The South American opossum, *Didelphis marsupialis*, from Brazil as another definitive host for *Sarcocystis speeri* Dubey and Lindsay, 1999

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**Summary**

The North American opossum, *Didelphis virginiana*, is a definitive host for at least 3 species of *Sarcocystis*: *S. falcatula* Stiles 1983, *S. neurona* Dubey, Davis, Speer, Bowman, de Lahunta, Granstrom, Topper, Hamir, Cummings, Suter 1991, and *S. speeri* Dubey and Lindsay 1999. In order to identify species of *Sarcocystis* in the South American opossum, *D. marsupialis*, *Sarcocystis* sporocysts from the intestines of a naturally infected opossum (*D. marsupialis*) from Brazil were fed to 4 gamma-interferon knockout (KO) mice, a nude mouse, and 2 budgerigars (*Melopsittacus undulatus*). All 4 KO mice became ill and 1 died 42 days post-feeding (p.f.) of sporocysts, 1 was killed 44 days p.f. because of neurological signs, and 2 were killed 52 and 53 days p.f. because of abnormal gaits. Numerous sarcocysts were seen in the skeletal muscles of all 4 KO mice and they were structurally identical to *S. speeri* seen in KO mice fed sporocysts from *D. virginiana* from the United States and *D. albiventris* from Argentina. The nude mouse was killed 41 days p.f. because it appeared weak; schizonts were seen in sections of its liver and sarcocysts were seen in sections of skeletal muscles. *Sarcocystis speeri* was cultured in bovine turbinate cells inoculated with liver homogenate from this mouse. *Sarcocystis neurona* was not demonstrable in tissues of mice. The two budgerigars remained asymptomatic and *S. falcatula* was not found in their tissues when they were killed 29 days p.i. This is the first report of *S. speeri* from *D. marsupialis*.

Key words: *Sarcocystis speeri*, sporocysts, schizonts, *Didelphis marsupialis*, opossum.

**Introduction**

The North American opossum (*Didelphis virginiana*) is a definitive host for at least 3 pathogenic species of *Sarcocystis*: *S. falcatula* (Box, Meier & Smith, 1984), *S. neurona* (Fenger et al. 1997; Dubey & Lindsay, 1998), and *S. speeri* (Dubey & Lindsay, 1999). The sporocysts of these 3 species are similar morphologically, but can be distinguished by their pathogenicity and infectivity to birds and immunodeficient mice, e.g. *S. falcatula* is not infective for mice, whereas *S. neurona* and *S. speeri* are not infectious for birds and *S. falcatula* is infective to birds (Marsh et al. 1997; Dubey & Lindsay, 1998; Dubey, Speer & Lindsay, 1998). Both *S. neurona* and *S. speeri* can induce encephalitis in mice associated with schizonts and merozoites (Dubey & Lindsay, 1999). Sarcocysts of *S. neurona* have not been detected in mice to date. However, sarcocysts were seen in gamma interferon knockout (KO) mice fed sporocysts of *S. speeri* (Dubey et al. 1998; Dubey & Lindsay, 1999). Recently, *S. speeri* and *S. falcatula* were reported from the South American opossum, *D. albiventris*, from Argentina (Dubey et al. 1999b, 2000a–d). We report *D. marsupialis* from Brazil as another definitive host for *S. speeri*.

**Materials and Methods**

*Sarcocystis* sporocysts were obtained from an adult *D. marsupialis* submitted to the University of São Paulo, Brazil as part of a study of the *Sarcocystis* species observed in opossums in South America. The intestine was removed, the epithelium scraped from the small intestine in to a blender, homogenized and digested in 10% commercial bleach solution for...
Table 1. *Sarcocystis speeri* infections in mice fed sporocysts from an opossum, *Didelphis marsupialis*

<table>
<thead>
<tr>
<th>Mouse type</th>
<th>Day p.i.*</th>
<th>Schizonts†</th>
<th>Sarcocysts†</th>
<th>Bradyzoites‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>KO 5880</td>
<td>Died 42</td>
<td>Brain</td>
<td>SKM, H, T</td>
<td>Not done</td>
</tr>
<tr>
<td>KO 5881</td>
<td>Killed 53</td>
<td>Not seen</td>
<td>SKM, H, T, O</td>
<td>Yes</td>
</tr>
<tr>
<td>KO 1248</td>
<td>Killed 52</td>
<td>Brain</td>
<td>SKM, H, T, O</td>
<td>Yes</td>
</tr>
<tr>
<td>KO 1249</td>
<td>Killed 44</td>
<td>Brain</td>
<td>SKM, T, O</td>
<td>Not done</td>
</tr>
<tr>
<td>Nude 6540</td>
<td>Killed 41</td>
<td>Liver</td>
<td>SKM</td>
<td>Not done</td>
</tr>
<tr>
<td>C57/Black</td>
<td>Killed 61</td>
<td>Not done</td>
<td>Not done</td>
<td>Negative</td>
</tr>
<tr>
<td>C57/Black</td>
<td>Killed 61</td>
<td>Not done</td>
<td>Not done</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Day post-inoculation.
† Results based on examination of tissue sections. H, heart; SKM, skeletal muscle; O, ocular muscles; T, tongue.
‡ Results based on acid-pepsin digestion.

Fig. 1. For legend see opposite.
Sarcocystis speeri from Didelphis marsupialis from Brazil

Fig. 2. Sarcocysts in sections of skeletal muscle of a KO mouse 53 days post-feeding of sporocysts from the opossum from Brazil. Haematoxylin and eosin stain. (A) Numerous sarcocysts (arrows) and focal inflammation (arrowheads) in abdominal muscle. (B) Longitudinal section of a sarcocyst. Note thin cyst wall (arrows). (C) Higher magnification of a sarcocyst showing cyst wall with villar protrusions (arrowheads), metrocytes (large arrow), and bradyzoites (small arrows).

10 min. After centrifugations to remove the bleach, the suspension was filtered through a series of metallic sieves (final exclusion 400 µm) and the sporocysts and debris concentrated by centrifugation (×400 g). The resulting material was stored in Hanks balanced salt solution (HBSS) containing 10 mg/ml streptomycin, 10000 U/ml penicillin, 500 U/ml myostatin, and 0.5 mg/ml fungizone. Sporocysts were shipped via air from São Paulo, Brazil to Beltsville, MD, USA. Sporocysts were stored at 4 °C until used in the present studies. At Beltsville sporocysts were fed to 4 KO mice (BALB/c-lfngtm1Tv), 1 nude (C57Bl/6JFH11-Nu) mouse and 2 C57/Bl mice obtained from Jackson Laboratories (Bar Harbor, ME, USA). Two captive budgerigars (Melopsittacus undulatus) were each fed the same dose of sporocysts fed to mice as described (Dubey & Lindsay, 1998). The budgerigars were killed 29 days post-feeding (p.f.) sporocysts.

Animals that were killed or died were necropsied. Portions of all internal organs, were fixed in 10% buffered formalin and processed for histology. Paraffin-embedded sections were cut at 5 µm and examined after staining with haematoxylin and eosin. For immunohistochemical staining, paraffin sections were reacted with anti-S. neurona and anti-S. speeri antibodies prepared in rabbits as described (Dubey & Lindsay, 1998, 1999; Dubey et al. 1999a). Briefly, anti-S. neurona serum was derived from an isolate obtained from a naturally infected opossum no. 8095 (Dubey et al. 1999a). The serum was diluted 1:10000 and it
does not react with *S. speeri* schizonts and sarcocysts or with any other related apicomplexans (Dubey & Hamir, 2000). The anti-*S. speeri* serum was obtained using an isolate from a naturally infected opossum from Argentina (Dubey & Lindsay, 1999). It was diluted 1:5000 and it does not react with *S. neurona* schizonts.

The carcasses of KO mice killed 52 and 53 days p.f. and of C57/Bl mice killed 73 days p.f. were homogenized in a blender, digested in acid pepsin solution, and the digest examined microscopically for *Sarcocystis* bradyzoites (Dubey, Speer & Fayer, 1989).

The nude mouse was killed 41 days p.f. Attempts were made to cultivate *S. speeri* from the liver of this mouse. Liver homogenate was inoculated onto bovine turbinate cells (BT cells, ATCC CRL 1390, American Type Culture collection, Rockville, MD, USA) as described (Dubey et al. 2000b).

Portions of skeletal muscle from KO mouse 5881 (Table 1) were fixed in 3% (v/v) glutaraldehyde in phosphate buffer and later processed for ultrastructural examination at the Center for Molecular Medicine and Infectious Diseases, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, USA. Tissues were post-fixed in 1% (w/v) osmium tetroxide, dehydrated in a series of ethanols, passed through 2 changes of propylene oxide, and embedded in PolyBed 812 resin (Polysciences Inc., Warrington, PA, USA). Thin sections were stained with uranyl acetate and lead citrate and examined with a JOEL-
RESULTS

All 4 KO mice fed sporocysts became ill around 40 days p.f. One mouse died 42 days p.f., and 3 were killed 44, 52, 53 days p.f. because they had neurological signs or had difficulty walking (Table 1). Mice that died or were killed 42 and 52 days p.f. had severe encephalitis associated with numerous schizonts and merozoites. In the mouse killed 52 days p.f., there was a large lesion in the caudal lobe of the cerebrum with necrosis of the neuropil and marked gliosis (Fig. 1A). Myriads of Sarcocystis schizonts in all stages of development were seen (Fig. 1B–H). There were groups of young round to oval-shaped small schizonts (Fig. 1D, E) that resembled metacysts. These groups of schizonts in similar stages of development probably resulted from merozoites from parent schizonts without leaving the host cell. This phenomenon is typical of S. falcata, S. neurona and S. speeri asexual development in cell culture (Lindsay et al. 1999; Dubey et al. 1999b, 2000b).

Sarcocysts were seen in all 4 KO mice. These sarcocysts were thin walled and had characteristic villar protrusions of S. speeri (Fig. 2). Numerous sarcocysts were seen in skeletal muscle including tongue, diaphragm, abdomen and ocular muscles. A few sarcocysts were seen in the myocardium. Bradyzoites were seen in pepsin digests of muscles of mice killed 52 and 53 days p.f. Sarcocystis zoites were not seen in muscle digests of the C57/Bl mice.

Schizonts in various stages of development were seen in cell culture by day 15 post-infection (p.i.). On day 28 p.i. several isolated groups of asexual development were seen. Occasionally, schizonts contained a residual body. The merozoites and schizonts were structurally similar to those of S. speeri cultured from the liver of D. albiventris from Argentina (Dubey et al. 2000).

Fourteen sarcocysts were examined using TEM. Metrocytes were the most abundant stage present in sarcocysts. The primary sarcocyst wall consisted of the parasitophorous vacuolar membrane and an underlying electron-dense layer. Villar protections came off the sarcocyst wall and contained only ground substance internally (Fig. 3). The ground substance was also present immediately below the primary sarcocyst wall and divided the sarcocyst into compartments. Bradyzoites were few in number but contained all the organelles typical of this stage.

Schizonts and merozoites in mice reacted positively with anti-S. speeri serum but not with anti-S. neurona serum. Neither schizonts nor sarcocysts were seen in tissues of the two budgerigars.

DISCUSSION

Schizonts observed in tissues of the KO and nude mice were structurally similar to those of S. speeri (Dubey & Lindsay, 1999). The results of the present study indicate the S. speeri occurs naturally in Brazil in D. marsupialis.

Sarcocystis neurona-induced equine protozoal encephalomyelitis (EPM) is a serious neurological disease of horses in North America, Brazil and Panama (Dubey et al. 1991; MacKay, 1997). The distribution of EPM closely parallels the range and distribution of the opossum, D. virginiana. Whether D. albiventris and D. marsupialis are also a host for S. neurona is unknown. One way to identify the presence of S. neurona in opossum faeces is by bioassay in gamma interferon knockout or nude mice (Dubey & Lindsay, 1998). The results of the present investigation indicate that D. marsupialis is another definitive host for S. speeri and should be considered when attempting to isolate S. neurona sporocysts from opossums in South America.

REFERENCES


