Life cycle of *Sarcocystis gongyli* Trinci 1911 in the skink *Chalcides ocellatus ocellatus* and the snake *Spalerosophis diadema*

A light and electron microscopic study

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**Abstract.** The life cycle of *Sarcocystis gongyli* was established and its stages were studied by means of light and electron microscopy. The life cycle of this parasite needs two hosts, the skink *Chalcides ocellatus ocellatus* as the intermediate host and the snake *Spalerosophis diadema* as the final host. Microscopically visible sarcocysts were observed in the skeletal muscles of naturally infected skinks (infection rate, 63%). These cysts measured 60–100 x 200–900 μm (mean, 85 x 600 μm). Typical mature cysts were bordered by a primary cyst wall folded into long, leaf-like protrusions, which reached approximately 2.4 μm in length and were never branched. The interior of the cyst contained both metrocyes and cyst merozoites that showed the typical characteristics of the Apicomplexa. Experimental transmissions of the parasite from naturally infected intermediate hosts (skinks) to final (definitive) hosts were carried out. Only the snake *Sp. diadema* (family Colubridae) shed fully sporulated oocysts of the *Isospora* type (each with two sporocysts). The prepatent period was 14 days and the patent period ended 60 days post-infection (p.i.). Gamogony and sporogony were found within the intestinal epithelial cells of the snake. Free sporocysts in fresh snake feces measured 9.1 x 10.6 μm on average. The fine structure of the gamogonic and sporogonic stages was studied. Gamogony occurred within the first 8 days p.i., giving rise to young oocysts in the epithelial cells of the small intestine.

**Materials and methods.**

Experimental animals used in this study included 60 skinks (*C. ocellatus ocellatus* Forskal) and 3 different species of snakes of the family Colubridae (*Spalerosophis diadema, Psammophis sibilans* and *Malpolon monspessulana*). The reptiles were captured at different localities in Giza province (Egypt) and were brought to the laboratory, where they were identified according to Marx (1968) and maintained singly in glass cages with sand and alluvium at room temperature (27°C ± 2). Skinks were fed regularly with insect larvae and snakes received laboratory-reared, coccidia-free mice.

To detect the incidence of natural *Sarcocystis* infection in the skinks, muscles of the different parts of the body were examined by naked eye for macroscopic forms of sarcocysts. At the same time, examination for microscopic cysts was done using the cryosection technique. Small pieces of heavily infected muscles were immediately fixed as described below. Fecal samples from the skinks and snakes were examined daily for coccidian oocysts, using the usual flotation technique (Long et al. 1976), for at least 1 month.
prior to experimental infection. For experimental transmission, sarcocysts from naturally infected skinks were fed up to 16 coccidia-free snakes (Sp. diadema, P. stibians and M. monspessulana). As controls, two non-infected snakes of each species were kept under the same conditions. These control animals never showed any evidence of coccidian infection during the period of experiment. To study the development of the parasite in the final host, two experimentally infected snakes were killed at 2, 4, 6, 8, 10, 12, 14 and 16 days post-infection (p.i.). The small intestine of these snakes was divided into three equal parts; the mucosa and submucosa of these parts were removed by scraping, fixed immediately and processed further for light and electron microscopy.

Light and transmission electron microscopy

For histological examination, infected tissues were fixed in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate buffer (pH 7.3) at 4°C for 24 h. Post-fixation was done in 2% OsO₄ in the same buffer. The specimens were dehydrated in graded ethanol, transferred to propylene oxide and finally embedded in Araldite (Serva) embedding medium. Semi- and ultrathin sections were cut on a Reichert Ultracut. Semithin sections were stained with methyl blue and azure A, whereas ultrathin sections were contrasted with uranyl acetate and lead citrate before examination in a Zeiss EM 9 S 2 transmission electron microscope.

Results

Sarcocysts in naturally infected skinks

Of 60 collected skinks (C. ocellatus ocellatus), 38 were found to be infected, giving an infection rate of 63%. Only microscopic mature and immature forms of sarcocysts occurred in the striated muscles of this host. Cysts were observed in different parts of the body, being concentrated in the tail and hind-limb muscles. The cysts measured 60–100 x 200–900 μm, with a mean of 85–600 μm. They were surrounded by a striated primary cyst wall 2–6 μm thick. The ground substance was usually found directly beneath the primary cyst wall. Many septa extended from this ground substance into the interior of the cyst, dividing it into numerous compartments that surrounded the parasites. These parasites were usually differentiated into metrocytes, which were located almost at the periphery underneath the ground substance, and the cyst merozoites, which usually filled the interior of the cyst (Fig. 1).

The fine structure of the primary cyst wall, which can often be used as an important criterion for Sarcocystis species determination (see Mehlhorn and Heydorn 1978), revealed that the cyst wall examined in the present study was regularly folded into long, non-branched, leaf-like protrusions with a remarkable leafstalk. These protrusions measured 1.9–3.0 μm (mean, 2.4 μm) in length. Cross sections of these protrusions were nearly spherical and measured 0.6–1.7 μm (mean, 1.2 μm) in diameter. The ground substance consisted mainly of fine, dense and homogeneous granules and contained neither filaments nor tubular elements (Figs. 6–8).

The primary cyst wall was characterized by the presence of knob-like, electron-dense elevations. A secondary cyst wall was never observed (Fig. 8). Fine structural characteristics of both metrocytes and merozoites were similar to those described for many other Sarcocystis species (see Mehlhorn and Heydorn 1978) and were in general not specific. The cyst stages were provided by the typical apicomplexan organelles. Metrocytes constantly underwent endodyogony, producing cyst merozoites that were infective for final hosts (Figs. 1, 5). These cyst merozoites were small, measuring 0.8–2.0 x 4.1–5.3 μm, with a mean of 1.5 x 4.6 μm (Fig. 4).

Experimental transmission and development in the final host

With respect to the obligatory heteroxenous prey-predator life cycle described for Sarcocystis species (Mehlhorn and Heydorn 1978), the final host for S. gongyli was expected to be among the natural predators of this skink. From field observations and their nutritional behaviour, many predators were potential final hosts for this parasite, such as snakes of the family Colubridae that live in the same area as the skinks.

In attempts to prove a skink-snake cycle, three colubrid snakes were infected that had been collected from the same location as the skinks. Prior to experimental transmission, all snakes used in the experiments had been proven to be coccidia-free. After ingestion of heavily infected muscles of the skinks, only Sp. diadema shedded sporulated oocysts and sporocysts after a prepatent period of 14 days p.i., which continued until 60 days p.i. (patent period). About 500000 sporulated oocysts and sporocysts were collected from feces and mucosal scrapings of an experimentally infected snake. Ellipsoidal free sporocysts (each with four sporozoites) that measured 9.1 x 10.6 μm and had a smooth, thick wall and a residual body (composed of numerous fine globules) were excreted. These sporocysts showed no Stieda body. Sporulated oocysts surrounded by a thin, smooth colorless wall (tightly adjacent to the two sporozoites) were also observed in the feces. Neither an oocyst residuum nor a polar granule occurred.

Histological sections of the intestinal mucosa of experimentally infected snakes showed that gamogonic and sporogonic stages had developed in the epithelial cells of the villi of the duodenum and ileum. In the snakes killed 2 days p.i., merozoites developed directly into gamonts; no asexual reproduction was observed. Zygotes (young oocysts) were found 8 days p.i.; they were spherical, with a central nucleus, and their oocyst wall consisted of two layers as seen by electron microscopy (Fig. 12).

Sporogony started 10 days p.i. Two sporoblasts were found within each oocyst. These oocysts measured 7.2–12.6 x 10.8–16.2 μm, with a mean of 11.8 x 14.6 μm. In the snakes killed 14 or 16 days p.i., fully sporulated oocysts measuring 10.3–11.6 x 14.2–17.4 μm (average, 11 x 15.9 μm) were observed in the epithelial cells as well as in the feces (Figs. 9–11). The fine structure of oocysts and sporocysts was identical to that of other sarcosporidia (Figs. 12, 13).