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Light and electron microscope study of *Sarcocystis sp.* from the fallow deer (*Cervus dama*)

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Abstract. By means of light and electron microscopy a study was made of *Sarcocystis* sp. from 11 fallow deer (*Cervus dama*). Cysts of *Sarcocystis* sp. were found in the tongue and abdominal muscle of 3 of 11 deer from forests near Bonn (FRG). These measured 212–560 µm in length and 54–120 µm in width and contained metrocytes and merozoites. The cyst wall, which had narrow band-like protrusions, is compared with other *Sarcocystis* sp. from Cervidae.

Introduction

In the last few years *Sarcocystis* of cervids (white-tailed deer, roe deer, red deer, wapiti, elk, moose) have been intensively studied (Erber et al. 1978; Hernandez-Rodriguez et al. 1981; Colwell and Mahrt 1981; Entzeroth 1982; Entzeroth et al. 1983; Speer and Dubey 1982; Dubey et al. 1983). Species descriptions have been based in part on cyst morphology and on life-cycle studies of this obligatory two-host parasite. The present paper describes the ultrastructure of *Sarcocystis* in the fallow deer (*Cervus dama*) and compares it with that of other *Sarcocystis* cysts in Cervidae. The nomenclature of *Sarcocystis* in Cervidae is discussed.

Material and methods

Eight fallow deer from a state forest (Kottenforst), one deer from a game farm (Münstereifel), and two deer from a game park (Rolandseck) were examined for *Sarcocystis* infections. Samples from tongue and abdominal muscle were dissected under a binocular microscope at 20–50x. Cysts were removed from the muscle and photographs taken with a Zeiss photomicroscope II. In an attempt to detect possible low infections muscle samples were digested in a 0.2% trypsin solution in phosphate-buffered saline and the digest was examined for merozoites by phase contrast microscopy.

For electron microscopy, pieces of tongue muscle containing cysts were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2–24 h, rinsed in the buffer, postfixed

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for 2 h in 1% uranyl acetate in 70% ethanol and two changes of propylene oxide, and mebedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM9 S-2 electron microscope.

Results

Of the 11 fallow deer examined, 3 were positive for *Sarcocystis* sp. Sarcocysts were found in one of two deer from a game park (Rolandseck) and in one of eight deer from the state forest (Kottenforst). The density of tissue infection was relatively low. In another of the Kottenforst deer, cysts were not found but merozoites were demonstrated by trypsin digestion of the tongue muscle. One deer from a game farm (Münstereifel) was negative. Isolated muscle cysts measured 212–510 μm in length and 43–120 μm in widt ($n=10$). These cysts were located within the sarcoplasm of the host cell and were differentiated to the stage in which mostly merozoites and only a few metrocytes were present. By light microscopy the cysts appeared thin-walled and internally divided by septa into compartments (Fig. 1, which contained the slender banana-shaped merozoites and shorter, thicker, less curved metrocytes (Fig. 2). Merozoites measured 11.5–16.5 μm in length and 3.3–4 μm in width (13.5×3.7 μm; $n=20$). Metrocytes were 10.0–11.0 × 4.0–4.5 μm (10.5×4.2 μm; $n=10$).

Electron micrographs revealed intracellular cysts. The primary cyst wall was made up of the former parasitophorous vacuole membrane with a border of elongated protrusions that were sloping, or both sloping and folded over (Figs. 4–6). The bases of the protrusions varied from narrow (87 nm, Fig. 5) to wide (390 nm, Fig. 6). Distally, the protrusions became narrowed, measuring 22 nm in width and consisting of parallel membranes with virtually no central core; their terminal ends had often formed fork-like branches (Figs. 5, 6). Determination of the total length and shape of the protrusions was difficult due to their tangential orientation and folding. According to our measurements these were up to 4.5 μm long. The primary cyst wall had an average thickness of 1.0 μm.

Study of isolated ultrathin sections and several serial sections gave the impression that the elongate protrusions were not simply a series of filaments or hair-like projections, but more like a series of flattened sacs. This was evident from the lack of any spherical tube-like structures that would otherwise be present in cross-sections.Thus, sac-like protrusions would appear flattened whether cut in cross-section, tangentially or longitudinally. The primary cyst wall membrane was reinforced by underlying osmiophilic material, thus appearing to be approximately twice as thick as the plasmalemma of the merozoites (see Fig. 6).

Between the bases of the elongate protrusions the limiting membrane followed a highly tortuous route giving rise to short, stubby protrusions 30 nm long, that in single thin sections appeared to occur in groups of two or three (Figs. 5, 6).

The ground substance formed a band 0.5–0.6 μm wide at the periphery of the cyst and extended into the wider bases of the protrusions (Fig. 6), and inward as fine septa that surrounded groups of merozoites and metro-

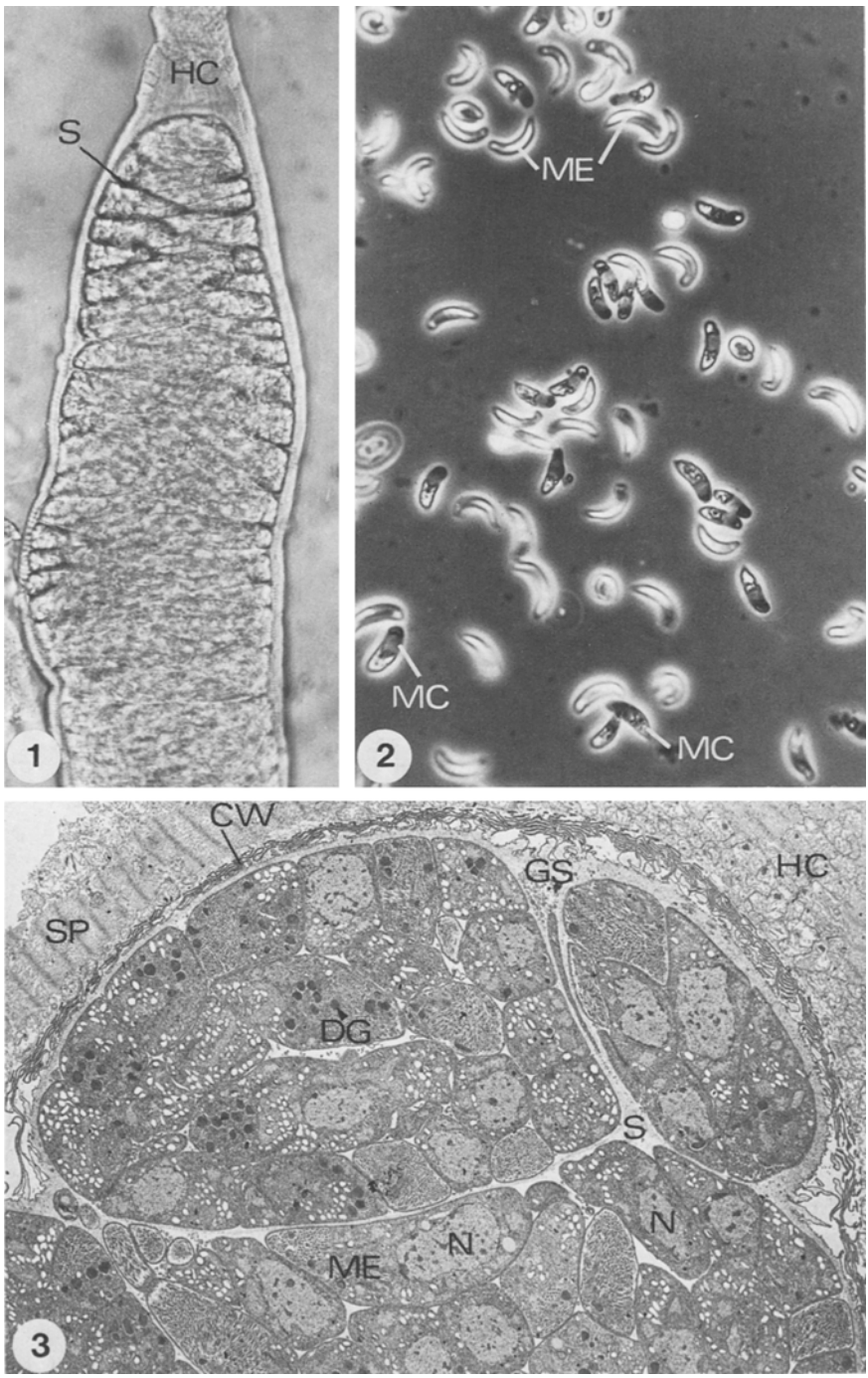
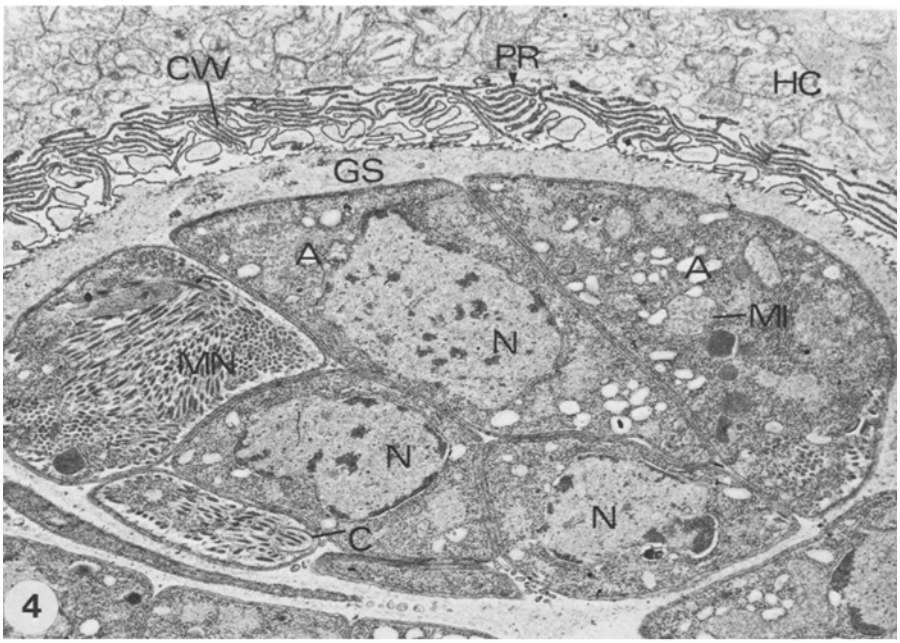
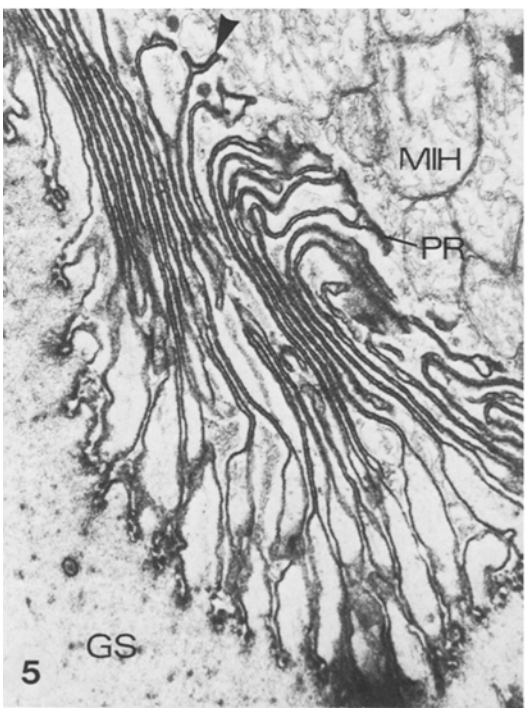


Fig. 1. A wet-mount preparation of a muscle cyst of *Sarcocystis* sp. from the tongue muscle of *Cervus dama*. The cyst is situated in a muscle cell (*HC*) and divided by septa (*S*) into compartments. Light micrograph. × 360

Fig. 2. Merozoites (*ME*) and metrocytes (*MC*) of *Sarcocystis* sp. from a cyst after trypsin digestion. Light micrograph, phase contrast. × 585

Fig. 3. Electron micrograph of a ultrathin section through the marginal part of a cyst of *Sarcocystis* sp. from *Cervus dama*. The cyst is situated in host cell (*HC*) sarcoplasm (*SP*) and is limited by a folded cyst wall (*CW*). Clusters of merozoites (*ME*), which contain a nucleus (*N*) and dense granules (*DG*), are surrounded by ground substance (*GS*) that forms





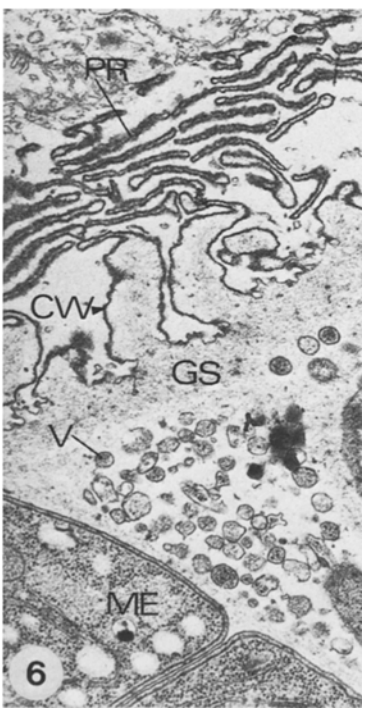


Fig. 4. Higher magnification of a portion of the cyst shown in Fig. 3. Note the highly folded cyst wall (*CW*) which forms band-like protrusions (*PR*) toward the host cell (*HC*). Beneath the ground substance (*GS*), merozoites with a nucleus (*N*), mitochondrion (*MI*), micronemes (*MN*), amylopectin (*A*), and conoid (*C*) are shown sectioned through different planes. ×9,000

Fig. 5. Section through the cyst wall of *Sarcocystis* sp. from *Cervus dama* showing band-like slender protrusions (*PR*) sometimes branched at the tip (*arrowhead*) adjacent to host cell mitochondria (*MIH*). ×20,700

Fig. 6. Tangential section through the cyst wall of *Sarcocystis* sp. with protrusions of the cyst wall (*PR*), ground substance (*GS*) containing vesicles (*V*) and merozoites (*ME*) underneath. ×20,700

cytes (Figs. 1, 3, 4). In some areas the homogeneous electron-pale ground substance contained membrane-bound vesicles that ranged in size from 85 to 150 nm.

The cysts were considered to be nearly mature, as evidenced by the presence of mostly merozoites and few metrocytes. Cyst merozoites had all of the fine structural features typically observed in these stages. These included a trimembranous pellicle, a posteriorly positioned nucleus (Fig. 3), amylopectin granules, micronemes, rhoptries, dense granules and a conoid (Figs. 3, 4).

Discussion

To the best of our knowledge no other ultrastructural studies on *Sarcocystis* sp. of *Cervus dama* have been made. The sarcocysts in the present study were characteristic of *Sarcocystis* species, having a primary cyst wall consisting of protrusions, under which was a granular ground substance giving rise to septa into the interior of the cyst.

The parasite stages in the cyst were nearly identical to those described by many previous authors (Scholtyseck et al. 1974; Mehlhorn and Heydorn 1978; Chobotar and Scholtyseck (1982). The dimensions of the *Sarcocystis* in the present study are in the range reported for cysts found in other members of the genus *Cervus*. These include *Sarcocystis cervicanis* from red deer in Spain (Hernandez-Rodriguez et al. 1981), *Sarcocystis* sp. from red deer in Hungary (Entzeroth et al. 1983), *Sarcocystis wapiti* from the North American wapiti (Speer and Dubey 1982), and *Sarcocystis sibyllensis* from the North American elk (Dubey et al. 1983). The host in each case is *Cervus elaphus* (Hanacki et al. 1982).

There are considerable differences, however, in the thickness and structure of cyst walls among the deer *Sarcocystis*. Basically cysts are described as "thick-walled" if they have prominent projections that in light microscopy give the wall a striated appearance or "thin-walled" if such projections are absent or reduced (Mehlhorn et al. 1975, 1976; Entzeroth 1982; Entzeroth et al. 1983; Dubey et al. 1983). The former may have walls that reach a thickness of 10 μm or more and the latter range from less than 1 μm up to 2 or 3 μm.

Walls of *Sarcocystis* in the present study were thin; on average 1.0 μm. Ultrastructurally the cyst wall with its band-like folds and thin wall in the present study resembled those of *Sarcocystis* sp. from the roe deer *Capreolus capreolus* (Schramlová and Blazek 1978; Entzeroth 1982, type 4), *Sarcocystis cervicanis* (Hernandez-Rodriguez et al. 1981) and *Sarcocystis* sp. (Entzeroth et al. 1983) both from red deer, *Sarcocystis wapiti* (Speer and Dubey 1982) from the wapiti and cyst type A from moose *Alces alces* (Colwell and Mahrt 1981). It is of interest that these cysts, with their closely similar morphology, all occur in hosts of the family Cervidae. Furthermore in those reports which also included life-cycle studies, the final hosts were shown to be members of the family Canidae. Since the mid-1970's it has become common practice to differentiate species of *Sarcocystis* in a given host by comparison of the morphological features of the cyst wall (Mehlhorn

et al. 1976; Mehlhorn and Heydorn 1978; Tadros and Laarman 1982; Dubey et al. 1983). When life-cycle studies revealed a relative lack of specificity in the final host, which supports gamogonous development, coupled with apparent stricter specificity in the intermediate host in which the cysts develop (Tadros and Laarman 1982; Dubey et al. 1983), cyst morphology assumed a prominent role in the identification and naming of species of *Sarcocystis*. The final host in the present study was not determined, but from the reports cited above describing similar cysts and life-cycles, it is possible that these cervid intermediate hosts may harbour a common species of *Sarcocystis*. It was on this basis that Entzeroth et al. 1983 suggest synonymizing *Sarcocystis cervicanis* (Hernandez-Rodriguez et al. 1981) and *Sarcocystis wapiti* (Speer and Dubey 1982) with *Sarcocystis grüneri* (Yakimoff and Sokoloff 1934). The latter name was given by Yakomoff and Sokoloff (1934) to sarcocysts of the maral (*Cervus elaphus)* and reindeer (*Rangifer tarandus*), primarily because they believed that similar sarcocysts from similar hosts (such as herbivores) were of the same species. Because Yakimoff and Sokoloff used *Sarcocystis grüneri* jointly for both hosts, Entzeroth et al. (1983) arbitrarily chose this name for *Sarcocystis* from red deer without being aware that in a second paper Yakimoff's (1936) use of *Sarcocystis grüneri* was based on descriptions of these parasites from reindeer. It seems proper therefore that *Sarcocystis grüneri* should be the valid name for cysts of the reindeer as apparently intended by Yakimoff (1936) and as stated by Levine and Tadros (1980).

It is, of course, also possible that each of the cysts described from cervids represents a different species with a common cyst morphology. This would be supported by the common perception that there is a high degree of host specificity in intermediate hosts (Tadros and Laarman 1982; Dubey et al. 1983). However, Fayer et al. (1982) have pointed out that *"Sarcocystis* species are thought to be highly specific for their intermediate host *despite the fact that few species have been studied"* (our emphasis). While the idea of strict host specificity to *Sarcocystis* in the intermediate host has some experimental support (Dubey 1980, reviewed by Fayer et al. 1982), other studies have shown that cross-transmissions between intermediate hosts are possible (Box and Duszynski 1978; Erber 1980; Crum et al. 1981; Fayer et al. 1982; Matuschka 1983).

To say that the nomenclature and taxonomy of *Sarcocystis* species of Cervids is unsettled is an understatement. It will take considerable efforts, including extensive transmission studies and possibly sharing of data among groups working concurrently with cervid's if order is to emerge.

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