Sarcocystis species in skeletal muscle of otter (*Lutra lutra*)

K. WAHLSTRÖM[1,2], T. NIKKILÄ[3] *and* A. UGGLA[2]*

[1] *Swedish Museum of Natural History, Stockholm, Sweden*
[2] *Department of Parasitology, National Veterinary Institute and Swedish University of Agricultural Sciences, PO Box 7073, S-750 07 Uppsala, Sweden*
[3] *Department of Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden*

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SUMMARY

Cysts of a *Sarcocystis* species were found in large numbers in skeletal muscle of an otter (*Lutra lutra*) which was raised in Norway and died in captivity in Sweden. This is the first report of *Sarcocystis* infection in the otter. The sarcocysts were 0·3–2·3 mm long and 0·06–0·25 mm wide. As judged by light microscopy the sarcocyst walls were thin (< 3 μm) with a serrated surface but without visible projections. By transmission electron microscopy, the sarcocyst wall measured 0·6–1·8 μm and had minute undulations covering the entire sarcocyst surface giving the wall a wavy appearance. Septa were indistinct. The sarcocysts contained few metrocytes and numerous bradyzoites. Sarcocysts were not found in 69 other otters subjected to necropsy in Sweden.

Key words: *Sarcocystis* sp., ultrastructure, otter, *Lutra lutra*.

INTRODUCTION

Parasites of the genus *Sarcocystis* (phylum Apicomplexa) have obligatory prey–predator 2-host life-cycles. The sexual cycle takes place in the intestinal mucosa of the definitive host, usually a carnivore, and results in the formation of sporocysts which are shed in the faeces. The asexual cycle which leads to the development of sarcocysts in muscular or neural tissues occurs in the intermediate host which is generally a herbivorous or omnivorous animal. Sarcocysts are only occasionally found in carnivores which thereby function as intermediate hosts. More than 130 species of *Sarcocystis* have been described, but only in less than half of these have their life-cycles been elucidated (for a review, see Dubey, Speer & Fayer (1989)).

The otter (*Lutra lutra*), belonging to the Mustelidae family, is a semi-aquatic mammal with mainly fish in its diet. In Sweden, as in many other parts of the world, the otter population has been reduced to very low numbers. The otter is now a protected species in Sweden, and, as part of a national conservation project, captive-bred otters are released into the wild to strengthen the population.

Infection with *Sarcocystis* species has not been previously described in the otter, which is a predatory animal. We report here the findings of muscular sarcocysts in an otter imported from Norway to the Swedish otter conservation project, and describe the structure of the sarcocysts.

* Corresponding author: Department of Parasitology, National Veterinary Institute and Swedish University of Agricultural Sciences, PO Box 7073, SE-750 07 Uppsala, Sweden.
Tel: +46 18 67 41 79. Fax: +46 18 30 91 62. E-mail: arvid.uggla@sva.se

MATERIALS AND METHODS

Animal

An adult female otter captured and imported from Norland, Norway, died before it was to be released into the wild in Sweden. The animal was necropsied as part of a larger study on the pathology of Swedish otters, comprising 70 cases up to date.

Post-mortem procedures

At necropsy, portions of heart, skeletal muscle and several other tissues were fixed in 10% neutral, buffered formalin. Tongue and oesophagus were not included. Tissues were then routinely processed and stained by haematoxylin and eosin (H&E). Sections of skeletal muscle were additionally stained by periodic acid–Schiff (PAS).

For electron microscopy, portions of skeletal muscle fixed in formalin as above were post-fixed in 2% osmium tetroxide in 0·1% phosphate buffer for 1 h at 4 °C, dehydrated in an ascending ethanol series and embedded in Epon (Agar Scientific Ltd, Stanstead, UK). Ultrathin sections were stained by Reinold's uranyl acetate–lead citrate (National Veterinary Institute, Uppsala, Sweden). A Philips EM 420 transmission electron microscope was used for examination and photography.

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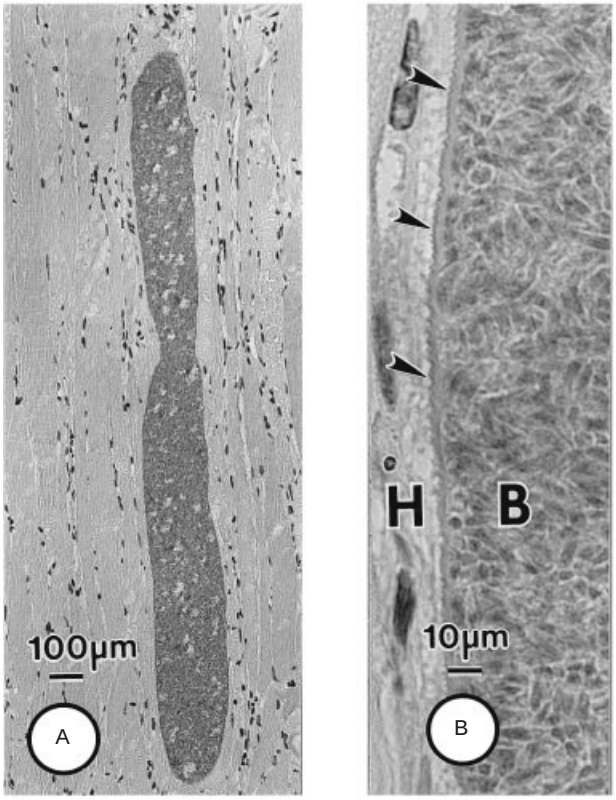

Fig. 1. (A and B) Sarcocyst (*Sarcocystis* sp.) from skeletal muscles of an otter (*Lutra lutra*). Haematoxylin and eosin. (A) Longitudinal section. (B) Higher magnification showing a thin cyst wall with minute serrations (arrowheads). H, host cell; B, bradyzoites.

RESULTS

Grossly, the otter was found to be in a poor condition; it was cachectic and anaemic, had a broken tail and had lost teeth. The cut surface of muscles had a motteled appearance with some haemorrhages. Microscopically, numerous sarcocysts were found in skeletal muscles. The muscles were partly heavily degenerated but there was no specific reaction adjacent to the sarcocysts (Fig. 1 A). The sarcocysts were localized in skeletal muscle from different parts of the body, but no sarcocysts were found in the heart or in other organs studied. Sarcocysts were not found in any of the other 69 otters necropsied.

The sarcocysts examined were structurally identical except for their dimensions, and all appeared fully developed. Sarcocyst walls were $< 3\ \mu$m thick and the sarcocyst surface appeared serrated but had no visible surface projections (Fig. 1 B). The sarcocysts studied ranged in length between 0·3 and 2·3 mm and they were 0·06–0·25 mm wide.

Ultrastructure

The sarcocysts were situated intracellularly in skeletal muscle cells. Microfolds and bulges were seen on the entire cyst surface, which gave the wall a wavy appearance (Fig. 2 A). The parasitophorous vacuolar membrane had regular, 60–80 nm rounded invaginations (Fig. 2 B). The cyst ground substance, 0·5 to 1·8 μm thick, extended into the interior of the cyst to form septa about 0·15 μm thick (Fig. 2 A).

There were only a few metrocytes, which were ovoid and more or less degenerate. The numerous bradyzoites, measuring about $8 \times 2\ \mu$m, were elongated and slightly curved with a pointed anterior and a rounded posterior end, bearing the characteristics of *Sarcocystis* bradyzoites (Dubey *et al*. 1989).

DISCUSSION

The sarcocysts and bradyzoites observed by light as well as electron microscopy showed all the morphological and ultrastructural features characteristic of *Sarcocystis* species (Dubey *et al*. 1989). The cyst wall was thin and smooth, and had ultrastructurally a wavy, finely undulating appearance. No cyst wall protrusions were seen. According to the classification by Dubey *et al*. (1989), the sarcocyst wall observed was structurally similar to that of so called type 1 cysts. Sarcocysts with a wall of this structure have been predominantly seen in rodents, for instance *S. rauschorum* in the varying lemming (*Dicrostonyx richardsoni*) and *S. muris* in the house mouse (*Mus musculus*), but also in larger animals including monkeys (Mehlhorn, Hartley & Heydorn, 1976; Dubey *et al*. 1989).

To our knowledge *Sarcocystis* infection has not previously been described in the otter. Other Mustelids act as definitive hosts for various *Sarcocystis* species, e.g. *S. putorii* in the European weasel (*Mustela nivalis*), ferret (*Putorius furo*), polecat (*Mustela putorius*) and stoat (*Mustela erminea*) (Tadros & Laarman, 1982; Dubey *et al*. 1989). The ferret was also an additional definitive host of *S. muris* (Rommel, 1979).

Sarcocysts are only occasionally found in the muscles of carnivores, e.g. the domestic dog and cat (Hill, Chapman & Prestwood, 1988), badger (*Meles meles*) (Odening *et al*. 1994), and mink (*Mustela vison*) (Ramos-Vara *et al*. 1997). The identity and life-cycles of these *Sarcocystis* species are not known. It has been suggested that the unusual infection of *Sarcocystis* in cats and dogs may be influenced by immunosuppression (Hill *et al*. 1988). The animal presented here was found to be in a poor state, but there was no definitive evidence that it had an impaired immune system.

Carnivores are usually at the end of food chains and do not form a normal part of the diet of larger predators. Muscular sarcocysts in carnivores therefore appear to constitute a dead end of the parasite's life-cycle (Tadros & Laarman, 1982). The otter has no known predator although there are observations

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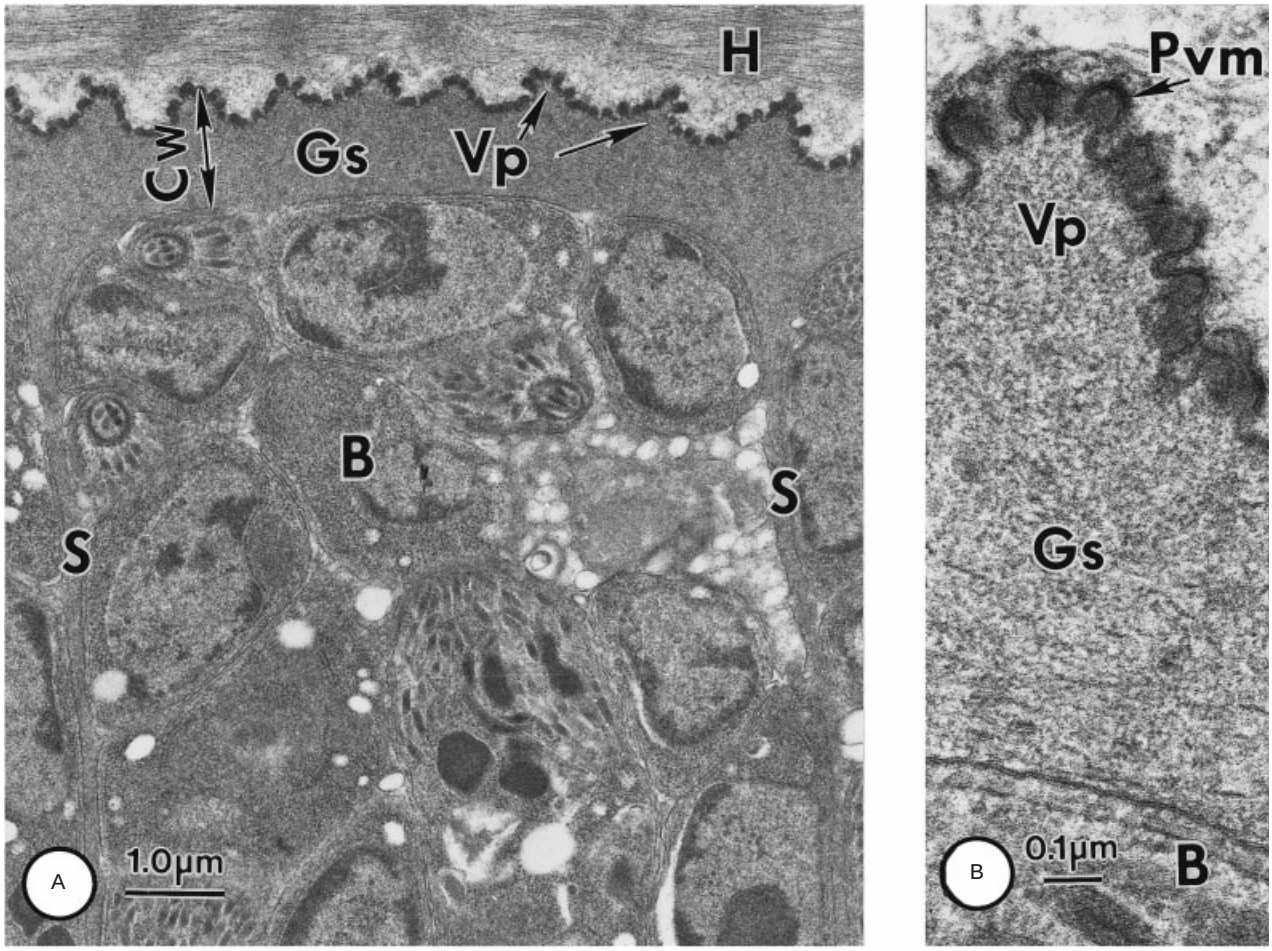

Fig. 2. (A and B) Transmission electron micrographs of sarcocyst (*Sarcocystis* sp.) from skeletal muscle of an otter (*Lutra lutra*). (A) Marginal part of sarcocyst located intracellularly in host myocyte (H) and containing groups of packed bradyzoites (B) separated by septa (S). Note thin cyst wall (Cw) with ground substance (Gs) and minute villar protrusions (Vp). (B) Higher magnification of the sarcocyst wall showing small, stubby villar protrusions (Vp), and the ground substance (Gs) with adjacent bradyzoite (B). Note the undulating outline of the parasitophorous vacuolar membrane (Pvm).

of eagles, sharks, wolves and foxes preying on otter (Chanin, 1988), and these and other animals that feed on carrion could thus be possible definitive hosts.

The ultrastructure of the sarcocyst wall is a major criterion for species differentiation in the genus *Sarcocystis* (Dubey *et al*. 1989). However, with the limited observations available there is at present not sufficient ground for the proposal of a new *Sarcocystis* species in the otter. The otter may be an intermediate host for a *Sarcocystis* species that completes its life-cycle in a not yet identified carnivore, or it may in this case have acted as an accidental host.

Formaldehyde fixed specimens and frozen muscle of the present case are deposited at the Swedish Museum of Natural History, Stockholm, Sweden (No. A89/5011).

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