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Life Cycle of *Sarcocystis tenella* in Sheep and Dog

M. Erber*
Institut für Vergleichende Tropenmedizin und Parasitologie, Leopoldstraße 5, D-8000 München 40, Federal Republic of Germany

Abstract. For *Sarcocystis tenella*, the second microscopic sarcocyst in sheep, the dog was shown to act as final host shedding sporocysts measuring 13.75–15.8 (14.8 ± 0.8) × 9.7–10.8 (10.1 ± 0.4) µm after a prepatent period of 8–13 days. The clinical signs and the course of experimental infections in sheep were most similar to *S. ovicanis*. After high doses of sporocysts sheep had temperatures up to 42° C, anaemia, and paresis; they finally died from haemorrhagic diathesis. The development of *S. tenella* in sheep was studied and it resulted in microscopic cysts in the musculature that measured 300–650 × 20–50 µm. They showed hair-like delicate protrusions of the cyst wall measuring 6–8 × < 0.5 µm, by which *S. tenella* could be clearly differentiated from *S. ovicanis* from day 60 p.i. onwards. The decreasing number of *S. tenella* through degeneration of cysts is suggested to be a self-cleaning process.

Introduction

The name *Miescheria tenella* was first used by Railliet (1886a)[1] and later changed to *Sarcocystis tenella* by Moulé (1888) describing microscopic cysts parasitizing in sheep musculature and measuring up to 500 µm in length and 60–100 µm in width (Table 1). Unfortunately, many scientists also used, and still use, the name *Sarcocystis tenella* for macroscopic sarcocysts measuring up to 15 mm in length and up to 6 mm in width. As Collins et al. (1979) pointed out, these cysts represent two different species, for which the cat acts as final host. For the 'fat' oesophageal cysts the above-mentioned authors re-used the term of Railliet (1886b): *Sarcocystis gigantea*[2]. Their ultrastructural photographs are nearly identical with those of *S. ovifelis* Heydorn et al. 1975 (Mehlhorn et al. 1976), and the name might therefore be a synonym. The second macroscopic species, the 'thin' one, they named

1 In a comment on a presentation by Moulé (1886)

2 In a lapsus linguae they wrote *S. gigantia*, but in a later publication (Collins and Charleston 1980) they used the correct term *S. gigantea*

* *Present address:* Liebigstr. 19, D-8000 München 22, West Germany

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Table 1. Nomenclature and properties of *Sarcocystis* spp. in sheep

Name	Author(s) and year	Morphological description	Final host
Balbiania gigantea	Railliet (1886b)	macroscopic cysts like wheat grains, peas or hazel-nuts of size	not determined
Sarcocystis ovifelis	Heydorn et al. (1975)	ovoid macroscopic cysts measuring up to 15 mm	cat
Sarcocystis ovifelis	Mehlhorn et al. (1976)	elmi: cyst wall has cauli-flower-like protrusions up to 4.5 µm in height with fibrils (microtubules)	cat
Sarcocystis gigantea	Collins et al. (1979)	'fat' macroscopic cysts, elmi: cyst wall has irregular and placentiform protrusions (villi), collagenous secondary cyst wall	cat
Sarcocystis medusiformis	Collins et al. (1979)	'thin' macroscopic cysts, elmi: cyst wall has rounded protrusions (villi) with snake-like filaments (i.e. protrusions of the protrusions), no secondary cyst wall	cat
Miescheria tenella	Railliet (1886a)	microscopic cysts measuring up to 500 µm in length and 60–100 µm in width, in sections thin- or thick-walled	not determined
Sarcocystis tenella	Moulé (1888)		not determined
Sarcocystis ovicanis	Heydorn et al. (1975) Boch et al. (1979)	microscopic cysts with 2–4 × 0.6–0.9 µm finger-like stable protrusions without fibrils forming a palisade-like cyst wall, in sections with a 1.5 to 2.0 µm thick radiated wall	dog
Sarcocystis spec.	Boch et al. (1979)	microscopic cysts with 5–11 × < 0.5 µm hair-like delicate protrusions forming a wavy cyst wall, in sections with a thin smooth wall	not determined
Sarcocystis tenella	present paper		dog

S. medusiformis, according to the distinctive structure of its cyst wall protrusions. The different staining properties of the cysts provide further evidence for the existence of two different macroscopic species; the 'fat' *S. gigantea* has PAS-positive secondary cyst walls, whereas the 'thin' *S. medusiformis* shows no PAS-reaction of the cyst walls (Moore 1980).

In sheep musculature two microscopic species also occur: one, with finger-like stable protrusions 2–4 µm long and 0.6–0.9 µm wide (Boch et al. 1979) forming a palisade-like cyst wall, is named *S. ovicanis* (Heydorn et al.

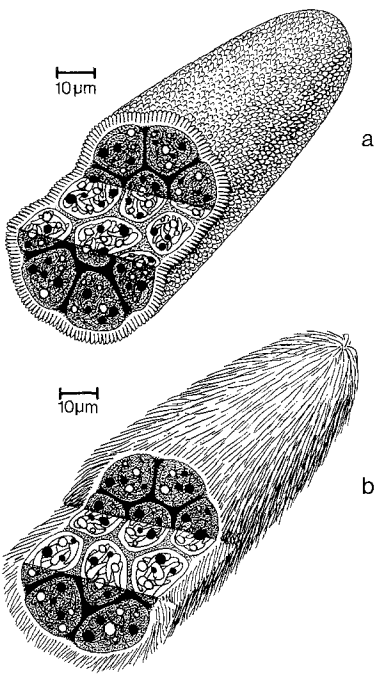

Fig. 1 a, b. Diagram of two isolated microscopic sarcocysts of sheep. **a** *Sarcocystis ovicanis* Heydorn et al. 1975. **b** *Sarcocystis tenella* (Railliet 1886a) Moulé 1888

1975; Fig. 1a), the other has delicate hair-like protrusions 5–11 µm long and <0.5 µm wide forming a wavy cyst wall and is unnamed (*S.* spec. Boch et al. 1979; Fig. 1b). The histological sections depicted by Moulé (1886) may be correlated to these two different microscopic species. Figure 3 in the report by Moulé (1886) shows cysts with thick walls and may represent what we now call *S. ovicanis*, whereas Fig. 5 shows a cyst with a thin or 'delicate' wall and may represent what Boch et al. (1979) described as *S.* spec. As the old name *Sarcocystis tenella*, i.e. delicate sarcocyst, is well-suited to the above-mentioned morphological properties, this name should be restricted to *S.* spec. Boch et al. 1979. The present paper describes some biological characteristics for this *Sarcocystis tenella* (Railliet 1886a) Moulé 1888.

Materials and Methods

Samples of sheep musculature (oesophagus and diaphragm) were collected during meat inspection in Munich municipal abattoir. For the detection of sarcosporidia and demonstration of isolated cysts, the samples were treated with the tryptic digestion and the glass-bead shaking methods as previously described (Erber 1977).

Infected mutton was fed to coccidia-free dogs (beagles) and cats 3–6 months old, conventionally reared and kept on a commercial diet (Altromin pellets and canned food) supplemented with fresh milk. The animals had access to tap water ad libitum. Faecal samples of cats and dogs were collected daily and examined for sporocysts by means of the flotation technique using $ZnCl_2NaCl$ solution (specific gravity 1.3 g/cm^3) and centrifuged 5 min at 800 g (Roto Silenta, Hettich), the examinations started 10 days before feeding and stopped 50 days after feeding.

Sporocysts, collected from the surface of the flotation fluid by a Pasteur pipette were washed twice in tap water and stored at 4 °C after adding some drops of 2.5% potassium

dichromate. To obtain a concentrated suspension of sporocysts one dog was killed after a starvation period of 1 day.

The small intestine was then cleaned of faecal content, homogenized (household homogenizer) and artificially digested in two different media. First the gut was incubated in 2 l pepsin medium (5 g pepsin 30,000 U/g; Merck No. 7190 and 5 ml 37% HCl in 1 l hot tap water) at 40° C for 4 h on a magnetic stirrer. Then it was washed twice with tap water and the sediment digested in 1 l trypsin medium (0.25% trypsin 1:250, Difco No. 0152–15 in PBS with 2 ml 1 N NaOH) at room temperature for 1 h on a magnetic stirrer. After two washings the sediment was stored at 4 °C with some ml of 2.5% potassium dichromate. The concentration of sporocysts was estimated by counting 5 µl of the suspension as previously described (Bergler et al. 1980).

Conventionally reared lambs (Merino) 4–6 months old were inoculated orally with sporocysts with a stomach tube. For parasitological control of the cyst development a piece of about 2 cm^3 was taken from the thigh after sedation (with 1 ml 2% xylazine-hydrochloride i.v., Rompun®, Bayer) and extradural anaesthesia (with 5–10 ml 2% lidocainhydrochloride, Xylocain®, Astra).

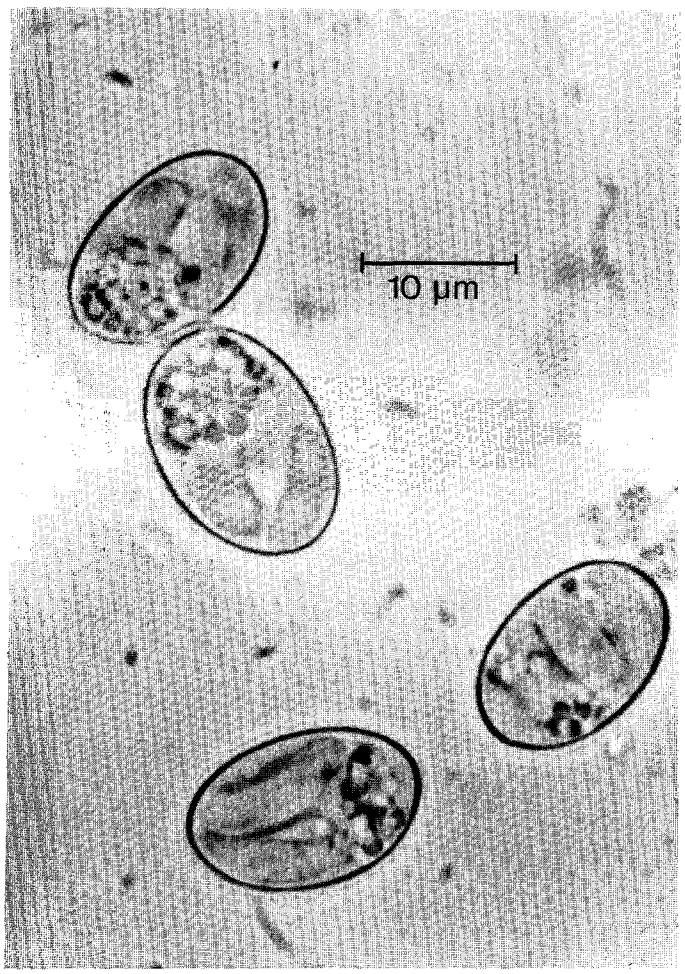

Fig. 2. Sporocysts of *S. tenella* (+ *S. ovicanis?*), shed by dog 82, unstained wet preparation

Results

On two consecutive days two dogs (dog 80 and dog 82) were fed mutton (oesophagus and diaphragm) containing *Sarcocystis tenella* (Fig. 1 b), whereas *S. ovicanis* (Fig. 1 a) was absent. They shed fully sporulated oocysts and sporocysts beginning on day 12 or 13 p.i. for 27 and 14 days respectively (Fig. 2). The sporocysts measured 13.75–15.8 $(14.8 \pm 0.8) \times 9.7$–$10.8$ (10.1 ± 0.4) µm ($n = 50$). Four cats fed mutton from the same lot shed no sporocysts. The excretion rate in dogs 80 and 82 was rather low and on some days only a few sporocysts were found. The collection of sporocysts from the faeces of dog 82 led to a final suspension of 10 ml at a concentration of 25,000 sporocysts/ml, which served as a source for the experimental infection of three lambs with *S. tenella* (Table 2).

Two lambs were dosed with 100,000 sporocysts each (from dog 82). They started a fever up to 42 °C on day 20 p.i. followed by anaemia (haematocrit levels decreased to values of 14%), anorexia and finally paresis. Lamb 316 died on day 28, lamb 315 on day 31 p.i.; both showed at necropsy general haemorrhagic diathesis, i.e. petechia at the whole internal body surface and seromucous fluid in the abdominal and chest cavities. In the liver, kidney and heart of both animals merozoites were seen by means of Giemsa stained impression smears. They measured 10–12×2–4 µm, showing a large dark stained nucleus and granula disseminated in the plasma. Neither in the striated muscles (tongue, oesophagus, diaphragm, abdomen) nor in the heart were metrocytes, cystozoites or sarcocysts evident.

Sheep 320 dosed with 15,000 sporocysts of dog 82 showed no clinical signs. It was slaughtered on day 168 p.i. From all muscles examined a

Table 2. Experimental infections of sheep with *Sarcocystis tenella*

Sheep	Source of sporocysts	Number of inoculated sporocysts	Clinical symptoms	d.p.i.		Schizonts/ merozoites in liver, kidney, heart	Cysts/ zoites in the musculature
				Died	Killed		
315	Dog 82, fed with mutton naturally infected with *S. tenella*	100,000	Beginning on d.p.i. 20: fever up to 42° C, anemia, anorexia, paresis	31		+	–
316	Dog 82	100,000		28		+	–
320	Dog 82	15,000	None		168	–	+
329	Dog 101, fed with sheep 320, 168 d.p.i. with *S. tenella*	60,000	Fever up to 41° C between d.p.i. 25 and 29		117	–	+

d.p.i. = day post inoculation; + = present; – = not present

Table 3. *Sarcocystis* in sheep 320, infected with 15,000 sporocysts of *S. tenella*, 168 d.p.i.

Sample from	Cystozoites demonstrated by trypsin digestion	Differentiation and number of cysts in fresh preparations (cover glass 50 × 24 mm) demonstrated by the glass-bead shaking method	
		S. tenella	*S. ovicanis*
Tongue	+ + + +	>100	0–2
Oesophagus	+ + + +	>100	0–2
Diaphragm	+ + + +	10–15	3–5
Abdominal muscles	+ + +	>100	0–1
Thigh muscles	+ + +	8–12	1–3
Heart	+ + +	4–6	2–4

+ + + = few cystozoites in every microscopic view
+ + + + = many cystozoites in every microscopic view

Table 4. Development of *S. tenella* in sheep 329 (60,000 sporocysts)

d.p.i.	Number of cysts[a]	Size of cysts and characterization of development	Remarks
35	–	–	
50	>50	60–250 × 15–20 µm, immature cysts, chambers not septated, metrocytes only, cyst wall <0.5 µm thick and without protrusions	
60	>100	100–350 × 20–25 µm, 2/3 of the cysts immature, 1/3 of the cysts with hair-like protrusions 5–6 × <0.5 µm, few cystozoites	additionally few *S. ovicanis* cysts
70	>50	100–650 × 20–30 µm, few immature cysts, most cysts with hair-like protrusions 6–8 × <0.5 µm, some with septated chambers	additionally many *S. ovicanis* cysts
117	10–20	300–650 × 20–50 µm, mature cysts containing masses of cystozoites and only few metrocytes in clearly recognizable septated chambers, few degenerated cysts without protrusions containing amorphous material	1/3 of the cysts *S. ovicanis* 2/3 of the cysts *S. tenella*

[a] In unstained fresh preparations (cover glass 50 × 24 mm), demonstrated by the glass-bead shaking method

great many *S. tenella* cysts and some *S. ovicanis* cysts were obtained (Table 3); schizonts or merozoites were not present in the internal organs (Table 2). About 2 kg infected muscles cut in cubes of 5–10 mm were fed to dog 101 on three consecutive days. Beginning on day 8 p.i. dog 101 shed fully sporulated oocysts and sporocysts in the same size range as mentioned above. The faecal excretion of parasites reached its maximum between days 13 and 23, then fell to low rates until day 48 when the faecal examination was stopped. Of these sporocysts gained through flotation 60,000 were orally

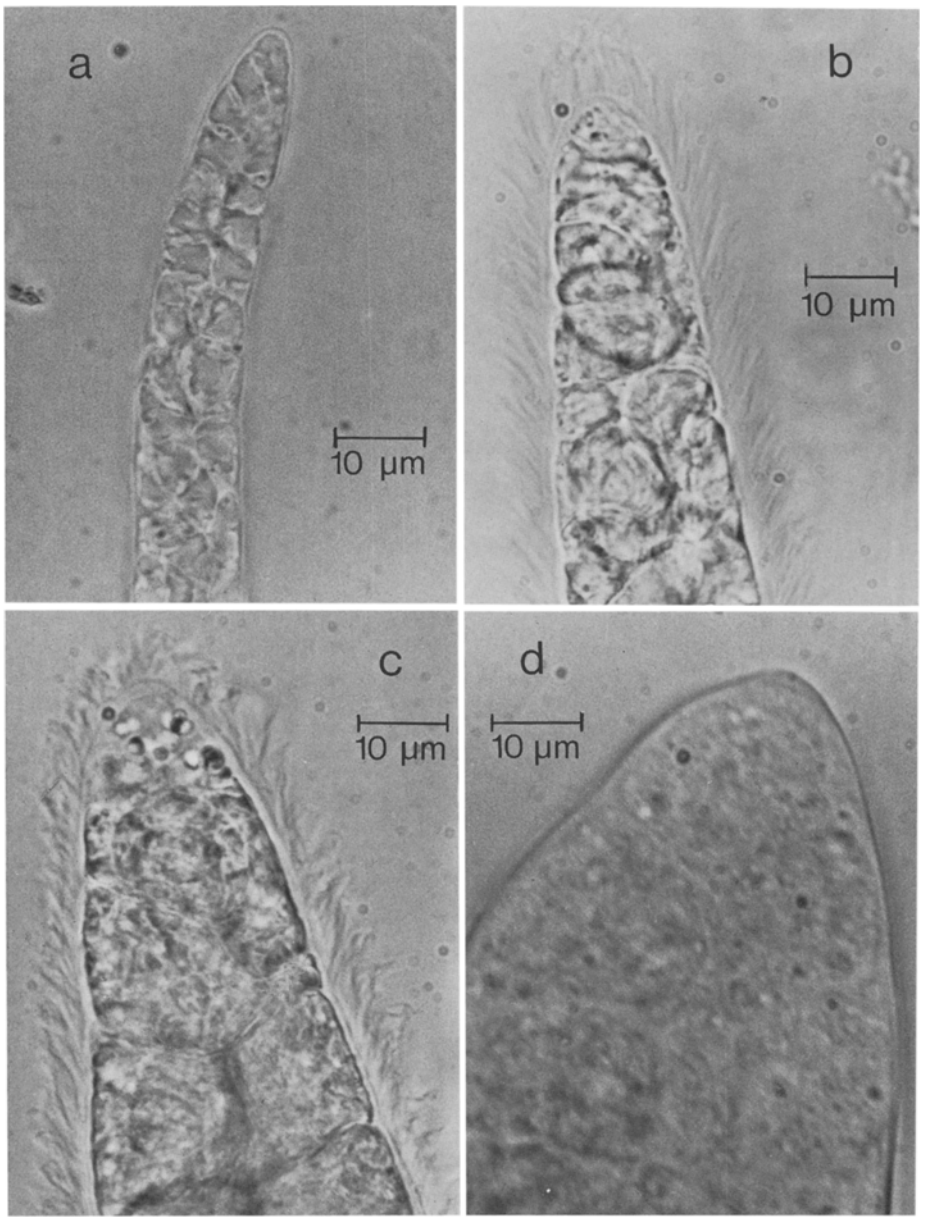

Fig. 3a, b, c, d. Development of *S. tenella* in sheep 329, unstained fresh preparations. **a** on day 50 p.i., immature cyst, **b** on day 60 p.i., maturing cyst, **c** on day 70 p.i., mature cyst, **d** on day 117 p.i., degenerated cyst

administered to sheep 329 (Table 2). Fever up to 41° C was evident between days 25 and 29, but no other clinical signs. Haematocrit values maintained a normal level.

To study the development of *S. tenella* cysts, several muscle biopsies were performed in sheep 329 (Table 4). Whereas on day 35 p.i. no parasites

were found, many immature cysts were found on day 50 p.i. They measured 60–250 µm in length, had a very thin cyst wall without protrusions and contained only metrocytes (Fig. 3a). On day 60 p.i. two-thirds of the cysts found in great numbers were immature, whereas one-third had 5–6 µm long hair-like delicate protrusions (Fig. 3b), which had increased in length on day 70 p.i. up to 8 µm and formed a wavy cyst wall (Fig. 3c). On day 117 p.i., when the animal was slaughtered, cysts were found up to 650 µm in length and 50 µm in width containing mainly masses of banana-shaped cystozoites (=merozoites) in clearly recognizable septated chambers, but also some degenerated cysts without protrusions of the wall and containing amorphous material (Fig. 3d). From day 60 onwards *S. ovicanis* cysts were also found. The proportion of *S. tenella* and *S. ovicanis* in all investigated muscle samples (tongue, oesophagus, diaphragm, heart, abdominal and thigh musculature) after slaughter was about 2:1. Dog 96, which was fed musculature of sheep 117 on five consecutive days, shed sporocysts in the same size range as mentioned above, beginning on day 9. On day 19 the dog was killed and the small intestine was peptically and tryptically digested, which resulted in 20 ml of a suspension containing 2,000,000 sporocysts/ml.

Discussion

It was demonstrated that the dog, which is already known to be final host for *S. ovicanis* (Rommel et al. 1974), also acts as final host for *S. tenella*, the other microscopic sarcocyst of sheep. Depending on the number of ingested sarcocysts, the observed prepatent period was 8–13 days. The shed sporocysts measured $14.8 \pm 0.8 \times 10.1 \pm 0.4$ µm, which is nearly the same size as measured for *S. ovicanis* with $14.8 \pm 0.8 \times 9.9 \pm 0.7$ µm (Heydorn et al. 1975). The clinical signs and the course of the infection in sheep also showed great similarities to *S. ovicanis* (Gestrich et al. 1974; Heydorn and Gestrich 1976; Leek et al. 1977) and, depending on the number of inoculated sporocysts, could lead to death. Nevertheless, *S. tenella* is a separate species, as proven by the studies of cyst development in sheep. Cysts of *S. tenella* were clearly different from those of *S. ovicanis* from day 60 p.i. onwards when they formed a wavy wall consisting of hair-like delicate protrusions.

The prevalence of *S. tenella* in infected sheep has already been studied in Southern Germany and the infection rate of 84.8% is surprisingly high (Boch et al. 1979). The ultrastructure of similar cysts is described in naturally infected sheep from the German Democratic Republic (Bergmann and Kinder 1975) and in experimentally inoculated sheep from Australia (O'Donoghue and Goebel 1981). I also found them in mutton imported from New Zealand (unpublished data). Therefore, it is most likely that in the many works dealing with the prevalence or the experimental transmission of microscopic sarcocysts in sheep *S. tenella* is overlooked or put together with *S. ovicanis* as one species. Surprisingly shows the first publication about *Sarcocystis* in sheep figures of both microscopic species (Moulé 1886).

It is not surprising that the experimental studies carried out here turned out to be a mixture of *S. tenella* and *S. ovicanis*, because the dog acts

as final host for both species from intermediate host sheep. Therefore, it is impossible to separate the two species by repeated cyclical transmissions. After the second series of experiments one-third of the cysts formed were found to be *S. ovicanis*.

The influence of *Sarcocystis* on the weight gain in fattening lambs was investigated using a mixture of *S. ovicanis* and *S. tenella* (Erber and Burgkart 1981). The inoculation of 25,000 sporocysts (about 12,500 sporocysts of each species) reduces the daily weight gain up to 28.5%, depending on the age of the lambs. Both microscopic sarcocysts in sheep may be of economic importance. The number of *S. tenella* decreased through the degeneration of cysts. This is perhaps a self-cleaning process by the intermediate host, which has already been described for *S. suicanis* (Erber and Geisel 1979), *S. equicanis/S. fayeri* (Erber and Geisel 1981) and *S. muris* where cellular reactions, e.g. mononuclear cells and macrophages, are demonstrated.

Sarcocystis tenella (Railliet 1886a) Moulé 1888

Intermediate Host
Domestic sheep (*Ovis aries*)
Cysts: Microscopic in size, 300–650 × 20–50 µm
Cysts wall: in unstained fresh preparations with numerous hair-like delicate protrusions, 5–11 × <0.5 µm.

Final Host
Domestic dog (*Canis lupus familiaris*)
Sporocysts: 13.75–15.8 (14.8 ± 0.8) × 9.7–10.8 (10.1 ± 0.4) µm
Prepatent period: 8–13 days
Pathogenicity: Sporocysts highly pathogenic for lambs in high doses, reduction of weight gain in low doses.

References

Bergler KG, Erber M, Boch J (1980) Untersuchungen zur Überlebensfähigkeit von Sporozysten bzw. Oozysten von *Sarcocystis, Toxoplasma, Hammondia* und *Eimeria* unter Labor- und Freilandbedingungen. Berl Münch Tierärztl Wochenschr 93:288–293

Bergmann V, Kinder E (1975) Unterschiede in der Struktur der Zystenwand bei Sarkozysten des Schafes. Mh Vet Med 30:772–774

Boch J, Bierschenck A, Erber M, Weiland G (1979) *Sarcocystis*- und *Toxoplasma*-Infektionen bei Schlachtschafen in Bayern. Berl Münch Tierärztl Wochenschr 92:137–141

Collins GH, Charleston WAG (1980) Studies on *Sarcocystis* species VII: the effect of temperature on the viability of macrocysts (*Sarcocystis gigantea*) of sheep. New Zealand Vet J 28:189–191

Collins GH, Atkinson E, Charleston WAG (1979) Studies on *Sarcocystis* species III: the macrocystic species of sheep. New Zealand Vet J 27:204–206

Erber M (1977) Möglichkeiten des Nachweises und der Differenzierung von zwei *Sarcocystis*-Arten des Schweines. Berl Münch Tierärztl Wochenschr 90:480–482

Erber M, Burgkart M (1981) Wirtschaftliche Verluste durch Sarkosporidiose (*Sarcocystis ovicanis* und *S.* spec.) bei der Mast von Schaflämmern. Prakt Tierarzt 62:422–427

Erber M, Geisel O (1979) Untersuchungen zur Klinik und Pathologie der *Sarcocystis-suicanis*-Infektion beim Schwein. Berl Münch Tierärztl Wochenschr 92:197–202

Erber M, Geisel O (1981) Vorkommen und Entwicklung von 2 Sarkosporidienarten des Pferdes. Z Parasitenkd 65:283–291

Gestrich R, Schmitt M, Heydorn AO (1974) Pathogenität von *Sarcocystis tenella*-Sporozysten aus den Fäzes von Hunden für Lämmer. Berl Münch Tierärztl Wochenschr 87:362–363

Heydorn AO, Gestrich R (1976) Beiträge zum Lebenszyklus der Sarkosporidien. VII. Entwicklungsstadien von *Sarcocystis ovicanis* im Schaf. Berl. Münch Tierärztl Wochenschr 89:1–5

Heydorn AO, Gestrich R, Mehlhorn H, Rommel M (1975) Proposal for a new nomenclature of the Sarcosporidia. Z Parasitenkd 48:73–82

Leek RG, Fayer R, Johnson AJ (1977) Sheep experimentally infected with *Sarcocystis* from dogs. I. Disease in young lambs. J Parasitol 63:642–650

Mehlhorn H, Hartley WJ, Heydorn AO (1976) A comparative ultrastructural study of the cyst wall of 13 *Sarcocystis* species. Protistologica 12:451–457

Moore S (1980) Two types of ovine *Sarcocystis* macrocysts distinguished by periodic acid-Schiff staining of the cyst walls. New Zealand Vet J 28:101–102

Moulé LT (1886) Psorospermies du tissu musculaire du mouton. Bull Mém Soc Centr Méd Vét 4:125–129, table with figures after p. 164

Moulé LT (1888) Des sarcosporidies et leur fréquence, principalement chez les animaux de boucherie. Soc Sci Arts Vitry-le-Francois 14:3–42

O'Donoghue PJ, Goebel E (1981) The schizogenous proliferation and tissue cyst development in sheep of *Sarcocystis* sp. from dogs; as revealed by light and electron microscopy. VI. Internat Congr Protozool Warsaw, Poland, Abstr 276

Railliet A (1886a) Comment on Moulé (1886) Psorospermies du tissu musculaires du mouton. Bull Mém Soc Centr Méd Vét 4:130

Railliet A (1886b) Psorospermies géantes dans l'oesophage et les muscles du mouton. Bull Mém Soc Centr Méd Vét 4:130–134

Rommel M, Heydorn AO, Fischle B, Gestrich R (1974) Beiträge zum Lebenszyklus der Sarkosporidien. V. Weitere Endwirte der Sarkosporidien von Rind, Schaf und Schwein und die Bedeutung des Zwischenwirtes für die Verbreitung dieser Parasitose. Berl Münch Tierärztl Wochenschr 87:392–396

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