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## SEROLOGIC SURVEY FOR PHOCID HERPESVIRUS-1 AND -2 IN MARINE MAMMALS FROM ALASKA AND RUSSIA

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**ABSTRACT:** Blood samples were collected from 1,042 marine mammals off the coast of Alaska (USA) and Russia during the period 1978 to 1994. Eight species of pinnipeds were represented. Sera were tested for presence of neutralizing antibodies to both the PB84 isolate of phocid herpesvirus-1 (PhHV-1) and the 7848/Han90 strain of phocid herpesvirus-2 (PhHV-2). Species-specific antibody prevalences ranged from 22% to 77% for PhHV-1 and 11% to 50% for PhHV-2. Species-specific antibody prevalences for PhHV-1 were greater than or equal to prevalences for PhHV-2. For both viruses and each host species, differences in antibody prevalences were not related to: (1) sex, (2) location of capture, or (3) year of collection. Antibody prevalence of PhHV-1 in walrus (*Odobenus rosmarus*) could be quantitatively predicted as a function of age. These two viruses have distinct biological properties and based on current data the epizootiology of the two viruses is different, as well. No evidence of herpesvirus-induced mortality has been detected in areas included in this survey. Based on results of this survey, neither PhHV-1 nor PhHV-2 are considered significant mortality factors in mammals which inhabit the marine environment off the coast of Alaska or Russia.

**Key words:** Marine mammals, phocid herpesvirus, serology, survey.

### INTRODUCTION

Phocid herpesvirus-1 (PhHV-1) is an alpha herpesvirus (Harder et al., 1996a). It was first identified in 1984 when it caused the deaths of 11 harbor seal (*Phoca vitulina*) pups in a nursery in the Netherlands (Osterhaus et al., 1985). Twelve additional pups which were ill during that epizootic subsequently recovered over the course of a 2-mo period (Borst et al., 1986). Mortality due to PhHV-1 infection has only been observed in: (1) neonates or (2) seals acutely infected with phocine distemper virus, or (3) seals which have been otherwise immunocompromised (A. D. M. E. Osterhaus, unpubl.).

Clinical signs of PhHV-1 disease included: (1) elevated body temperature, (2) inflammation of the oral mucosa, (3) nasal discharge, (4) coughing, (5) vomiting, (6) diarrhea, (7) anorexia, and (8) 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).

There is experimental evidence that: (1) harbor seals can be infected by means of intranasal instillation of PhHV-1 or direct contact with infected animals, and (2) virus is shed in nasal and ocular discharge from naturally- and experimentally-infected animals (Horvat et al., 1989; Harder et al., 1996b). Presumably, natural transmission occurs by means of aerosols or direct contact, as in other alpha herpesvirus infections.

A second seal herpesvirus type has been isolated from: (1) a captive California sea lion (*Zalophus californianus*) (Kennedy-Stoskopf et al., 1986), (2) free-ranging adult harbor seals from the North Sea (Lebich et al., 1994), and (3) free-ranging harbor seals from the North Atlantic offshore of the United States (Harder et al., 1996a). These isolates are collectively known as phocid herpesvirus-2 (PhHV-2). Based on nucleotide sequence data, PhHV-2 is a

gamma-herpesvirus (Harder et al., 1996a). There is no evidence that PhHV-2 causes clinical disease in pinnipeds.

Blood was collected from 49 free-ranging harbor seals which were involved in the 1988 phocine distemper virus (PDV) epizootic in the North Atlantic. Based on serologic tests, approximately half of these seals had been exposed to PhHV-1 (Frey et al., 1989). Despite this high antibody prevalence, PhHV-1 was isolated from only four of 112 harbor seals which died during the 1988 PDV epizootic (Frey et al., 1989).

Herpesviruses have also 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 Prince William Sound, Alaska. One male had lesions on the penis and prepuce. Intranuclear inclusion bodies typical of a 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 (G. M. Zaucha, pers. comm.). Hepatic and adrenal necrosis were found in 12 harbor seal pups which died at a rehabilitation center in Sausalito, California (USA) during 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 (*Phocoena phocoena*) (Kennedy et al., 1992), California sea lion (Kennedy-Stoskopf et al., 1986), and sea otter (*Enhydra lutris*) (Harris et al., 1990).

Serum antibody prevalence for PhHV-1 was 100% in 25 apparently healthy Weddell seals (*Leptonychotes weddelli*) and three apparently healthy crabeater seals (*Lobodon carcinophagus*) from the eastern Weddell Sea (Harder et al., 1991). Neu-

tralizing 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).

The purpose of this study was to determine the serum antibody prevalence of PhHV-1 and PhHV-2 in marine mammal populations off the coast of Alaska and Russia.

## MATERIALS AND METHODS

Animals were collected by various investigators during studies of marine mammal population biology and ecology. Collection areas included portions of southeastern Alaska, the Gulf of Alaska, eastern and western Bering Sea, Chukchi Sea, and Beaufort Sea (Fig. 1). The following descriptive data were recorded for each animal: (1) sex, (2) date and (3) location. Age data were collected only for walruses (*Odobenus rosmarus*). Age determination for walruses was based on: (1) body conformation, (2) tusk size, and (3) facial characteristics (Fay, 1982). For the purpose of comparison, samples were grouped by year of collection. Year of collection does not necessarily reflect year of exposure.

Blood was routinely drawn from the extradural vein, caudal gluteal vein or rear flipper. Samples were allowed to clot and then centrifuged. Serum was transferred to sterile vials. Sera were initially stored at  $-12^{\circ}\text{C}$  for several months and then transferred to  $-40$  to  $-46^{\circ}\text{C}$  for periods lasting from several months to several years until the time of testing.

Microneutralization assays were used to detect antibodies against both PhHV-1 (Osterhaus et al., 1985) and PhHV-2 (Lebich et al., 1994). The PB84 isolate of PhHV-1 was grown in seal kidney cells (SeKC) (Osterhaus et al., 1985). The 7848/Han90 strain of PhHV-2 was propagated in Crandall Rees feline kidney (CrFK) cells (Harder et al., 1996a). Both isolates were obtained from harbor seals from the North Sea (Osterhaus et al., 1985; Lebich et al., 1994). One hundred mean tissue culture infective doses ( $\text{TCID}_{50}$ ) of virus was used in each test. Results were evaluated by development of viral cytopathic effect after 3 days for PhHV-1 and 7 days for PhHV-2.

Under normal test protocol, samples which neutralize PhHV-1 or PhHV-2 at a serum dilution of 1:5 or greater are considered indicative of previous natural exposure to the virus (Osterhaus et al., 1985; Lebich et al., 1994). For the current study, a higher threshold dilution of 1:20 was selected. Samples that met or exceeded a titer of 20 will be referred to as positive. All others will be referred to as neg-

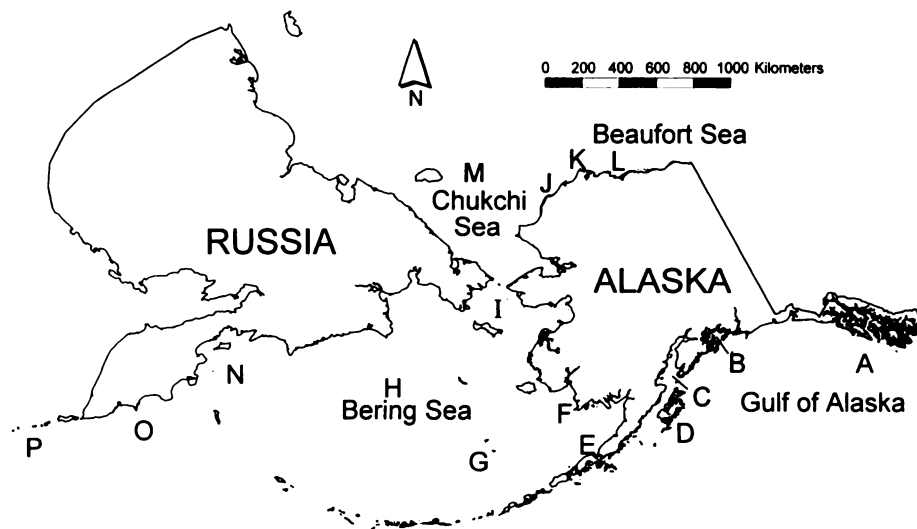


FIGURE 1. Location of collection sites for marine mammals included in phocid herpesvirus-1 serologic survey. A, Southeast Alaska (134°W, 56°N); B, Prince William Sound (147°W, 61°N); C, Cook Inlet (152°W, 60°N); D, Kodiak Island (153°W, 57°N); E, Alaska Peninsula (160°W, 56°N); F, Kuskokwim Bay (163°W, 59°N); G, Pribilof Islands (170°W, 57°N); H, Bering Sea (174°W, 62°N); I, Bering Strait (169°W, 66°N); J, Northwest Alaska (164°W, 70°N); K, Barrow (157°W, 71°N); L, Prudhoe Bay (148°W, 71°N); M, Chukchi Sea (172°W, 69°N); N, Karaginsky Gulf (164°E, 58°N); O, Southeast Kamchatka Peninsula (160°E, 53°N); P, Kuril Islands (150°E, 46°N).

ative. This change in threshold was implemented in order to reduce the impacts of potential nonspecific neutralizing or cytotoxic substances on test interpretation. The higher threshold may have lead to an underestimation of actual antibody prevalences.

For walrus, a generalized linear model with a logit link (McCullagh and Nelder, 1989) was used to determine if there was a significant dependence of serum antibody prevalence on the following variables: (1) age, (2) sex, and (3) year of collection. Antibody response is a binary response variable. Age was treated as a continuous variable. Sex and year of collection were categorical. Geographic location was not included in any logit models for predicting antibody status of walruses. With one exception, samples were collected from unique locations each year. Therefore, year of collection is confounded with location in the modeling process. All main and interaction effects of these variables were evaluated. 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 value (McCullagh and Nelder, 1989). The version 6.10 SAS statistical software package was used to fit the model with maximum likelihood parameter estimates (SAS Institute, Cary, North Carolina, USA).

Sex and year-of-collection data for harbor seal, sea lion, and spotted seal (*Phoca largha*) were also subjected to the logit modelling process for PhHV-1. Ages were not available for these species. The chi-square test (Snedecor and Cochran, 1980) was used to determine if PhHV-2 antibody prevalence for ribbon seals (*Histiophoca fasciata*), spotted seals, or harbor seals was related to sex of the animal.

## RESULTS

Serum antibody prevalences for PhHV-1 ranged from 22% to 77% (Table 1). Serum antibody prevalences for PhHV-2 ranged from 11% to 50%. In most species, prevalence for PhHV-1 was higher than for PhHV-2. From 11% to 34% of each species had been exposed to both viruses.

For walruses, neither sex nor year of collection had a significant effect on antibody prevalence for PhHV-1 ( $P > 0.05$ ). The only factor retained in the model was age ( $P = 0.04$ ). Age is a continuous variable. Therefore, the final model is equivalent to logistic regression. The model can be used to predict the probability of a pos-

TABLE 1. Serum antibody prevalence of phocid herpesvirus-1 (PhHV-1) and phocid herpesvirus-2 (PhHV-2) in selected marine mammal species sampled off the coast of Alaska and Russia, 1978 to 1994.

Species	Years of collection	Locations collected <sup>a</sup>	Prevalences <sup>b</sup>			
			PhHV-1	PhHV-2	PhHV-1 and PhHV-2	Negative
Walrus ( <i>Odobenus rosmarus</i> )	1981-1987	F,I,M	189/341 (55%)	98/341 (29%)	61/341 (18%)	115/341 (34%)
Northern fur seal ( <i>Callorhinus ursinus</i> )	1980	G	53/154 (34%)	62/154 (40%)	22/154 (14%)	61/154 (40%)
Harbor seal ( <i>Phoca vitulina</i> )	1978-1994	A,B,C,D,E,G	95/124 (77%)	52/124 (42%)	42/124 (34%)	19/124 (15%)
Spotted seal ( <i>Phoca largha</i> )	1978-1993	H,I,J,K,N,O,P	23/32 (72%)	5/32 (16%)	5/32 (16%)	9/32 (28%)
Ribbon seal ( <i>Histiophoca fasciata</i> )	1978-1979	H	7/24 (29%)	7/24 (29%)	5/24 (21%)	15/24 (63%)
Steller sea lion ( <i>Eumetopias jubatus</i> )	1978-1993	A,B,C,D,E,G	11/22 (50%)	5/22 (23%)	4/22 (18%)	10/22 (45%)
Bearded seal ( <i>Erignathus barbatus</i> )	1978-1990	H,K	11/18 (61%)	3/18 (17%)	2/18 (11%)	6/18 (33%)
Ringed seal ( <i>Pusa hispida</i> )	1978-1992	H,L,K	2/4 (50%)	2/4 (50%)	1/4 (25%)	1/4 (25%)

<sup>a</sup> A, Southeast Alaska; B, Prince William Sound; C, Cook Inlet; D, Kodiak Island; E, Alaska Peninsula; F, Kuskokwim Bay; G, Pribilof Islands; H, Bering Sea; I, Bering Strait; J, Northwest Alaska; K, Barrow; L, Prudhoe Bay; M, Chukchi Sea; N, Karaginsky Gulf; O, Southeast Kamchatka Peninsula; P, Kuril Islands.

<sup>b</sup> Number of samples with significant levels of antibody/total number of samples tested (% positive).

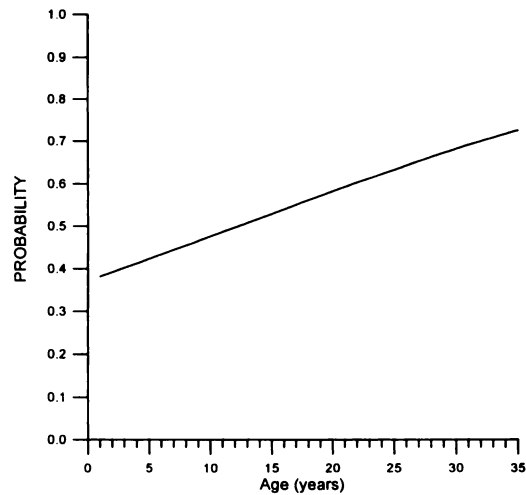


FIGURE 2. Relationship between walrus (*Odobenus rosmarus*) age and predicted probability of a walrus serum sample exceeding threshold titer ( $\geq 20$ ) for phocid herpesvirus-1.

itive test result as a function of age (Fig. 2):  $\pi(\text{age}) = \exp(-0.5249 + 0.0428 \times \text{age}) / 1 + \exp(-0.5249 + 0.0428 \times \text{age})$ ; where  $\exp(\times)$  is the exponential function (base  $e$ ).

For harbor seals, spotted seals, and sea lions, the only factor which had an effect on prevalence was sex. Sex is a categorical variable. Therefore, the final model is equivalent to a chi-square contingency table. Prevalence was higher in females for all three species (103 of 138 for female harbor seals versus 90 of 151 for males; 13 of 25 versus two of 23 for sea lions; and 17 of 22 versus 19 of 31 for spotted seals. These sex-specific differences were statistically significant only for harbor seals ( $P = 0.007$ ) and sea lions ( $P = 0.001$ ).

Antibody prevalence of PhHV-2 in all species was not related to: 1) sex, 2) age, or 3) year of collection. Antibody prevalence of PhHV-2 for walruses was not a function of age.

## DISCUSSION

Cross reactivity is a common problem in serologic surveys. Antibodies produced as a result of exposure to one disease agent may react with a closely-related agent.

These circumstances can produce false positive results. Cross neutralization is negligible for most alpha-/gamma-herpesvirus pairs such as equine herpesviruses-1 and -2, and varicella zoster virus and Epstein Barr virus (Plummer et al., 1973; Honess and Watson, 1977). In our study, many sera had high titers against either PhHV-1 or PhHV-2. These results concur with a previous study of marine mammals from European waters (Lebich et al., 1994). Therefore, sera with significant titers against either PhHV-1 or PhHV-2 are believed to represent prior natural exposure to the individual virus. Sera with titers against both PhHV-1 and PhHV-2 are believed to represent natural exposure to both viruses.

No phocid herpesvirus isolates are available from the area included in the current survey. Therefore, isolates of European origin were used. Pinnipeds in the current study area may be exposed to viruses which are closely-related, but antigenically-distinct from those found in European waters. Antibody produced in response to these hypothetical viruses may neutralize European strains of PhHV-1 or PhHV-2 less efficiently. These circumstances could yield false negative results. There are minor variations between European isolates of PhHV-1 (Frey et al., 1989). However, there were no significant antigenic differences between isolates of European and United States origin (Harder et al., 1996a). Therefore, all positive serologic test results are considered indicative of PhHV-1 and/or PhHV-2 exposure.

Two general taxonomic groups were represented in the survey: (1) phocids—ringed seal (*Pusa hispida*), spotted seal, harbor seal, bearded seal (*Erignathus barbatus*) and ribbon seal; and (2) odobenids and otariids—walrus, Steller sea lions (*Eumetopias jubatus*), and northern fur seal (*Callorhinus ursinus*). Prevalences for PhHV-1 ranged from 22% to 77% for the phocids and 34% to 54% for the odobenids and otariids. The three highest prevalences were seen in phocid seals. However,

there was no clear-cut pattern among or between the two groups. Current antibody prevalences for PhHV-1 (Table 1) are in the range previously reported for hooded seals (*Cystophora cristata*) and harp seals (*Phoca groenlandica*) from the White Sea (Stuenkel et al., 1994) and for European harbor seals (Frey et al., 1989). Prevalences for PhHV-2 ranged from 11% to 50% for the phocids and 19% to 44% for the odobenids and otariids. The two highest prevalences were seen in phocids.

Antibody prevalences for PhHV-1 were generally higher than for PhHV-2 (Table 1). Transmission of the highly contagious PhHV-1 is primarily via the respiratory route (Osterhaus et al., 1985; Harder et al., 1996b). Routes of transmission for PhHV-2 are unknown. The most effective method of isolating PhHV-2 is via cocultivation of leukocytes (Lebich et al., 1994). Apparently, the virus is highly cell-associated under natural conditions. This conclusion is supported by cell culture under laboratory conditions (Lebich et al., 1994). The cell-associated nature of PhHV-2 makes airborne transmission unlikely. Alternate routes of transmission for PhHV-2 may produce lower transmission rates than for PhHV-1. These lower rates of transmission may be reflected in the lower antibody prevalences observed in the current survey.

Harbor seals and Steller sea lions live primarily in the subarctic and temperate regions. In contrast, walrus and the remainder of the seal species live primarily in the arctic. There was no discernible pattern of prevalence between these two groups. Apparently, exposure of marine mammals to PhHV-1, PhHV-2, or related herpesviruses has been: (1) common, (2) geographically widespread, and (3) long term.

The logit model can be used to quantitatively predict antibody prevalence of PhHV-1 as a function of age for walrus (Fig. 2). This model apparently reflects: (1) moderate exposure rates at an early

age, and (2) continual opportunity for exposure throughout life.

Differential sex-specific mortality rates could contribute to the higher sex-specific antibody prevalence in female harbor seals, spotted seals, and sea lions. If mortality is higher in males than in females, then females would constitute a greater proportion of the older age cohorts. The age effect observed in walrus (Fig. 2) is evidence that prevalence would also be higher in older age harbor seals, spotted seals and sea lions. Unfortunately, age data were not available for these three species. Therefore, the hypothesis could not be tested.

*Herpesvirus canis* infection in neonatal domestic dogs is often fatal (Carmichael, 1970). Passive transfer of maternal antibody can mitigate the impact of infection. Dogs more than 2 to 3 wk of age are usually able to survive infection (Carmichael, 1970). This change is attributed to development of an effective system of thermoregulation. Fatal PhHV-1 infections occur only in seals with immature or compromised immune systems. Other cohorts develop transient respiratory disease. Presumably, similar mechanisms determine the outcome of alpha herpesvirus infection in all species.

During the sample collection period, populations of the following species have declined: (1) Steller sea lion (Merrick et al., 1987), (2) northern fur seal (Fowler, 1990), and (3) harbor seal (Pitcher, 1990). No cases with clinical signs of PhHV-1 infection have been reported for either Steller sea lions or northern fur seals. A single case of fatal PhHV-1 infection in a harbor seal was recently reported from the coast of Washington (G. M. Zaucha, pers. comm.). Thus, there is no apparent relationship between declines of these species and exposure to PhHV-1 or a related herpesvirus.

Based on census data, populations of both walrus (Gilbert, 1989) and polar bear (*Ursus maritimus*) (Amstrup et al., 1986) seem stable. The geographic range of sea

otters in Alaskan waters has expanded in recent decades, apparently a reflection of increased population size (Rotterman and Simon-Jackson, 1988). Population status for other species is unknown.

Based upon serum antibody prevalences reported here, marine mammals in the waters of Alaska and Russia are commonly exposed to PhHV-1, PhHV-2, or related herpesviruses. Apparently, herpesviruses have become enzootic in these waters. There have been no documented herpesvirus epizootics in these host populations. However, PhHV-1 is capable of causing morbidity and mortality in nonimmunocompetent seals. Therefore, future epizootics of clinical disease are possible.

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