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CLINICAL PATHOLOGY AND ASSESSMENT OF PATHOGEN EXPOSURE IN SOUTHERN AND ALASKAN SEA OTTERS

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ABSTRACT: The southern sea otter (*Enhydra lutris nereis*) population in California (USA) and the Alaskan sea otter (*E. lutris kenyoni*) population in the Aleutian Islands (USA) chain have recently declined. In order to evaluate disease as a contributing factor to the declines, health assessments of these two sea otter populations were conducted by evaluating hematologic and/or serum biochemical values and exposure to six marine and terrestrial pathogens using blood collected during ongoing studies from 1995 through 2000. Samples from 72 free-ranging Alaskan, 78 free-ranging southern, and (for pathogen exposure only) 41 debilitated southern sea otters in rehabilitation facilities were evaluated and compared to investigate regional differences. Serum chemistry and hematology values did not indicate a specific disease process as a cause for the declines. Statistically significant differences were found between free-ranging adult southern and Alaskan population mean serum levels of creatinine kinase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, calcium, cholesterol, creatinine, glucose, phosphorous, total bilirubin, blood urea nitrogen, and sodium. These were likely due to varying parasite loads, contaminant exposures, and physiologic or nutrition statuses. No free-ranging sea otters had signs of disease at capture, and prevalences of exposure to calicivirus, *Brucella* spp., and *Leptospira* spp. were low. The high prevalence (35%) of antibodies to *Toxoplasma gondii* in free-ranging southern sea otters, lack of antibodies to this parasite in Alaskan sea otters, and the pathogen's propensity to cause mortality in southern sea otters suggests that this parasite may be important to sea otter population dynamics in California but not in Alaska. The evidence for exposure to pathogens of public health importance (e.g., *Leptospira* spp., *T. gondii*) in the southern sea otter population, and the naïveté of both populations to other pathogens (e.g., morbillivirus and *Coccidiodes immitis*) may have important implications for their management and recovery.

Key words: *Brucella,* calicivirus, *Coccidiodes immitis,* hematology, *Leptospira,* morbillivirus, sea otter, serology, serum chemistry, *Toxoplasma gondii.*

INTRODUCTION

Historically sea otters (*Enhydra lutris*) numbered up to 300,000 in a contiguous Pan-Pacific range but were found only in scattered, remnant populations by the early 1900s due to excessive hunting (Kenyon, 1969). The Alaskan sea otter (*E. lutris kenyoni*) subspecies, found in coastal waters from the western Aleutian Islands to southeast Alaska (USA), is comprised of three stocks (southeast, southcentral, and southwest) which together accounted for over 100,000 individuals (90% of the world sea otter population) by the 1980s (Riedman and Estes, 1990). Since last surveyed in 1992, the southwest stock in the Aleutian archipelago has experienced a severe decline in the central portion of the range of unknown magnitude and extent, but of at least 70% (Estes et al., 1998). The southern sea otter subspecies (*E. lutris nereis*), presently found in nearshore waters from Pt. Conception, California (USA), in the south, to Año Nuevo, California, in the north, recovered from hunting more slowly than Alaskan stocks (Riedman and Estes, 1990). After a high of 2,377 animals in 1995, the population of the southern sea otter began to decline and numbered only 2,090 animals during a spring count in 1999 (United States Fish and Wildlife Service, 2003). These recent population declines in southern and Alaskan sea otters have raised concerns about causes of mortality in both subspecies (Estes et al., 1998;

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United States Fish and Wildlife Service, 2003).

Assessments of differences in the subspecies have primarily focused on taxonomy, behavior, and ecology (Riedman and Estes, 1990). Prior health assessments of sea otter populations consisted of baseline serum chemistry and hematology values, evaluated in the late 1980s for southern sea otters (Williams and Pulley, 1983; Rebar et al., 1996), limited serologic testing for antibodies to canine morbillivirus (Ham-Lamme et al., 1999) and *Toxoplasma gondii* (Miller et al., 2002a, b) in Alaskan and southern sea otters, and a necropsy program of beach-cast southern sea otters (Thomas and Cole, 1996; Miller et al., 2002b). The necropsy program determined that almost 40% of freshly dead beach-cast southern sea otters died from disease, with the leading causes including protozoal encephalitis and acanthocephalan peritonitis (Thomas and Cole, 1996). Another 10% were emaciated at death and no specific cause for their debilitation could be identified (Thomas and Cole, 1996). Unfortunately, it has not been possible to compare causes of death between free-ranging southern and Alaskan sea otters because of the difficulty of recovering freshly dead sea otters from the remote areas of Alaska. Gaining information about serum chemistry and hematology values and exposure of Alaskan and southern sea otters to potential infectious agents during the declines may provide updated baseline data for future management programs and assist in future studies regarding the impact of these diseases on the populations.

Our objective was to conduct a comparative cross-sectional study of clinical pathology values (hematology and serum chemistries) and determine exposure to pathogens in Alaskan and southern sea otters to contribute to the investigation of recent declines in the two populations. We compared serum chemistry values between free-ranging Alaskan and southern adult sea otters and serum chemistry and hematology values between free-ranging southern adult and pup sea otters. We also determined and compared exposure to canine distemper virus (as a screen for morbillivirus), calicivirus, *Leptospira interrogans* (serovars pomona, hardjo, gryppotyphosa, and icterohaemorrhagiae), *Brucella* spp., *T. gondii,* and *Coccidiodes immitis* between free-ranging Alaskan, free-ranging southern, and debilitated southern sea otters in rehabilitation facilities.

MATERIALS AND METHODS

Blood samples for serum and whole blood analyses were obtained from wild-caught free-ranging sea otters in Alaska and California, as well as stranded sea otters (for pathogen exposure only) brought to the Monterey Bay Aquarium (MBA) and The Marine Mammal Center (TMMC) rehabilitation centers in central California. Free-ranging animals were captured as part of several different sea otter population studies by personnel of the United States Geological Services, Biological Resources Division and the California Department of Fish and Game, Office of Spill Prevention and Response (OSPR), under permit authority from the United States Fish and Wildlife Service (Permit #PRT MA672724-9). Captures of free-ranging Alaskan sea otters occurred from July through September 1997 in the western Aleutian Islands (51°83′N, 176°51′W; $n=61$) and Elfin Cove, southeast Alaska (58°20′N, 136°35′W; $n=11$). Captures of free-ranging southern sea otters were from July 1995 through March 2000 in Monterey Bay, California (36°52′–36°62′N and 121°91′–121°94′W, $n=78$). Individual animals or mother/pup pairs were captured in hand-held Wilson traps, dip nets, or tangle nets (Ames et al., 1986). Free-ranging southern sea otters captured prior to 1996 and one captured after 1996 were manually restrained for sample collection. All free-ranging sea otters in Alaska and most southern sea otters captured after 1996 were immobilized with an intra-muscular injection of fentanyl (Sigma Chemical Company, St. Louis, Missouri, USA) at 0.22 mg/kg and diazepam (Abbott Laboratories, North Chicago, Illinois, USA) at 0.11 mg/kg for blood collection. Anesthesia was reversed with an intramuscular injection of naltrexone at 0.44 mg/kg (Wildlife Pharmaceuticals, Fort Collins, Colorado, USA). Wild captured, unweaned California pups were immobilized with isoflurane (IsoFlo, Abbott Laboratories, North Chicago, Illinois) administered via facemask and then endotracheally to effect. A similar isoflurane regimen was utilized

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for some rehabilitated animals with the addition of a pre-anesthetic intra-muscular administration of diazepam (Abbott Laboratories, North Chicago, Illinois) at 0.5 mg/kg and oxymorphone (Numorphan, Endo Pharmaceuticals, Inc., Chadds Ford, Pennsylvania, USA) at 0.3 mg/kg approximately 30 min prior to the isoflurane administration. Effects of the oxymorphone were reversed by administration of naloxone (Baxter Pharmaceutical Products, New Providence, New Jersey, USA) at 0.03 mg/kg intramuscularly. Moribund stranded otters were restrained manually without chemical sedation. Sera samples from animals in rehabilitation (n=41) were collected at admission to the rehabilitation center in order to reflect exposure to pathogens in the wild.

A complete physical examination was performed on all captured and rehabilitated animals, during which animals were weighed to the nearest 0.1 kg, their age class and sex determined, and physical abnormalities noted. Age classes were pup (0–6 mo), juvenile (6–12 mo), subadult (1–3 yr), or adult (>3 yr) based on pelage, tooth wear, weight, length, and reproductive status (Kenyon, 1969).

Blood samples were collected from the proximal femoral, popliteal, or jugular vein. Aliquots were immediately placed into evacuated tubes (Vacutainer, Becton-Dickinson and Company, Franklin Lakes, New Jersey) coated with ethylenediaminetetraacetic acid (EDTA) for hematologic determination or serum clot tubes for serum biochemical analysis. Serum clot tubes were centrifuged within 4 hr of collection and serum separated and frozen at −80 C for up to 1 yr until analyzed. For Alaskan samples, serum was aliquoted into plastic screw top vials and frozen in liquid nitrogen for transport to California and subsequent freezing for up to 1 yr at −80 C. Due to the remoteness of the Alaskan study site, complete hematologic analyses were not performed; however packed cell volumes were determined, and peripheral blood smears were made and examined for abnormalities. Complete blood counts, including differential white blood cell counts on whole blood (Brown, 1980), were performed within 24 hr of collection for the southern animals.

Complete blood counts, differential white blood cell counts, and serum biochemical analyses of 17 analytes were performed by IDEXX Veterinary Services Incorporated, Sacramento, California (free-ranging southern sea otter samples), and the Veterinary Medical Teaching Hospital (VMTH), University of California, Davis, California (serum biochemical analysis only for Alaskan sea otter samples). Complete blood counts were done on a Baker 910 (ABX Diagnostics, Allentown, Pennsylvania) for free-ranging southern sea otters. Serum biochemical analyses were performed at IDEXX on a Hitachi 747 analyzer (Hitachi Instruments Incorporated, Indianapolis, Indiana, USA) and at VMTH on a Hitachi 717 analyzer (Boehringer Mannheim Corporation, Indianapolis, Indiana).

Screening for morbillivirus was conducted by testing for exposure to canine distemper by enzyme linked immunosorbent assay (ELISA) specific for purified canine distemper virus (Snyder Hill strain, ATCC VR-526) as previously described (Ham-Lamme et al., 1999) by the Marine Mammal Immunology Laboratory at the University of California, Davis, California. Positive (seropositive dog serum, VMRD, Inc., Pullman, Washington, USA) and negative controls (sham wells) were used. Based on a standard curve developed by Ham-Lamme et al. (1999), signals of more than twice background were classified as positive.

Presence of calicivirus antibody was detected by ELISA using two different calicivirus antigens, a calicivirus recombinant protein (*Escherichia coli* lac z fusion protein) and a cesium chloride banded antigen (SMSV-5, -13, and -17) as previously described by Smith et al. (1998a), on samples submitted to the Calicivirus Laboratory, School of Veterinary Medicine, Oregon State University (Corvallis, Oregon, USA). Based on the work by Smith et al. (1998a), samples were considered positive at or above a titer of 1:100.

Microagglutination tests using antigens for *Leptospira interrogans* serovars pomona, hardjo, grippotyphosa, and icterohaemorrhagiae (Galton et al., 1962) were conducted at the California Animal Health and Food Safety Laboratory System, Davis, California. Titers were determined positive at or above a titer of 1:100 in accordance with methods established for California sea lions (*Zalophus californianus*; Colagross-Schouten et al., 2002).

A commercially available buffered antigen agglutination test for *Brucella abortus* strain 1119-3 (Rose Bengal Card Test, Becton Dickinson, Cockeysville, Maryland, USA) was used to screen sera for presence of cross-reacting anti-*Brucella* species antibodies (Metcalf et al., 1994). Samples were classified as positive upon agglutination. The Rose Bengal Card Test has been shown to be 100% sensitive for use in other marine mammal species but to lack specificity relative to indirect ELISA (Ross et al., 1996).

Sera were tested for antibodies to *T. gondii* using an indirect fluorescent antibody test (IFAT) validated for use in sea otters (Miller et al., 2002a) and used in an assessment of *T. gondii* infection in southern sea otters in relation to environmental variables (Miller et al.,

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2002b). The IFAT was performed as previously described with the optimal cut-off for maximal sensitivity and specificity set at 1:320 based on the validation work of Miller et al. (2002a).

A quantitative agar gel immunodiffusion test was used to detect antibodies to *Coccidiodes immitis* (Pappagianis, 1988) in samples submitted to the Microbiology and Mycology Laboratory (School of Medicine, University of California, Davis). Formation of a band of identity resulted in a sample being classed as positive.

The mean, standard deviation, and range (minimum–maximum) for each hematologic and serum biochemical value were calculated for adult ($n \leq 49$) and pup ($n \leq 19$) sea otters from California and for each serum biochemical value for adult sea otters from Alaska ($n \leq 54$). Unpaired *t*-tests (Zar, 1996) were used to compare serum biochemical values for sea otters from different locations within Alaska (western Aleutian, southeast Alaska). Two-factor analysis of variance (ANOVA; Zar, 1996) was used to compare hematological and serum biochemical values for sea otters of different sex (M=male, F=female) and group location (free-ranging Alaskan and southern) for adult sea otters and for sex and age (adults and pups) for southern sea otters. If necessary, data were logarithmically transformed to meet the assumption of normality, and analyses were conducted using the transformed variable. Due to potential effects of hemolysis on analytes (Coles, 1986), severely hemolyzed samples were excluded from serum chemistry and hematology analyses and from reported means and ranges.

Not all samples were tested for each potential pathogen because of lack of sufficient serum or sample contamination. The χ^2 test (Zar, 1996) or, when expected cells were less than five, the Fisher exact test (Fisher, 1935) were used to assess differences between locations for prevalence of antibodies when different from zero. Confidence intervals (95% CI) were generated for prevalences by location using the binomial distribution. All statistical analyses were performed with Statview 5.0 (Cary, North Carolina, USA).

RESULTS

Samples from 54 adult Alaskan, 49 adult southern, and 19 pup southern sea otters were submitted for serum chemistry and hematology comparisons. Samples from juveniles and subadults were not used for serum chemistry and hematology comparisons due to small sample sizes for these two age groups.

Medical abnormalities were noted in three western Aleutian males during physical examinations. One had abscesses on its rear flippers; one had superficial lacerations on its rear flippers, and one had an abscess on its lower jaw. Three western Aleutian and 20 southern females had recently healed nasal wounds due to mating, consisting of closed lesions and punctures on their nasal pads, and 10 southern females had similar, but fresh (bleeding or scabbed over) nasal wounds.

Location differences

There were no significant differences between western Aleutian and southeast Alaskan sea otters in mean sera analytes tested. Thus for subsequent group analyses, western Aleutian and southeast Alaskan animals were grouped together for analyses as Alaskan.

Free-ranging southern sea otters had significantly lower serum creatinine kinase (location: $F_{1,101}=12.60$, $P=0.0008$) and alkaline phosphatase (location: $F_{1,101}=4.64$, $P=0.03$) activities and calcium (location: $F_{1,101}=79.06$, $P<0.0001$), glucose (location: $F_{1,101}=21.96$, $P<0.0001$), phosphorus (location: $F_{1,101}=86.79$, $P<0.0001$), total bilirubin (location: $F_{1,97}=80.98$, $P<0.0001$), and sodium (location: $F_{1,101}=33.97$, $P<0.0001$) concentrations compared to free-ranging Alaskan sea otters (Table 1). Free-ranging Alaskan sea otters had significantly lower creatinine (location: $F_{1,101}=52.61$, $P<0.0001$) and blood urea nitrogen (location: $F_{1,100}=53.89$, $P<0.0001$) concentrations (Table 1). For a few analytes, means for males and females varied by location. For alanine aminotransferase (location*sex: $F_{1,99}=7.16$, $P=0.009$; mean±standard deviation for Alaskan female [AF]: 210±75 IU/l, Alaskan male [AM]: 194±60 IU/l, southern female [SF]: 252±56 IU/l, southern male [SM]: 173±54 IU/l) and aspartate aminotransferase levels (location*sex: $F_{1,99=7.8,}$ $P=0.006$; AF: 167±80 IU/l, AM: 188±64 IU/l, SF: 222±67 IU/l, SM: 171±65 IU/l) differences were primarily due to higher sera con-

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TABLE 1. Mean, standard deviation (SD) and range (minimum–maximum) of individual serum biochemical analytes for free-ranging adult Alaskan and adult and pup southern sea otters.

	Free-ranging adult Alaskan sea otters		Free-ranging adult southern sea otters		Free-ranging pup southern sea otters	
Analyte	*n*	Mean (SD) range	*n*	Mean (SD) range	*n*	Mean (SD) range
Creatinine kinase (IU/l)	54	1362 (1119)[a] 147–3394	49	843 (811)[a,b] 147–3394	19	984 (355)[b] 453–1906
Total protein (g/dl)	54	6.9 (0.7) 5.8–9.3	49	6.9 (0.8)[b] 2.6–8.1	19	5.8 (0.5)[b] 4.9–6.7
Globulin (g/dl)	54	4.3 (0.5) 3.3–5.8	49	4.3 (0.5)[b] 3.4–6.0	19	3.0 (0.5)[b] 2.2–4.0
Albumin (g/dl)	54	2.6 (0.3) 2.2–3.6	49	2.7 (0.2)[b] 2.1–3.0	19	2.8 (0.2)[b] 2.4–3.0
Alkaline phosphatase (IU/l)	54	112 (35) 62–190	49	98 (27)[b] 42–166	19	198 (60)[b] 107–348
Alanine aminotransferase (IU/l)	54	205 (71) 100–435	49	230 (65) 77–350	19	181 (34) 113–244
Aspartate aminotransferase (IU/l)	54	173 (75)[a] 75–495	49	208 (70)[a] 113–413	19	168 (54) 92–348
Calcium (mg/dl)	54	9.2 (0.7)[a] 8.3–12.0	49	8.0 (0.6)[a,b] 6.9–10.2	19	8.8 (0.5)[b] 7.9–9.8
Cholesterol (mg/dl)	54	174 (57) 120–319	49	164 (54) 73–290	19	187 (35) 135–286
Creatinine (mg/dl)	53	0.4 (0.2)[a] 0.2–1.1	49	0.6 (0.07)[a,b] 0.4–0.7	19	0.4 (0.05)[b] 0.3–0.5
Glucose (mg/dl)	54	148 (46)[a] 43–294	49	112 (26)[a,b] 65–188	19	127 (28)[b] 96–200
Phosphorous (mg/dl)	54	5.9 (1.3)[a] 2.4–8.8	49	3.8 (0.9)[a,b] 1.8–5.6	19	7.3 (1.5)[b] 3.5–10.1
Total bilirubin (mg/dl)	54	0.2 (0.1)[a] 0–0.7	49	0.1 (0.04)[a] 0.0–0.2	19	0.1 (0.02) 0.1–0.2
Blood urea nitrogen (mg/dl)	54	51 (11)[a] 30–82	49	67 (12)[a,b] 36–91	19	53 (10)[b] 40–81
Sodium (mmol/l)	54	157 (10)[a] 139–202	49	148 (4)[a,b] 138–155	19	147 (3)[b] 143–152
Potassium (mmol/l)	54	4.3 (0.5) 3.4–5.6	49	4.4 (0.4)[b] 3.7–5.6	19	4.2 (0.3)[b] 3.7–5.0
Chloride (mmol/l)	54	115 (7) 96–142	49	112 (9) 105–171	19	111 (2) 107–116

[a] Adult Alaskan and southern sea otter means within a row are significantly different ($P \leq 0.05$).
[b] Adult and pup southern sea otter means within a row are significantly different ($P \leq 0.05$).

centrations in free-ranging southern sea otter females. Alaskan males had a higher mean globulin concentration (location*sex: $F_{1,99}=7.30$, $P=0.008$; AF: 4.2±0.5 g/dl, AM: 4.6±0.8 g/dl, SF: 4.3±0.5 g/dl, SM: 4.2±0.7 g/dl), while mean cholesterol concentration was higher in females than males and within males it was higher in Alaskan males (location*sex: $F_{1,99}=7.68$, $P=0.007$; AF: 186±62 mg/dl, AM: 146±63 mg/dl, SF: 185±46 mg/dl, SM: 110±29 mg/dl). Males from both locations had significantly higher creatinine (sex: $F_{1,100}=6.26$, $P=0.01$; females: 0.47±0.15 mg/dl, males: 0.56±0.13 mg/dl), potassium (sex: $F_{1,101}=6.50$, $P=0.01$; females 4.3±0.4 mmol/l, males: 4.5± 0.4 mmol/l), and sodium (sex: $F_{1,10}=6.52$, $P=0.01$; females: 151±6 mmol/l, males: 156±13 mmol/l) concentrations than females.

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Age differences

Free-ranging adult southern sea otters had significantly higher mean total protein (age: $F_{1,66}$=28.64, P<0.0001), globulin (age: $F_{1,66}$=73.76, P<0.0001), creatinine (age: $F_{1,66}$=84.15, P<0.0001), blood urea nitrogen (age: $F_{1,66}$=22.73, P<0.0001), and potassium (age: $F_{1,66}$=6.35, P=0.01) concentrations, but lower creatinine kinase (age: $F_{1,66}$=7.06, P=0.01) and alkaline phosphatase (age: $F_{1,66}$=86.32, P<0.0001) activity and albumin (age: $F_{1,66}$=4.88, P=0.03), calcium (age: $F_{1,66}$=20.86, P<0.0001), glucose (age: $F_{1,66}$=5.80, P=0.02), and phosphorus (age: $F_{1,66}$=89.45, P<0.0001) concentrations than free-ranging pups (Table 1). Adults had a higher mean sodium concentration (age: $F_{1,66}$=5.42, P=0.02), and males of both age groups had higher sodium concentrations than females (sex: $F_{1,66}$=8.87, P=0.004). Males in both age groups also had lower aspartate aminotransferase levels (sex: $F_{1,66}$=6.96, P=0.01). Adult southern females had higher alanine aminotransferase activity than adult southern males and southern pups (age*sex: $F_{1,65}$=6.30, P=0.01; female adult [FA]: 222±67 IU/l, male adult [MA]: 171±65 IU/l, female pup [FP]: 172±37 IU/l, male pup [MP]: 166±64 IU/l). Adult southern males had lower cholesterol activity than adult females and southern pups (age*sex: $F_{1,65}$=15.69, P=0.0002; FA: 185±46 mg/dl, MA: 110±29 mg/dl, FP: 184±24 mg/dl, MP: 189±40 mg/dl).

Adults also had higher mean red blood cell count (age: $F_{1,64}$=6.37, P=0.01), hemoglobin concentration (age: $F_{1,64}$=39.30, P<0.0001), hematocrit (age: $F_{1,64}$=23.34, P<0.0001), mean cell volume (age: $F_{1,64}$=30.40, P<0.0001), mean cell hemoglobin (age: $F_{1,64}$=49.53, P<0.0001), and mean cell hemoglobin concentration (age: $F_{1,64}$=5.32, P=0.02) but lower lymphocyte count (age: $F_{1,64}$=8.92, P=0.005) than pups (Table 2). While adult southern males had higher monocyte counts than adult southern females, female southern pups had the highest mean monocyte count of all four age-sex classes (age*sex: $F_{1,63}$=9.11, P=0.004; FA: 415±318×1,000/ul, MA: 507±278×1,000/ul, FP: 837±497×1,000/ul, MP: 350±272×1,000/ul).

Serology

Variable numbers of sera samples were submitted for each serologic test due to limited availability of individual samples from each group (Table 3). There were no detectable antibodies to canine distemper virus (n=187) or *C. immitis* (n=176) in any of the individuals tested (Table 3). In southern sea otters presented for medical treatment and rehabilitation, prevalence of antibodies to calicivirus was 2.5% (1/40: 1/14 adult, 0/6 subadult, 0/2 juvenile, 0/18 pup; confidence interval [CI]=0.7–9.7%), to *Leptospira* spp. was 10.0% (4/40: 1/13 adult, 1/6 subadult, 1/2 juvenile, 1/19 pup; CI=2.8–22.0%), to *Brucella* spp. was 2.7% (1/37: 0/12 adult, 0/6 subadult, 0/2 juvenile, 1/17 pup; CI=0.7–10.2%) and to *T. gondii* was 27.3% (9/33: 4/11 adults, 4/6 subadults, 0/2 juvenile, 1/14 pup; CI=8.9–46.5%; Fisher's exact P-value=0.01 for subadults vs. pups). In free-ranging southern sea otters, prevalence of exposure to *Leptospira* spp. was 1.6% (1/63: 1/40 adult, 0/4 subadult, 0/3 juvenile, 0/16 pup; CI=0.4–5.5%), to *Brucella* spp. was 5.9% (4/68: 2/41 adult, 1/4 subadult, 0/3 juvenile, 1/20 pup; CI=1.6–14.0%), and to *T. gondii* was 35% (27/77: 24/49 adults, 2/6 subadults, 0/1 juvenile, 1/21 pup; CI=12.2–52.9%; Fisher's exact P-value=0.0003 for adults vs. pups; some of the same individuals were tested and results included in Miller et al. 2002a). Results from an additional six sera samples from southern sea otters (4/40 rehabilitation, 2/74 free-ranging, all pups) were classified as suspect for exposure to calicivirus because they were not positive at the cut-off titer of 1:100 but were positive at 1:50. Of the six pathogens evaluated, free-ranging Alaskan sea otters were found to have antibodies only to *Brucella* spp. with 7.7% testing positive, (5/65; 5/54 adults, 0/7 subadults,

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TABLE 2. Mean, standard deviation (SD), and range (minimum-maximum) for individual hematologic parameters for southern sea otter adults and pups.

	Free-ranging southern sea otters			
	Adults		Pups	
Value	*n*	Mean (SD) range	*n*	Mean (SD) range
Red blood cells (×10⁶)	48	5.1 (0.5)[a] 2.6–5.9	18	4.7 (0.4)[a] 3.7–5.3
Hemaglobin (g/dl)	48	18.7 (2.2)[a] 12.6–20.5	18	16.3 (1.6)[a] 12.6–20.5
Hematocrit (%)	48	55.5 (5.4)[a] 29.4–64.5	18	47.0 (4.0)[a] 38.8–57.9
Mean cell volume (fl)	48	108.8 (5.3)[a] 94.0–119.2	19	100.4 (4.5)[a] 94–110
Mean cell hemoglobin (pg)	48	37.4 (2.4)[a] 34.7–43.2	18	34.7 (1.4)[a] 33.2–39.0
Mean cell hemoglobin concentration (g/dl)	48	35.4 (1.1)[a] 32.6–37.5	18	34.6 (1.2)[a] 32.5–37.1
White blood cells (×1,000/ul)	48	7.2 (1.9) 4.2–13.3	18	7.6 (1.8) 4.9–10.6
Segmented neutrophils (×1,000/ul)	48	3.7 (1.1) 1.7–8.6	18	3.5 (0.15) 1.5–7.3
Segmented neutrophils (%)	48	52 (10) 29–81	18	45 (9) 30–69
Lymphocytes (×1,000/ul)	48	2.2 (1.1)[a] 0.7–6.3	18	3.0 (0.18)[a] 1.0–6.3
Lymphocytes (%)	48	30 (10) 12–53	18	40 (10) 20–59
Monocytes (×1,000/ul)	48	0.4 (0.3) 0.07–1.4	18	0.5 (0.1) 0.0–1.9
Monocytes (%)	48	6 (4)[a] 1–20	18	7 (5) 0–18
Eosinophils (×1,000/ul)	48	0.8 (0.5) 0–1.8	18	0.6 (0.08) 0–1.5
Eosinophils (%)	48	11 (7) 0–28	18	8 (4) 0–14
Platelet (×1,000/ul)	22	242 (95) 135–524		not available

[a] Means within a row are significantly different ($P \leq 0.05$).

0/3 juveniles, 0/1 pup; CI=2.2–17.3%). Positive *Brucella* results were found for both Alaskan stocks evaluated with four positives from the 46 adults tested from the western Aleutians and one positive from the eight adults tested from southeast Alaska.

DISCUSSION

Results of the hematologic and serum biochemical health evaluation did not reveal a specific disease process in the sea otters examined or a potential cause of the decline of the sea otter populations. The average serum biochemical and hematologic values for both Alaskan and southern sea otters in our study fell within published ranges for southern sea otters described before the recent decline period (Williams and Pulley, 1983; Rebar et al., 1996). However, a variety of significant serum biochemical differences between free-ranging adult southern and Alaskan sea otters were identified that may be reflective of differing environmental conditions. The elevated alanine aminotransferase and aspartate aminotransferase activities, creatinine and blood urea nitrogen

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TABLE 3. Prevalence of antibodies to six pathogens in free-ranging Alaskan, free-ranging southern, and debilitated southern sea otters.

Pathogen	Free-ranging Alaskan sea otters No. positive/ No. tested (% positive)	Free-ranging southern sea otters No. positive/ No. tested (% positive)	Debilitated southern sea otters in rehabilitation No. positive/ No. tested (% positive)
Canine distemper virus	0/72 (0)	0/75 (0)	0/40 (0)
Calicivirus	0/72 (0)	0/74 (0)[a]	1/40 (3)[a]
Leptospira interrogan[b]	0/72 (0)	1/63 (2)	4/40 (10)
Brucella spp.	5/65 (8)	4/8 (6)	1/37 (3)
Toxoplasma gondii[c]	0/65 (0)	27/77 (35)	9/33 (27)
Cocidiodes immitis	0/69 (0)	0/75 (0)	0/32 (0)

[a] 4/40 (10%) of the rehabilitation southern sea otters and 2/74 (3%) of the free ranging southern sea otters were suspect for exposure to calicivirus at 1:100 dilution and positive at 150 dilution.
[b] Antibodies to *L. interrogans* serovars were found in one free-ranging southern sea otter (hardjo), three debilitated southern sea otters (*hardjo*), and one debilitated southern sea otter with equally high positive titers to serovars hardjo and pomona.
[c] Results for some of these samples were included in Miller et al. (2002a).

concentrations, as well as the lower creatinine kinase and alkaline phosphates activity, and calcium, cholesterol, glucose, phosphorous, total bilirubin, and sodium concentrations in free-ranging southern compared to Alaskan sea otters may have been due to differences in analytical equipment, parasite exposure and load, physiologic and nutritional status, or exposure to environmental contaminants.

Interlaboratory variation in sample handling and processing reagents can contribute to differences in values for analytes (Ross et al., 1998). The laboratories used for serum chemistry analyses used two very similar machines with only minor differences in reagents (Werner, pers. comm.). The use of two different laboratories was not by design, but rather the result of several agencies handling sea otter samples prior to the initiation of this collaborative study. Sample handling and shipping were carefully controlled which likely reduced potential contributions of these factors to analyte variability. However, it is possible that the use of two different laboratories contributed slightly to the location differences, as creatinine kinase, alanine aminotransferase, aspartate aminotransferase, and blood urea nitrogen can be affected by analytical equipment differences.

Emaciation and infection with disease-causing acanthocephalan and protozoal parasites is common in stranded southern sea otters (Thomas and Cole, 1996; Kreuder et al., 2003). Unfortunately, specific serology or fecal analyses for acanthocephalan infections in sea otters are not available for antemortem diagnostics or, for *Profilicolis* spp., infections are nonpatent (Miller, unpubl. data), thus it is not possible to determine population exposure prevalences. For *T. gondii,* presence of serum antibodies is likely evidence of infection in humans and this may be the case for southern sea otters. Thus many of the sampled southern sea otters may have had subclinical *T. gondii* infections as indicated by the 35% serologic prevalence. In contrast, none of the Alaskan sea otters sampled showed evidence of exposure to *T. gondii.* Dogs with toxoplasmosis have elevated serum levels of alanine aminotransferase and aspartate aminotransferase (Coles, 1986). Therefore, it is possible that infection with *T. gondii* in southern sea otters contributed to higher activities for these two analytes in southern animals.

Prey availability studies were conducted in the locations of the current study several years before the recent declines and may no longer be relevant. However, a general difference in diet between Alaskan

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and southern sea otters is the inclusion of epibenthic fish in the diet of Alaskan sea otters, whereas southern sea otters tend to concentrate on macroinvertebrates (Riedman and Estes, 1990). Thus, the lower glucose, cholesterol, phosphorus, and sodium concentrations and higher blood urea nitrogen concentration in southern sea otters may have been due to dietary differences between the two populations. Male sea otters may forage further offshore in deeper water which may provide access to different and larger prey items (Riedman and Estes, 1990). These dietary differences between males and females may have contributed to the lower cholesterol and higher creatinine, potassium, and sodium in male sea otters in both populations.

Sea otters exhibit site fidelity and may be affected by localized contamination from land-based runoff of polycyclic aromatic hydrocarbons (PAHs), pesticides, and fertilizers (Riedman and Estes, 1990). Exposure to contaminants may result in clinical chemistry changes, depending on the contaminant and route of exposure (Coles, 1986). Organopathies and associated serum chemistry changes have been noted in mink exposed to contaminants. Chronic low-level PAH contamination of feed resulted in increased alanine aminotransferase and aspartate aminotransferase activities and serum cholesterol concentration in mink (Mazet et al., 2000). Induction of liver microsomal enzyme activity in mammals has been associated with many pesticides and environmental pollutants (Smith, 1991). Fatty livers and kidney degeneration in mink exposed to polychlorinated biphenyls (PCBs) have been documented (Aulerich and Ringer, 1977), and elevated serum levels of triglycerides, bilirubin, and cholesterol have been found in humans chronically exposed to PCBs (Tokunaga et al., 1999). Hepatotoxic effects of DDT exposure have also been noted for nonhuman primates (Takayama et al., 1999).

Southern sea otters have exhibited higher levels of organochlorine pesticides (DDT and related compounds) and lower values, but more variable fingerprints, for PCBs than Alaskan sea otters (Nakata et al., 1998; Bacon et al., 1999). Mortality due to disease in southern sea otters has been associated with elevated tissue levels of DDT (Nakata et al., 1998). Thus, the higher levels of alanine aminotransferase, aspartate aminotransferase, and blood urea nitrogen in southern sea otters may have been related to chronic low level exposure to DDT, while PCB exposure in Alaskan sea otters may have led to elevations in total bilirubin and cholesterol in that population.

In addition to location differences for serum biochemical values, some clinically and biologically useful associations with sex and age were noted. Sexual dimorphism in sea otters is quite marked, with adult female sea otters being approximately 30% smaller than males (Kenyon, 1969). Females also undergo physiologic stresses of pregnancy and lactation for much of their adult life, and may suffer nasal lacerations during mating (Riedman and Estes, 1990). Liberation of fat stores in the liver, associated with pregnancy and lactation, may have contributed to elevations in alanine aminotransferase in adult female southern sea otters, as has been observed in nursing adult ferrets (Williams et al., 2000). The sex and age-related cholesterol differences in southern sea otters may have been related to dietary differences or thyroid activity, as seen in the dog (Coles, 1986).

Differences in serum biochemical and hematologic values for adult compared to pup southern sea otters in California follow a similar pattern to those seen in terrestrial mammals (Willard et al., 1989). Lower levels of total protein and globulin and higher lymphocyte and monocyte counts in pups may be related to a developing immune system, while elevated levels of alkaline phosphatase and phosphorus are likely due to the rapid bone growth in a pup (Coles, 1986). The lower levels of

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alanine aminotransferase and creatinine in pups could be due to muscle metabolism for lactation in adult females or sex and age related long-term exposure to low concentrations of contaminants, as mentioned above. Similarly, higher blood urea nitrogen and creatinine in adults may be related to their higher protein intake as adults. Barring severe muscle damage or necrosis, in domestic animals serum alanine aminotransferase levels within a species are also proportional to body size (Coles, 1986), and this may also be the case in sea otters. Lower red blood cell counts, hemoglobin and hematocrit concentration, mean cell volume, and mean cell hemoglobin were seen in young animals. This may be due to an iron deficient milk diet and the requirement for a high rate of erythropoiesis to match the growth rate.

Despite concerns that the slow recovery in southern sea otters may be related to high levels of disease (Thomas and Cole, 1996), this study is the first to focus on serologic assessment and comparison of southern to Alaskan sea otters for exposure to a panel of potential pathogens. The marine and terrestrial pathogens evaluated were chosen because they have been linked to reproductive failure, die-offs, and disease in sympatric marine mammal species (Kinne, 1985; Thomas and Cole, 1996) and serologic tests exist that have been validated for use in sea otters or shown to work well across species lines. Other pathogens known to cause reproductive failure or die-offs in marine mammals, such as influenza, could not be assessed because serologic tests were not available that met these criteria.

Morbilliviruses have caused mass mortalities (often with associated increases in beach-cast carcasses) of marine mammals in the Atlantic Ocean, Lake Baikal, and the North Sea but have not been isolated from a marine mammal from the Pacific (Duignan, 1999). However, morbilliviral nucleic acid has been detected by polymerase chain reaction (PCR) in tissues from stranded common dolphins (*Delphis capensis*) in California (Reidarson et al., 1998). In light of the catastrophic decline of the Alaskan Aleutian Islands population, we chose to evaluate exposure to canine distemper virus and cross-reacting morbilliviruses. Biologists were present on several of the Aleutian islands during the catastrophic decline and did not find higher than expected numbers of carcasses during routine beach surveys (Estes, unpubl. data). Because serologic exposure was not detected and no clinical or pathologic cases have been identified, there is presently no evidence to implicate morbilliviruses in either population decline. Due to the susceptibility of the Mustelidae to morbilliviral infections (Aiello, 1998) and the potential for massive die-offs to result from exposure, periodic testing for exposure to this virus should continue. Screening of animals to be released into the wild from captivity or those temporarily brought into captivity for translocation purposes is imperative.

Caliciviruses are ubiquitous marine viruses (Smith et al., 1998b). Smith et al. (1976) found a relatively high prevalence (>90%) of exposure to various caliciviruses in their serologic survey of pinnipeds and cetaceans, with no associated outbreaks of disease. The lower seroprevalence in sea otters, relative to pinnipeds, suggests potential differences in pathogen transmission dynamics or host susceptibility. Continued monitoring for exposure to these viruses is encouraged because caliciviruses have zoonotic potential (Barlough et al., 1986), have caused outbreaks of vesicular disease in sympatric pinnipeds (Gage et al., 1990; Van Bonn et al., 2000), and have been associated with other disease or illness in pinnipeds, including premature parturition.

Identification of southern sea otters with antibodies to *Leptospira* spp. is the first evidence of exposure in sea otters. Outbreaks of leptospirosis in California sea lions and phocids have been documented with associated morbidity and mortality (Gulland et al., 1996; Stamper et al.,

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1998), and serologically positive animals have been found in populations of Alaskan and California marine mammals sympatric with sea otters (Smith et al., 1977; Gulland et al., 1996). The two sea otters in this study with the highest titers were rehabilitation cases sampled at admission and euthanatized on the same day due to a poor prognosis for recovery. However, their gross pathologic and histologic findings were not consistent with renal disease (Thomas, pers. comm.). The lack of evidence of exposure to *Leptospira* spp. in free-ranging Alaskan sea otters, low prevalence in free-ranging southern sea otters, and higher prevalence without evidence of renal disease in rehabilitated southern sea otters may be the result of regional exposure differences or *Leptospira* spp. pathogenicity. The positive cases in this study suggest that continued monitoring and testing for additional *Leptospira* serovars in rehabilitated and free-ranging southern sea otters would be prudent given that leptospirosis is a serious zoonotic disease with various serovars differing in clinical importance.

While *Brucella* has not been isolated from sea otters, monitoring of *Brucella* exposure status is recommended based upon the human health implications of this zoonotic pathogen and evidence from this study for exposure in both Alaskan and southern sea otters. *Brucella* spp. cause reproductive failure in many terrestrial species (Nielsen and Duncan, 1990). Molecular characterization of *Brucella* strains isolated from marine mammals indicate these newly discovered *Brucella* isolates may be several species corresponding to diverse marine mammal hosts (Bricker et al., 2000), but the significance for population health is unknown. Serologic studies suggest that exposure to *Brucella* species is quite variable for pinniped and cetacean species, with evidence for exposure in the North Sea, northwestern Atlantic, the Arctic ocean, and northeastern Pacific (Ewalt et al., 1994; Foster et al., 1996; Ross et al., 1996; Forbes et al., 2000). For harbor seals (*Phoca vitulina*), a species sympatric with sea otters along the Pacific coast, prevalences ranged from 3.1% in the St. Lawrence Estuary, Canada, to 21% for seals from a Pacific Vancouver Island population, Canada, (Nielson et al., 2001). In our study, the low prevalence of antibodies to *Brucella* spp. may indicate incidental exposure in sea otters from other marine mammal species. Further work to isolate and characterize the *Brucella* strain in sea otters would help in understanding the contribution of this pathogen to sea otter population dynamics.

Confirmation of *T. gondii* infection in live-sampled otters was not possible by non-invasive methods other than serology. However, previous studies showed good correlation of IFAT results with *T. gondii* infection status of carcasses (Miller et al., 2002a). Age was significantly correlated with *T. gondii* seropositivity and *T. gondii* is known to be a major cause of mortality in southern sea otters (Kreuder et al., 2003). Thus the high seroprevalence to *T. gondii* infection in free-ranging southern compared to Alaskan sea otters, the increasing seroprevalence with age in free-ranging and debilitated sea otters, and the low seroprevalences for the other pathogens supports the concerns of Kreuder et al. (2003) regarding the epidemiologic contribution of *T. gondii* to the poor recovery in southern sea otters.

The cause-specific mortality for coccidioidomycosis in California sea otters was 6% (6/90) from 1992 through 1994, with cases occurring in the winter or summer between Morro Bay and Pismo Beach, California (Thomas et al., 1996). The lack of antibodies in tested animals in this study may reflect the relative naïveté and susceptibility to, and potential for subsequent fatal infection of sea otters with this terrestrial pathogen. Conversely, the failure to detect antibodies to *C. immitis* may have been due to temporal (seasonal and annual) and geographic exposure variability, because most clinically affected animals in the past have presented at the

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southern end of the sea otter range. Since none of the sea otters sampled for this study were from the southern end of the range, 10 archived sera samples from 1992 from animals from this area were assessed for exposure to *C. immitis* using the agar gel immunodiffusion test. None were positive to *C. immitis.* Interestingly, coccidioidomycosis is considered a disease of immunosuppression in humans, and clinical expression of this disease in sea otters may lend evidence to the immunosuppression theory of reduced recovery in southern sea otters (Thomas and Cole, 1996).

In summary, even though sampling occurred spatially and temporally coincident with the declines in both populations, significant perturbations in hematologic and serum biochemical values were not identified that could implicate a cause for the declines. However, the location differences in clinical pathologic values and serology support the theory that disease may be playing a more important role in the poor recovery and recent decline in the southern sea otter population than is likely in Alaska. The absence or low prevalence of antibodies to pathogens with the potential to cause high mortality in sea otters suggests these populations may be susceptible to epidemics of disease if such pathogens were introduced. Therefore, further monitoring activities, including serologic testing and carcass examination, should be continued to assess the potential role of disease in the populations' declines.

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