Research Note

Muscular Sarcocystis in a Dog

BYRON L. BLAGBURN,1 KYLE G. BRAUND,2 KAREN A. AMLING,2 AND MARIA TOIVIO-KINNUCAN1

1 Department of Pathobiology, College of Veterinary Medicine, Auburn University, Alabama 36849-5519
2 Neuromuscular Laboratory of the Scott-Ritchey Research Unit, College of Veterinary Medicine, Auburn University, Alabama 36849-5519

ABSTRACT: A sarcocyst is described from the biceps femoris muscle of a dog using both light and transmission electron microscopy. The sarcocyst was spherical to ovoid and measured 47 × 52 μm inclusive of the wall. The wall was palely eosinophilic, approximately 2.3 μm at its widest margin, and 0.9 μm at its narrowest margin. Cyst wall projections were barely visible in histological sections. Ultrastructurally, they appeared as irregularly spaced electron dense projections, measuring up to 1.5 μm long and 0.9 μm wide. The electron dense granular layer of the wall was approximately 0.7 μm thick. Interior septa were visible as electron dense lines that appeared to compartmentalize numerous irregularly arranged bradyzoites. Bradyzoites were elongate with discernible apical complexes and with posterior nuclei. Metrocytes were not seen. The sarcocyst did not appear to elicit an inflammatory response and was considered an incidental finding.

KEY WORDS: canine, dog, Sarcocystis, muscle.

Sarcocystis spp. are traditionally viewed as obligatorily heteroxenous coccidia that normally undergo vascular merogony and muscular cystogenesis in intermediate hosts, and gametogony, sporogony, and sporulation in definitive hosts (Fayer, 1980). The presence of Sarcocystis cysts in the muscles of a carnivore, normally a definitive host, is perplexing. This unusual and infrequent occurrence is the basis of the present report.

A 4-yr-old male (castrated) dog was presented to a veterinary clinic in the southeastern United States for physical and neurological examination because of ataxia and limb stiffness. Biopsies of biceps femoris (BF), triceps brachii, and temporalis muscles were submitted to 1 of the authors (K.G.B.) for histomorphological and histochemical evaluations. Frozen sections were prepared as previously described (Braund et al., 1978) and stained with a battery of stains including the periodic acid-Schiff (PAS) reaction. Following the discovery of what appeared to be a cyst of Sarcocystis species in a section of BF, additional frozen samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sections at 5 μm, and stained with hematoxylin and eosin (H&E) for additional light microscopic examination. Formalin-fixed BF muscle was prepared for electron microscopy using the following procedure: frozen sections were air-dried at room temperature, fixed in 10% formalin, stained with H&E, and left in distilled water. Sections were examined using a light microscope and the area containing the sarcocyst demarcated using a diamond pen. Sections were then postfixed in 1% osmium tetroxide in Millonig’s buffer, pH 7.3, for 1 hr, dehydrated in an ethanol series, and embedded in Spurr resin (Polysciences Inc., Warrington, Pennsylvania). Resin flooded slides, elevated by toothpicks, were oven-cured overnight at 70°C. The area of the section containing the sarcocyst was detached using a single-edge razor blade. This portion of the section was then glued onto a Spurr dummy block (Aron Alpha Quick Setting Adhesive, Ted Pella Inc., Tustin, California). Thin sections were cut using an Ultratome III, LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined using a Philips 301 electron microscope.

The single cyst observed in an H&E-stained cross section of the BF using light microscopy was spherical to ovoid and measured approximately 47 × 52 μm inclusive of the cyst wall (Fig. 1). The wall was palely eosinophilic, approximately 2.3 μm at its widest margin, and 0.9 μm at its narrowest margin. Outer cyst wall projections were barely visible in histological sections. Septate projections of the cyst wall into the interior of the cyst were visible as clear or dark lines in PAS- and H&E-stained sections, respectively. Bradyzoites were moderately to intensely eosinophilic in H&E-stained sections, and were irregularly arranged in groups bordered by septa. The same cyst stained using the PAS reaction revealed a cyst wall and interior septa that were PAS-negative. Bradyzoites were magenta and were arranged as described for H&E-stained...
Figures 1, 2. Cyst of *Sarcocystis* from a frozen biopsy of biceps femoris muscle of a dog. 1. Photomicrograph of cyst showing primary cyst wall (CW), septum (S), and bradyzoites (Bz). Hematoxylin and eosin. 2. Electron micrograph of the cyst wall prepared from formalin-fixed, frozen biopsy of biceps femoris muscle. Note villous projections (VP) from the primary cyst wall.
sections. Ultrastructurally, the primary cyst wall contained numerous irregularly spaced villous projections up to 1.5 μm long and 0.9 μm wide (Fig. 2). An electron dense granular layer, approximately 0.7 μm thick, was present just beneath the villous projections. This electron dense granular substance was also present in many of the villous projections. In some areas, a parasitophorous vacuole was present adjacent to the primary cyst wall. Septate projections of the cyst wall were visible as electron dense lines that appeared to compartmentalize the numerous irregularly arranged bradyzoites. Bradyzoites were elongate with discernible apical complexes and with posterior nuclei. Metrocytes were not seen. The presence of the sarcocyst in the BF muscle was considered an incidental finding.

Morphological characteristics of the observed cyst are consistent with those of Sarcocystis spp. (Dubey, 1977). The presence of sarcocysts in the muscles of a dog is intriguing. Previous reports of canine muscular sarcocystiasis included recovery of an apparent sarcocyst in esophageal muscles of a dog from India (Sahasrabudhe and Shah, 1966) and in the myocardium of a dog from the United States (Hill et al., 1988). In neither case was an inflammatory reaction associated with the sarcocysts. Carnivorous hosts from which muscular cysts of Sarcocystis spp. have been recovered include: humans, domestic dogs, domestic cats, leopards, raccoons, whales, black bears, viperid snakes, pythons, buzzards, weasels, skunks, badgers, genets, and mongooses (Bhatavdekar and Purohit, 1963) and in the myocardium of a dog from the United States (Hill et al., 1988). In another case was an inflammatory reaction associated with the sarcocysts. Carnivorous hosts from which muscular cysts of Sarcocystis spp. have been recovered include: humans, domestic dogs, domestic cats, leopards, raccoons, whales, black bears, viperid snakes, pythons, buzzards, weasels, skunks, badgers, genets, and mongooses (Bhatavdekar and Purohit, 1963) and in the myocardium of a dog from the United States (Hill et al., 1988). Several explanations may be forwarded for the presence of muscle cysts in what would be considered a definitive host for Sarcocystis spp. Lack of rigid host specificity for Sarcocystis stages in the intermediate host would allow sporocysts of Sarcocystis species to infect more than a single intermediate host species. Recent evidence supports decreased intermediate host specificity for certain Sarcocystis species (Box and Duszynski, 1978). Sarcocystis spp. may form intestinal and extraintestinal stages in the same host. This has been reported recently for Sarcocystis galloti infecting Canarian lizards (Matuschka and Bannert, 1987). In addition, domestic canids may serve as intermediate hosts for Sarcocystis spp. whose definitive host preys upon canids. Certain canids might prey upon homologous members of the species or upon closely related canids. It was reported, for example, that coyotes will prey upon other coyotes (Andrews and Boggess, 1978). Further, over an 8-yr period, the Patuxent Wildlife Research Center, Laurel, Maryland, confirmed 24 depredations of domestic dogs by wolves. Of 19 carcasses that were subsequently checked, 14 were partially or fully eaten. Wolves are also known to kill and consume other wolves (Dr. Steven H. Fritts, Patuxent Wildlife Research Center, Laurel, Maryland, pers. comm.).

Because muscle cyst morphology is not considered taxonomically definitive, it was not possible to determine the species to which the sarcocyst belongs. The cyst described herein is morphologically similar to those described previously from cats (Everitt et al., 1987; Kirkpatrick et al., 1987; Hill et al., 1988). Comparisons cannot be made with the cysts previously described from dogs, because electron micrographs were not included with either report.

We gratefully acknowledge Rebecca A. Cole for her assistance. This is College of Veterinary Medicine Publication No. 2047.

Literature Cited


The Growth of Hymenolepis diminuta in Five Strains of Mice

K. T. PEARLSTEIN,1 M. A. POEHLMAN,2 AND G. D. INSLER1

1Department of Biology, Adelphi University, Garden City, New York 11530
2Department of Biology, Nassau Community College, Garden City, New York 11530

ABSTRACT: The kinetics of growth and subsequent rejection pattern of the intestinal cestode, Hymenolepis diminuta, were examined in 5 different inbred strains of mice. Six 8-wk-old females from the strains C57/B1, A, Balb/c, DBA, and C3H/He were infected with 5 cysticercoids via a stomach tube. The effects of infection were examined by autopsy on days 6, 8, 10, 12, and 14 postinfection. Parameters examined were worm number, worm length, worm biomass, spleen weight, liver weight, and white blood cell differentials. There were no significant differences in spleen or liver weights or white blood cell differentials among the different strains. However, there were significant differences in development as assessed by worm number, length, and biomass. Autopsy indicated that worms established and grew normally in all mice by day 6, but between days 10 and 14 most worms were lost. Worms recovered from different strains demonstrated different growth profiles. For example, worms that were isolated from C3H/He mice exhibited a high recovery, long length, and large biomass when compared to worms isolated from C57/B1 mice.

KEY WORDS: Hymenolepis diminuta, cestode, growth, development, mice, inbred strains.

The rat cestode, Hymenolepis diminuta, is a non-invasive intestinal tapeworm that lives as an adult in a variety of rodent hosts. When mice are given a primary infection of H. diminuta, they respond by rejecting the worms. These events are known to be immunologically mediated (Hopkins, 1980). The influence of the strain of mouse on rejection of H. diminuta has not been fully explored. Limited studies have examined Swiss albino, CFLP, Porton, Balb/c, C57, and nude mice (Weinmann, 1966; Hopkins et al., 1972; Isaac et al., 1975; Bland, 1976; Andreasen et al., 1978; Isaac, 1983). In separate trials, each of these strains was shown under different circumstances independently to reject H. diminuta. However, simultaneous infections were not performed within 1 laboratory, so it is difficult to assess if there is differential growth and rejection of H. diminuta among mouse strains.

The purpose of this investigation was to examine the kinetics of growth and subsequent rejection pattern of H. diminuta in 5 strains of mice. Parameters examined were white blood cell differentials, spleen and liver weights, and worm number, length, and biomass.

Tapeworm-free, 6- to 8-wk-old female mice of the inbred strains designated A/J, Balb/cByJ, C3H/HeJ, C57/Bl/6J, and DBA/2J were obtained from the Jackson Laboratory (Bar Harbor, Maine). They were maintained under conventional laboratory conditions. They were supplied with mouse breeding diet (Purina mouse chow) and water ad libitum.

Cysticercoids of H. diminuta approximately 32 days old were dissected from adult flour beetles, Tribolium confusum, in distilled water as described by Insler and Roberts (1976). Five cysticercoids in 0.3 ml of distilled water were administered to each of 25 mice of each strain by intragastric intubation. Control mice received 0.3 ml of distilled water.