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Sporocysts Isolated from the Southern Copperhead (*Agkistrodon contortrix contortrix*) Produce *Sarcocystis montanaensis*-like Sarcocysts in Prairie Voles (*Microtus ochrogaster*)

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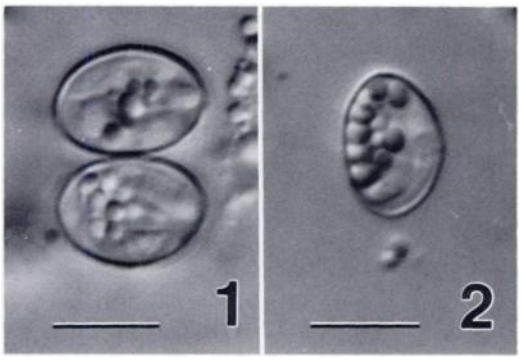
ABSTRACT: Sporulated oocysts and free sporocysts of a *Sarcocystis* sp. were isolated from the feces of a southern copperhead (*Agkistrodon contortrix contortrix*) collected in Arkansas (USA). Twenty sporocysts measured 11.2 by 8.5 μm, lacked a Stieda body, and had four sporozoites and a granular sporocyst residuum. Sarcocysts similar to those of *Sarcocystis montanaensis* were present in the tongues of prairie voles (*Microtus ochrogaster*) inoculated orally with 800 sporocysts 128 days previously. Sarcocysts were thin-walled, divided into compartments by septa, and had electron dense projections (0.14 μm) on the primary cyst wall. Infection was not pathogenic for prairie voles under the conditions of this study. No infections were observed in ICR strain laboratory mice (*Mus musculus*) or white-footed mice (*Peromyscus leucopus*) following oral inoculation of 800 sporocysts.

Key words: *Sarcocystis montanaensis*, life cycle, sarcocyst, sporocyst, snake, copperhead, *Agkistrodon contortrix contortrix*, vole, *Microtus ochrogaster*.

Dubey (1983) described two new species of *Sarcocystis* from naturally infected meadow voles (*Microtus pennsylvanicus*) and long-tailed voles (*M. longicaudatus*), collected in Montana (USA). Thin-walled sarcocysts were named *S. montanaensis*, and the thick-walled sarcocysts *S. microti*. Sarcocysts of *S. montanaensis* were found in three of 31 (10%) meadow voles and one of eight (13%) long-tailed voles examined, while sarcocysts of *S. microti* were found in one of 31 (3%) meadow voles and one of eight long-tailed voles. An attempt to infect a laboratory raised cat with vole tissues containing both *S. montanaensis* and *S. microti* was unsuccessful (Dubey, 1983).

In present study, we demonstrate that southern copperheads (*Agkistrodon contortrix contortrix*) are definitive hosts for a *Sarcocystis* species that produces sarcocysts in the tongue of experimentally infected prairie voles (*M. ochrogaster*) and that these sarcocysts are indistinguishable from *S. montanaensis* described from voles (Dubey, 1983).

Feces containing sporulated oocysts and free sporocysts of a *Sarcocystis* sp. (Figs. 1, 2) were collected from a naturally infected adult female southern copperhead [Arkansas State University Museum of Zoology (ASUMZ 15311), State University, Arkansas 72467, USA] obtained from Perry County, Arkansas, USA (34°54′N, 93°08′W) and shipped in 2.5% (w/v) potassium dichromate solution to the Division of Biology (Kansas State University, Manhattan, Kansas 66506, USA). Twenty sporocysts measured 9.8 to 12.2 × 8.0 to 9.0 μm (mean, 11.2 by 8.5 μm) and had a shape index of 1.2 to 1.4 (mean, 1.3). The sporocyst wall was about 0.5 μm thick, lacked a Stieda body and enclosed four sporozoites that were arranged around a granular sporocyst residuum. One side of the sporocyst was slightly flattened. Sporocysts were washed free of potassium dichromate by centrifugation in tap water, counted in a hemocytometer, and 800 sporocysts were orally inoculated into each of two 8-week-old ICR strain laboratory mice (*Mus musculus*), white-footed mice (*Peromyscus leucopus*) and prairie voles (*Microtus ochrogaster*). Sporocysts were 11-days-old when used for inoculations. All



FIGURES 1, 2. Nomarski interference-contrast photomicrographs of *Sarcocystis* sp. in the feces of a southern copperhead. 1. An oocyst containing 2 sporocysts. Bar = 10 μm. 2. A free sporocyst. Bar = 10 μm.

experimentally inoculated prairie voles and white-footed mice were the progeny of animals that had been raised in the laboratory for at least three generations.

All inoculated rodents were killed 128 days postinoculation and squash preparations were made from the heart, diaphragm, and tongue of each animal. Clinical disease resulting from experimental inoculations was not observed in any rodents. Sarcocysts were found only in the prairie voles. Sarcocysts in squash preparations were elongate, divided into sections by septa and measured up to 800 μm. Portions of infected tongue were fixed in 3% glutaraldehyde in phosphate buffer (pH 7.2) and sent to the Department of Pathobiology, Auburn University, Alabama 36849, USA. Samples were processed for transmission electron microscopy (TEM) and examined in a Philips 301 transmission electron microscope operating at an accelerating voltage of 60 kV.

A single sarcocyst was observed with TEM (Figs. 3 to 5). The sarcocyst wall was classified as a Type 1 sarcocyst wall according to the guidelines suggested by Dubey et al. (1989). The parasitophorous vacuole (PV) membrane was ornamented with numerous electron dense projections and composed the primary cyst wall. The projections were present as distinct knob-like structures with an electron dense upper portion and a lower portion composed only of the unit membrane of the PV (Figs. 3, 4). Often, the projections appeared to blend together over various portions of the cyst wall (Fig. 4) and in some sections, due to orientation, there appeared to be holes in the primary cyst wall (Fig. 5). The projections were about 0.14 μm in thickness and the cyst wall about 0.8 μm. Electron dense ground substance was present directly beneath the PV membrane and formed septa that divided the sarcocyst into compartments (Fig. 3). A few metrocytes were present near the periphery of the sarcocyst. Bradyzoites possessed all structures typical of this developmental stage. Additionally, it was notable that micronemes were present both in the anterior and posterior portions of the bradyzoites (Fig. 3).

Matuschka (1986) experimentally infected three species of voles (*M. arvalis*, *M. oeconomus*, and *M. guentheri*) with sporocysts of *S. clethrionomyelaphis* isolated from the extremely rare Aesculapian snake (*Elaphe longissima*) located in southern Germany. Sarcocysts of *S. clethrionomyelaphis* have 3 to 5 μm hair-like protrusions on the primary cyst wall (Mehlhorn and Matuschka, 1986) which make it clearly different from the sarcocysts seen in prairie voles in our study.

The sarcocysts observed in our study resemble the sarcocysts of *S. crotali* that develop in experimentally infected laboratory mice fed sporocysts isolated from Mojave rattlesnakes, *Crotalus scutulatus scutulatus* (Enzeroth et al., 1985). However, we could not infect ICR strain laboratory mice with sporocysts isolated from southern copperheads. In addition, the sporocysts observed in our study are larger than those of *S. crotali*.

The present study represents the second time that *Sarcocystis* sp. has been reported from copperheads. Wacha and Christiansen (1975) reported isosporan oocysts from two of two osage copperheads (*A. contortrix phaeogaster*) in Iowa (USA). The sporocysts in their study were smaller

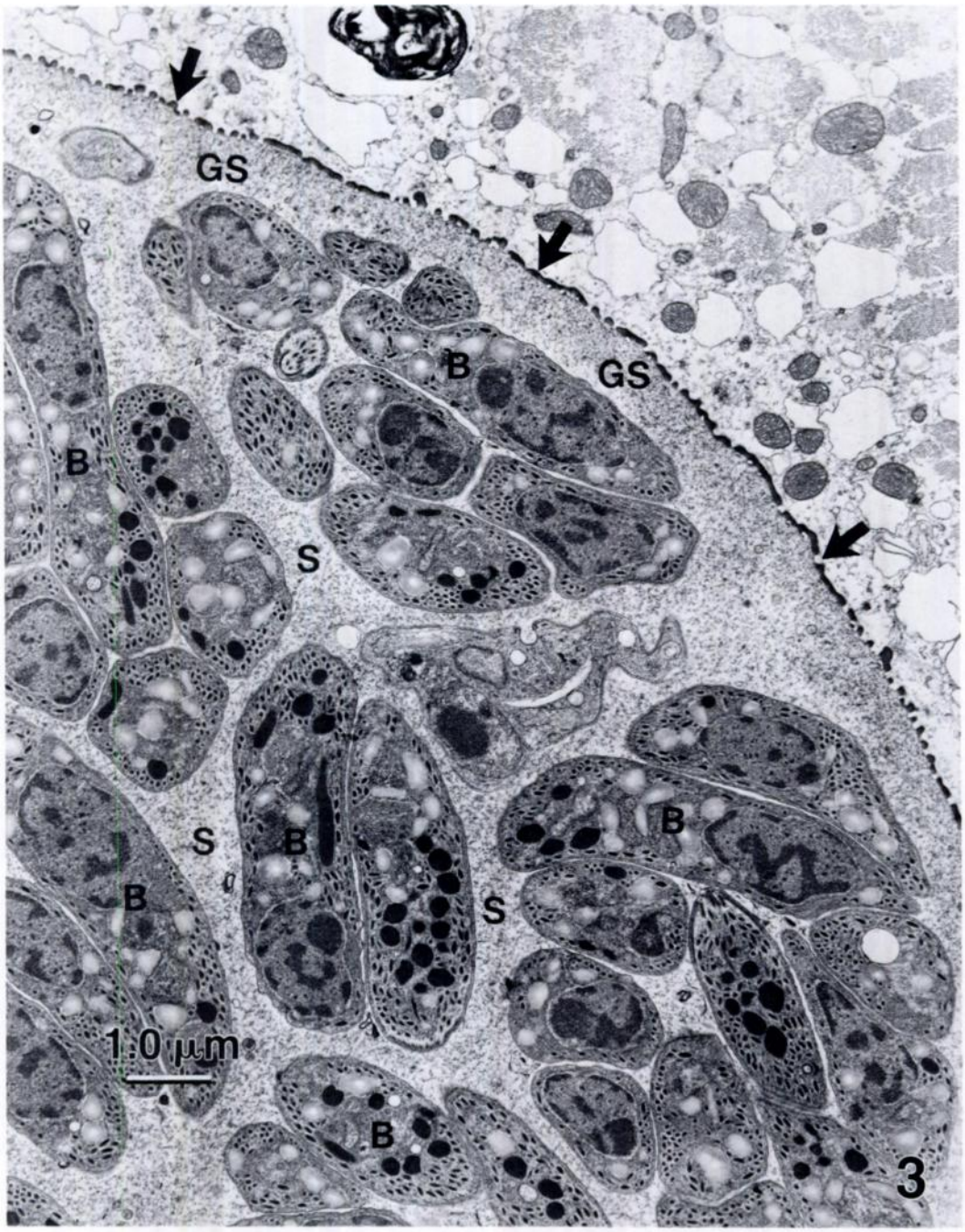
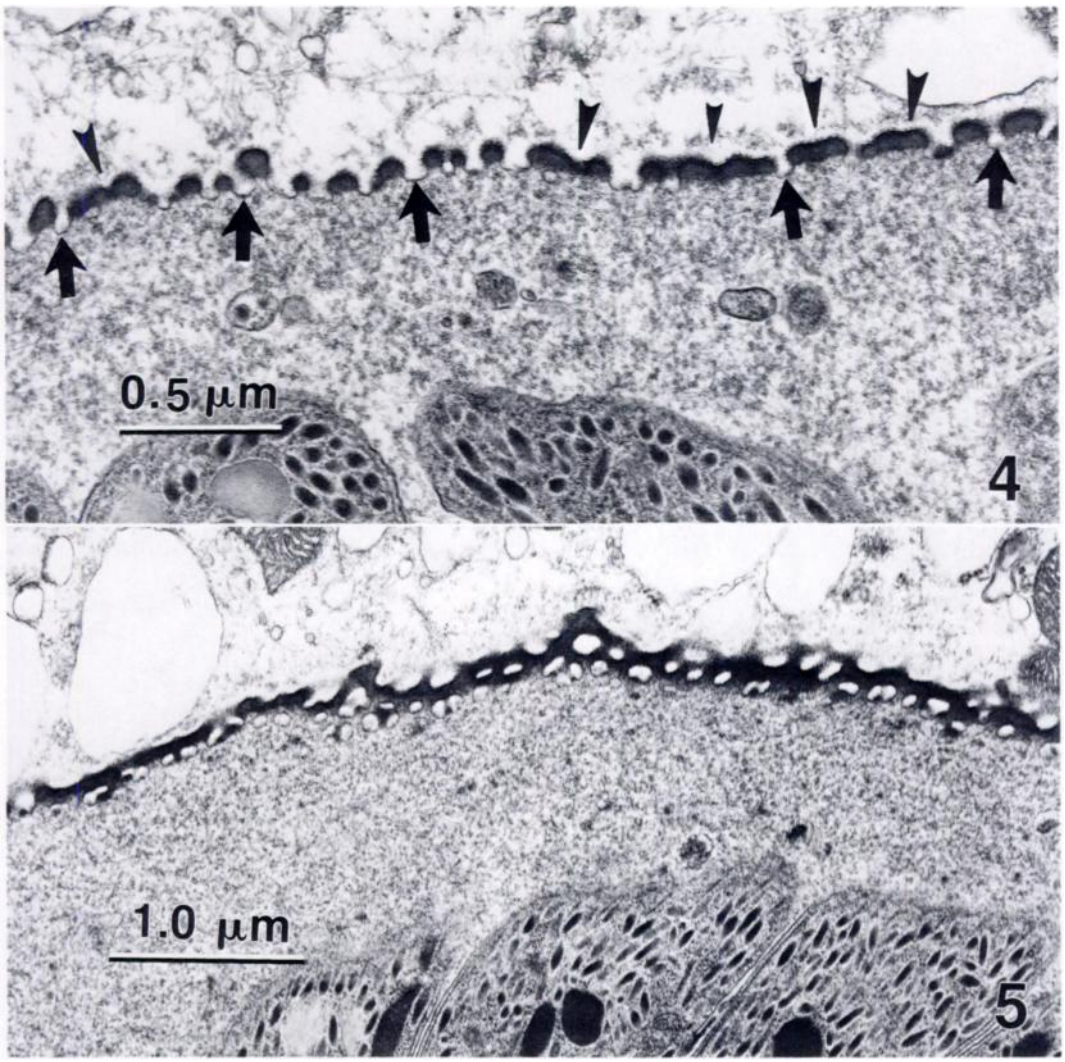


FIGURE 3. Transmission electron micrograph of a sarcocyst in an experimentally infected prairie vole. Note the ornamented primary cyst wall (arrows), ground substance (GS), bradyzoites (B), and septa (S). Uranyl acetate and lead citrate. Bar = 1 μm.



FIGURES 4, 5. Transmission electron micrographs of the sarcocyst wall of *Sarcocystis* species in a prairie vole. 4. Section through a sarcocyst showing knob-like projections on the primary cyst wall. Note that the projections are composed only of the parasitophorous vacuole membrane at the base (arrows), also note that some projections appear to be fused (arrow heads). Uranyl acetate and lead citrate. Bar = 0.5 µm. 5. Tangential section through a sarcocyst. Note that the holes in the primary cyst wall are artifacts of the angle of sectioning and represent sections through the base of the projections. Uranyl acetate and lead citrate. Bar = 1 µm.

(10.2 × 7.7 µm versus 11.2 × 8.5 µm) and probably represent a separate species. However, the shape index was identical for sporocysts in both studies (1.3). These authors (Wacha and Christiansen, 1975) also noted that two of two massasaugas (*Sistrurus catenatus*) from Iowa were infected. The sporocysts from these snakes measured 11.4 × 8.6 µm, nearly identical to the size we observed in the present study. No transmission studies were reported for either isolate (Wacha and Christiansen, 1975). Interestingly, the massasauga does not range any farther to the west than central Colorado (USA) nor does its range extend as far east as Arkansas (Minton, 1983). However, its range overlaps with the southern copperhead to the east and with the prairie rattlesnake (*C. viridis viridis*) to the west (Conant, 1975). The prairie

rattlesnake is present in Montana (USA) and may be a suitable definitive host for the *Sarcocystis* sp. observed in the present study.

The structure of sarcocysts and bradyzoites observed in the present study is indistinguishable from that reported by Dubey (1983) for *S. montanaensis* found in naturally infected *M. pennsylvanicus* and *M. longicaudatus*. Although we were able to experimentally infect *M. ochrogaster*, this vole does not occur in Perry County, Arkansas where the definitive host was collected. Another microtine, the woodland vole (*M. pinetorum*), has been reported from Perry County (Sealander and Heidt, 1990) and may be a suitable intermediate host.

Definitive proof that the species seen in the present study is *S. montanaensis* would require feeding naturally infected meadow or long-tailed voles to uninfected southern copperheads, isolating sporocysts from these snakes and reproducing infections in prairie voles.

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