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CONFIRMATION OF *SARCOCYSTIS JAMAICENSIS* SARCOCYSTS IN IFN-γ GENE KNOCKOUT MICE ORALLY INOCULATED WITH SPOROCYSTS FROM A RED-TAILED HAWK (*BUTEO JAMAICENSIS*)

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KEY WORDS

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Sarcocystis jamaicensis
Merozoites
Schizonts
Encephalitis
Red-Tailed Hawk
Buteo jamaicensis
Sporocysts

ABSTRACT

Here, we report confirmation of sarcocysts of *Sarcocystis jamaicensis* in an experimental intermediate host, IFN-γ gene knockout (KO) mice orally inoculated sporocysts from its natural definitive host, a red-tailed hawk (*Buteo jamaicensis*) (RTH). A RTH submitted to the Carolina Raptor Center, Huntersville, North Carolina, was euthanized because it could not be rehabilitated and released. Fully sporulated sporocysts from intestinal scrapings of the RTH were orally fed to 2 laboratory-reared outbred Swiss Webster mice (SW; *Mus musculus*) and to 2 KO mice. The sporocysts were infective for KO mice but not to SW mice. Both SW mice remained asymptomatic, and neither schizonts nor sarcocysts were found in their tissues when euthanized on day 54 post-inoculation (PI). The KO mice developed neurological signs and were necropsied 38–54 days PI. Schizonts/merozoites were found in both KO mice euthanized and they were confined to the brain. The predominant lesion was meningoencephalitis. Microscopic sarcocysts were found in muscles of both KO mice. When viewed with light microscopy, the sarcocyst wall appeared thin (<1 μm thick) and smooth. Ultrastructural details of sarcocysts are described.

Species of *Sarcocystis* have a 2-host life cycle, with asexual development in the extra-intestinal tissues of an intermediate host (often an herbivore) and sexual reproduction in the intestine of the definitive host (a carnivore). The definitive host becomes infected by ingesting tissues of the intermediate hosts containing mature sarcocysts. Bradyzoites released from these sarcocysts penetrate the lamina propria of the small intestine and undergo fertilization to form oocysts within a day. Oocysts sporulate in situ and sporulated oocysts or sporocysts released from oocysts are excreted in feces. The intermediate host becomes infected by ingesting food or water contaminated with sporocysts. Sporozoites released from sporocysts initiate asexual multiplication, first as schizonts and then as sarcocysts (Dubey et al., 2016).

Sarcocystis infections are common in the skeletal muscles (Lindsay and Blagburn, 1999) and intestines (Lindsay and Blagburn, 1989; Yabsley et al., 2009) of raptors in the United States but little is known of their life cycles. Recently, we reported a new species of *Sarcocystis*, *Sarcocystis jamaicensis*, with red-tailed hawks (RTHs; *Buteo jamaicensis*) as the natural definitive host and the IFN-γ gene knockout (KO) mice as an experimental intermediate host. The KO mice inoculated with sporocysts from the hawk developed schizont-associated encephalitis and a few sarcocysts in their muscles (Verma et al., 2017). However, the ultrastructural description of sarcocysts was based on specimens from a deparaffinized block of formalin-fixed muscle. Thus, the description of sarcocyst was preliminary because the specimen was suboptimal for electron microscopic observations. Here, we confirm the ultrastructure of sarcocysts from an optimally fixed sample.

A female adult RTH with a leg injury was found in Mecklenburg County near Charlette, North Carolina and admitted to the Carolina Raptor Center, Huntersville, North Carolina. It was alive, lying down with its eyes closed, and had slightly labored breathing but no movement.

It died and was examined the same day. At necropsy, the liver and spleen were pale and the pancreas was almost white. A black,

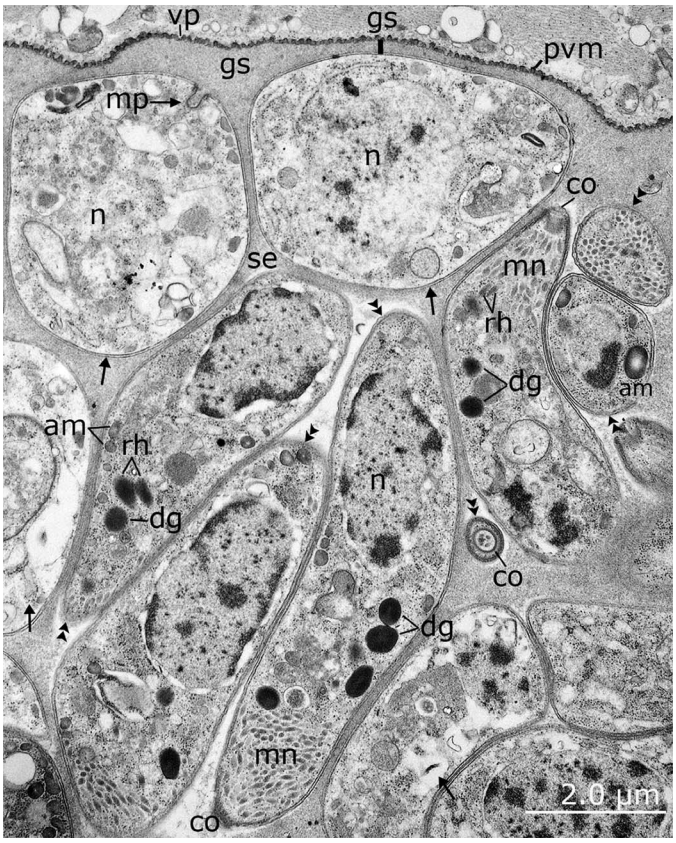

Figure 1. Transmission electron microscopy of *Sarcocystis jamaicensis* sarcocyst in semitendinosus muscle of knockout mouse 54 days post-inoculation. Note metrocytes (arrows), bradyzoites (double arrowheads), amylopectin granules (am), conoid (co), dense granules (dg), ground substance layer (gs), micronemes (mn), micropore (mp), nucleus (n), parasitophorous vacuolar membrane (pvm), rhoptries (rh), septum (se), and villar protrusion (vp).

pasty material was present from the esophagus to the intestines. The cause of death was not determined. The intestinal tract was removed, refrigerated at 4 C, and transported to the Zoonotic Protozoal Diseases Laboratory at the Virginia Maryland College of Veterinary Medicine, Blacksburg, Virginia. Sporocysts were collected separately from the intestinal scrapping of the RTH using methods described previously (Lindsay et al., 2017). Some of these sporocysts were used to attempt in vitro culture in African green monkey kidney (CV-1) cells using methods previously described (Lindsay et al., 2017). Excystation occurred, and schizonts were observed developing in CV-1 cells within 2 wk, but the culture died out and was discarded after 2 mo of observations.

Sporocysts were also sent to the Animal Parasitic Diseases Laboratory (APDL), U.S. Department of Agriculture, Beltsville, Maryland for further experimentation. At APDL, sporocysts were inoculated orally into 2 Swiss Webster (SW) mice (*Mus musculus*) and 2 IFN-γ gene KO mice. Complete necropsies were performed and portions of heart, lung, spleen, tongue, eye, brain, kidney, liver, intestine, muscles, and other organs were fixed in 10% neutral buffered formalin. Fixed tissue samples were cut into sections (2.5 × 0.7 cm), placed in cassettes, embedded in paraffin, and sectioned at 5 µm thick. Tissue sections were stained with hematoxylin and eosin and observed using light microscopy.

A piece of hind limb muscle from the KO mouse euthanized day 54 post-inoculation (PI) was fixed in glutaraldehyde and processed for transmission electron microscopy (TEM). Sections stained with uranyl acetate and lead citrate were examined and imaged at 80 kV with a Hitachi HT-7700 transmission electron microscope (Hitachi High Technologies America Inc., Dallas, Texas).

Both inoculated KO mice became ill and had evidence of neurological disease; both were dragging their rear limbs and were necropsied on day 38 and 54 PI. The SW mice remained asymptomatic and were also necropsied on day 54 PI. In both KO mice, the predominant lesion was meningoencephalitis associated with schizonts and merozoites as previously described (Verma et al., 2017). Additionally, there was mild hepatitis in both KO mice but protozoa were not identified. A few sarcocysts were found in limb muscles of both KO mice; they were thin-walled and identical to those described by Verma et al. (2017). A total of 9 sarcocysts (2 in the heart and 7 in limb muscles) were seen in the KO killed day 38 PI. Five sarcocysts were in sections of limb muscles of the KO mouse killed day 54 PI.

Four sarcocysts from the KO mouse examined day 54 PI were studied ultrastructurally; they were microscopic. The parasitophorous vacuolar membrane (pvm) of the sarcocyst wall was wavy, lined by a thin electron-dense layer (edl) 8.5 nm thick, and had villar protrusions (vp). The vp were stump-shaped, close together, distributed at uneven distances, varied in sizes, and appeared to have a stalk at the bottom and an expanded tip; they were up to 100 × 50 nm (Fig. 1). The vp lacked microtubules and electron dense granules. The pvm invaginated toward the sarcocyst interior. The ground substance layer (gs) beneath the pvm was smooth, up to 1 µm thick, and continued into the interior of the sarcocyst as septa (Fig. 2). All 4 sarcocysts appeared to be at the same stage of maturity and contained metrocytes and bradyzoites. Some metrocytes and bradyzoites were juxtaposed with gs; at these locations, the gs was only 200 nm thick. The gs was smooth and contained only a few electron-dense granules (Fig. 2).

By TEM, the metrocytes were electron-lucent, a mean size of 3.49 × 4.42 µm (range 3.7–5.0 × 2.2–4.0 µm, n = 7), and contained very few organelles. They had a large nucleus, a micropore, a long mitochondrion, a conoid, and few amylopectin granules. Several metrocytes were dividing by endodyogeny. The bradyzoites had a mean size of 2.01 × 7.57 µm (range 6.4–9.0 × 1.7–2.2 µm, n = 7) and contained a conoid, numerous haphazardly arranged micronemes, 2 rhoptries, several dense granules, a long mitochondrion, and a subterminal nucleus.

The natural intermediate host for *S. jamaicensis* is unknown. The description of sarcocyst provided here should facilitate the search for a missing part of the life cycle of *S. jamaicensis* when putative hosts are encountered.

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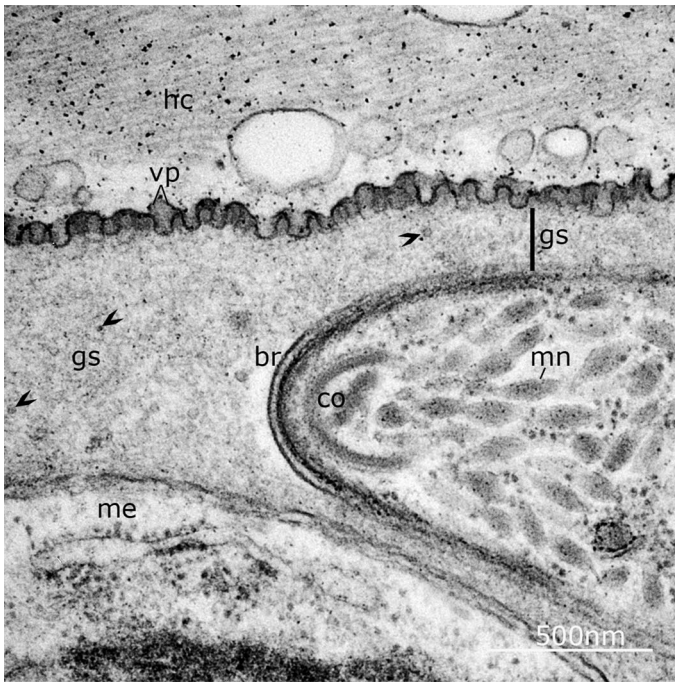

Figure 2. Details of the sarcocyst wall of *Sarcocystis jamaicensis* from the same knockout KO mouse as in Figure 1. The sarcocyst has an undulating surface with short villar protrusions (vp) and smooth ground substance layer (gs) with few granules (arrowheads). A bradyzoite (br) and a metrocyte (me) are juxtaposed with gs. Note the conoid (co) and numerous micronemes (mn) in the bradyzoite.

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