

## Life Cycle of *Sarcocystis* between Poikilothermic Hosts. Lizards are Intermediate Hosts for *S. podarcolubris* sp. nov, Snakes Function as Definitive Hosts

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The role of the Western Whip snake *Coluber viridiflavus* was demonstrated as a definitive host for *Sarcocystis podarcolubris* sp. nov. of the Italian Wall lizard *Podarcis sicula* and the Tyrrhenian Wall lizard *Podarcis tiliguerta*. Sporocysts (9.58 × 6.94 μm) of *S. podarcolubris* from a naturally infected snake *C. viridiflavus* were fed to a *Sarcocystis* free lizard *P. sicula* and via arthropods *Musca domestica* to another *Sarcocystis* free lizard *P. tiliguerta*. About 3–4 months later sarcocysts could be detected in both lizards. The cysts measured 90–130 μm × 450–550 μm. The cyst wall had 2.5–3 μm long villus like protrusions. The sausage-shaped bradyzoites measured circa 7.7 × 2 μm. Refeeding of the experimentally infected lizards to the snake led to a renewed shedding of sporocysts after a prepatency of 12–15 days.

### Introduction

While many mammals have become known as intermediate or final hosts of *Sarcocystis* infections, only few investigations have been made in *Sarcocystis* species of reptiles. Up to the present the developmental biology of only three *Sarcocystis* species from reptiles are outlined by experimental investigations [1–5]. In these cases rodents have been described as intermediate and serpents as definitive hosts. Some other papers are limited to isolated descriptions of cysts found in reptiles [6–13]. A recent note reports failure to find a rodent as intermediate host for *Sarcocystis* sporocysts excreted from the River Jack *Bitis nasicornis* [14]. The present paper describes for the first time transmission experiments and life cycle studies on a reptile-reptile (lizard-snake) cycle in *Sarcosporidia*. The study was undertaken to elucidate the live cycle of *Sarcocystis* sporocysts excreted by a Western Whip snake *Coluber viridiflavus* from Sardinia, Italy.

### Materials and Methods

The source of the *Sarcocystis* species investigated in this study was a Western Whip snake *Coluber*

*viridiflavus* caught near Lago Baratz, Sardinia, Italy. The 70 cm long snake was maintained in a isolated cage at 25 ± 2 °C and fed *Sarcocystis* free white mice *Mus musculus* only. All fecal samples from the *C. viridiflavus* were checked for Coccidia by the ZnCl<sub>2</sub>/NaCl flotation technic. Italian Wall lizards, *Podarcis sicula* and Tyrrhenian Wall lizards *Podarcis tiliguerta*, were captured near Sassari, Sardinia. They were all caged separated from one another in a different room from the snake and fed on diverse arthropods. Flies, *Musca domestica* were trapped in a piggery in Berlin, Germany kept single in small glas tubes and fed with sugar solution. Sporocysts were enriched and isolated from snake fecal suspension by screening after flotation with ZnCl<sub>2</sub>/NaCl solution. The sporocysts were washed by centrifugation in water and stored in 2.5% Potassiumdichromate at 4 °C. The Sporocysts were counted in a Mc Master chamber. Before inoculation, the sporocysts suspension was washed again twice in water. Each of three NMRI-mice were administered a suspension containing about 500 sporocysts orally by an inoculation needle attached to a 1 ml disposable syringe. Another three NMRI-mice were treated in the same way with about 20000 sporocysts each. One from each group of mice was killed on the 80th day p.i. the remaining four on the 104th day p.i. Skeletal muscles, heart and brain were examined for cysts in fresh press preparations. Skeletal muscles were mixed separately with PBS in a mixer and the mixture checked on microscopic mounts. To make sure that the experimental lizards were not infected with sarcocysts each animal was scrutinized by biopsy. One Tyrrhenian wall lizard *P. tiliguerta* was orally infected by an inoculation needle with ca. 250 sporocysts. A biopsy was made on the tail and the hind limb on the 102nd day p.i. The lizard was killed on the 129th day p.i. The Italian wall lizard *P. sicula* was infected using flies *M. domestica* as phoretic hosts. For this purpose ten flies which had starved for 24 h were fed drops of a sugar solution containing 10000 Sporocysts/0.2 ml. Two or three drops of the sporocyst containing sugar solution were given to each fly by an Eppendorf-pipette till 0.2 ml had been consumed. Following this the flies were fed within one hour to the lizard, which had fasted since 48 h. This lizard was killed on the 95th day p.i. Skeletal muscles, heart and brain of the lizard were examined for sarcocysts as mentioned above for mice. The rest of the peeled carcass of *P.*

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*sicula* was fed to the Western Whip snake *C. viridiflavus* which was no longer excreting sporocysts. Sporocysts, cysts and bradyzoites were measured and photographed using a Zeiss photomicroscope.

## Results

In the feces of a Western Whip snake *C. viridiflavus* from Sardinia numerous sporocysts of a *Sarcocystis* species were found (Fig. 1, 2). The average size of the sporocysts was  $9.58 \times 6.94$  ( $9.20-10.73 \times 6.13-7.67$ )  $\mu\text{m}$ . After the oral application of either 500 or 20000 of these sporocysts to each of three NMRI-mice *M. musculus* no symptoms of illness and no fatalities occurred. The examination of skeletal muscles, heart and brain for sarcocysts was also

negative. On the 80th and 104th day p.i. neither macroscopic nor microscopic cysts could be detected.

The biopsy of the experimentally infected lizard *P. tiliquerta* on day, 102 p.i. was successful. Skeletal muscles from the base of the tail as well as from the hind limb were sarcocyst positive (Fig. 3). This lizard was killed on the 129th day p.i. Cysts were seen in all muscles but not in the brain. The experimentally (via flies) infected lizard *P. sicula* killed on day 95 p.i. was more heavily infected than the *P. tiliquerta*. In all muscles tested for Sarcosporidia cysts could be discovered. The cysts found in both lizards were circa 90–130  $\mu\text{m}$  wide and up to 450–550  $\mu\text{m}$  long. The cysts contained numerous banana-shaped bradyzoites of about 7.7  $\mu\text{m}$  long and circa 2  $\mu\text{m}$  broad. The cyst wall had small villus-like processes. The cyst wall protrusions measured 2.5–3  $\mu\text{m}$  in length. The positive trial in which a lizard was infected using *M. domestica* as phoretic arthropod hosts demonstrated another conceivable way of *Sarcocystis*-infections in lizards. Twelve days after feeding the experimentally infected *P. sicula* to the *C. viridiflavus* the snake excreted a few unsporulated oocysts. First sporulated oocysts and free sporocysts were seen on the 13th, and 15th days p.i. respectively. Prepatency lasted for 12–15 days, while the patent infection prevailed for more than three months.

## Taxonomic summary

*Sarcocystis podarcicolubris* sp. nov.

Type host: Western Whip snake *Coluber viridiflavus*.

Intermediate host: Italian Wall lizard *Podarcis sicula*. Tyrrhenian Wall lizard *Podarcis tiliquerta*.

Type localita: Sardinia Italy.

Prepatency: 12–15 days.

Patent period: several months.

## Diagnosis:

Sporocysts: The ellipsoidal sporocysts are commonly free of an oocyst wall and without a Stieda body. The sporocyst wall is 0.5  $\mu\text{m}$  thick. A sporocyst residuum is present. It is compact and spherical consisting of small refractile globules. The sporocysts measure  $9.58 \times 6.94$  ( $9.20-10.73 \times 6.13-7.67$ )  $\mu\text{m}$ . The sporozoites are elongate banana-shaped, with one end slightly pointed.

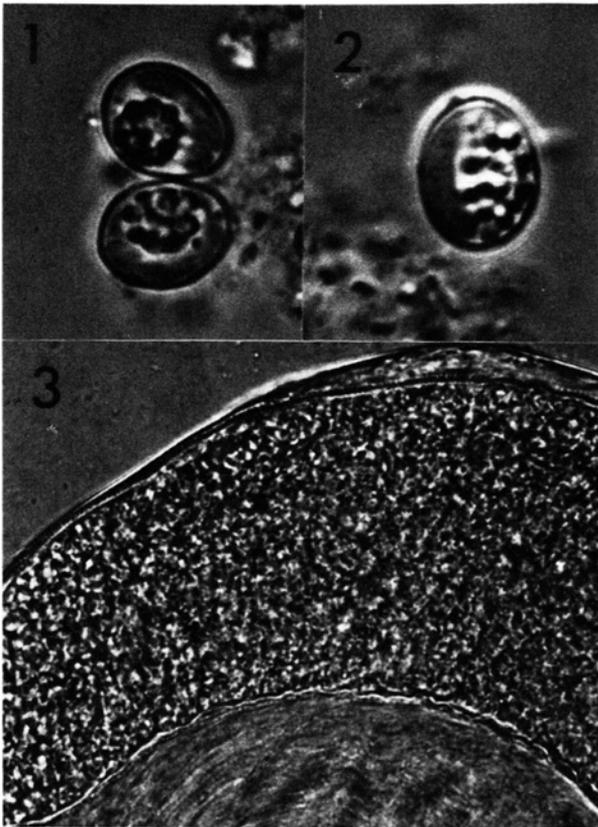


Fig. 1. Sporulated oocyst of *Sarcocystis podarcicolubris* sp. nov. (unstained) 2000 $\times$ .

Fig. 2. Sporocyst of *Sarcocystis podarcicolubris* sp. nov. (unstained) 2000 $\times$ .

Fig. 3. Sarcocyst of *Sarcocystis podarcicolubris* sp. nov. from *Podarcis sicula* 95th day p.i. (unstained) 500 $\times$ .

Sarcocysts: The cysts found 3–4 months after infection in muscles are usually about 90–130  $\mu\text{m}$  in width and ca. 450–550  $\mu\text{m}$  long. Small villus-like protrusions of the cyst wall measure 2.5–3  $\mu\text{m}$ . The sausage-shaped bradyzoites are ca. 7.7  $\mu\text{m}$  long and about 2  $\mu\text{m}$  broad with a pointed anterior and a rounded posterior end.

## Discussion

Intramuscular cysts of a *Sarcocystis* species were first described by Lühe in 1900 (cited after [10] from the Common Wall lizard *Podarcis muralis* (former *Lacerta muralis*). Babudieri in 1932 redescribed the species and gave the scientific name *Sarcocystis lacertae* [10]. Since the predator-prey type of life cycle in *Sarcocystis* has recognized only as recently as 1972 by Heydorn and Rommel, no final host is known for *S. lacertae* [15, 16]. In spite of this the cysts of *S. podarcolubris* reported in the present study also differ in other respects from the descriptions by Lühe and Babudieri for the cyst of *S. lacertae* [10]. The muscle-cyst of *S. lacertae* are larger than those described in the present study and measure 1  $\times$  1.8–2 mm in diameter. Mature cysts of *S. podarcolubris* on the 102nd day p.i. only reached 450–550  $\mu\text{m}$  in length and 90–130  $\mu\text{m}$  in width. No striation, villus like protusions or similar characteristics, as found for *S. podarcolubris* in the present investigation, have been described for the

cyst wall of *S. lacertae*. No scientific name was given in a description of cysts from *P. muralis* by Senaud and Puytorac in 1965. Their results come close to the present findings, with cysts of 480  $\mu\text{m}$  in length and 100–200  $\mu\text{m}$  in width, with villus-like cyst-wall protrusions of 2.5–3  $\mu\text{m}$  in length. On the other hand, the bradyzoites they described are slightly smaller (4–6  $\times$  1–1.5  $\mu\text{m}$ ) than the mean of 7.7  $\times$  2  $\mu\text{m}$  in the present study. If they described the same species as in this paper, a third intermediate host for *S. podarcolubris* has to be assumed.

In defining a new species in *Sarcocystis* the naming of one intermediate and one final host is usually regarded as essential [17, 18]. Because this is fulfilled in the present study the name *S. podarcolubris* has given in conformity with the proposal for a new nomenclature of Sarcosporidia by Heydorn *et al.* and Frenkel *et al.* [17, 18].

The feasibility of transmission by arthropods as vectors for *Sarcocystis* sporocysts was tested recently by Smith and Frenkel [19] for *S. muris* and some other Coccidia and by Häfner [20] for *S. singaporensis*. Arthropods exposed to feces containing sporocysts were fed to the intermediate hosts and subsequently induced infections [19, 20]. The epidemiological role of arthropod vectors is also demonstrated for *S. podarcolubris* in the present study. It can be concluded that coprophageous arthropods play an important part in spreading *Sarcocystis* species, especially in animals like lizards, which feed almost exclusively on arthropods.

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