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Short Communication

Acute onset of encephalomyelitis with atypical lesions associated with dual infection of *Sarcocystis neurona* and *Toxoplasma gondii* in a dog

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ABSTRACT

A two-year-old male, neutered, basset hound-beagle mix with progressive neurological impairment was examined postmortem. Grossly, the dog had multiple raised masses on the spinal cord between nerve roots. Microscopically, the dog had protozoal myeloencephalitis. *Toxoplasma gondii* and *Sarcocystis neurona* were detected in the CNS by immunohistochemistry and polymerase chain reaction (PCR). Sarcocysts in formalin-fixed muscle were negative for *Sarcocystis* by PCR. Banked serum was negative for *T. gondii* using the modified agglutination test, suggesting an acute case of *T. gondii* infection or immunosuppression; however, no predisposing immunosuppressive diseases, including canine distemper, were found. To the authors' knowledge, this is the first report of dual *T. gondii* and *S. neurona* infection in a dog.

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1. Introduction

Toxoplasma gondii and *Sarcocystis neurona* are both apicomplexan parasites that can cause neurological lesions in animals. In dogs, *T. gondii* generally does not cause primary infection unless preceded by an immunosuppressive disease, such as canine distemper; however, infection with *S. neurona* as a sole pathogen can lead to significant lesions (Dubey and Odening, 2001; Dubey et al., 2006). Transmission of *T. gondii* and *S. neurona* occurs by ingestion of oocysts in contaminated soil, food, or water or by ingestion of tissue cysts in undercooked meat. *T. gondii* infection occurs in many species of wild birds and mammals, particularly those that are carnivorous or that have ground dwelling behavior (Dubey and Odening, 2001; De Thoisy et al., 2003; Dubey, 2008). Although dual infections with *T. gondii* and *S. neurona* have been documented in multiple species (Dubey and Odening, 2001; Gerhold et al., 2005; Thomas et al., 2007), reports are infrequent and sporadic. To the authors' knowledge, this is the first report of a case of dual *T. gondii* and *S. neurona* infection in a dog that was *T. gondii* seronegative and had atypical pathological findings associated with the apicomplexa infection.

Abbreviations: DAB, 3,3′-diaminobenzidine; HE, hematoxylin & eosin; MAT, modified agglutination test; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

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2. Case report

A two-year-old male, neutered, basset hound-beagle mix was referred to the University of Tennessee Veterinary Medical Center in May 2011 for acute onset of non-ambulatory paraparesis. The dog was adopted from a shelter two weeks prior and reported to have always had an atypical pelvic limb gait. On initial presentation, the dog was bright, alert, and responsive, with vital parameters within normal limits. Neurologic exam showed left pelvic limb paralysis with intact superficial pain sensation and right pelvic limb paresis with normal spinal reflexes in both legs. Pain was elicited on palpation of the cranial lumbar spine. Initial complete blood count, chemistry panel, and urinalysis results showed a mild eosinophilia (1350/μL) but were otherwise unremarkable. A sugar fecal flotation was negative for oocysts and helminth eggs. Magnetic resonance imaging (MRI) of the thoracolumbar spine was performed under general anesthesia. The MRI showed two areas of focal spinal cord swelling from T4 to T6 and L2 to L4. In these areas there was asymmetric parenchymal T2-hyperintensity with associated contrast enhancement that appeared predominantly intramedullary. Cerebrospinal fluid collected from the lumbar cistern contained 70 nucleated cells per microliter (predominantly lymphocytes with lower numbers of monocytoid cells and rare neutrophils and eosinophils) and elevated protein (260 mg/dL). All tests for infectious disease were negative and included polymerase chain reaction (PCR) testing of urine for canine distemper virus; serum antibody titers for *Rickettsia rickettsii*, *Ehrlichia canis*, *Borrelia burgdorferi*, and *Neospora caninum*; serum antigen testing for *Cryptococcus* spp.; and urine antigen testing for *Blastomyces* spp. While awaiting the results of these tests, the dog was treated with supportive care, pain medications, and prednisone (0.5 mg/kg orally twice daily). The dog lost voluntary motor function in the right pelvic limb about 48 h after presentation; treatment with doxycycline (4.5 mg/kg orally twice daily) and clindamycin (10.7 mg/kg orally twice daily) was initiated. The patient then lost deep pain sensation in both pelvic limbs about 4 days after presentation; based on clinical worsening and negative infectious disease testing, immunosuppressive therapy with prednisone (1.1 mg/kg orally twice daily) and cytarabine arabinoside (100 mg/m^2 subcutaneously once) was initiated. The following day, the dog appeared depressed and to be in more pain. The owner elected euthanasia and a necropsy was performed.

Upon necropsy, the dog had a body condition score of 3 out of 5. There was marked skeletal muscle atrophy over the lumbosacral region, along both pelvic limbs, and involving the epaxial skeletal muscle beginning at the mid-thoracic region and extending caudally. Vertebral column prominence was increased throughout this affected region. The spinal cord contained two raised, tan, semicircular, soft extramedullary masses, one at T5–T6 (Fig. 1 A) and one at L2–L3. The mass at T5–T6 was $6 \times 6 \times 4$ mm, and the mass at L2–L3 was $1.3 \times 7 \times 4$ mm. Lesions were present between nerve roots. No lesions were noted in the vertebral column or vertebral canal. The cerebrum, cerebellum, and brainstem were grossly normal. On cut section, both mass locations within the spinal cord contained multiple raised, tan to white, expansile, poorly demarcated masses (Fig. 1A, inset).

Portions of the brain, eye, trachea, lung, skeletal muscle, heart, kidney, testis, liver, spleen, esophagus, small intestine, and large intestine were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 3 mm, and stained with hematoxylin and eosin for light microscopy. Portions of the same tissues were collected and frozen at −20 °C.

Microscopically the thoracic cord showed multifocal infiltration by lymphocytes, as well as fewer plasma cells and macrophages in the Virchow Robbins spaces and the meninges (Fig. 1B). White matter fiber tracts were multifocally forming chains of dilated myelin sheaths (Wallerian degeneration) with multifocal central macrophages (digestion chambers) (Fig. 1B). Activated microglial cells were increased. On cross section, inflammation described extended into the left dorsal funiculus and randomly throughout the remaining funiculi (Fig. 1B). Increased numbers of dilated myelin sheaths, digestion chambers, and white matter vacuoles (spongiosis) were seen throughout these funiculi (Fig. 1B).

The thoracic spinal cord contained a large area of pyogranulomatous inflammation and necrosis that extended along and effaced the dorsal lateral funiculi and focally through the meninges, forming a dome shape mass lesion (Fig. 1C). Pyogranulomatous inflammation consisted of numerous neutrophils, 15–25-μm diameter protozoal cysts containing numerous 2-μm-diameter zoites, free 2-μm-diameter zoites, karyorrhectic debris, macrophages with occasional intrahistiocytic zoites, and fewer multinucleated giant cells, lymphocytes, and plasma cells (Fig. 1C). Numerous lymphocytes and plasma cells infiltrated and expanded Virchow Robbins spaces. Tracking along and expanding the meninges in between two cerebellar folia were numerous macrophages, with fewer lymphocytes, plasma cells, and neutrophils and rare hemosiderophages. Similar inflammation tracked along the remaining meninges, albeit rarely, and was seen randomly throughout the brainstem, where there were also increased numbers of activated microglial cells and activated astrocytes. A focally extensive section of neuropil contained increased numbers of active microglial cells and activated astrocytes with multifocal perivascular, and random infiltrates of lymphocytes and macrophages were seen in the internal capsule. There were multifocal regionally extensive areas of gray matter vacuolation. Neurons along the dentate gyrus of the hippocampus and randomly throughout the neuropil were shrunken, angular, and hypereosinophilic (necrotic). Sections of skeletal muscle contained individual myocytes that were multifocally small (atrophy), vacuolated to hypereosinophilic (degeneration and necrosis, respectively), and surrounded by clear space (edema) (Fig. 1D). Expanding a focal myocyte was a 50-μm-diameter protozoal cyst with numerous 2-μm-diameter bradyzoites. The cyst wall was 3 μm thick circumferentially. Sections of affected lumbar spinal cord were stained for *T. gondii*, *S. neurona*, and *N. caninum* using immunohistochemical procedures as previously described (Gerhold et al., 2005). Staining of cyst zoites was strong for *S. neurona* (Fig. 1E), present but less intense for *T. gondii*