Evaluation of cardiac lesions and risk factors associated with myocarditis and dilated cardiomyopathy in southern sea otters (Enhydra lutris nereis)

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Objective—To describe cardiac lesions and identify risk factors associated with myocarditis and dilated cardiomyopathy (DCM) in beach-cast southern sea otters.

Animals—Free-ranging southern sea otters.

Procedure—Sea otters were necropsied at the Marine Wildlife Veterinary Care and Research Center from 1998 through 2001. Microscopic and gross necropsy findings were used to classify sea otters as myocarditis or DCM case otters or control otters. Univariate, multivariate, and spatial analytical techniques were used to evaluate associations among myocarditis; DCM; common sea otter pathogens; and potential infectious, toxic, and nutritional causes.

Results—Clusters of sea otters with myocarditis and DCM were identified in the southern aspect of the range. Risk factors for myocarditis included age, good body condition, and exposure to domoic acid and Sarcocystis neurona. Myocarditis associated with domoic acid occurred predominantly in the southern part of the range, whereas myocarditis associated with S neurona occurred in the northern part of the range. Age and suspected previous exposure to domoic acid were identified as major risk factors for DCM. A sample of otters with DCM had significantly lower concentrations of myocardial L-carnitine than control and myocarditis case otters.

Conclusions and Clinical Relevance—Cardiac disease is an important cause of death in southern sea otters. Domoic acid toxicity and infection with S neurona are likely to be 2 important causes of myocarditis in sea otters. Domoic acid–induced myocarditis appears to progress to DCM, and depletion of myocardial L-carnitine may play a key role in this pathogenesis. (Am J Vet Res 2005;66:289–299)
the Picornaviridae family, similar to cosxackievirus, is an important cause of severe myocarditis and acute heart failure in a range of species, including nonhuman primates, pigs, rodents, marsupials, and elephants.  

Dilated cardiomyopathy has been recognized in dogs with certain breed predilections; however, cardiac inflammation is not a common feature of this condition in dogs, and most cases are classified as idiopathic. Familial cardiomyopathy has been linked to inherited l-carnitine deficiency in humans, dogs, and Syrian hamsters. Cardiomyopathy has been induced in rodents fed l-carnitine–deficient diets. Cats are susceptible to cardiomyopathy when fed commercial diets deficient in taurine; however, severe cardiac inflammation is not a common feature of this condition. Vitamin E and selenium deficiency cause a complex disorder in young swine and other animals that involves myocardial necrosis and hemorrhage. A complete investigation of risk factors for cardiac disease in sea otters must include evaluation of potential causal factors for myocarditis and DCM that have been described in other species, while prioritizing pathogens with a reasonable probability of occurring in wild carnivores in the marine environment. Availability of appropriate veterinary diagnostic tests must also be considered. Furthermore, cardiac disease in otters may be linked to disease entities that have already been described in this population, such as Toxoplasma gondii, Sarcocystis neurona, and domoic acid intoxication. Toxoplasmosis is an important cause of myocarditis and congestive heart failure in humans infected with HIV and immune-suppressed heart transplant patients. Myocarditis attributable to toxoplasmosis has also been reported in marine mammals, including a captive California sea lion (Zalophus californianus), a northern fur seal (Callorhinus ursinus), and domoic acid intoxicated northern sea otter stranded in Florida. Sarcocystis neurona has been implicated as a cause of myocarditis in raccoons, and S. neurona sarcocysts have been identified in sea otter myocardium. Domoic acid, a marine toxin produced by Pseudonitzschia australis, is a common cause of death in sea lions, and heart lesions (including myocardial pallor, myocardial hemorrhage, and fibrous epicarditis) were detected in addition to myocardial inflammation. Control otters included all otters with minimal or no myocardial inflammation. Dilated cardiomyopathy case otters included all otters with grossly enlarged, dilated atria and ventricles noted by the pathologist at necropsy and without cardiac lesions (n = 27). 

Sea otters were classified as cases or controls on the basis of 2 separate case definitions for cardiac disease. Myocarditis case otters included all otters with mild to severe, nonsuppurative (lymphocytic) myocardial inflammation on microscopic examination of H&E-stained cardiac tissue. Myocarditis control otters included all otters with minimal or no myocardial inflammation. Dilated cardiomyopathy case otters included all otters with grossly enlarged, dilated atria and ventricles noted by the pathologist at necropsy and without cardiac lesions (n = 27).
DCM did not have gross cardiac chamber enlargement and myocarditis and were therefore the same individuals classified as myocarditis control otters. Otters without DCM, but with myocarditis, were excluded from the DCM case-control analyses of potential risk factors to prevent misclassification of myocarditis case otters as control otters if myocarditis and DCM are actually part of the same disease process.

Classification of demographic and environmental risk factors—Each otter's stranding date and location were recorded at the time of carcass recovery. Stranding location was assigned consecutive values to the nearest 0.5-km increment along a smoothed California coastline. Otters were classified by age on the basis of tooth eruption at necropsy as juveniles (those with milk teeth) and adults (all adult dentition). Body condition was determined by the amount of subcutaneous fat detected at necropsy, and otters were classified as having good body condition if abundant to moderate subcutaneous fat was detected. Otters classified with poor body condition had scant to no subcutaneous fat. Adult females were examined to determine if they were lactating at the time of death. Nose wounds (presumably incurred during mating) detected at necropsy were classified as recent and severe or minor if wounds were small, old, or absent.

Sample collection and evaluation of possible causes of myocarditis and DCM—Representative samples from all major tissues were placed in neutral-buffered 10% formalin at necropsy. Blood was collected from the heart and major vessels and centrifuged at 25,000 rpm for 10 minutes. The resulting upper (serum) fraction was aliquoted into cryotubes and stored in liquid nitrogen or at −80°C until used for laboratory analysis. In addition, 30- to 50-g samples of myocardium, liver, and pectoral muscle were collected from a subset of otters, placed in aluminum foil or plastic bags, and frozen at −80°C until used for analysis.

Disease agents that have been previously recognized in sea otters and linked to cardiac disease in other species were evaluated in every otter with available samples. Exposure to *T gondii* was evaluated by use of a previously validated indirect immunofluorescent antibody test (IFAT) on all available serum (n = 84). A positive cutoff titer of ≥ 1:320 serum dilution was used, which maximizes sensitivity and specificity of this test. Exposure to *S neurona* was also evaluated by use of an IFAT (n = 83). Because the specificity and sensitivity of this test to *S neurona* in sea otters are not known, 3 different positive cutoff titers (≥ 80, ≥ 320, and ≥ 640) were evaluated independently as potential risk factors for myocarditis and DCM. In addition, immunohistochemistry was performed to evaluate sea otter myocardium for the intracellular protozoal stages with polyclonal antiserum to *T gondii* and *S neurona*, as described. Immunohistochemical stains were applied to 3-μm paraffin sections of cardiac tissue from 12 myocarditis case otters (including 10 DCM case otters), 5 otters seropositive for *T gondii* (1 myocarditis case otter and 4 control otters), 5 otters seropositive for *S neurona* (3 myocarditis case otters and 2 control otters), and 6 otters seronegative for *T gondii* and *S neurona* (1 myocarditis case otter and 5 control otters). A quantitative real-time polymerase chain reaction (PCR) assay to detect *T gondii* and *S neurona* RNA was applied to cryopreserved brain and myocardium from 6 myocarditis case otters and 2 control otters to detect *T gondii* and *S neurona*—specific, single-stranded RNA in cardiac tissues. The PCR assay for *T gondii* was performed as previously described and the PCR assay for *S neurona* (Genbank accession No. U07812) was developed according to the same protocols. Otters with varied serologic responses to both parasites were evaluated, including 4 myocarditis case otters seropositive for *T gondii* (including 3 cases with DCM as well), 1 myocarditis case otter seropositive for *S neurona*, 1 control otter seropositive for *S neurona*, 1 seronegative myocarditis case otter with DCM, and 1 control otter seronegative for both parasites.

Additional potential infectious risk factors for cardiac disease in humans and terrestrial animals that have not been recognized as pathogens in sea otters were initially evaluated by use of serum from a subset of ≥ 16 age-matched myocarditis case otters (including 6 DCM case otters). Because none of these serologic tests have been validated in sea otters, positive cutoff titers indicative of previous infection in other species were used. Pathogen exposure was performed by use of a guinea pig complement fixation test for *Chlamydia psittaci* with a positive cutoff titer ≥ 1:40, a hemagglutination inhibition test for canine parovirus-2 (CPV-2) with a positive cutoff titer ≥ 1:40, and serum neutralization tests for canine adenovirus-1 (CAV-1) and EMCV with a positive cutoff titer ≥ 1:32. The microscopic agglutination test was used to detect exposure to *Leptospira interrogans* serovars pomona, hardjo,icterohaemorrhagiae, grippotyphosa, and canicola by use of a positive cutoff titer ≥ 1:100. Because of seropositive responses on initial screening, the sample size for *L interrogans* serovar testing was later increased to include 12 control otters and the sample size for EMCV was increased to include 10 additional myocarditis case otters and 13 control otters. Because EMCV has been detected in chronically infected seronegative pigs, an immunomagnetic reverse transcriptase-polymerase chain reaction (RT-PCR) technique designed to detect EMCV was applied to cryopreserved brain or myocardial samples from 9 DCM case otters, 7 myocarditis case otters, and 13 control otters.

Potential noninfectious causes of cardiomyopathy included nutritional deficiencies and toxicant exposure. Tissue concentrations of the essential nutrients vitamin E, selenium, taurine, and carnitine were evaluated in tissue samples from 9 age-matched myocarditis case otters (including 7 DCM case otters) and 9 control otters. Selenium concentrations in livers of sea otters were determined by inductively coupled plasma spectrometry with hydride generation. Vitamin E concentrations in livers were determined by use of high-performance liquid chromatography with fluorescence detection. Taurine concentrations in livers were measured in milligrams per gram of wet weight of liver, as previously described. Total t-carnitine concentration was measured in nanomoles per milligram of noncollagen protein (NCP) skeletal muscle to minimize error caused by muscle atrophy, myofiber loss, or fibrosis. Frozen cardiac tissues from 4 myocarditis case otters (3 with DCM) and 1 control otter were also analyzed for taurine and t-carnitine concentrations, as previously described. To increase sample size for t-carnitine measurements, myocardium samples from 10 additional myocarditis case otters (with 4 DCM case otters) and 5 control otters were later obtained from sea otters necropsied after June 2001. The same criteria for classification of cardiac disease status were applied to those cases to ensure comparability of data.

Domoic acid is rapidly cleared after ingestion and presently available laboratory techniques would not be useful for assessing past exposure to domoic acid in necropsied sea otters. Therefore, sea otters stranded within a temporal and spatial vicinity of sea otters identified with acute death attributable to domoic acid intoxication were classified as having suspected previous exposure to domoic acid. From 1998 through June 2001, domoic acid intoxication was identified as the primary cause of death in 4 sea otters. Domoic acid exposure was confirmed in those otters by detection of domoic acid in urine and gastrointestinal contents by use of a receptor-binding assay and when possible, results were confirmed by liquid chromatography-tandem mass spec-
Sea otters were classified as having suspected dacaric acid exposure if they were stranded 1 week before and as much as 12 weeks after a sea otter with confirmed acute dacaric acid intoxication, provided their stranding location was within 50 km of the stranding location for an otter with acute dacaric acid intoxication. Twelve weeks was chosen as a cutoff for exposure to dacaric acid to account for possible environmental persistence of dacaric acid in sea otter prey, as detected in razor clams, or for long-term postexposure effects, as reported in rodent models and California sea lions.

Statistical analyses—Associations between myocarditis and DCM and various individual, demographic, and pathogen risk factors were evaluated by use of a 1-sided $\chi^2$ test, Fisher exact test, and the odds ratio (OR). Confounding and effect modification were evaluated for significant associations by stratifying on secondary risk factor variables and comparing the OR for individual strata. If confounding on the stratified variables was determined to be substantial (>10% of the OR), the adjusted Mantel-Haenszel test OR was reported.

The nutritional factors (vitamin E, selenium, taurine, and carnitine concentrations) measured for a subset of case and control otters (n = 39), and gross lesions consistent with DCM were observed in 11% of otters (10) included in the study. For otters with histologically confirmed myocarditis, gross findings at necropsy included orange-white streaking of the ventricular myocardium (9/39) and congestive heart failure (18/39) characterized by pulmonary edema in conjunction with pleural effusion, hepatomegaly and centrilobular hepatic congestion, or peritoneal effusion. The inflammatory infiltrate in otters with myocarditis was multifocal to diffuse and was most concentrated in the subepicardial and subendocardial myocardium. Inflammatory cells were observed in both the atrial and ventricular myocardium. The distribution of histopathologic findings common to myocarditis and DCM case otters was determined (Table 1).

All DCM case otters included in the study had lymphocytic myocarditis, which was considered severe in 6 of 10 DCM case otters. Gross cardiac enlargement in otters with DCM ranged from mild (4/10) to moderate (3/10) and severe (3/10). Orange-white myocardial streaking was detected in 7 of 10 otters with DCM. All otters with DCM had pulmonary edema and pleural effusion, most (9/10) had hepatomegaly and hepatic congestion, and 2 had marked peritoneal effusion. Three DCM case otters with severe myocardial inflammation evaluated by immunohistochemical lymphocyte markers had predominantly T-cell infiltrates. One myocarditis case otter with lymphocytic inflammation and intracellular T gondii had equal numbers of B and T cells, as did 1 otter with fatal shark-bite wounds and mild supplicative myocardial inflammation.

Univariate evaluation of risk factors—Myocarditis was more common in adult than juvenile otters (36/39 myocarditis case otters were adults, com-

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mild myocardial inflammation</th>
<th>Moderate myocardial inflammation</th>
<th>Severe myocardial inflammation</th>
<th>Lymphocytic ganglioneuritis</th>
<th>Myofiber necrosis</th>
<th>Interstitial fibrosis</th>
<th>Myocardial congestion</th>
<th>Myofiber vacuolization</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>2/10</td>
<td>2/10</td>
<td>6/10</td>
<td>2/10</td>
<td>0/10</td>
<td>6/10</td>
<td>3/10</td>
<td>6/10</td>
</tr>
</tbody>
</table>

Vacuolization may have been a consequence of tissue autolysis.
pared with 29/56 control otters; \( P < 0.001 \), and DCM was detected only in adult otters (\( P = 0.004 \)). Both myocarditis and DCM were distributed evenly among males and females. Myocarditis was more common in otters found dead in good nutritional body condition (19/27) than in otters with poor body condition (19/67; \( P < 0.001 \)). Like most beach-cast otters, most DCM case otters (8/10) were in poor or emaciated body condition. Adult otters with DCM were 12.5 times as likely to have severe and recent nose wounds than control otters (Mantel-Haenszel OR 95% CI, 2.2 to 71.4; \( P = 0.002 \)). Means for heart measurements and weights obtained from myocarditis case, DCM case, and control otters did not differ significantly, possibly because of low statistical power.

Seroprevalence to \( T \) gondii; \( S \) neurona; EMCV; CPV-2; CAV-1; \( Chlamydia \) psittaci; and \( L \) interrogans serovars pomona, hardjo, icterohaemorrhagiae, grippotyphosa, and canicola among myocarditis case, DCM case, and control otters was determined (Table 2). Results of serologic tests for EMCV, \( T \) gondii, \( S \) neurona, and all 5 \( L \) interrogans serovars were positive in some myocarditis case otters, and testing of control otters was warranted to rule out an association with cardiac disease. Only seropositivity to \( T \) gondii and \( S \) neurona was significantly associated with myocarditis. Otters that were seropositive to \( T \) gondii were 3.5 times as likely to have myocarditis as seronegative otters (\( P = 0.008 \)), and otters that were seropositive to \( S \) neurona with titers \( \geq 1:320 \) were 3.6 times as likely to have myocarditis as seronegative otters (\( P = 0.013 \)). Seropositivity to \( S \) neurona at the \( \geq 1:80 \) titer cutoff was not associated with myocarditis, whereas seropositivity at the \( \geq 1:640 \) titer did not differ from the \( \geq 1:320 \) titer cutoff in significance or degree of association with myocarditis. Seropositivity to \( T \) gondii was associated with DCM, with seropositive otters being 7.0 times as likely to have DCM than seronegative control otters (\( P = 0.052 \)). All otters with DCM were seronegative for \( S \) neurona. Because exposure to both \( T \) gondii and \( S \) neurona was associated with stranding location in univariate analyses and sample size for exposure to these pathogens was sufficient in myocarditis case and control otters, the association between seropositivity and myocarditis was stratified by location. Exposure to both protozoal parasites was significantly associated with myocarditis in only the most northern portion of the sea otter range (from Pacifica to Moss Landing), even though carcass retrieval for study otters was evenly distributed in the 4 location categories evaluated. In this northern region, otters seropositive for \( T \) gondii were 9.6 times as likely (stratified OR 95% CI, 1.1 to 119.9) to have myocarditis than were seronegative otters. Otters seropositive for \( S \) neurona were 15.0 times as likely (stratified OR 95% CI, 1.6 to 191.0) to have myocarditis than were seronegative otters.

Results of immunohistochemical staining for \( T \) gondii were negative for all sea otter myocardium examined, including the 5 otters seropositive for \( T \) gondii. Results of immunohistochemical staining for \( S \) neurona were positive in the myocardium for 3 of 4 myocarditis case otters seropositive for \( S \) neurona. Myocardium in 2 of these otters seropositive for \( S \) neurona contained merozites that had positive results for whole parasite staining with anti-\( S \) neurona serum. Only sarcocysts were detected in the third otter, which had scattered granular staining of bradyzoites and variable but faint staining of cyst walls. Weak staining of sarcocysts could be attributable to another \( Sarcocystis \) sp, but a similar pattern of staining for bradyzoites was detected in raccoons experimentally infected with \( S \) neurona. The PCR assay detected \( S \) neurona RNA in brain tissue but not the myocardium in 1 of 2 \( S \) neurona

### Table 2—Seroprevalence of specific pathogens in beach-cast southern sea otters with myocarditis or DCM, and otters with minimal or no myocardial inflammation (controls).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Toxoplasma gondii</th>
<th>Sarcocystis neurona</th>
<th>EMCV</th>
<th>CPV-2</th>
<th>CAV-1*</th>
<th>Chlamydia psittaci*</th>
<th>Leptospira interrogans serovar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>24/48</td>
<td>74/47</td>
<td>9/13</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1/12</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>28/36</td>
<td>14/36</td>
<td>12/25</td>
<td>0/10</td>
<td>0/7</td>
<td>0/5</td>
<td>0/12</td>
</tr>
<tr>
<td>DCM</td>
<td>7/8</td>
<td>0/8</td>
<td>3/6</td>
<td>0/6</td>
<td>0/5</td>
<td>0/3</td>
<td>0/6</td>
</tr>
</tbody>
</table>

*Serologic test for canine adenovirus-1 (CAV-1) had 3 indeterminate results. "Serologic test for \( Chlamydia \) psittaci" had 11 indeterminate results.


**Table 3—Nonparametric 90% reference limits for nutritional parameters measured in beach-cast southern sea otters.**

<table>
<thead>
<tr>
<th>Nutritional parameters</th>
<th>Reference limit</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E (mg/kg wt liver)</td>
<td>11.6–84.9</td>
<td>29</td>
</tr>
<tr>
<td>Selenium (mg/kg wt liver)</td>
<td>0.62–4.06</td>
<td>29</td>
</tr>
<tr>
<td>Taurine (mg/g liver)</td>
<td>0.57–2.48</td>
<td>26</td>
</tr>
<tr>
<td>l-carnitine (nmol/g NCP skeletal muscle)</td>
<td>0.62–5.93</td>
<td>40</td>
</tr>
</tbody>
</table>

*Concentrations of vitamin E in liver were lower in otters in good body condition, compared with otters in thin body condition. Concentrations of selenium in liver were higher in otters in the northern part (Pacifica to Moss Landing) of the sea otter range, compared with the remainder of the range. NCP = Noncollagen protein.

**Table 4—Median (range) of l-carnitine (nmol/g NCP) in cardiac and skeletal muscle from beach-cast southern sea otters with myocarditis or DCM and otters with minimal or no myocardial inflammation (controls).**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Controls</th>
<th>Myocarditis</th>
<th>DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-carnitine in cardiac muscle (n = 20)</td>
<td>3.55a</td>
<td>3.95b</td>
<td>1.83b</td>
</tr>
<tr>
<td>Median</td>
<td>(3.90–5.04)a</td>
<td>(3.26–4.78)b</td>
<td>(1.10–2.60)b</td>
</tr>
<tr>
<td>l-carnitine in skeletal muscle (40)</td>
<td>1.88</td>
<td>2.76</td>
<td>3.56</td>
</tr>
<tr>
<td>Median</td>
<td>(0.30–4.79)</td>
<td>(0.80–11.48)</td>
<td>(0.62–5.29)</td>
</tr>
</tbody>
</table>

*Within a row, values with different superscript letters were significantly (\( P < 0.05 \)) different.
seropositive control otters tested and detected T gondii RNA in myocardial tissue but not brain tissue from 1 myocarditis case otter seropositive for T gondii out of 3 otters seropositive for T gondii. The RT-PCR technique used to detect EMCV RNA was negative for all otters tested, including those otters that were seropositive to EMCV.

The correlate created here to estimate past domoic acid exposure was highly associated with both myocarditis and DCM. Similar to T gondii and S neurona, prevalence of domoic acid exposure varied greatly among otters in the various geographic regions, and exposure to domoic acid was only significantly associated with myocarditis in 1 stranding location. All 6 otters suspected of being exposed to domoic acid from San Simeon to Morro Bay had myocarditis, compared with 2 myocarditis case otters out of 15 suspected unexposed otters (P = 0.001). Exposure to domoic acid was perfectly correlated with DCM in this region because all 4 DCM case otters had a history of domoic acid exposure and all 13 control otters were not suspected of being exposed to domoic acid (P < 0.001).

Nonparametric reference limits for vitamin E and selenium in liver tissue, taurine and carnitine in skeletal muscle, and taurine in cardiac muscle were determined (Table 3). Concentrations of vitamin E in liver were lower in otters with good body condition, compared with otters in thin body condition (P = 0.022), whereas concentrations of t-carnitine in skeletal muscle were higher in otters with good body condition, compared with otters in thin body condition (P = 0.005). Concentrations of selenium in liver differed significantly by stranding location (P = 0.017), with higher concentrations in the northern part of the sea otter range (median selenium concentration from Pacifica to Moss Landing was 2.82 mg/kg wet weight of liver, compared with 1.63 mg/kg in the remainder of the range). Concentrations of t-carnitine in cardiac muscle of DCM case otters were lower than myocarditis case otters and control otters (P = 0.002); in fact, the range for cardiac t-carnitine concentration for all DCM case otters was less than the range for myocarditis case otters and control otters (Table 4). Concentrations of t-carnitine in skeletal muscle were not significantly different among DCM case otters, myocarditis case otters, and control otters.

Spatial and temporal cluster analyses—A high-risk spatial-temporal cluster of myocarditis was detected in a 57-km section of the southern part of the sea otter range extending from 5 km south of Morro Bay to Pismo Beach (centered at 35.141 N latitude, 120.652 W longitude) from May 18, 2000 to April 18, 2001 (Figure 1). All 8 sea otters stranded in this area during this period had myocarditis, which was 2.4 times the rate of occurrence expected if this condition was randomly distributed along the coast (P = 0.071). Sea otter carcasses were not retrieved from the remote and rocky 140-km section of coastline in the center of the sea otter range; therefore, no inferences could be made about the prevalence of cardiac disease in this area.

Purely temporal high-risk clusters of myocarditis and DCM were also identified from July 13 through August 9, 2000. All 6 sea otters that were recovered during this period had myocarditis, which was 2.4 times the rate of occurrence expected if myocarditis was distributed randomly during the period of study (P = 0.004). Three of these 6 myocarditis case otters had DCM, which was 6.7 times the expected rate of occurrence for DCM during this period (P = 0.002). A purely spatial low-risk cluster of myocarditis was detected in a 25-km section of the range in Monterey Bay from Seaside to Pacific Grove (centered at 36.661 N, 121.825 W), where only 2 of 20 stranded sea otters had myocarditis (P = 0.095).

Multivariate analysis of risk factors—Age at death, sex, body condition at death, the presence of nose wounds at death, stranding location, stranding date, exposure to T gondii, exposure to S neurona, and suspected exposure to domoic acid were evaluated for associations with myocarditis and DCM by use of logistic regression modeling. Because of the large number of otters with myocarditis, the interaction terms for T gondii, S neurona, and domoic acid exposure by the...
south (vs north) stranding location could also be evaluated for an association with myocarditis. Of all variables and interaction terms evaluated, age, body condition, exposure to *S. neurona*, and exposure to domoic acid were significantly associated with myocarditis and this model had overall good fit (Hosmer-Lemeshow $\chi^2 = 3.18; P = 0.868$, Table 5). The log odds ($\log_{e}$) of myocarditis was predicted by use of the following logistic model:

$$\log_{e} \frac{P(x)}{1 - P(x)} = -3.61 + 1.80(\text{adult at death}) + 1.85 \quad \text{(good body condition)} + 2.24(\text{exposure to } S \text{ neurona}) + 2.36 \quad \text{(exposure to domoic acid)} + 0.35(\text{south stranding location}),$$

where $P(x) =$ probability of an otter having myocarditis at death. Otters exposed to *S. neurona* were >9 times as likely to have myocarditis than unexposed otters, and otters exposed to domoic acid were >11 times as likely to have myocarditis than unexposed otters, with all other variables being equal.

Although the main effect for the southern stranding location and the interaction terms for *S. neurona* and domoic acid exposure by southern stranding location did not significantly predict myocarditis, the stratified univariate analyses were consistent with modification of the effect of *S. neurona* and domoic acid by stranding location. Therefore, stranding location was forced into the logistic model, and separate logistic models were created for northern and southern stranding sea otters to determine if these subpopulations differed in risk factors for myocarditis. For northern stranding sea otters, only exposure to *S. neurona* was predictive of myocarditis, whereas exposure to domoic acid and good body condition were significantly predictive of myocarditis in the south (Table 5).

Because all DCM case otters were adults at death, all juvenile otters (n = 20) were excluded from the logistic model to prevent perfect correlation of predictor and outcome variables. Therefore, only 39 otters could be included in the logistic regression model for DCM. The only risk factor significantly associated with DCM in sea otters was suspected exposure to domoic acid. Sea otters suspected to have been exposed to domoic acid were 31.5 times as likely to have DCM than otters that were not suspected to have been exposed to domoic acid (asymptotic OR 95% CI, 4.4 to 226.5; Hosmer-Lemeshow $\chi^2 < 0.01; P > 0.999$). The exact estimation procedure yielded an OR of 26.9 with slightly wider CI (exact OR 95% CI, 3.4 to 384.4). The Loge of DCM was predicted by use of the following asymptotic logistic model:

$$\log_{e} \frac{P(x)}{1 - P(x)} = -2.20 + 3.45(\text{exposure to domoic acid}),$$

where $P(x) =$ probability of an otter having dilated cardio-myopathy at death.

### Discussion

The pathologic and risk factor findings reported here suggest that DCM is an advanced stage of myocarditis in sea otters. Many pathologic features, such as myocardial discoloration, congestive heart failure, interstitial fibrosis, dystrophic mineralization, and vascular congestion of myocardium, were commonly detected in both myocarditis and DCM case otters but were more common and more severe in DCM case otters. Exposure to both domoic acid and *S. neurona* was a risk factor for myocarditis in sea otters, whereas only exposure to domoic acid was a risk factor for DCM. Although establishing causal inferences from a purely observational cross-sectional study can be difficult, associations in a multivariate-adjusted analysis of the magnitude reported here for *S. neurona* and domoic acid are evidence of a direct association between exposure and disease. The fact that there are at least 2 important causes of myocarditis in sea otters is not unexpected, given that nonsuppurative inflammation is a common and nonspecific response to myocardial injury. Most likely, myocarditis associated with previous domoic acid exposure progresses to DCM, perhaps after repeated or prolonged exposure to domoic acid. In the sea otters evaluated in this report, myocarditis associated with *S. neurona* did not progress to DCM because none of the DCM case otters were seropositive for *S. neurona*. Because this condition may be rapidly fatal, otters may be more likely to die from meningoencephalitis associated with *S. neurona* before developing advanced cardiac disease or myocarditis may be the end point for cardiac lesions caused by *S. neurona*.

Geographic differences in the association between myocarditis and the proposed causal agents, *S. neurona* and domoic acid, are also supportive of their associations with cardiac disease. Domoic acid exposure was highly associated with myocarditis in the southern aspect of the sea otter range, with exposed otters being 55 times as likely to have died with myocarditis than unexposed otters, and this association was not detected in the northern part of the range. Although *Pseudonitzschia* blooms have occurred throughout central California, a particularly toxic bloom occurred off the coast of San Luis Obispo county in central California in June and July 2000 with high concentrations of *P. australis* and a size-

### Table 5—Odds ratios and 95% confidence limits (CI) for risk factors associated with myocarditis in beach-cast southern sea otters shown for all stranding locations and for north and south stranding locations separately.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Combined stranding locations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult age</td>
<td>6.0</td>
<td>1.24–29.51</td>
<td>0.026</td>
</tr>
<tr>
<td>Good body condition</td>
<td>6.4</td>
<td>1.65–24.58</td>
<td>0.007</td>
</tr>
<tr>
<td>Exposure to <em>S. neurona</em></td>
<td>9.4</td>
<td>2.27–38.78</td>
<td>0.002</td>
</tr>
<tr>
<td>Suspected exposure to domoic acid</td>
<td>10.6</td>
<td>2.32–48.53</td>
<td>0.002</td>
</tr>
<tr>
<td>South stranding location</td>
<td>1.4</td>
<td>0.43–4.71</td>
<td>0.567</td>
</tr>
<tr>
<td><strong>North stranding location only</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult age</td>
<td>9.4</td>
<td>0.85–104.80</td>
<td>0.068</td>
</tr>
<tr>
<td>Good body condition</td>
<td>2.9</td>
<td>0.46–13.43</td>
<td>0.287</td>
</tr>
<tr>
<td>Exposure to <em>S. neurona</em></td>
<td>9.1</td>
<td>1.68–49.68</td>
<td>0.011</td>
</tr>
<tr>
<td>Suspected exposure to domoic acid</td>
<td>3.1</td>
<td>0.52–30.57</td>
<td>0.324</td>
</tr>
<tr>
<td><strong>South stranding location only</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult age</td>
<td>1.6</td>
<td>0.10–25.10</td>
<td>0.746</td>
</tr>
<tr>
<td>Good body condition</td>
<td>3.0</td>
<td>1.45–62.21</td>
<td>0.028</td>
</tr>
<tr>
<td>Exposure to <em>S. neurona</em></td>
<td>10.6</td>
<td>0.75–151.5</td>
<td>0.081</td>
</tr>
<tr>
<td>Suspected exposure to domoic acid</td>
<td>55.7</td>
<td>2.75–1,127</td>
<td>0.009</td>
</tr>
</tbody>
</table>
able epidemic of domoic acid toxicosis in California sea lions that extended through November 2000. In our study, intramuscular rosettes were confirmed in the myocardium from myocarditis case otters seropositive for S. neurona by use of immunohistochemistry but not DCM case otters. Nonsuppurative myocarditis associated with S. neurona merozoites and schizonts in the myocardium has also been documented in adult raccoons. Furthermore, results of an experimental trial indicate that S. neurona was detected within myocardial lesions in raccoons 1 to 3 weeks after infection of S. neurona sporesysts. The severity of myocarditis in most otters exposed to S. neurona was in the mild to moderate range. Only 1 otter exposed to S. neurona had severe myocarditis; however, this adult otter was concurrently infected with T. gondii.

Exposure to T. gondii was not significantly associated with myocarditis or DCM in the multivariate analyses. Whereas T. gondii zoites have been detected in the myocardium on H&E sections and T. gondii was detected in myocardial tissue from 1 of 6 myocarditis case otters by use of the PCR assay, T. gondii zoites were not detected by immunohistochemistry in myocarditis or DCM case otters. Because T. gondii was highly correlated with myocarditis and DCM in the univariate analysis, it is difficult to rule out T. gondii infection as a possible third cause of cardiac disease. Most likely, the nonstratified univariate association between T. gondii serologic response and cardiac disease is at least partly confounded by age and location. Exposure to T. gondii is highest in adults and in otters sampled in the southern part of the sea otter range, and both adult age and stranding location in the south were highly correlated with myocarditis and DCM. Extensive exposure to both T. gondii and domoic acid in sea otters in the Morro Bay area may make it difficult to distinguish their respective roles in contributing to myocardial lesions. It is also intriguing that both S. neurona and T. gondii were strongly associated with myocarditis in otters that were stranded from Pacifica to Moss Landing in the univariate analyses. Otters in this area that were seropositive to T. gondii were more commonly also seropositive to S. neurona (9/15) than otters that were seronegative to T. gondii (3/9), suggesting that sea otters may become exposed to both parasites through common sources or that once sea otters are infected with 1 parasite, they are more vulnerable to infection with the other. Although dual infections with both protozoal parasites further confound our ability to evaluate their association with myocarditis, T. gondii was never significantly associated with myocarditis once the effects of S. neurona and domoic acid were incorporated into the analyses.

Although our finding that exposure to domoic acid is a substantial risk factor for myocarditis was largely unanticipated, this association is biologically plausible. Cardiac lesions, including discoloration of myocardium and myocarditis, have been described in sympatric sea lions with domoic acid intoxication. Furthermore, domoic acid has been detected in common sea otter prey such as razor clams (Silqua patula), mussels (Mytilus spp.), Dungeness crab (Cancer magister), and sand crabs (Emerita analoga). Domoic acid exerts its neurotoxicity by binding primarily to N-methyl-D-aspartate (NMDA) glutamate receptors in the CNS, particularly the hippocampus, causing increased intracellular calcium and eventually neuron cell death. Domoic acid is structurally similar to the excitatory neurotransmitter, glutamate, and its analogs, such as kainic acid, but has as much as 3 times the binding affinity of kainic acid and as much as 100 times the binding affinity of glutamate.

Cardiovascular effects from domoic acid have not been as well investigated as neurologic effects, but results of several studies suggest a potential link between glutamate excitotoxicity and cardiac function. Ionotropic NMDA glutamate receptors have been detected in vagal preganglionic neurons in the medulla oblongata that project to the heart and intramural ganglia, nerve fibers, and the conducting system of the heart in rats and monkeys. Stimulation of glutamate receptors in the dorsomedial hypothalamus elevates heart rate in rats. Administration of kainic acid directly into the paraventricular hypothalamus of rats results in tachycardia, fulminating hypertension, and cardiac death, whereas administration of NMDA caused both bradycardia and tachycardia and myocardial necrosis with multifocal mononuclear inflammation. Another known structural analog of endogenous glutamate, monosodium glutamate, is believed to be responsible for the chest pains and palpitations reported in humans after monosodium glutamate ingestion. Similar to that seen with glutamate toxicity and its analogs, excitotoxicity of preganglionic neurons, intracardial ganglia, and the interconnecting plexus by domoic acid intoxication could substantially alter cardiac rhythm and function, possibly causing excitotoxin-induced myocardial necrosis and inflammation. If glutamate receptors in myocardium respond to domoic acid exposure in the same manner as neuronal glutamate receptors, myocardial cell death from increased intracellular calcium concentrations would be the expected outcome of domoic acid toxicity. Although the role of intracellular calcium in cardiotoxicity is poorly understood, intracellular calcium overload is involved in the cardiotoxic effects of the chemotherapeutic agent doxorubicin, which is prevented by glutamine administration. A direct cardiotoxic effect involving cardiac ganglia is supported by the frequency with which sea
neurons. Of these, mitochondrial toxicity and adverse effects on energy production are of particular interest.

In the context of mitochondrial toxicity, carnitine plays a crucial role. Carnitine is a lipid-soluble substance that shuttles long-chain fatty acids across the inner mitochondrial membrane, enabling the oxidation of fatty acids for energy production. For example, during long-term treatment with the chemotherapeutic agent adriamycin, serum carnitine concentrations have been observed to decrease, leading to carnitine deficiency and energy production limitations in the myocardium. The energy loss is particularly significant as it is required for transportation of necessary long-chain fatty acids to support metabolic processes.

Carnitine is also a critical component in the regulation of metabolic pathways and energy production. It is involved in the transport of fatty acids into mitochondria for beta-oxidation, which is a key process in energy production, particularly in tissues with high energy demands like the heart. Deficiency in myocardial concentrations of carnitine is associated with impaired energy production and can lead to cardiomyopathy, a condition affecting the heart muscle.

In cases of myocarditis, the heart muscle is inflamed. In such cases, myocardial carnitine concentrations are significantly lower compared to control otters, suggesting possible primary carnitine depletion in myocardium. This depletion is commonly associated with impaired energy production and can contribute to the development of cardiomyopathy. In otters with myocarditis, carnitine deficiency is often observed, highlighting its importance in maintaining heart function.

Moreover, the toxic effects of domoic acid, a neurotoxin present in certain species of phytoplankton, have been studied in otters. Long-term exposure to domoic acid is associated with myocarditis and DCM. A recent study found carnitine treatment to be an effective approach against glutamate toxicity, demonstrating its potential role in preventing or treating myocarditis.

Despite the evidence pointing to the role of carnitine in myocardial health, there are still uncertainties regarding the causal pathway toward development of cardiomyopathy. Further research is needed to clarify the role of carnitine in the pathogenesis of myocarditis and DCM in sea otters. The understanding of the interplay between factors such as domoic acid exposure, carnitine deficiency, and the development of heart disease in otters could provide insights into the mechanisms of cardiomyopathy and guide future interventions.
ly the role of preferred prey availability in the frequency of these diseases, and assist in management decisions and conservation of this threatened species.

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g. Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, Calif.
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d. National Oceanic Atmospheric Administration, Center for Coastal Environmental Health and Biomolecular Research, Marine Biotoxin Program, Charleston, SC.
f. Intercooled Stata 8.0, Stata Corp, College Station, Tex.
g. SaTScan, version 3.1, software for the spatial and space-time scan statistics, Kullendorf M and Information Management Services Inc, Silver Spring, Md.

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