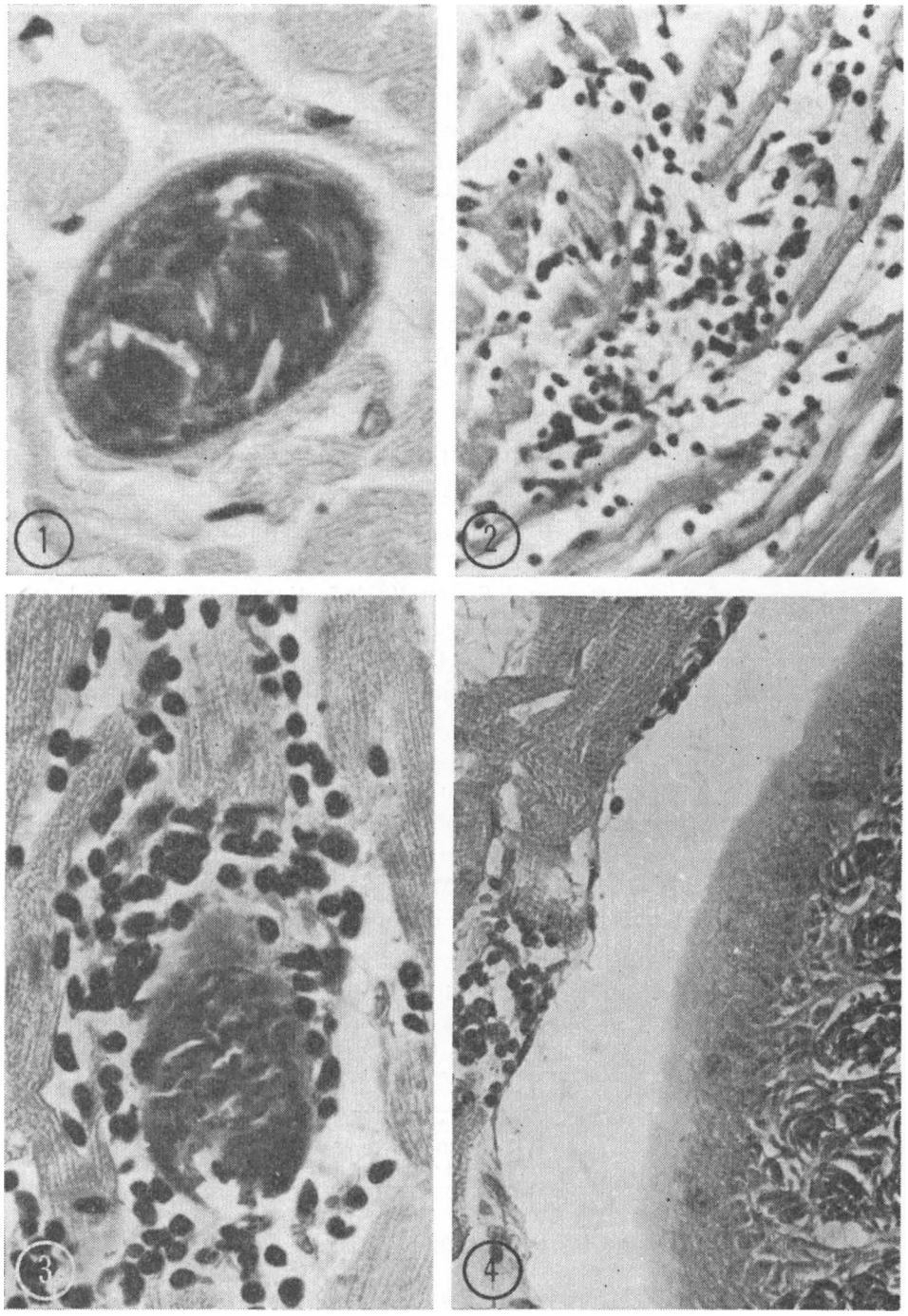

Figure 1. S. tenella cyst in the heart muscles. Striations in the cyst wall. HE × 1050.

Figure 2. Representative small focus of mononuclear cell infiltration in the heart muscles. HE × 475.

Figure 3. S. tenella cyst with severe mononuclear cell infiltration and incipient degeneration. HE × 740.

Figure 4. S. gigantea with characteristic wall structures and metrocytes beneath the capsule. Slight mononuclear cell infiltration. HE × 475.

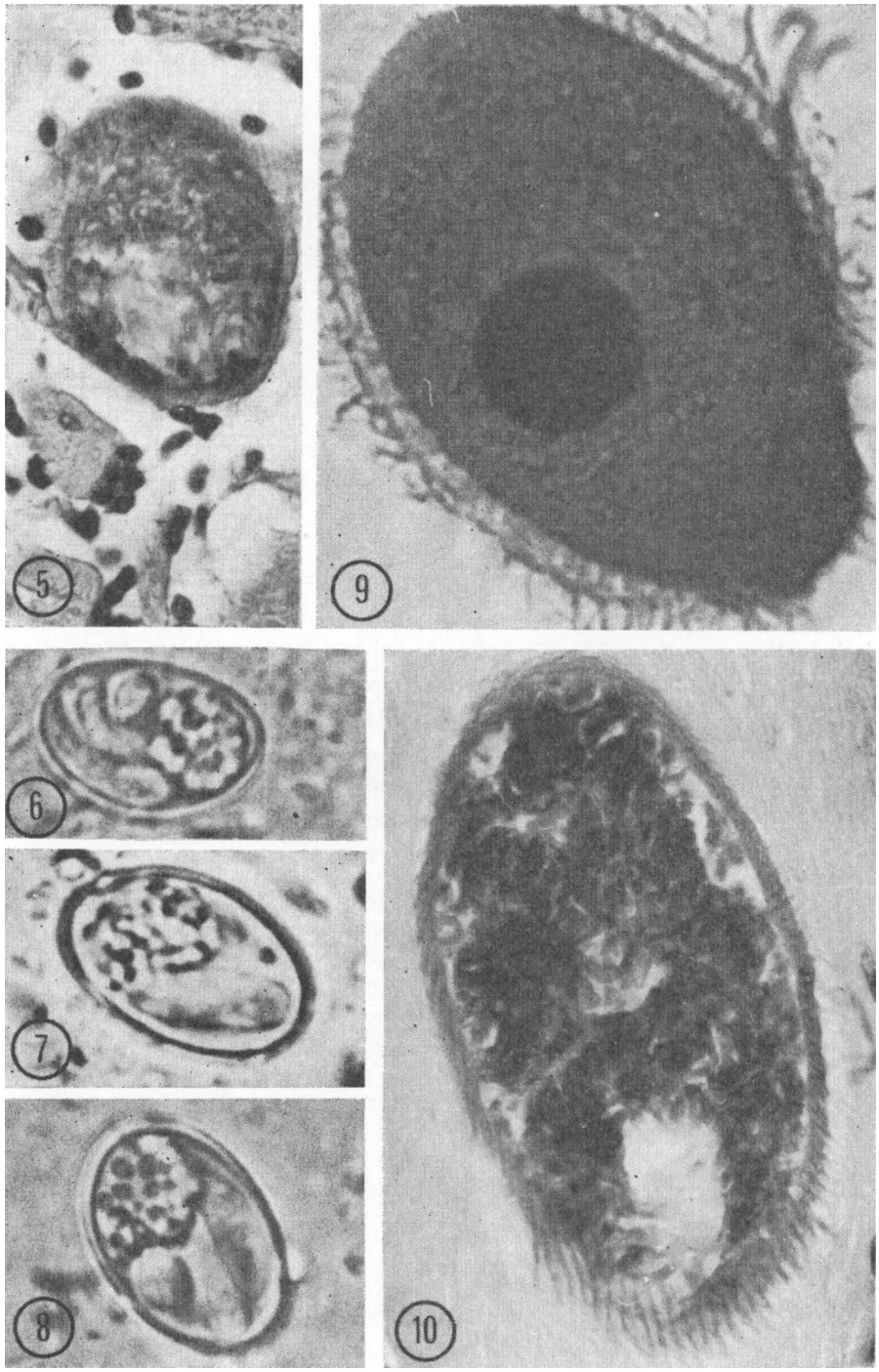

Figure 5. Necrotic S. tenella cyst with slight mononuclear cell infiltration. HE × 760.
Figure 6. S. gigantea sporocyst from cat. × 2500.
Figure 7. S. tenella sporocyst from fox. × 2500.
Figure 8. S. tenella sporocyst from dog. × 2500.
Figure 9. Ciliate with double-contoured wall and a distinct macronucleus. HE × 2500.
Figure 10. A third type of Sarcocystis sp. in the diaphragm. Thick cyst wall with prominent radial striations. HE × 760.

technique, 52.7 % on trichinoscopy, and 65.1 % on histology. Three samples (2.9 %) in Group I infected with S. gigantea were negative for S. tenella on histology. The total Sarcocystis infection rate on histology was 86.4 % in Group I. Mixed infection of S. tenella and S. gigantea was demonstrated in 27.2 % of the samples in Group I and in 11.6 % in Group II.

Cyst wall characteristics of S. tenella and S. gigantea are shown in Fig. 1 and Fig. 4 respectively.

The proportional infection rate of S. tenella in the heart, oesophagus, and diaphragm is presented in Table 2. The differences in S. tenella infection rates between the heart and the diaphragm and between the heart and the oesophagus were significant in Group I ($P < 0.0005$) and Group III ($P < 0.05$), but not in Group II. The severity of S. tenella infection in the organs investigated is demonstrated in Fig. 11. Score 1 infection predominated in the organs investigated in all 3 groups. Score 2 infection was found in the heart in all 3 groups, with the highest frequency in Group II (34.3 %), lower frequency in Group III (23.1 %), and lowest in Group I (16.3 %). In the diaphragm score 2 infection was recorded in Group II only (9.3 %). In the oesophagus score 2 occurred in Group I (4.3 %) and Group II (9.3 %). Score 3 infection was only found in the heart in Group I.

Table 2. Proportional infection rate of S. tenella in the heart, oesophagus, and diaphragm in Groups I, II, and III.

	Group I		Group II		Group III	
Organ investigated	Number of positive samples	%	Number of positive samples	%	Number of positive samples	%
Heart	76	88.4	28	87.5	9	69.2
Diaphragm	35	40.7	19	59.4	4	30.7
Oesophagus	37	43.0	22	68.7	4	30.7

The size of 50 S. gigantea cysts averaged 4.4 mm, range 1—10 mm. S. tenella cysts measured about 40 µm by 145 µm. The cyst wall proper measured 2—3 µm and displayed radial striations. In Group III in 3 separate samples a few thick-walled Sarcocystis cysts, measuring on average 138 µm by 165 µm, were observed in the diaphragm. The wall proper measured 5—6 µm and dis-

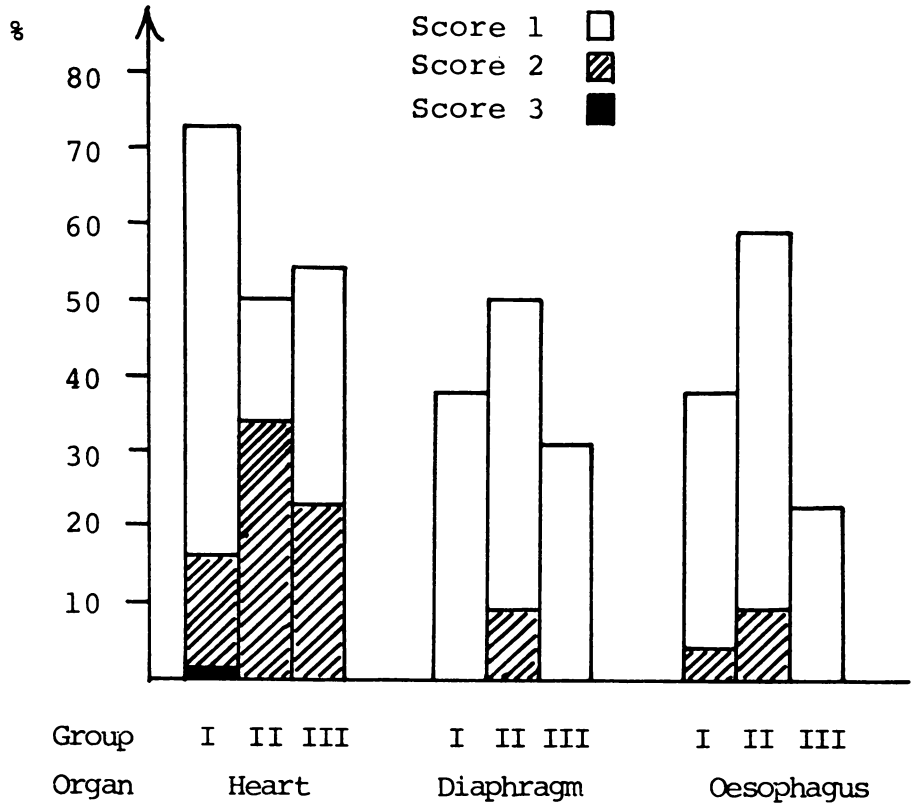

Figure 11. Severity of S. tenella infection in the heart, diaphragm, and oesophagus expressed as percentage of positive samples.
Score 1: Sarcocysts present in sections.
Score 2: 1—5 sarcocysts per field of view.
Score 3: 6—10 sarcocysts per field of view.

played prominent radial striations (Fig. 10). In another 3 samples ciliates, measuring on average 38 µm by 49 µm and with a distinct macronucleus, were found (Fig. 9). The ciliate cysts were not accompanied by inflammatory cell reaction.

Sarcocystis appeared randomly distributed in the sections. S. tenella cysts with degenerated cyst wall structures could be found in the muscle samples surrounded by inflammatory cells (Fig. 3), and sometimes degenerated cysts with sparse inflammatory reaction were observed (Fig. 5). Mononuclear cell infiltrates were also found around S. gigantea cysts (Fig. 4).

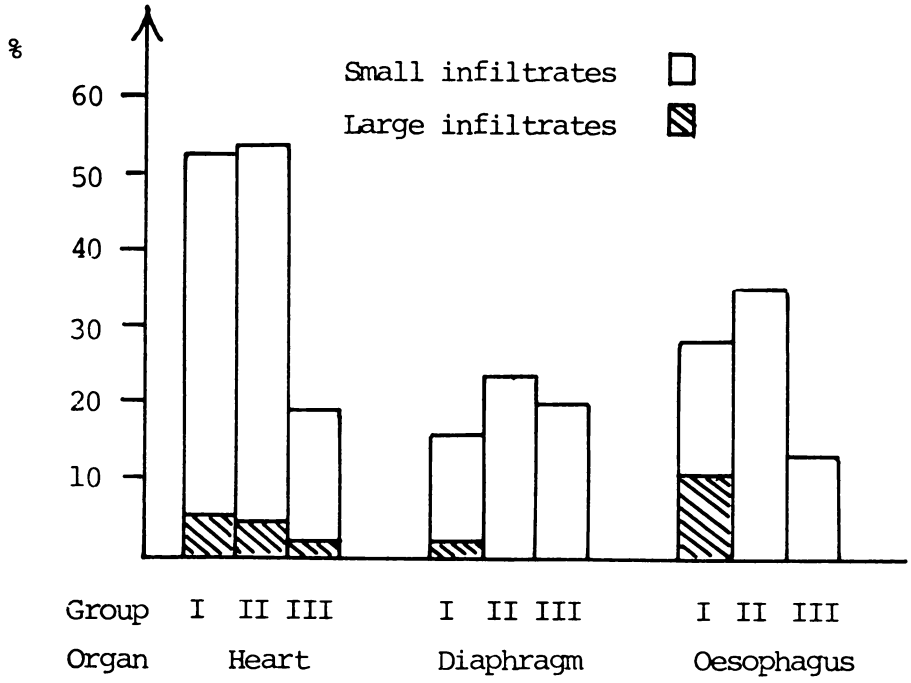

Figure 12. Interstitial mononuclear cell infiltrates expressed as percentage of the total number of samples.
Small infiltrates: Aggregates of few mononuclear cells.
Large infiltrates: Aggregates of many mononuclear cells. Incipient muscular necrosis.

Interstitial mononuclear cell infiltrates were found focally in sections from all 3 organs (Fig. 2). The cells were mainly lymphocytes and macrophages, with occasional neutrophils and a few eosinophils. The frequency is presented in Fig. 12. Necrosis of muscle fibres was inconstant. There was a positive correlation between myocardial Sarcocystis infection and the occurrence of mononuclear cell infiltrates ($r = 0.8933$, $P < 0.0001$).

In the myocardium, vascular lesions characterized by intimal musculoelastic or fibroelastic proliferations and concurrent vacuolization of the media were often found in all 3 groups. The morphology and prevalence rate of these changes will be dealt with in a separate paper.

The results of experimental infection of 1 cat, 2 dogs, and 2 foxes are presented in Table 3. Sporocysts were not detected in the faeces from the controls. The feline sporocysts (Fig. 6)

Table 3. Infectivity for dogs and foxes of tissues from sheep naturally infected with S. tenella and S. gigantea, and for a cat fed S. gigantea macrocysts.

Animal	Prepatent period, days	Patent period, number of days with sporocyst shedding, continuous	intermittent	Sporocyst size µm
Dog No. 1	11	31	53	14×10
„ „ 2	10	52	40	
Fox No. 1	17[a]	38	29	14×10
„ „ 2	17[a]	42[b]	45	
Cat	11	52	24	12×8

[a] No sample 15 and 16 days after infection.
[b] One day negative within the period.

showed the characteristics of S. gigantea. Sporocysts found in the fox (Fig. 7) and canine faeces (Fig. 8) were morphologically identical.

DISCUSSION

Cyst morphology and cyst wall criteria for both S. gigantea and S. tenella used in the present investigation are in accordance with *Mehlhorn & Heydorn* (1978) and *Markus* (1978). The occurrence of Sacrocysts infection recorded by histological examination corresponds with results reported from other countries (*Bierschenk* 1979, *Leguia & Herbert* 1979). There was an increasing frequency for both sarcocyst species with increasing age. With respect to S. gigantea infection, no positive samples were found in the lamb group. The overall frequency of S. gigantea in our material was 18.1 %, as compared to 14.5 % reported in an American investigation (*Seneviratna et al.* 1975), 2.6 % with macrocysts (*Boch et al.* 1979) in South Germany, and as high as 81.5 % in Wales (*Farmer et al.* 1978). Husbandry practices and age estimations of the animals infected are probably essential in this connection, since the feline host is mostly restricted to the farm houses and its close surroundings. The size of the feline population is also influential. Owing to close taxonomical relationship between S. gigantea and Toxoplasma gondii and the same feline-ovine life cycle, a similar distribution of the two parasites would be expected. *Waldeland* (1976) found 10—15 % of lambs and 25—37 % of mature sheep positive for

T. gondii, using peptic digestion of skeletal muscles and inoculation into mice. These figures correspond fairly well with 30 % of S. tenella positive ewes in our material, but differ from the S. gigantea negative lamb group. The longer duration of S. gigantea life cycle may be of importance in this context. S. tenella infection rate was 63.92 % in a German report (*Boch et al.* 1978), and there was a predominance of a thick-walled sarcocyst species that occurred in 84.8 % of the samples. In the Netherlands *Kruijf & Bibo* (1976) found 18.0 % of 8-months-old sheep infected with S. tenella, as compared to 25.0 % in Group III in our material.

With respect to S. tenella infection rate, there is a sharp increase from 25.0 % in the lambs to 74.4 % in the yearlings, while the increase from yearlings to older age groups is less dramatic. Winter feeding probably contributes towards the infection rates recorded for both S. gigantea and S. tenella, but obviously the infection pressure, especially for S. tenella, is high on the pastures. Sheep are probably also strongly exposed to the ingestion of sporocysts because of their grazing habits, as suggested for toxoplasma oocysts by *Waldeland* (1976). Further, the survival capacity of Sarcocystis sporocysts is of great importance (*Bergler et al.* 1980). Both dogs and foxes are known to be final hosts of S. tenella (*Ashford* 1977, *Levine* 1977, *Fayer* 1980), and this has also been demonstrated in the present material. The prevalence of Sarcocystis sporocysts in dogs and the red fox (Vulpes vulpes) has been found by *Farmer et al.* (1978) in Wales to be 36.5 % in sheepdogs and 17.0 % in foxes, but a similar survey is not available in this country.

When the 3 methods used were compared, histology and the homogenization technique gave almost identical results in Group I. In Group III the discrepancy between the results of histology and the homogenization method is marked. Trichinoscopy gave lower results than the homogenization technique in all 3 groups. In Group III half of the cases positive for S. tenella infection on trichinoscopy were confirmed on histology. According to *Boch et al.* (1978), in cattle only animals with heavy Sarcocystis infection will be detected on trichonoscopy. Trypsin digestion is considered superior to other methods for the detection of sarcocysts (*Erber* 1977). The enzymatic digestion techniques have not been evaluated against the methods used in our material. Most of the discussion in this paper is based on histology alone.

Infection rates differ among organs in the samples investigated. In all 3 groups the heart was the organ with the heaviest sarcocyst load. In Group I less than the half of the sarcocyst positive cases were confirmed in sections from the diaphragm and/or the oesophagus. Similar results are demonstrated in Group III, while in Group II the infection rates in the diaphragm and the oesophagus were 59.4 % and 68.7 % respectively, as compared to 87.5 % in the heart. Possibly a higher infection rate of S. tenella could be obtained by using 2 more sections from the heart and excluding sections from the diaphragm and the oesophagus. It is therefore reasonable to select the heart as the indicator organ for estimations of S. tenella infection.

Quantitation of the S. tenella infection, except in Group II, revealed a higher score for sarcocysts in the heart than for the diaphragm and oesophagus. This is of some importance for detection of S. tenella infected animals, but also reveals age-related differences in distribution and grade of infection.

Interstitial infiltrates of mononuclear cells were found in an unexpectedly high frequency in all 3 organs. Small infiltrates consisting of few mononuclear cells predominated. *Leek et al.* (1977) found in experiments with lambs that a mild to moderate mononuclear cell infiltration was localized along the perivascular connective tissues of the musculature, and that the cellular response was multifocal without apparent association with developmental sarcocysts. Except for sarcocysts and a few ciliate cysts, no other parasites were seen in the sections in the present material, and other agents capable of inducing similar changes have not been evaluated.

The occurrence of a ciliate in the heart muscle was found incidentally. No further efforts have been made to identify these parasites.

The thick-walled Sarcocystis sp. found in the diaphragm were few in number. They differ from sarcocysts previously described with respect to cyst wall thickness and striations, but possess characteristics in common with thick-walled Sarcocystis sp. described by *Boch et al.* (1979).

Sporocyst production in the final hosts is in accordance with the results reported in other investigations and conforms to criteria given by *Dubey* (1976), *Levine* (1977), and *Levine & Tadros* (1980). Dogs and foxes in our experiment shed identical sporocysts in the faeces with different prepatent periods, varying

from 10—11 days in dogs to 17 days in the silver foxes. In dogs *Rommel et al.* (1974) reported a prepatent period of 8—9 days, as compared to 15 days observed by *Ford* (1974), while *Ashford* (1977) found sporocysts in the red fox (Vulpes vulpes) 9—10 days after feeding meat containing microscopic sarcocysts. In the present work, patent periods were 84 and 92 days in the dogs, and 67 and 87 days in the silver foxes. *Rommel et al.* (1974) observed a patent period of more than 6 weeks duration. *Ford* reported low numbers of sporocysts 60 days after feeding of infective material and no sporocysts after 90 days. In the cat the prepatent period was 11 days, and the patent period lasted 76 days in our experiment. *Rommel et al.* (1972) found a prepatency of 12—13 days. The patent period in the latter report varied from 3—35 days.

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SAMMENDRAG

Undersøkelser vedrørende naturlig forekommende Sarcocystis-infeksjon hos sau i Sørvest-Norge.

I alt 198 tilsynelatende normale saueslakt ble undersøkt med henblikk på forekomst av Sarcocystis-infeksjon og patologiske forandringer i prøver fra hjerte, mellomgolv og spiserør.

Forekomsten av S. gigantea var 18,2 % med følgende gruppefordeling: søyer 30,0 %, årsgamle 11,6 %, lam 0. Forekomsten av S. tenella var 65,1 % med tilsvarende gruppefordeling: søyer 83,5 %, årsgamle 74,4 % og lam 25,0 %.

En tredje Sarcocystis type med tykk vegg ble påvist i mellomgolv hos 3 dyr.

Fokale mononukleære interstitielle infiltrater ble påvist i myocard hos 37,9 %, i mellomgolv hos 19,6 % og i spiserør hos 31,3 %. Forekomsten av Sarcocystis-infeksjon og interstitielle infiltrater var positivt korrelert ($P < 0{,}0001$).

Hund og rev som ble fôret med naturlig infisert muskulatur fra sau og en katt som ble gitt fridissekerte S. gigantea cyster, utskilte sporocyster typiske for henholdsvis S. tenella og S. gigantea.

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