FOLIA PARASITOLOGICA 46: 99-102, 1999

# Extraintestinal stages of coccidia in liver of Schneider's skink *Eumeces schneideri* (Sauria: Scincidae) from Northern Egypt

**Břetislav Koudela**[1] and **David Modrý**[1,2]

[1]Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic;

[2]Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic

Key words: extraintestinal tissue cysts, liver, sporozoite, crystalloid body, *Isospora,* Reptilia

**Abstract.** An extraintestinal coccidian parasite was identified in Schneider's skinks *Eumeces schneideri* Daudin, 1802. Numerous tissue cysts were found in melanomacrophage aggregations in the liver of six of ten examined skinks. No tissue cysts were found in other tissues. Tissue cysts were 22-26 × 9-13 μm and contained a single sporozoite. Sporozoites were 10-13 × 2-4 μm, and contained a single nucleus, homogeneous inclusion and PAS positive granules, and were surrounded by PAS negative, 1.5-3.0 μm thick cyst wall. Transmission electron microscopy revealed that the tissue cyst wall was composed of granular material and the sporozoites contained crystalloid body with regular arrangements of units. Appearance of tissue cyst and structure of crystalloid body indicate that Schneider's skinks represent a paratenic host for non-determined *Isospora* species.

Schneider's skinks *Eumeces schneideri* Daudin, 1802 are widely distributed in Northern Africa and in the Near and Middle East. Only two species of coccidia, *Eimeria baltrocki* Daszak et Ball, 1991 and *Cryptosporidium saurophilum* Koudela et Modrý, 1998 have been described from these common and abundant lizards to date (Daszak and Ball 1991, Koudela and Modrý 1998). Herein we present a description of tissue cysts with extraintestinal coccidian stages found in the liver of *E. schneideri.*

## MATERIALS AND METHODS

A group of adult Schneider's skinks *Eumeces schneideri* originally captured in Northern Egypt were imported by pet-reptile importer into the Czech Republic. Additionally, two other specimens were collected during a herpetological and parasitological survey in Jordan (Petra) and Syria (Maalulu). Clinically affected or moribund lizards were euthanised and necropsied. Prior necropsy, blood collection was performed aseptically by clipping tissue in the tail and thin blood smears were prepared. Air dried smears were fixed in methyl alcohol and stained with Giemsa. At necropsy, tissue samples of the stomach, duodenum, small and large intestine, cloaca, heart, lung, liver, gall bladder and kidney were fixed in 10% buffered formalin. Fixed tissues were processed for light microscopy using standard methods. Paraffin sections were stained with haematoxylin and eosin (HE), periodic acid Schiff (PAS) and Gomori-Grocott trichrome stain.

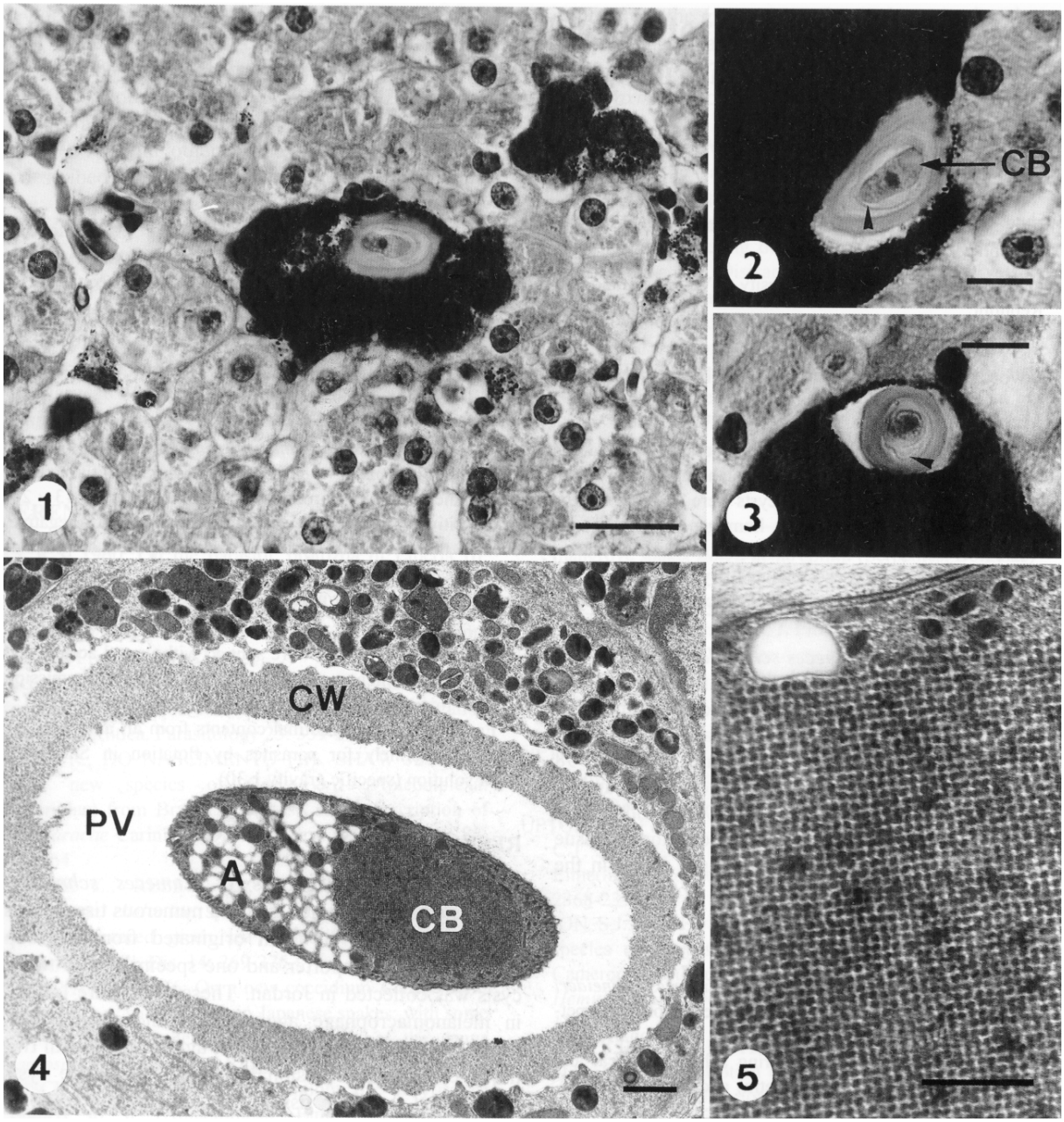
For transmission electron microscopy, a portions of liver were fixed in 2.5% glutaraldehyde in cacodylate buffer (0.1M, pH 7.4) at 4°C and post-fixed in 1% osmium tetroxide in the same buffer. Specimens were washed three times in the same buffer, dehydrated in graded alcohols and embedded in Durcupan. Thin sections were stained with uranyl acetate and lead citrate and then examined with a JEOL 1010 transmission electron microscope.

Fresh faeces and intestinal contents from all animals were screened routinely for parasites by flotation in Sheather's sugar solution (specific gravity 1.30).

## RESULTS

Of ten adult specimens of *Eumeces schneideri* necropsied and examined, six had numerous tissue cysts in the liver. Five of them originated from a group imported by pet importer and one specimen with tissue cysts was collected in Jordan. These cysts were located in melanomacrophage aggregations and most aggregations contained one tissue cyst; aggregations with two tissue cysts were occasionally found. No tissue cysts were found in other tissues. In histological sections, tissue cysts were 22-26 × 9-13 μm and contained a centrally located single sporozoite (Figs. 1-3). Neither multinucleate stages nor sexual coccidian stages were found in melanomacrophage aggregations. The tissue cyst wall was PAS and Gomori-Grocott negative and measured 1.5-3 μm. Centrally located sporozoites were crescent shaped and were 10-13 × 2-4 μm. They contained a centrally located nucleus and homogenous eosinophilic cytoplasmic inclusion posteriorly to the nucleus in HE stained sections (Fig. 2). Membranous projections were often observed between cyst wall and sporozoites (Figs. 2, 3). Sporozoites contained several cytoplasmic PAS positive granules, but the tissue cyst wall was PAS negative.

Address for correspondence: B. Koudela, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic. Phone: ++420 38 777 5408; Fax: ++420 38 47743; E-mail: koudela@paru.cas.cz




**Figs. 1-5.** Light and transmission electron microscopic appearance of tissue cysts in the liver of *Eumeces schneideri*. **Fig. 1.** Tissue cyst in a melanomacrophage aggregation in the liver. HE. **Fig. 2.** Longitudinal section of tissue cyst showing centrally located sporozoite with faint staining homogeneous inclusion considered to be the crystalloid body (CB) and membranous projections between cyst wall and sporozoites (arrowhead). HE. **Fig. 3.** Cross section of tissue cyst showing membranous projections between cyst wall and sporozoites (arrowhead). **Fig. 4.** Electron micrograph of tissue cyst in the liver. Note the thick tissue cyst wall (CW) beneath the parasitophorous vacuole (PV), crystalloid body (CB) and amylopectin granules (A). TEM. **Fig. 5.** Regular arrangement of units of the crystalloid body. TEM. Scale bars: Fig. 1 = 20 μm; Figs. 2, 3 = 10 μm; Fig. 4 = 1 μm; Fig. 5 = 300 nm.

The presence of tissue cysts in the liver was not associated with inflammation or granulomatous reaction. A moderate steatosis was observed in four of eight skinks examined. Histological examination revealed coccidian developmental stages along the gall bladder epithelium and both sporulated and unsporulated oocysts in the lumen of the gall bladder of four skinks with tissue cysts in the liver. The same

coccidian stages were also observed in three skinks without liver cysts. Additionally, cryptosporidia were found in histological sections of small intestine from five of ten necropsied skinks.

Ultrastructurally, the cyst wall was limited by the parasitophorous vacuole membrane and was composed of granular material containing electron-dense bodies and tubular structures. The tubular structures were also found in the lumen of the tissue cyst. Centrally located sporozoites contained numerous spherical electron-lucent amylopectin granules and electron-dense bodies. They were more concentrated in the anterior region of the sporozoite where also elongated rhoptries and numerous micronemes were found (Fig. 4). The number of rhoptries could not be determined. The middle part of the sporozoite had well defined nucleus and posterior part contained a prominent spherical crystalloid body composed of regularly arranged, electron-dense, spherical units about 30 nm in diameter (Figs. 4, 5). Crystalloid bodies were not membrane bound (Fig. 5). Mitochondria, Golgi complex and smooth and rough endoplasmic reticula were also detected.

No blood parasites were detected in the Giemsa stained blood smears. Examination of faeces by flotation in Sheather's sugar solution revealed that four of six skinks with tissue cysts passed also oocysts of *Eimeria baltrocki*, two specimens passed cryptosporidial oocysts and all of them oxyurid ova.

## DISCUSSION

The tissue stages observed in the liver of Schneider's skinks had the typical appearance of coccidian sporozoites and were very similar to extraintestinal stages of feline and canine *Isospora* species found in cats, dogs and paratenic hosts, and to *Isospora belli* Wenyon, 1923 found in humans (Dubey and Frenkel 1972, Dubey 1975, Michiels et al. 1994, Restrepo et al. 1987, Lindsay et al. 1997). No *Isospora* species has been reported from Schneider's skink to date. In the present study, we did observe coccidian developmental stages along the gall bladder epithelium and both sporulated and unsporulated oocysts in the lumen of the gall bladder and oocysts in faeces of seven skinks. However, only four skinks of those had tissue cysts in the liver. These results show that the presence and number of tissue cysts in the liver did not correspond with findings of coccidian developmental stages in gall bladder epithelium and oocysts in faeces. Moreover, morphology of passed oocysts was identical with the description of *Eimeria baltrocki* (Daszak and Ball 1991).

Extraintestinal *Isospora* stages were most often found in mesenteric lymph nodes, but other organs such as spleen, liver and bronchial and mediastinal lymph nodes can be infected (Dubey and Frenkel 1972, Lindsay et al. 1997). In the present study, the tissue cysts were observed in melanomacrophages aggregations in liver and we did not find them in other tissues examined. Melanomacrophages are large melanin bearing cells with phagocytic function in the heamatopoietic and certain other soft tissues of lower vertebrates and are occasionally described as Kupffer cells (Scalia et al. 1988). The extraintestinal stages of *I. belli* were also observed within Kupffer cells or within macrophages located in portal areas (Michiels et al. 1994).

In this study, we observed extraintestinal stages as single sporozoite in tissue cysts. This is in agreement with reports for canine and feline *Isospora* species and *I. belli* in humans (Dubey 1975, Dubey and Frenkel 1972, Restrepo et al. 1987, Michiels et al. 1994, Lindsay et al. 1997). Our results of the histochemical stainings (PAS and Gomori-Grocott) were identical to reports of histochemical examination of *I. belli* tissue cyst (Michiels et al. 1994, Lindsay et al. 1997).

Results of TEM examination of tissue cysts in the present study revealed organelles typical for coccidian sporozoites. The most prominent structure in the posterior part of sporozoites was spherical crystalloid body composed of regularly arranged units. The sporozoites of mammalian *Isospora* species contain one or two crystalloid bodies, that are composed of regularly arranged units, whereas the sporozoites of *Eimeria* species contain one or two homogenous electron-dense refractile bodies (Chobotar and Scholtyseck 1982). Our findings of TEM examination of tissue cysts are comparable with the TEM descriptions of extraintestinal stages of feline *I. felis* (Mehlhorn and Markus 1976) and canine *I. ohioensis* (Dubey and Mehlhorn 1978), both in the mesenteric nodes of the mouse, and extraintestinal stages of *I. belli* in AIDS patients (Restrepo et al. 1987, Michiels et al. 1994, Lindsay et al. 1997). Lindsay et al. (1997) noted that the presence of crystalloid body in the coccidian developmental stages does not conclusively demonstrate that they are sporozoites because crystalloid bodies are also found in *I. suis* merozoites and therefore, possibly, in other *Isospora* species merozoites. However, we observed the extraintestinal stages always as a single parasite without any characters of merogony.

The parasites described here had the appearance of *Isospora* sporozoites of feline and canine *Isospora* species found in mice. Mice, rats, hamsters, dogs, cats, cattle, sheep and camels have been shown to be paratenic-transport hosts for canine and feline *Isospora* species (Frenkel and Dubey 1972, Dubey 1975, Dubey and Mehlhorn 1978, Fayer and Frenkel 1979, Hilali et al. 1992). After ingestion of sporulated oocysts by a paratenic host, sporozoites invade extraintestinal tissues, encyst there and persist there for a long time. The definitive host can become infected by ingesting

the tissue cysts. Schneider's skinks are widely distributed throughout desert and semi-desert ecosystems of Northern Africa and the Near East and due to their relative abundance and large size often serve as a prey for many carnivorous predators, including cats and dogs. We suppose that extraintestinal stages found in the liver of Schneider's skinks are dormant coccidian sporozoites and that Schneider's skink represents a paratenic host for non-determined *Isospora* species of some carnivorous higher vertebrate.

**Acknowledgements.** This study was supported by the Grant Agency of the Czech Republic, Grant No. 508/95/0273. We thank Veronika Schacherlová and Petra Eliašová for preparing tissue samples for histology and electron microscopy.

## REFERENCES

CHOBOTAR B., SCHOLTYSECK E. 1982: Ultrastructure. In: P.L. Long (Ed.), Biology of the Coccidia. University Park Press, Baltimore, pp. 102-165.

DASZAK P., BALL S.J. 1991. Five new species of *Eimeria* (Apicomplexa: Eimeriidae) from lizards. Syst. Parasitol. 20: 141-147.

DUBEY J.P. 1975: Experimental *Isospora canis* and *Isospora felis* infection in mice, cats, and dogs. J. Protozool. 22: 416-417.

DUBEY J.P., FRENKEL J.K. 1972. Extra-intestinal stages of *Isospora felis* and *I. rivolta* (Protozoa: Eimeriidae) in cats. J. Protozool. 19: 89-92.

DUBEY J.P., MEHLHORN H. 1978: Extraintestinal stages of *Isospora ohioensis* from dogs in mice. J. Parasitol. 64: 689-695.

FAYER R., FRENKEL J.K. 1979. Comparative infectivity for calves of oocysts of feline coccidia: *Besnoitia, Hammondia, Cystisospora, Sarcocystis*, and *Toxoplasma.* J. Parasitol. 65: 756-762.

FRENKEL J.K., DUBEY J.P. 1972: Rodents as vectors for feline coccidia, *Isospora felis* and *Isospora rivolta*. J. Infect. Dis. 125: 69-72.

HILALI M., NASSAR A.M., EL-GHAYSH A. 1992: Camel (*Camelus dromedarius*) and sheep (*Ovis aries*) meat as a source of dog infection with some coccidian parasites. Vet. Parasitol. 43: 37-42.

KOUDELA B., MODRÝ D. 1998: New species of *Cryptosporidium* (Apicomplexa: Cryptosporidiidae) from lizards. Folia Parasitol. 45: 93-100.

LINDSAY D.S., DUBEY J.P., TOIVIO-KINNUCAN M.A., MICHIELS J.F., BLAGBURN B.I. 1997: Examination of extraintestinal tissue cysts of *Isospora belli.* J. Parasitol. 83: 620-625.

MEHLHORN H., MARKUS M.B. 1976: Electron microscopy of stages of *Isospora felis* of the cat in the mesenteric lymph node of the mouse. Z. Parasitenkd. 51: 15-24.

MICHIELS J.F., HOFMAN P., SAINT PAUL M.C., BOISSY C., MONDAI V., LEFICHOUX Y., LOUBIER R. 1994: Intestinal and extraintestinal *Isospora belli* infection in an Aids patient. A second case report. Pathol. Res. Pract. 190: 1089-1093.

RESTREPO C., MACHER A.M., RADANY E.H. 1987: Disseminated extraintestinal isosporiasis with acquired immune deficiency syndrome. Am. J. Clin. Pathol. 87: 536-542.

SCALIA M., GEREMIA E., CORSARO C., SANTORO C., SCIUTO S., SICHEL G. 1988: The extracutaneous pigmentary system: evidence for the melanosynthesis in Amphibia and Reptilia liver. Comp. Biochem. Physiol. 89B: 715-717.

Received 10 September 1998

Accepted 11 November 1998