DISEASE AGENTS IN STELLER SEA LIONS IN ALASKA: A REVIEW AND ANALYSIS OF SEROLOGY DATA FROM 1975-2000

By

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DIRECTOR'S FOREWORD

Saint's Bread and Sick Sea Lions

St Nicholas of Tolentino (1245-1305) invented a miraculous healing bread that is still baked at his shrine in central Italy on his Saints day (10th September). The bread healed children and animals — hence St Nicholas is known as the patron saint of sick animals. The same bread was also said to have put out a fire in the Doges palace in Venice. St Nicholas was a strict vegetarian who fasted a lot and led a busy and blameless life. He was an effective and unassuming peacemaker in a nasty civil war, who instructed those whom he helped to "Say nothing of this". It is also said that he was once gave his blessing to a roast goose, which came back to life and flew out of the window. However, St Nicholas never encountered a sick sea lion, so was never able to put his bread to the ultimate test.

Little is known about the diseases to which Steller sea lions are susceptible. This is primarily due to the lack of dead and ailing sea lions available to examine for pathology. The remoteness and vastness of the regions occupied by these animals as well as the low human populations in these areas have made it extremely difficult to study the diseases of Steller sea lions. Other species of sea lions are known to suffer from a wide range of diseases caused by viruses such as influenza viruses, morbilliviruses, herpesviruses and bacteria such as Leptospira (an infection that causes renal failure). They are also susceptible to a variety of parasites including tapeworm, lungworm, stomach worm and other intestinal parasites, as well as nasal mites. The question is whether such diseases were involved in the decline of Steller sea lions in Alaska.

This report collates the results of serological tests of archived blood samples collected from Steller sea lions in Alaska over three decades — from the mid-1970s to the 1990s. Tests covered a wide range of viral and bacterial disease-causing organisms.

And the results? The pattern of Steller sea lion decline is consistent with a disease outbreak, but no 'smoking gun' was found. Further sleuthing is needed.

The Fisheries Centre Research Reports publishes results of research work carried out, or workshops held, at the UBC Fisheries Centre. The series focuses on the multidisciplinary problems of fisheries management (including marine mammals), and aims to provide a synoptic overview of the foundations, themes and prospects for current research. Fisheries Centre Research Reports are recorded in the Aquatic Sciences and Fisheries Abstracts and are distributed to appropriate workshop participants or project partners. A full list of the reports is published at end of this issue. All reports are available as free PDF downloads from the Fisheries Centre's Web site www.fisheries.ubc.ca, while paper copies are available for a modest cost-recovery charge.

***Saint Nicholas of Tolentino,* as painted in 1507 by Pietro Perugino (1450—1523), who trained with Leonardo da Vinci and Piero della Francesca in Florence. Perugino painted frescos on the newly-built Sistine chapel walls in 1481 and, three and a half centuries later, inspired the Pre-Raphaelites. St Nicholas looks a bit wan (the fasting no doubt), but his Latin text affirms "I am your servant and your son's holy shield". (Note 'ancille' can also mean 'goose', so I leave the alternative translation to the reader).**

Galleria Nazionale d'Arte, Rome, oil on panel, 79 x62 cm

Tony J. Pitcher
Professor of Fisheries
Director, UBC Fisheries Centre

ABSTRACT

Results of serology studies conducted from 1975-1996 on Alaskan populations of Steller sea lions (*Eumetopias jubatus*) were synthesized and supplemented with analyses of archived sera to assess the chronological and spatial patterns of exposure to disease agents and the role that infectious disease may have played in the decline of Steller sea lions in the Gulf of Alaska and Aleutian Islands. Serum samples were obtained during three periods (1970s, 1980s and 1990s) and were tested for exposure to *Leptospira interrogans*, caliciviruses, *Chlamydophila psittaci, Brucella sp*, morbilliviruses, influenza A, *Toxoplasma gondii*, phocid herpesviruses and canine parvovirus. Testing for these agents and canine adenoviruses 1 and 2 continued through 2000. In most cases, conclusions cannot be drawn about chronological changes in the prevalence of disease agents during the decline of Steller sea lions because the samples were not collected from all regions in each time period, nor from sufficient numbers of animals in each age class. In addition, samples were not all analyzed by the same laboratories, were not stored under controlled conditions, were not tested for the same disease agents, and assays were not validated for Steller sea lions.

There is no convincing evidence of significant exposure to influenza A, morbilliviruses, *Brucella abortus*, canine parvovirus and *Leptospira sp*. However, there is evidence of exposure to a herpesvirus, *C. psittaci,* caliciviruses, *T. gondii* and canine adenovirus in regions of both increasing and decreasing sea lion abundance. As these agents are either present throughout the areas examined, or were not evident in all of the animals examined, it is unlikely that these disease agents caused the population decline of sea lions by epidemic mortality. However, as the number of samples tested for morbillivirus is low, and the assays used have not been validated for Steller sea lions, exposure to a morbillivirus during the peak of the decline cannot be completely ruled out from the data available.

Some pathogens become endemic and interact with malnutrition or predation to decrease survival or reproduction—therefore preventing recovery of depleted populations. In other species, *C. psittaci*, herpesviruses, adenoviruses, and *T. gondii* are more readily expressed as clinical diseases when individuals are stressed. It is possible that these agents could be contributing to the lack of recovery by causing undetected mortality and morbidity, or by reducing fecundity and juvenile survival rates. A systematic disease agent monitoring protocol should therefore be initiated to adequately test for disease agents in different time periods and regions.

Serological studies are limited in that they only assess immunological response following exposure to infectious agents. They do not give information on the prevalence of disease agents, or on presence of clinical disease. Further studies should be aimed at detecting infectious agents directly, and determining their association with morbidity and mortality, as well as changes in host population dynamics.

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INTRODUCTION

There are two genetically distinct populations of Steller sea lions (*Eumetopias jubatus*) in Alaska termed the eastern and western stocks (Bickham et al. 1996, NMFS 1998). The western population of Steller sea lions has declined since the late 1970s, while the eastern population has increased (Braham et al. 1980, Loughlin et al. 1992, Trites and Larkin 1996, Calkins et al. 1997). The decline appeared to first occur in the eastern Aleutians and spread west through the Aleutians and east through the Kodiak region, the Gulf of Alaska and Prince William Sound. The dividing line between the decreasing and increasing populations is Cape Suckling (144°W longitude).

The leading hypothesis to explain the decline of western Steller sea lions in the Gulf of Alaska and Aleutian Islands is that young and recently weaned animals are dying in unusually high numbers due to inadequate nutrition (Alaska Sea Grant 1993, DeMaster and Atkinson 2002, Trites and Donnelly 2003). Such food shortages could be the result of commercial fisheries and/or natural changes in the ecosystem (NRC 1996, Rosen and Trites 2000). Other possible causes of the sea lion decline include increased incidence of parasites and disease, predation by killer whales (*Orcinus orca*), meteorological changes, pollution and toxic substances, entanglement in marine debris, and incidental and intentional take by man (Loughlin 1998). Data to assess each of the possibilities are limited and the cause of the decline remains unknown.

Reproductive failure could be a significant factor in the decline of Steller sea lions. Biologists have noted aborted fetuses during winter months (Calkins and Goodwin 1988), and birth rates of 55-65% (Calkins and Goodwin 1988, Pitcher et al. 1998). These birth rates are unusually low compared to typical birth rates of 79-95% observed in other otariid species (Trites and York 1993, Boyd et al. 1995). Reproductive failure can be caused by exposure to infectious diseases, stress and toxins, by poor nutrition and genetic defects. Disease can reduce reproduction in many species by decreasing litter size, or increasing abortions and juvenile mortality, all of which can have major impacts on the dynamics of wild populations (Cameron 1947, Scott 1988, Anderson and May 1991, McCallum 1994, Grenfell and Gulland 1996). For example, *Chlamydophila psittaci* (formerly *Chlamydia psittaci*—Eldson 2002) infections have resulted in declines in population abundance (e.g., koala bears *Phascolarctos cinereus*—Brown and Grice 1984). Disease can also reduce populations directly by decreasing survival. The impacts of massive mortalities during epizootics are well documented, such as the 1989 and 2002 phocine distemper virus (PDV) epizootic in northern Europe that killed approximately 18,000 harbor seals (*Phoca vitulina*) each (Harwood 1990, Jensen *et al.* 2002). Such large-scale mortality may dramatically decrease host population size and increase susceptibility to extinction from stochastic events (Harwood and Hall 1990). Epizootics generally result from the introduction of a novel pathogen into an immunologically naive population, although factors altering host immunity can also trigger epizootic events (Spalding and Forrester 1993). In the case of Steller sea lions, the pattern of decline spreading from an initial area is consistent with the introduction of a disease agent into the population.

Serum has been collected from Steller sea lions in Alaska since the 1970s. Some of this serum was analyzed close to the time of collection, and showed that Steller sea lions were potentially exposed to a variety of disease agents including *C. psittaci*, caliciviruses, herpesviruses, and *Toxoplasma gondii*. Other serum samples were archived for extended periods of time. The goal of our study was to summarize and consolidate the results of these historical serologic studies, and to supplement them with additional analyses of archived and currently collected sera to assess the chronologic and spatial patterns in exposure to several disease agents. Looking at patterns of exposure may provide insight into the role of infectious diseases in the decline of Steller sea lions.

METHODS

SAMPLE COLLECTIONS

Blood samples were obtained during three periods: 1970s, 1980s and 1990s. During October 1975-March 1977, whole blood was collected from 93 sea lions shot in the Gulf of Alaska (GOA) (Calkins and Pitcher 1982). From 1985-1986, the National Marine Fisheries Service (NMFS) and the Alaska Department of Fish and Game (ADF&G) collected samples from 178 animals from the Gulf of Alaska from Dixon Entrance to Unimak Pass (Calkins and Goodwin, 1988). Southeast Alaska (SEA) was considered the area south and east of Cape Yakataga to Dixon Entrance; the central Gulf was from Cape Yakataga to Wide Bay on the AK Peninsula; and the western Gulf was the area from Wide Bay to Unimak Pass**;** and the Kodiak area was defined as

the Kodiak Archipelago, Shelikof Strait, the coastal waters of the Kenai Peninsula from Wide Bay to Cape Douglas. This sample of sea lions was shot with a high-powered rifle, and was heavily biased towards adult females to facilitate reproductive studies. Blood samples were obtained immediately after shooting from freely bleeding external bullet wounds, or from the heart, body cavity, or major blood vessels after the body cavity was opened. Some of these serum samples were tested at the time for *Leptospira interrogans*, San Miguel sea lion virus (SMSV) and *C. psittaci*. During the 1990s, serum samples were collected from animals anesthetized with Telazol® via dart gun or isoflurane for other studies (Heath et al. 1997).

In all cases, blood samples were centrifuged at low speed. Fresh serum was harvested and frozen at –10°C and subsequently sent to the specified laboratories or stored in either a walk-in freezer or ultracold freezer.

SAMPLE ANALYSES

Chlamydophila psittaci

Dr. James Evermann at Washington Animal Disease Diagnostic Laboratory (WADDL) analyzed 104 samples obtained from the Gulf of Alaska in 1985 and 1986 for *C. psittaci* using standard complement fixation technique for serum antibody detection (Wasserman and Levine 1961). Titers $\geq$32 were considered positive, while those $\geq$128 were considered indicative of a recent infection (Evermann, pers. comm.). Sera collected in the 1990s and 2000 by Drs. T. Spraker, F. Gulland and K. Burek were submitted to The National Veterinary Services Laboratory (NVSL) for complement fixation and were considered 'suspicious' at titers of 10, and strongly suggestive of recent exposure at $\geq$ 20. In addition to these historical and current samples, we submitted 152 archived sera samples for retrospective analysis to NVSL.

Some of the sera at both laboratories reacted specifically with both the test antigen and the negative control, indicating a non-specific reaction. We excluded these from the analysis of percent positive. We also used data from original laboratory sheets and spreadsheets rather than from the reports by Calkins and Goodwin (1988) and Spraker and Bradley (1996) due to discrepancies between the reports and data sheets.

Caliciviruses (SMSV's)

Historically, 93 sera samples obtained in 1975-1977 and 143 obtained in 1985-1986 in the Gulf of Alaska, 5 sera from 1976 from the Bering Sea and 27 in 1986 from Southeast Alaska were submitted to Dr. A.W. Smith at Oregon State University, Corvallis, Oregon for calicivirus serology. Serologic testing by serum neutralization and results were reported by Barlough (1987) and Calkins and Goodwin (1988). In 1992/93, 14 animals from the Gulf of Alaska and 25 from Southeast Alaska were submitted to NVSL for calicivirus analysis. Titers $\geq$ 20 were considered positive.

Retrospectively, 35 of the archived sera samples were tested by Dr. Smith for SMSV-5 and McAllistair serotypes using serum neutralization. All 212 samples in Dr. Smith's archive and 141 samples collected between 1998-2000 were also tested using a group specific ELISA. For the archived samples, the antigen was CsCl SMSV-5,13,17. Sera were initially tested at a dilution of 1:100 and the secondary antibody was protein A. Sera were considered positive at a corrected O.D. >0.200 and when the O.D. of serum versus virus was $\geq$ 2X the serum control. For the samples past 1998, the antigen was a calicivirus specific recombinant protein (CKSD3A#1) at 1 µg/ml. For a control, samples were also tested against the fusion tag portion (CKS) of the recombinant protein at 1 µg/ml. Sera were initially tested at the 1:100 dilution. The secondary antibody was protein A alkaline phosphatase (sigma P9650) at 1:800. The colorizer was blue phos (KPL) and the plates were read at 650 nm. Serum were considered positive if the corrected optical density (O.D. of CKSD3A#1 minus CKS) was $\geq$0.100 with the O.D. of the antigen $\geq$2X the O.D. of the serum control.

Phocid Herpesvirus-1

Serum microneutralization (SN) tests were used to detect antibodies against phocid herpesvirus-1 (PhHV-1) and PhHV-2 at Erasmus Universiteit Rotterdam (Sheffield and Zarnke 1997, Zarnke *et al.* 1997). There were 187 animals tested for just PhHV-1, and 22 were tested against both. Data from the 22 tested for both were published by Zarnke *et al.* (1997). These were from all regions of Alaska, and titers $\geq$20 were considered positive. The PB84 isolate of PhHV-1 was grown in seal kidney cells (seKC) (Osterhaus et al. 1985). One-hundred mean tissue culture infective doses (TCID50) of virus were used and samples $\geq$20 were referred to as positive.

In 1998-2000, 88 serum samples from Southeast Alaska and 45 from Prince William Sound were tested for phocid herpesvirus-1 antibodies using an indirect enzyme-linked immunosorbent assay (iELISA). Phocid herpesvirus-1 (PHV-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 ultracentrifuged over a 30% w/v sucrose cushion. The microtiter plates (Pro-bind, Falcon, Beckton Dickinson, Franklin Lakes, NJ, USA) were coated overnight at 4°C with purified virus. Steller sea lion serum samples were initially tested at a 1:100 dilution. Antibody binding was detected by sequential incubation with protein A horseradish peroxidase conjugated to strepavidin (Zymed, San Francisco, CA, USA) and *O*-phenylinediamine dihydrochloride (Sigma, St Louis, MO, USA) producing a color-change proportional to the herpesviral antibody present in the samples. Optical densities were read at 490nm with a UV max kinetic microplate reader and results analyzed using Softmax software, version 3.0 (Molecular Device, Menlo Park, CA, USA). Samples with O.D. values of $\geq$3X the negative control for that plate (data not shown) were assigned titers of $\geq$100. O.D. values > 0.600 were considered positive.

Canine adenoviruses 1 and 2

Forty-six samples from Prince William Sound and 33 from Southeast Alaska were collected and tested in 2000 for canine adenoviruses 1 and 2 by serum neutralization at Cornell University Diagnostic Laboratory using a threshold titer > 4.

Marine morbilliviruses

Two hundred and six archived samples collected in 1979-1994 were tested by Dr. Jerry Saliki at Oklahoma State University for canine distemper virus (CDV), phocine distemper virus (PDV), porpoise morbillivirus (PMV) and dolphin morbillivirus (DMV) by serum neutralization. Titers $\geq$8 were considered positive. Samples collected in 1998-2000 were tested by serum neutralization or a competitive ELISA (cELISA) for CDV and PDV at OK State (Saliki and Lehenbauer 2001) or an ELISA at UC Davis.

Toxoplasma gondii

Twenty-five samples collected between 1978 and 1994 were tested by the Parasite Biology and Epidemiology laboratory, in Beltsville, MA for evidence of exposure to *T. gondii* using a modified agglutination test (MAT). Six animals were tested retrospectively from 1985/1986 that had recently aborted and 27 pups and juveniles from Southeast Alaska collected in 1998-2000 were tested for *Toxoplasma gondii* using latex agglutination at OK State University. At OK State University, $\geq$64 was considered positive and <64 suspicious. Thirty-three animals from Southeast Alaska were tested by MAT at CVDLS in 1991. Values $\geq$16 were considered positive and $\leq$16 suspicious for exposure.

Leptospira sp.

Sera collected from 63 sea lions in the Gulf of Alaska (1975-1977) were analyzed in 1978 for antibodies against *Leptospira interrogans*, serovar *pomona* by D. Ritter of Fairbanks, Alaska (Calkins and Pitcher 1982). One hundred and thirty-seven serum samples obtained in 1985 and 1986 were submitted to Dr. A. B. Thiermann at the Central Plains Area National Animal Diseases Center in Ames, Iowa for serology using an eight-way test. Sera from 30 animals collected in 1997 from Southeast Alaska were tested at the California Veterinary Diagnostic Laboratory Services (CVDLS) for exposure to five serovars, and pups were also tested for exposure to *L. p. bratislava*. Thirty-two archived and 128 current (1998-2000) samples were analyzed at Oklahoma State University for exposure to seven serovars. A standard microscopic agglutination microtiter (MAT) procedure was used (Cole et al. 1979), and samples with titers $\geq$100 were considered positive.

Influenza A

Twenty-seven samples collected in 1978- 1994 from all areas were tested for influenza A virus using a double agar immunodiffusion assay at the School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin. Seventy-five samples from all areas were tested retrospectively at the National Veterinary Services Laboratory (NVSL) by agar gel immunodiffusion (AGID).

Brucella spp.

Twenty-six samples collected from 1978 to 1994 from all areas were tested for *Brucella spp*. using an indirect enzyme linked immunosorbent assay (iELISA) at CVL (Bacteriology Department, Surrey KT, United Kingdom). One hundred and eighty six samples were tested retrospectively at Oklahoma State by a *Brucella abortus* card test, which was followed by a complement fixation test and a Rivanol test. Thirty-one samples collected in 1997 in Southeast Alaska were analyzed at CVDLS by standard plate agglutination with a threshold titer of 25.

Canine parvovirus

Fourteen samples collected in 1989 from the Kenai, Kodiak, and Prince William Sound (PWS) areas were tested for canine parvovirus antibody by immunofluorescent antibody test at Colorado State. The threshold titer was 8. Thirty-two samples from Prince William Sound, western Aleutians and Southeast Alaska from 1994-1997 were tested retrospectively by immunofluorescent antibody test at Oklahoma State. The threshold titer was 5. Forty-six samples from Prince William Sound and 33 from Southeast Alaska were collected and tested in 2000 at Cornell University laboratory using the Hemagglutination inhibition test (HAI/2-ME) with a threshold titer of 4.

RESULTS

Chlamydophila psittaci

Looking at historical data from WADDL, 71% of 1-5 yr olds (n=14) and 94% of adults (n=33) sampled in the Gulf of Alaska in 1985 were antibody positive for *C. psittaci*. Antibodies were also detected in one adult animal, but not in one fetus and eight pups tested by NVSL in Kodiak and CSE in 1992-1993 (Table 1). In southeast Alaska, 100% of adult females in the 1992/3 (n=19) were *C psittaci* antibody positive (Table 1) at a titer of 10. All fetuses (n=31) and pups (n=9) were negative serologically for *C. psittaci* (from all regions and years combined).

Since antibody prevalence was highest in adults in the historical data, adults were targeted in the retrospective analyses. In this analysis, 50% of adults (n=4) and 33% of the subadult males (n =3) from the Bering Sea and Aleutian Islands were positive for *C. psittaci* at 10 or higher in the 1970s (Table 1). This compares to 87% (n=15) in the 1980s, and 80% (n=5) in the 1990s. These are not significant differences. In the Gulf of Alaska, 64% of the adults sampled in the 1970s (n=36), 45% in the 1980s (n=22) and 89% in the 1990s (n=9) were antibody positive for *C. psittaci*. No serum was available for southeast Alaska animals in the 1970s. However, samples from the 1980s were 48% (n=31) positive and 84% (n=19) in the 1990s among adult sea lions (Table 1). Thirty three percent (n=6) of fetuses from Southeast Alaska were positive at a titer of 10. Significantly fewer serum samples tested positive at the 20 level. In all regions combined, only 16 of the adults were male, the rest were female. With such small numbers, they were combined with the females. Fifty six percent (9/16) of the adult males were positive at 10 and none at 20.

In current serology studies juveniles and pups in both Prince William Sound and Southeast Alaska were tested (Table 2), and antibody prevalence was very similar in the two regions. Using the more rigorous threshold level of 20, individuals began to be positive around 6-7 months and the antibody prevalence increased with age (Table 3).

Results from 10 samples tested both historically at WADDL and retrospectively by NVSL were not consistent (Table 4). There were three major differences in the results from the two laboratories. First, WADDL's positives were found to be negative or suspect by NVSL, while WADDL's non-specific samples were generally classified as suspect by NVSL. Second, titers were very different. At WADDL, higher titers were common. For the NVSL retrospective positive samples titers were generally quite low (10 to 40) in the Bering Sea region and the Gulf of Alaska, while 19% (n=16) of the animals in southeast Alaska had titers $\geq$80. Sera of the animals from Southeast Alaska that had higher titers were stored for the least amount of time. Whether these differences were due to different analytical procedures at the laboratories, or sample degradation is difficult to determine. Because of the appearance of the retrospective samples and a history of freeze/thaw problems, we suspect a sample degradation problem. Another difference was the variability in the percentage of samples that reacted non-specifically (i.e., 6% of 394 samples at NVSL were non-specific compared to 26% of 104 samples at WADDL).

Caliciviruses (SMSV's)

Historically as many as 13 serotypes of caliciviruses were included in the survey between 1975 and 1995 including SMSV-1, SMSV-2, SMSV-4, SMSV-5, SMSV-6, SMSV-7, SMSV-8, SMSV-10, SMSV-13, SMSV-14, and Walrus, Cetacean and bovine calicivirus (BCV). Only SMSV-5, -6, -10 and -13 were tested on sufficient samples for any comparison between areas and times (Table 5). In the Bering sea / Aleutians in the 1970s, 20% (3) adult females and 100% (2) of subadult males were positive serologically to SMSV-5. In the Gulf of Alaska, the prevalence of SMSV-5 in non-fetal animals was 44% (n=93) in the 1970s, 13% in 1985, 23% in 1986, and 0% in the 1990s. In southeast Alaska, the prevalence was 33% in 1986 and 4% (n=25) in the 1990s. Animals with antibodies to this serotype, as well as serotypes 6, 10, and 13, were present in both declining and stable populations. Others were tested too sporadically for a reasonable comparison. Samples were all negative for SMSV-4 and Walrus serotypes.

All of the archived serology samples tested for calicivirus antibodies at OSU (Dr. A. W. Smith) were negative for SMSV-5 and McAllistair serotypes by serum neutralization (Table 11). Two individuals, one from the Gulf of Alaska and one from Southeast Alaska, were positive by group specific ELISA. Many of these samples had been previously tested using serum neutralization to the panels of calicivirus. The negative results were not in keeping with the earlier findings and may reflect sample degradation or possibly lack of sensitivity of the ELISA.

In contrast, samples tested from 1998-2000 by group specific ELISA were 22% positive in Prince William Sound and 18% in Southeast Alaska (Table 2). Except for the 9 and 11 month old animals that were 0% positive (n= 13), age did not appear to be a significant factor (Table 3). One pup (n= 35) tested by serum neutralization in the Gulf of Alaska was positive to SMSV-5 at a titer of 10.

Phocid Herpesvirus-1

Historically, adult animals in all three regions were commonly positive for phocid herpesvirus 1 (Table 10) antibodies by serum neutralization. The only exceptions to this were adult animals in Southeast Alaska in the early 1990s. Pups and juvenile animals sampled in 2000 and tested by an ELISA had similar prevalences of herpesvirus antibodies from Prince William Sound and Southeast Alaska, and antibody prevalence increased sharply with age around 11 months old (Table 3).

Canine adenoviruses 1 and 2

Of the 79 pups and juveniles tested for canine adenoviruses 1 and 2 between 1998-2000, the percentage positive was similar between Prince William Sound and Southeast Alaska for both CAV-1 and CAV-2 (Table 2). Twenty-three were positive to CAV-1, 19 to CAV-2. Fifteen of these were double positives. In 17 cases, CAV-1 had a higher titer than CAV-2. In 9 cases, CAV-2 had higher titers.

Morbilliviruses

A small amount of historical data presented by Sheffield and Zarnke (1997) has since been determined to be unreliable due to inconsistencies in the laboratory reports and was disregarded.

Of the 206 archived samples analyzed retrospectively at OK State, 0.4% (1) had positive titers for CDV (canine distemper virus), 5% (11) for PDV (phocine distemper virus), 2.3% (5) for PMV (porpoise morbillivirus) and 2% (4) for DMV (dolphin morbillivirus). There were positive individuals in the Bering Sea in the 1970s and 80s, in the Gulf of Alaska in the 1970s, and 1980s and one positive animal in Southeast Alaska in the 1990s (Table 7). Most of the positives were low (<16), and were often to only one serotype (Table 8). The only higher titer (64) was in one individual in the Bering Sea in the 1980s. Since many of the OK State titers were low and to only one serotype, these were most likely false positives. In ongoing testing by a competitive ELISA for CDV and PDV antibody from 1998-2000 in Prince William Sound and Southeast Alaska, all samples have been negative (Table 11).

Toxoplasma gondii

Although very small numbers of samples have been tested, adult animals in all three regions were commonly positive for *T. gondii* antibodies (Table 9). All 5 samples from animals that had recently aborted were negative for *Toxoplasma gondii* antibodies using latex agglutination.

Leptospira spp.

In the Gulf of Alaska, none of the sea lions sampled in the 1970s tested positive at 100 for *Leptospira interrogans* (n=63, Table 11). Ten years later, 2% (n=137) were positive serologically. Comparable samples were not available from the increasing population in southeast Alaska. However, no positive serology for *Leptospira interrogans* was detected in Southeast Alaska in 1997-2000 (n=158) (Table 11).

Of the three animals from the Gulf of Alaska that were positive in the 1980s, one was a 12-year-old female who had recently aborted. She was positive to serovar *icterohaehorrhagiae* at 100. Another female (nulliparous 3-year-old) had a titer of 100 to *grippotyphosa*, and the third (a pregnant 5-year-old female) was positive to *bratislava* at a titer of 200.

None of the 32 archived samples submitted to OK State University for the standard microscopic agglutination microtiter procedure tested positive for *L. interrogans* (Table 11).

Influenza A

All 102 animals tested either historically or retrospectively for influenza A virus antibodies were negative (Table 11).

Brucella abortus

Over 300 animals tested for *Brucella abortus* or *B. spp* antibodies (Table 11) were negative. The only exception was one adult female from Southeast Alaska.

Canine parvovirus-2

Forty-six animals have been tested either historically or retrospectively for CPV-2 antibodies by IFA (Table 11) and all were negative. Of the 79 animals tested for CPV-2 by HAI in 2000, two were positive at low titer (one in Prince William Sound and one in Southeast Alaska).

DISCUSSION

Since 1975, Steller sea lions have been tested serologically for exposure to *Brucella sp*, *Chlamydophila psittaci*, morbilliviruses, influenza A, *Toxoplasma gondii*, canine parvovirus, caliciviruses, *Leptospira sp*. and phocid herpesviruses. Of these disease agents, positive antibody reactions have been most prevalent to *C. psittaci,* caliciviruses, herpesvirus, and *T. gondii*. All others were either not detected or were detected at very low frequencies. Tests for canine adenoviruses were first conducted in 1998. Positive antibody reactions were found to both CAV-1 and CAV-2 at moderate frequencies in both Prince William Sound and Southeast Alaska.

Chlamydophila psittaci

C. psittaci can produce reproductive failures including abortion, stillbirths, and birth of weak offspring in sheep and goats (Papp et al. 1993), abortion and respiratory infections in people (Hyde and Benirschke 1997), and conjunctivitis and subclinical to severe bronchointerstitial pneumonia in cats, cattle, sheep, goats, birds and horses (Dungworth 1993). *C. psittaci* infections in koalas are associated with clinically evident conjunctivitis ("pink eye"), rhinitis, cystitis, and genital inflammation (Canfield et al. 1991). Expression of disease, particularly in birds, is thought to be stress related. *C. psittaci* is transmitted by aerosols originating either from infected placentas or tissues of aborted fetuses or by ingestion of contaminated feed (Papp et al. 1993). The respiratory form is transmitted either from aerosols of fecal material or from the respiratory tract of other infected animals.

It is not known whether *Chlamydophila* cause disease in Steller sea lions, but it is clear that Steller sea lions have a high antibody prevalence to a *C. psittaci*-like organism (Table 1). Sea lions appear to become exposed, as they become antibody positive between 1-5 years of age, with the prevalence of positive individuals increasing to >60% in adults (Tables 1 and 2). Prevalence of antibodies appears to be higher in the spring during pupping and breeding than in October (Calkins and Goodwin 1988). *C. psittaci*-induced lesions were not noted on any of the animals that were examined (Calkins and Goodwin 1988).

In assessing *C. psittaci* serology from NVSL, it is debatable whether the less rigorous threshold titer of 10 should be applied when dealing with an animal for which the test has not been standardized, or whether the more rigorous 20 level should be used. False positives are more likely to be picked up at 10 due to reactions with other organisms, such as gram-negative organisms. However, a related organism for which the test was not specifically developed, possibly unique to Steller sea lions, may also be picked up. In the retrospective analyses, the threshold of 10 correlated better with the results of WADDL that was performed close to the time of collection. We found evidence of exposure in all regions of Alaska at both 10 and 20 thresholds—but we do not know a) what exact organism or organisms caused the reactions, b) at what levels the tests were accurate, or c) whether or not any disease is associated with infection with *C. psittaci*.

Of all the disease agents tested for in Steller sea lions, *C. psittaci* appears to be the most prevalent, occurring in 50-90% of the adults tested. Unfortunately, it is difficult to draw meaningful conclusions about chronologic changes in the prevalence of *C. psittaci* because serum was gathered from different regions and times, and was tested at different laboratories using different criteria. However, it is clear that animals in the thriving eastern stock were exposed to *C. psittaci* at a high frequency during the 1990s and that *C. psittaci* or a *C. psittaci*-like organism appears to have been circulating in this area since the 1980s.

There are a number of shortcomings to the *C. psittaci* analysis. The most significant is that if you only look at samples analyzed close to the time of collection, the data are not directly comparable because of differences in the laboratories used. Most of the data for the Gulf of Alaska came from WADDL. NVSL in Ames Iowa generated other data and used different threshold titers. When sera that had been tested by WADDL in the 1980s were reanalyzed retrospectively by NVSL, there was no consistent relationship. It is difficult to say whether this is a factor of different laboratories, different methods,

or due to sample degradation. However, the older samples grossly appeared abnormal and at least with the Anchorage Alaska Department of Fish and Game samples, there was a history of possible freeze thaws and storage at suboptimal temperatures. Also, many of the tests for other disease agents conducted retrospectively were largely negative, even when previously positive. This suggests that sample degradation was the major problem and that the retrospective results are highly suspect.

Caliciviruses (SMSV's)

Caliciviruses have been shown to cause abortion, vesicular dermatitis, and arthritis in a number of species. The best-known syndrome in the veterinary field is a vesicular or ulcerative disease--vesicular exanthema of swine (VES), which occurs in pigs, ruminants, and marine mammals. Direct losses in pigs include weight loss, diminished growth rate, abortion, death of suckling pigs and decreased milk production (Studdert 1978). Vesicular exanthema is characterized by formation of vesicles (blisters) on the snout, mouth, non-haired skin and feet in pigs. The pathogenesis of abortion has not been determined, but is most likely secondary to severe, systemic, febrile disease of the sow (Studdert 1978). In cats, caliciviruses can cause respiratory disease, mostly upper respiratory diseases with occasional pneumonia (Gillespie and Scott 1973), fatal hemorrhagic disease and abortion with some strains. Caliciviruses are also an important cause of pediatric viral gastroenteritis in humans (Caul 1996). Morbidity in domestic species varies between 10-100% and appears to be related to the virus type, host and environmental factors.

Caliciviruses have been recovered from two disease conditions in marine mammals: vesicular lesions on the flippers of California sea lions and northern fur seals, and cases of abortion and premature pupping among California sea lions (Barlough et al. 1987), fur seals, and northern elephant seal (A.W. Smith, pers. comm.).

Antibody prevalence to a variety of caliciviruses appears to be widespread in Steller sea lions (Table 5). Although the overall prevalence rates between Kodiak and Southeast Alaska were similar, they had a different pattern of serotypes. Different serotypes may well have different levels of pathogenicity (Smith 2000). This bears further scrutiny. Calkins and Goodwin (1988) found a higher prevalence for SMSV-5 and SMSV-13 in Southeast Alaska than in the Kodiak area. Prevalence rates were lowest among the nulliparous females when females were grouped into nulliparous, pregnant or post partum, and reproductively failed (Calkins and Goodwin 1988).

The major shortcoming of the calicivirus analysis is that different serotypes were tested for in different years and regions, making detailed comparisons between years or regions impossible. The original data from Southeast Alaska animals are also missing (n = 26). The only indication that they were tested is a chart in the report by Calkins and Goodwin (1988). It would be useful to try and determine if there were particular serotypes present within the affected versus unaffected areas and whether this corresponded to either lesions or incidences of abortion.

Another shortcoming of the calicivirus analysis is that all of the 212 archived serology samples we tested for caliciviruses retrospectively were negative for SMSV-5 and group specific ELISA, even though some of these had previously tested positive by serum neutralization. This is not in keeping with the earlier findings and most likely reflects sample degradation. Again, as with the *C. psittaci* results, these findings make the retrospective results suspect.

Phocid Herpesvirus (PhHV-1)

Phocid herpesvirus 1 (PhHV-1) is an alpha-herpesvirus. The alpha-herpesviruses in general tend to be skin or mucosal pathogens and have the capacity to establish latent infections typically in the sensory ganglia. Many herpesviruses activate during times of stress, with development of clinical signs and increased transmission. Latently infected carriers are therefore an important source of infection for susceptible animals (Gaskell and Willoughby 1999). All infected, clinically recovered animals may be carriers.

Clinical signs in seals naturally infected with PhHV1 range from subclinical infection, through mild upper respiratory diseases and severe pneumonia (Osterhaus *et al.* 1988, Harder *et al.* 1996), to generalized infection, with almost 50% mortality (Osterhaus et al. 1985). During a natural outbreak among juvenile harbor seals in a Dutch sanctuary in 1984, 11 of 23 (48%) animals died. Natural transmission presumably occurs by means of aerosols or direct contact as in other alpha-herpesvirus infections (Zarnke et al. 1997). Because of the inability to experimentally produce such severe disease as observed in the original outbreak, the exact role or pathogenicity of herpesvirus infection alone is controversial.

Mortality due to PhHV-1 infection has only been observed in neonates and seals acutely infected with phocine distemper virus or otherwise immunocompromised (Zarnke *et al.* 1997). Therefore exposure to PhHV-1 alone may not be enough to result in disease in adult animals, but could act in conjunction with another stressor. PhHV-1 antibody positive animals have been found throughout the range of the Alaskan Steller sea lions. Thus it is unlikely that PhHV-1 was the primary cause of mortality in Steller sea lions. However, it could potentially cause disease secondary to other stresses. Since we know nothing of the actual herpesvirus, but just serological response, there could even be genetic differences and therefore different pathogenicities of the herpesviruses in the different regions.

Canine adenoviruses 1 and 2 (CAV-1 and –2)

Adenoviruses in general cause diseases in a wide range of animal species (Wood 2001) resulting in high mortality. However, clinical adenovirus disease is usually sporadic and limited to neonates or immunologically compromised individuals (Fenner et al. 1993). CAV-1, the cause of infectious canine hepatitis (ICH), can cause severe diseases in dogs and other canids, and is ubiquitous. Fatalities occur in younger animals but are rare in dogs older than 2 years of age. In dogs, diseases can range from a mild febrile illness with pharyngitis and tonsillitis, to vomiting, melena, high fever, abdominal pain, petechiation, and death. There is necrosis in the liver with presence of typical intranuclear inclusion bodies. Focal interstitial nephritis may be present and corneal edema ("blue eye") may develop late in the course of the disease. Immune mediated glomerulonephritis may also be a sequela to the disease. In foxes with CAV-1, neurologic signs often develop in the fulminant cases with a 10-25% mortality rate (Zarnke 1981). A captive polar bear was reported with probable ICH. American black bears are also known to be susceptible to disease.

CAV-2 causes a mild to moderate tracheobronchitis and appears to be involved in the kennel cough complex in which a primary viral infection followed by secondary bacterial infection results in persistent tracheobronchial inflammation. Occasionally there can be a bronchiolotis, however naturally occurring pulmonary disease caused by adenovirus in dogs is mostly found in conjunction with canine distemper or other conditions causing immunologic impairment.

There are several reports of adenovirus infection in marine mammals. Acute hepatic necrosis was described in California sea lions (Britt *et al.* 1979, Dierauf *et al.* 1981) and thought to be due to an adenovirus. Adenoviruses have also been isolated from sei (*Balaenoptera borealis*) and bowhead (*Balaena mysticetus*) whales.

Our results indicate that Steller sea lions have been exposed to an adenovirus. Adenoviruses CAV-1 and 2 in particular are very cross-reactive, so the reaction to both indicates exposure to an adenovirus, not necessarily to two different ones. Sea lions may even have their own type of adenovirus. Exposure appears to have occurred in both Prince William Sound and Southeast Alaska at a similar rate and pattern (Table 2). There are no data on historical exposure pattern or whether any disease is associated with exposure to the adenovirus.

Morbilliviruses

Morbilliviruses are highly contagious and frequently cause epizootic disease in previously unexposed host populations. Morbilliviruses include canine distemper, which affects terrestrial carnivores and Baikal seals; measles which affects man; and rinderpest and Peste des Petits Ruminants virus which affect ruminants. In addition, there are now a series of viruses infecting marine mammals including phocine distemper virus (PDV), dolphin morbillivirus (DMV), and porpoise morbillivirus (PMV). These viruses cause multisystemic diseases including pneumonia, conjunctivitis, encephalitis, enteritis, abortion and death.

Morbillivirus epizootics have been documented six times in aquatic mammals since 1988 when they were first isolated. Affected animals include European harbour seals and grey seals (*Halichoerus grypus*) in 1988 and 2002, Baikal seals (*Phoca siberica*) in Siberia from 1987-1988, striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea from 1990-1992 (Domingo et al. 1990) and bottlenose dolphins (*Tursiops truncatus*) along the eastern coast of the United States from 1987-1988, and in the Gulf of Mexico from 1993-1994 (Kennedy 1998). Approximately 18,000 harbour seals and a few hundred grey seals died in 1988 and again in 2002 (Heide-Jorgensen and Harkonen 1992, Jensen *et al.* 2002).

Small numbers of the Steller sea lions sampled were antibody positive to CDV, PDV, PMV and DMV (Tables 7 and 8). However, we suspect these were false positives. Most of the positive titers in the retrospective samples were at the

threshold of 8, and were generally to one serotype (Table 8). To be convincing enough to say that these animals were exposed to a morbillivirus in an area without known morbilliviruses, titers would have to be higher, and positive results between PMV/DMV and CDV/PDV should have been paired since these are believed to be closely related. Ongoing testing of animals from 1998 - 2000 using ELISAs and serum neutralization was also uniformly negative.

Toxoplasma gondii

T. gondii is an obligate intracellular apicomplexan protozoan parasite that infects a wide variety of mammals and birds and has a worldwide distribution. Members of the Felidae are considered to be the only definitive host. A range of intermediate hosts are infected by oocytes shed in the feces of cats. Transplacental infection and abortion also commonly occurs in sheep and goats, rarely in swine and humans. Carnivores become infected by ingesting cysts in the tissues of infected animals.

Systemic toxoplasmosis occurs most often in young animals, especially immunologically immature neonates, in immunocompromised hosts or in species such as marsupials and highly arboreal primates which probably evolved in isolation from cats (Barker et al. 1993). Because of the association with debilitated immune function, systemic toxoplasmosis is seen in conjunction with morbillivirus infection, ehrlichiosis and lymphosarcoma. Infected animals with a functioning immune system will often develop chronic or dormant form of toxoplasmosis characterized by the formation of cysts in the brain, skeletal muscle, and myocardium. In fulminant systemic toxoplasmosis, clinical signs vary depending on organ involved and include fever, lethargy, anorexia, ocular and nasal discharges, respiratory distress. Meningoencephalitis is associated with incoordination, circling, tremors, opisthotonus, convulsions, and paresis. Typical lesions include interstitial pneumonitis, focal hepatic necrosis, lymphadenitis, myocarditis, nonsuppurative meningoencephalitis (Kennedy and Miller 1993).

In marine mammals, this parasite and the associated meningoencephalitis is reported as an "emerging disease" in southern sea otters (*Enhydra lutris nereis*) (Jessup et al. 2000) and harbour seals (Chechowitz et al. 2000). Approximately 40% of mortalities in necropsied fresh sea otter carcasses are due to infectious diseases and parasites (Thomas and Cole 1996). A significant proportion of those are toxoplasma positive with associated meningoencephalitis, and over 70% of the sea otters tested were positive for antibody to *T. gondii*. In other marine mammals, *T gondii* is seen occasionally and in dolphins is often associated with morbillivirus infection (Domingo *et al.* 1992, DiGuardo *et al.* 1995) or suspected immuno-suppression (Van Pelt and Dietrich 1973, Inskeep *et al.* 1990).

Toxoplasmosis has been diagnosed in two free-ranging beluga whales from the St. Lawrence estuary where 27% of the belugas tested were antibody positive to *T. gondii* by modified agglutination test (Mikaelian et al. 2000). There are also reports of fatal toxoplasmal meningoencephalitis in a west Indian manatee (Buergelt and Bonde 1983), a northern fur seal (Holshuh et al. 1985), in stranded bottlenosed dolphins (Cruickshank *et al.* 1990, Inskeep *et al.* 1990), spinner dolphin (Migaki et al. 1990) and striped dolphins (Domingo et al. 1992).

In the Steller sea lions tested, a variable number of adult animals were positive. However, this appears to be true throughout the range and was not restricted to the area of decline. These positives were at a relatively low titer and without demonstration of toxoplasma-associated diseases, and the reactions could be non-specific or cross reactive reactions (false positives).

Leptospira spp.

Leptospira spp. cause a variety of syndromes in pinnipeds, including septicemia, often with hemorrhage or hemolytic jaundice, hepatitis, interstitial nephritis and abortion or stillbirth (Leighton and Kuiken 2001). Leptospirosis occurs regularly in California sea lions with high rates of morbidity and mortality. Between 1981 and 1994, one-third of the several thousand stranded sea lions that were examined in California had clinical leptospirosis, and 71% of the affected animals died (Gulland et al. 1996).

Serologic testing of Steller sea lions collected in the Gulf of Alaska during the 1970s (63 animals) and 1980s (137 animals) detected only three positive animals. These were all at a low titer and could be non-specific or cross reactive. Nor were lesions typical of leptospirosis noted on these animals (Calkins and Goodwin 1988). Ongoing monitoring in the 1990s did not detect any positive individuals. Significant exposure to *Leptospira interrogans* does not appear to have occurred among these Steller sea lions.

Influenza

Influenza is known to have caused mass die-offs in a number of marine mammal populations (Webster *et al.* 1981, Geraci *et al.* 1982). All 125

samples from Steller sea lions tested negative for this disease .

Brucella abortus

Brucella abortus is an important cause of abortion in domestic ruminants, dogs (Carmichael and Kenney 1968) and people. It also causes arthritis and tendonitis in caribou and has been detected in marine mammals. Of the Steller sea lion sera tested to date (Table 11), only one animal from Southeast Alaska tested positive for *Brucella abortus*. Continued monitoring with larger sample sizes is required to detect low levels of infection and/or the introduction of this disease into Steller sea lion populations.

Canine parvovirus

Canine parvovirus–2 (CPV-2) is a member of the closely related feline parvovirus subgroup. This group includes feline parvovirus, mink enteritis virus, fox parvovirus, raccoon parvovirus, canine parvovirus–2 and raccoon dog parvovirus. The HAI test used would most likely have picked up any of these closely related viruses. It is debatable whether the IFA used earlier in these studies would reveal positive animals because there are some species-specific conjugates in this test that may not cross-react with the sea lion antibodies.

CPV-2 causes two syndromes in canids: a myocarditis in pups under 4 months of age and a gastroenteritis. The virus acts by infecting and killing rapidly dividing cells. In young puppies, myocytes are affected, and death often results from the myocarditis. In older animals, lymphocytes are first infected and damaged, followed by infection and destruction of the proliferative (crypt) cells in the intestinal lining. This can result in severe diarrhea, dehydration, and pyrexia, and can result in death. The severity of the signs depends on many factors depending on age, nutritional status, concomitant parasitic, bacterial or viral infections (Barker and Parrish 2001).

Parvoviruses are very hardy, surviving for months and are very stable when frozen. Transmission is by fecal-oral route, mainly through ingestion of virus from the environment, rather than by direct contact with infected animals (Reif 1976). In naïve populations, an epizootic can occur with significant mortality in all age classes (Mason et al. 1987), affecting population size (Hesterbeek and Roberts 1995). In populations in which it is enzootic, most disease would be expected to occur in juveniles exposed to CPV following decline of maternal antibodies at about 2 months of age (Mason *et al.* 1987). Host range includes the Felidae, Canidae, Procyonidae, Mustelidae, Ursidae. Viverridae are also suspected of being susceptible to parvoviruses of FP subgroup (Barker and Parrish 2001).

There are no reports of otariids being affected by parvoviruses. In our animals, all samples were negative by IFA. A very small percentage of animals were positive when tested by HAI. Animals in both populations were affected (one each), but the numbers are so small, it is difficult to draw meaningful conclusions.

Did Disease Cause the Decline of Steller Sea Lions?

The 80% decline in the population of Steller sea lions in the Gulf of Alaska and Aleutian Islands through the 1980s was rapid, and may have begun in the eastern Aleutian Islands before spreading east and west along the chain (Braham *et al.* 1980, Trites and Larkin 1996). This pattern of decline is consistent with a disease outbreak, but no sea lion bodies were recovered and no lesions were noted. Failure to recover corpses could be due to the remoteness of the haulout sites, scavenger activity and the enormous expanse of ocean occupied by Steller sea lions, rather than the fact that there were no corpses to be found.

Disease agents found previously in marine mammals include caliciviruses, *Brucella sp*, influenza A, morbilliviruses, herpesviruses, *Leptospira interrogans*, and *Chlamydophila psittaci*. The morbilliviruses and influenza A are two particularly virulent diseases that have caused major die-offs in marine mammals (Webster *et al.* 1981, Geraci *et al.* 1982, Domingo *et al.* 1990, Heide-Jorgensen and Harkonen 1992, Callan *et al.* 1995, Kennedy 1998). However, neither of these disease agents were detected at significant levels among the Steller sea lions sampled.

Antibodies to *Chlamydophila psittaci*, calici-viruses, herpesviruses, adenoviruses and *T. gondii* were detected at moderate to high frequencies in Steller sea lions in areas of the decline and also in areas of the thriving populations. An argument can be made that these disease agents had the potential to contribute to some of the changes noted in the population decline (i.e., abortions, lower birth rates and higher mortalities of juveniles).

Nutritional stress is widely considered to be the most likely underlying cause of the decline of Steller sea lions in the Gulf of Alaska and Aleutian Islands (Alaska Sea Grant 1993, DeMaster and

Atkinson 2002, Trites and Donnelly 2003). However, there are no signs that Steller sea lions starved to death. Rather, it appears that the declining population consumed larger amounts of lower quality prey (i.e., primarily walleye pollock and Atka mackerel) compared to the higher diversity of energy-rich prey consumed by the growing population in southeast Alaska (i.e., herring, sandlance, salmon, rockfish, etc.) (Merrick *et al.* 1997, Sinclair and Zeppelin 2002, Trites 2003). Differences in diet means that young sea lions in the declining populations must consume between 35% and 80% more than in southeast Alaska (Rosen and Trites 2000, Winship and Trites 2003). Young animals may not be able to meet this increased energy demand and may be more susceptible to disease. Other factors such as differences in genetic susceptibility to disease and exposure to contaminants could make an animal more susceptible to disease.

Future Research

The serology samples collected since 1975 were always taken tangential to other sea lion research. Disease has never been a primary area of Steller sea lion research and has never been conducted in a systematic manner. Thus, serology samples have for the most part been too few to draw meaningful conclusions, or were collected over such a wide range of differing regions to confound interpreting the data. People often seem to rely on archived samples for possible future studies. However, we have found that opportunities can be lost with this approach to monitoring disease due to inconsistencies in laboratories and questionable sample quality due to degradation over time. The best that can be said from the serology data collected to date from Steller sea lions is which diseases were more prevalent than others, but little can be reliably concluded about changes over time or differences between regions.

A systematic protocol should be initiated to screen for diseases using serology. Such analyses are already ongoing by the Alaska Department of Fish and Game. It needs to be designed to track changes in pathogen exposures both over time and between regions. Serology of the declining population can be compared with those of the "healthy" population, producing a natural control group for animal comparison. This is important because contaminant levels, reproductive rates, starvation, prey abundance, and exposure to diseases can be quantified, but the significance of each is difficult to determine due to uncertainties about what is normal in a "healthy" population, and whether these factors cause population effects. In addition to serology, some of the newer genetic techniques should be explored to detect and describe the disease agents, and determine whether they correlate with actual diseases.

The most important disease agents to consider are those most likely to affect individual Steller sea lions in a manner that impacts their populations. Disease agents are more likely to affect closely related animals in a similar manner, though some agents can cross species lines. Newly introduced agents of moderate to high pathogenicity may affect a population due to lack of immunity in the population. Therefore it is important to look for agents over time and across different regions.

Based on the data collected to date, the most important disease agents to continue investigating are *C. psittaci*, caliciviruses, *Toxoplasma gondii,* herpesviruses and adenoviruses. Questions that could be addressed include patterns of exposure, rates and conditions of transmission, genetic characteristics or the actual disease agents and most importantly whether there is evidence of disease through the examination of captured individuals and recovered carcasses. *Leptospira interrogans*, *Brucella spp*., influenza A, morbilliviruses and parvoviruses were not detected in high levels. However they should continue to be screened for since Steller sea lions appear to be immunologically naïve and introduction of one of these agents could cause significant effects on the population recovery.

Disease can undoubtedly alter the vital rates of Steller sea lions, but the overall contribution to the decline of Steller sea lions cannot be properly evaluated with the available data. While it is safe to conclude that disease was not the smoking gun behind the population decline, it may well have been a significant contributing factor.

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Table 1. Historical and retrospective prevalence (%) of antibodies to *C. psittaci* in Steller sea lions from different regions. The number in parentheses (n) indicate sample size. The first letter in the superscript indicates the age class of the animals sampled, the second letter indicates the sex of animals sampled. The laboratory and data type are listed under the region (R = retrospective; Hx = historical). Threshold was 10 at NVSL and 32 at WADDL.

Year collected	Bering Sea	Gulf of Alaska	Kodiak		Southeast Alaska	
	NVSL-R	NVSL- R	WADDL – HX	NVSL – Hx	NVSL- R	NVSL - HX
1970s	33% (3) $^{S, M}$ 50 % (4) $^{A, F}$	64% $^{4A, M / 32 A, F}$	--	--	--	--
1980s	87 % (15) $^{U, U}$	45% (22) $^{5A, M /17A, F}$	0 % (30) ^{FT} 71 % (14) ^{S} 94 % (33) ^{A}	--	33 % (6) ^{FT} 48 % (31) $^{5A, M/ 26 A, F}$	--
1990s	80% (5) $^{2 A, M / 3 A, F}$	89 % (9) $^{A, F}$	--	0 % (1) ^{FT} 0 % (8) ^{P} 100% (1) $^{AD, F}$	84% (19) $^{A, F}$	0% (1) ^{P} 100% (19) $^{A, F}$

FT = Fetus; P = Pup; S = Subadult; A = Adult; U = Unknown; M = Male; F = Female

Table 2. Current serology data on 1998-2000 for *C. psittaci* (complement fixation), calicivirus and PhHV-1 (ELISAs) in pups and juveniles organized by region (Prince William Sound and Southeast Alaska.

Region	*C. psittaci* (CF $\geq$20)	Calicivirus (ELISA)	PhHV-1 (ELISA)	CAV-1 (SN)	CAV-2 (SN)
PWS	15% (40)	22% (46)	24% (45)	30% (46)	26% (46)
SEA	12% (91)	18% (95)	10% (88)	27% (33)	21% (33)

Table 3. Current serology data on 1998-2000 for *C. psittaci* (complement fixation), calicivirus and PhHV-1 (ELISA), CAV-1 and –2 in pups and juveniles organized by age.

Ages	*C. psittaci* CF at $\geq$20	Calicivirus ELISA	Herpesvirus ELISA	CAV-1 SN	CAV-2 SN
2-4 months	0% (61)	20% (55)	2% (53)	15% (27)	19% (27)
6-7 months	4 % (22)	24% (29)	4% (24)	--	--
9 or 11 months	22 % (9)	0% (13)	38% (13)	--	--
1-2 years	36% (39)	20% (44)	30% (43)	36 % (52)	27 % (52)

Table 4. Comparison of WADDL's historical *C. psittaci* serology results to NVSL retrospective data.

SAMPLE NUMBER	WADDL TITER	WADDL RESULT	NVSL TITER	NVSL RESULT
404	64	P	0	N
406	256	NS	10	S
426	64	P	0	N
427	256	P	10	S
434	256	P	0	N
440	256	NS	10	S
449	256	NS	0	N
450	256	P	10	S
453	128	NS	10	S
464	256	NS	10	S

Table 5. Historical calicivirus results. Prevalence (%) of antibodies to calicivirus SMSV-5, 6, 10 and 13 by serum neutralization in Steller sea lions from different regions in Alaska. Threshold was 20. Number in parentheses (n) = sample size. The first letter in superscript indicates age class of the animals sampled, the second letter indicates the sex of animals sampled.

	Bering Sea / Aleutians		Gulf of Alaska / Kodiak				Southeast Alaska			
	SMSV-5	SMSV-6,10,13	SMSV-5	SMSV-6	SMSV-10	SMSV-13	SMSV-5	SMSV-6	SMSV-10	SMSV-13
1970s	100% (2) S, M 20% (3) A, F	0% (2) S, M 20% (3) A, F	44% (93) U,U	--	--	--	--	--	--	--
1980s	--	--	10% (29) Ft 13% (74) S/A 23% (40) NFt	0% (29) Ft 7% (74) S/A 10% (40) NFt	10% (29) **Ft** 31% (74) S/A 8% (40) NFt	3% (29) **Ft** 12% (74) S/A 23% (40) NFt	33% (27) 1S, 1P, 25 AD	11% (27) NFt	15% (27) NFt	33% (27) NFt
1990s	--	--	0 % (14) 1Ft/10P/3U	0 % (14) Ft/P/U	--	0 % (14) Ft/P/U	4% (25) 1 P, 2U, 1S, 21A	8% (25)NFt	--	20% (25)NFt

FT = Fetus; P = Pup; S = Subadult; A = Adult; NFt = non fetus (mixed ages); U = Unknown; M = Male; F = Female

Table 6. Serotypes of calicivirus reacted to by Steller sea lions.

Site	Year	Age group	Number tested	SMSV- 1	2	5	6	7	8	10	13	BCV
Bering Sea (eastern)	1976	S	2	1	--	2	0	0	0	0	0	0
		A	3	0	--	1	1	0	0	1	1	1
Gulf of Alaska/ Kodiak	1975-1978	NFT	93	--	60	41	---	--	--	--	--	--
	1985	FT	29	0	--	3	0	1	0	3	1	0
		S/A	74	5	--	10	5	2	2	23	9	2
	1986	NFT	40	--	--	9	4	--	--	3	9	--
	1992-1993	1 Ft, 3 10P, 3 U	14	0	0	0	0	0	0	--	0	--
Southeast Alaska	1986	1P, 1S, 25 A	27	--	--	9	3	--	--	4	9	--
	1992-1993	1P, 2 U 1S, 21 A	25	1	3	1	2	0	1		5	

FT = Fetus; P = Pup; S = Subadult; A = Adult; NFt = non fetus (mixed ages); U = Unknown; M = Male; F = Female

Table 7. Simplified retrospective morbillivirus data from Oklahoma State. All are serum neutralization for two-5 yr olds (S) and adults only. In some cases, the ages are not known (Bering Sea and Kodiak, 1978). Numbers in parentheses are numbers tested.

Time frame	Virus	Bering Sea	Kodiak / Gulf of Alaska	Southeast Alaska
1970s	CDV	0%	0%	--
	PDV	33% (3)	10%(32)	--
	DMV	33% (3)	11% (32)	--
	PMV	33% (3)	10% (32)	--
1980s	CDV	6% (16)	0% (56)	0% (28)
	PDV	0%	11% (56)	0% (28)
	DMV	0%	0% (56)	0% (28)
	PMV	6% (16)	0% (56)	0% (28)
1990s	CDV	0%	0% (3)	0% (23)
	PDV	0%	0% (3)	0% (23)
	DMV	0%	0% (3)	0% (23)
	PMV	0%	0% (3)	0% (23)

Table 8. Comparative morbillivirus serum neutralization titers at OK State. This includes retrospective and current positive individuals.

Region	Capture year	Age (yrs)	Sex	CDV Result	CDV titer	PDV result	PDV titer	PMV result	PMV titer	DMV result	DMV titer
Bering Sea Region	1979	A	F	N	0	N	0	P	16	N	0
	1979	S	M	N	0	N	0	N	0	P	16
	1979	A	F	N	0	P	16	N	0	N	0
	1986	U	F	P	64	T	32	P	24	T	32
Gulf of Alaska, Kodiak, Prince William Sound, Kenai (2-6)	1976	8	F	N	0	P	8	N	0	N	0
	1977	12	M	N	0	N	0	T	8	P	8
	1977	8	F	N	0	T	8	P	16	N	0
	1977	6	F	N	0	N	0	N	0	P	8
	1977	8	F	N	0	P	8	N	0	N	0
	1977	6	F	N	0	N	0	P	16	P	8
	1977	14	F	N	0	P	8	P	8	T	8
	1978	12	F	N	0	P	8	N	0	N	0
	1985	11	F	N	0	P	8	N	0	N	0
	1985	9	F	N	0	P	8	N	0	N	0
	1985	13	F	N	0	P	16	N	0	N	0
	1985	7	F	N	0	P	8	N	0	N	0
	1985	6	F	N	0	P	8	N	0	N	0
	1985	14	F	N	0	P	8	N	0	N	0
Southeast Alaska	1998	0.13	M	N	0	N	0	P	16	P	16

S = Subadult; A = Adult

Table 9. Historical, retrospective and current prevalence (%) of antibodies *Toxoplasma gondii* by modified latex agglutination in Steller sea lions from different regions in Alaska. Percentages indicated include suspicious and positive titers. Number in parentheses (n) indicates sample size. The first letter in superscript indicates age class of the animals sampled. The second letter indicates the laboratory used.

Decade	Bering Sea	Kodiak / Prince William Sound	Southeast Alaska
1970s	67% (3) $^{A, B}$	0% (2) $^{S, B}$	--
1980s	--	0% (6) $^{A, Fa, O}$	--
	--	0% (3) $^{S, B}$	--
	--	0% (4) $^{A, B}$	--
1990s	--	0% (1) $^{P, B}$	0% (8) $^{P, B}$
	--	50% (4) $^{A, B}$	83% (6) $^{A, B}$
	--	--	0% (25) ^{P,C}
	--	--	12% (8) $^{A, C}$
	--	--	30% (23) $^{P, O}$
	--	--	25% (4) $^{S, O}$

P = Pup; S = Subadult 1-5 yr ; A = adult; Fa = female recently aborted
O = Oklahoma State University; B = Beltsville; C = CVDLS

Table 10. Historical data on prevalence (%) of antibodies to phocid herpesvirus-1 in Steller sea lions from different regions by serum neutralization. Number in parentheses (n) indicates sample size. The superscript indicates age class of the animals sampled.

Year collected	Bering Sea /Aleutians	Kodiak	Prince William Sound	Southeast Alaska
1970s	100% (2) ^{S} 100 % (3) ^{A}	50 % (12) ^{S} 50 % (4) ^{A}	-	-
1980s	0 % (12) ^{U}	21 % (19) ^{S} 30 % (10) ^{A}	17 % (6) ^{S} 0 % (1) ^{A}	100 % (1) ^{P} 16 % (19) ^{A}
1990s	-	0 % (27) ^{P} 23 % (13) ^{A}	-	0 % (21) ^{P} 0 % (35) ^{A}

P = Pup; S = Subadult 1-5 yr; A = adult; U = unknown

Table 11. Tests with negligible or negative results.

Disease agent	Lab	Type of test	Year collected	Data type	Region	# tested
Leptospira interrogans	UAF	MAT – primarily Leptospira interrogans ser. pomona	1975-1978	Hx	GOA	63 (2U, 38S, 23AD)
	NVSL	MAT – 8-way	1985-1986	Hx	E. GOA	137 (29Ft, 28S, 80AD)
	CVDLS	MAT --5 way plus Bratislava on pups	1997	C	SEA	30 (24P, 6AD)
	OK State	6-way MAT	1997	C	Bering sea	11 (P)
			1998-2000	C	PWS	46 (26P, 20S)
				C	SEA	82 (65P, 17S)
Morbillivirus	OK State	Competitive ELISA	2000	C	PWS	46 (26P, 20S)
				C	SEA	33 (14P, 19S)
	OK State	SN	1998	C	SEA	33 (32P,1S)
	UC-Davis	ELISA	1998/2000	C	SEA	53 (50P, 3 S)
Influenza	UW — Madison	DAID	1978-1994	Hx	All areas	27
Brucella sp	CVL	Indirect ELISA	1978-1994	Hx	All areas	26
	CVDLS	Standard plate agglutination with threshold of 1:25	1997	C	SEA	31
	OK State	BACT test 1 CF Test 2 Rivanol Test 3	1998-2000	C	PWS	46
				C	SEA	94
Canine parvovirus (CPV-2)	CO State	IFA	1989	Hx	PWS, Kodiak, Kenai	14
	Cornell	Hemagglutination inhibition (HAI/2-ME)	2000	C	PWS	46
				C	SEA	33

Type of Test: MAT = microagglutination test; ELISA = enzyme linked immunosorbent assay; SN = serum neutralization; DAID = double agar gel immunodiffusion ; AGID= agar gel immunodiffusion; BACT = brucella abortus card test; CF =- complement fixation; IFA = immunofluorescent assay

Laboratory: UAF = University of Alaska – Fairbanks; NVSL = National Veterinary Services Laboratory; CVDLS= California Veterinary Diagnostic Laboratory; OK State = Oklahoma State University; UC-Davis = University of California – Davis

Age and sex: U = Unknown; FT = Fetus; P = Pup; S = Subadult; A = Adult; F = Female