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ORIGINAL PAPER

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# A light and electron microscope study of *Sarcocystis mitrani* (sp. nov.) infecting the skink *Scincus mitranus* in the central region of Saudi Arabia

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**Abstract** The prevalence of *Sarcocystis* infection among skinks, *Scincus mitranus*, was studied for the first time. Grossly macroscopic sarcocysts were found to infect the skeletal muscles of the skink (infection rate: 4.16%). Fecal examination for the presence of sporocysts was negative in this study. Sarcocysts were studied using light and transmission electron microscopes. Mature sarcocysts measuring 0.05–0.3×0.5–1.8 mm (mean 0.15×1.2 mm) were observed. The characteristic primary cyst wall, with long, finger-like, non-branched and non-stalked protrusions, is described. The ground substance gives rise to numerous thick septa dividing the interior of the cyst into chamber-like compartments. Zoites, including metrocytes and merozoites, were found to have the main architecture of Apicomplexa. Peculiarities of these elements and the importance of the primary cyst-wall ultrastructure for identification and specification of *Sarcocystis* are discussed. Secondary cyst wall was completely absent. Alterations in the infected host cell were observed.

## Introduction

The elucidation of the life cycle of *Sarcocystis* has stimulated intensive studies of the natural rate of incidence of sarcosporidiosis in domesticated and wild animals worldwide. More than 189 species belonging to the genus *Sarcosystis* have been described (Tenter 1995; Odening 1998). Some species of these parasites were proved to be highly pathogenic to their hosts, including man (Mehlhorn and Heydorn 1978; Buxton 1998; Dubey et al. 2000).

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Reptiles have been shown to be an intermediate and/or final host for *Sarcocystis* infection (Matuschka 1981, 1987; Mehlhorn and Matuschka 1986; Abdel-Ghaffar et al. 1990, 1994; Laison and Paperna 2000). The rat–snake life cycle was first described for *Sarcocystis* by Rzepczyk (1974), while other lizard–snake life cycles were elucidated by Matuschka (1987).

The reptile fauna of Saudi Arabia was very poorly known until quite recently. Nowadays, vast numbers of varieties of reptiles are recognized in the fauna of Saudi Arabia (Arnold 1986). Some are important either because they are fatally poisonous animals or because they are food for some inhabitants. Despite many years of zoological research in Saudi Arabia, details of the biology and ecology of many reptiles and their parasites are scarce and fragmented.

The sand-fish lizard *Scincus mitranus* is found throughout most of the sand dunes of Arabia (Al-Sadoon 1988). Together with other lizards, such as the Dhub (*Uromastyx aegypticus*), this skink may be a source of food for some desert inhabitants. Very few parasitological studies of coccidian infection among reptiles have been done in Saudi Arabia. The only studies of coccidial infection among reptiles are those of Kasim et al. (1993) and Alyousif and Al-Rasheid (2001), who described the eimerian oocysts recovered from the skinks *S. mitranus* and *Mabuya aurata* respectively. The present study investigates the prevalence of natural infection with *Sarcocystis* and the ultrastructural characteristics of the parasite infecting the skink *Scincus mitranus* that lives in the central region of Saudi Arabia.

## Materials and methods

### Sample collection

A total of 48 adult skinks *Scincus mitranus* (Scincidae, Lacertalia) were collected by hand from the sand dunes at different localities around Riyadh city, in the central region of Saudi Arabia. Animals were brought to the laboratory at the Zoology Department, College of Science, King Saud University in Riyadh, where they were

identified according to Arnold (1986) and maintained in individual glass cages, with sand and alluvium, at room temperature (27 ± 2 °C). They were regularly fed with insect larvae during the examination period.

To detect the prevalence of natural *Sarcocystis* infection, fecal samples from the skinks were daily examined for coccidian oocysts and sporocysts for 2 weeks, using the usual flotation technique (Long et al. 1976). After that, animals were dissected and fresh skeletal muscles from different parts of the body were examined with the naked eye for macroscopic forms of sarcocysts. Cryosections were examined microscopically for cysts . Small pieces of heavily infected muscles were fixed as described below.

### Light and transmission electron microscopy

For histological examination, infected tissues were fixed in 3% (v/v) glutaraldehyde buffered with 0.1 M sodium cacodylate buffer (pH 7.3) at 4 °C for 24 h. Fixed materials were then air-freighted to the Zoomorphology, Cell Biology and Parasitology Institute, Duesseldorf University, Germany, where they were processed for examination by transmission electron microscopy. Post-fixation was done in 2% $OsO_4$ in the same buffer. The specimens were dehydrated in graded ethanol, transferred to propylene oxide and finally embedded in Araldite (Serva) embedding medium. Semi-thin and ultra-thin sections were cut on a Reichert Ultracut. Semi-thin sections were stained with methylene blue and azure A, whereas ultra-thin sections were contrasted with uranyl acetate and lead citrate before examination in a Zeiss EM9S2 transmission electron microscope.

For light microscopic examination, fixed materials were dehydrated in an ascending series of alcohol, cleared in butanol, and embedded in paraplast. Sections (3–5 μm) were prepared and stained with hematoxylin and eosin. Stained sections were examined using a Zeiss photoresearch microscope.

## Results

Grossly visible macroscopic sarcocysts were detected in the skeletal muscles of different parts of the body. The intensity of infection varied from low to massive, depending on the site of infection. These sarcocysts were intensively concentrated in the tail muscles, followed by the hind- and fore-limbs. Out of the 48 adult skinks collected and examined, two animals were found to be infected (infection rate: 4.16%). Cysts measured 0.05–0.3×0.5–1.8 mm (mean 0.15×1.2 mm). However, fecal examination of collected animals showed that they were devoid of coccidian oocysts or sporocysts, which indicates that this skink serves as a natural intermediate host only for *Sarcocystis*. Examination of fresh preparations and paraffin sections of sarcocysts by light microscopy revealed the presence of a thick cyst wall, with villous protrusions. The interior of the cyst was clearly divided into numerous chamber-like compartments, separated from each other by wide detectable septa. The compartments contained the different zoites inside the cyst (Fig. 1). Only mature sarcocysts were observed in the present study.

Transmission electron microscopic examination of different sarcocysts from the naturally infected skinks showed similar features. A thick primary cyst wall (1.8–5.2 μm), with long, finger-like villous, non-stalked and non-branched protrusions with blunt ends,

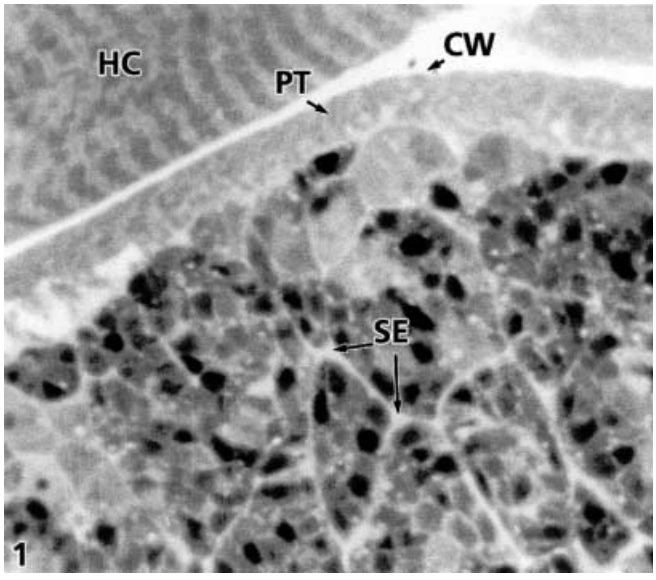


**Fig. 1** Photomicrograph of cross-section through a mature sarcocyst of *Sarcocystis mitrani*. Note the cyst wall with its protrusions and the wide septa dividing the interior of the cyst (×400). *HC* Host cell, *PT* protrusions of the primary cyst wall, *CW* primary cyst wall, *SE* septum

arranged mostly in oblique wave-like forms were observed (Fig. 2). These protrusions measured 1.6–3.1 μm (mean 2.5 μm) in length. In cross-section, they were spherical to oval in form and measured 0.7–1.6 μm (mean 1.2 μm) in diameter. The outer surface of the primary cyst wall and all the arising protrusions were not smooth in texture, but had a serrated appearance. This serration appeared to be formed of regular successive knob-like elevations of the thickened parasitophorous vacuole membrane, separated from each other by thin-wall small invaginations. When sectioned appropriately, these thin invaginations appeared as holes in the primary cyst wall (Fig. 2). The interior of the protrusions was occupied by dense microtubular structures, which were mostly concentrated centrally, leaving a translucent space just beneath the outer surface of each protrusion. These microtubular structures extended to the ground substance at the base of the protrusion.

Small invaginations of the primary cyst wall were irregularly distributed through the interior of the cyst. A relatively thick homogenous layer (the ground substance) measuring 0.2–0.8 μm, with an average thickness of 0.45 μm, was observed just underneath the primary cyst wall (Fig. 2). This ground substance had an amorphous structure consisting mainly of fine, dense, homogenous granules, and contained neither filaments nor tubules, except at the bases of the protrusions where the microtubular elements were extended (Fig. 2). However, large osmiophilic granules were observed scattered throughout this ground substance.

Arising from the ground substance and towards the interior of the cyst, numerous thick septa divided the

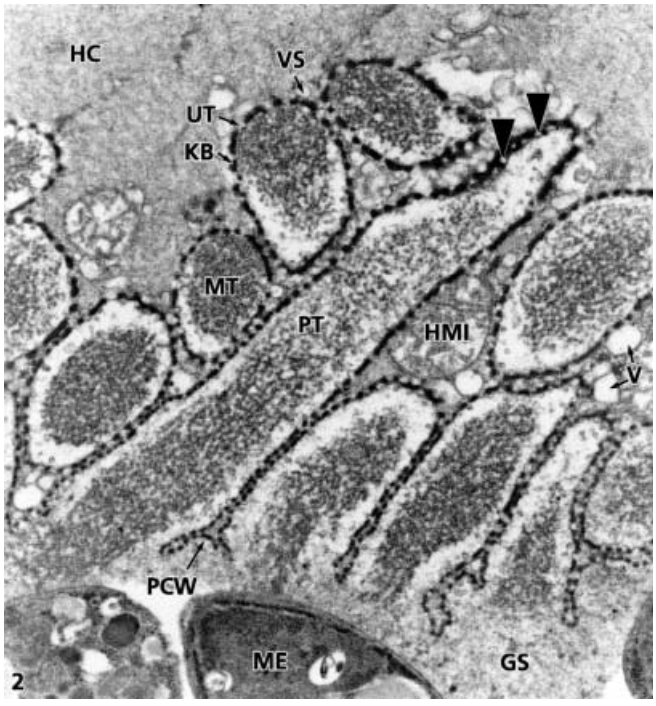


**Fig. 2** Transmission electron micrograph of *Sarcocystis mitrani* within its intermediate host. Section through a sarcocyst inside a muscle fibre showing the primary cyst wall, with protrusions in the longitudinal section showing the thick, knob-like elevations. Note the ornamentations of the primary cyst wall and holes (*arrowheads*) that are due to the plane of sectioning (×50,000). *HC* Host cell, *VS* vesicles, *UT* unthickened invaginations of the primary cyst wall, *KB* knob-like elevations from the primary cyst wall, *MT* microtubules, *PT* protrusions of primary cyst wall, *HMI* host cell mitochondria, *PCW* primary cyst wall, *ME* merozoite, *GS* ground substance

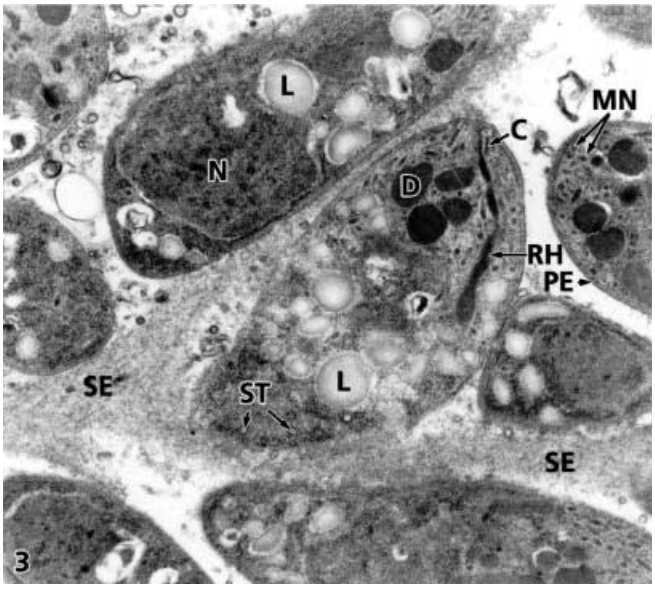


**Fig. 3** Transmission electron micrograph of enlarged part of the cyst interior, showing the fine structural characteristics of the cyst merozoites and the wide septa dividing the cyst (×17,200). *L* Lipid inclusions, *N* nucleus, *D* dense bodies, *ST* subpellicular microtubules, *SE* septum, *C* conoid, *MN* micronemes, *RH* rhoptries, *PE* pellicle

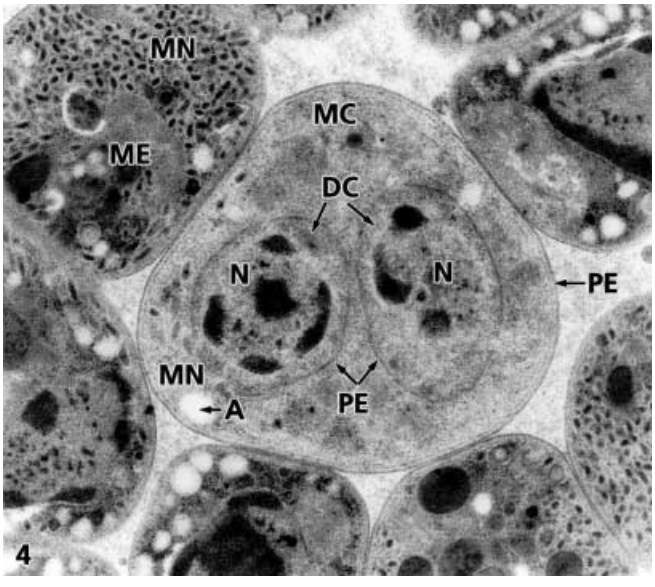


**Fig. 4** Transmission electron micrograph of metrocytes with developing merozoites (×21,500). *MN* Micronemes, *ME* merozoite, *MC* metrocyte, *DC* daughter cell, *N* nucleus, *A* amylopectin granules, *PE* pellicle

interior of the cyst into chamber-like compartments containing the different zoites. These septa had the same structure of the amorphous ground substance. In some sections, the beginning of the septum was somewhat thicker than the ground substance itself.

Zoites of mature cysts examined were distributed in the stroma of each chamber. These zoites were differentiated into a few metrocytes and some of their remnants; the majority of zoites were merozoites. The fine structural characteristics of both metrocytes and merozoites were similar to those described for many other Sarococystis species. Merozoites had the typical apicomplexan organelles, while metrocytes were typically globular in shape, with clear double membrane pellicles, and constantly underwent division by endodyogeny (Fig. 4). Merozoites in the present study were characterized by their short length, reaching 5.2 μm, and were 2.0 μm in width; this is somewhat shorter and wider than other described merozoites (Fig. 3). In addition to the apical complex characteristics, reserve food materials, including mainly lipid inclusions and a few amylopectin granules, were found scattered randomly below the apical complex and at the posterior end of each merozoite (Fig. 3, Fig. 4).

Regarding the host–parasite relationship, striations of the surrounding muscle fibres were mostly lost, and clusters of vacuoles and vesicles were formed between the successive cyst-wall protrusions inside the host cell (Fig. 2). Some host-cell mitochondria with damaged cristae were concentrated near the primary cyst wall. A secondary cyst wall was completely absent.

## Discussion

Despite the elucidation of the obligatory heteroxenous (prey–predator) life cycle of the genus *Sarcocystis*, host specificity and species characterization, particularly for the final host, among Sarcosporidia are contradictory and confusable. Close similarity in the fine structural features of the sexual stages, and overlapping of sporocysts dimensions and characteristics of *Sarcocystis* infecting different hosts has been recorded (see Mehlhorn and Heydorn 1978; Tadros and Laarman 1978, 1982; Abdel-Ghaffar et al. 1990). This is also true of *Sarcocystis* species that infect reptiles, where the dimensions and characteristics of the sprocysts recovered from 11 naturally infected species of snakes were found to overlap(Paperna and Finkelman 1998). Moreover, the fine structure of the endogenous stages, including the size and contents of the wall-forming bodies, also conforms with the same developmental stages of *Sarcocystis* species studied from other snakes and mammalian definitive hosts (Paperna and Finkelman 1996).

On the other hand, sarcocysts (infecting the muscles) of all species of the genus *Sarcocystis* studied in their intermediate hosts have a characteristic cyst wall (Scholtyseck et al. 1974; Mehlhorn and Heydorn 1978). The ultrastructural characteristics of these sarcocysts, particularly the primary cyst wall and its protrusions, indicate the clear and definite specificity and variability of these structures among the different *Sarcocystis* species and their infected hosts. This has been proved by many previous studies (Mehlhorn et al. 1976; Mehlhorn and Heydorn 1978; Mehlhorn and Matuschka 1986; Abdel-Ghaffar et al. 1990, 1994; Lindsay et al. 2000; Sakran 2000). Even within the same host the architecture of the primary cyst wall and its protrusions were found to change with the age of the sarcocyst (Tadros and Laarman 1978). Therefore, the ultrastructural details of the primary cyst wall and the protrusions of sarcocysts are the most important criteria for the identification of *Sarcocystis* species.

In the present study, the skink *Scincus mitranus* was proved to be the intermediate host for *Sarcocystis mitrani*. Macroscopic sarcocysts were detected in the muscles of naturally infected skinks, but fecal examinations for coccidian sprocysts were completely negative. The low infection rate (4.16%) recorded in the present study in comparison with other studies may be due to the small number of animals examined, the abundance of the final host, or the behavior of the skink.

Regarding *Sarcocystis* species parasitizing the muscular tissues of the family Scincidae, Tadros and Laarman (1978) found cysts in the skink, *Mabuya carinata*; however, these cysts were not confirmed as a *Sarcocystis* species. The site of the cysts and the characteristics of the cyst wall led to a classification within the genus *Besnoitia*. At the same time, Munday et al. (1979) and Abdel-Ghaffar et al. (1990) described *Sarcocystis* cysts in the muscles of the skinks *Leiolopisma metallica* and *Chalcides ocellatus ocellatus* respectively. Macroscopic forms of sarcocysts were also detected in the muscles of skinks of the genus *Agama* (Sakran 2000).

The present ultrastructural study has revealed that the primary cyst wall is regularly folded in long finger-like villous protrusions with a serrated appearance. The villi are never branched and have microtubular elements in their interior. These elements extend to the ground substance at the bases of the protrusions only. This structure is specific and quite different from that previously described for other reptilian *Sarcocystis* species (Matuschka and Mehlhorn 1984; Matuschka et al. 1987; Abdel-Ghaffar et al. 1990, 1994; Sakran 2000). The ground substance and extended thick septa dividing the interior of the cyst into several compartments, described in this investigation, were also peculiar. These thick and wide septa contrast with other very thin and small ones (which were invisible by light microscopy) that have been seen in other species; they are believed to give more stability to the macroscopic cysts (Mehlhorn and Heydorn 1978; Lindsay et al. 2000).

With respect to the zoites occupying the interior of the cysts (the metrocytes and merozoites), in spite of the general agreement with the typical fundamental structures of apicomplexan motile stages, including the apical complex elements (pellicle, conoid, rhoptries, micronemes and micropores etc.), there are differences regarding the measurements of the parasites and the reserve food materials observed in the present study. Merozoites in the present study were clearly shorter and wider than those described for emierian merozoites, and even for other sarcocyst merozoites. Micronemes were concentrated at one side, only in the anterior end, whereas the nucleus was posteriorly located. Reserve food materials were mainly lipid inclusions, and amylopectin granules were rarely detected.

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