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Sarcocystis canis Associated Hepatitis in a Steller Sea Lion (*Eumetopias jubatus*) from Alaska

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ABSTRACT: *Sarcocystis canis* infection was associated with hepatitis in a Steller sea lion (*Eumetopias jubatus*). Intrahepatocellular protozoal schizonts were among areas of necrosis and inflammation. The parasite was genetically identical to *S. canis* and is the first report in a Steller sea lion, indicating another intermediate host species for *S. canis*.

Sarcocystis infections have been identified in various marine mammals, including a report of *Sarcocystis canis* associated hepatitis in a California sea lion (*Zalophus californianus*; Dubey et al. 2003), a striped dolphin from Spain (*Stenella coeruleoalba*), a Hawaiian monk seal (*Monachus schauislandi*; reviewed in Dubey et al. 2006), and a *S. canis*-like organism in two polar bears (*Ursus maritimus*, Garner et al. 1997). Additionally, *S. canis*-associated hepatitis has been diagnosed in a chinchilla (*Chinchilla laniger*), a horse (*Equus caballus*), and a black bear (*Ursus americanus*). *Sarcocystis*-associated hepatitis can be severe and often is lethal in presumably aberrant hosts.

Steller sea lions (*Eumetopias jubatus*) are protected marine mammals present off the coast of Alaska. Alaska has thousands of kilometers of coastline, of which humans frequent only a small portion. Finding marine mammal carcasses can be challenging, and is more likely to occur in areas around cities where resources for carcass discovery and retrieval are more readily available. *Sarcocystis* spp. infections could originate from many sources across the range of the Steller sea lion, and the definitive host for *S. canis* has yet to be determined. The publication of cases in previously unreported species and geographic locations can aid in the search to determine a definitive host species for this pathogen. Here we report fatal *S. canis* hepatitis in a Steller sea lion from a remote area of Alaska.

A Steller sea lion was found floating in Muir Inlet at Glacier Bay National Park on 17 April 2010 (58°48′36.8″N; 136°05′08.2″W). The carcass appeared fresh and was secured on the beach the next day. A field necropsy was performed 5 days after it was found (22 April 2010) under Marine Mammal Protection Act permit 932-1905/MA-009526. The animal was estimated to weigh between 1,100 to 1,500 kg, was in good to average body condition, and was 243 cm long. There were no external scars or lesions aside from significant scavenging on the carcass. The liver did not show gross evidence of hepatitis and the animal was not icteric. The stomach was empty except two rectangular stones. The small intestines contained scant, dark brown, liquid feces and were highly infected with thorny-headed worms (acanthocephalans). The intestinal mucosa was normal in appearance, but petechial hemorrhages were present on the serosal surfaces and mesenteries. The kidneys were bilaterally congested, as were the mesenteric lymph nodes.

Sections of heart, lung, liver, adrenal gland, spleen, kidney, intestine, esophagus, and cartilage were fixed in 10% neutral buffered formalin. Paraffin-embedded sections were cut to 5 μm, stained with hematoxylin and eosin, and examined microscopically.

Histologically, the liver was congested and had multifocal areas of necrosis with hemorrhage, fibrin thrombi, pyknotic

debris, and rare neutrophils. Scattered necrotic hepatocytes contained *Sarcocystis* merozoites and schizonts, some arranged in rosettes. Rarely, cells consistent with Kupffer cells contained single merozoites (Fig. 1). Small numbers of lymphocytes, plasma cells, and neutrophils were seen in portal tracts.

The spleen contained foamy macrophages in the red pulp with evidence of erythrophagocytosis. Renal tubules were lined with foamy epithelial cells and scattered tubules contained orange, granular casts indicative of pigmentary nephrosis. The lung and intestine were congested and had rare fibrin thrombi in alveolar capillaries. These findings are consistent with intravascular hemolysis and probable disseminated intravascular coagulopathy. There was excessive mucus production and submucosal gland proliferation in a small section of lung, suggesting lungworm infection.

Acanthocephalans were evident on cross section of the intestine and were identified as *Corynosoma villosus*, a common parasite in piscine prey of Steller sea lions in Alaska (Moles and Heintz 2007).

Small sections of frozen liver samples were used for PCR amplification and sequencing of a 1012 base-pair segment of 18S ribosomal DNA at the US Department of Agriculture, Animal Parasitic Diseases Laboratory in Beltsville, Maryland (Rosenthal et al. 2008). Evolutionary analysis of the DNA sequence identified *S. canis* (Fig. 2), which was genetically identical to that found in a case of sarcocystosis in a polar bear from a zoo in Anchorage, Alaska (Dubey et al. 2006). The next closely related species was *Sarcocystis arctosi* of brown bears (*Ursus arctos*). The organism was genetically much different from *Sarcocystis neurona*, which is more common in marine mammals (Dubey et al. 2003).

Exposure of Steller sea lions to *S. canis* could happen along several thousand kilometers of beaches, bays, or ocean. Detection in this nonterrestrial species might suggest a definitive host that utilizes

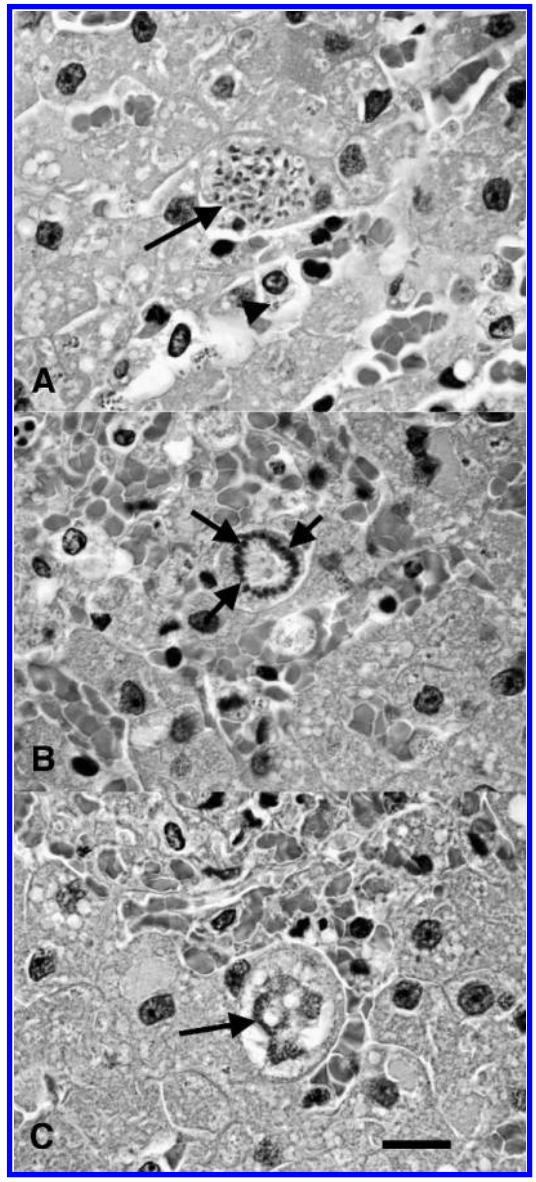

FIGURE 1. Photomicrographs of hematoxylin & eosin-stained, formalin-fixed, and paraffin-embedded sections of liver demonstrating *Sarcocystis canis* organisms in a free ranging Steller sea lion (*Eumetopias jubatus*) from southeast Alaska, USA found 17 April 2010. Bar=25 µ. (A) Numerous merozoites filling a hepatocyte (arrow). A possible Kupffer cell contains a single zoite (arrowhead). (B) A mature schizont with merozoites arranged around the periphery forming a rosette (arrows). (C) A schizont beginning to degenerate (arrow).

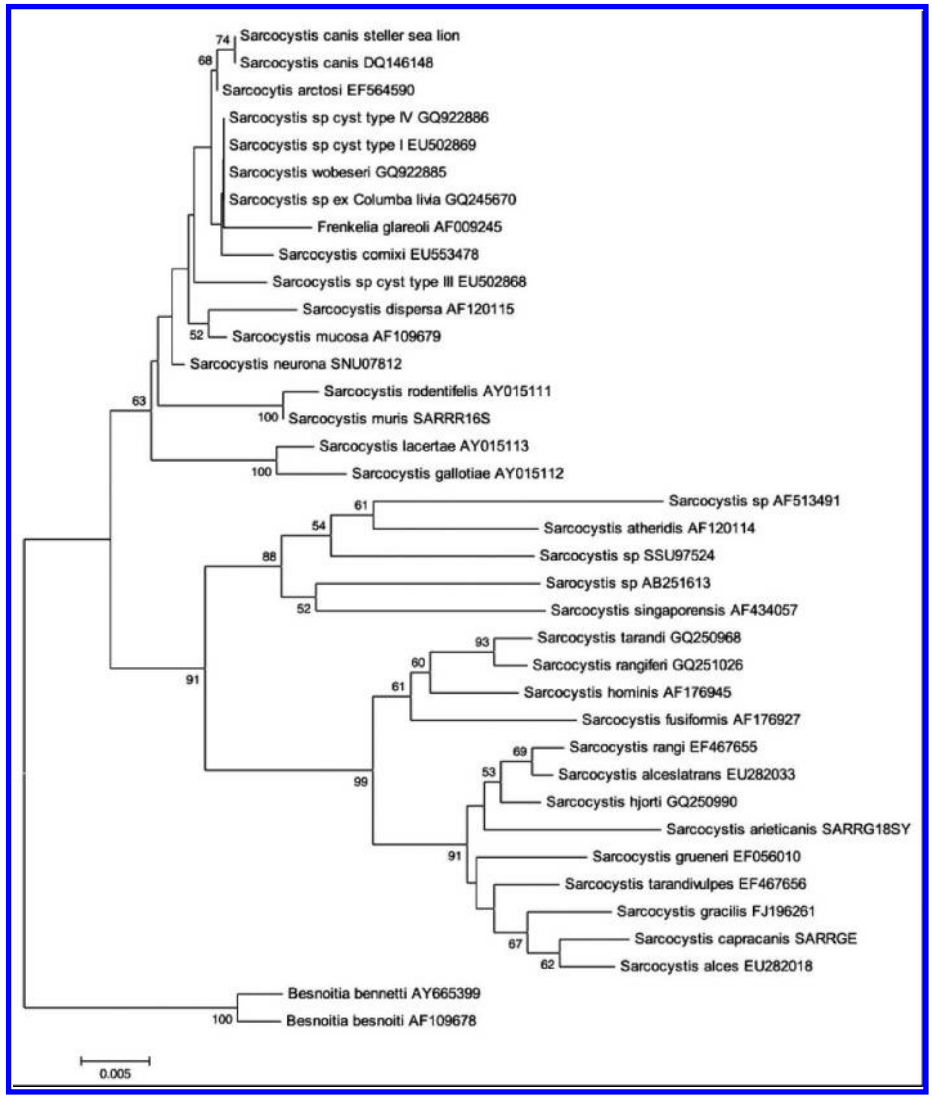

FIGURE 2. Small subunit tree showing evolutionary relationships of *Sarcocystis* spp. taxa conducted in MEGA5. The evolutionary history was inferred using the Minimum Evolution (ME) method (Rzhetsky and Nei 1993). The optimal tree with the sum of branch length = 0.30911564 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. The ME tree was searched using the Close-Neighbor-Interchange algorithm at a search level of 0. The Neighbor-joining algorithm was used to generate the initial tree. The analysis involved 37 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 766 positions in the final dataset.

marine and terrestrial environments. Because the definitive host has not been determined, reports of incidences of disease can assist in determining the definitive host species. The California sea lion has been suggested as both a definitive and intermediate host for several coccidian parasites (Colegrove et al. 2011); this supports the possibility of a marine definitive host for *S. canis*, but not sea

lions. It is difficult to determine the extent of infection in a species with a wide geographic range such as the Steller sea lion. They can travel up to ~3,500 km in a year (L. Jemison, Alaska Department of Fish and Game, pers. comm.), ample distance to visit several geographic areas (and different ecosystems) in a year. Due to the remote nature of beaches and bays across Alaska, many other cases could have occurred without discovery.

The cause of mortality in this animal was a severe necrotizing multifocal *S. canis*-associated hepatitis and is the first report in a Steller sea lion. Increasing reports in marine mammals raises the question as to the significance of sarcocystosis in pinniped populations as a whole, especially those experiencing population declines, such as Steller sea lions (Horning and Mellish 2012) and Hawaiian monk seals (Gerber et al. 2011).

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