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INFECTIOUS DISEASE AND THE DECLINE OF STELLER SEA LIONS (*EUMETOPIAS JUBATUS*) IN ALASKA, USA: INSIGHTS FROM SEROLOGIC DATA

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ABSTRACT: Serologic data were examined to determine whether infectious disease may have played a role in the decline of Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska and Aleutian Islands, USA. Available published data, unpublished data, and recent collections (1997–2000) were compared and reviewed. Data were stratified by geography to compare the declining western Alaskan population in the Aleutian Islands through eastern Prince William Sound to the increasing population in southeastern Alaska. Prevalences of antibodies from the 1970s to the early 1990s were noted for *Leptospira interrogans, Chlamydophila psittaci, Brucella* spp., phocid herpesvirus-1, and calciviruses. Serum samples collected from 1997–2000 were tested for antibodies to these agents as well as to marine mammal morbilliviruses, canine parvovirus, and canine adenovirus-1 and -2. Conclusions could not be drawn about changes in antibody prevalence to these agents during the decline of Steller sea lions, however, because data were incomplete or not comparable as a result of inconsistencies in testing techniques. Despite these shortcomings, results provided no convincing evidence of significant exposure of Steller sea lions to morbilliviruses, *Brucella* spp., canine parvovirus, or *L. interrogans.* Steller sea lions have been exposed to phocid herpesviruses, caliciviruses, canine adenovirus, and *C. psittaci* or to cross-reactive organisms in regions of both increasing and decreasing sea lion abundance. Based on similar antibody prevalence estimates from the increasing and decreasing populations, these agents are unlikely to have been the primary cause of the population decline. They may have contributed to the decline or impeded population recovery, however, because of undetected mortality and morbidity or reductions of fecundity and body condition in animals under other stresses. Systematic monitoring for disease agents and their effects is needed to determine whether infectious disease currently plays a role in the decline and lack of recovery of Steller sea lions.

Key words: Adenovirus, calicivirus, *Chlamydophila psittaci,* herpesvirus, morbillivirus, serology, Steller sea lion

INTRODUCTION

The eastern and western populations of Steller sea lions (*Eumetopias jubatus*) are genetically distinct and geographically separated at Cape Suckling, Alaska (60°N, 144°W) (Bickham et al., 1996). The western population of Steller sea lions has declined since the late 1970s and is listed under the U.S. Endangered Species Act as being endangered (Trites and Larkin, 1996; Loughlin, 1998). In contrast, the eastern population has increased and is listed as being threatened. What has caused the western population to decline is the subject of considerable debate (DeMaster and Atkinson 2002; National

Research Council 2003; Trites and Donnelly 2003).

The western population declined by ~70% from the late 1970s through the 1980s and dropped another 40% through the 1990s. The decline appears to have begun in the eastern Aleutian Islands and spread west through the central Aleutians and east through the Kodiak Island region, the Gulf of Alaska, and Prince William Sound (PWS). Relatively low birth rates were observed during this period, as were aborted fetuses during winter months (Calkins and Goodwin, 1988; Pitcher et al., 1998). Demographic data further suggest that mortality rates of juvenile sea lions may have increased between the 1970s and 1980s (York, 1994) and that reproductive failures and increased adult mortality may have occurred during the 1990s (Holmes and York, 2003).

Factors that potentially contribute to the decline of Steller sea lions include malnutrition, disease, predation by killer whales, climate change, exposure to toxic substances, entanglement in marine debris, and incidental as well as intentional take by humans (Loughlin, 1998; Trites and Donnelly, 2003). Unfortunately, data to assess each of these possibilities are limited. The spatial and temporal patterns of the rapid initial decline are consistent with a disease outbreak, but no sea lion carcasses have been noted or recovered. Failure to find carcasses may be a result of the remoteness of the breeding (rookery) and resting (haul-out) sites and the enormous expanse of ocean occupied by Steller sea lions, sinking of carcasses, or removal of sick and dead animals by predators or scavengers.

The goal of the present study was to examine spatial and temporal patterns in antibody prevalence of Steller sea lions in Alaska to determine whether infectious diseases identified in northern Pacific pinnipeds may have played a role in the population decline of these sea lions. From the 1970s to the present, serum samples have been tested for antibodies to canine adenoviruses (CAVs), morbilliviruses, caliciviruses, canine parvovirus, phocid herpesvirus (PhHV)-1, *Chlamydophila psittaci, Leptospira interrogans,* and *Brucella abortus.* Available published data, unpublished data, and recent collections (1997–2000) were compared and reviewed.

MATERIALS AND METHODS

During the 1970s and 1980s, sea lions were killed under permit with high-powered rifles. Whole blood was collected from freely bleeding external bullet wounds or from the heart, body cavity, or major blood vessels after the body cavity was opened. Results of other analyses performed on collected tissue samples have been reported by Calkins and Goodwin (1988). During the 1990s, as described by Bossart et al. (2001), blood samples were collected from the digital or caudal gluteal veins from live animals anesthetized with tiletamine and zolazepam (Heath, 1996) or with isoflurane (Heath et al., 1997). Sera were separated and frozen at −10 C in the field and then stored at −20 C or −70 C until tested.

Animals were assigned to age classes based on size, analysis of growth layers of teeth, or time of year when captured based on an estimated pupping date of early June. Pups were animals <1 yr, and juveniles were 1–2 yr. Subadults were 3–5 yr, and adults were >5 yr.

Samples obtained from the Kodiak Island area in 1985 were tested for antibodies to *C. psittaci* using a standard complement fixation technique for serum antibody detection at the Washington Animal Disease Diagnostic Laboratory (WADDL) (Wasserman and Levine, 1961). Titers ≥32 were considered to be positive, and titers ≥128 were considered to be indicative of a recent infection. Sera collected in the 1990s and 2000 from PWS and southeastern Alaska (SEA) were submitted to the National Veterinary Services Laboratory (NVSL; Ames, Iowa, USA) for complement fixation and considered to be "suspicious" at titers of 10 and strongly suggestive of recent exposure at titers ≥20. At both laboratories, some of the sera reacted with both the test antigen and the negative control, indicating a nonspecific reaction. These were excluded from the analysis.

Sera from 1986 from both the western population around Kodiak Island and the Gulf of Alaska, and from the eastern population in SEA were tested for antibodies to San Miguel sea lion virus (SMSV) serotype-5, -6, -10, and -13 using microtiter serum neutralization tests at Oregon State University, Corvallis, Oregon, USA (Barlough et al 1987). Forty animals from

the western population and 26 from the eastern population (including pups, subadults, and adults) were tested.

Samples collected between 1998 and 2000 were tested for antibodies to caliciviruses using a group-specific enzyme-linked immunosorbent assay (ELISA). The antigen was a calicivirus-specific recombinant protein (CKSD3A #1) at 1 μg/ml. To determine background binding, samples also were tested against the fusion tag portion (CKS) of the recombinant protein at 1 μg/ml. The secondary antibody was protein A alkaline phosphatase (P9650, Sigma, St Louis, Missouri, USA) diluted at 1:800. The colorizer was blue phos (KPL), and the plates were read at 650 nm. Sera were initially tested at a 1:100 dilution. Sera were considered to be positive if the corrected optical density (OD; determined as OD of CKSD3A#1−OD for CKS) was >0.100 with the OD of the antigen >2× the OD of the serum control.

Serum samples (n=185) collected in the 1980s were tested for antibodies to PhHV-1 by serum neutralization at 60 median tissue culture infective doses ($TCID_{50}$) at Erasmus Universiteit, Rotterdam, The Netherlands. A subset of these data consisting of 22 animals also tested for PhHV-2 were reported in Zarnke et al. (1997), but no information concerning location or age of the animals was included in that report. Antibody titers >20 were considered to be positive. In 1998–2000, 133 samples were tested for PhHV-1 using an indirect ELISA (iELISA) at the University of California, Davis, California, USA. Phocid herpesvirus-1 (Pacific isolate, HS950) (King et al., 1998) was propagated in Crandell-Rees Feline Kidney cells (CrFK) and purified by standard methods using cell disruption, clarification, and finally, ultracentrifugation over a 30% (w/v) sucrose cushion. The microtiter plates (Pro-bind, Falcon, Becton Dickinson, Franklin Lakes, New Jersey, USA) were coated overnight at 4 C with purified virus. Serum samples initially were tested at a 1:100 dilution. Antibody binding was detected by sequential incubation with protein A horseradish peroxidase conjugated to streptavidin (Zymed, San Francisco, California, USA) and O-phenylinediamine dihydrochloride (Sigma) producing a color change proportional to the herpesvirus antibody in the samples. Optical densities were read at 490 nm with an ultraviolet max kinetic microplate reader and the results analyzed using Softmax software, version 3.0 (Molecular Devices, Menlo Park, California, USA). Samples with OD values of >3× the negative control for that plate (data not shown) were assigned titers of >100, and OD values of >0.600 were considered to be positive.

Seventy-nine samples collected in the year 2000 from PWS and SEA were tested for antibodies to CAV-1 and CAV-2 by serum neutralization at Cornell University Diagnostic Laboratory, Ithaca, New York, USA, using a threshold titer of ≥4. Briefly, this was a standard microserum neutralization test done in 96-well plates. Twofold serial dilutions of test serum were mixed with an equal volume of CAV-1 and CAV-2 containing 30–300 $TCID_{50}$. The serum-virus mixture was maintained at room temperature for 1–1.5 hr. Following incubation, a 50-μl volume of MDCK cells was added to each well. The plates were incubated at 37 C in 5% CO_2 for 4 days. Neutralization was determined by the absence of CAV-1 cytopathology in the test wells.

In the 1970s, sera of 63 animals (38 juveniles, 23 adults, and two of unknown age) from PWS and Gulf of Alaska were tested at the University of Alaska, Fairbanks, Alaska, USA (Ritter, pers. comm.), for antibodies to *L. interrogans.* In the 1980s, 137 animals (29 fetuses, 28 juveniles, and 80 adults) were tested from the same area by the NVSL. In the late 1990s, 11 pups from the Bering Sea, 46 animals from PWS (26 pups and 20 juveniles), and 112 animals (89 pups, 17 juveniles, and six adults) from SEA were tested at Oklahoma State University (Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, Oklahoma, USA) or California Veterinary Diagnostic Laboratory (CVDLS; Davis, California, USA). A standard microscopic agglutination microtiter test (Colagross-Schouten et al., 2002) was used with a threshold titer of >100. Serovars tested against included *L. i. pomona, L. i. grippotyphosa, L. i. bratislava, L. i. canicola,* and *L. i. hardjo* by all laboratories; *L. i. tarasovi* and *L. i. autumnalis* also were included at the NVSL.

Thirty-three samples collected in 1998 in SEA were tested by serum neutralization to four marine mammal morbilliviruses using a standard protocol at Oklahoma State University. Seventy-nine samples collected in 2000 from PWS and SEA were tested by a competitive ELISA for antibodies to canine distemper virus and phocine distemper virus at Oklahoma State University as described by Saliki and Lehenbauer (2001). Fifty-three samples collected in SEA between 1998 and 2000 were tested by ELISA at the University of California, Davis, as described by Ham-Lammé et al. (1998).

Historically, 26 sera collected from 1978–94 from the Bering Sea to SEA were tested for antibodies to *Brucella* spp. using an iELISA at Central Veterinary Laboratories, Bacteriology Department, Surrey KT, United Kingdom. Thirty-one sera from SEA collected in 1997 were tested at CVDLS using a standard plate

TABLE 1. Prevalence (%) of complement fixation antibodies to *Chlamydophila psittaci* in Steller sea lions from different regions of Alaska, USA.[a]

Year collected	Kodiak Island (WADDL)	PWS (NVSL)	SEA (NVSL)
1985	0% (30)^{FT}		
	100% (2)^{J}	—	—
	67% (12)^{S}		
	94% (33)^{A}		
1992–2000		0% (1)^{FT}	—
	—	7% (30)^{P}	1% (71)^{P}
		22% (18)^{J}	48% (21)^{J}
		100% (1)^{AF}	64% (25)^{AF}

[a] Numbers in parentheses represent the sample size. Letters indicate the age class and the sex of animals sampled. Threshold titer was 32 at Wahington Animal Disease Diagnostic Laboratory (WADDL) and 20 at National Veterinay Services Laboratory (NVSL). A subset of the Kodiak data was reported by Calkins and Goodwin (1988). A = adult; F = female; FT = fetus; J = juvenile; P = pup; PWS = Prince William Sound; S = subadult; SEA = southeastern Alaska.

agglutination test with a threshold titer of 25. Forty-six samples from PWS and 94 from SEA collected from 1998–2000 were tested at Oklahoma State University using a card test (Nicoletti, 1967).

Seventy-nine samples from PWS and SEA were collected and tested in 2000 by the Cornell University Diagnostic Laboratory for canine parvovirus-2 using the hemagglutination inhibition test (HAI) with a threshold titer of 4. Methods have been described previously by Carmichael et al. (1980).

When sample sizes were adequate, antibody prevalence in animals from different regions and decades were compared using Fisher's exact test with Sigma Stat 2.30 (SPSS, Chicago, Illinois, USA), with statistical significance assumed to be at $P<0.05$.

RESULTS

Chlamydophila psittaci

Historical data from 1985 concerning the western population of Steller sea lions in Kodiak Island and some data from 1992–2000 in PWS and SEA were available. Antibodies to *C. psittaci* were detected in Steller sea lions from each region during all time periods (Table 1). Prevalence was highest in adults, with 64–100% seropositive. Of adult animals tested by WADDL, eight were male and 25 were female. With the 1992–2000 data, antibody prevalence estimates for pups and juveniles sampled from PWS and SEA were not significantly different (Table 1). Antibody prevalence increased with age in both areas (Table 2). Considerable variation was observed between the proportion of samples classified as nonreactors at NVSL (6% of 394) and WADDL (26% of 104).

Caliciviruses

Animals collected in 1986 were tested for antibodies to SMSV-5, -6, -10, and -13 by serum neutralization. Animals in both populations had evidence of exposure to

TABLE 2. Current data (1998–2000) on prevalence (%) of antibodies for *Chlamydophila psittaci* by complement fixation, calicivirus and phocid herpesvirus-1 (PhHV-1) by enzyme-linked imunsorbent assay (ELISA), and canine adenoviruses-1 and -2 (CAV-1 and -2) by serum neutralization in Steller sea lion pups and juveniles by age.[a]

Age	*C. psittaci*	Calicivirus	PhHV-1	CAV-1	CAV-2
2–4 mo	0% (61)	20% (55)	2% (53)	15% (27)	19% (27)
6–7 mo	4% (22)	24% (29)	4% (24)	—	—
11 mo	22% (9)	0% (13)	38% 913)	—	—
1–2 yr	36% (39)	20% (44)	30% (43)	37% (52)	27% (52)

[a] The threshold titer for *C. psittaci* was ≥20. Numbers in parentheses represent sample size.

TABLE 3. Historic and current data on prevalence (%) of antibodies to phocid herpesvirus-1 in Steller sea lions from different regions of Alaska, USA, using serum neutralizations (SN) at Rotterdam, The Netherlands, and enzyme-linked immunosorbent assay (ELISA) at University of California, Davis, California, USA.[a]

Decade	Test	Bering Sea/Aleutians	Kodiak Island	PWS	SEA
1970s	SN	100% (2)^{S}	50% (12)^{S}	—	—
		100% (3)^{A}	50% (4)^{A}		
1980s	SN	0% (12)^{U}	21% (19)^{S}	17% (6)^{S}	100% (1)^{P}
			30% (10)^{A}	0% (1)^{A}	16% (19)^{A}
Early 1990s	SN	—	0% (27)^{P}	—	0% (21)^{P}
			23% (13)^{A}		0% (35)^{A}
1998–2000	ELISA			20% (25)^{P}	3% (65)^{P}
				30% (20)^{J}	30% (23)^{J}

[a] Numbers in parentheses represent sample size. Letters indicate age class of the animals sampled. A subset of the historic data, along with data on phocid herpesvirus-2, was presented in Zarnke et al. (1997). A = adult; J = juvenile; P = pup; S = subadult; U = unknown.

each of these serotypes (Calkins and Goodwin, 1988). Antibody prevalence estimates for SMSV-5, -6, -10, and -13 from sea lions (n=40) representing the western stocks were 23, 10, 8, and 23%, respectively. Antibody prevalence estimates for SMSV-5, -6, -10, and -13 from sea lions (n=26) representing the eastern stocks were 33, 11, 15, and 33%, respectively. Significant differences in antibody prevalence observed in eastern and western stocks were not detected. For the 1998–2000 ELISA data, a significant difference was not detected between calicivirus antibody prevalence in pups and juveniles from PWS (22%) and SEA (18%). Prevalence did not appear to increase with age in animals between 2 mo and 2 yr of age (Table 2).

Phocid Herpesvirus-1

Historically, adult animals in all three regions tested positive for PhHV-1 antibodies by serum neutralization; however, antibodies were not detected in either the Aleutians in the 1980s or SEA in the early 1990s (Table 3). Only one of 49 pups sampled was positive for antibodies to PhHV-1 by serum neutralization. Examining more recent ELISA data, 20% of pups tested positive in PWS, and 3% tested positive in SEA (Table 3). Antibodies were detected in 30% of juveniles in both PWS (n=20) and in SEA (n=23). Pups became antibody positive as early as 2 mo of age, and antibody prevalence abruptly increased to 38% by 11 mo of age (Table 2). The difference in prevalence between the pups in PWS and those in SEA most likely related to a difference in the sample structure, with the PWS sample being composed of a higher proportion of older pups than the SEA sample.

Canine Adenoviruses

Regarding the 79 pups and juvenile animals tested for exposure to CAV-1 in 2000, prevalence rates were similar between PWS (30% of 46) and SEA (27% of 33). With CAV-2, 26% of 46 animals in PWS and 21% of 33 animals in SEA were antibody positive. Animals tested seropositive for both viruses at 2–4 mo, but prevalence increased with age (Table 2). Of the 23 CAV-1 and 19 CAV-2 antibody-positive animals, 15 were positive to both CAV-1 and CAV-2. In nine cases, titers were higher for CAV-1 than for CAV-2, whereas in five cases, CAV-2 titers were higher and in one case titers were equal.

Leptospira interrogans

Only three of 358 animals tested positive for antibodies to *L. interrogans* using the microagglutination test. The three positive animals were sampled in the Gulf of Alaska during the 1980s. One was a 12-yr-old female that had recently aborted and

was positive for *L. i.* serovar *icterohaemorrhagiae* at a titer of 100. Another female was nulliparous, 3 yr of age, and had a titer of 100 to *L. i. grippotyphosa.* A third female was pregnant, 5 yr of age, and positive to *L. i. bratislava* at a titer of 200.

Morbilliviruses

One 2-mo-old animal was positive for both dolphin morbillivirus and porpoise morbillivirus at titers of 16 by serum neutralization. This animal was one of 33 from SEA tested in 1998, and the same animal was negative by ELISA at the University of California, Davis. All other samples from 1998–2000 tested by a competitive ELISA for canine distemper virus and phocine distemper virus antibodies were negative.

Brucella abortus

All 197 animals tested for antibodies to *B. abortus* were negative except for one adult female from SEA that was sampled in 1986.

Canine parvovirus-2

Of the 79 animals tested for canine parvovirus-2 by HAI in the year 2000, two were positive at low titers. Both were juvenile animals, one from PWS and one from SEA.

DISCUSSION

Disease can increase mortality and cause reproductive failure through abortions, stillbirths, neonatal mortality, reduced fecundity, and reduced conception rates, all of which can have major impacts on the dynamics of wild populations (Scott, 1988; Gulland, 1995). The impacts of mass mortalities on marine mammal populations are well documented, such as the 1988 and 2002 phocine distemper virus epizootics in northern Europe that each killed approximately 18,000 harbor seals (*Phoca vitulina*) (Harwood 1990; Jensen et al., 2002). Such large-scale mortality may decrease host population size, making them susceptible to extinction from stochastic events (Harwood and Hall, 1990). Epizootics usually result from the introduction of a novel pathogen into an immunologically naive population, although factors altering host immunity also can act as a trigger (Spalding and Forrester, 1993). Effects of disease on reproduction of wild populations are less well documented. However, *Brucella* spp. and *C. psittaci* (formerly *Chlamydia psittaci*) (Eldson, 2002) can cause abortions and infertility, resulting in declines of host abundance (Cameron, 1947; Carmichael and Kenney, 1968; Brown and Grice, 1984), and parasitic nematodes can decrease breeding success by lowering the rate of juvenile survival (Hudson, 1986).

Because no program has existed for recovering Steller sea lion carcasses in Alaska, little is known about the prevalence of infectious diseases in this species. *Chlamydophila psittaci* was isolated from an aborted Steller sea lion fetus (Spraker and Bradley, 1996). A calicivirus was isolated from the rectum of a healthy sea lion pup in Oregon (Skilling et al., 1987); however, associated lesions or clinical illness were not present. Examination of other species of marine mammals in Alaska also has been limited for similar reasons, but existing serologic data from a number of species, including Steller sea lions, indicate that several infectious diseases are present in Alaskan waters. Published serologic data from Steller sea lions are restricted to a small number of samples ($n=27$) tested for antibodies to influenza A (Danner et al., 1998), samples collected in mid-1980s from Kodiak Island and the Bering sea that were tested for antibodies to several calicivirus serotypes (Barlough et al., 1987), and animals tested for antibodies to PhHV-1 and -2 (Zarnke et al., 1997). In all these cases, the number of samples was small, and the samples were not stratified by region.

Limited serologic data are available from other marine mammals in Alaska and adjacent arctic as well as subarctic areas. Antibodies to influenza A as detected by

double immunodiffusion have been noted in a ringed seal (*Phoca hispida*) in Alaska (Danner, 1998), and antibodies have been detected in 25% out of 903 ringed seals tested by a competitive ELISA in arctic Canada (Nielsen et al., 2001). Beluga whales (*Delphinaterus leucas*) in arctic Canada also were seropositive at 1.2% prevalence (Nielsen et al., 2001). Influenza A viruses have been the cause of mass mortalities in marine mammals (Geraci et al., 1982). Serologic testing of free-ranging Pacific walrus (*Odobenus rosmarus divergens*) in Alaska during 1994–96 detected low titers and low prevalence of antibodies to *L. interrogans* serovars, an 18% prevalence of calicivirus antibodies, and a 21 % prevalence of influenza A antibodies; antibodies to *Brucella* spp. and phocine distemper virus were not detected (Calle et al., 2002). Antibodies to *Toxoplasma gondii* have been reported in walruses, sea lions, harbor seals, ringed seals, bearded seals (*Erignathus barbatus*), and spotted seals (*Phoca largha*) tested in Alaska (Dubey et al., 2003). Antibodies to *Neospora caninum* have been found in California sea lions, walruses, harbor seals, and ringed seals. Antibodies to both PhHV-1 and -2 have been described in a variety of marine mammals from Alaska and Russia, including walruses, northern fur seals (*Callorhinus ursinus*), harbor seals, spotted seals, ribbon seals (*Histriophoca fasciata*), bearded seals, ringed seals, and Steller sea lions (Zarnke et al., 1997).

A number of infectious agents have been identified in pinniped populations in the northeastern Pacific Ocean; these populations have ranges that overlap that of the eastern Steller sea lion population. Caliciviruses, which are associated with vesicular and hemorrhagic diseases as well as with abortion in a variety of species (Smith, 2000), have been isolated from California sea lions of southern California and associated with cases of abortion and premature pupping. Herpesviruses have been detected in dead harbor seal pups with adrenal necrosis in California (Gulland et al., 1997) and in California sea lions with urogenital cancer (Lipscomb et al., 2000; King et al., 2002). *Toxoplasma gondii* causes abortion and multisystemic fulminant disease in some species and encephalitis in California sea otters (*Enhydra lutris*) (Cole et al., 2000) and harbor seals (Miller et al., 2001). *Leptospira interrogans* var. *pomona* has been isolated from California sea lions and northern fur seals and associated with reproductive failure and mortality in these otariids (Vedros et al., 1971; Smith et al., 1974). Brucellosis is associated with reproductive failure in a wide range of terrestrial and aquatic wildlife species, and a marine *Brucella* sp. has been isolated recently from marine mammals, including harbor seals in the northern Pacific with pneumonia (Garner et al., 1997; Foster et al., 2002). *Brucella* sp. infections have been associated with abnormal testes and uterus in common minke whales (*Balaenoptera acutorostrata*) and Bryde's whales (*Balaenoptera edeni*) (Ohishi et al., 2003) and with chronic meningoencephalitis in live-stranded striped dolphins (*Stenella coeruleoalba*) (Gonzalez et al., 2002). Abortions caused by placentitis have been associated with *B. cetacea* infection in bottlenose dolphins (*Tursiops truncatus*) (Miller et al., 1999).

In the present study, only limited interpretation of serologic data in the context of regional differences or chronologic changes in antibody prevalence was possible. Because much of the data were historic, sampling often was limited and did not occur at the same time in all regions. For example, data for *C. psittaci* were only available from Kodiak Island in the 1980s—after this population began to decline. Corresponding data for the 1980s were unavailable for PWS and SEA populations. Also, different laboratories and testing protocols were used over time, and even when the same test was used, threshold values for defining what was serologically positive often differed. These factors prevented direct comparison of data between regions and over time.

Despite these temporal and spatial limitations, these data do provide some insight regarding the epidemiology of these diseases and their potential role in the population decline. It was evident that *C. psittaci,* or a closely related agent, was endemic in both stocks. Evidence of exposure has been found since 1985–86 on Kodiak Island and since the early 1990s in the thriving population. Whether *C. psittaci* causes disease in Steller sea lions is not known, but it does produce reproductive failures, including abortion, stillbirth, and birth of weak offspring in sheep and goats (Papp, 1993); abortion and respiratory infections in people (Hyde and Benirschke, 1997); and infertility in koalas (Canfield et al., 1991). Steller sea lions appeared to become exposed to a *C. psittaci*–like agent between 1 and 5 yr of age, with the prevalence of positive individuals increasing to >60% in adults. The presence of antibodies in 1- to 5-yr-old animals that have not returned to the breeding sites (rookeries) indicates that exposure occurs not only on the rookeries but also on the nonbreeding sites (haul-outs).

Serologic data on caliciviruses are available from Barlough et al. (1987) and a technical report by Calkins and Goodwin (1988); these have been summarized by Burek et al. (2003). From these reports, it was evident that Steller sea lions had been exposed to a wide variety of caliciviruses in all regions of Alaska and that exposure had started at an early age. The patterns of exposure to different serotypes appear to vary between regions. Not all samples were tested for exposure to the same serotypes, however, making regional and chronologic comparisons difficult. A very similar rate of serologically positive animals was found between the western and eastern animals in our 1998–2000 data using a group-specific ELISA. Results from this test, however, give no information regarding potential differences in prevalence between serotypes. Pathogenicity of caliciviruses in other species varies by serotype (Smith, 2000). Further studies on the association between this organism and reproductive failure in Steller sea lions is warranted given that exposure to caliciviruses appears to be widespread, a calicivirus has been isolated from a Steller sea lion (Skilling et al., 1987), and these viruses are known to cause abortions in other species. Future studies should aim to determine differences in the prevalence of SMSV serotypes by region, both by serology and by characterization of the circulating viruses, either by isolation or polymerase chain reaction (PCR).

Exposure to PhHV-1, or to a closely related herpesvirus, occurred throughout the regions tested, with the antibody prevalence directly related to the age of the animal. Because PhHV-1 is a virus of phocid seals, Steller sea lions are more likely infected with a closely related virus, because most α-herpesviruses tend to be species specific. The effect of this herpesvirus on otariids, specifically Steller sea lions, is unknown. In harbor seal neonates and seals acutely infected with phocine distemper virus or otherwise immunocompromised, PhHV-1 can cause mortality and is associated with abortions (Osterhaus et al., 1985; Gulland et al., 1997). Further studies should be undertaken to characterize this herpesvirus genetically and to describe any differences in genotypes and pathology of the herpesvirus(es) by region.

Adenoviruses cause diseases in humans and in a wide range of animal species (Woods, 2001). Some adenoviruses are capable of causing epizootics, resulting in high mortality. Usually, however, clinical adenoviral disease is sporadic and limited to neonates or immunologically compromised individuals (Fenner et al., 1999). Several reports have appeared of adenovirus infection in marine mammals. Acute hepatic necrosis was described in California sea lions (Britt et al., 1979; Dierauf et al., 1981) and, based on electron microscopy, was thought to be caused by an adenovirus. Viral culture has not been successful, and the extent of cross-reactivity of serologic tests for CAVs to the sea lion

adenovirus is unknown. Adenoviruses also have been isolated from sei (*Balaenoptera borealis*) (Smith and Skilling, 1979) and bowhead (*Balaena mysticetus*) whales (Smith et al., 1987). Our results indicate that Steller sea lions were exposed to an adenovirus and that exposure appears to have occurred in both PWS and SEA. The serologic reaction most likely was a cross reaction between the CAVs and an adenovirus that is endemic in the sea lions, but viral culture is needed to confirm this. The disease potential of adenoviral infections in Steller sea lions is unknown.

Significant exposure to *L. interrogans* does not appear to have occurred in Steller sea lions in Alaska. This was unexpected, because leptospirosis is common in California sea lions and northern fur seals. Although they do not typically share rookeries, northern fur seals, northern elephant seals, and California sea lions occasionally are seen at some Steller sea lion rookeries and haul-outs. Of the three serovars to which antibodies were detected, only *L. i. grippotyphosa* has been reported previously in marine mammals (Stamper et al., 1998), and antibodies to the common serotype *L. i. pomona* in California sea lions and northern fur seals (Gulland et al., 1996) were not detected. The reason for this difference in serovars is unclear. It may result from false positives (because the titers were at threshold), or it may result from exposure of Steller sea lions to a different source of *L. interrogans,* such as from terrestrial wildlife.

Exposure to *Brucella* spp. also appears to be insignificant. It is possible, however, that the methods used in the present study did not detect infected animals, because validation of these tests specifically for marine mammals requires further investigation (Foster, 2002).

Morbillivirus epizootics can cause severe mortality (Kennedy, 1998). They have been documented regularly in marine mammals since 1988, when they were first isolated. One pup sampled in SEA in 1998 had low antibody titers to the porpoise and dolphin morbilliviruses by serum neutralization. With only one individual testing positive, it seems highly unlikely that morbilliviruses were present in the population, and this most likely was a false-positive result. This animal was negative by ELISA. In an exposed population, a range of titers should be observed. Testing of recent samples from 1998–2000 using a competitive ELISA has been uniformly negative. Molecular testing (PCR) of archived tissues may indicate whether morbillivirus antigen was circulating in the population during the peak of the decline in the 1980s and merits further consideration.

Canine parvovirus-2 is a member of the feline parvovirus subgroup, in which a number of closely related viruses affect a range of carnivore species (Barker and Parrish, 2001). It causes two syndromes in canids: myocarditis in pups <4 mo of age, and gastroenteritis in older animals. The severity of the signs depends on many factors, including age, nutritional status, and concomitant infections (Barker and Parrish, 2001). In naïve populations, an epizootic can occur with significant mortality in all age classes. For populations in which it is enzootic, most disease would be expected to occur in juveniles exposed to canine parvovirus following the decline of maternal antibodies at ~2 mo of age (Barker and Parrish, 2001). To our knowledge, no reports of otariids being affected by parvoviruses have appeared. In the present study, two animals tested positive by HAI at low titers, indicating possible exposure; however, positive animals were from both declining and stable populations.

In summary, no serologic evidence from the limited data currently available supports the possibility that an epidemic occurred during the rapid decline of Steller sea lions from the late 1970s to the 1980s. Some of the data leading to this conclusion are questionable, however, and sample sizes are insufficient to exclude this possibility completely. Exposure to a number of endemic disease agents suggests that in-

fectious disease could play a role in the current lack of recovery by Steller sea lions. Nothing is known about the prevalence of these endemic disease agents other than the prevalence of antibodies to them. Likewise, their potential for causing disease in Steller sea lions remains undocumented.

A systematic protocol should be established to screen for infectious diseases using both gross and histologic examination of carcasses, culture and molecular techniques to identify organisms, and serology. Serologic assays need to be validated for Steller sea lions, and careful sample banking procedures need to be instituted to prevent degradation of sera. Monitoring for the endemic disease will be done with consideration of sample selection by age matching and of regional differences. Monitoring for the major epidemic disease agents, including marine mammal morbilliviruses and influenza A, needs to be continued, both because pinnipeds appear to be particularly susceptible to these diseases and because these appear to be naïve populations.

Other disease agents of interest that could be looked for serologically in Steller sea lions include canine coronavirus, which has been detected serologically in Alaskan wolves (Zarnke et al., 2001); porcine circoviruses, which have been detected by PCR in sea lion feces (Skilling, pers. comm.); PRRSV, *Coxiella burnetti, N. caninum, Sarcocystis neurona,* and *T. gondii,* all of which cause abortion and systemic disease in other species; and *Salmonella* spp., *Campylobacter* spp., and *Erysipelothrix rhusiopathiae,* which cause of systemic disease in other marine mammals.

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