Sarcocystis spp. and Sarcocystosis

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Abstract

Sarcocystis species are apicomplexan protozoans that have 2 host life cycles. The muscular sarcocyst occurs in the intermediate host. The parasite develops in a parasitophorous vacuole in the myocyte. The structure of the sarcocyst is used in identification. The sarcocyst is infectious for the definitive host when eaten. Sporocysts are excreted by the definitive host.

Key words: sarcocyst, protozoan, myocyte, bradyzoite, life cycle.

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Sarcocystis spp. are members of the protozoan phylum Apicomplexa. The phylum contains the coccidial and malarial parasites of man and animals. The phylum obtains its name from the assemblage of organelles that are present in the anterior end of invasive stages and collectively form the apical complex [7]. The apical complex is involved in the entrance of parasites into host cells.

All *Sarcocystis* spp. have obligatory heteroxenous (2-host) life cycles. *Sarcocystis* was first observed in 1843 by F. Miescher in a house mouse [10]. The muscles of the mouse contained "milky white threads" which came to be known as Miescher's tubules. Later a species was found in the pig and named *Synchytrium miescherianum* by Kühn in 1865. The name was subsequently changed to *Sarcocystis miescheriana* by Labbé in 1899. Therefore the correct type species is *Sarcocystis miescheriana* (Kühn, 1865) Labbé 1899. It was not until the early 1970's that the two-host life cycle was described [15, 29, 30].

Sarcocystis Species Life Cycle

The *Sarcocystis* life cycle requires two hosts. Carnivores or omnivores are usually the definitive host while omnivores and herbivores are usually intermediate hosts. Certain species of lizards can serve as both the definitive and intermediate host for the same *Sarcocystis* sp. [23]. The intermediate host becomes infected by ingestion of sporocysts (resistant stages) from the environment. Sporocysts contain 4 infective sporozoites. The sporozoites excyst from the sporocysts in the intestinal tract. The sporozoites leave the intestinal tract and undergo first-generation merogony in endothelial cells of arteries, usually in mesenteric lymph nodes [10]. A second generation of merogony occurs in capillaries or small arteries in many tissues throughout the body. The meronts are usually most numerous in glomeruli of the kidneys. The merozoites from the last generation are released into the circulation and are occasionally found intracellularly in unidentified mononuclear cells [10]. Limited multiplication may occur at this stage of infection. Eventually these merozoites will penetrate cardiac and striated muscle cells and develop into the sarcocyst stage which contains bradyzoites (Figures 1-4). Occasionally, sarcocysts are observed in brains of infected animals. The first- and second-generation meronts develop directly in the host cell cytoplasm, whereas the bradyzoites develop within the myocyte in a parasitophorous vacuole.

The structure of the sarcocyst is used in identification of *Sarcocystis* species. The developing sarcocyst contains a stage called a metrocyte that divides by endodyogeny (binary fission) to produce bradyzoites. Metrocytes are not infectious for definitive hosts. A mature sarcocyst may contain thousands of bradyzoites and may be grossly visible. The presence of grossly visible sarcocysts of *S. gigantea* in sheep and *S. hirsuta* in cattle is a cause for condemnation of the carcass [9, 10]. Carcass condemnation results in economic losses for ranchers. The structure of the sarcocyst wall is usually characteristic for a species within a host (see below).

When sarcocysts containing bradyzoites are ingested by an appropriate definitive host, the bradyzoites are liberated from the sarcocyst and penetrate cells of the small intestine. Goblet cells are most often parasitized and, as development proceeds, the cells containing the macrogamete stages (female gamete) undergo lysis and enter the lamina propria where further development and maturation occurs. The microgametes (male gametes) usually remain in goblet

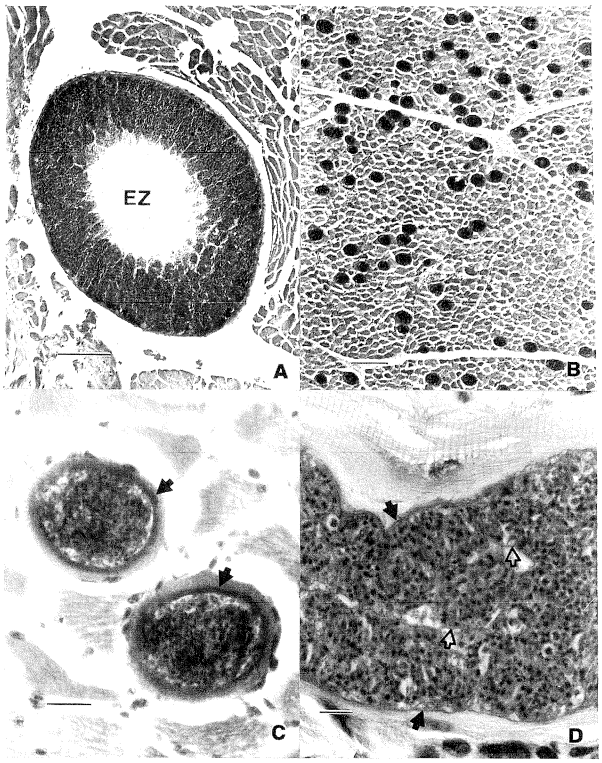

Figure 1. Photomicrographs of sarcocysts. A, Section through a grossly visible sarcocyst of a Sarcocystis *species in the breast muscles of a naturally infected parrot. Note the empty central zone (EZ), Bar = 100 μm. B, Numerous sarcocysts of* Sarcocystis falcatula *in the breast muscle of a naturally infected cockatiel, Bar = 100 μm. C, Two sarcocysts of* Sarcocystis falcatula *demonstrating thick sarcocyst walls (arrows), Bar = 25 μm. D, Thin-walled (arrows) sarcocyst of* Sarcocystis montanaensis *in the tongue of an experimentally infected prairie vole. Note the septa (open arrows), Bar = 10 μm.*

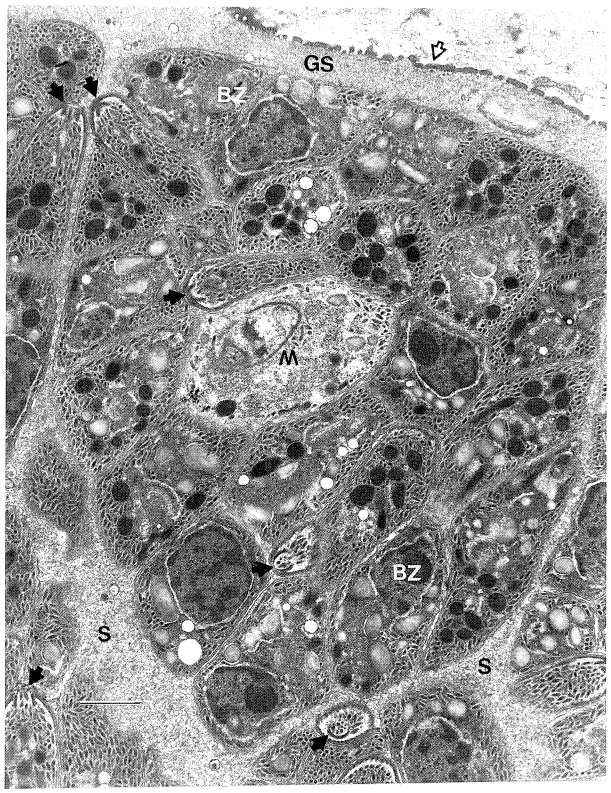

Figure 2. Transmission electron micrograph of a portion of a sarcocyst of Sarcocystis montanaensis *in the tongue of an experimentally infected prairie vole. Note the ground substance (GS) that is under the primary cyst wall (open arrow) and that the ground substance gives rise to the septa (S) in the internal portion of the sarcocyst. The conoids (arrows) of several bradyzoites (BZ) are apparent, as is a single metrocyte (M) in the center of this group of bradyzoites, bar = 1.0 μm.*

cells during development. Eventually, fertilized macrogametes sporulate in the lamina propria to produce an oocyst that contains 2 sporocysts. The oocyst wall usually ruptures as the sporocysts exit the lamina propria in to the lumen of the intestinal tract where they are eventually voided in the feces as single sporocysts.

Identification of *Sarcocystis* sp.

The structure of the sarcocyst wall is the most important feature used in species identification (Figures 1-3). Light microscopic examination can be used to distinguish some species within an intermediate host, although examination with transmission electron microscopy (TEM) is often necessary (Figures 2, 3). New species should be described based on the ultrastructure of the sarcocyst and transmission studies, if possible. Dubey et al. [10] described the features of sarcocysts from various animals using TEM and determined that 24 basic types were present. The sarcocyst wall may be relatively thin and simple consisting of only minor modifications of the parasitophorous vacuole membrane to form the primary cyst wall (PCW) (Figures 2, 3). However, the PCW of some species is highly complex and may contain specific modifications such as branches, folding, villar-like protrusions (Figure 3), mushroom-like protrusions, or other modifications. The modifications may contain microtubules, microfilaments, or electron dense bodies or granules. The structure of the PCW of these complex type sarcocysts usually changes with the age of the sarcocyst. The PCW is immediately under layed by

electron-dense ground substance. The ground substance produces septa that divide the sarcocysts into compartments that are composed of groups of bradyzoites and occasionally metrocytes (Figures 1, 2). Only a few *Sarcocystis* species do not have true septa (i.e., *S. arieticanis*) but the parasites still appear to be in compartments. A secondary cyst wall is rarely present (i.e. *S. gigantea* of sheep) and is composed of fibrillar material of host cell origin that encloses the parasitized myocyte [25].

Biology of sarcocysts

Sarcocysts have been observed in smooth and striated myocytes, Purkinje fibers of the heart, and in neural cells in the brain. The most common location is in striated muscle fibers and there appears to be no preference for type I or type II fibers [28]. Degenerative changes may be observed in myocytes after merozoite invasion but the parasites quickly adapt to the myocyte and little alteration is usually observed in infected myocytes due to the presence of intact mature sarcocysts [27].

The gross, light, and ultrastructural appearance of a sarcocyst can vary with age and location. The macroscopic sarcocysts of *S. gigantea* of sheep are elongate when in the diaphragm, while those in the esophagus are globular. Some species of *Sarcocystis* avoid certain muscle-organ types. For example, *S. muris* of the mouse, *S. gigantea* of sheep, and *S. hirsuta* of cattle do not develop in the heart.

***Sarcocystis* look-alikes**

Muscular tissue cysts of *Toxoplasma gondii* and *Hammondia hammondi* may resemble sarcocysts but have thin tissue cyst walls and never have septa or a secondary cyst wall [24]. Groups of tachyzoites of *Neospora caninum* can be observed myocytes but they have no true cyst wall and no septa. The cysts of *Besnoitia* sp. may be seen in muscle tissues but are in connective tissue cells and not myocytes. The nuclei of *Besnoitia* infected host cells are hyperplastic and hypertrophic. *Besnoitia* cysts do not have septa.

Pathogenesis and immunity

Most *Sarcocystis* infections in naturally infected animals are subclinical. In general, dog transmitted species tend to be more pathogenic than cat transmitted species. The precystic stages generally produce the clinical signs and lesions associated with sarcocystosis. Lesions are dependent on the numbers of sporocysts ingested and are associated with tissue necrosis, damage to the vascular endothelium, and the cascade of events that follows.

Hemorrhage can be observed on skeletal muscles and heart in acute infections. Microscopic lesions consist of vasculitis, hemorrhage and necrosis of myocytes associated with penetration of myocytes by merozoites. Sarcocysts may undergo degeneration in which case they may be encircled by mononuclear cells, neutrophils, eosinophils, and occasionally giant cells (Figure 4). However, it is not unusual to observe sarcocysts with no associated inflammatory response.

Animals develop immunity to *Sarcocystis* infections. Immunity is species specific, and vaccination with one species does not protect against heterologous challenge. Cell mediated immunity is probably more important than humoral immunity. Sporozoites and merozoites probably are more immunogenic than are bradyzoites.

The presence of sarcocysts is not necessary for the maintenance of protective immunity.

Sarcocystis infections can result in immunosuppression [17]. Both antibody and cell mediated immune responses to unrelated antigens are suppressed during primary infection but cell mediated immune responses are affected more profoundly. Preexisting immunity is not affected by *Sarcocystis* infection.

Muscular *Sarcocystis* infections in humans and other primates

Reported cases of muscular *Sarcocystis* infection in humans have come from individuals from India and Southeast Asia, with fewer reports in humans from Central and South America, Europe, Africa, China and the United States of America [10]. *Sarcocystis lindemanni* was the name originally given for the parasite in human muscle but its validity is questionable. Seven distinct structural types of sarcocysts have been observed in human tissues. Several are similar to those observed in other primates [3]. In one case the sarcocysts were macroscopic in size. Sarcocysts have been observed in skeletal muscles and occasionally heart. Lesions generally are not associated with the presence of the sarcocysts [3].

Muscular *Sarcocystis* infection in nonhuman primates is common [20, 26]. One survey reported sarcocysts in sections of muscle from 79 (21%) of 375 wild-caught Old and New World monkeys, although the prevalence is probably higher. About 4 structural types of cysts have been described. Two species *S. kortei* (thick-walled) and *S. nesbitti* (thin-walled) have been named. Myocarditis and myositis have been reported in infected monkeys but its association with *Sarcocystis* infection is unclear.

Muscular sarcocystis infections in cats and dogs

Sarcocysts of *Sarcocystis* species have been found in a number of felids including domestic cats [13, 14, 15, 16, 21] and wild felids [1, 8, 11, 18, 22]. The ultrastructural features of sarcocysts from different feline hosts are indistinguishable. Dubey et al. [11] gave the name *Sarcocystis felis* to the parasite. Identification is based on observing the sarcocysts in muscle tissue. Sarcocysts have been observed in heart, tongue, masseter, esophagus, diaphragm and biceps femoris. In muscle squash preparations, sarcocysts are up to 1 cm in length [18]. In tissue sections, sarcocysts can approach 2,000 µm, are septate, and have regularly spaced, finger-like projections from the tissue cyst wall. No inflammatory response is associated with the tissue cysts. Definitive identification of *S. felis* is based on observation of the ultrastructural features of the tissue cyst wall.

Muscular *Sarcocystis* infection in dogs is apparently less common than in cats [5, 19, 31]. The sarcocysts have been

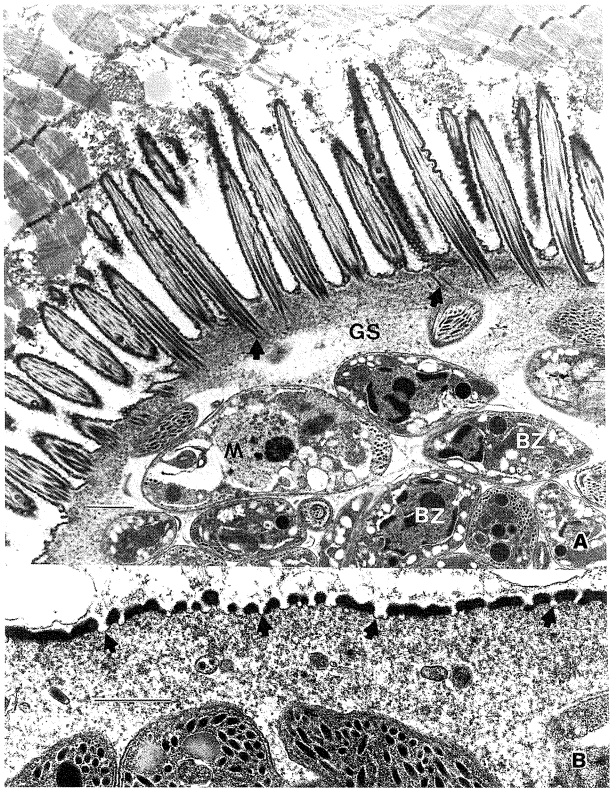

Figure 3. Transmission electron micrographs of sarcocyst walls. A, Sarcocystis falcatula *sarcocyst (Type 11 classification) demonstrating villar projections that contain microtubules (arrows). Note the ground substance (GS) below the sarcocyst wall, the metrocyte (M), and the bradyzoites (BZ), Bar = 1.0 μm. B,* Sarcocystis montanaensis *sarcocyst (Type 1 classification) demonstrating electron dense knob-like projections on the primary sarcocyst wall. Note that the wall is composed only of the parasitophorous vacuole membrane at the base (arrows), Bar = 0.5 μm.*

observed in esophagus, heart, and biceps femoralis. No inflammatory response is associated with these sarcocysts. The ultrastructure of the sarcocyst appears similar to that observed in cats [5] but few sarcocysts have been studied.

Muscular *Sarcocystis* infections in domestic animals

Clinically, abortion is the most important disease manifestation of *Sarcocystis* infection in domestic animals [10].

The presence of macroscopic sarcocysts and the subsequent condemnation of sheep and cattle carcasses is also an economic consideration of sarcocystosis. The potential transmission of *S. suihominis* from pigs to humans and *S. hominis* from cattle to humans is also a potential public

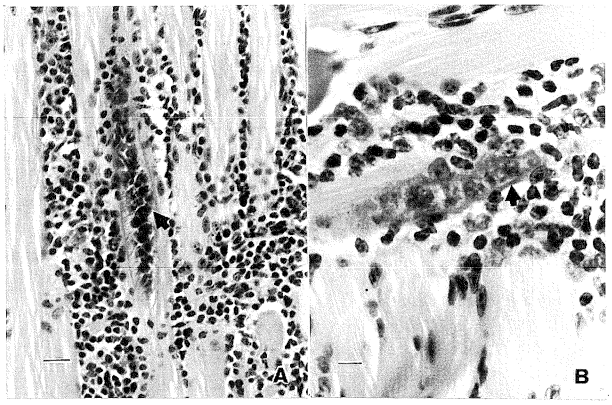

Figure 4. Sarcocystis cruzi *in the tongue of an experimentally infected calf. A, Mononuclear cell infiltrate in myocytes and adjacent to a sarcocyst (arrow), Bar = 25 μm. B, Mononuclear cells infiltrating a sarcocyst (arrow), Bar = 10 μm.*

health concern affecting pork and beef producers in countries where these parasites are present.

Cattle are intermediate hosts for 3 species of *Sarcocystis*. *Sarcocystis cruzi* is a potential bovine pathogen and is the most prevalent bovine species. *Sarcocystis hirsuta* and *S. hominis* also use cattle as intermediate hosts but are only slightly pathogenic.

Domestic sheep harbor 4 species of *Sarcocystis*. *Sarcocystis tenella* is the most prevalent and most pathogenic. *Sarcocystis arieticanis*, *S. gigantea* and *S. medusiformis* are also observed in sheep. *Sarcocystis medusiformis* is a macroscopic, cat-transmitted species that has been observed only in Australia and New Zealand.

Domestic goats are intermediate hosts for 3 species of *Sarcocystis*. *Sarcocystis capracanis* is the most prevalent and pathogenic. *Sarcocystis hircicanis* and the macroscopic *S. moulei* are relatively nonpathogenic and less prevalent.

Pigs are intermediate hosts for 2 or possibly 3 species of *Sarcocystis*. *Sarcocystis miescheriana* is the most prevalent and most pathogenic. *Sarcocystis suihominis* is less prevalent and less pathogenic. *Sarcocystis porcifelis* has been reported to have a porcine-feline life cycle but little else is known about it. This species has only been observed in former Soviet Union.

The number and identity of valid *Sarcocystis* species in horses, donkeys, and zebras are not known with certainty. Four species have been named and all are transmitted by dogs. They are *S. asinus*, *S. bertrami*, *S. equicanis*, and *S. fayeri*. Both thin-walled and thick-walled types of sarcocysts have been found.

Sarcocystis infection and eosinophilic myositis

Eosinophilic myositis (EM) occurs most frequently in cattle and less often in sheep, pigs, and horses [10]. It is a specific inflammatory condition due to the accumulation

of eosinophils, and can be found in almost any striated muscle. Eosinophilic myositis appears as 2 distinct types of lesions. The first is seen on the surface of muscles and usually has a greenish appearance and can be up to 15 mm long and 3 mm wide. The second is up to 15 cm long, firm, and bright green to pale yellow. Sarcocysts have been found in animals with the first type of EM lesion leading to the postulation that they are associated with EM. However, EM has not been reproduced in animals under experimental conditions. In addition, EM occurs much less frequently than does *Sarcocystis* infection. Presently, the connection between EM and the presence of sarcocysts is unclear [10].

Muscular *Sarcocystis* infections in birds

Sarcocystis species are known to infect domestic poultry in several areas of the world, but are apparently of little disease importance. Wild birds are also infected frequently. *Sarcocystis falcatula* is a parasite that utilizes opossums as definitive hosts and several species of Passeriform and Psittaciform birds as intermediate hosts [6]. Occasional outbreaks of disease due to *S. falcatula* have been reported in outdoor aviaries.

Sarcocystis species in laboratory animals and cultured cells

Many aspects of the biology of *Sarcocystis* have been examined in ruminant, porcine, and rodent models. In addition to their expense, large animals are often naturally infected, and as such are not suitable for many laboratory studies. The *S. muris* mouse-feline model has been used successfully by researchers to investigate many aspects of the biology of the parasite. Other rodent based laboratory models are used less frequently. Research on *Sarcocystis* species is hampered because the definitive hosts produce few sporocysts.

The sexual stages and the precystic stages of several *Sarcocystis* species have been grown in cell cultures [15, 32]. The precystic stages of *S. cruzi* can be continuously grown in cell culture [2]. The sarcocyst stage has not as yet been grown in cell culture.

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References

[1] Anderson AJ, Greiner EC, Atkinson CT, Roelke ME: Sarcocysts in Florida bobcats (*Felis rufus floridanus*). *J Wildl Dis* 1992; 28: 116-120.

[2] Andrews CA, Fayer R, Dubey JP: Continuous in vitro cultivation of *Sarcocystis cruzi*. *J Parasitol* 1990; 76: 254-255.

[3] Beaver PC, Gadgil RK, Morera P: *Sarcocystis* in man: A review and report of five cases. *Am J Trop Med Hyg* 1979; 28: 819-844.

[4] Bhatavedkar MY, Purohit BL: A record of sarcosporidiosis in lion. *Indian Vet J* 1963; 40: 44-45.

[5] Blagburn BL, Braund KG, Amling KA, Toivio-Kinnucan M: Muscular *Sarcocystis* in a dog. *Proc Helminthol Soc Wash* 1989; 56: 207-210.

[6] Box ED, Smith JH: The intermediate host spectrum on a *Sarcocystis* species of birds. *J Parasitol* 1982; 68: 668-673.

[7] Current WL, Upton SJ, Long PL: Taxonomy and Life cycles, in Long PL (ed.), *Coccidiosis of Man and Domestic Animals*, CRC Press Inc, Boca Raton, Florida, 1990, pp. 1-16.

[8] Dubey JP, Bwangamoi O: *Sarcocystis felis* (Protozoa: Sarcocystidae) from the African lion (*Panthera leo*). *J Helminthol Soc Wash* 1994; 61: 113-114.

[9] Dubey JP, Leek RG, Fayer R: Prevalence, transmission, and pathogenicity of *Sarcocystis gigantea* of sheep. *JAVMA* 1986; 188: 151-154.

[10] Dubey JP, Speer CA, Fayer R: *Sarcocystosis of animals and man*. CRC Press Inc., Boca Raton, Florida, 1989, pp 215.

[11] Dubey JP, Hamir AN, Kirkpaterick CE, Todd KS, Rupprecht CE: *Sarcocystis felis* sp. n. (Protozoa: Sarcocystidae) from the bobcat (*Felis rufus*). *J Helminthol Soc Wash* 1992; 59: 227-229.

[12] Dubey JP, Udtujan RM, Cannon L, Lindsay DS: Condemnation of beef because of *Sarcocystis hirsuta* infection. *JAVMA* 1990; 196: 1095-1096.

[13] Edwards JF, Ficken MD, Luttgen PJ, Frey MS: Disseminated sarcocystosis in a cat with lymphosarcoma. *JAVMA* 1988; 193: 831-832.

[14] Everitt JE, Basgall ED, Hooser SB, Todd KS: *Sarcocystis* sp. in the striated muscle of domestic cats, *Felis catus*. *Proc Helminthol Soc Wash* 1987; 54: 279-281.

[15] Fayer R: Gametogeny of *Sarcocystis* in cell culture. *Science* 1972; 175: 65-67.

[16] Fiori MG, Lowndes HE: Histochemical study of *Sarcocystis* sp. intramuscular cysts in gastrocnemius and soleus of the cat. *Parasitol Res* 1988; 75: 123-131.

[17] Gill HS, Charleston WAG, Moriarty KM: Immunosuppression in *Sarcocystis muris*-infected mice: Evidence for suppression of antibody and cell-mediated immune responses. *Immunol Cell Biol* 1988; 66: 209-214.

[18] Greiner EC, Roelke ME, Atkinson CT, Dubey JP, Wright SC: *Sarcocystis* sp. in muscles of free-rang-

ing Florida panthers and cougars (*Felis concolor*). *J Wildl Dis* 1989; 25: 623-628.

[19] Hill JE, Chapman WL, Prestwood AK: Intramuscular *Sarcocystis* sp. in two cats and a dog. *J Parasitol* 1988; 74: 724-727.

[20] Karr SL, Wong MM: A survey of *Sarcocystis* in nonhuman primates. *Lab Anim Sci* 1975; 25: 641-645.

[21] Kirkpaterick CE, Dubey JP, Goldschmidt MH, Saik JE, Schmitz JA: *Sarcocystis* sp. in muscles of domestic cats. *Vet Pathol* 1986; 23: 88-90.

[22] Kluge JP: Trichinosis and sarcosporidiosis in a puma. *Bull Wildl Dis Assoc* 1967; 3: 110-111.

[23] Matuschka FR, Bannert B: Cannibalism and autotomy as predator-prey relationship for monoxenous Sarcosporidia. *Parasitol Res* 1987; 74: 88-93.

[24] Mehlhorn H, Frenkel JK: Ultrastructural comparison of cysts and zoites of *Toxoplasma gondii*, *Sarcocystis muris*, and *Hammondia hammondi* in skeletal muscle of mice. *J Parasitol* 1980; 66: 59-67.

[25] Mehlhorn H, Hartley WJ, Heydorn AO: A comparative ultrastructural study of the cyst wall of 13 *Sarcocystis* species. *Protistologica* 1976; 2: 451-467.

[26] Mehlhorn H, Heydorn AO, Janitschke K: Light and electron microscopical study on sarcocysts from muscles of the Rhesus monkey (*Macaca mulatta*), babbon (*Papio cynocephalus*) and tamerin (*Saguinus* (= *Oedipomidas*) *oedipus*). *Z Parasitenkd* 1977; 51: 165-178.

[27] O'Toole DO: Experimental ovine sarcocystosis: Sequential ultrastructural pathology in skeletal muscle. *J Comp Path* 1987; 97: 5160.

[28] Powell EC, Pezeshkpour G, Dubey JP, Fayer R: Types of myofibers parasitized in experimentally induced infections with *Sarcocystis cruzi* and *Sarcocystis capracanis*. *Am J Vet Res* 1986; 47: 514-517.

[29] Rommel M, Heydorn AO: Beiträge zum Lebenszyklus der Sarkosporidien III. *Isospora hominus* (Railliet und Lucet, 1891) Wenyon, 1923, eine Dauerform der Sarkosporidien des Rindes und des Schweins. *Berl Münch Tierärztl Wschr* 1972; 85: 143-145.

[30] Rommel M, Heydorn AO, Gruber F: Beiträge zum Lebenszyklus der Sarkosporidien I. Die Sporozyste von *S. tenella* in den Fäzes der Katze. *Berl Münch Tierärztl Wschr* 1972; 85: 101-105.

[31] Sahasrabudhe VK, Shah HL: The occurrence of *Sarcocystis* sp. in the dog. *J Protozool* 1966; 13: 531.

[32] Speer CA, Dubey JP: Vascular phase of *Sarcocystis cruzi* cultured in vitro. *Can J Zool* 1986; 64: 209-211.