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Sarcocystis neurona schizonts-associated encephalitis, chorioretinitis, and myositis in a two-month-old dog simulating toxoplasmosis, and presence of mature sarcocysts in muscles

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ABSTRACT

Sarcocystis neurona is an unusual species of the genus *Sarcocystis*. Opossums (*Didelphis virginianus*, *D. albiventris*) are the definitive hosts and several other species, including dogs, cats, marine mammals, and horses are intermediate or aberrant hosts. Sarcocysts are not known to form in aberrant hosts. *Sarcocystis neurona* causes fatal disease in horses (Equine Protozoal Myeloencephalitis, EPM). There are numerous reports of fatal EPM-like infections in other species, usually with central nervous system signs and associated with the schizont stage of *S. neurona*. Here, we report fatal disseminated *S. neurona* infection in a nine-week-old golden retriever dog from Mississippi, USA. Protozoal merozoites were identified in smears of the cerebrospinal fluid. Microscopically, lesions and protozoa were identified in eyes, tongue, heart, liver, intestines, nasal turbinates, skeletal muscle and brain, which reacted intensely with *S. neurona* polyclonal antibodies. Mature sarcocysts were seen in sections of muscles. These sarcocysts were ultrastructurally similar to those of *S. neurona* from experimentally infected animals. These data suggest that the dog is another intermediate host for *S. neurona*. Data suggest that the dog was transplacentally infected.

1. Introduction

Sarcocystis neurona, *S. canis*, *Toxoplasma gondii*, and *Neospora caninum* are related apicomplexans that can cause systemic illness in many species of animals, including dogs (Dubey et al., 2006). Species of *Sarcocystis* usually have a two-host, prey-predator life cycle, with herbivores as intermediate hosts and carnivores as definitive hosts (Dubey et al., 1989). The intermediate host becomes infected with *Sarcocystis* species by ingesting sporocysts or oocysts, or both, excreted in the feces of the definitive host. After a brief period of schizogony, the parasite encysts in tissues and forms sarcocysts. The definitive host becomes infected by ingesting sarcocysts encysted in the tissues of intermediate hosts.

S. neurona is an unusual species of the genus *Sarcocystis*. Opossums (*Didelphis virginianus*, *D. albiventris*) are the definitive host and several other species, including dogs, cats, marine mammals, and horses serve as intermediate

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or aberrant hosts (Dubey et al., 2001a, 2006). Although mature sarcocysts may form in intermediate hosts, they are not formed in aberrant hosts. There are several reports of severe *S. neurona*-like infections in other species, usually with central nervous system signs associated with the schizont stage (Dubey et al., 2001a; Britton et al., 2010). Immature *S. neurona*-like sarcocysts have been reported in the muscle from a dog with myositis; however, the cause of myositis in that case was not determined because schizonts were present only in the muscle interstitium (Vashisht et al., 2005). Here we report systemic sarcocystosis in a dog that was most likely acquired transplacentally.

2. Materials and methods

2.1. Naturally infected dog

A nine-week-old golden retriever dog was referred to the College of Veterinary Medicine (CVM), Mississippi State University in 1998 with a complaint that the dog had seizures. Significant clinical history included a recent vaccination and prior treatment with corticosteroids for juvenile cellulitis. Because of poor prognosis the puppy was euthanized. Historical questioning revealed that the pup was one of 11 littermates. One littermate had been euthanized due to seizures, but was not fully examined at time of necropsy. Another littermate had a history of a head tilt that apparently resolved. The remaining eight littermates appeared normal.

The dam and both of the euthanized puppies were necropsied at CVM; all three were negative for antibodies to *T. gondii* and *N. caninum*. The puppy in this case report, was negative for *Rickettsia rickettsii* (cause of Rocky Mountain Spotted fever), and ehrlichiosis based on serum testing. Its CSF distemper titer was 1:64. No other information is available as the original files have now been destroyed.

2.2. Histopathologic examination

Tissues from only one pup (described here) were studied histologically. Samples of brain, heart, lungs, spleen, liver, tongue, turbinates, skeletal muscle, intestines, pancreas, lymph nodes, eyes, thymus, and thyroid were fixed in 10% neutral buffered formalin and processed for routine histology. Paraffin-embedded sections were cut at 5 μm, stained with hematoxylin and eosin (H and E) and examined microscopically. Tissues were also submitted to the Animal Parasitic Diseases Laboratory (APDL), Beltsville, Maryland in 1998 for diagnosis.

2.3. Immunohistochemical (IHC) examination

De-paraffinized sections were reacted with antibodies to *T. gondii*, *N. caninum*, and *S. neurona* as described (Lindsay and Dubey, 1989; Dubey and Hamir, 2000; Hamir and Dubey, 2001; Dubey et al., 2006). The specificity and preparation of antibodies to *S. neurona* (Dubey et al., 1999), *N. caninum* and *T. gondii* (Lindsay and Dubey, 1989) were as described.

2.4. Transmission electron microscopy (TEM)

In 2013 portions of the cerebrum and tongue of the pup were de-paraffinized, fixed in glutaraldehyde, post fixed in osmium, processed for TEM, and examined in a JEOL, JEM 1400 electron microscope.

3. Results

Gross lesions consisted of mild to moderate generalized lymphadenopathy, thymic atrophy, and excessive amounts of CSF fluid. *Toxoplasma*-like protozoal tachyzoites were seen within mononuclear cells in the CSF by two of the authors (JPD, SB); however, this slide is no longer traceable and was likely discarded along with the original files.

Microscopically, lesions were seen in the brain, eyes, heart, liver, small intestine, pancreas, nasal turbinates, skeletal muscle and tongue. The brain had multifocal severe granulomatous meningoencephalitis with necrosis. Multiple, random, severe foci of necrosis and inflammation in cerebral gray and white matter often extended into the adjacent meninges. Inflammatory infiltrates in areas of severe malacia, consisted predominantly of macrophages, intermingled with lymphocytes, plasma cells, and fewer eosinophils and neutrophils. Neovascularization both within malacic foci and adjacent neuropil was marked, and there was marked edema and lymphoplasmacytic perivascular cuffing. Heavy leptomeningeal infiltrates consisted predominantly of lymphocytes and plasma cells and intermittent eosinophils (Fig. 1). Although it was difficult to identify protozoa in H and E sections, organisms were numerous in sections reacted with *S. neurona* antibody by IHC (Fig. 1A–F). Most organisms were merozoites or developing schizonts (Fig. 1D–F).

Within the eyes, there was bilateral endophthalmitis with severe granulomatous chorioretinitis, retinal necrosis and detachment, and mild anterior uveitis. Protein and small numbers of macrophages were in the anterior chamber. The retina was extensively undermined by a subretinal exudate consisting of macrophages, scattered giant cells, and some eosinophils. This was associated with extensive retinal necrosis and detachment (Fig. 1G and 2). The retina had generalized atrophy of inner retinal layers or complete effacement and disorganization of residual retinal layers with granulomatous infiltrates and similar infiltrates extending into the vitreous. The detached part of the retina was focally adhered to the posterior capsule of the lens. Predominantly lymphocytes and plasma cells and fewer macrophages infiltrated and effaced the tapetum lucidum and extended throughout the choroid. A few lymphocytes and plasma cells infiltrated the iris and ciliary body. Numerous *S. neurona* merozoites and developing schizonts were seen in all layers of the retina (Fig. 1H and I). A few merozoites were seen in the choroid.

Two types of microscopic lesions were seen in the tongue. First, there were granulomas and foci of necrosis associated with schizonts and merozoites (Fig. 3A–D). Fibrous connective tissue and intermittent striated muscle fibers encapsulating adipose tissue, identified as the lyssa, was infiltrated by mononuclear cells. Individual merozoites and immature schizont-like structures were