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Electron microscopical studies on cysts of *Sarcocystis arieticanis* within cardiac muscle of naturally infected sheep

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Abstract Transmission electron microscopy was used to the cysts and their walls of *Sarcocystis arieticanis* within cardiac muscle of naturally infected sheep. The general ultrastructural features of the cysts, previously described by other authors, were confirmed. The cyst walls of *S. arieticanis* and the size and shape of the protrusions at different locations on the cyst wall were explained in detail. The cysts were 35–62.5 μm × 52.5–162.5 μm in size and the cyst wall had different-shaped protrusions. Aged cysts that were localized in the heart and differences in the morphology of their protrusions were noticed.

Introduction

Four species of *Sarcocystis*; (*S. arieticanis*, *S. gigantea*, *S. medusiformis*, *S. tenella*) have been detected in sheep (Dubey et al. 1989). Numerous ultrastructural reports (Mehlhorn et al. 1975; Munday and Obendorf 1984; Obendorf and Munday 1987; O'Toole 1987; Dubey et al. 1988) have shown that the sarcocyst wall of *Sarcocystis* spp varies, from being relatively simple to highly complex. It is well known that the ultrastructure of the sarcocyst wall is a useful criterion for distinguishing *Sarcocystis* spp. There is uniformity of opinion that the sarcocyst wall of *S. arieticanis* has characteristic, hair-like, long filamentous protrusions. The villar protrusions lack microtubules and thus are folded over the sarcocyst wall (Heydorn and Mehlhorn 1987). There are reports describing the fine structure of cyst structures and their wall in *S. gigantea* (Munday and Obendorf 1984), *S. medusiformis* (Obendorf and Munday 1987) and *S. tenella* (Mehlhorn et al. 1975; Vlemmas et al. 1989). However, ultrastructural studies of *S. arieticanis* are very limited (Heydorn and Mehlhorn 1987; Dubey et al. 1988). Some authors have studied *S. arieticanis* in Australia (Savini et al. 1993), Europe (Boch et al. 1979; Heydorn 1985; Hajtos et al. 2000), Japan (Saito et al. 1996), New Zealand (Pomroy and Charleston 1987), Turkey (Ozturk and Kucukerden 1996; Sevinc et al. 2000) and the USA (Dubey et al. 1988), mainly by abattoir surveys. Beside these, there is no report on electron microscopic studies in natural cases of *S. arieticanis* infection of cardiac muscle. For this reason, we report the fine structure of *S. arieticanis* and its wall in natural cases.

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Materials and methods

Individual, microscopic sarcocysts from the cardiac muscles were collected from three sheep suspected of enteric clostridial infections. They were fixed in 10% buffered neutral formalin for light microscopy and 2.5% glutaraldehyde in cocodylate buffer (pH 7.4) for transmission electron microscopy. Later, specimens were processed conventionally and embedded in paraffin and Epon. Semithin and ultrathin sections were stained with toluidine blue, or with uranyl acetate and lead citrate. Sections were observed with a Zeiss EM-9S electron microscope.

Results and discussion

Sarcocysts were located within the cardiac muscle fibers and there was no evidence of any pathological changes or reaction against the cysts. Microscopic cysts were identified on the basis of differences in cyst wall morphology and septa. Some cysts were bounded by a thin and minutely undulating cyst wall, with delicate septa that was not seen more clearly. Other cysts were bounded by a relatively thick and undulating cyst wall, with septa. In light microscopy, protrusions of the cyst wall were not seen in sections stained with hematoxylin and eosin, although they were detected as blurs, when stained with toluidine blue. All of the cysts were similar in size, ranging over 35.0–62.5 μm in width and

52.5–162.5 μm in length. It was not possible to identify every sarcocyst in the tissue sections, because of the angle of the sarcocyst sectioned or other alterations to sarcocyst structure that occurred during fixation and tissue processing or were due to cardiac muscle morphology.

Ultrastructurally, thin-walled cysts were readily identifiable by light microscopy, having a cyst wall <1 μm (0.07–0.26 μm) in thickness and septa (0.005 μm). The primary cyst wall (PCW) of the cysts consisted of a parasitophorous vacuolar membrane with an inner, electron-dense layer immediately beneath it. At various locations around the sarcocyst surface, the PCW formed different-shaped protrusions (Figs. 1, 2), which were snake-like (Fig. 1), trapezoidal (Fig. 2) or a mixture of shapes; and their sizes were 0.07–0.21 μm × 0.50–1.20 μm in snake-like and 0.14–0.24 μm × 0.87–1.30 μm in trapezoidal shapes. The tips of the base part of trapezoidal protrusions were 0.57–0.68 μm in width. Protrusions turned to the surface of the sarcocyst were continued immediately as hair-like structures above the base protrusions, so that region was exhibited almost parallel to the sarcocyst surface. A granular layer (0.01–0.21 μm thick) was located between the PCW and the zoites of the sarcocyst. The sarcocyst contained mainly bradyzoites and a few metrocytes near the margin of the cyst. The bradyzoites (1.94–2.33 μm × 4.67–6.46 μm) and metrocytes (0.13–0.21 μm × 0.17–0.34 μm) were characteristically the same as other *Sarcocystis* spp.

Relatively thick-walled cyst walls were 0.77–0.92 μm in thickness. Bright septa were 0.04–0.18 μm in width. The cyst wall was undulating in shape and showed thin, finger-like protrusions (0.08–0.25 μm × 1.54–1.92 μm) arising from the apex of the PCW. Those protrusions continued as hair-like structures. There was an almost uniformly thick granular layer (0.46–0.72 μm) beneath the PCW and some metrocytes immersed in the granular layer were also noted. Septa arising from the granular layer traversed the sarcocyst, separating it into compartments (3.48–7.15 μm × 10.45–13.51 μm) contained bradyzoites and metrocytes. Degenerated materials, debris, (Fig. 3) and bradyzoites (1.98–2.32 μm × 4.73–7.16 μm) with fully developed organelles were observed within the compartments of the cyst (Fig. 4).

The cyst walls had protrusions with two distinct regions: a snake-like, trapezoidal or thin, finger-like base, and thin, thread-like final segments. The distal, final segments were often folded and parallel to the sarcocyst surface. The basal part of the protrusions also had coarse granules.

The occurrence of *S. arieticanis* has been reported in sheep from Turkey (Ozturk and Kucukerden 1996; Sevinc et al. 2000). They recorded that prevalences in the esophagus, diaphragm and intercostal muscles were 18.8% (Ozturk and Kucukerden 1996) and, in the esophagus, 33.89% (Sevinc et al. 2000). *S. arieticanis* was much less common than *S. tenella* in these two reports. Also, numerous authors have studied *S. arieticanis* (Heydorn 1985; Pomroy and Charleston 1987;

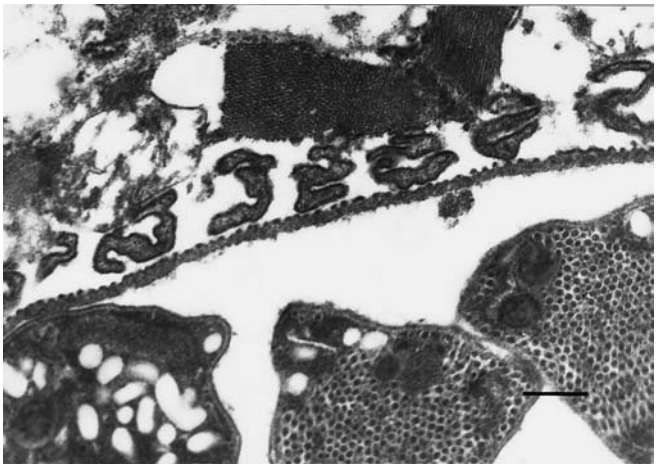

Fig. 1 The cyst wall of *Sarcocystis arieticanis*, showing the base of the snake-like protrusions. *Bar* 0.132 μm

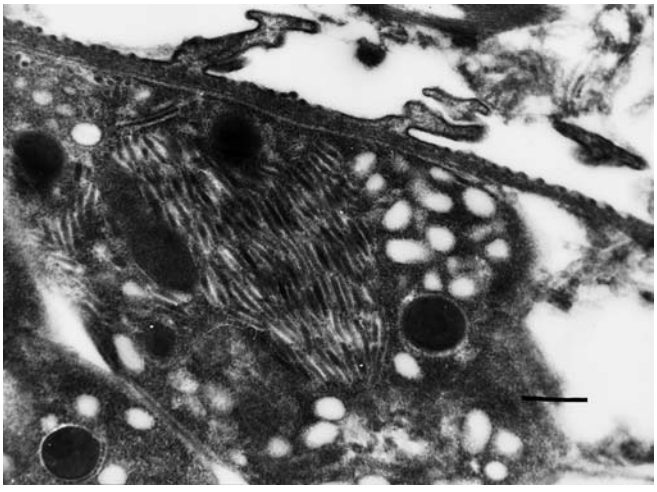

Fig. 2 Trapezoidal protrusions of the cyst wall of *S. arieticanis*. *Bar* 0.120 μm

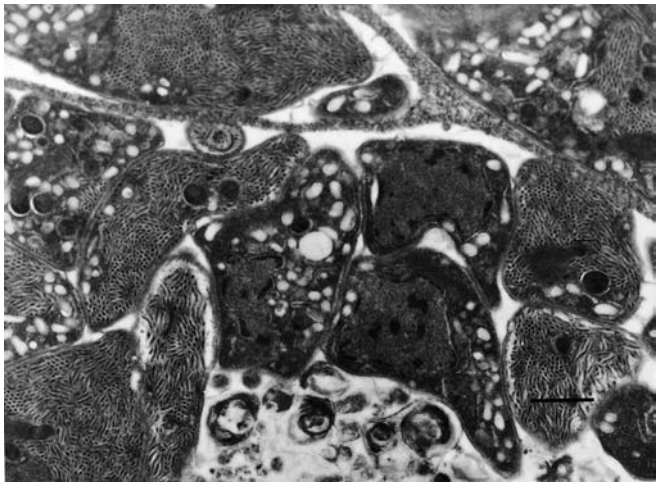

Fig. 3 Degenerated materials are located at the center and bradyzoites at the peripheral part of compartments. *Bar* 0.122 μm

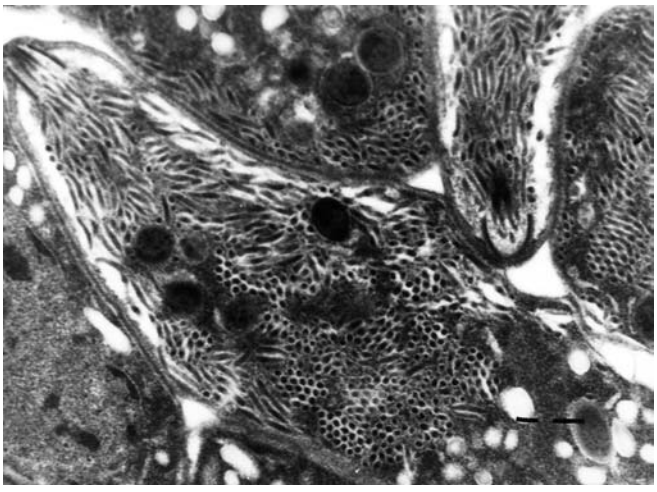

Fig. 4 Completely developed bradyzoites can be seen. *Bar* 0.247 μm

Dubey et al. 1988; Savini et al. 1993; Saito et al. 1996; Hajtos et al. 2000), mainly as abattoir surveys. There are very many differences in these reports, such as prevalence, size and morphology. These may be related to the age of the sheep surveyed and the techniques applied. Our observations are based on histological and electron microscopic examinations, whereas others made their observations on live preparations. Therefore, we could not identify all the sarcocysts in tissue sections stained with hematoxylin and eosin or with toluidine blue.

The cyst wall of *S. arieticanis* was similar in appearance to electron micrographs presented for the same species by Heydorn and Melhorn (1987) and Dubey et al. (1988). However, there were some morphological differences (e.g. sizes, shape of protrusions). The morphology of sarcocysts can change very considerable with age; and therefore the identification of cysts of unknown age on morphological grounds alone should be approached with caution (Dubey et al. 1989). Moreover, fixation and tissue processing have also caused some morphological alterations. Cyst walls may also differ to a certain degree, depending on the muscle tissues they are developing in, e.g. protrusions of cyst walls may be folded in older cysts, especially in heart muscle. Moreover, *S. arieticanis* cysts are described mainly from the esophagus, diaphragm, tongue and intercostal muscles (Dubey et al. 1989). In our case, all cysts were localized in heart muscle. Therefore, it was difficult to decide definitely whether the cysts we found in heart muscle of naturally infected sheep were cysts of *S. arieticanis* or whether they were cysts of a "new" species. Personally, we believe that they were cysts of *S. arieticanis*.

Thus, cyst walls are only one criterion for species differentiation, but little differences cannot be used as a single criterion for species discrimination. Transmission electron microscopic studies are also necessary.

References

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

Dubey JP, Lindsay DS, Speer CA, Fayer R, Livingstone CW Jr (1988) *Sarcocystis arieticanis* and other *Sarcocystis* species in sheep in the United States. J Parasitol 74:1033–1038

Dubey JP, Speer CA, Fayer R (1989) Sarcocystosis of animals and man. CRC Press, Boca Raton

Hajtos I, Glavits R, Palfi V, Kovacs T (2000) Sarcocystosis with neurological signs in a breeding-sheep stock (in Hungarian). Magy Allatorv Lapja 122:72–78

Heydorn AO (1985) Zur Entwicklung von *Sarcocystis arieticanis* n.sp. Berl Muench Tieraerztl Wochenschr 98:231–241

Heydorn AO, Mehlhorn H (1987) Fine structure of *Sarcocystis arieticanis* Heydorn, 1985 in its intermediate and final hosts (sheep and dog). Zentralbl Bakteriol Parasitenk Infektionskr Hyg Abt1: Reihe A 264:353–362

Mehlhorn H, Heydorn AO, Gestrich R (1975) Licht- und elektronenmikroskopische Untersuchungen an Cysten von *Sarcocystis ovicanis* Heydorn et al. (1975) in der Musculatur von Schafen. ZParasitenkd 48:83–93

Munday BL, Obendorf DL (1984) Morphology of *Sarcocystis gigantea* in experimentally infected sheep. Vet Parasitol 16:193–199

Obendorf DL, Munday BL (1987) Experimental infection with *Sarcocystis medusiformis* in sheep. Vet Parasitol 24:59–65

O'Toole D (1987) Experimental ovine sarcocystosis: sequential ultrastructural pathology in skeletal muscle. J Comp Pathol 97:51–60

Ozturk G, Kucukerden N (1996) The prevalence of *Sarcosystis* species on sheep slaughtered at the Elazig Meat and Fish Co. abattoir (in Turkish). Etlik Vet Mikrobiol Derg 8:74–83

Pomroy WE, Charleston AG (1987) Prevalance of dog-derived *Sarcocystis* spp in some New Zealand lambs. NZ Vet J 35:141–142

Saito M, Shibata Y, Itagaki H (1996) *Sarcocystis arieticanis* of sheep in Japan (Protozoa: Apicomplexa). Jpn J Parasitol 45:290–294

Savini G, Dunsmore JD, Robertson ID, Seneviratna P (1993) *Sarcocystis* spp in Western Australian sheep. Aust Vet J 70:152–154

Sevinc F, Altinoz F, Uslu U, Aldemir OS (2000) The prevalence of *Sarcocytis* species in sheep (in Turkish). Vet Bil Derg 16:75–79

Vlemmas I, Kanakoudis G, Tsangaris TH, Theodorides I, Kaldrymidou E (1989) Ultrastructure of *Sarcocystis tenella* (*Sarcocystis ovicanis*). Vet Parasitol 33:207–217