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ULTRASTRUCTURAL STUDY OF *SARCOCYSTIS* SPECIES IN SHEEP AND GOATS

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Summary

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Four lambs and four kids were infected with *Sarcocystis* cysts, obtained from 2 experimentally invaded dogs. They were subsequently euthanized at the 100th day after the infection. Muscle samples from the tongue, heart, forelimb and hindlimb were obtained and studied by electron microscopy. *Sarcocystis* macrocysts were further collected from 20 sheep in 2 slaughterhouses in Stara Zagora. Parts of the cysts were prepared for electron microscopical investigation.

The studies showed three *Sarcocystis* spp. in sheep - *S. tenella* (*S. ovicans*), *S. arieticanis* and *S. gigantea* (*S. ovifelis*) and two in goats – *S. capracanis* and *S. hircicanis*.

The morphological features of cyst protrusions, the primary cyst wall and the presence or lack of a secondary cyst wall, specific for *Sarcocystis* in sheep and goats, were described.

Key words: goat, *Sarcocystis*, sheep

INTRODUCTION

Cysts, containing banana-like cells, were first discovered by Miescher (1843) in murine muscles. Because of the specific localization of those cysts in the muscle fiber, there are called *Sarcocystis*. The parasites are found in reptiles, birds and mammals – an evidence for their cosmopolitan occurrence. The highest percentages of *Sarcocystis* invasion are reported in cattle, sheep, goats, swine and some wild animal species (Piekarski et al., 1961; Senaud, 1967; Kaliner, 1975).

Using experimental models of infection, it is established that several *Sarcocystis* species parasitize in the various intermediate hosts. They form cysts that could be differentiated only by their ultrastructure. Levin (1983), Heydorn and Mehlhorn (1987), Dubey et al. (1989) observed four *Sarcocystis* species in sheep, three species in goats and three – in cattle.

In Bulgaria, the incidence of *Sarcocystis* in various animal species is studied by Meshkov (1975) and Raikov et al. (1989). In sheep, the variety and the biology of *Sarcocystis* species is established through biological experiments (Meshkov, 1973; 1979; Arnaudov and Belchev, 1988; Ivanov, 1992). On the other side, there are no reported attempts for species-related differentiation of *Sarcocystis* encountered in this country by ultrastructural studies.

The aim of the present study was to investigate the species affiliation of *Sarcocystis* in sheep and goats by electron microscopy in experimentally and naturally infected animals.

MATERIALS AND METHODS

Each of 4 two-month-old lambs and 4 two-month-old kids were experimentally infected by 50 000 *Sarcocystis* sporocysts. The latter were obtained from two experimentally infected donor dogs. One of them was fed meat from a naturally invaded sheep and the other – with meat from a naturally infected goat. The method of obtaining a *Sarcocystis* sporocyst culture and for infection of animals is described in a previous study of ours (Ivanov, 1998). The animals were housed in conditions excluding subsequent sarcocyst infections. At post infection day 100, both lambs and kids were euthanized. Samples for detection of the presence of *Sarcocystis* were obtained from the tongue, heart, forelimb and hindlimb muscles.

All materials were fixed by immersion in 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2). The fixation was performed in a refrigerator for 24 hours at 4 ^{o}C. Samples were washed in the same buffer, followed by a postfixation in 2% osmium tetroxide for 2 h. The material was dehydrated in an ethanol series and embedded in Durcupan ACM (Fluka). Ultrathin sections were cut with an ultramicrotome Ultracut E Reichert-Jung, put on copper meshes and double-stained with uranyl acetate for 30 min and lead acetate for 10 min. The sections were observed by Opton EM 109 electron microscope.

Macrocysts were collected from 20 sheep in two slaughterhouses in Stara Zagora. The animals originated from various settlements in the region. Material for study was sampled from oesophageal, pharyngeal, tongue, intercostal, diaphragmal and abdominal muscles. Parts from the cyst wall were cut and prepared for the ultrastructural examination as described above.

RESULTS AND DISCUSSION

Sarcocystis microcysts were observed in all studied muscle samples from lambs. They contained only mature cells (cystozoites), filling the interior of the cysts and had no secondary cyst wall. Morphologically, two kinds of *Sarcocystis* microcysts were differentiated. The one had long (≈ 3.5 μm) and wide (≈ 0.5 μm) protrusions of the primary cyst wall. They were located in close proximity, were palisade-like, contained no microtubules and possessed structural plaques at their top. There were septa protruding from the primary cyst wall towards the interior of cysts, forming compartments where cystozoites were situated (Fig. 1). The other kind of cysts were with long (5–9 μm) and thin, hair-like protrusions. They were fold and lied upon the primary cyst wall. No septa were observed (Fig. 2).

Heydorn et al. (1975), Mehlhorn et al. (1975) and Mehlhorn and Heydorn (1978) described only one *Sarcocystis* species in sheep and goats, where the definitive host was the dog, proposing to name them *Sarcocystis ovicanis* and *S. capracanis* respectively.

The ultrastructural studies of microcysts from experimentally and naturally invaded sheep (Mehlhorn et al., 1976; Levin, 1983; Heydorn and Mehlhorn, 1987 and Dubey et al., 1989) revealed two types of *Sarcocystis* microcysts. They were differentiated by the primary cyst wall and the morphology of cyst protrusions. The first kind, characterized by palisade-like (up to 3.5 μm) protrusions was determined as *S. tenella* (*S. ovicanis*) and the other, with hair-like protrusions (5-9 μm) – as *S. arieticanis*. Comparing the morphological features observed in this study with those previously reported, we consider that the studied microcysts belonged to the *Sarcocystis tenella* (*S.*

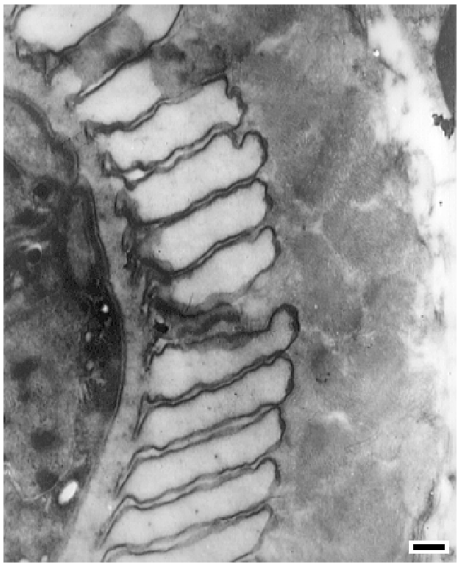

Fig. 1. *Sarcocystis tenella* (*S. ovicanis*): palisade-like protrusions of the primary cyst wall. Bar = 0.5 μm.

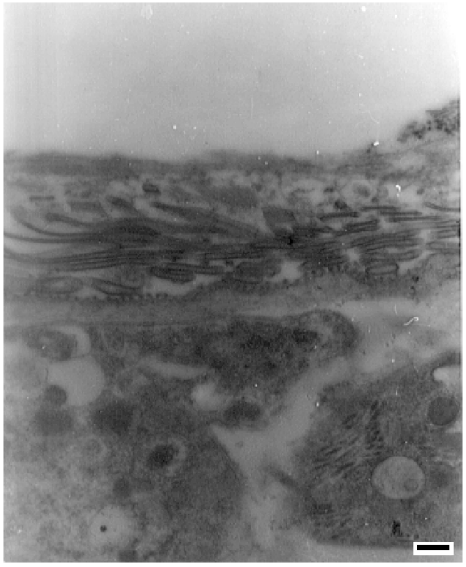

Fig. 2. *Sarcocystis arieticanis*: hair-like protrusions of the primary cyst wall. Bar = 0.5 μm.

ovicanis) and *S. arieticanis* species.

The ultrastructural studies of *Sarcocystis* macrocysts collected from sheep showed that they were from a single species. It possessed protrusions (height ≈ 4 μm) resembling a cauliflower. Apart from the primary, the cysts had a secondary cyst wall as well. They contained septa and cystozoites located only on the periphery whereas the central zones were empty (Fig. 3). Heydorn et al. (1975), Mehlhorn et al. (1975) and Mehlhorn and Heydorn (1978) repoted *Sarcocystis*, forming macroscopically visible cysts in sheep. The definitive host of this species – *S. gigantea* (*S. felis*), is the cat and it forms its cysts in the muscle tissue of the oesophagus, the pharynx and the diaphragm. Giving a description of *S. gigantea* in sheep, Levin (1983), Heydorn and Mehlhorn (1987) and Dubey et al. (1989) state that this *Sarcocystis* species forms macrocysts with a secondary cyst wall and protrusions resembling cauliflower with a height up to 4 μm. In our ultrastructural studies of ovine macrocysts, we observed similar morphological features and therefore, we determined them as belonging to the *S. gigantea* species.

The results obtained from the study of kid muscle samples showed the presence of two types *Sarcocystis* cysts. The first one possessed finger-like protrusions with a length of about 3 μm and thickness 0.5 μm located nearby. The primary cyst wall was with a thickness of ≈ 2 μm. Multiple septa were penetrating into the interior of the cysts (Fig. 4). The second kind *Sarcocystis* cysts had thin filamentous protrusions up to 5 μm long. They lied on the primary cyst wall (Fig. 5). In both types, there was no secondary cyst wall.

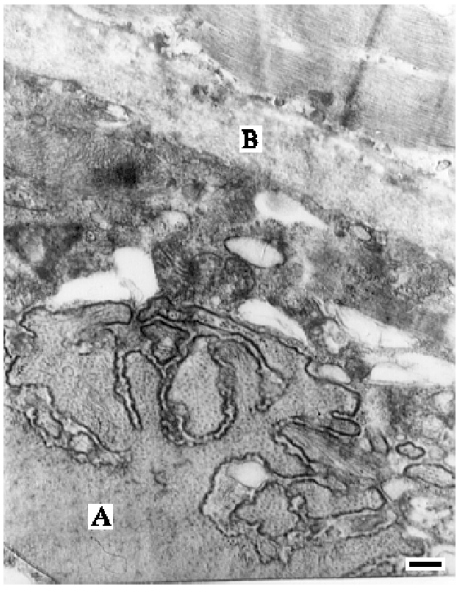

Fig. 3. *Sarcocystis gigantea* (*S. ovifelis*): A – cauliflower-like protrusions of the primary cyst wall; B – secondary cyst wall. Bar = 0.5 μm.

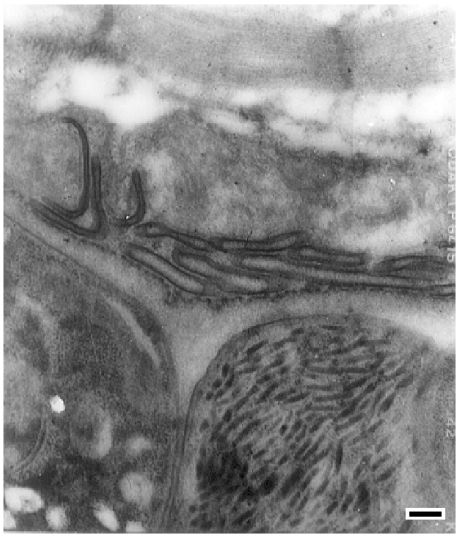

Fig. 5. *Sarcocystis hircicanis*: filamentous protrusions of the primary cyst wall. Bar = 0.5 μm.

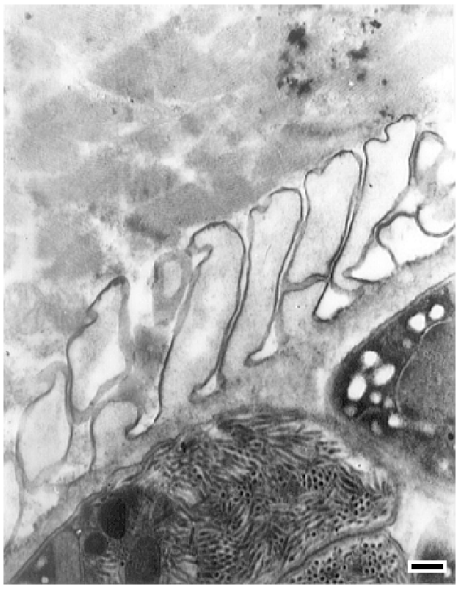

Fig. 4. *Sarcocystis capracanis*: finger-like protrusions of the primary cyst wall. Bar = 0.5 μm.

The morphological features of *Sarcocystis* microcysts in goats are described by means of electron microscopy by Levin (1983), Heydorn and Mehlhorn (1987), and Dubey et al. (1989). The authors reported two *Sarcocystis* types: *S. capracanis* and *S. hircicanis*. The results of our ultrastructural studies were completely analogous to those of forementioned authors and thus, we determined the microcysts in goats as belonging to the *Sarcocystis capracanis* and *Sarcocystis hircicanis* species.

According to Heydorn et al. (1975), Mehlhorn et al. (1975), and Mehlhorn and Heydorn (1978), *S. ovicanis* and *S. capracanis* microcysts are encountered in the whole striated musculature including the heart of infected animals. The *S. ovicanis* and *S. ovifelis* species (in sheep) and *S. capracanis* (in goats) have also been de-

tected in our previous experiments in both naturally or experimentally infected animals (Ivanov, 1992; 1995; 1998). With these trials and the present ultrastructural study, we observed for the first time in Bulgaria three *Sarcocystis* species in sheep (*S. tenella*, *S. arieticanis* and *S. gigantea*) and two species in goats (*S. capricanis* and *S. hircicanis*).

CONCLUSIONS

The present study identified three *Sarcocystis* species in sheep in Bulgaria - *S. tenella* (*S. ovicans*), *S. arieticanis* and *S. gigantea* (*S. ovifelis*). The first two species formed microcysts in animal musculature and the third one – macrocysts.

In goats, two microcyst *Sarcocystis* species were observed – *S. capracanis* and *S. hircicanis*.

The morphological features of cyst protrusions, the primary cyst wall and the presence or lack of a secondary cyst wall were specific for *Sarcocystis* in sheep and goats. They could be used in future ultrastructural studies for species-related differentiation of *Sarcocystis* in those animal species.

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