Journal of Wildlife Diseases, 47(3), 2011, pp. 579–592

ASSESSMENT OF CLINICAL PATHOLOGY AND PATHOGEN EXPOSURE IN SEA OTTERS (*ENHYDRA LUTRIS*) BORDERING THE THREATENED POPULATION IN ALASKA

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ABSTRACT: Northern sea otter (*Enhydra lutris kenyoni*) abundance has decreased dramatically over portions of southwest Alaska, USA, since the mid-1980s, and this stock is currently listed as threatened under the Endangered Species Act. In contrast, adjacent populations in south central Alaska, USA, and Russia have been stable to increasing during the same period. Sea otters bordering the area classified in the recent decline were live-captured during 2004–2006 at Bering Island, Russia, and the Kodiak Archipelago, Alaska, USA, to evaluate differences in general health and current exposure status to marine and terrestrial pathogens. Although body condition was lower in animals captured at Bering Island, Russia, than it was at Kodiak, USA, clinical pathology values did not reveal differences in general health between the two regions. Low prevalences of antibodies (<5%) were found in Kodiak, USA, and on Bering Island, Russia, to *Toxoplasma gondii*, *Sarcocystis neurona*, and *Leptospira interrogans*. Exposure to phocine herpesvirus-1 was found in both Kodiak, USA (15.2%), and Bering Island, Russia (2.3%). Antibodies to *Brucella* spp. were found in 28% of the otters tested on Bering Island, Russia, compared with only 2.7% of the samples from Kodiak, USA. Prevalence of exposure to *Phocine distemper virus* (PDV) was 41% in Kodiak, USA, but 0% on Bering Island, Russia. Archived sera from southwest and south-central Alaska dating back to 1989 were negative for PDV, indicating exposure occurred in sea otters in Kodiak, USA, in recent years. Because PDV can be highly pathogenic in naïve and susceptible marine mammal populations, tissues should be examined to explore the contribution of this virus to otter deaths. Our results reveal an increase in exposure to pathogens in sea otters in Kodiak, Alaska, USA, since the 1990s.

Key words: Alaska, *Brucella*, *Enhydra lutris*, phocine distemper, Russia, sea otter, serosurvey.

INTRODUCTION

The northern sea otter (*Enhydra lutris kenyoni*) is found along the Pacific coast of Washington, USA; Canada; and throughout coastal Alaska, USA, to the tip of the Aleutian Islands (Estes, 1990). The range of the Asian sea otter (*Enhydra lutris lutris*) extends from the Commander Islands, along the coast of Kamchatka, to the Kuril Islands, Russia, then to northern Japan (Kornev and Korneva, 2006). Northern sea otter abundance has decreased by 50% across southwest Alaska, USA, since the mid-1980s and by up to 95% across the Aleutian Archipelago (Estes et al., 2005). The eastward extent of the decline is to the west of Castle Cape

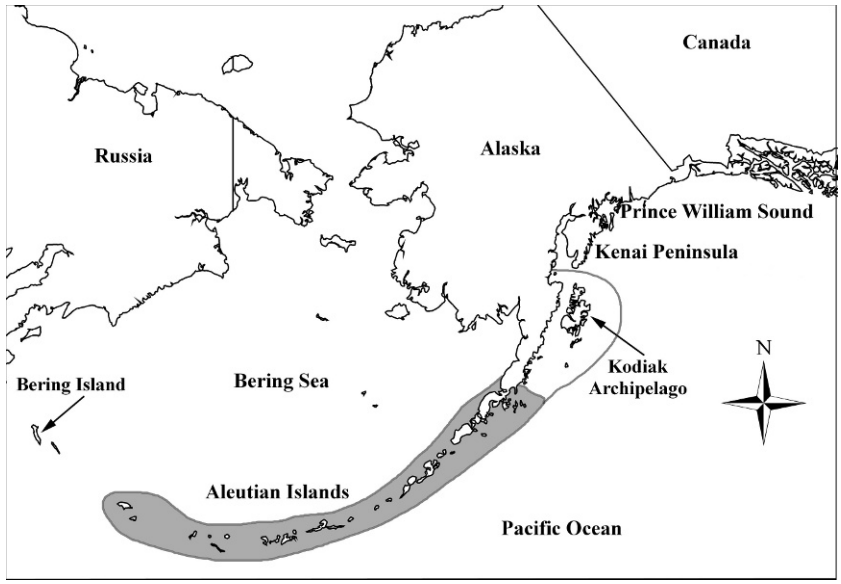

FIGURE 1. Capture locations (arrows) of free-ranging sea otters (*Enhydra lutris kenyoni*) 2004–2006. The solid line delineates the stock of northern sea otters in Alaska, USA, that is listed as *threatened* under the US Endangered Species Act; the shaded area corresponds to the region experiencing severe population decline.

on the Alaska, USA, peninsula (Burn and Doroff, 2005), approximately 250 km west of the Kodiak, USA, archipelago population (Fig. 1), which appears to be stable (USFWS, 2008). The US Fish and Wildlife Service listed the southwest stock as *threatened* under the Endangered Species Act in 2005. The causes for these population declines are not completely understood (primarily because of the difficulties recovering carcasses in remote areas with rugged coastlines), but increased predation pressure from killer whales (*Orcinus orca*) has been implicated (Estes et al., 1998). In contrast, the Russian population on the Commander Islands, at the western extent of the Aleutian Island chain, approximately 420 km west of Attu, Alaska, USA (Fig. 1), has been stable to increasing during the same period, presumably near carrying capacity (Kornev and Korneva, 2006).

Studies of health and disease were performed during the mid to late 90s in Alaska, USA, and California, USA. Health assessments in Prince William Sound from 1992 to 1993 were follow-up to the *Exxon Valdez* oil spill in 1989 (Ballachey et al., 2003). A survey was also performed to compare health and pathogen exposure in the southern sea otters off California, USA, to northern sea otters captured in the western Aleutians and southeast Alaska, USA, in 1997 (Hanni et al., 2003). Neither of the previous studies identified biologically significant differences in clinical pathology values associated with specific disease processes or decreased survival. Although antibodies to *Toxoplasma gondii*, *Leptospira interrogans*, and *Brucella* spp. were found in sea otters off California, USA, a low prevalence to *Brucella* spp. only was detected in otters from both Alaskan stocks examined. Lack of evidence of exposure to most potential marine and terrestrial pathogens, suggests the population in Alaska, USA, may be susceptible to epidemics if such pathogens were introduced. There are limited data for otters in other regions of their range.

Our goal was to capture and sample animals at the eastern and western extents of the threatened population to assess

general health, to examine differences in clinical pathology (hematology and serum chemistry) and evidence of exposure to 10 marine and terrestrial pathogens. Free-ranging sea otters were sampled in two regions that appear to be stable: Bering Island, Russia, a population at or near carrying capacity (Kornev and Korneva, 2006), and Kodiak Archipelago, Alaska, USA, a population below carrying capacity (von Biela et al., 2009), bordering the area classified in the recent decline. Updated health screening of live sea otters may provide important information for population management and recovery, and examination of health and pathogen exposure in these stable peripheral populations would be helpful for conducting postdecline assessments should those populations decline in the future.

MATERIALS AND METHODS

Animals and samples

Sea otters were captured on the beach on the northwest cape haul-out site (55°11′24″N, 166°8′24″E; $n=58$) on Bering Island, Russia (Fig. 1), with hand-held dip nets (Ames et al., 1983) in March 2004 and 2005, and were transported in wire cages on snow machines to the field camp for processing. Sea otters ($n=31$) were also captured in Russia in July 2006 using tangle nets (Ames et al., 1983) at scattered locations along the northwestern Bering Island, Russia, coast. In Alaska, USA, sea otters were captured using tangle nets at nine sites in the Kodiak Archipelago (between 57°40′48″N, 152°19′48″W and 58°24′36″N, 152°31′12″W; $n=74$; Fig. 1) in August 2004 and July 2005. Following capture, animals were weighed and immobilized with an intramuscular injection of 0.22 mg/kg fentanyl citrate (Central Avenue Pharmacy, Pacific Grove, California, USA; Monson et al., 2001) and 0.2 mg/kg midazolam hydrochloride (Hospira Inc., Lake Forest, Illinois, USA; M. J. Murray, pers comm.). Fentanyl was reversed with an intramuscular injection of naltrexone hydrochloride at 0.4 mg/kg (Zoopharm, Laramie, Wyoming, USA).

Complete physical examinations were performed on all animals, including morphometric measurements, age-class and sex determinations, overall body condition, coat condition, tooth condition and wear, and descriptions of lesions, scars, or wounds. Age classes were defined as pup (0–12 mo), juvenile (1–3 yr), or adult (>4 yr) based on pelage, tooth aging, tooth wear, weight, length, and reproductive status (Bodkin et al., 2000). Animals were tagged on the hind flippers using plastic Temple® tags (Temple Tag, Inc., Temple, Texas, USA). Blood samples were collected from the jugular vein (Bossart et al., 2001) and placed into Vacutainer tubes (Vacutainer, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) coated with ethylenediaminetetraacetic acid (EDTA) for hematologic analysis, into serum separator tubes for serum chemistry and serologic analysis, and into sodium heparin for measurement of hemoglobin concentration. Whole blood was allowed to clot at room temperature, then centrifuged, and serum was aliquoted and stored at −20 C and later at −80 C until analysis.

Hematology and serum chemistry

Because of the remoteness of the study sites, complete hematologic analyses were performed using manual methods and included leukocyte (white blood cell [WBC] count; Unopette kit 365856, Becton-Dickinson) and erythrocyte (red blood cell [RBC] count; Unopette kit 365851, Becton-Dickinson), and hematocrit findings, and peripheral blood smears prepared for differential WBC counts. Tubes containing sodium heparin and serum separator tubes were centrifuged, and serum and plasma were separated and frozen at −20 C in the field, and then at −80 C until analysis. Hemoglobin concentration was measured spectrophotometrically (Rea et al., 1998), allowing for the calculation of erythrocyte indices, including mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Differential WBC counts and serum chemistry analyses of 15 analytes were performed by Quest Diagnostics (Quest Diagnostics Incorporated, Portland, Oregon, USA).

Serologic analyses

We screened for marine calicivirus, *Toxoplasma gondii*, *Sarcocystis neurona*, *Brucella* spp., morbillivirus (*Canine distemper virus* [CDV], *Phocine distemper virus* [PDV], porpoise morbillivirus, and *Dolphin morbillivirus*), phocine herpesvirus-1 (*Phocid herpesvirus 1*), and *Leptospira* spp. Calicivirus antibody was detected by serum neutralization using San Miguel sea lion virus (calicivirus serotype 1) at the National Veterinary Diag-

nostic Laboratory (NVSL, Ames, Iowa, USA). Samples with a titer of greater than 1:8 were considered positive.

Sera were tested for antibodies to *T. gondii* and *S. neurona* using an indirect fluorescent antibody test (IFAT) validated for *T. gondii* in sea otters at the University of California, Davis, California, USA. The IFAT was performed as described (Miller et al., 2002) with the optimal cutoff for maximal sensitivity and specificity set at 1:320. The cutoff for *S. neurona* was also set at 1:320, but that test has not been validated in sea otters.

Exposure to *Brucella* spp. was measured using a competitive enzyme-linked immunosorbent assay (cELISA) at the Mystic Aquarium and Institute for Exploration, Mystic, Connecticut, USA (Meegan et al., 2010). The assay was developed using a whole-cell antigen of a strain of marine *Brucella* species isolated from a harbor seal (*Phoca vitulina*), characterized and available commercially through NVSL (case 04-0281, accession 311585). The test was developed to screen sera from marine mammal species for the presence of antibodies against marine-origin *Brucella* spp. All samples were screened at a 1:10 dilution, and the percentage of inhibition was measured. Samples with >30% inhibition were considered positive. This test has not been validated in sea otters; however, based on comparisons with culture-confirmed cases, specificity and sensitivity for cetacean samples were 73% and 100%, respectively, and for pinnipeds 77% and 67%, respectively (Meegan et al., 2010). Additionally comparison testing revealed the marine-origin cELISA was more sensitive for detecting anti-*Brucella* antibodies in marine mammal samples than was the *B. abortus* tests available.

Sera were submitted to Oklahoma Animal Disease Diagnostic Laboratory (Stillwater, Oklahoma, USA) for testing for antibodies to *Leptospira* spp., phocine herpesvirus-1 and morbillivirus (CDV, PDV, porpoise morbillivirus, dolphin morbillivirus). Standard microscopic agglutination microtiter procedure was used to test for exposure to *Leptospira interrogans* (serovars pomona, hardjo, grippotyphosa, icterohaemorrhagiae, and bratislava; Colagross-Schouten et al., 2002). Titers >1:100 were determined to be positive in accordance with methods established for California sea lions (*Zalophus californianus*). Exposure to phocine herpesvirus-1 was tested using a serum neutralization assay that detects antibodies against the Atlantic isolate (PB84) grown in seal kidney cells in accordance with methods by Osterhaus et al. (1985), which detects antibodies against the Atlantic isolate of phocine herpesvirus-1. Samples were considered positive if they had a titer of >1:16 (twice the cutoff reported by the laboratory). This cutoff was chosen because the assay has not been validated for use with sea otters. Screening for morbilliviruses exposure was performed by serum neutralization (Saliki and Lehenbauer, 2001); samples were considered positive if titers were ≥1:16, again twice the cutoff reported by the laboratory as described above.

Archived sera were also obtained from live-captured sea otters and harbor seals in Alaska for morbillivirus antibody testing: 119 (104 adults, 15 juveniles) sea otters captured 1991–2005 (Table 1) and 42 (21 adults, 14 juveniles, 7 pups) harbor seals captured in Cook Inlet and Kachemak Bay, Alaska, USA, by the Polar Ecosystems Program, National Marine Mammal Laboratory as a part of ongoing research, 2005–2006. Archived samples had been stored at −80 C.

A subset of samples ($n=10$) from both Kodiak, USA, and Bering Island, Russia, was submitted to the Animal Health Diagnostic Center, College of Veterinary Medicine at Cornell University for CDV testing to compare diagnostic test results. These samples were tested by serum neutralization and again a titer of ≥1:16 was considered positive.

Statistical analysis

Because age-related differences in condition and clinical pathology values have been noted among sea otters (Hanni et al., 2003) and were confirmed in this study, comparisons are not presented between age classes and regions but, instead, within each age class for the two regions. The mean, standard deviation, and range for each hematologic and serum chemistry value were calculated for each age class for both study regions. Unpaired *t*-tests (Zar, 1996) were used to compare hematology and serum chemistry values for the two regions within an age class (juveniles, adults). Two-factor analysis of variance (ANOVA; Zar, 1996) was used to compare serum chemistry values for sea otters of different sex and region for adults and for juveniles ($P<0.05$). Comparison between sexes for hematologic parameters was not performed because data were only available from two female otters from Bering Island, Russia. When necessary, data were logarithmically transformed to meet the assumption of normality. Transformed variables were used in subsequent analyses. Because of the small size of the sample of captured pups from both locations, the data from that age class were not included in the analyses.

TABLE 1. Numbers of archived serum samples from northern sea otters (*Enhydra lutris kenyoni*) collected throughout a broad geographic range of Alaska, USA, in 1989–2005, and tested for antibodies against morbilliviruses.

Year	Location	Female		Male		Total
		Adult	Juvenile	Adult	Juvenile	
1989	Kenai Peninsula, *Exxon Valdez* oil spill area	6		5		11
1989	Kodiak Island, *Exxon Valdez* oil spill area	10	3	1	2	16
1991	Western Aleutians, Adak Island	9		4		13
1992	Western Aleutians, Amchitka Island	6	4	5		15
1992	Western Prince William Sound	5		5		10
1995	Western Aleutians, Shemya Island			4		4
1998	Western Prince William Sound	7	3	3		13
2001	Western Prince William Sound	7	3	6		16
2004	Western Aleutians, Adak Island	6		1		7
2004	Western Aleutians, Attu Island	1		1		2
2004	Western Aleutians, Shemya Island	1				1
2004	Western Aleutians, Amchitka Island	1				1
2005	Eastern Prince William Sound	5		5		10
Total		64	13	40	2	119

Probability values were adjusted to control for the effect of multiple comparisons using the Bonferroni correction (Bonferroni, 1936).

The χ^2 test (Zar,1996) or, when an expected cell was less than five, Fisher's exact test (Fisher, 1935) was used to assess differences in antibody prevalence among locations, age classes, and sexes. When appropriate, strength of association was estimated by the odds ratio. Statistical analyses were performed with SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

RESULTS

We collected 163 samples from 2004 to 2006 (74 from Kodiak, USA; 89 from Bering Island, Russia). Complete hematology was performed on samples from 62 otters from Kodiak, USA (2004 and 2005), and 29 from Bering Island, Russia (2004). Serum chemistry analysis was conducted on 72 and 89 samples collected from the two areas, respectively, and included all years of the study. Samples from all otters were submitted for serologic analysis, but not all were tested for each pathogen because of insufficient serum.

Abnormalities, including broken teeth, nose scars, superficial lacerations, and punctures, were noted in animals from both study areas. Although not statistically significant, body condition was lower in animals from Bering Island, Russia (mean mass/length ratios [kg/cm] for adults: males=0.226, females=0.182) compared with Kodiak, USA: males=0.240, females=0.197; Monson, 2009). Particularly on Bering Island, Russia, body condition values were lowest in 2004 (captures conducted on haul-out sites in late winter, $P<0.0001$) and highest in 2006 (captures conducted in open water in summer; Monson, 2009).

Clinical pathology

Juvenile otters in Kodiak, USA, had a higher mean lymphocyte count than those on Bering Island, Russia ($P<0.05$; Table 2). Means for adult otters from Kodiak, USA, were significantly higher for total WBC count, neutrophil, lymphocyte and monocyte counts, and mean corpuscular volume ($P<0.05$; Table 2). Adults from Bering Island, Russia, had higher mean eosinophil count and mean cell hemoglobin concentration (Table 2).

There were also significant differences among overall serum chemistry analytes when comparing the two regions by age class ($P<0.05$; Table 3). Juveniles from Kodiak, USA, had higher mean chloride,

TABLE 2. Mean, SD, and range for hematology parameters by age class for free-ranging sea otters (*Enhydra lutris kenyoni*) from the Kodiak Archipelago, Alaska (AK), USA, and the Bering Island, Russia (RU), captured 2004–2006.

	Pups[a]		Juveniles[a]				Adults[a]			
	AK (n=6)		AK (n=20)		RU (n=14)		AK (n=36)		RU (n=15)	
Value	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Red blood cell (10^6/μl)	4.9 (0.4)	4.3–5.4	5.1 (0.9)	4.0–7.5	5.3 (0.8)	4.4–7.3	4.7 (0.9)	3.0–7.5	5.5 (0.7)	4.4–6.5
Hemoglobin (g/dl)	17.3 (1.8)	14.2–19.7	15.0 (4.8)	6.8–22.6	18.3 (3.6)	14.5–21.4	16.1 (4.1)	9.7–21.0	18.9 (1.3)	16.6–21.1
Hematocrit (%)	54 (6)	45–63	54 (3)	50–59	54 (4)	44–63	55 (5)	45–63	55 (6)	51–60
Mean cell volume (fl)	110.7 (12.8)	89.5–124.4	108.8 (17.3)	71.6–139.8	102.2 (15.1)	73.6–133.3	119.0 (19.9)[c]	70.5–189.2	100.5 (12.2)[c]	83.6–126.4
Mean cell hemoglobin (pg)	35.4 (4.0)	28.3–38.5	29.6 (9.6)	15.4–45.1	34.9 (5.4)	27.2–49.2	34.6 (9.6)	14.9–61.4	34.5 (4.2)	25.5–41.8
Mean cell hemoglobin concentration (g/dl)	32.0 (1.2)	30.9–34.0	27.5 (7.9)	13.8–39.3	34.2 (2.1)	29.7–37.7	29.0 (6.1)[c]	19.9–37.8	34.4 (2.1)[c]	30.2–37.8
White blood cell (10^3/μl)	6.7 (0.9)	5.5–7.9	9.1 (2.1)	4.8–14.5	6.8 (2.2)	3.9–9.4	9.9 (2.1)[c]	7.4–16.5	7.3 (3.3)[c]	2.9–16.6
Neutrophils/μl	3,817 (950)	2,525–5,092	5,199 (1,362)	2,976–7,770	4,352 (1,570)	2,488–6,688	6,025 (1,636)[c]	2,772–10,744	3,784 (1,443)[c]	969–6,124
Neutrophils (%)	58 (16)	33–76	57 (13)	33–75	64 (7)	51–76	61 (12)	33–86	54 (16)	34–88
Lymphocytes/μl	2,685 (1,304)	1,474–4,743	3,285 (1,541)[b]	1,050–6,240	1,546 (883)[b]	516–4,021	3,083 (1,388)[c]	865–7,425	1,634 (840)[c]	389–2,992
Lymphocytes (%)	39 (14)	22–62	34 (12)	10–65	23 (8)	6–43	31 (12)	10–54	23 (11)	7–46
Monocytes/μl	204 (176)	0–383	392 (257)	0–1,015	435 (328)	167–1,376	296 (259)	0–945	336 (160)	165–800
Monocytes (%)	3 (3)	0–6	4 (2)	0–7	7 (4)	2–16	3 (2)[c]	0–9	5 (2)[c]	1–8
Eosinophils/μl	13 (32)	0–79	492 (612)	0–2,048	473 (378)	0–1,376	458 (458)[c]	0–1,485	1,566 (2,030)[c]	0–8,110
Eosinophils (%)	1 (0.5)	0–1	5 (6)	0–21	7 (5)	0–16	5 (5)[c]	0–15	18 (13)[c]	0–49
Basophils/μl	0	12 (38)	0–155	0	5 (20)	0–93	6 (25)	0–97		
Basophils (%)	0	1 (0.5)	0–2	0	1 (0.5)	0–1	1 (0.5)	0–1		

[a] Age classes were defined as pup (0–12 months), juvenile (1–3 years), or adult (>4 years) based on pelage, tooth aging, tooth wear, weight, length, and reproductive status.

[b] Juvenile sea otter means within a row are significantly different between regions (P<0.05).

[c] Adult sea otter means within a row are significantly different between regions (P<0.05).

TABLE 3. Mean, SD, and range for serum chemistry analytes by age class for free-ranging sea otters (*Enhydra lutris kenyoni*) from the Kodiak Archipelago, Alaska (AK), USA, and the Bering Island, Russia (RU), captured 2004–2006.

	Pups[a]				Juveniles[a]				Adults[a]			
	AK (n=6)		RU (n=2)		AK (n=25)		RU (n=31)		AK (n=41)		RU (n=56)	
Value	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Total protein (g/dl)	5.6 (0.4)	4.8–5.9	6.2 (0.5)	5.8–6.7	6.4 (0.5)[b]	5.2–7.5	5.9 (0.5)[b]	4.9–6.6	6.8 (0.5)[c]	6.0–7.8	6.4 (0.4)[c]	5.5–7.3
Globulin (g/dl)	2.6 (0.3)	2.0–2.9	3.4 (0.5)	2.9–3.8	3.6 (0.5)[b]	2.6–5.0	3.1 (0.4)[b]	2.3–3.7	4.1 (0.5)[c]	3.3–5.2	3.9 (0.4)[c]	2.8–4.7
Albumin (g/dl)	3.0 (0.2)	2.8–3.2	2.9 (0.1)	2.8–2.9	2.8 (0.2)	2.5–3.3	2.8 (0.2)	2.2–3.1	2.7 (0.2)[c]	2.4–3.1	2.6 (0.2)[c]	2.1–3.1
Alkaline phosphatase (IU)	417.0 (172.2)	262–723	153.3 (30.5)	123–184	182.6 (148.2)[b]	45–656	103.7 (47.3)[b]	39–200	101.4 (47.3)	41–321	99.6 (59.0)	47–460
Alanine aminotransferase (IU)	260.8 (164.4)	123–586	194.3 (34.1)	155–215	161.6 (80.6)	75–455	154.2 (37.4)	90–249	159.4 (62.3)	78–364	166.1 (77.3)	76–564
Aspartate aminotransferase (IU)	310.0 (184.7)	122–587	171.7 (22.9)	146–190	239.4 (189.3)	95–931	152.5 (56.6)	67–364	272.3 (221.1)	107–1154	238.8 (168.1)	103–1,043
Calcium (mg/dl)	9.7 (0.5)	9.1–10.5	9.0 (0.1)	8.9–9.1	8.8 (0.4)	7.8–9.4	8.6 (0.4)	8.1–10.1	8.5 (0.5)	7.8–10.7	8.4 (0.4)	7.9–9.5
Creatinine (mg/dl)	0.2 (0.1)	0.1–0.3	0.3 (0.1)	0.3–0.4	0.3 (0.1)	0.2–0.5	0.4 (0.2)	0.2–0.7	0.4 (0.1)[c]	0.2–0.7	0.6 (0.2)[c]	0.3–0.9
Glucose (mg/dl)	120.2 (33.1)	65–167	140.3 (21.4)	116–156	129.9 (27.0)	65–176	111.0 (29.3)	66–176	123.3 (29.6)	61–200	142.1 (32.1)	41–215
Total bilirubin (mg/dl)	0.2 (0.1)	0.1–0.3	(0.1)	0.1–0.2	0.2 (0.1)	0.1–0.3	0.2 (0.1)	0.1–0.5	0.2 (0.1)	0.1–0.3	0.2 (0.1)	0.1–0.3
Blood urea nitrogen (mg/dl)	39.2 (14.1)	29–67	55.0 (17.3)	36–70	42.6 (9.6)[b]	25–64	56.6 (14.8)[b]	29–86	46.9 (10.2)[c]	29–75	61.3 (14.9)[c]	31–112
Sodium (mmol/l)	151.8 (1.6)	151–155	150.3 (1.5)	149–152	152.3 (1.4)	150–155	151.2 (3.2)	142–158	153.1 (1.8)	150–157	151.9 (2.4)	148–159
Potassium (mmol/l)	4.6 (0.5)	4.2–5.6	4.0 (0.9)	3.0–4.7	4.0 (0.4)	3.2–4.9	4.2 (0.3)	3.5–4.7	4.1 (0.3)	3.4–5.0	4.1 (0.4)	3.3–5.0
Chloride (mmol/l)	115.5 (3.0)	111–120	114.3 (2.1)	112–116	116.6 (2.0)[b]	113–120	114.1 (3.2)[b]	104–120	115.9 (2.2)	111–120	115.4 (4.1)	109–128
γ-Glutamyl transpeptidase (IU/l)	11.8 (5.2)	3–18	—		12.8 (7.3)	5–37	—		12.0 (3.9)	6–28	—	

[a] Age classes were defined as pup (0–12 months), juvenile (1–3 years), or adult (>4 years) based on pelage, tooth aging, tooth wear, weight, length, and reproductive status.

[b] Juvenile sea otter means within a row are significantly different between regions ($P<0.05$).

[c] Adult sea otter means within a row are significantly different between regions ($P<0.05$).

TABLE 4. Comparison of antibody prevalences in free-ranging sea otters (*Enhydra lutris kenyoni*) from the Kodiak Archipelago, Alaska (AK), USA; and the Bering Island, Russia (RU), captured 2004–2006.

Pathogen	AK, No. positive/No. tested (%)	RU, No. positive/No. tested (%)
Toxoplasma gondii	2/74 (2.7)	4/89 (4.5)
Sarcocystis neurona	2/74 (2.7)	0/89 (0)
Calicivirus (serotype 1)	0/65 (0)	0/89 (0)
Canine distemper virus	0/73 (0)	0/89 (0)
Phocine distemper virus	30/73 (41.0)	0/89 (0)
Dolphin morbillivirus	0/72 (0)	0/58 (0)
Porpoise morbillivirus	0/72 (0)	0/58 (0)
Phocine herpesvirus-1	11/72 (15.3)	2/88 (2.3)
Leptospira spp.[a]	1/72 (1.4)	2/89 (2.3)
Brucella spp.	1/72 (1.4)	25/89 (28.1)

[a] Low positive titers (1:200) to *L. hardjo.*

total protein, globulins, and alkaline phosphatase (ALP) concentrations; and juveniles on Bering Island, Russia, had higher blood urea nitrogen. Adults from Kodiak, USA, had higher total protein, globulin, and albumin concentrations; whereas adults on Bering Island, Russia, had higher BUN and creatinine. Although not significantly different between groups, the maximum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values were high in adults from both regions. There were no significant differences in serum chemistry analytes between juvenile males and females in either region; however, a few parameters varied by sex in adults. Males from both regions had greater sodium ($F_{1,97}=10.73$, $P=0.01$), whereas adult females from Kodiak, USA, had higher ALT ($F_{1,97}=14.36$, $P<0.001$) and AST ($F_{1,97}=18.73$, $P<0.001$) concentrations. Finally, adult males from Kodiak, USA, had higher chloride values than did females, whereas on Bering Island, Russia, females had increased chloride values ($F_{1,97}=15.71$, $P<0.001$).

Serology

Antibody prevalences were compared for 10 pathogens in otters from both study regions (Table 4). No detectable antibodies were found against *San Miguel sea lion virus*, and low prevalences were found to *T. gondii* and *Leptospira* spp. in both areas. Prevalence of antibodies to *T. gondii* was 2.6% in Kodiak, USA (two adult females with titers of 1:1,280 and 1:2,560) and 4.5% on Bering Island, Russia (two adult males and two adult females, titers ranged from 1:320 to 1:10,240), whereas prevalence to *Leptospira interrogans* was 1.3% (one adult female) and 2.3% (two adult males) from Kodiak, USA, and Bering Island, Russia, respectively, all with low titers of 1:200 to serovar hardjo. Two adult females from Kodiak, USA, had low titers to *S. neurona* (1:320).

Antibody to phocine herpesvirus-1 was detected in both areas, with animals from Kodiak, USA (15.2%), more than seven times (95% CI=1.6–33.9) more likely to be positive than were those from Bering Island, Russia (2.3%). Low titers ranged from 1:24 to 1:32. Both positives from Bering Island, Russia, were adult males, and an equal proportion of animals of all age classes and both sexes captured in Kodiak, USA, were positive (Table 5). Antibodies were also detected in both groups to *Brucella* spp., but otters from Bering Island, Russia (28.1%), were 21 times (95% CI=2.8–161.9) more likely to be positive than were otters from Kodiak, USA (1.3%), where the one positive otter was an adult male. Antibody prevalence on Bering Island, Russia, increased with age

TABLE 5. Prevalence of antibodies by age class in sea otters (*Enhydra lutris kenyoni*) from the Kodiak Archipelago, Alaska (AK), USA, and the Bering Island, Russia (RU), 2004–2006.

Pathogen	AK (%)			RU (%)		
	Pup[a] (n=6)	Juvenile[a] (n=27)	Adult[a] (n=40)	Pup[a] (n=2)	Juvenile[a] (n=31)	Adult[a] (n=56)
Phocine distemper virus	50	44.4	32.5	0	0	0
Phocine herpesvirus-1	16.7	14.8	17.2	0	0	3.6
Brucella spp.	0	0	2.5	0	16.1	35.7

[a] Age classes were defined as pup (0–12 months), juvenile (1–3 years), or adult (>4 years) based on pelage, tooth aging, tooth wear, weight, length, and reproductive status.

($P<0.05$; Table 5), with males and females equally likely to be positive.

No evidence of exposure was found to morbilliviruses on Bering Island, Russia. In contrast, although no antibodies were found for any of the cetacean morbilliviruses, a prevalence of 41.1% (30/73) was measured for PDV antibodies in samples from otters from Kodiak, USA. Repeated testing confirmed the PDV results, and the subset sent to Cornell University for CDV testing were negative (all positive [n=6] or suspect positive [n=4] for antibodies against PDV). Phocine distemper titers were low but were >1:16 and up to 1:48. Positive reactions below our cutoff of 1:16 were noted in an additional 17 otters (titers of 1:12) that were considered indeterminate or suspect. Phocine distemper virus antibodies were found in all age classes, did not increase significantly with age (Table 5), and an equal proportion of males and females were positive. Results for all archived sea otter (Table 1) and harbor seal samples from Alaska, USA, submitted for testing for antibodies against all four morbilliviruses were negative.

DISCUSSION

Hematology and serum chemistry results did not reveal major differences that would indicate a difference in sea otter health between the two study areas. Although variations in some parameters were found, the means did not fall outside published ranges for sea otters (Bossart et al., 2001; Hanni et al., 2003), and the differences were not significant physiologically. However, a few individuals had increased values for a small number of analytes compared with published values. The maximum eosinophil count in otters from Bering Island, Russia, was more than five times that reported in the literature and, although not pathognomonic for parasitism, may be indicative of increased parasite loads. Although no association was found between increased eosinophils and positive *T. gondii* serology, the presence of other parasitic infections could have been the cause; however, that was not examined. Two adult otters, one from each area, accounted for the maximum ALP, ALT, and AST values. All other values were within expected ranges, but both were older adults, suggesting that they may have had chronic/active ongoing liver disease resulting in hepatocellular leakage of the enzymes. Lastly, ALP concentrations in pups and juveniles captured in Kodiak, USA, were higher than published values. This condition could be due to rapid bone growth in these young animals, which has been documented in other species (Coles, 1986), or, although less likely, could be an indicator of underlying liver disease. Differences in sample handling, processing, and storage may have also contributed to differences among these values.

The negative results for San Miguel sea lion virus were consistent with findings by Hanni et al. (2003). Although caliciviruses are common marine mammal pathogens and evidence of exposure has been

reported in Steller sea lions (*Eumetopias jubatus*) in the region (Burek et al., 2005), this pathogen does not appear to be of concern in these sea otters. The low prevalences of antibodies to *T. gondii*, *S. neurona*, and *Leptospira interrogans* represent a small increase in prevalence compared with those reported by Hanni et al. (2003) for otters in Alaska, USA, since 1997. Leptospirosis commonly causes morbidity and mortality in California sea lions and northern fur seals (*Callorhinus ursinus*; Smith et al., 1977; Gulland et al., 1996), as does *T. gondii* and *S. neurona* in southern sea otters (Kreuder et al., 2003), but there has been little evidence of these diseases in sea otters in Alaska, USA.

The significantly higher prevalence of antibodies to phocine herpesvirus-1 in otters captured in Kodiak, USA, is of interest. Phocine herpesvirus infections commonly occur in young harbor seals, sometimes associated with morbidity and mortality (Goldstein et al., 2004). Reactivation of this virus also occurs in adult and subadult seals during times of stress. An unidentified herpesvirus has been circulating in otters in Alaska, USA, at least since the late 1980s when herpesviral inclusions were seen in tissues from animals that died in Prince William Sound during the *Exxon Valdez* oil spill in 1989 (Lipscomb et al., 1994). Because the test used in this study was developed for use in harbor seals to detect antibodies to phocine herpes, it is likely that cross-reactive antibodies were detected to a related herpesvirus in these otter samples, rather than exposure to phocine herpes.

Almost one-third of the sea otters sampled on Bering Island, Russia, had antibodies against *Brucella* spp., in contrast to only one otter from Kodiak, USA. *Brucella* spp. infections have been correlated with reproductive failure in terrestrial mammals, but the pathogenicity of that infection is not well understood in marine species (Maratea et al., 2003). Prevalence increased with age, as has been reported for *Brucella* spp. exposure in other marine mammals (Zarnke et al., 2006). That increase may be associated with increased sexual activity with maturation. It is unclear why, in contrast, there is a low prevalence of exposure in otters from Kodiak, USA, but that is consistent with results that have been reported for other regions of Alaska (Hanni et al., 2003). It is unknown whether the presence of antibodies correlates with disease. *Brucella*-like organisms have been isolated from marine mammal species worldwide including a number of pinnipeds: grey seals (*Halichoerus grypus*), harp seals (*Phoca groenlandica*), ringed seals (*Phoca hispida*), and hooded seals (*Cystophora cristata*), as well as from parasites found in Pacific harbor seals (Garner et al., 1997; Forbes et al., 2000; Foster et al., 2001); thus a possible route of exposure may be through contact with infected seals. Other possible routes may be similar to those proposed for other marine mammals, including maternal transmission, physical trauma, ingestion, or through an undetermined parasite intermediate (Foster et al., 2001). Alternatively, as otters haul-out regularly on beaches on Bering Island, Russia (Burdin, pers. obs.), exposure to the pathogen could have occurred through contact with terrestrial mammals, such as caribou (*Rangifer tarandus*), domestic cattle (*Bos taurus*), and feral goats (*Capra hircus*) that also inhabit the island. Brucellosis, caused by *Brucella suis* type 4, is a serious problem in domestic reindeer (*Rangifer tarandus*) in parts of Russia, and wild caribou are considered to be a reservoir (Klein, 1980). The increased prevalence could also be due to cross-reactivity with antibodies to other Gram-negative bacteria. Although not a part of this study, evaluation of cross-reactivity of the marine *Brucella*-based cELISA with other Gram-negative bacteria (Meegan et al., 2010) found that it did not occur with *Yersinia enterocolitica* O:9 antibody-positive sera; however, cross-reactivity was found with *B. abortus*– and *B. melitensis*–positive sera. Thus, because the ma-

rine *Brucella* cELISA does cross-react with other terrestrial *Brucella* spp., it is unclear whether exposure to a marine or terrestrial strain was detected.

A high prevalence of antibodies against PDV was found in otters tested in Kodiak, USA. To confirm that exposure was to PDV and not to CDV, a subset of PDV antibody-positive samples were sent to an independent laboratory to test for CDV antibodies and were negative, whereas repeated testing for PDV antibodies was positive. A recent study corroborated this serologic finding by documenting PDV nucleic acid in nasal secretions from 10% (8/77) of the same otters (Goldstein et al., 2009). Five of these otters also had antibodies to PDV, thus the molecular findings supported the serologic results further confirming the presence of PDV infection in this population. Phocine distemper virus caused outbreaks of disease in harbor seals in northern Europe in 1988 and 2002 (Hall et al., 1992; Jensen et al., 2002) and has been associated with seal deaths on the East Coast of the United States and Canada (Duignan et al., 1995). Although viruses, such as PDV, can be highly pathogenic in naïve and susceptible marine mammals, there is frequently serologic evidence of exposure and infection (i.e., high or increasing titers) following a viral epidemic in individuals from the population that survived the infection (Duignan et al., 1995; Ohishi et al., 2001). Although the titers in these sea otters were relatively low, the complete lack of serologic evidence of exposure in the archived samples (sea otters from this study including animals from southwest and south-central Alaska, USA, and those reported by Hanni et al.) was striking. Because the PDV fragment detected in the Alaskan otters was identical to that of the 2002 Atlantic isolate (Goldstein et al., 2009), and serologic surveys of marine mammals in Alaska before 2000 revealed that these species had not been exposed to PDV, the evidence indicates that exposure to PDV likely occurred in sea otters since 2000. Because terrestrial Arctic species from Canada have also been exposed to PDV (Philippa et al., 2004), the risk for exposure from predatory and scavenging North Pacific Ocean carnivore species must not be overlooked. Two of four animals captured in 2004 in the Fox Islands of the eastern Aleutians ($n=1$) and along the southern Alaska Peninsula ($n=3$) were also positive (samples not included in this study because of the small sample size), indicating that exposure may not be limited to the Kodiak, USA, region. Additional testing to examine archival tissues molecularly for presence of the virus is needed to better describe the timeline of virus emergence. Although we found no evidence of exposure to PDV on Bering Island, Russia, morbillivirus exposure has been documented in Russia. *Canine distemper virus* caused a mass die-off of Baikal seals (*Phoca sibirica*) in Lake Baikal, Siberia, Russia, in 1988 and in Caspian seals (*Phoca caspica*) in the Caspian Sea, Russia, in 2000 (Mamaev et al., 1995; Kennedy et al., 2000), but phocine distemper has not been documented in marine mammals in the Russian Far East.

In summary, although serum chemistries and hematology did not detect differences in general health between otters captured in the two regions, they provide baseline values for otters in these regions and may be used for comparison in the future should these populations experience declines. We demonstrated an increase in exposure to pathogens compared with previous work evaluating otters captured in 1997 in southwest and southeast Alaska, USA. Of note is the disparity in prevalence of exposure to pathogens between Kodiak, USA, and Bering Island, Russia, in particular to PDV in otters in the eastern Aleutians and Kodiak Archipelago. In contrast to Bering Island, Russia, which is separated by ~250 km of open water, Kodiak Island is <50 km from the Alaska Peninsula, thus there is continuous habitat out to the eastern

Aleutian Islands allowing for increased contact among individuals. It is unclear what role phocine distemper might play in Alaskan sea otter health or population dynamics. Conceivably, infection could cause both direct mortality and, like other morbilliviruses, could predispose otters to lethal infections by bacterial or protozoal opportunists. Our findings do not confirm or deny those speculations, although morbidity related to disease might make sea otters more susceptible to predation, or predation and disease could independently contribute to increased mortality. Continued surveillance in Alaska, USA, is needed to gain an understanding of the effects of phocine distemper on the recovery of the threatened stock in southwest Alaska, USA.

ACKNOWLEDGMENTS

This work was funded by the US Fish and Wildlife Service, Alaska SeaLife Center, US Geological Survey, National Marine Mammal Laboratory, and the California Department of Fish and Game and was conducted under the DMA permits MA041309-2 and MA740507-2. Thanks to personnel from the US Fish and Wildlife Service, US Geological Survey, Alaska SeaLife Center, Marine Mammal Protection Division, Sevvostrybvod, 'Komandorsky' Reserve, Monterey Bay Aquarium, Oklahoma Animal Disease Diagnostic Laboratory, and University of California at Davis, especially: D. Jenski, H. Coletti, M. Yourkowski, J. de la Bruere, T. Fischbach, K. Kloecker, J. Estes, T. Tinker, M. Terwilliger, B. Hatfield, J. Ames, M. Harris, G. Benthal, V. Nikulin, S. Zagrebelny, V. Fomin, D. Utkin, E. Dubovi, A. Melli, A. Packham, L. Rea, P. Rivera, J. Saliki. A special thanks also to P. Boveng and J. Richmond for their generous help in testing of the harbor seal samples. We also thank J. Mazet and M. Murray for thoughtful review of this manuscript.

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Submitted for publication 12 January 2010.
Accepted 18 March 2011.