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Short communication

Sarcocystis neurona retinochoroiditis in a sea otter (*Enhydra lutris kenyoni*)

J.P. Dubey[a,*], N.J. Thomas[b]

[a] United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, MD 20705-2350, USA
[b] Department of Interior, United States Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711, USA

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ABSTRACT

Sarcocystis neurona is an important cause of fatal disease in sea otters in the USA. Encephalitis is the predominant lesion and parasites are confined to the central nervous system and muscles. Here we report retinochoroiditis in a sea otter (*Enhydra lutris kenyoni*) found dead on Copalis Beach, WA, USA. Salient lesions were confined to the brain and eye. Multifocal nonsuppurative meningoencephalitis was present in the cerebrum and cerebellum associated with *S. neurona* schizonts. The retina of one eye had a focus of inflammation that contained numerous *S. neurona* schizonts and merozoites. The focus extended from the retinal pigment epithelium inward through all layers of the retina, but inflammation was most concentrated at the inner surface of the tapetum and the outer retina. The inner and outer nuclear layers of the retina were disorganized and irregular at the site of inflammation. There was severe congestion and mild hemorrhage in the choroid, and mild hemorrhage into the vitreous body. Immunohistochemistry with *S. neurona*-specific polyclonal rabbit antibodies stained schizonts and merozoites. To our knowledge this is the first report of *S. neurona*-associated retinochoroiditis in any naturally infected animal.

1. Introduction

Sarcocystis neurona is an important cause of a fatal disease, initially named equine protozoal myeloencephalitis (EPM), of horses in the Americas (Dubey et al., 2001a). An EPM -like disease has been reported in many hosts including marine mammals, dogs, cats, raccoons, and mink. Of all its natural hosts, EPM is probably most common and most severe in sea otters (Lindsay et al., 2000; Kreuder et al., 2003; Thomas et al., 2007; Miller et al., 2010). Recently, *S. neurona* infection was associated with a large scale die off in sea otters in California (Miller et al., 2010). In April 2004, 40 southern sea otters (*Enhydra lutris neresis*) were found beached on the coast line near Morro Bay, California. Sixteen of these 40 otters were investigated in detail for causes of mortality (Miller et al., 2010). Protozoal meningoencephalitis was the predominant lesion and *S. neurona* was identified in 15 of these 16 otters; there was no mention of lesions in eyes. Here, we report the first case of *S. neurona*-associated retinochoroiditis in any naturally infected animal.

2. Materials and methods

2.1. Necropsy and histological examination

A female subadult sea otter (115 cm long, tail 30 cm) was found dead 29 April 2010 on the north end of Copalis Beach, WA, USA. A necropsy was performed the next day. Samples of brain, pituitary gland, trigeminal nerve, heart,

* Corresponding author. Tel.: +1 301 504 8128; fax: +1 301 504 9222.
E-mail address: jitender.dubey@ars.usda.gov (J.P. Dubey).

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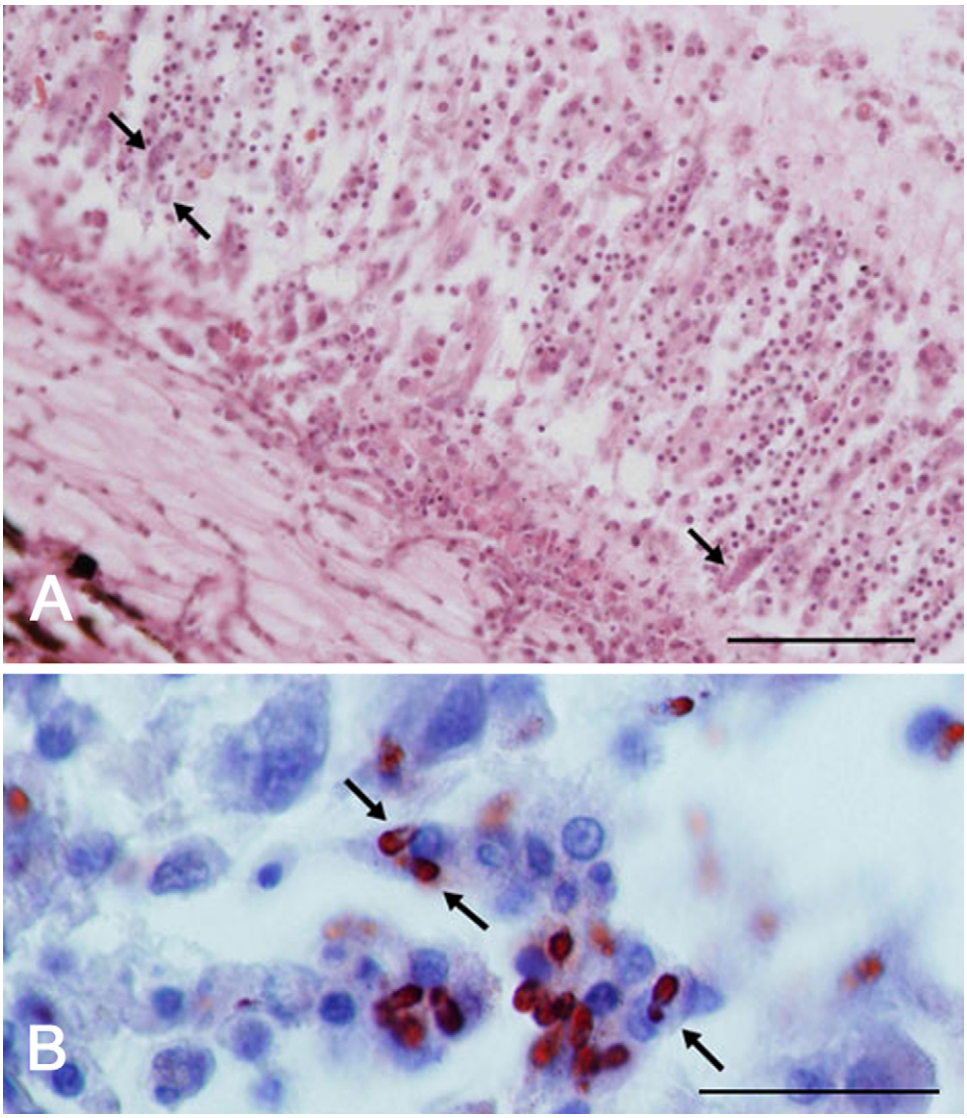

Fig. 1. (A) Histological section of one eye of the sea otter. Note the inflammatory focus with *S. neurona* (arrows) barely visible at this magnification. HE stain, bar = 100 μm. (B) Several intracellular *S. neurona* merozoites (arrows) in retinal cells of the same eye. Immunohistochemical reaction with *S. neurona*-specific polyclonal rabbit antibodies, bar = 35 μm.

lung, tracheobronchial lymph node, thymus, thyroid gland, 2 inguinal lymph nodes, liver, kidney, spleen, adrenal gland, pancreas, bile duct, stomach, 4 intestinal sections, skeletal muscle including tongue, diaphragm and abdominal wall, and 1 eye were fixed in 10% buffered formalin. The second eye was fixed in Bouin's fixative. Paraffin-embedded sections were examined after staining with hematoxylin and eosin (HE). Selected sections were examined immunohistochemically with polyclonal rabbit antibodies specific to *Toxoplasma gondii* and *S. neurona* as described (Thomas et al., 2007).

3. Results

The animal was in good body condition and had no significant gross lesions.

Salient lesions were confined to the brain, heart, skeletal muscle, and one eye. Neural lesions consisted of moderate to severe multifocal nonsuppurative meningoencephalitis in the cerebrum and cerebellum associated with immature and mature protozoal schizonts, and extracellular merozoites. Immunohistochemistry for canine distemper virus was negative in brain. Protozoa reacted positively with *S. neurona* antibodies and were negative for *T. gondii*.

A moderate number of *Sarcocystis* tissue cysts (sarcocysts) were present within myofibers in all skeletal muscle sections. Sarcocysts contained metrocytes and bradyzoites and were morphologically similar to *S. neurona* sarcocysts. A moderate number of acutely necrotic or degenerate myofibers were accompained by mild mononuclear cell accumulations.

A small loose infiltrate of lymphocytes was present in the endocardium of the interventricular septum. The right atrium had a thick-walled, mature sarcocyst within a myofiber near the atrioventricular junction.

The retina of the Bouin's-fixed eye had a focus of inflammation that contained numerous protozoa (Fig. 1A). The focus extended from the retinal pigment epithelium inward through all layers of the retina, but inflammation was most concentrated at the inner surface of the tapetum and in the outer retina. Cells in the tapetum were predominantly small lymphocytes, while the aggregate at its inner surface included macrophages, lymphocytes and small numbers of neutrophils with mild hemorrhage. Macrophages were most common in the outer and inner nuclear and plexiform layers and ganglion cell layer. The inner and outer nuclear layers of the retina were disorganized and irregular at the site of inflammation. There was severe congestion and mild hemorrhage in the choroid, and mild hemorrhage into the vitreous body. Immature, and mature schizonts, and individual merozoites were seen in the lesion in the HE-stained section. No inflammation was seen in the optic nerve or the meninges of the optic nerve. The lesion and protozoa were not seen in histological recut sections of the same eye. Therefore, we removed the coverslip from the original HE section with the lesion and subjected it to immunohistochemistry with *S. neurona* antibodies; numerous merozoites and relatively few schizonts were present in the lesion (Fig. 1B).

4. Discussion

Sarcocystis neurona has an unusual life cycle with opossums (*Didelphis* spp.) as the only known definitive hosts but many intermediate hosts (Dubey et al., 2001a). This is unlike other *Sarcocystis* species that parasitize herbivores and rodents (Dubey et al., 1989). Raccoons (*Procyon lotor*), sea otters, skunks (*Mephitis mephitis*), and armadillos (*Dasypus novemcinctus*) are its natural intermediate hosts (Cheadle et al., 2001a,b; Dubey et al., 2001b,c), and *S. neurona*-like sarcocysts were found in one horse (Mullaney et al., 2005). Many aspects of the life cycle of *S. neurona* are unknown. The route of migration of the parasite in the horse is unknown because it has not been possible to find *S. neurona* stages in experimentally infected horses (Fenger et al., 1997; Cutler et al., 1999; Saville et al., 2001). Complete development of the asexual stages of the parasite is known only from the raccoons fed *S. neurona* sporocysts (Stanek et al., 2002). In raccoons fed *S. neurona* sporocysts, individual organisms, interpreted to be sporozoites, were found in blood and tissues 1–5 days p.i. From 7 to 15 days p.i., schizonts were found in many tissues, including eyes on 7 and 10 days p.i. Sarcocysts were formed in muscles beginning 22 days p.i. Sarcocysts matured by 77 days p.i. and laboratory-raised opossums fed experimentally infected raccoon muscles shed *S. neurona* sporocysts (Stanek et al., 2002). Horses fed *S. neurona* sporocysts developed EPM but parasites were not histologically demonstrated in equine tissues (Fenger et al., 1997; Saville et al., 2001; Sofaly et al., 2002).

The gamma gene interferon knock out (KO) mouse has been used as another experimental animal to study the biology of *S. neurona* because of difficulties of housing raccoons in parasite-free conditions (Dubey, 2001). In KO mice fed *S. neurona* sporocysts, sporozoites excysted from sporocysts and invaded intestinal epithelium. Between 1 and 3 days p.i., organisms were seen in the intestines, and these were considered to be sporozoites. Between 4 and 11 days p.i., organisms were seen in several visceral tissues. Beginning 13 days p.i., *S. neurona* was seen consistently in the brain. Of 28 KO mice examined 20–62 days p.i., *S. neurona* schizonts were seen in the brain of all 28, lungs of 14, heart of 8, and eyes of 3; sarcocysts were never seen in KO mice (Dubey, 2001).

In our previous studies on the causes of mortality in sea otters that were submitted to the National Wildlife Health Center, Madison, WI, USA, eyes were not routinely examined for lesions or protozoa (Thomas et al., 2007). Of those sea otters examined in laboratories in California, there is no mention of eye lesions due to protozoa (Kreuder et al., 2003; Miller et al., 2009, 2010). Therefore, it is uncertain whether *S. neurona* infection of the eye in sea otters is rare or common. Studies in raccoons and KO mice indicate that *S. neurona* can parasitize eyes, and eyes should be included in tissues examined for *S. neurona* infection in animals.

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