From the Department of Internal Medicine I, Division of Parasitology, Norwegian College of Veterinary Medicine, Oslo, Norway.

# THE FOX AS DEFINITIVE HOST FOR SARCOCYSTIS SP. GJERDE, 1984 FROM SKELETAL MUSCLE OF REINDEER (RANGIFER TARANDUS)

WITH A PROPOSAL FOR SARCOCYSTIS TARANDIVULPES N. SP. AS REPLACEMENT NAME

# Bjørn Gjerde

GJERDE, B.: The fox as definitive host for Sarcocystis sp. Gjerde, 1984 from skeletal muscle of reindeer (Rangifer tarandus). With a proposal for Sarcocystis tarandivulpes n. sp. as replacement name. Acta vet. scand. 1984, 25, 403—410. — Skeletal muscle of 5 wild reindeer was examined for sarcocysts and used for experimental infection of 6 foxes. Skeletal and cardiac muscle of another reindeer were only examined for sarcocysts. The skeletal muscle of all animals was infected with Sarcocystis sp.. In 2 of the animals cysts of S. hardangeri were also present. The single heart examined contained only cysts of S. grupper:

Four foxes given skeletal muscle containing apparently only cysts of Sarcocystis sp., started shedding Sarcocystis sporocysts, measuring

of Sarcocystis sp., started shedding Sarcocystis sporocysts, measuring on average  $13.6\times9.8~\mu m$ , after a prepatent period of 10-12 days. Two foxes given skeletal muscle containing cysts of both Sarcocystis sp. and S. hardangeri shed similar sporocysts, measuring on average  $13.5\times9.7~\mu m$ , after a prepatent period of 10-12 days. Based on the results from the present and previous investigations, Sarcocystis sp. is considered to have foxes (Vulpes vulpes and Alopex lagopus) and dogs (Canis familiaris) as definitive hosts, becoming the second species of Sarcocystis with a known reindeer/Canidae life cycle. The name Sarcocystis tarandivulpes n. sp. is proposed as a replacement name for Sarcocystis sp. Gjerde, 1984 from skeletal muscle of reindeer. muscle of reindeer.

life cycle; dogs; Canidae; intermediate host.

The reindeer in Norway has been found to be the intermediate host for 5 species of Sarcocystis (Protozoa, Sporozoa) (Gjerde 1984 a, b). Four of these species usually parasitize only skeletal muscle, whereas the fifth species apparently only occurs in the heart. S. grueneri from cardiac muscle has been found to have the fox and the dog as definitive hosts (*Gjerde & Bratberg* 1984). The same carnivores also shed Sarcocystis sporocysts after being fed skeletal muscle of domestic reindeer. However, as at least 3 species, none of which was S. grueneri (*Gjerde* 1984 a), occurred simultaneously in the skeletal muscle given, the infective species could not be determined.

In the present paper the outcome of an experimental feeding of foxes with skeletal muscle infected with only 1 species of Sarcocystis is reported, and a replacement name is proposed for Sarcocystis sp. described from skeletal muscle of reindeer by *Gierde* (1984 a, b).

# MATERIALS AND METHODS

Muscle tissue from 6 wild reindeer from Hardangervidda in southern Norway was used in the present investigation. Samples of the abdominal muscles from 4 animals (Nos. 1—4), and samples of the muscles of the scapula and shoulder of 1 animal (No. 5) were examined for sarcocysts and used for experimental feeding of 6 foxes. Samples of the abdominal muscles, the muscles of the scapula and shoulder, the oesophagus and the heart of 1 animal (No. 6) were only examined for sarcocysts.

Figures 1—4. Sarcocystis tarandivulpes n. sp.; fresh preparations of sarcocysts and sporocyst.

Figure 1. Part of cyst from the abdominal muscles. Note short cyst wall protrusions (p), giving the cyst outline a serrated appearance. × 1000.

Figure 2. Part of cyst from the oesophagus. Note difference in size from cyst in Fig. 1. The cyst wall protrusions (p) are evident. × 1000.

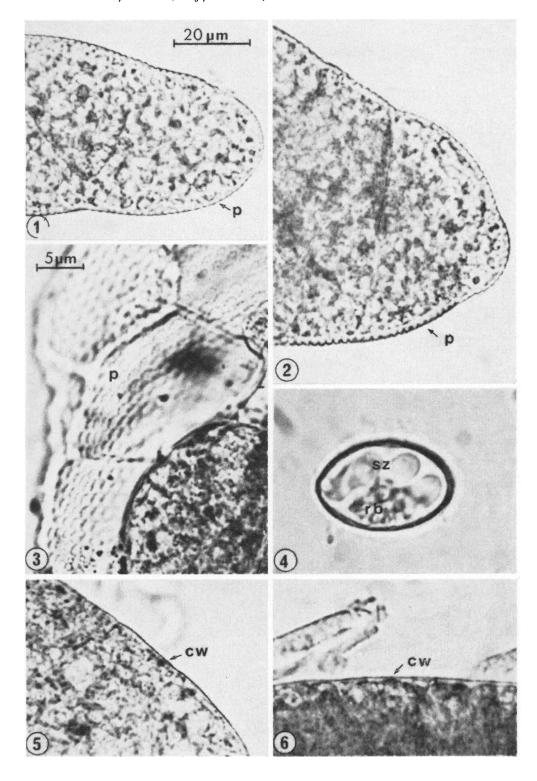
Figure 3. Part of disrupted cyst from the oesophagus. Note numerous, tightly packed cyst wall protrusions (p).  $\times$  2500.

Figure 4. Sporocyst from the faeces of a fox in experiment 1. Note sporozoites (sz) and sporocystic residual body (rb). × 2500.

Figure 5. Part of a cyst of S. grueneri from cardiac muscle. Note thin and smooth cyst wall (cw) without cyst wall protrusions. × 1000.

Figure 6. Part of S. grueneri-like cyst from the oesophagus. Note thin and smooth cyst wall (cw).  $\times$  1000.

Bjørn Gjerde: The fox as definitive host for Sarcocystis sp. Gjerde, 1984 from skeletal muscle of reindeer (Rangifer tarandus).



Fresh muscle tissue was examined both grossly and under a stereoscopic microscope for the presence of sarcocysts. Cysts were isolated and further examined under a light microscope with regard to size, shape and cyst wall structure as described previously (*Gjerde* 1984 a).

For experimental feeding, 6 conventionally reared, individually caged, coccidia-free blue foxes (Alopex lagopus) were used. Each fox was given about ½ kg of muscle tissue in a single feeding. In experiment 1, each of 2 foxes was given a pooled sample from reindeer Nos. 1—3, while each of 2 foxes was given a sample from reindeer No. 4. In experiment 2, each of 2 foxes was given a sample from reindeer No. 5. Faeces were collected daily for nearly 4 weeks, beginning at the day of the experimental feeding, and examined for sporocysts by means of a flotation technique using a NaCl/ZnCl<sub>2</sub>-solution.

The size of sporocysts and mature sarcocysts was measured with an ocular micrometer.

### RESULTS

Examination of muscle tissue for sarcocysts

The findings in reindeer Nos. 1—5 have been described in detail previously (*Gjerde* 1984 b), and will only be mentioned briefly. In the abdominal muscles of reindeer Nos. 1—4, only cysts of Sarcocystis sp., i.e. slender cysts with short, knob-like cyst wall protrusions (about 1 µm long and wide), were found. In the muscles of the scapula and shoulder of reindeer No. 5, cysts of both Sarcocystis sp. and S. hardangeri were found. The latter species had macroscopic, ovoid to cylindrical cysts surrounded by a layer of fibrous material, and with irregularly spaced tongue-like cyst wall protrusions.

In reindeer No. 6, the muscles of the scapula harboured some cysts of S. hardangeri and a few cysts of Sarcocystis sp., while the abdominal muscles contained relatively many cysts of Sarcocystis sp. (Fig. 1), but only a few cysts of S. hardangeri. The cysts of the latter species were typically located just beneath the muscle fascia, close to the junction of muscle and tendon or aponeurosis. The cysts of Sarcocystis sp. in the abdominal muscles measured on average  $970~(610-2000)\times76~(45-130)~\mu m$ ; n=37.

From the oesophagus 36 cysts or parts thereof were isolated and the cyst wall structure studied. Of these, 35 cysts possessed

the short, knob-like protrusions characteristic of Sarcocystis sp. (Figs. 2 and 3), while 1 cyst seemed to be without protrusions and thus had a similar cyst wall structure as S. grueneri (Fig. 6). The cysts of Sarcocystis sp. in the oesophagus measured on average 1320  $(700-2550) \times 195 \ (90-320) \ \mu m$ ; n=21, while the S. grueneri-like cyst measured  $1190 \times 130 \ \mu m$ .

In cardiac muscle of reindeer No. 6, cysts of S. grueneri, i.e. cysts without cyst wall protrusions, occurred in large numbers (Fig. 5). The cysts measured on average 425 (260—575)  $\times$  110 (60—145)  $\mu$ m; n=15.

# Experimental feeding of foxes with skeletal muscle

The 4 foxes in experiment 1 started shedding Sarcocystis sporocysts on days 10, 11, 12 and 12, respectively, after the experimental feeding, while the 2 foxes in experiment 2 started shedding sporocysts on days 10 and 12, respectively, post infection. Some of the foxes also shed a few thin-walled Sarcocystis oocysts during the first days of patency. Sporocysts were still being shed when the experiments ended on days 25 (Exp. 1) and 23 (Exp. 2) after infection, i.e. after a patent period of 14—16 days.

The sporocysts were fully sporulated when shed, and each sporocyst contained 4 elongate sporozoites and a granular sporocystic residual body (Fig. 4). The sporocysts shed by the foxes in experiment 1 measured on average  $13.6 \pm 0.5$  (11.9 - 14.8)  $\times$  9.8  $\pm$  0.4 (8.8 - 10.8) µm; n=405. The mean length to width ratio was 1.38. The 4 foxes in the experiment shed sporocysts of almost identical average size.

The sporocysts shed by the 2 foxes in experiment 2 measured on average 13.5 (11.9—15.2)  $\times$  9.7 (8.4—10.6) µm; n=71. Their mean length to width ratio was 1.39. There was no significant difference between the size of the sporocysts shed in experiment 1 and 2.

## DISCUSSION

Gjerde & Bratberg (1984) described 2 types of thick-walled and 1 type of thin-walled cysts from sections of skeletal muscle of domestic reindeer. In sections of cardiac muscle they found only thin-walled cysts, which they assumed belonged to the same species as the thin-walled cysts in skeletal muscle. Thus, they assigned the old name S. grueneri to the thin-walled cysts in

both cardiac and skeletal muscle. However, when fresh preparations of micro-isolated cysts were examined, the thin-walled cysts could be further differentiated as cysts of 2 different species parasitizing cardiac and skeletal muscle, respectively (*Gjerde* 1984 a). The name S. grueneri was confined to the species having cysts without cyst wall protrusions in cardiac muscle, whereas Sarcocystis sp. was used to designate the species having cysts with very short, knob-like protrusions in skeletal muscle.

Gjerde & Bratberg (1984) also found that the feeding of either cardiac or skeletal muscle separately to foxes and dogs, induced the shedding of Sarcocystis sporocysts by the carnivores, indicating that the single type of cysts in the heart, and at least 1 of the 3 types of cysts in skeletal muscle had been infectious. However, when it appeared that the thin-walled cysts in cardiac and skeletal muscle belonged to 2 different species, some of the statements made concerning their definitive hosts were no longer valid. While it still was true that S. grueneri (from cardiac muscle) had the fox and the dog as definitive hosts, any of the species occurring in skeletal muscle (S. rangiferi, S. tarandi, Sarcocystis sp.) could have caused the sporocyst shedding when this type of muscle tissue was given. However, the feeding of isolated cysts of S. rangiferi to foxes and dogs did not result in sporocyst shedding (Gjerde & Bratberg 1984), indicating that these carnivores were unsuitable hosts for this species.

In the present investigation the experimental feeding of foxes with skeletal muscle of wild reindeer harbouring cysts of Sarcocystis sp. only (experiment 1), or cysts of both Sarcocystis sp. and S. hardangeri (experiment 2), induced the shedding of sporocysts. In experiment 1 there is every reason to believe that Sarcocystis sp. was the infectious species, while in experiment 2 both Sarcocystis sp. and S. hardangeri could have been so. However, the possibility of 1 or more other infectious species being present in the muscle tissue used in experiment 1, cannot be entirely ruled out, although it is not very likely from our present knowledge.

Cysts of S. grueneri, the other species with a fox/reindeer life cycle, have so far not been detected with certainty in skeletal muscle (*Gjerde* 1984 a, b). In the present investigation a S. grueneri-like cyst was only found in the oesophagus, but not in the abdominal muscles or the muscles of the scapula and shoulder. Such a strong predilection for certain muscle sites is

also known from other Sarcocystis species. In naturally infected cattle, for instance, S. cruzi has a distinct predilection for cardiac muscle, to the exclusion of S. hominis and S. hirsuta, which have a predilection for the muscles of the lower oesophagus (Tadros & Laarman 1982). Reindeer infected with S. hardangeri seem to harbour few cysts of this species in the abdominal muscles (Gjerde, unpublished observations and present investigation), and an infection with S. rangiferi and S. tarandi has so far not been seen in wild reindeer from Hardangervidda.

Furthermore, it is not likely that a very light and undetected infection with any of these species alone could have caused the shedding of a detectable number of sporocysts. Consequently, Sarcocystis sp. must have been the main or single contributor to the sporocyst shedding observed in experiment 1, establishing the fox as definitive host for this species. The sporocysts shed by the foxes in experiment 2 in the present investigation, and those passed by silver foxes, blue foxes and dogs given skeletal muscle of domestic reindeer (*Gjerde & Bratberg* 1984), must therefore also have been, wholly or in part, sporocysts of Sarcocystis sp.

The reindeer is thus considered to be the intermediate host for 2 different species of Sarcocystis completing their life cycle in Canidae. A corresponding infection of the same intermediate host with 2 species having Canidae as definitive hosts has also been found in sheep (Erber 1982), goat (Heydorn & Unterholzner 1983), horse (Erber & Geisel 1981) and roe deer (Erber et al. 1978). It would, however, have been desireable to have the present and previous findings confirmed by experimental infection of Sarcocystis-free reindeer with sporocysts derived from Canidae fed Sarcocystis-infected cardiac and skeletal muscle, respectively. So far, it has not been possible to carry out such experiments at our department.

S. grueneri and Sarcocystis sp. have a similar prepatent period of 11 days (*Gjerde & Bratberg* 1984) and 10—12 days, respectively. The sporocysts shed by foxes given skeletal muscle of domestic reindeer have been found to be slightly larger than those shed by foxes given cardiac muscle (*Gjerde & Bratberg* 1984, *Gjerde*, unpublished observations), suggesting that Sarcocystis sp. has slightly larger sporocysts than S. grueneri.

It is known that several Sarcocystis species may have various, but usually closely related definitive hosts (*Tadros & Laarman* 1982). Both S. grueneri and Sarcocystis sp. of reindeer seem to

undergo gametogony in different Canidae (*Gjerde & Bratberg* 1984). In Norway, the red fox (Vulpes vulpes), of which the silver fox is a colour mutant, is considered to be the most important definitive host for these 2 species, being the most common canine carnivore in the reindeer areas. The name Sarcocystis tarandivulpes n. sp. is therefore proposed as a replacement name for Sarcocystis sp. described by *Gjerde* (1984 a, b) previously, and in the present paper.

More research is needed to further elucidate the life cycle of S. tarandivulpes and S. grueneri, and to determine the difference between the 2 species in cyst ultrastructure and type of muscle tissue invaded. It would also be of interest to determine the pathogenicity of these 2 species for the reindeer, as several other species of Sarcocystis undergoing gametogony in Canidae are known to cause severe and even fatal disease during their early development, i.e. the schizogonic proliferation in the vascular endothelium, in the intermediate host (Tadros & Laarman 1982).

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### SAMANDRAG

Rev som endevert for Sarcocystis sp. Gjerde, 1984 frå skjelettmuskulaturen hos rein (Rangifer tarandus). Med eit framlegg om Sarcocystis tarandivulpes n. sp. som nytt namn.

Fersk skjelettmuskulatur frå 5 villreinar var undersøkt for sarcocyster og nytta til eksperimentell fôring av 6 blårevar. Skjelett- og hjertemuskulatur av ein annan villrein vart berre undersøkt for sarcocyster. Fire revar som hadde fått skjelettmuskulatur som berre inneheldt cyster av Sarcocystis sp., tok til å skilja ut Sarcocystis-sporocyster etter ein prepatentperiode på 10—12 dagar. Sporocystene målte i gjennomsnitt  $13.6\times9.8~\mu m$ . To revar som hadde fått skjelettmuskulatur som inneheldt cyster av både Sarcocystis sp. og S. hardangeri, skilde ut sporocyster som målte  $13.5\times9.7~\mu m$  etter ein prepatentperiode på 10-12 dagar.

På grunnlag av resultata frå denne og tidlegare granskingar konkluderer ein med at Sarcocystis sp. frå skjelettmuskulaturen hos rein har hund og rev som endevertar. Ein gjer framlegg om at namnet S. tarandivulpes n. sp. avløyser den tidlegare nytta nemninga for denne arta. S. tarandivulpes er den andre arta hos rein, ved sida av S. grueneri frå hjertemuskulaturen, som har dyr av hundeslekta som endevertar. Dei 2 artene har om lag same prepatenstid og sporocyster av om lag same storleik.

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Reprints may be requested from: B. Gjerde, the Norwegian College of Veterinary Medicine, Box 8146, Dep., N-0033 Oslo 1, Norway.