Development of the Ox–Cat Cycle of *Sarcocystis hirsuta*

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**ABSTRACT:** The ox–cat cycle of *Sarcocystis hirsuta* was studied by killing 18 calves between 7 and 110 days after inoculation (DAI) with sporocysts from cats. At 7 DAI, sporozoites were found free and within leukocytes in the lumen of arteries associated with mesenteric lymph nodes and in endothelial cells of mesenteric arteries. First-generation meronts were found 7–23 DAI in arteries associated with mesenteric lymph nodes and intestines. Meronts matured between 7 and 10 DAI. First-generation meronts were 37.2 × 22.3 μm and contained more than 100 merozoites, which measured 5.1 × 1.2 μm. Merozoites measuring 5.4 × 1.5 μm were found free in the peripheral blood of a calf 11 DAI. Second-generation meronts were found in capillaries of striated muscles and heart 15–23 DAI; meronts matured between 15 and 16 DAI. Second-generation meronts were 13.9 × 6.5 μm and contained three to 35 merozoites that measured 4 × 1.3 μm. Second-generation individual merozoites were seen within myofibers and in tissue macrophages in muscles 16–23 DAI. Sarcocysts formed between 25 and 75 DAI in striated muscles, but not in heart; esophagus was the most heavily infected organ. At 30 DAI, a sarcocyst contained two metrocytes. At 62 DAI, sarcocysts were up to 550 μm long and 35 μm wide and contained only metrocytes; the wall was up to 3 μm thick and cross-striated. Bradyzoites developed between 62 and 75 DAI. Mature sarcocysts were up to 800 μm long and the wall was up to 6 μm thick. Sarcocysts became infective for cats at 75 DAI. Cats shed sporocysts and oocysts 8–10 days after ingesting infected muscles.

Of the three species of *Sarcocystis* that occur in the ox (*S. cruzi* [*S. bovicanis*], *S. hirsuta* [*S. bovifelis*], and *S. hominis* [*S. bovihominis*]), developmental stages of only *S. cruzi* are known (for review see Levine and Ivens, 1981). In this report the development of *S. hirsuta* is described. Clinical signs and lesions will be reported separately.

**Materials and Methods**

**Inoculation of calves**

Sporocysts of *S. hirsuta* were obtained originally by feeding tongue and esophagus of a naturally infected cow from the Montana State University Agricultural Experiment Station to a specific-pathogen-free (SPF) cat. Sporocysts were collected from intestinal scrapings and stored in a balanced salt–antibiotic mixture (Dubey, 1980, 1981). Two experiments were performed.

In experiment 1, seven 8–12-week-old calves were killed 75, 88, 89, 104, 110, 170, and 220 days after oral inoculation (DAI) with 1 million, 1 million, 250,000, 1.4 million, 5,000, 5,000, and 5,000 sporocysts, respectively, and their muscles were fed to 22 SPF cats.

In experiment 2, 11 1–2-week-old calves were necropsied 7, 10, 15, 16, 20, 23, 30, 35, 42, 62, and 82 DAI with 15, 25, 1, 15, 25, 15, 15, 1, 1, 3, and 1 million sporocysts, respectively. Two 7-day-old calves served as uninoculated controls, and were killed 30 and 82 days after the initiation of the experiment.

Inoculated calves were housed separately from uninoculated calves for 7 DAI, and their excreta were incinerated to kill sporocysts that might have passed unexcysted in their feces. They were fed milk until 6–8 weeks of age and then were fed grain and hay. Calves in experiment 1 were housed outdoors and had access
to hay, grain, and pasture. Calves in experiment 2 were housed individually indoors.

**Examination for parasitemia**

In experiment 2, blood (7 ml) was drawn from each inoculated calf into vacuum tubes containing ethylenediaminetetraacetic acid twice weekly and on the day of necropsy. Smears of buffy coat were air dried, fixed with methanol, stained with Giemsa’s stain, and examined as described (Dubey, 1982a). Smears were considered negative when parasites were not seen in about 1 ml of blood.

**Necropsy of calves**

Calves were killed by electrocution, exsanguinated, and necropsied immediately. Portions of bone marrow, brain, spinal cord, eyes, pituitary, salivary and adrenal glands, thymus, lungs, heart, diaphragm, spleen, kidneys, liver, gallbladder, urinary bladder, omentum, rumen, reticulum, omasum, abomasum, small and large intestines, esophagus, skeletal muscle, lymph nodes (superficial cervical, mandibular, retropharyngeal, mediastinal, hepatic, gastric, mesenteric, subiliac), cerebrum, cerebellum, pons, medulla, and tongue were fixed in 10% Millonig’s buffered formalin (MBF). In two calves necropsied 7 and 10 DAI, sections of every ½ m of intestines were examined. Selected tissues were also fixed in Bouin’s fluid (BF), in Helly’s fixative (HF), or in 1% glutaraldehyde and 4% formaldehyde (GF) mixture.

Paraffin-embedded sections were cut at 5 μm. Selected tissues were embedded in glycol methacrylate and sectioned at 3 μm. Sections were stained with hematoxylin and eosin (HE), Heidenhain’s iron hematoxylin (IH), or periodic acid–Schiff’s hematoxylin (PASH).

**Inoculation of cats**

Cats used in this study were obtained from the SPF cat colony maintained in the Veterinary Research Laboratory, Montana State University, Bozeman, Montana. They were never fed raw meat until used in experiments. Cats were fed ground muscles of experimentally inoculated calves over a period of 1–7 days, and their feces were examined for sporocysts after sugar flotation (Dubey, 1976).

**Results**

**First-generation meronts**

At 7 DAI, sporozoites were found free and within leukocytes in the lumen of arteries at the periphery of mesenteric lymph nodes (Figs. 1–3). In HE-stained sections, sporozoites had a central nucleus with a very small nucleolus, a pink-staining area anterior to the nucleus, and a pale area posterior to the nucleus.

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**Figures 1–4.** 1. Cross section of an artery in mesentery of a calf, 7 DAI with 15 million *Sarcocystis hirsuta* sporocysts. Note sporozoites in a leukocyte close to the endothelial lining (arrowhead), in close approximation to endothelial lining (arrow), and within endothelial lining (arrowhead). Host cells containing meronts and sporozoites are enlarged and protruding into the arterial lumen. BF, PASH; 5 μm ×400. 2. Enlargement of the area marked in Figure 1. Note two sporozoites (arrowheads) and two
meronts (arrow, one is out of focus) in a single host cell in the left corner and several young meronts in the right corner. PAS-positive granules are larger in sporozoites than in meronts. ×1,000. 3. A sporozoite (arrow) in a leukocyte in mid-lumen of a mesenteric artery of calf in Figure 1. The leukocyte nucleus (arrowhead) is out of focus. Note subterminal nucleus of the sporozoite. BF, HE. ×1,000. 4. A 3-μm section of mesenteric artery of the calf in Figure 1. Note that the host cell nucleus (arrowhead) and a young meront (arrow) are enclosed within a membrane. In the adjoining cell there are three meronts. Note different-sized nucleoli. HF, IH. ×1,000.
Figures 5–9. 5–7. First-generation meronts in 3-μm sections of arteries in mesenteric lymph nodes of a calf, 10 DAI with 25 million sporocysts. ×1,000. 5. Immature meront (arrow) protruding into arterial lumen. Note thick covering, several prominent nucleoli, and dispersed chromatin. Another meront with differentiated nuclei is lying free in the arterial lumen. HF, IH. 6. Meront with differentiated nuclei and enlarged host-cell nucleus (arrow) covered by a thick capsule. BF, HE. 7. Mature meront with merozoites arranged randomly and in a row at the periphery. BF, HE. 8, 9. Merozoites (arrows) in smear of blood from the jugular vein of a calf, 11 DAI with 15 million sporocysts. Methanol, Giemsa’s stain. ×1,000. Note three nuclei in Figure 9.

(Fig. 3). In PASH-stained sections, a large PAS-positive area and several small granules were found in sporozoites (Fig. 3). Sporozoites appeared to be within endothelial cells, and some lay across the length of the host cell. Six longitudinally cut sporozoites were 5.6 × 2.5 μm (5–7 × 2–3). The next stage was a round to ovoid, uninucleate meront with a central nucleus (Figs. 2, 4). The nucleolus in young meronts was larger than that in the sporozoite, and PAS-positive granules
Figures 10–17. Second-generation meronts and merozoites in 3-μm sections of heart of a calf, 16 DAI with 15 million sporocysts. All meronts are located in capillaries. ×1,000. 10, 16, 17, HF, HE; 11–15, BF, IH. 10. Uninucleate meront (arrow). Note prominent nucleolus. 11. Immature meront with undifferentiated nucleus or nuclei. 12. Immature meront with differentiating nuclei. 13. Multinucleated meront. 14. Cross section of a mature meront. Both ends of merozoites have unstained areas, thus the vacuolated appearance of the meront. 15. Mature meront with longitudinally sectioned merozoites. 16. Ruptured meront with spilled merozoites. The capillary lumen has not yet collapsed. 17. One extracellular merozoite (arrow) and two merozoites (arrowheads) within a tissue macrophage.

decreased with the development of meronts (Fig. 2). Meronts were 9.5 × 5.8 μm (4–14 × 3.5–7; N = 16) and contained up to five nucleoli. It was difficult to determine whether these were separate nucleoli or part of a single nucleus. Infected host cells were hypertrophied and meronts were seen both below and above the host-cell nucleus. One host cell contained two meronts and two sporozoites. Some arteries were heavily parasitized, whereas most of them were not infected.

At 10 DAI, meronts were in arteries within mesenteric lymph nodes, mesentery, and intestines. In the small intestine, the middle part was the most heavily infected; meronts were not seen in the first and the last meter segments. More meronts were in intestinal arteries than in arteries in the mesentery. The host covering (capsule) was thicker in immature meronts than in mature meronts (Figs. 5–7). Chromatin in the undifferentiated nucleus of meronts was dispersed and granular, and it condensed as nuclei became differentiated. Nuclei in meronts were sometimes arranged in groups. The number of nuclei in meronts was difficult to count, but appeared to be more than 100. Meronts with undifferentiated nuclei were 22.7 × 12.1 μm (10–35 × 3–18; N = 10) and with differentiated nuclei were 37.2 × 22.3 μm (28–56 × 17–40; N = 30). Merozoites were arranged peripherally
as well as randomly (Fig. 7). Several immature and mature meronts, and merozoites, were free in the arterial lumen. Merozoites in sections were $5.1 \times 1.2 \mu m$ ($5-6.5 \times 1-1.5; N = 9$) and in smears were $6.3 \times 1.4 \mu m$ ($5.5-7 \times 1-1.5; N = 12$). Mature meronts were PAS-negative, and there was no residual body.

At 15, 16, 20, and 23 DAI, very few meronts were seen, and these were in arteries associated with mesenteric lymph nodes.

**Parasitemia**

Merozoites were found in the peripheral blood of a calf at 11 DAI with 15 million sporocysts. All 15 merozoites seen were free in plasma. Merozoites were $5.4 \times 1.5 \mu m$ ($5-6 \times 1-2; N = 13$) and contained a subterminal nucleus (Fig. 8). One merozoite had three nuclear lobes or three separate nuclei (Fig. 9).

**Second-generation meronts**

Second-generation meronts were found in capillaries of heart, thigh, diaphragm, tongue, and eye 15–23 DAI; most of them were in the heart. The youngest uninucleate meront was $5 \times 3.5 \mu m$. In immature meronts, the chromatin was dispersed and there were one to four nucleoli either in separate nuclei or in lobes of a single nucleus. Meronts with differentiated nuclei were $13.9 \times 6.5 \mu m$ ($7-45 \times 3-11; N = 55$) and the number of nuclei was 12.4 (3–35; $N = 55$). Merozoites in meronts were loosely arranged, with spaces between them. Meronts were PAS-negative. Ten longitudinally cut merozoites averaged $4 \times 1.5 \mu m$; most of them were $3 \times 1.5 \mu m$. Merozoites had a vesicular central nucleus and two clear areas at both ends in sections stained with hematoxylin; thus, meronts appeared vacuolated (Fig. 14).

**Individual merozoites in tissues**

Individual merozoites outside of meronts were seen 16–23 DAI in muscles where second-generation meronts developed. Some merozoites appeared to be within myofibers. One to three merozoites were seen in tissue macrophages, especially around blood vessels (Fig. 17). Intraleukocytic merozoites had a vesicular nucleus and were structurally similar to second-generation meronts. One merozoite appeared to have two nuclei. Nuclei in extracellular merozoites were often pyknotic.

**Sarcocysts**

The esophagus was the most heavily infected organ, followed by tongue, eye muscles, and muscles of legs. Sarcocysts were not found in the heart. At 30 and 35 DAI, single sarcocysts ($7 \times 5 \mu m$) containing two metrocytes were found in sections of skeletal muscle (Fig. 18). At 42 DAI, one sarcocyst measuring $90 \times 14 \mu m$ was found in a section of tongue; its wall was thin and contained only metrocytes. At 62 DAI, sarcocysts were up to $550\ \mu m$ long and $35\ \mu m$ wide (Fig. 19); the wall was $1.5-3.0\ \mu m$ thick and cross-striated. In sarcocysts at 62 DAI, only metrocytes were seen, which were about $5 \times 3.5\ \mu m$ in sections. At 82 and 89 DAI, the sarcocyst wall was $3-6\ \mu m$ thick and both bradyzoites and metrocytes were seen. At 105 and 110 DAI, sarcocysts were up to $800\ \mu m$ long and $60\ \mu m$ wide; the sarcocyst wall was up to $6\ \mu m$ thick and contained mostly bradyzoites.

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Figures 18–21.  18. Sarcocyst in 3-μm section of thigh muscle of calf 595, 30 DAI with 15 million sporocysts. BF, HE. ×1,000. 19. Immature sarcocyst in 5-μm section of tongue of a calf, 62 DAI with 3 million sporocysts. HF, HE. ×100. 20. Sarcocyst in 3-μm section of esophagus of a calf 110 DAI with 5,000 sporocysts. Note cross-striated PAS-negative wall and hundreds of PAS-positive bradyzoites. BF. ×1,000. 21. A 3-μm section of small intestine of a cat, 12 days after it was fed meat infected with Sarcoctis hirsuta. Note two sporocysts in the lamina propria. Arrowheads point to sporozoites. BF, HE. ×1,000.
Bradyzoites in sections were about $13 \times 3 \ \mu m$ and contained several large PAS-positive granules; the sarcocyst wall was PAS-negative (Fig. 20).

Both thin-walled sarcocysts ($S.\ cruzi$) and thick-walled ($S.\ hirsuta$) sarcocysts were found in calves of the first experiment. Only thick-walled sarcocysts were found in the calves of the second experiment.

**Gametogony**

Sarcocysts were infectious to cats at 75 DAI. Cats shed sporocysts 8–10 days after ingesting infected meat. The prepatent period was 7 days in two cats, 8 days in eight cats, and 9 days in 10 cats. The two cats fed heart of calves killed 75 and 88 DAI did not shed sporocysts, whereas those fed other muscles from the same calf did. Oocysts were located in the lamina propria and in the villar epithelial tips of small intestine of cats (Fig. 21). In intestinal scrapings of cats, sporulated oocysts were $17.1 \times 12.7 \ \mu m$ ($16-18 \times 11-14; \ N = 10$). They contained sporocysts measuring $12.8 \times 8.4 \ \mu m$ ($11-14 \times 7-9; \ N = 20$), each with four elongate sporozoites. A Stieda body was absent. The sporocystic residuum varied from being compact to being a few scattered granules. Living sporozoites were $8.2 \times 1.9 \ \mu m$ ($7.5-9 \times 1.5-2; \ N = 10$), with anterior pointed end. In sections of sporozoites, the nucleus was subterminal and there were several PAS-positive granules (Fig. 21).

**Control calves**

No parasites were found in the calf killed 30 DAI. A few immature sarcocysts were found in the other calf killed 82 DAI, probably acquired from the $S.\ hirsuta$-infected calf housed next to it.

**Discussion**

This is the first study of the intravascular development of $S.\ hirsuta$. After the sporocysts excyst in the gut of an ox, the sporozoites reach the mesenteric arteries by an unknown route. The mechanism of transport and penetration of endothelial lining of mesenteric arteries is unknown. It is probable that sporozoites are carried in the leukocytes to the site of their development within the endothelial lining. The PAS reaction proved useful in tracing the development of sporozoites into meronts, because PAS-positive granules found in sporozoites disappeared during the development of first-generation meronts. The type of host cell parasitized was not determined. It is suspected that merogony occurs in host cells between endothelium and tunica intima.

Parasitemia in $S.\ hirsuta$ infection was transient and of low degree. The size of merozoites and the timing indicate that parasitemia was due to first-generation merozoites.

The occurrence of second-generation meronts only in muscles is unusual when compared to occurrences of the other species of $Sarcocystis$ in domestic animals (for review see Levine and Ivens, 1981). The occurrence of merozoites in macrophages in muscles suggests that the second-generation merozoites are transported locally from capillaries to myofibers; this might explain why second-generation merozoites were not found in peripheral blood.

Sarcocysts formed between 25 and 30 DAI. At 62 DAI, the sarcocyst wall was thick and cross-striated. Photographs published by Boch et al. (1978) indicate
Table 1. Comparison of developmental stages of *S. cruzi* and *S. hirsuta*.

<table>
<thead>
<tr>
<th>First-generation meronts</th>
<th><em>S. cruzi</em> (ox-coyote)</th>
<th><em>S. hirsuta</em> (ox-cat)</th>
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<tbody>
<tr>
<td>Location</td>
<td>Several organs</td>
<td>Mesenteric and intestinal arteries</td>
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<tr>
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<td>7–26</td>
<td>7–23</td>
</tr>
<tr>
<td>Peak development (DAI)</td>
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<td>10</td>
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<tr>
<td>Size of meronts (µm)</td>
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<td>&gt;100</td>
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<tr>
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<td>5.1 × 1.2</td>
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<td>Striated muscles, heart</td>
</tr>
<tr>
<td>Duration (DAI)</td>
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<td>15–23</td>
</tr>
<tr>
<td>Peak development (DAI)</td>
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<td>3–35</td>
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<tr>
<td>Wall (µm)</td>
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<tr>
<td>Heart</td>
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<tr>
<td>Striated muscles</td>
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<td>Yes</td>
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<tr>
<td>Smooth muscles</td>
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that the wall becomes cross-striated between 50 and 61 DAI; no other details were given. Gestrich et al. (1975) described the ultrastructure of *S. hirsuta* sarcocysts in two calves killed 98 and 160 DAI. At 98 DAI, sarcocysts contained both metrocytes and bradyzoites, but only bradyzoites were present at 160 DAI.

Cats shed sporocysts 8–10 days after ingesting meat infected with *S. hirsuta*. The number of sporocysts shed was much smaller than that shed by canines ingesting *S. cruzi* (Fayer, 1977; Dubey, 1980), suggesting that domestic cats are not a very good host of *S. hirsuta*. These observations in experimental infections are similar to those in cats fed beef naturally infected with *S. hirsuta* (Dubey and Streitel, 1976). Gametogony of *S. hirsuta* in the present study was similar to that reported by Heydorn and Rommel (1972). The prepatent period and the morphology of sporocysts and sarcocysts indicate that the parasite studied in Germany by Heydorn et al. (1975) was the same as in the present study.

Newborn calves were used in experiment 2 of this study in order to minimize natural infections. Weaned calves in experiment 1 housed outdoors became infected with *S. cruzi*. Natural infections with *S. cruzi* were not found in control calves used in the present study and in 10 other newborn calves killed in other projects on sarcocystosis and toxoplasmosis in our laboratory.

The development of *S. hirsuta* in newborn calves is compared in Table 1 with the development of the ox–coyote cycle of *S. cruzi* studied under identical con-
ditions in this laboratory (Dubey, 1982b). Differences and similarities are apparent. One striking difference is the absence of second-generation meronts of *S. hirsuta* in kidneys, whereas those of *S. cruzi* occur predominantly in renal cortex. Also, sarcocysts of *S. hirsuta* were not found in heart, whereas those of *S. cruzi* occur predominantly in heart.

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Literature Cited


