Serologic Survey for Exposure to Marine and Terrestrial Pathogens in Seals of the Bering Sea

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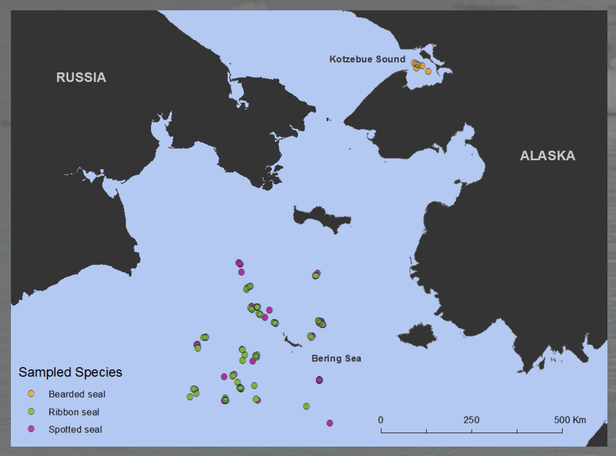
Abstract

Little is known about the health of and exposure to diseases in ice-associated seals of the Bering Sea, which include ribbon (*Histriophoca fasciata*), spotted (*Phoca largha*), and bearded seals (*Erignathus barbatus*). A warming climate and diminished sea ice could have significant effects on the health of these seals; therefore, baseline data on health parameters and exposure to disease may provide a reference for detecting future climate-related changes in health. To assess exposure to several common marine and terrestrial pathogens, ribbon and spotted seals were sampled at the edge of the pack ice of the Bering Sea during spring of 2007-2010, and bearded seals were sampled in the pack ice in Kotzebue Sound, Alaska , as they moved from breeding areas in the Bering Sea to foraging areas in the Chukchi Sea, during summer 2009, 2011, and 2012. Serologic tests were conducted to test for antibodies against phocine herpesvirus-1 (PhHV-1), *Leptospira interrogans*, *Toxoplasma gondii, Sarcocystis neurona*, influenza A, *Brucella* spp., *Coxiella burnetii*, and phocine distemper virus (PDV). Spotted seals were positive for antibodies to PhHV-1 (76.6%), influenza A (10.6%), *Brucella* spp. (8.5%), *Coxiella burnetii* (8.3%), and PDV (43.9%), while ribbon seals tested seropositive for PhHV-1 (9.5%), influenza A (4.8%), *Coxiella burnetii* (8.0%), and PDV (42.3%), and a small sample of bearded seals tested seropositive for PhHV-1 (33.3%) and PDV (100%). All species were seronegative for antibodies to *Leptospira interrogans*, *Toxoplasma gondii*, and *Sarcocystis neurona*. Overall, antibody prevalences for pathogens in these ice seals were fairly low, with the exception of the high proportion of spotted seals that were seropositive for PhHV-1 and high proportions of all three species that were seropositive for PDV, indicating widespread exposure to these two pathogens.



Capture locations of ribbon and spotted seals from the Bering Sea and bearded seals from the Chukchi Sea

Methods

We used a variety of serological tests to determine whether ribbon, spotted, and bearded seals were positive for antibodies to eight pathogens (**Table A**). We calculated antibody prevalences for pathogens in each species (**Figure A**).

We conducted additional testing for *Brucella* spp. following the serological test. PCR was performed on samples with positive and inconclusive titers to detect *Brucella* spp. DNA, and then a species-specific PCR and a multiple-locus variable-number tandem repeat analysis on 15 loci (MLVA-15) were conducted on samples with sufficient *Brucella* DNA to identify how the *Brucella* spp. in the ice seal samples related to known *Brucella* species.

We used a combination of ordinal and logistic regression to analyze prevalence data (**Table A**). We used ordinal regression for pathogens whose serological tests were summarized as nominal responses (e.g., "positive", "inconclusive", or "negative"), and logistic regression for pathogens whose serological tests resulted in binary responses (i.e., "positive" or "negative"). Although seldom used in wildlife epidemiology, we viewed ordinal regression as a more natural framework for exploiting the richer set of nominal responses (as opposed to the usual approach of treating inconclusive test results as if they were negative responses).

We analyzed seal prevalence data as a function of species, age class, and sex, and used AIC to judge whether combinations of these predictors affected prevalence of different pathogens. We also conducted chi-square contingency tests using simpler binary responses to compare our results with previous studies.

Table A. Serologic tests used to determine antibody prevalences for each pathogen and statistical method used to evaluate factors contributing to differences in prevalence levels.

Pathogen	Serologic test	Statistical method
PhHV-1	Indirect enzyme-linked immunosorbent assay (ELISA)	Ordinal regression
Influenza A	Blocking-type ELISA	Logistic regression
Brucella spp.	*Brucella* microagglutination test	Ordinal regression
Coxiella burnetii	Indirect fluorescent antibody test	Logistic regression
Phocine distemper virus	Serum neutralization test	Not analyzed yet
Leptospirosis interrogans serovars	Microscopic agglutination test	N/A*
Toxoplasma gondii	Indirect fluorescent antibody test	N/A
Sarcocystis neurona	Indirect fluorescent antibody test	N/A

*All species were negative for antibodies to *Leptospirosis interrogans, Toxoplasma gondii,* and *Sarcocystis neurona*.

Results

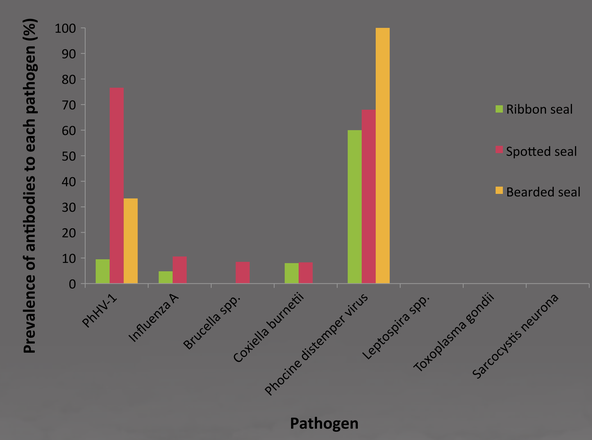


Figure A. Antibody prevalences measured in ribbon and spotted seals, 2007-2010, and in bearded seals, 2009-2012, from the Bering Sea to eight potential pathogens.

Table B. Number of samples positive for antibodies and sample sizes for pathogens tested for each seal species.

Pathogen	Ribbon seals No. positive/No. tested	Spotted seals No. positive/No. tested	Bearded seals No. positive/No. tested
PhHV-1	6/63	36/47	2/6
Influenza A	3/63	5/47	0/6
Brucella spp.	0/57	4/47	0/5
Coxiella burnetii	2/25	2/24	0/4
Phocine distemper virus	22/52	18/41	3/3
Leptospira spp.	0/57	0/47	0/6
Toxoplasma gondii	0/63	0/47	0/6
Sarcocystis neurona	0/63	0/47	0/6

Table C. Observed prevalences of antibodies to PhHV-1, influenza A, and PDV, and sample sizes for ribbon, spotted, and bearded seals, by age class.

	Ribbon seals, % (No. positive/No. tested)			Spotted seals, % (No. positive/No. tested)			Bearded seals, % (No. positive/No. tested)		
Pathogen	YOY	Subadult	Adult	YOY	Subadult	Adult	YOY	Subadult	Adult
PhHV-1	0 (0/19)	6.7 (1/15)	17.2 (5/29)	70.4 (19/27)	78.6 (11/14)	100 (6/6)	(0/0)	20 (1/5)	100 (1/1)
Influenza A	10.5 (2/19)	0 (0/15)	3.4 (1/29)	11.1 (3/27)	0 (0/14)	33.3 (2/6)	(0/0)	0 (0/5)	0 (0/1)
Phocine distemper virus	30 (6/20)	36.4 (4/11)	57.1 (12/21)	27.2 (6/22)	69.2 (9/13)	50 (3/6)	(0/0)	100 (2/2)	100 (1/1)

Table D. Results from logistic or ordinal regression models and from chi-square tests. Model results indicate predictor variables that best described differences in prevalences. Chi-square results indicate variables that produced significantly different prevalences.

Pathogen	Model results	Chi-square results
PhHV-1	Species Slight age effect	Species
Influenza A	Slight age effect	None
Brucella spp.	None	None
Coxiella burnetii	None	None
Phocine distemper virus	Not analyzed yet	Age class

PhHV-1

- Antibody prevalence was much higher in spotted seals than ribbon or bearded seals (**Figure A, Table C, D**).
- The slight age effect was shown by higher prevalences in older age classes for all three species (**Table D**).

Influenza A

- The most highly ranked models indicated a slight age effect and predicted subadults to have lower prevalences than for adults or young-of-the-year (**Table C, D**).
- However, this conclusion should be viewed with caution because observed differences among age classes may just be the result of sparse data (i.e., an intercept-only model was within 2.0 ΔAIC points of the top model.

Phocine distemper virus

- The chi-square test indicated that prevalence was significantly affected by age class (X^2=7.95, df=2, p=0.019) (**Table C, D**). PDV data have not yet been analyzed with logistic regression.

Brucella spp.

- Sample sizes were too small to determine if minor differences in the models were biologically meaningful.

PCR and MLVA-15 results

- 1 ribbon seal and 7 spotted seals were positive for *Brucella* spp. DNA.
- MLVA-15 assays produced a full profile for 1 spotted seal and partial profiles for 1 ribbon and 1 spotted seal.
- Both the MLVA-15 cluster analysis and *Brucella* species-specific PCR concluded that the *Brucella* spp. in these ice seals was more similar to non-marine *Brucella* species, *B. abortus* and *B. ovis*.

Discussion

Our findings indicate that these ice seal species have been exposed to multiple pathogens. However, exposure varied greatly with each pathogen, with high exposure to PhHV-1 and PDV but low or zero antibody prevalences for the other six pathogens. We did not observe any clinical signs of disease.

Using a new method of ordinal regression allowed us to include inconclusive antibody measurements when analyzing the PhHV-1 and *Brucella* data, which increased our sample size for both pathogens, particularly for PhHV-1. In addition to increasing sample size, ordinal regression also incorporates classification uncertainty into the models, providing a more complete analysis.

Low prevalences of antibodies to known zoonoses (*Brucella* spp., influenza A, and *Coxiella burnetii*), were found in ribbon and spotted seals. The *Brucella* spp. DNA found in these ice seals was most closely related to *B. abortus*, which is one of the main human brucellosis pathogens (Franco et al. 2007).

Marine mammal health is closely related to human health in these areas because many Alaska Native communities depend on marine mammals for the subsistence harvest. Although exposure to zoonoses in ribbon and spotted seals was low in this study, further research and monitoring of disease in these populations is needed because knowledge of disease exposure and health of these seals is particularly important for communities in northern Alaska.

References

Franco, M. P., M. Mulder, R. H. Gilman, and H. L. Smits. 2007. Human brucellosis. The Lancet Infectious Diseases 7:775-786.

Acknowledgments

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