Acta vet. scand. 1984, *25*, 205—212.

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

SARCOCYSTIS INFECTION IN WILD REINDEER (RANGIFER TARANDUS) FROM HARDANGERVIDDA IN SOUTHERN NORWAY: WITH A DESCRIPTION OF THE CYSTS OF SARCOCYSTIS HARDANGERI N. SP.

By

Bjørn Gjerde

GJERDE, B.: *Sarcocystis infection in wild reindeer (Rangifer tarandus) from Hardangervidda in southern Norway: With a description of the cysts of Sarcocystis hardangeri n. sp.* Acta vet. scand. 1984, *25*, 205—212. — Fresh preparations of micro-isolated sarcosysts from skeletal muscle of 5 wild reindeer were examined by light microscopy. Slender, spindelshaped cysts measuring 821 × 60 μm, and having short knob-like cyst wall protrusions were found in all animals. In 1 animal cysts different in structure from the cysts of the 4 previously known Sarcocystis spp. of reindeer were found. These cysts are considered to be cysts of a new Sarcocystis sp. of reindeer, for which the name Sarcocystis hardangeri has been proposed.

S. hardangeri n. sp. had macroscopic, ovoid to cylindrical cysts measuring 1667 (900—2570) × 819 (450—1575) μm. The cysts were surrounded by a 8—10 μm thick layer of fibrillar material. After removal of this layer, relatively few and irregularly spaced, slanting protrusions became visible. The 20—30 μm long protrusions were tongue-like, and were lying close to the surface of the cyst.

Cysts of S. grueneri, S. rangiferi and S. tarandi were not demonstrated in the 5 wild reindeer examined.

cyst structure; cyst wall structure; intermediate host.

The wild and the domestic reindeer in Norway belong to the same subspecies, Rangifer tarandus tarandus L., within the genus Rangifer. Wild reindeer are distributed only in certain mountain regions in southern Norway, while domestic reindeer are found mainly in the middle and northern parts of the country (*Krafft* 1981).

In previous papers (*Gjerde & Bratberg* 1984, *Gjerde* 1984) 4 Sarcocystis species infecting the domestic reindeer in northern Norway have been described. In the present paper a report is given of Sarcocystis infection in wild reindeer from Hardangervidda in southern Norway, and the cysts of S. hardangeri n. sp. are described.

MATERIALS AND METHODS

Samples of skeletal muscle were obtained from 5 wild reindeer shot in the eastern part of Hardangervidda in the winter of 1983 during a research project conducted by the Directorate of Wildlife and Freshwater Fish (DVF), the Game Research Division, Trondheim. Samples of the abdominal muscles were obtained from 4 animals (8 months to 3 years old), and the entire upper forelimb were obtained from 1 animal (age unknown).

Fresh muscle tissue was examined both grossly and under a stereo microscope for the presence of sarcocysts. Cysts were isolated and examined under a light microscope as described by *Gjerde* (1984).

A regression analysis was performed on the values of cyst length and maximum width (diameter) to get an impression of the growth pattern of the cysts. The mean length to width ratio (L/W) was calculated from the individual ratios.

RESULTS

Infection with Sarcocystis sp.

Micro- to macroscopic, slender, spindelshaped cysts (Fig. 1) were found in the muscle tissue of all 5 animals examined. The cysts measured on average 821 ± 237 (475—1380) µm in length

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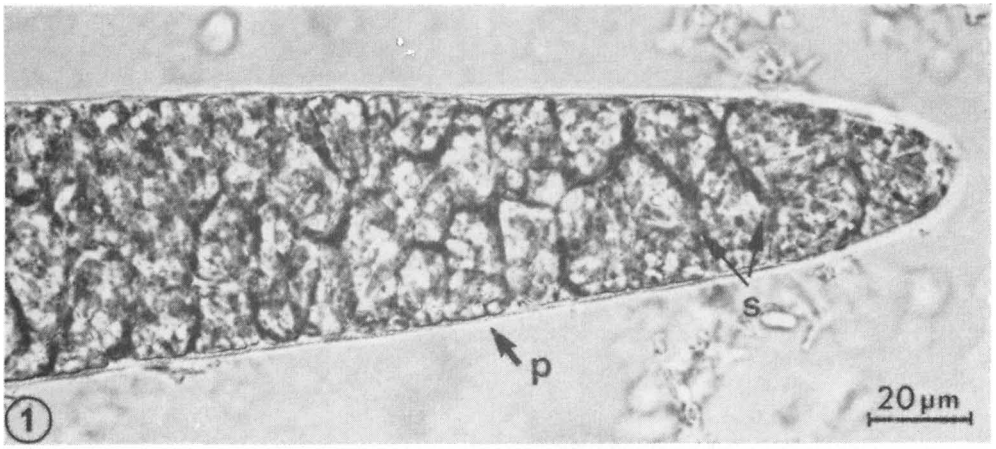
Figure 1. Sarcocystis sp. Part of a micro-isolated cyst. Note slender cyst with thin septa (s) and knob-like protrusions (p) of the cyst wall. Fresh preparation. × 680.

Figures 2 — 7. Sarcocystis hardangeri n. sp.; fresh preparations of cysts and cystozoites. In Figs. 3—6 the fibrillar layer has been removed.

Figure 2. Oviform cyst surrounded by a layer of fibrillar material (fl). The large diameter of the cyst makes the interior appear dark on the photomicrograph. × 68.

Figure 3. Part of cyst. Note tongue-like protrusions (p) covering an otherwise smooth cyst wall (cw). × 425.



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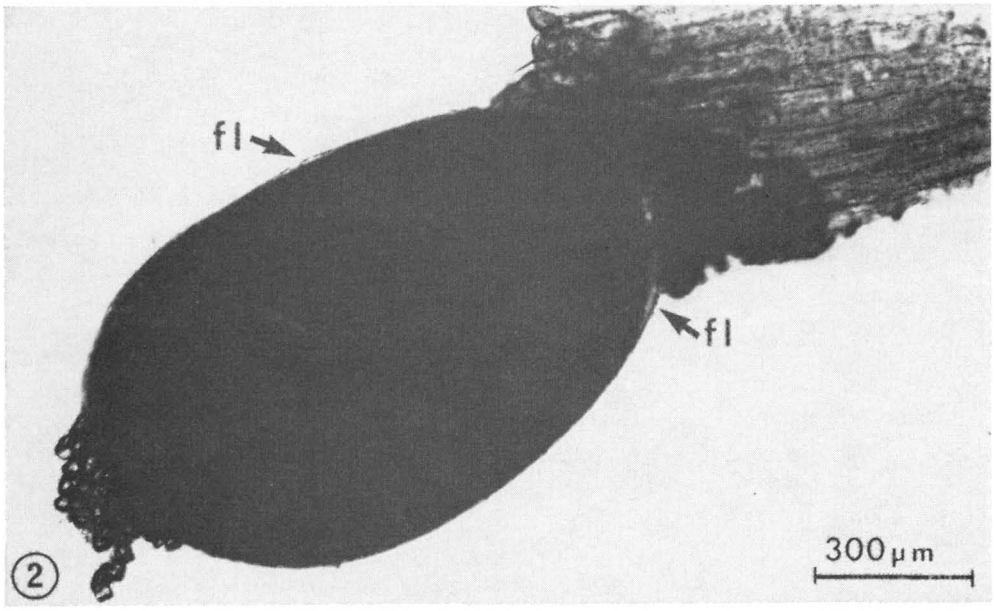

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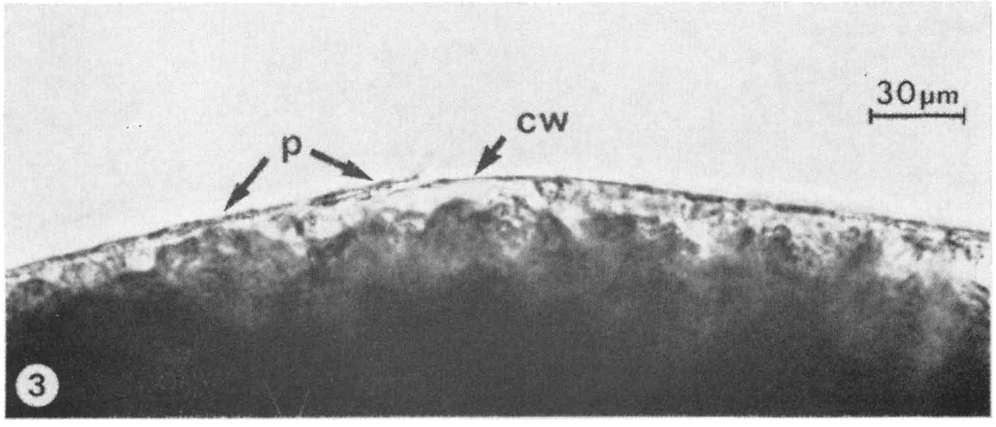

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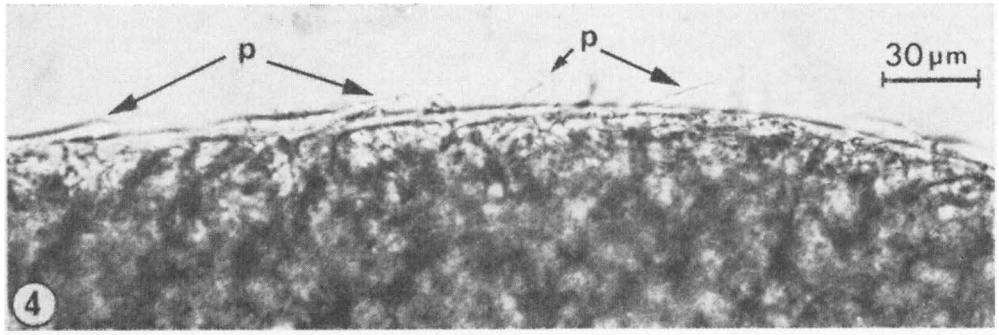
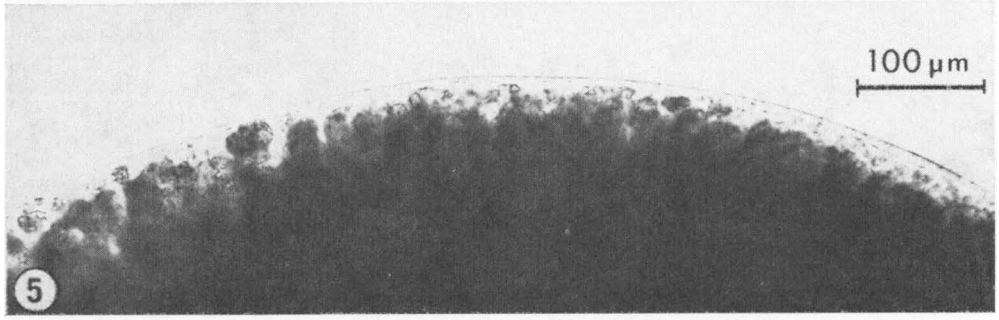
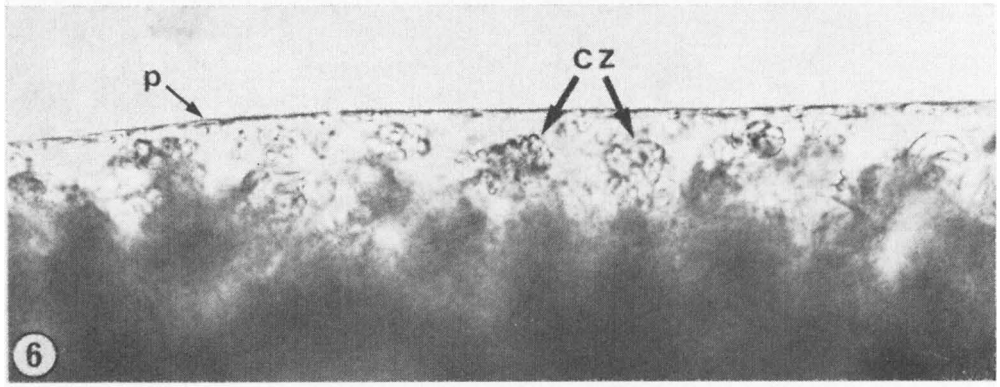

p
p
30 µm
4


100 µm
5


p
cz
6


7

(the mean ± the standard deviation), and 60 ± 16 (35—100) μm in width; n=37. Their mean length to width ratio was 14.16.

The cyst wall had short (≤ 1 μm), knob-like protrusions, giving the cyst wall an indented appearance (Fig. 1). On the basis of their structure these cysts were identified as cysts of Sarcocystis sp. described from skeletal muscle of domestic reindeer by *Gjerde* (1984).

Infection with Sarcocystis hardangeri n. sp.

In the muscles on the scapula and shoulder of 1 reindeer both cysts of Sarcocystis sp. and cysts with a markedly different structure were found. The latter cysts were macroscopic in size, appearing as small, white grains in the muscles. In the superficial muscles on the scapula and shoulder the cysts seemed to be located mainly in the muscle fibers lying just beneath the epimysium and fascia, and often at the end of muscles close to the junction of muscle and tendon. In the deep muscles on the scapula the cysts were located close to the attachment of muscle to bone, i.e. close to the periost.

The cysts were ovoid, elliptical or cylindrical (more or less oviform) in shape (Fig. 2). They measured on average 1667 ± 446 (900—2570) μm in length, and 819 ± 191 (450—1575) μm in width; n=44. Their mean length to width ratio was 2.07 (1.18—3.51). The sample regression of width on length showed that the width (maximum diameter) increased on an average 0.230 μm per μm increase in length.

The cysts were surrounded by a 8—10 μm thick layer of fibrillar material (Fig. 2). This layer would frequently rupture during the process of micro-isolation, releasing the cyst through the tear, while the fibrillar layer was left as an empty shell.

Figure 4. Part of cyst. The protrusions (p) are projecting slightly from the surface of the cyst, giving the cyst outline a ragged appearance. × 425.

Figure 5. Part of cyst. The protrusions are lying so close to the surface of the cyst that they cannot be seen at this magnification. × 170.

Figure 6. Part of cyst. The protrusions (p) are lying in close contact with the surface of the cyst and are difficult to detect. Note clusters of cystozoites (cz) within cyst. × 425.

Figure 7. Cystozoites of S. hardangeri. × 425.

When the fibrillar layer had been removed, the cyst wall proper could be seen to have relatively few and irregularly spaced protrusions (Figs. 3 and 4). The protrusions were tongue-like in shape (resembling linear or lanceolate leaves). They were 20—30 μm long, about 2 μm thick, and 4—7 μm wide, tapering at the distal end. The slanting protrusions were lying close to the surface of the cyst. In some cysts the distal end of the protrusions projected slightly from the surface of the cyst, giving the cyst outline a ragged appearance (Figs. 3 and 4). In other cysts the protrusions at full length were lying in close contact with the surface of the cyst, making them difficult to detect, and giving the impression of a cyst wall without any protrusions (Figs. 5 and 6). Between the protrusions the cyst wall seemed to be smooth (Fig. 6).

The interior of the cysts was divided into numerous small chambers by rather conspicuous septa. The chambers contained banana-shaped crystozoites measuring on average 12.1 (11.2—12.9) × 3.2 (2.8—3.5) μm; n=15 (Fig. 7).

The cysts described above could not be identified as cysts of any of the 3 previously known Sarcocystis spp. of reindeer, and thus represent cysts of a new species, for which the name Sarcocystis hardangeri is proposed.

Table 1. Characteristics of the cysts of 5 Sarcocystis spp. infecting the reindeer in Norway.

Sarcocystis species	Host	Cyst length (in μm)	Cyst width (in μm)	L/W	Protrusions	Fibril. layer
S. hardangeri n. sp.	W.r.	1667 (900—2570)	819 (450—1575)	2.07	Tongue-like, 20—30 μm	Yes
Sarcocystis sp. (S)	W.r.	821 (475—1380)	60 (35—100)	14.16	Short, knob-like,	No
Sarcocystis sp. (S)	D.r.	916 (450—1415)	64 (40—135)	15.00	Short, knob-like	No
S. grueneri (H)	D.r.	581 (240—1160)	137 (45—325)	4.57	None	No
S. tarandi	D.r.	999 (450—2206)	75 (40—255)	14.08	Finger-like, 9.2×2.2 μm	No
S. rangiferi	D.r.	2106 (836—4740)	403 (135—810)	5.31	Finger-like, 13.2×6.7 μm	Yes

W.r. = wild reindeer; D.r. = domestic reindeer
S = in skeletal muscle; H = in the heart

In Table 1 some of the characteristics of the 2 types of sarcocysts found in wild reindeer in the present investigation are presented, along with the characteristics of the cysts found in domestic reindeer by *Gjerde* (1984).

DISCUSSION

In the present investigation of skeletal muscle from wild reindeer 2 types of sarcocysts were found. In a given species of intermediate host, mature cysts displaying distinctly different structure, especially of their walls (size and shape of protrusions if present, presence of a fibrillar layer), are considered to belong to different species of Sarcocystis specific to that particular intermediate host (*Mehlhorn & Heydorn* 1978). As the wild and domestic reindeer can be considered to be identical in a parasitological context, a direct comparison can be made between the cysts found in the present investigation and hose found in domestic reindeer by *Gjerde* (1984).

Thus, the slender, spindelshaped cysts occurring in all 5 wild reindeer examined, can be classified as cysts of the Sarcocystis sp. previously found in skeletal muscle of domestic reindeer (compare Table 1). The macroscopic, ovoid to cylindrical cysts surrounded by a fibrillar layer, on the other hand, displayed a structure distinguishing them from the cysts of the previously known Sarcocystis spp. of reindeer (Table 1). Consequently, these cysts represent cysts of a new Sarcocystis sp. of reindeer, for which the name Sarcocystis hardangeri is proposed.

The name is derived from the name of the geographical area, Hardangervidda (the largest alpine plane in northern Europe), from which the wild reindeer first recognized to be infected with this species, originated. The Hardangervidda wild reindeer area is the largest and most densly populated wild reindeer area in Norway, with a total wild reindeer population of more than 20,000 animals (*Krafft* 1981).

Objections may be raised to the statement that S. hardangeri is a reindeer species, as it was only found in 1 animal. This species was not described from domestic reindeer in northern Norway by *Gjerde & Bratberg* (1984) or by *Gjerde* (1984). However, in recent investigations (*Gjerde*, unpublished observations) a few domestic reindeer from northern Norway have also been found to harbour cysts of this species. It is also possible that the

"rice grain cysts resembling Balbiania" (i.e. S. gigantea of sheep) found by *Hadwen* (1922) in a few reindeer/caribous in Alaska were cysts of S. hardangeri, but they might as well have been cysts of S. rangiferi, the second species with macroscopic cysts in reindeer. The smaller, more prevalent sarcocysts found by *Hadwen* (1922) in cardiac and skeletal muscle, seem to have been cysts of S. grueneri and Sarcocystis sp., respectively.

From the results of the present investigation and previous investigations (*Gjerde & Bratberg* 1984, *Gjerde* 1984) it can be stated that the reindeer in Norway is the intermediate host for 5 species of Sarcocystis. Although cysts of S. grueneri, S. rangiferi and S. tarandi were not found in the present investigation, these species might well occur in wild reindeer from Hardangervidda, as only samples of skeletal muscle from 5 animals were examined. Cysts of S. grueneri have previously only been found in cardiac muscle of reindeer (*Gjerde* 1984).

Sarcocystis species have an obligatory two-host life cycle. Differences in the prevalence of some of the species between wild and domestic reindeer, distributed in different geographical areas, might therefore be expected, as the required definitive hosts might not be equally distributed in the different areas. So far, the definitive hosts of Sarcocystis sp., S. hardangeri, S. rangiferi and S. tarandi are unknown, while S. grueneri has the fox (and probably the dog) as definitive host (*Gjerde & Bratberg* 1984).

S. hardangeri and S. rangiferi both have cysts surrounded by a fibrillar layer (Table 1). However, the differences in the size and shape of the cysts of these 2 species, and in the structure of the cyst wall, make it easy to differentiate between them. Thus, the cysts of S. rangiferi have numerous, erect, finger-like protrusions that are visible when the cysts are surrounded by their fibrillar layer (*Gjerde* 1984). The cysts of S. hardangeri, on the other hand, have relatively few, slanting, tongue-like protrusions that become visible only after removal of the fibrillar layer.

Whether the fibrillar layer of S. hardangeri represents a secondary cyst wall as defined by *Mehlhorn & Heydorn* (1978), i.e. being of host origin and enclosing the entire parasitized host cell, could not be determined by the methods employed in the present investigation. The answer to this question will have to await the results of the electron microscopic examination of the cysts.

ACKNOWLEDGEMENTS

The author would like to thank I. Catrinius and H. Giltvedt at the Photographic Department, the Norwegian College of Veterinary Medicine for preparation of the photomicrographs.

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SAMANDRAG

Sarcocystis-infeksjon hos villrein frå Harangervidda: Med ei skildring av cystene til S. hardangeri n. sp.

Prøvar av skjelettmuskulatur frå 5 villreinar frå Hardangervidda vart undersøkt for sarcocyster både makroskopisk og ved hjelp av eit stereomikroskop. Cyster vart isolert og vidare undersøkt under eit lysmikroskop for artsdifferensiering. Funna hos villrein vart direkte samanlikna med tidlegare funn hos tamrein, då dei begge tilhøyrer same underart innanfor slekta Rangifer.

I alle dei 5 undersøkte dyra fann ein cyster like cystene til Sarcocystis sp. frå skjelettmuskulatur hos tamrein. I eitt av dyra (der ein bog vart undersøkt) fann ein cyster som var ulike cystene til dei 4 tidlegare kjende Sarcocystis-artene hos rein. Dette var såleis cyster til ei ny art, som ein har gitt namnet S. hardangeri.

S. hardangeri n. sp. hadde makroskopiske, eggforma cyster. I dei overflatiske musklane på scapula fann ein gjerne cystene like under muskelfascien, nær overgangen mellom muskel og sene. I dei djupe musklane med direkte feste på scapula fann ein cystene like over periost. Cystene målte i gennomsnitt 1667 (900—2570) × 819 (450—1575) µm.

Cystene var omgjevne av eit 8—10 µm tjukt fibrillært lag. Etter at dette laget var fjerna, kunne ein sjå at cysteveggen hadde tungeliknande protrusjonar som stod med relativt stor og varierande avstand

seg imellom. Protrusjonane var 20—30 μ lange, om lag 2 μm tjukke og 4—7 μm breie med spiss ende. Protrusjonane stod anten på skrå litt opp frå cysta, eller dei låg tett inntil cysteoverflata i heile si lengde.

Cyster av S. grueneri, S. rangiferi og S. tarandi vart ikkje påvist i dei 5 undersøkte dyra.

(Received April 6, 1984).

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