

Diagnosis and treatment of *Sarcocystis neurona*-induced myositis in a free-ranging California sea lion

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Case Description—An underweight, lethargic adult female California sea lion (*Zalophus californianus*) became stranded along the California shore and was captured and transported to a rehabilitation hospital for assessment and care.

Clinical Findings—Initial physical assessment revealed the sea lion was lethargic and in poor body condition. Active myositis was diagnosed on the basis of concurrent elevations in activities of alanine aminotransferase and creatine kinase detected during serum biochemical analysis. Infection with *Sarcocystis neurona* was diagnosed after serologic titers increased 4-fold over a 3-week period. Diagnosis was confirmed on the basis of histopathologic findings, positive results on immunohistochemical staining, and results of quantitative PCR assay on biopsy specimens obtained from the diaphragm and muscles of the dorsal cervical region.

Treatment and Outcome—Anticoccidial treatment was instituted with ponazuril (10 mg/kg [4.5 mg/lb], PO, q 24 h) and continued for 28 days. Prednisone (0.2 mg/kg [0.09 mg/lb], PO, q 12 h) was administered for 2 days and then every 24 hours for 5 days to treat associated inflammation. At the end of treatment, the sea lion was clinically normal, alanine aminotransferase and creatine kinase values were within reference limits, and antibody titers against *S neurona* had decreased 6-fold. The sea lion was released approximately 3 months after becoming stranded.

Clinical Relevance—*S neurona*-induced myositis was diagnosed in a free-ranging California sea lion. On the basis of the successful treatment and release of this sea lion, anticoccidial treatment should be considered for marine mammals in which protozoal disease is diagnosed. (*J Am Vet Med Assoc* 2012;240:324–328)

An adult female California sea lion (*Zalophus californianus*) was observed ashore in Marin County, Calif, on January 1, 2010. Assessment of the sea lion the next day indicated it was lethargic and had multiple minor skin wounds. The sea lion was captured and transported to the Marine Mammal Center in Sausalito, Calif, for a health assessment and medical care.

Physical examination of the sea lion revealed that it was alert and inquisitive, was 155 cm (94 inches) in standard body length (ie, measured from nose to tail while in sternal recumbency; standard body length is used to estimate the age of sea lions), and was moderately underweight at 41 kg (90.2 lb).¹ It had multiple skin lacerations < 1 cm long along both pelvic extremities and several foci of hyperpigmented skin along the trunk and left cervical region. Reduced lung

ABBREVIATIONS	
ALT	Alanine aminotransferase
CK	Creatine kinase
IHC	Immunohistochemical
qPCR	Quantitative PCR

sounds were detected bilaterally via thoracic auscultation. Blood samples were collected via venipuncture of the caudal gluteal vein for a CBC, serum biochemical analysis, and serum archiving.

Results of the CBC and biochemical analysis were clinically unremarkable, except for a moderate increase in ALT activity (341 U/L; reference range,² 28 to 94 U/L) and mild hyperkalemia (5.6 mmol/L; reference range,² 4.1 to 5.1 mmol/L). Increases in serum ALT activity are

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The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of UCAR, NOAA, or the US Department of Commerce.

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typically associated with hepatocellular damage in dogs and cats.³ In California sea lions, cardiac and skeletal muscle and liver have high ALT activity; therefore, an increased ALT value is not a liver-specific finding in this species.⁴ Although hyperkalemia in marine mammals is an indication of severe disease processes such as renal failure and hypoadrenocorticism, it is also commonly caused by hemolysis and physical exertion.^{2,5}

Treatment with amoxicillin (24 mg/kg [10.9 mg/lb], PO, q 12 h for 10 days) was initiated for the skin lesions, and the sea lion was given 1 L of lactated Ringer's solution, SC, every 24 hours for 2 days. A fecal sample from the sea lion had positive results when tested for pulmonary nematodes (*Parafilaroides decorus*) and gastric roundworms (*Anisakidae* spp), which are common in California sea lions and can be pathogenic.^{1,6,7} Anti-inflammatory medication is often included in regimens for the treatment of lungworms in stranded pinnipeds to reduce inflammation and exacerbation of clinical signs associated with dying parasites.⁸ Therefore, in addition to a single dose of ivermectin^a (0.2 mg/kg [0.09 mg/lb], PO), carprofen^b was administered (4.3 mg/kg [1.95 mg/lb], PO, q 24 h, for 5 days). During this treatment period, the sea lion maintained a good appetite and gradually gained weight.

Three weeks after admission, a CBC and serum biochemical analysis were repeated as part of routine follow-up monitoring and prerelease screening. The CBC results remained within reference limits, but the serum biochemical analysis revealed an increase in ALT activity to 410 U/L and a marked increase in CK activity (9,206 U/L; reference range,² 80 to 1,058 U/L). Creatine kinase is a sensitive indicator of muscle damage⁹ and, in sea lions, is reported to be at highest concentrations in skeletal and cardiac muscle.⁴ The marked increase in CK activity and persistent mild increase in ALT activity suggested substantial muscle damage or necrosis in this sea lion. Differential diagnoses for myositis in this sea lion included infectious disease, nutritional deficiency, exertional myopathy, immune-mediated myositis, toxicosis, hypothyroidism, and hyperadrenocorticism. Active protozoal myositis was the leading differential diagnosis because there was a lack of abnormal clinical examination findings and diagnostic results consistent with other diagnoses on the differential list. Protozoal myositis was also suspected because it is commonly diagnosed in marine mammals necropsied at the Marine Mammal Center. Archived serum samples were submitted to the School of Veterinary Medicine at the University of California-Davis for protozoal serologic testing. Indirect fluorescent antibody titers against *Toxoplasma gondii* and *Neospora caninum* were < 1:40. Antibody titers against *Sarcocystis neurona* were 1:5,120 and 1:20,480 on day 1 and day 25 after capture, respectively. These results were consistent with active sarcocystosis.

Biopsy specimens were collected and evaluated to confirm the diagnosis of active sarcocystosis. Anesthesia was induced in the sea lion by administration of isoflurane in 100% oxygen via face mask. The sea lion was then intubated with an endotracheal tube and positioned in dorsal recumbency. The surgical table was tilted so that the head of the sea lion was elevated 30° to facilitate a laparoscopic approach to the diaphragm.

Spontaneous respiration was maintained throughout the procedure. A pneumoperitoneum was established with a Veress-type needle and insufflators,^c and a laparoscope portal was then created immediately cranial to the umbilicus. A 10-mm 30° forward-angle rigid endoscope^c was used to visually examine the visceral surface of the diaphragm and perform a cursory exploration of the abdomen. A 5-mm instrument sheath was inserted pericostally in the left cranial abdominal quadrant, and full-thickness biopsy specimens of the left and right sides of the diaphragmatic crura were obtained with insulated biopsy forceps. Pale streaks were widely disseminated throughout the diaphragm. No other intra-abdominal pathological lesions were observed, and skin at the portal incisions was closed with a simple horizontal mattress pattern by use of 2-0 braided silk.^d

After routine closure of the endoscope and instrument portals, the sea lion was positioned in sternal recumbency. The skin of the dorsal aspect of the cervical region was aseptically prepared, and the skin and fascia overlying the cleidocervicalis muscle were sharply incised. Approximately 1 cm³ of the cleidocervicalis muscle was excised as a biopsy specimen. The biopsy incision was closed in 2 layers with a simple continuous pattern in the subcutaneous tissue and a Ford interlocking pattern in the skin by the use of 3-0 polydioxanone^e.

During the anesthetic period, the sea lion received 400 mL of lactated Ringer's solution and dexamethasone sodium phosphate (2 mg/kg, [0.9 mg/lb], IV) via an indwelling catheter in the right jugular vein to reduce a potential inflammatory reaction to sarcocysts in the muscle tissue. Prior to extubation, the sea lion was given butorphanol tartrate (0.2 mg/kg, IM) and ceftiofur crystalline-free acid^f (6.6 mg/kg [3.0 mg/lb], SC). The sea lion had an uncomplicated recovery from anesthesia and resumed eating within 4 hours. Ten days after surgery, the sea lion was manually restrained and the sutures at the laparoscopic portals were removed.

Subsections of biopsy specimens were placed in neutral-buffered 10% formalin and submitted to the Zoological Pathology Program at the College of Veterinary Medicine of the University of Illinois at Urbana-Champaign for histologic examination. Additional subsections were submitted as fresh tissue to the School of Veterinary Medicine at the University of California-Davis for protozoal culture and isolation attempts and parasite identification by use of a qPCR assay.

Histologic examination and qPCR assays of biopsy specimens revealed infection with a protozoal organism that appeared consistent with *S. neurona*. A single sarcocyst was observed within a myofiber in a section of skeletal muscle. The cyst was approximately 35 µm in diameter; had a thick, partially septate wall; and contained 3 to 5 crescent-shaped basophilic bradyzoites (1 µm in diameter). Bradyzoites within the tissue cyst had weak staining to a polyclonal anti-*S. neurona* antibody during IHC assay. Both cervical and diaphragm muscle sections had moderate to marked lymphoplasmacytic and histiocytic myositis with degeneration, necrosis, and regeneration of myofibers. The cervical skeletal muscle was more severely affected than that of the diaphragm. Tissue specimens from the cervical muscle and diaphragm had positive

results for *S. neurona* when tested by use of a qPCR assay. Attempts to culture protozoal organisms from biopsy specimens were unsuccessful.

After the diagnosis of protozoal myositis was confirmed, prednisone was administered (0.2 mg/kg, PO, q 12 h for 2 days and then q 24 h for 5 days) to reduce the inflammatory response anticipated from antiprotozoal treatment, and ponazuril[®] (10 mg/kg [4.5 mg/lb], PO, q 24 h) was administered for 28 days. Two weeks after surgery, clinicians detected a moist cough in the sea lion and moderate swelling at the biopsy site on the dorsal aspect of the cervical region. Administration of doxycycline (3.5 mg/kg [1.6 mg/lb], PO, q 12 h) was initiated, and treatment was continued for 2 weeks. Doxycycline was indicated because of its expected spectrum of activity against common pathogens (including mycoplasmas) that affect sea lions.¹⁰ Ten days after beginning administration of ponazuril, ALT and CK values had decreased (312 U/L and 1,012 U/L, respectively) and the antibody titer against *S. neurona* was 1:10,240.

During the course of antiprotozoal treatment, the sea lion resumed appropriate activity for a pinniped in rehabilitation and continued to gradually gain weight, reaching ideal body condition by the end of treatment. Serum ALT and CK values returned to the reference range (72 U/L and 246 U/L, respectively), and the antibody titer against *S. neurona* was 1:640 at the end of ponazuril administration. The sea lion was released back into the wild 82 days after being captured.

Discussion

To our knowledge, the sea lion described here represents the first report of myositis associated with *S. neurona* infection in an otariid species. The lethargy observed at the time of stranding was likely a result of muscle pain caused by the myositis, the clinical manifestation of which may have been alleviated with anti-inflammatory medication. Tissue cysts of *Sarcocystis* spp have been detected in muscle tissue of 3 California sea lions and a northern fur seal (*Callorhinus ursinus*) during necropsy, but in each case, the tissue cysts were considered incidental findings because there was no concurrent inflammation.¹¹⁻¹⁴ The previously described¹¹⁻¹⁴ *Sarcocystis* spp tissue cysts found in skeletal muscle were all considered to be distinct from *S. neurona* on the basis of disease syndrome, parasite morphology, and results of IHC analysis. The parasite was associated with clinical disease (acute hepatic necrosis) in only 1 sea lion.¹² In the sea lion described here, the protozoal parasite in the cervical skeletal muscle was indistinguishable from *S. neurona* on the basis of structural characteristics of the protozoal cyst, results of IHC staining, serologic titers, and results of qPCR assay. Characterization of additional parasite stages was not possible because parasite culture from tissue specimens was unsuccessful. It is possible the sea lion was infected with an *S. neurona*-like parasite that cross-reacted with *S. neurona* during IHC analysis and serologic testing. Only 1 genetic locus was evaluated, and although the qPCR test was specific for *S. neurona* on the basis of sequences currently deposited in GenBank,¹ a closely related but undescribed *Sarcocystis* sp that infects sea

lions may have a highly homologous sequence at the genetic locus that was amplified and sequenced in this report.

Mild lymphocytic myositis associated with *Sarcocystis* spp tissue cysts was observed in skeletal muscle and the diaphragm of a white-sided dolphin (*Lagenorhynchus acutus*).¹⁵ Although *Sarcocystis* spp tissue cysts have been observed in many other species of marine mammals, they have been considered incidental findings because inflammation was not associated with the cysts.¹⁶⁻²¹ In contrast, the sea lion described here had moderate to severe myositis detected during histologic examination. This finding, along with the clinical signs and substantial increases in ALT and CK activities, suggested the myositis was generalized. Although uncommon, clinically relevant myositis characterized by lymphohistiocytic inflammation has been described in a dog infected with *S. neurona*²² and in horses infected with *Sarcocystis fayeri*.²³ Myositis has also been reported in another dog,²⁴ humans,²⁵ and livestock^{26,27} infected with other *Sarcocystis* spp, but the inflammation associated with sarcocysts in the skeletal muscle was predominantly eosinophilic and pyogranulomatous. Differences in the inflammatory response to sarcocystosis could be caused by species-specific differences in parasite pathogenicity, immunocompetency of the infected animal, and comorbid conditions, including secondary autoimmune inflammatory processes.

Ponazuril is an anticoccidial drug effective against apicomplexan parasites in multiple species.²⁸⁻³⁰ It has been approved by the FDA to treat equine protozoal myeloencephalitis caused by *S. neurona*. Ponazuril was used to successfully treat a captive Pacific harbor seal (*Phoca vitulina*) infected with *S. neurona*.³¹ That harbor seal had neurologic signs similar to those described in other harbor seals^{32,33} with *S. neurona* infections, the most important of which were associated with cerebellar involvement. The harbor seal was initially treated with ponazuril (5 mg/kg [2.3 mg/lb], PO, q 24 h); however, improvement in clinical signs plateaued after treatment for 2 weeks, and the dosage was increased to 10 mg/kg. Adjunct treatment with additional anticoccidial drugs was instituted after a relapse of clinical signs. The authors of that report³¹ speculated that initial treatment with ponazuril at 10 mg/kg, PO, every 24 hours may have shortened the clinical course of the disease, thus providing guidance for the treatment of the sea lion described here. No adverse effects were observed during the course of treatment in the sea lion reported here.

For both the captive harbor seal in the other report³¹ and the sea lion reported here, anticoccidial treatment was instituted early in the course of the disease, which likely contributed to therapeutic success. In another report,¹¹ southern sea otters (*Enhydra lutris nereis*) and harbor seals with *S. neurona* infections were frequently unresponsive to treatment with anticoccidial drugs once neurologic signs were apparent, and a high fatality rate was observed in the early stages of anticoccidial treatment. Stranded sea otters and harbor seals with neurologic signs likely had advanced disease and comorbid conditions that complicated treatment and potential recovery. For the sea lion described here, myositis was diagnosed early in the course

of the disease. In contrast to previous reports of marine mammals with *S neurona* infections, this sea lion did not display any signs attributable to CNS involvement. However, neither CSF nor CNS tissue was available for diagnostic testing, and the presence of *S neurona* in CNS tissue could not be ruled out.

Muscle biopsy specimens that contained sarcocysts were collected 38 days after admission. In general, bradyzoites of *Sarcocystis* spp form 75 days after inoculation.²⁶ Given this timeline and the serum antibody titer of 1:5,120 against *S neurona* on the day of admission, the sea lion reported here was most likely infected before being captured and admitted to the rehabilitation facility. Although the indirect fluorescent antibody test has not been validated in California sea lions, the 4-fold increase in antibody titer in the 3 weeks following admission was indicative of a recent and active *S neurona* infection and not simply previous exposure. The antibody titers recorded during the course of diagnosis and treatment of the myositis in the sea lion reported here were consistent with antibody titers against *S neurona* in other marine mammals with confirmed infections.^{11,31,33}

Sarcocystis neurona has a complex 2-host life cycle, with a multitude of intermediate hosts, but the opossum (*Didelphis virginiana*) is the only known definitive host.²⁹ A high prevalence of exposure to *S neurona* has been reported for several marine mammal species, which suggests there is exposure via environmental contamination with sporocysts originating from opossum feces.^{11,13} Freshwater runoff has been linked to other important protozoal diseases in marine mammals and humans^{34–36} and has been proposed as a means by which sporocysts from opossum feces are transported from land to sea.¹¹ Unidentified intermediate hosts in the marine environment may exist, thereby increasing the potential for exposure in marine mammal species through the marine food web.^{11,13} Vertical transmission is an important route of infection for *T gondii* in humans³⁶ and has been confirmed for *T gondii* in terrestrial and marine mammals.^{11,37,38} Vertical transmission of *S neurona* has not been reported to our knowledge; however, the identification of *S neurona* infection in a 1-week-old Pacific harbor seal pup suggests that there may be transplacental or transmammary transmission.³²

Diagnosis of *S neurona*-associated myositis in the stranded California sea lion reported here is supported by our clinical and pathological findings of generalized myositis, increasing antibody titers against *S neurona*, and molecular confirmation of *S neurona* infection in muscle tissue as well as a positive clinical response to anticoccidial treatment. The clinical disease associated with *S neurona* infection in this California sea lion differed from that of previously reported *S neurona* infections in marine mammals and reflected the presence of the pathogen and associated inflammation in skeletal muscle, as opposed to neuronal tissue. Aggressive administration of anticoccidial medication and early intervention likely contributed to the successful treatment of this sea lion and its subsequent release back into the wild.

- a. Rimadyl, Pfizer Animal Health, New York, NY.
b. Noromectin, Norbrook Laboratories Ltd, Station Works, Newry, County Down, Northern Ireland.

- c. Karl Storz Veterinary Endoscopy-America Inc, Goleta, Calif.
d. 2-0 silk, Arrow International, Research Triangle Park, NC.
e. PDS II, Ethicon Inc, Somerville, NJ.
f. Excede, Pfizer Animal Health, New York, NY.
g. Marquis 15% wt/wt, Bayer Corp, Shawnee Mission, Kan.
h. Olineka T, University of California-Davis, Davis, Calif: Personal communication, 2010.

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From this month's AJVR

Evaluation of circulating concentrations of glucose homeostasis biomarkers, progesterone, and growth hormone in healthy Elkhounds during anestrus and diestrus

Malin Mared et al

Objective—To investigate whether circulating concentrations of biomarkers of glucose homeostasis, progesterone, and growth hormone in healthy female Elkhounds differ during diestrus and anestrus and compare those findings with data from dogs of other breeds.

Animals—22 healthy female dogs of Elkhound breeds (known to have a high incidence of diestrus-associated diabetes mellitus) and 18 healthy female non-Elkhound dogs.

Procedures—For each dog, a blood sample (12 mL) was collected once during anestrus and once 2 to 8 weeks after cessation of estrual bleeding. Serum or whole blood samples were analyzed for glucose, growth hormone, insulin-like growth factor-1, C-peptide, fructosamine, and glycated hemoglobin A1c concentrations. Homeostasis model assessments (HOMAs) of pancreatic beta-cell function and insulin secretion were calculated.

Results—In Elkhounds, C-peptide concentration and the HOMA for beta-cell function (markers of insulin secretion) were higher in samples obtained during diestrus, compared with findings in samples obtained during anestrus. The HOMA for insulin sensitivity was lower (albeit not significantly) during diestrus than it was during anestrus in Elkhounds. Markers of insulin secretion and sensitivity were similar during anestrus and diestrus in the dogs of other breeds. Serum progesterone concentrations were greater during diestrus than during anestrus in Elkhounds and non-Elkhound dogs. All other variables did not differ between diestrus and anestrus within or between the 2 breed groupings.

Conclusions and Clinical Relevance—Results provided evidence that circulating insulin concentrations during diestrus are higher than those during anestrus in Elkhounds, which could contribute to development of diestrus-associated diabetes mellitus. (*Am J Vet Res* 2012;73:241–246)



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