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SEROLOGIC SURVEY FOR *BRUCELLA* SPP., PHOCID HERPESVIRUS-1, PHOCID HERPESVIRUS-2, AND PHOCINE DISTEMPER VIRUS IN HARBOR SEALS FROM ALASKA, 1976–1999

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ABSTRACT: Harbor seals (*Phoca vitulina richardsi*) were captured in the coastal regions of Southeast Alaska, Gulf of Alaska, Prince William Sound (PWS), and Kodiak Island during 1976–1999. Blood was collected from 286 seals. Sera were tested for evidence of exposure to *Brucella* spp., phocid herpesvirus-1 (PhoHV-1), phocid herpesvirus-2 (PhHV-2), and phocine distemper virus (PDV). Antibody prevalence rates were 46% (46/100) for *Brucella* spp., 93% (225/243) for PhoHV-1, 0% (0/286) for PhHV-2, and 1% (2/160) for PDV. Antibody prevalence for *Brucella* spp. was directly related to host age. Antibody prevalence for PhoHV-1 was higher in PWS as compared to the other three regions. No evidence of mortality attributable to these four agents was observed during the course of this study. Based on the results of this survey, none of these agents is considered a significant mortality factor in harbor seals from the four regions of coastal Alaska included in the study.

Key words: Alaska, *Brucella* sp., harbor seals, phocid herpesvirus, phocine distemper virus, *Phoca vitulina richardsi*, pinnipeds, serology.

INTRODUCTION

Harbor seals, *Phoca vitulina richardsi*, inhabit the coastal waters of Alaska from Southeast Alaska northward to Prince William Sound (PWS) and the Kodiak Archipelago, and westward through the Aleutian Islands and Bristol Bay (Hoover-Miller, 1994). Once considered abundant, the number of harbor seals in parts of Alaska has declined greatly since the mid-1970s (Hoover-Miller, 1994). Specifically, numbers declined by 85% on Tugidak Island (south of Kodiak Island) during 1976–1988 (Pitcher, 1990), and by 63% in PWS during 1984–1997 (Frost et al., 1999). The status of harbor seals in other parts of the Gulf of Alaska is uncertain, though numbers are believed to have declined in the greater Kodiak Island area through the 1980s, prior to increasing over the last decade (Small et al., 2003). Numbers of harbor seals have increased

or remained stable in Southeast Alaska and Bristol Bay. The causes for the decline in harbor seal numbers have not been identified (Pitcher, 1990; Small et al., 2003). Possible factors that may be affecting seal numbers include: 1) reduced prey availability, either by natural changes in the marine environment or as a result of commercial fishing; 2) human-caused mortality, either through harvest or by incidental take in fisheries; 3) disease; 4) pollutants; and 5) predation by killer whales (*Orcinus orca*) and possibly sleeper sharks (*Somniosus pacificus*) (Sease, 1992). Some of the decline in PWS was caused by the *Exxon Valdez* oil spill (Frost et al., 1994), but numbers were already declining before the spill (Pitcher, 1989).

Harbor seals are found primarily within 50–60 km of shore (Lowry et al., 2001; Small et al., 2005), where they feed, haul out to rest, give birth, care for their young, and molt. Females give birth to single

pups once a year, usually in the first half of June in Alaska. Pups are weaned when they are 3–6 wk old. Adult females breed about 2 wk after their pups are weaned, and give birth about 11 mo later. Female harbor seals first become pregnant when they are 3–7 yr old (Hoover-Miller, 1994). Most information about the foods of harbor seals was collected in the mid-1970s (Pitcher and Calkins, 1979). The major prey in PWS and the Gulf of Alaska include pollock (*Theragra chalcogramma*), octopus/squid (class Cephalopoda), capelin (*Mallotus villosus*), Pacific cod (*Gadus macrocephalus*), and herring (*Clupea pallasii*).

Brucella was first isolated from the organs of four common seals (*Phoca vitulina*), two harbor porpoises (*Phocoena phocoena*), and one common dolphin (*Delphis delphis*) originating from the Scottish coasts (Ross et al., 1994). *Brucella* has been reported to cause abortion in bottlenose dolphins (*Tursiops truncatus*) in the USA (Ewalt et al., 1994; Miller et al., 1999). In addition, serologic evidence of *Brucella* infection exists in Atlantic walrus (*Odobenus rosmarus rosmarus*) and ringed seals (*Phoca hispida*) of Arctic Canada (Nielsen et al., 1996). Evidence of *Brucella* infection in Pacific harbor seals (*Phoca vitulina richardsi*), California sea lions (*Zalophus californianus*), and harbor porpoises in Puget Sound, Washington, USA has also been reported (Payeur et al., 1998). In the North Pacific, *Brucella*-associated testicular granulomas in common minke whales (*Balaenoptera acutorostrata*) have been reported. Molecular characterization of DNA isolated from testes that grossly had granular lesions showed that *Brucella* from North Pacific minke whales was different from terrestrial and North Atlantic marine mammal *Brucella* strains (Ohishi et al., 2004). In the Southern Hemisphere, detection of anti-*Brucella* antibodies in Weddell seals (*Leptonychotes weddellii*) from Cape Shirreff, Antarctica (Blank et al., 2002) suggests the possibility of widespread infection. *Brucella* spp. have been isolated from

numerous species of terrestrial animals, and they cause abortion in the majority of these species (Corbel and Morgan, 1975). It is therefore possible that *Brucella* spp. infections can also cause reproductive failure in marine mammals.

Phocid herpesvirus-1 (PhoHV-1; *Vari-cellovirus*, Alphaherpesvirinae) was first identified in 1984 when it caused the deaths of 11 captive harbor seal pups in a nursery in the Netherlands (Osterhaus et al., 1985). Mortality caused by PhoHV-1 infection has been observed only in captive animals, including neonates, seals acutely infected with phocine distemper virus (PDV), or seals that have been otherwise immunocompromised.

Clinical signs of PhoHV-1 disease included elevated body temperature, inflammation of the oral mucosa, nasal discharge, coughing, vomiting, diarrhea, anorexia, and lethargy (Visser et al., 1991). Duration of the illness ranged from 1 to 6 days (Borst et al., 1986). Interstitial pneumonia and necrosis of hepatic parenchyma were the primary histologic lesions. Less significant changes were also observed in kidneys, spleen, and lymph nodes (Borst et al., 1986).

Virus is shed in nasal and ocular discharges from naturally- and experimentally-infected animals (Horvat et al., 1989; Harder et al., 1997). Presumably, natural transmission occurs by means of aerosols or direct contact, as in other alpha herpesvirus infections.

Serum samples from 49 free-ranging harbor seals that were involved in the 1988 PDV epizootic in the North Atlantic yielded a PhoHV-1 antibody prevalence of approximately 50% (Frey et al., 1989). Despite this high antibody prevalence, PhoHV-1 was isolated from only four of 112 harbor seals that died during the 1988 PDV epizootic (Frey et al., 1989).

A second herpesvirus, phocid herpesvirus-2 (PhHV-2), has been isolated from a captive California sea lion (Kennedy-Stoskopf et al., 1986), free-ranging adult harbor seals from the North Sea (Lebich

et al., 1994), and free-ranging harbor seals from the North Atlantic offshore of the United States (Harder et al., 1996). Based on nucleotide sequence data, PhHV-2 is a gamma-herpesvirus (Harder et al., 1996). There is no evidence that PhHV-2 causes clinical disease in pinnipeds.

Herpesviruses have been implicated in recent fatal and nonfatal infections of harbor seals in the North Pacific. Twenty-six harbor seals were collected during the investigation of the 1989 *Exxon Valdez* oil spill in PWS, Alaska, USA. One male had lesions on the penis and prepuce. Intracellular inclusion bodies typical of herpesvirus were observed during histologic examination of the lesions (Spraker et al., 1994). The death of a single harbor seal off the coast of Washington, USA, in 1990 was attributed to a herpesvirus. This diagnosis was based upon gross lesions, light microscopy, and electron microscopy. Hepatic and adrenal necrosis were found in 12 harbor seal pups that died at a rehabilitation center in Sausalito, California, USA, in 1990. Herpesvirus virions were detected in these necrotic areas by use of electron microscopy (Lowenstine et al., 1992).

Herpesviruses have also been detected in other Northern Hemisphere marine mammal species, including harbor porpoise (Kennedy et al., 1992), California sea lion (Kennedy-Stoskopf et al., 1986), sea otter (*Enhydra lutris*) (Harris et al., 1990), and beluga (*Delphinapterus leucas*) (Barr et al., 1989).

A previous serologic survey of several marine mammal species (including harbor seals) from Alaskan waters revealed exposure to both PhoHV-1 and PhHV-2 (Zarnke et al., 1997). Serum antibody prevalence for PhoHV-1 was 100% in 25 apparently healthy Weddell seals and three apparently healthy crabeater seals (*Lobodon carcinophagus*) from the eastern Weddell Sea (Harder et al., 1991). Neutralizing antibodies against PhHV-2 have been detected in a small cohort of harbor seals of the North Sea but not in Weddell seals of Antarctica (Lebich et al., 1994).

Phocine distemper virus (*Morbillivirus*, Paramyxoviridae) was first isolated from harbor seals following the 1988 epizootic off northwestern Europe (Osterhaus et al., 1990). Clinical signs included respiratory distress and oculonasal discharge (Kennedy et al., 1989). The primary post-mortem finding was severe pneumonia (Kennedy et al., 1989).

An estimated 18,000 seals died during the 1988 PDV epizootic (Osterhaus et al., 1990). Harp seals (*Phoca groenlandica*) have been implicated as a reservoir species and a possible source of PDV for this major die-off (Duignan et al., 1994). Subsequent investigations indicate continued transmission of PDV in the waters of northwestern Europe (Visser et al., 1993). In 2002, approximately 22,000 seals died in a second PDV epidemic in northwestern Europe (Müller et al., 2004).

Several thousand Lake Baikal seals (*Phoca sibirica*) died in Russia in 1987. Retrospective investigation implicated canine distemper virus as the cause of this mortality (Grachev et al., 1989).

Recent research revealed morbillivirus exposure in additional European species, including gray seal (*Halichoerus grypus*) (Harder et al., 1991), harbor porpoise (Kennedy, 1990), and Mediterranean striped dolphin (*Stenella coeruleoalba*) (van Bresse et al., 1991).

Identical or closely-related morbilliviruses have been identified in recent years from several species on the Atlantic coast of North America, including harbor seal (Duignan et al., 1993), harp seal (Daoust et al., 1993), walrus (Duignan et al., 1994), and ringed seal (Duignan et al., 1997).

The primary objective of this study was to determine the serum antibody prevalence of *Brucella* spp., PhoHV-1, PhHV-2, and PDV in harbor seals from four regions of coastal Alaska. The secondary objective was to determine the relationship between antibody prevalence and host sex, age, year of capture, and geographic region.

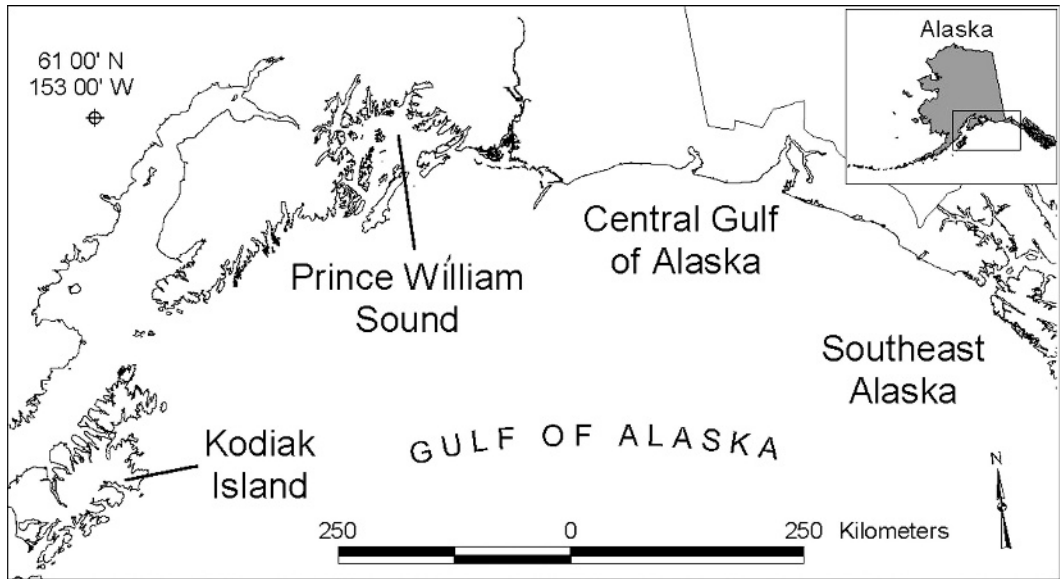


FIGURE 1. Capture areas for harbor seals included in serologic survey, 1976–1999.

MATERIALS AND METHODS

Seals were captured by various investigators during 1976–1999 in conjunction with studies of harbor seal population biology and ecology. Study areas included portions of Southeast Alaska, the Gulf of Alaska, PWS, and Kodiak Island (Fig. 1). Standard descriptive data (sex, location, and date of capture) were recorded for each animal. Animals were assigned to one of four age categories: pup, yearling, subadult, and adult. Age determination was based on body length and weight (Lowry et al., 2001). For the purpose of data analysis, samples were grouped by year of collection, which does not necessarily reflect year of exposure.

Blood was drawn from the extradural vein and allowed to clot. Serum was transferred to sterile vials. Sera were initially stored at -12°C for up to several months and then transferred to -40 to -46°C for several months to several years before testing.

Sera were tested for evidence of exposure to *Brucella* spp. by means of both competitive (MacMillan et al., 1990) and indirect enzyme-linked immunosorbent assay (ELISA) (Alton et al., 1988). The competitive ELISA (cELISA) described by MacMillan et al. (1990) was used at a single dilution. Briefly, microtiter plates coated with lipopolysaccharide (LPS) extracted from *B. melitensis* (strain 16M) were prepared and $20\ \mu\text{l}$ of the test serum was added, followed immediately by $100\ \mu\text{l}$ of a dilution of an anti-*Brucella* LPS monoclonal-peroxidase conjugate. After incu-

bation at room temperature, while shaking at 160 rpm, the plates were washed and the chromogen, o-phenylenediamine dihydrochloride, and the substrate, 3% hydrogen peroxide, were added. The color development was measured at 405 nm using a microtiter plate spectrophotometer. The optical density (OD) of the test samples was expressed as a percentage of the mean OD of the wells to which a negative control serum had been added. The negative control serum was obtained from a sheep that was clear of any clinical signs of disease and that was negative to all the standard serologic tests for brucellosis. Samples with an OD of less than 30% were classified as positive, and samples with an OD of more than 30% were classified as negative.

The indirect ELISA (iELISA) was based on that described by Alton et al. (1988) but differed in a number of respects. The iELISA requires anti-immunoglobulin conjugate with specificity for the immunoglobulin isotypes of the species under test. Such reagents were not available, but protein A has been shown to bind to the IgG of a range of marine mammals (Eliasson et al., 1988; Sikkema, 1989). The antigen was as described for the cELISA. The conjugate was a protein A-peroxidase conjugate. In this case the OD of the test samples was expressed as a percentage of the mean OD of the wells to which a positive control serum had been added. The positive control serum was obtained from a pig infected with *Brucella suis*. This sample was chosen as the control

because it is detected using protein A. Samples with an OD of more than 30% were classified as positive, and samples with an OD of less than 30% were classified as negative.

Sera meeting the cELISA and iELISA criteria will be referred to as positive. In the absence of sera either from animals known to be free of *Brucella* spp. infection, or from animals from which *Brucella* spp. had been isolated, it is impossible to set the diagnostic thresholds for the tests with certainty. Therefore, thresholds were selected on the basis of experience gained in testing a wide range of terrestrial mammals for brucellosis from Great Britain.

Antibodies to PhoHV-1, PhHV-2, and PDV were detected using the serum neutralization test (SNT). The test was performed using the PDV1-2-6A strain of PDV, the A92-10/4 isolate of PhoHV-1, and the A92-10/5 isolate of PhHV-2. The viruses were grown in either Vero cells (PDV and PhHV-2) or Crandell feline kidney (CRFK) cells (PhoHV-1). The SNT was performed following a modification of a microtiter method (Rossiter et al., 1985) as previously described for morbilliviruses (Saliki et al., 1993). Briefly, for PDV, serial twofold dilutions of heat-inactivated sera were made in duplicate columns of 96-well plates using the alpha modification of Eagle's minimum essential medium, starting at a 1:4 dilution. For PhoHV-1 and PhHV-2, a single 1:4 dilution was made in duplicate wells. An equal volume (25 μ l) of each virus, containing about 100 median tissue culture infective doses (TCID₅₀) of the appropriate virus, was added to corresponding appropriate plates. This yielded a starting dilution of 1:8 for PDV and a single dilution of 1:8 for PhoHV-1 and PhHV-2. The virus-serum mixtures were incubated at 37 C for 1 hr in 5% CO₂, and a Vero or CRFK cell suspension (150 μ l containing 10⁴ cells/well) was added to PDV/PhHV-2 and PhoHV-1 plates, respectively. The plates were incubated at 37 C in 5% CO₂ for 4 days. The test was read by examining cell monolayers under an inverted microscope for virus-specific cytopathic effects (CPE). Antibody titers for PDV were expressed as the reciprocal of the highest dilution of serum that completely neutralized CPE in duplicate wells. Results for PhoHV-1 and PhHV-2 were scored as positive (absence of CPE in both wells) or negative (presence of CPE in one or both wells) at 1:8 dilution.

A generalized linear model (McCullagh and Nelder, 1989) with repeated measures was used to determine whether there was significant dependence of antibody prevalence on host characteristics: age, sex, year, or geographic region. The model used a logit link

with a binomial distribution. Serologic test result is a binary response variable. Year was treated as a continuous variable. Age was treated as a categorical variable with four classes: pup, yearling, subadult, and adult. Geographic area was treated as a categorical variable with four classes: Kodiak, central Gulf of Alaska, PWS, and southeast Alaska. Sex was also treated as a categorical variable. All main and interaction effects of these variables were examined. During the modeling process, all higher-order terms were removed from the model if they did not substantially ($P > 0.05$) increase the fit of the model based on the deviance function compared to a chi-squared distribution (McCullagh and Nelder, 1989). The GENMOD procedure of the SAS statistical software package, version 8.02 (SAS Institute, Cary, North Carolina, USA), was used to fit the model with maximum likelihood parameter estimates.

RESULTS

Not all sera were suitable for all test procedures. In addition, some sample volumes were exhausted during the initial stages of testing. Thus, sample sizes were not consistent for all agents.

Overall antibody prevalences for all four agents are presented in Table 1. The most striking feature was the difference in prevalence for PhoHV-1 (225/243, 93%) as compared to PhHV-2 (0/286, 0%).

For *Brucella* spp., the fitted model for antibody prevalence included a single significant covariate, age. Substantial differences in antibody prevalence were found between the adult, subadult and yearling cohorts (Table 2). These differences were not statistically significant. However, prevalences for all three cohorts were significantly different from the prevalence for pups. Hence, we collapsed age into two classes: pups and nonpups. A difference in prevalence between nonpups (44/82, 54%), and pups (2/18, 11%) was detected ($P = 0.0044$).

The fitted model for PhoHV-1 antibody prevalence included a single significant covariate, geographic region. We examined the four geographic regions and determined that prevalences for Kodiak, central Gulf of Alaska, and southeast

TABLE 1. Serum antibody prevalence for four microbial disease agents in harbor seals (*Phoca vitulina richardsi*) from coastal regions of Alaska.

Agent	Antibody prevalence ^a
<i>Brucella</i> spp.	46/100 (46)
Phocid herpesvirus-1	225/243 (93)
Phocid herpesvirus-2	0/286 (0)
Phocine distemper virus	2/160 (1)

^a Number positive/number tested (%).

Alaska were not significantly different from each other, but they were all significantly different from PWS (Table 3). Hence, we collapsed geographic region into two classes, PWS and the other three regions. A significant difference ($P=0.0188$) was detected between prevalence rates for the three regions (106/120, 88%) and PWS (119/123, 97%).

Statistical modeling was not done for PhHV-2, for which all animals were negative, or for PDV, for which only two of 160 animals were positive.

DISCUSSION

The iELISA and cELISA have been used to test terrestrial mammals with none of the problems associated with the conventional complement fixation test, such as anticomplementary and prozone reactions (Nielsen et al., 1985). Both ELISAs are comparable with regards to sensitivity and specificity, but the cELISA is the preferred method because it is easier to perform. Other organisms may cause cross-reactions in serologic diagnostic tests for *Brucella* (Corbel et al., 1984).

TABLE 2. Age-specific serum antibody prevalence for *Brucella* spp. bacteria in harbor seals (*Phoca vitulina richardsi*) from coastal regions of Alaska.

Age category	Antibody prevalence ^a
Pup	2/18 (11)
Yearling	7/10 (70)
Subadult	17/25 (68)
Adult	20/47 (43)

^a Number positive/number tested (%).

TABLE 3. Location-specific serum antibody prevalence for phocid herpesvirus-1 in harbor seals (*Phoca vitulina richardsi*) from four regions of coastal Alaska.

Region	Antibody prevalence ^a
Prince William Sound	119/123 (97)
Kodiak Island	36/40 (90)
Central Gulf of Alaska	39/44 (89)
Southeast Alaska	31/36 (86)

^a Number positive/number tested (%).

However, recent isolations of *Brucella* spp. from marine mammals (Ross et al., 1996) increase the likelihood that they are indeed the cause of the positive test results. Further research is needed to evaluate the sensitivity and specificity of serologic test methods currently being used for potential *Brucella* sp. in a wide variety of marine mammal species.

The interactive behavior of pinniped species, especially during haulout periods, should be considered as an opportunity for transmission. The isolation of *Brucella* from reproductive organs in marine mammals (Miller et al., 1999) suggests venereal transmission as a possible route of infection. Maternal transfer may occur congenitally or while pups are suckled, as observed in terrestrial mammals. Young, sick, and malnourished seal pups may be predisposed to a variety of diseases, including *Brucella*, and exposure to pollutants may also reduce resistance. Another potential means of transfer is through parasites. The reported case of *Brucella* infection in *Parafilaroides* lungworm species from a Pacific harbor seal and *Pseudalius inflexus* lungworms from a juvenile male harbor porpoise from which *Brucella* was also recovered demonstrated this to be a potential route (Garner et al., 1997; Perrett et al., 2004).

The reproductive conditions associated with *Brucella* sp. infection in terrestrial mammals have been well documented. Effects of *Brucella* sp. infection in marine mammals are not fully understood. *Brucella* sp. has been isolated from the

reproductive tissues of marine mammals (Foster et al., 2002). Bottlenose dolphins have aborted in captivity (Ewalt et al., 1994; Miller et al., 1999). In addition, high serum antibody prevalences for *Brucella* sp. have been reported for a variety of species. Thus, *Brucella* sp. may indeed have a significant effect on the population dynamics of some marine mammal species.

The 54% antibody prevalence reported for *Brucella* sp. in the adult cohort in the current study is comparable to the 49% reported for common seals inhabiting Scottish waters (Foster et al., 2002). However, the age profile of that study was not reported. Thus, the two values are not directly comparable.

Brucella has also been isolated from the lymph nodes of four ringed seals off Baffin Island and one harp seal from the Gulf of St Lawrence, Canada. This was the first confirmed report of *Brucella* in marine mammals from Canada and the first report of the organism in ringed and harp seals. Ages of the animals were not stated (Forbes et al., 2000).

Antibody prevalences of 4% (10 of 248) for ringed seals and 12% (7 of 59) for walrus were reported in a study at eight locations in the Canadian arctic (Nielsen et al., 1996). Samples from seals ranging in age from 0 to 18 yr were collected at three widely distant sites. The random distribution of positive animals may have indicated sporadic exposure from another enzootically-infected phocid, or from a predator such as an arctic fox (*Alopex lagopus*). Limited epizootics may have occurred in the areas where these positive seals were found. A comparable situation may also have existed regarding the single population of walrus in Fox Basin.

Prevalence of 35% was reported for a sample of 16 Antarctic fur seals (*Arctocephalus gazelle*) and one Weddell seal from the Southern Hemisphere. Unfortunately, once again no age data were available for these animals (Retamoil et al., 2000). Few serologic surveys

reported low antibody prevalence. A prevalence of 0% was found for 45 Lake Baikal seals (Ross et al., 1996), assessed as a landlocked species. Introduction of *Brucella* sp. could have a major impact on the health of this population.

Low antibody prevalence in the pup cohort may have been related to age. All pups included in the survey were between 2 and 5 wk of age. Pups in this age range were probably still suckling, though some may have been recently weaned. If their mothers had been previously exposed to *Brucella* sp., the pups may have acquired passive immunity via antibodies in the colostrum. Potential exposure of pups to natural infection (aborting adult females, venereal transmission, etc.) would be unlikely.

Test results demonstrate that serum antibody prevalence of PhoHV-1 is high in harbor seals throughout the range of this study. These results concur with previous studies from both the Atlantic and Pacific coasts of the United States (Zarnke et al., 1997; Goldstein et al., 2003).

Antibody prevalence for PhoHV-1 was higher in seals from PWS than from other areas studied. Reasons for this difference are unclear. The harbor seal population in PWS has declined >60% since the late 1980s (Frost et al., 1999; Ver Hoef and Frost, 2003). Many seals were exposed to oil, as well as increased human activity, following the *Exxon Valdez* oil spill in 1989 (Frost et al., 1994; Lowry et al., 1994). Spill activities and the oil itself may have caused reactivation of latent PhoHV-1 infections. Sea otters held in captivity after the spill experienced herpes outbreaks that were attributed to stress (Harris et al., 1990; Spraker et al., 1994).

Food limitation associated with an oceanic regime shift has been suggested as a possible cause of harbor seal population decline in both PWS and the Gulf of Alaska during the 1970s and 1980s (Frost et al., 2001). If food-related stress resulted in activation of latent PhoHV-1 infection,

we would have expected antibody prevalences to be higher both near Kodiak and in PWS, because both populations experienced severe population declines. However, antibody prevalence was only elevated in the PWS population. The seal population near Kodiak is now increasing, whereas the PWS population continues to decline (Small et al., 2003; Ver Hoef and Frost, 2003). However, there is no indication that the PWS decline is caused by food limitation (Hastings et al., 2004; Small et al., 2005; Frost, pers. comm.). Young seals captured in PWS during 1997–2000 were larger and had more body fat than seals captured elsewhere in their range (Burns et al., 2005; Frost and Iverson, pers. comm.). Other possible causes of the population decline, such as hunting or predation, have no obvious relationship to antibody prevalence.

Conversely, the 0% antibody prevalence for PhHV-2 in the current study is in marked contrast to the 42% (52/124) prevalence reported previously for the same geographic area (Zarnke et al., 1997). Serologic tests for these two studies were performed in different laboratories utilizing different reagents and procedures.

Serologic evidence of exposure to PDV is widely distributed among harbor seal populations in North America marine waters (Duignan et al., 1993). However, antibody prevalence varies widely. The two samples reported as positive for PDV in this study are believed to be “false positives”; antibody titers barely exceeded the minimum threshold. Additional tests could not be conducted because samples were exhausted during the original test. Morbilliviruses typically spread widely through immunologically-naïve populations, and the current results suggest that the harbor seal populations in Alaskan waters had not been recently exposed to PDV prior to this study.

Field personnel have rarely reported signs of clinical disease in harbor seals during the period of this study (Spraker

et al., 1994). Population levels of harbor seals declined in some regions of the Gulf of Alaska during the 1970s and 1980s. However, no massive die-offs were reported. Over the last decade the seal population near Kodiak has increased steadily (yet abundance remains low), whereas the PWS population continues to decline, but a slower rate. The current survey has provided no link (either geographically or chronologically) between antibody prevalence of the four disease agents and changes in the dynamics of harbor seal populations. For these reasons, we do not believe that any of the four agents included in this survey represent significant sources of mortality for harbor seals in the coastal waters of Alaska.

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LITERATURE CITED

- ALTON, G. G., L. M. JONES, R. D. ANGUS, AND J. M. VERGER. 1988. Techniques for the brucellosis laboratory. Institut National de la Recherche Agronomique, Paris, France.
- BARR, B., J. L. DUNN, M. D. DANIEL, AND A. BAMFORD. 1989. Herpes-like viral dermatitis in a beluga whale (*Delphinapterus leucas*). *Journal of Wildlife Diseases* 25: 608–611.
- BLANK, O., P. RETAMAL, P. ABALOS, AND D. TORRES. 2002. Detection of anti-*Brucella* antibodies in Weddell seals (*Leptonychotes weddellii*) from Cape Shirref, Antarctica. *Archivos de Medicina Veterinaria* 34 (1): 117–122.

- BORST, G. H. A., H. C. WALVOORT, P. J. H. REIJNDERS, J. S. VAN DER KAMP, AND A. D. M. E. OSTERHAUS. 1986. An outbreak of herpesvirus infection in harbor seals (*Phoca vitulina*). *Journal of Wildlife Diseases* 2: 1–6.
- BURNS, J. M., D. P. COSTA, K. J. FROST, AND J. T. HARVEY. 2005. Development of body oxygen stores in harbor seals: effects of age, mass, and body composition. *Physiological and Biochemical Zoology* 78: 1057–1058.
- CORBEL, M. J., AND W. J. MORGAN. 1975. Proposal for minimal standards for descriptions of new species and biotypes of the genus *Brucella*. *International Journal of Systemic Bacteriology* 25: 83–89.
- , F. A. STUART, AND R. A. BREWER. 1984. Observations on serological cross-reactions between smooth *Brucella* species and organisms of other genera. *Developments in Biological Standardisation* 56: 341–348.
- DAOUST, P.-Y., D. M. HAINES, J. THORSEN, P. J. DUGNAN, AND J. R. GERACI. 1993. Phocine distemper in a harp seal (*Phoca groenlandica*) from the gulf of St. Lawrence, Canada. *Journal of Wildlife Diseases* 29: 114–117.
- DUGNAN, P. J., S. SADOVE, J. T. SALIKI, AND J. R. GERACI. 1993. Phocine distemper in harbor seals (*Phoca vitulina*) from Long Island, New York. *Journal of Wildlife Diseases* 29: 465–469.
- , J. T. SALIKI, D. J. ST. AUBIN, J. A. HOUSE, AND J. R. GERACI. 1994. Neutralizing antibodies to phocine distemper virus in Atlantic walrus (*Odobenus rosmarus*) from arctic Canada. *Journal of Wildlife Diseases* 30: 90–94.
- , O. NIELSEN, C. HOUSE, K. M. KOVACS, N. DUFFY, G. EARLY, S. SADOVE, D. J. ST. AUBIN, B. K. RIMA, AND J. R. GERACI. 1997. Epizootiology of morbillivirus infection in harp, hooded, and ringed seals from the Canadian Arctic and western Atlantic. *Journal of Wildlife Diseases* 33: 7–19.
- ELIASSON, M., A. OLSSON, E. PALMCRANTZ, K. WIBERG, M. INGANAS, B. GUSS, M. LINDERBERG, AND M. UHLEN. 1988. Chimeric IgG-binding receptors engineered from staphylococcal protein A and streptococcal protein G. *Journal of Biological Chemistry* 263: 4323–4327.
- EWALT, D. R., J. B. PAYEUR, B. M. MARTIN, D. R. CUMMINS, AND W. G. MILLER. 1994. Characteristics of a *Brucella* species from a bottlenose dolphin (*Tursiops truncatus*). *Journal of Veterinary Diagnostic Investigation* 6: 448–452.
- FORBES, L. B., O. NIELSEN, L. MEASURES, AND D. R. EWALT. 2000. Brucellosis in ringed seals and harp seals from Canada. *Journal of Wildlife Diseases* 36: 595–598.
- FOSTER, G., A. P. MACMILLAN, J. GODFROID, F. HOWIE, H. M. ROSS, A. CLOECKAERT, R. J. REID, S. BREW, AND I. A. P. PATTERSON. 2002. A review of *Brucella* sp. infection of sea mammals with particular emphasis on isolates from Scotland. *Veterinary Microbiology* 90: 563–580.
- FREY, H. R., B. LIESS, L. HAAS, H. LEHMANN, AND H. J. MARSCHALL. 1989. Herpesvirus in harbour seals (*Phoca vitulina*): Isolation, partial characterization and distribution. *Journal of Veterinary Medicine Series B* 36: 699–708.
- FROST, K. J., L. F. LOWRY, E. SINCLAIR, J. VER HOEF, AND D. C. MCALLISTER. 1994. Impacts on distribution, abundance, and productivity of harbor seals. In *Marine mammals and the Exxon Valdez*, T. R. Loughlin (ed.). Academic Press, Inc., San Diego, California, pp. 97–118.
- , ———, AND J. M. VER HOEF. 1999. Monitoring the trend of harbor seals in Prince William Sound, Alaska, after the Exxon Valdez oil spill. *Marine Mammal Science* 15: 494–506.
- , M. A. SIMPKINS, AND L. F. LOWRY. 2001. Diving behavior of harbor seals in Prince William Sound, Alaska. *Marine Mammal Science* 17: 813–834.
- , ———, R. J. SMALL, AND L. F. LOWRY. Development of diving by harbor seal pups in two regions of Alaska: use of the water column. *Marine Mammal Science* 42: In press.
- GARNER, M. M., D. M. LAMBOURNE, S. J. JEFFRIES, P. HALL BRIGGS, J. C. RHYAN, D. R. EWALT, L. M. POLZIN, AND N. F. CHEVILLE. 1997. Evidence of *Brucella* infection in *Parafilaroides* lungworms in a Pacific harbour seal (*Phoca vitulina richardsi*). *Journal of Veterinary Diagnostic Investigation* 9: 298–303.
- GOLDSTEIN, T., F. M. D. GULLAND, B. M. ALDRIDGE, J. T. HARVEY, T. ROWLES, D. M. LAMBOURNE, S. J. JEFFRIES, L. MEASURES, P. K. YOCHER, B. S. STEWART, R. J. SMALL, D. P. KING, J. L. STOTT, AND J. A. K. MAZET. 2003. Antibodies to phocine herpesvirus-1 are common in North American harbor seals (*Phoca vitulina*). *Journal of Wildlife Diseases* 39: 487–494.
- GRACHEV, M. A., V. P. KUMAREV, L. V. MAMAEV, V. L. ZORIN, L. V. BARANOVA, N. N. DENIKINA, S. I. BELIKOV, E. A. PETROV, V. S. KOLESNIK, R. S. KOLESNIK, V. M. DOROFEEV, A. M. BEIM, V. N. KUDELIN, F. G. NAGIEVA, AND V. N. SIDOROV. 1989. Distemper virus in Baikal seals. *Nature* 338: 209–210.
- HARDER, T. C., J. PLOTZ, AND B. LIESS. 1991. Antibodies against European phocine herpesvirus isolates detected in sera of Antarctic seals. *Polar Biology* 11: 509–512.
- , M. HARDER, H. W. VOS, K. KULONEN, S. KENNEDY-STOSKOPF, B. LIESS, M. J. G. APPEL, AND A. D. M. E. OSTERHAUS. 1996. Characterization of phocid herpesviruses type-1 and type-2 as putative α - and γ -herpesviruses of North American and European pinnipeds. *Journal of General Virology* 77: 27–35.
- , H. VOS, R. L. DE SWART, AND A. D. OSTERHAUS. 1997. Age-related disease in re-

- current outbreak of phocid herpesvirus type-1 infections in a seal rehabilitation centre: evaluation of diagnostic methods. *The Veterinary Record* 140: 500–503.
- HARRIS, R. K., R. B. MOELLER, T. P. LIPSCOMB, J. M. PLETCHER, R. J. HAEBLER, P. A. TUOMI, C. R. MCCORMICK, A. R. DEGANGE, D. MULCAHY, AND T. D. WILLIAMS. 1990. Identification of a herpes-like virus in sea otters during rehabilitation after the T/V *Exxon Valdez* oil spill. *In* Sea otter symposium: Proceedings of a symposium to evaluate the response effort on behalf of sea otters after the T/V *Exxon Valdez* oil spill into Prince William Sound, Anchorage, Alaska, K. Bayha and J. Kennedy (eds.). Biological Report 90(12). US Fish and Wildlife Service, Anchorage, Alaska, pp. 366–368.
- HASTINGS, K. K., K. J. FROST, M. A. SIMPKINS, G. W. PENDLETON, U. G. SWAIN, AND R. J. SMALL. 2004. Regional differences in diving behavior of harbor seals in the Gulf of Alaska. *Canadian Journal of Zoology* 82: 1755–1773.
- HOOVER-MILLER, A. A. 1994. Harbor seal (*Phoca vitulina*) biology and management in Alaska. Final Report. Contract No. T75134749 to US Marine Mammal Commission, Washington, D.C., 45 pp. Available from: MMC, 4340 East-West Hwy Suite 905, Bethesda, Maryland 20814.
- HORVAT, B., T. WILHAUS, H. R. FREY, AND B. LIESS. 1989. Herpesvirus in harbour seals (*Phoca vitulina*): Transmission in homologous host. *Journal of Veterinary Medicine Series B* 36: 715–718.
- KENNEDY, S. 1990. A review of the 1988 European seal morbillivirus epizootic. *The Veterinary Record* 127: 563–567.
- , J. A. SMYTH, P. F. CUSH, P. DUIGNAN, M. PLATTEN, S. J. MCCULLOUGH, AND G. M. ALLAN. 1989. Histopathologic and immunohistochemical studies of distemper in seals. *Veterinary Pathology* 26: 97–103.
- , I. J. LINDSTEDT, M. M. MCALISKEY, S. A. MCCONNELL, AND S. J. MCCULLOUGH. 1992. Herpesviral encephalitis in a harbor porpoise (*Phocoena phocoena*). *Journal of Zoo and Wildlife Medicine* 23: 374–379.
- KENNEDY-STOSKOPF, S., M. K. STOSKOPF, M. A. ECKHAUS, AND J. D. STRANDBERG. 1986. Isolation of a retrovirus and a herpesvirus from a captive sea lion. *Journal of Wildlife Diseases* 22: 156–164.
- LEBICH, M., T. C. HARDER, H. R. FREY, I. K. G. VISSER, A. D. M. E. OSTERHAUS, AND B. LIESS. 1994. Comparative immunological characterization of type-specific and conserved B cell epitopes of pinniped, felid and canid herpesviruses. *Archives of Virology* 136: 335–347.
- LOWENSTINE, L. J., L. J. GAGE, D. M. SMITH, AND C. FITZGERALD. 1992. Mortality in neonatal Pacific harbor seals (*Phoca vitulina richardsi*) from the California coast: Possible role of a herpes-like virus infection. *In* World Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases 12th international symposium proceedings: New and emerging infectious diseases. B. Osburn, G. Castrucci and C. Shore (eds.). University of California–Davis, Davis, California, 8–12 September, pp. 243–247.
- LOWRY, L. F., K. J. FROST, AND K. W. PITCHER. 1994. Observations of oiling of harbor seals in Prince William Sound. *In* Marine mammals and the *Exxon Valdez*, T. R. Loughlin (ed.). Academic Press, San Diego, California, pp. 209–225.
- , ———, J. M. VER HOEF, AND R. A. DELONG. 2001. Movements of satellite-tagged subadult and adult harbor seals in Prince William Sound, Alaska. *Marine Mammal Science* 17: 835–861.
- MACMILLAN, A. P., I. GREISER-WILKE, V. MOENNIG, AND L. A. MATHIAS. 1990. A competition enzyme immunoassay for brucellosis diagnosis. *Deutsche Tierärztliche Wochenschrift* 97 (2): 83–85.
- MCCULLAGH, P., AND J. A. NELDER. 1989. Generalized linear models. 2nd Edition. Chapman and Hall, London, United Kingdom, 511 pp.
- MILLER, W. G., L. G. ADAMS, T. A. FICHT, N. F. CHEVILLE, J. B. PAYEUR, D. R. HARLEY, C. HOUSE, AND S. H. RIDGWAY. 1999. *Brucella*-induced abortions and infections in bottlenose dolphins (*Tursiops truncatus*). *Journal of Zoo and Wildlife Medicine* 30: 100–110.
- MÜLLER, G., P. WOHLSEIN, A. BEINEKE, L. HAAS, I. GREISER-WILKE, U. SIEBERT, S. FONFARA, T. HARDER, M. STEDE, A. D. GRUBER, AND W. BAUMGÄRTNER. 2004. Phocine distemper in German seals, 2002. *Emerging Infectious Diseases* 10: 723–725.
- NIELSEN, K. H., L. KELLY, D. GALL, P. NICOLETTI, AND W. KELLY. 1985. Improved competitive enzyme immunoassay for the diagnosis of bovine brucellosis. *Veterinary Immunology and Immunopathology* 46: 285–291.
- NIELSEN, O., K. NIELSEN, AND R. E. A. STEWART. 1996. Serological evidence of *Brucella* spp. exposure in Atlantic walrus (*Odobenus rosmarus rosmarus*) and ringed seals (*Phoca hispida*) of Arctic Canada. *Arctic* 49: 383–386.
- OHISHI, K., K. TAKISHITA, M. KAWATO, R. ZENITANI, T. BANDO, Y. FUJISE, Y. GOTO, S. YAMAMOTO, AND T. MARUYAMA. 2004. Molecular evidence of new variant *Brucella* in North Pacific common minke whales. *Microbes and Infection* 6: 1199–1204.
- OSTERHAUS, A. D. M. E., H. YANG, H. E. M. SPIJKERS, J. GROEN, J. S. TEPPEMA, AND G. VAN STEENIS. 1985. The isolation and partial characterization of a highly pathogenic herpesvirus from the harbour seal (*Phoca vitulina*). *Archives of Virology* 86: 239–251.
- , J. GROEN, H. E. M. SPIJKERS, H. W. J. BOERDERS, F. C. G. M. UYTDEHAAG, P. DE VRIES,

- J. S. TEPEPMA, I. K. G. VISSER, M. W. G. VAN DE BILDT, AND E. J. VEDDER. 1990. Mass mortality in seals caused by a newly discovered morbillivirus. *Veterinary Microbiology* 23: 343–350.
- PAYEUR, J. B., L. CARPENTER, D. R. EWALT, M. M. GARNER, S. J. JEFFRIES, D. M. LAMBOURNE, B. NORBERG, L. POLZIN, AND J. C. RHYAN. 1998. Evidence of *Brucella* species infection in Pacific harbour seals (*Phoca vitulina richardsi*), Californian sea lions (*Zalophus californianus*), and harbour porpoises (*Phocoena phocoena*) in Puget Sound, Washington. Proceedings of the ARC-Onderstepoort, OIE international congress with WHO cosponsorship on anthrax, brucellosis, CBPP, clostridial and mycobacterial diseases, Berg-en-Dal, Kruger National Park, South Africa, 9–15 August, pp. 173–177.
- PERRETT, L. L., C. E. DAWSON, N. DAVISON, AND S. QUINNEY. 2004. *Brucella* infection of lungworms from a harbor porpoise. *The Veterinary Record* 154: 800.
- PITCHER, K. W. 1989. Harbor seal trend count surveys in southern Alaska, 1988. Final Report. Contract MM4465852-1 to US Marine Mammal Commission, Washington, D.C., 15 pp.
- . 1990. Major decline in number of harbor seals, *Phoca vitulina richardsi*, on Tugidak Island, Gulf of Alaska. *Marine Mammal Science* 6: 121–134.
- , AND D. G. CALKINS. 1979. Biology of the harbor seal, *Phoca vitulina richardsi*, in the Gulf of Alaska. US Department of Commerce, NOAA, Outer Continental Environmental Assessment Program Final Report 19: 231–310.
- RETAMOIL, P., O. BLANK, P. ABALOS, AND D. TORRES. 2000. Detection of anti-*Brucella* antibodies in pinnipeds from Antarctic territory. *The Veterinary Record* 146: 166–167.
- ROSS, H. M., G. FOSTER, R. J. REID, K. L. JAHANS, AND A. P. MACMILLAN. 1994. *Brucella* species infection in sea-mammals. *The Veterinary Record* 134: 359.
- , K. L. JAHANS, A. P. MACMILLAN, R. J. REID, P. M. THOMPSON, AND G. FOSTER. 1996. *Brucella* species infection in North Sea seal and cetacean populations. *The Veterinary Record* 138: 647–648.
- ROSSITER, P. B., D. M. JESSETT, AND W. P. TAYOR. 1985. Microneutralisation systems for use with different strains of peste des petits ruminants virus and rinderpest virus. *Tropical Animal Health and Production* 17: 75–81.
- SALIKI, J. T., G. LIBEAU, J. A. HOUSE, C. A. MEBUS, AND E. J. DUBOVI. 1993. Monoclonal antibody-based enzyme-linked immunosorbent assay for specific detection and titration of peste-des-petits-ruminants virus antibody in caprine and ovine sera. *Journal of Clinical Microbiology* 31: 1075–1082.
- SEASE, J. L. 1992. Status review of harbor seals (*Phoca vitulina*) in Alaska. National Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, Washington. 74 pp.
- SIKKEMA, W. D. 1989. An Fc-binding protein. *American Biotechnology Laboratory* 7: 42.
- SMALL, R. J., G. W. PENDLETON, AND K. W. PITCHER. 2003. Trends in abundance of Alaska harbor seals, 1983–2001. *Marine Mammal Science* 19: 344–362.
- , L. F. LOWRY, J. M. VER HOEF, K. J. FROST, R. A. DELONG, AND M. J. REHBERG. 2005. Differential movements by harbor seal pups in contrasting Alaska environments. *Marine Mammal Science* 21: 671–694.
- SPRAKER, T. R., L. F. LOWRY, AND K. J. FROST. 1994. Gross necropsy and histopathological lesions found in harbor seals. In *Marine mammals and the Exxon Valdez*, T. R. Loughlin (ed.). Academic Press, San Diego, California, pp. 281–311.
- VAN BRESSEM, M-F., I. K. G. VISSER, M. W. G. VAN DE BILDT, J. S. TEPEPMA, J. A. RAGA, AND A. D. M. E. OSTERHAUS. 1991. Morbillivirus infection in Mediterranean striped dolphins (*Stenella coeruleoalba*). *The Veterinary Record* 129: 471–472.
- VER HOEF, J. M., AND K. J. FROST. 2003. A Bayesian hierarchical model for monitoring harbor seal changes in Prince William Sound, Alaska. *Environmental and Ecological Statistics* 10: 201–209.
- VISSER, I. K. G., J. S. TEPEPMA, AND A. D. M. E. OSTERHAUS. 1991. Virus infections of seals and other pinnipeds. *Reviews in Medical Microbiology* 2: 105–114.
- , E. J. VEDDER, H. W. VOS, M. W. G. VAN DE BILDT, AND A. D. M. E. OSTERHAUS. 1993. Continued presence of phocine distemper virus in the Dutch Wadden Sea seal population. *The Veterinary Record* 133: 320–322.
- ZARNKE, R. L., T. C. HARDER, H. W. VOS, J. M. VER HOEF, AND A. D. M. E. OSTERHAUS. 1997. Serologic survey for phocid herpesvirus-1 and -2 in marine mammals from Alaska and Russia. *Journal of Wildlife Diseases* 33: 459–465.

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