Evaluation of viruses and their association with ocular lesions in pinnipeds in rehabilitation

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Abstract

Objective To assess whether corneal lesions in stranded pinnipeds were associated with viral infections, and to identify the potential pathogen(s) associated with the lesions.

Animals studied Twenty-nine California sea lions (*Zalophus californianus*), 18 northern elephant seals (*Mirounga angustirostris*), and 34 Pacific harbor seals (*Phoca vitulina richardsii*).

Procedure DNA and RNA were extracted from ocular swabs, corneal tissue, and aqueous humor and screened for herpesvirus, adenovirus, poxvirus, and calicivirus families by PCR.

Results The results indicated a high overall prevalence of viruses, with adenoviruses and herpesviruses detected in all three host species. Three novel adenoviruses (PhAdV-1, PhAdV-2, OtAdV-2) and two novel herpesviruses (PhHV-6, OtHV-4) were detected. There were no statistical differences in the prevalence of viral infection or coinfection among groups of individuals with or without corneal lesions, nor were lesion type, onset, or presence of concurrent disease significantly associated with a viral infection.

Conclusions The results suggested that viral presence in ocular tissues was common, not significantly associated with ocular disease and thus should not preclude release of an otherwise healthy animal. We could not confirm a correlation of virus presence with lesion due to the high percentage of virus-positive, clinically normal animals. This implied that seals and sea lions can have ocular tissues infected with several viruses without having readily evident associated lesions. This difficulty in correlating viral presence, particularly herpesviruses, with ocular lesions was also a common finding in studies with terrestrial species and highlighted the difficulty of confirming a virus as a primary pathogen in ocular lesions.

Key Words: corneal opacity, keratitis, ocular lesion, sea lion, seal, virus

INTRODUCTION

Ocular lesions are common in captive, stranded, and free-ranging pinnipeds.[1–5] Corneal lesions have been documented in captive and stranded pinnipeds including keratitis, conjunctivitis, corneal opacities, edema, ulceration, infection, inflammation, perforation, and scarring.[1,3,4,6–10] In a recent study, corneal lesions were found to be the most frequently observed ocular lesion in captive and in free-ranging pinnipeds.[5] This is important as corneal disease can cause significant visual obstruction, can lead to corneal perforation, and may also lead to secondary uveitis and subsequent cataract and/or glaucoma formation.[11]

Keratitis and conjunctivitis in terrestrial species have been attributed to a variety of causes, including bacteria and viruses. *Staphylococcus* spp., *Streptococcus* spp., and *Pseudomonas* spp. are common secondary corneal pathogens in domestic dogs, cats, and horses,[12,13] and *Moraxella* sp. is a

primary cause of ulcerative keratitis in cows, sheep, and goats.[14] Adenoviruses, such as canine adenovirus 1, can cause corneal edema in many species of canids, mustelids, and ursids.[15] Several herpesviruses, including herpes simplex virus 1, feline herpesvirus 1, bovine herpesvirus 4, and equine herpesvirus 2, can cause recurrent keratitis, conjunctivitis, and corneal scarring.[16–19] Additionally, feline calicivirus is a common cause of conjunctivitis in cats[20,21] and poxvirus infections have been described in cervids and bovids with keratitis and conjunctivitis.[22–25]

Potential for an infectious etiology of corneal lesions in pinnipeds has not been comprehensively investigated to date. Previous studies have focused on environmental factors such as UV radiation, water quality, and salinity.[4,26] In some facilities, changes to the environment have decreased the severity of ocular problems.[7,8,10] However, as bacteria and viruses are important causes or complications of corneal lesions in other species,[19,27–29] they likely also act as primary and secondary corneal pathogens in pinnipeds. California sea lions (*Zalophus californianus*) with systemic otarine adenovirus 1 infection had concurrent ocular lesions including ulcerative keratitis, corneal edema, iridocyclitis, and conjunctivitis.[30] In another study, ocular samples collected from California sea lions with necrotizing keratitis tested positive for a previously unknown herpesvirus.[9] Furthermore, both gram-negative and gram-positive bacteria have been cultured from ocular samples collected from pinnipeds with keratitis, corneal ulceration, and conjunctivitis, including *Pseudomonas* spp., *Escherichia coli*, *Proteus* spp., *Morganella morganii*, *Streptococcus viridans*, and *Staphylococcus aureus*.[31–33] However, none of these studies included control comparisons where samples from clinically normal eyes were also evaluated; thus, further examination of the association between the presence of infectious organisms and corneal lesions was needed.

The purpose of this study was to assess whether corneal lesions in stranded pinnipeds were associated with viral infections and to identify the potential pathogen(s) associated with the lesions. Ocular swabs, corneal tissue, and aqueous humor were collected from California sea lions, northern elephant seals (*Mirounga angustirostris*), and Pacific harbor seals (*Phoca vitulina richardsii*) with and without corneal lesions and examined for the presence of adeno, herpes, pox, and caliciviruses as species from these viral families have been previously documented in pinnipeds[30,34–36] and have been associated with ocular lesions in other species.[14,15,17,20,22–25,28]

MATERIALS AND METHODS

Animals and samples

Ocular swabs were collected using sterile rayon-tipped swabs (MicroPur; PurFybr, Inc., Munster, IN, USA) from 29 California sea lions (14 juvenile, 15 adult; 9 male, 20 female), 18 northern elephant seals (all weaned pups; 10 male, 8 female), and 34 Pacific harbor seals (all pups; 18 male, 16 female) that stranded along the California coast from Mendocino to San Luis Obispo and were admitted for rehabilitation to The Marine Mammal Center in Sausalito, California, from June 2008 to November 2009. Sex was determined by genital morphology. Age groups were defined as pup (<1 year), juvenile (1–4 years), and adult (>4 years). The reasons for stranding varied from trauma, malnutrition, domoic acid toxicosis, maternal separation, and leptospirosis to ophthalmic conditions such as a painful eye and trauma.[37] All animals received a physical examination performed by a veterinarian upon admission. The two study groups included (i) animals with clinically apparent ocular lesions at admission as well as animals that developed ocular lesions during rehabilitation and (ii) animals that died during the study period but had normal ophthalmic examinations as controls. If ocular lesions were present at admission, samples were collected during the admission examination to determine what infections were present in animals upon entry to the rehabilitation center; if an animal developed ocular lesions while at the facility, samples were collected at the time lesions were clinically evident. Corneal tissue, aqueous humor samples, and ocular swabs were obtained upon postmortem examination from controls and animals with ocular lesions that died during rehabilitation. All samples were immediately placed into sterile cryovials and frozen at −70 °C until processed for analysis.

Upon admission, animals were housed alone prior to sample collection at admission examinations, while those that developed lesions during rehabilitation were housed in pens with pools (pools averaged 8 feet in diameter by 4–6 feet in depth; groups of three to six animals). Cement walls (up to 90 cm in height) between pens reduced contact and cross-contamination among animals housed in adjacent pens. All pools were maintained on a closed salt water system with an average turnover rate of 45 min per pool, and water was maintained between 59 and 67 °F, pH 8.1 and 8.4, and a salinity of 20 and 25 ppt (but up to 32 ppt as requested by a veterinarian for treatment of clinical conditions requiring increased salinity). Ozone concentrations were monitored continually and adjusted by administering ozone gas for use as an oxidizer as water conditions required. The primary mechanical filtration system used pressurized sand filters with protein fractionation for removal of nonsoluble oils. Pens were cleaned twice daily with either 10% Nolvasan solution or 10% bleach solution. Animals were moved out of pens when they were cleaned with bleach, but to reduce handling animals remained on the pen floor when pens were cleaned with Nolvasan, but were moved away from areas being disinfected. Bleach was used in footbaths at all pen entrances and to clean hallways between pens. All volunteers and staff wore gloves, protective slickers, and waterproof boots, which were cleaned with bleach after leaving each pen. Utensils, carriers, nets, and all other equipment were disinfected with Nolvasan or bleach after each use.

Clinical categorizations

Once included in the study, each animal received a complete ophthalmic adnexal and corneal evaluation performed by a veterinarian that included forms for standardization of sampling and lesion evaluation. The ophthalmic examination had categories for describing the appearance of the eyelid, conjunctiva, sclera, corneal surface texture, the presence of blepharospasm, corneal opacity, or discharge, and whether the animal behaved as if it could see. Further details of an intraocular examination were not included on the exam form as intraocular examination was very limited due to intense miosis of all patients as is typical of pinnipeds in ambient light. Clinical, diagnostic, and histopathologic (if applicable) information was obtained from animal health records retrospectively. Categorization of the data included determining the lesion type of four ocular lesion types, whether the lesions were active or inactive (such as a scar), the disposition of the animal, and whether there was an underlying concurrent disease. The four ocular lesion types were as follows: keratoconjunctivitis (including keratitis, conjunctivitis, or both), intraocular, probable traumatic, and controls. The posterior segment was not routinely evaluated and therefore excluded from the categories. The broad categories were selected based on common viral lesions in terrestrial species (i.e., keratoconjunctivitis for herpesviruses, uveitis/bilateral nonulcerative corneal edema for adenoviruses) and the information that was present in the records. Trauma was often witnessed or determined by secondary lesions such as perforations, fight wounds, or gunshot. The authors (LW, KF) that reviewed records were masked to results of the viral testing.

Bacterial culture and cytology

Conjunctival swabs were collected for bacterial culture from seven Pacific harbor seals with ocular lesions from the inferior conjunctival palpebral fornix using a dry sterile cotton-tipped applicator, without prior application of proparacaine, and maintained under refrigeration for 12–24 h in Amies transport media until submission for aerobic culture and sensitivity at the UC Davis Microbiology Laboratory. Bacterial culture and sensitivity were reviewed at 24 and 48 h, and the presence and amount of bacteria was recorded; all processing was performed as per standard protocol at UC Davis Microbiology Laboratory. Conjunctival cytology was collected from one Pacific harbor seal with ocular lesions from the inferior conjunctival palpebral fornix using a Dacron swab (Becton, Dickinson and Company, East Rutherford, NJ, USA), without prior application of proparacaine. The slide was stained with Giemsa stain and examined at The Marine Mammal Center under light microscopy.

Histopathology

If an animal in the study died, both eyes were removed within 6 h of death, placed in 10% neutral-buffered formalin, and submitted for histopathology to the Comparative Ocular Pathology Laboratory of Wisconsin or the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, at the University of Illinois. Tissues from six California sea lions, one northern elephant seal, and six Pacific harbor seals were included for histopathologic evaluation.

Molecular analysis

DNA and RNA were extracted from ocular swabs using the DNeasy Blood and Tissue kit (Qiagen Inc., Valencia, CA, USA) and TRIzol reagent (Invitrogen Corp., Carlsbad, CA, USA), and from cornea and aqueous humor samples using the QiAmp DNA mini kit and RNeasy micro kit (Qiagen Inc.). cDNA was transcribed from extracted RNA using the Superscript III First Strand kit (Invitrogen Corp.). All extracted DNA and RNA samples were screened for selected viral families including herpes, adeno, pox, and caliciviruses by polymerase chain reaction (PCR).[30,34,35,38–42] Ferritin and β-actin primers were used to test the quality of the DNA and RNA. Results were visualized on a 1.5% agarose gel, and bands of correct size were excised and purified using the Qiaquick kit (Qiagen Inc.). Purified PCR products were cloned (pCR4-TOPO vector; Invitrogen Corp.) and sequenced (ABI 3730 Capillary Electrophoresis Genetic Analyzer; Applied Biosystems, Inc., Foster City, CA, USA).

Sequences were identified and confirmed using the nucleotide and translated nucleotide BLAST search tools within the National Center for Biotechnology Information (NCBI) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Plasmid and primer sequences were removed, and chromatograms were analyzed for ambiguities using GENEIOUS PRO v5.3.6 software (Biomatters Ltd., Auckland, New Zealand). Nucleotide sequences were aligned using the CLUSTALW plugin in Geneious Pro (Biomatters Ltd.), and consensus sequences were generated. Nucleotide sequences were obtained from the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide) for phylogenetic analysis, and predicted amino acid sequences were derived from the nucleotide sequences using Geneious Pro (Biomatters Ltd.). Novel viral fragments were named in accordance with the conventional nomenclature set out by the International Committee on Taxonomy of Viruses (ICTV) for each virus family. Alignments were performed using the CLUSTALW plugin in Geneious Pro (Biomatters Ltd.) for adeno and herpesviral sequences using partial predicted amino acid fragments of the DNA polymerase gene, and for calicivirus sequences using homologous nucleic acid fragments of the capsid coding region A. Caliciviruses were aligned using nucleic acid sequences due to the high similarity between viral fragments. Pairwise alignments for poxviruses were performed to confirm sequence identity.

Phylogenetic analysis

Adenovirus phylogenetic analysis was performed using PHYLIP v3.69.[43] Distances were measured with PROTDIST

using the Categories model, and trees were constructed using Fitch with global rearrangements. Seqboot generated 1000 resamplings of the data for bootstrap analysis, and a consensus tree was produced using the CONSENSE program. Bayesian analyses were performed for the herpesvirus and calicivirus multiple alignments using MRBAYES v3.1.2[44] with mixed amino acid substitution models for the herpesvirus alignment and mixed nucleic acid substitution models for the calicivirus alignment. Both analyses had gamma distributed rate variation and a proportion of invariant sites. Four heated chains with a chain length of 1 000 000 for the herpesvirus analysis, and 2 000 000 for the calicivirus analysis, were run with a subsampling frequency of 100 and 200, respectively. The first 10% were discarded as burn-in. All trees were unrooted and visualized using the program FIGTREE v1.3.1 (Institute of Evolutionary Biology, University of Edinburgh [http://tree.bio.ed.ac.uk]).

Statistical analysis

Multiple variables were identified and evaluated for association with ocular disease using chi-square tests of independence or Fisher's exact tests. The variables included the following: species, sex, age class (if different age classes were present), acquisition of the lesion during rehabilitation, concurrent or underlying systemic disease, and virus (es) presence. Additionally, analyses were performed to evaluate for associations between virus-positive samples and all other variables. Associations between virus-positive samples and species, sex, age class (if different age classes were present), presence of ocular lesion, lesion type, lesion onset (lesion present upon admission or acquired during rehabilitation), and concurrent disease were assessed using the chi-square test of independence or Fisher's exact test. Logistic regression was used to test for differences in prevalence of viral infection between species while adjusting for the confounding effects of age.

Differences in disposition for individuals with and without ocular lesions and by viral infection status (overall and for each pathogen separately) were evaluated using the chi-square test of independence. For this assessment, the categories for death and euthanasia were combined for comparison to the category for release back to the wild. Logistic regression was used to assess for differences in the odds of release, adjusted for concurrent disease, for individuals with and without lesions. Ninety-five percent confidence intervals were estimated for select prevalence estimates, and a level of significance of 0.05 was used for all statistical tests. Statistical analyses were performed using R statistical software.[45]

RESULTS

Clinical categories

The study included 51 individuals with ocular lesions and 30 individuals with clinically normal eyes: 62% (18/29) of the California sea lions, 61% (11/18) of the northern elephant seals, and 65% (22/34) of the Pacific harbor seals had ocular lesions (Table 1). Thirty-five percent (28/81) of animals in the study had ocular lesions and were sampled upon admission, while the rest developed lesions while in the rehabilitation facility. Of all animals admitted for rehabilitation over the study period (June 2008 to November 2009), 1.4% (28/2047) were admitted with ocular lesions, vs. 1.1% (23/2047) that developed ocular lesions at some point during rehabilitation.

Of 18 California sea lions with ocular lesions, most individuals (72%, 13/18) had lesions due to keratoconjunctivitis, and fewer had intraocular (6%, 1/18) or probable traumatic (22%, 4/18) lesions. Eighty-three percent (15/18) of the lesions in California sea lions were characterized as active lesions. All intraocular (100%, 1/1) and probable traumatic (100%, 4/4), and most of the keratoconjunctivitis lesions (77%, 10/13) were active.

Of 11 northern elephant seals with ocular lesions, most individuals had lesions due to keratoconjunctivitis (82%, 9/11). A single individual had an intraocular lesion (9%, 1/11), and another had a probable traumatic lesion (9%, 1/11). All intraocular (100%, 1/1) and probable traumatic (100%, 1/1) lesions and most keratoconjunctivitis lesions (89%, 8/9) were characterized as active lesions.

Most Pacific harbor seals had ocular lesions associated with keratoconjunctivitis (73%, 16/22) as opposed to intraocular (18%, 4/22) or probable traumatic (9%, 2/22) lesions. All intraocular (100%, 4/4) and probable traumatic (100%, 2/2) lesions and most keratoconjunctivitis (94%, 15/16) were characterized as active lesions.

A significantly greater proportion of Pacific harbor seals (77%, 17/22) acquired lesions during the rehabilitation process compared to northern elephant seals (27%, 3/11) and California sea lions (17%, 3/18) ($P < 0.01$ and $P < 0.001$, respectively). Fifty-three percent (27/51) of the individuals with ocular lesions were released back to the wild.

Concurrent disease

Concurrent diseases in animals with and without ocular lesions included domoic acid toxicosis (11), septicemia (6), omphalophlebitis (5), malnutrition (8), enteritis (5), pneumonia (11), pulmonary congestion and edema (2), verminous pneumonia (5), meningitis (2), central neurological disease (4), dermatitis (4), otitis (3), trauma (6), abscess (2), renal disease (3), urinary tract infection, epididymitis, cholecystitis, myocardial edema, herpesvirus, head trauma, congenital cardiac defect, heart disease, DIC, dental disease, gastric ulcer, seizure, neoplasia, and leptospirosis. Some animals had no concurrent disease, and some had multiple. Concurrent disease was significantly less prevalent in individuals with ocular lesions ($P = 0.001$), and a higher proportion of individuals with concurrent disease either died or were euthanized compared to individuals without concurrent disease ($P < 0.001$). The odds of

Table 1. Prevalence of known and novel herpesviruses, adenoviruses, poxviruses, and caliciviruses detected by PCR in ocular swabs, corneal tissue, and aqueous humor collected from California sea lions, northern elephant seals, and Pacific harbor seals with and without ocular lesions admitted to The Marine Mammal Center, Sausalito, CA from 2008 to 2009

	California sea lion			Northern elephant seal			Pacific harbor seal		
Virus	Total (n = 29)	Lesion (n = 18)	No Lesion (n = 11)	Total (n = 18)	Lesion (n = 11)	No Lesion (n = 7)	Total (n = 34)	Lesion (n = 22)	No Lesion (n = 12)
Otariid herpesvirus 1	0% 0/29	0% 0/18	0% 0/11	–	–	–	–	–	–
Otariid herpesvirus 2	72.4% 21/29	77.8% 14/18	63.6% 7/11	–	–	–	–	–	–
Otariid herpesvirus 4	3.4% 1/29	5.6% 1/18	0% 0/11	–	–	–	–	–	–
Phocid herpesvirus 1	–	–	–	–	–	–	20.6% 7/34	13.6% 3/22	33.3% 4/12
Phocid herpesvirus 2	–	–	–	–	–	–	2.9% 1/34	0% 0/22	8.3% 1/12
Phocid herpesvirus 3	–	–	–	66.7% 12/18	63.6% 7/11	71.4% 5/7	–	–	–
Phocid herpesvirus 6*	–	–	–	16.7% 3/18	18.2% 2/11	14.3% 1/7	2.9% 1/34	4.5% 1/22	0% 0/12
Otarine adenovirus 1	3.4% 1/29	0% 0/18	9.1% 1/11	–	–	–	–	–	–
Otarine adenovirus 2	3.4% 1/29	0% 0/18	9.1% 1/11	–	–	–	–	–	–
Phocine adenovirus 1	–	–	–	55.6% 10/18	63.6% 7/11	42.8% 3/7	–	–	–
Phocine adenovirus 2	–	–	–	–	–	–	8.8% 3/34	9.1% 2/22	8.3% 1/12
Poxvirus	0% 0/29	0% 0/18	0% 0/11	0% 0/18	0% 0/11	0% 0/7	–	–	–
Harbor seal parapoxvirus 2	–	–	–	–	–	–	8.8% 3/34	9.1% 2/22	8.3% 1/12
Calicivirus	–	–	–	0% 0/18	0% 0/11	0% 0/7	0% 0/34	0% 0/22	0% 0/12
San Miguel sea lion virus	6.9% 2/29	0% 0/18	18.2% 2/11	–	–	–	–	–	–

Novel viruses are in bold.
*Identical in northern elephant seals and Pacific harbor seals.

release for individuals with ocular lesions compared to individuals without lesions were not significant after adjusting for concurrent disease. The majority of animals in the study were treated with antibiotics. Sixty-seven percent (20/30) of control animals were treated with systemic antibiotics for concurrent disease. Of those with ocular lesions, 43% (22/51) were treated with systemic antibiotics and 41% (21/51) were treated with systemic and topical antibiotics. Topical antibiotics were administered three or four times per day under manual restraint when animals were being handled for other purposes (e.g., tube feeding, examinations), and included gentamicin ophthalmic solution, ofloxacin ophthalmic solution, and neomycin polymyxin bacitracin ophthalmic ointment. Systemic antibiotics administered either orally or via intramuscular injection to treat ocular lesions included enrofloxacin and penicillin.

Bacterial culture and cytology

Conjunctival swabs were submitted for bacterial culture from seven Pacific harbor seals.

Organisms grown on conjunctival culture included *Pseudomonas aeruginosa* (4/7), *E. coli* sp. (3/7), nonhemolytic *Streptococcus* sp. (2/7), hemolytic *Streptococcus* sp. (1/7), *Moraxella*-like sp. (1/7), *Klebsiella pneumoniae* (1/7), *Corynebacterium* sp. (1/7), *Psychrobacter phenylpyruvica* (2/7), *Bisgaardia hudsonensis* (1/7), and *M. morganii* (1/7). Cytology in the one Pacific harbor seal indicated the presence of a yeast infection.

Histopathology

Histopathologic examinations were performed on eight animals with ocular lesions and three controls. Six of the affected animals were Pacific harbor seals, three of which had chronic neutrophilic ulcerative keratitis with necrotic stroma and secondary anterior uveitis and anterior synechia. No bacteria were identified associated with the ulceration in any of these animals, but all animals had received topical antibiotics. One Pacific harbor seal with significant trauma to the eye had lesions consistent with a penetrating injury. Two Pacific harbor seals had healed inactive

corneal ulcers. One California sea lion had unilateral *phthisis bulbi* secondary to a suspect blunt trauma, and one northern elephant seal had a chronic healed corneal ulcer with stromal loss and fibrosis.

Molecular analysis

Virus presence, as detected by PCR, in ocular samples from animals with and without lesions included 47 (58%, 95% CI: 47–68%) individuals that tested positive for at least one virus (Table 1). The overall prevalence of viral infection was 79% (23/29, 95% CI: 62–91%) in California sea lions, 78% (14/18, 95% CI: 55–93%) in northern elephant seals, and 29% (10/34, 95% CI: 16–46%) in Pacific harbor seals. Viral infection was more prevalent in the adult ($P < 0.01$) and juvenile ($P < 0.01$) age classes compared to pups. Species differences in prevalence were not significant after adjusting for the effects of age class. Nineteen individuals (23%, 19/81) tested positive for at least one virus upon admission, and an additional 28 individuals (35%, 28/81) tested positive during rehabilitation. Of those that tested positive, about half of the California sea lions (52%, 12/23) and northern elephant seals (43%, 6/14) tested positive upon admission, while the majority of Pacific harbor seals (90%, 9/10) tested positive during rehabilitation. Viral coinfection was present in 20% (16/81, 95% CI: 12–30%) of individuals and was more prevalent in northern elephant seals (50%, 9/18, 95% CI: 28–72%) compared to California sea lions (14%, 4/29, 95% CI: 5–30%) and Pacific harbor seals (9%, 3/34, 95% CI: 3–22%) ($P < 0.05$ and $P < 0.01$). There were no significant differences in prevalence of viral infection or coinfection between individuals with and without corneal lesions, by lesion type or onset, or presence of concurrent disease.

Adenoviral DNA was detected in ocular samples from all three species, although a higher prevalence was detected in northern elephant seals (56%, 10/18) compared to California sea lions (7%, 2/29) ($P < 0.001$) and Pacific harbor seals (9%, 3/34) ($P < 0.001$) (Table 1, Fig. 1). Otarine adenovirus 1 (OtAdV-1; GU979536) and a novel California sea lion adenovirus, otarine adenovirus 2 (OtAdV-2; JX244189), were each detected in one individual (each 3.4%, Table 1) both without ocular lesions, from an ocular swab and corneal tissue, respectively. BLAST analysis indicated that OtAdV-2 was distinct from the recently identified OtAdV-1 in California sea lions[30] with 71% amino acid identity, and most closely related to tree shrew adenovirus 1 (AF258784) and alpaca adenovirus 1 (AlAdV-1; FJ711592), each with 73% amino acid identity. OtAdV-2 also shared 73% amino acids with another novel adenovirus detected in this study, from northern elephant seals, phocine adenovirus 1 (PhAdV-1; JX244191).

PhAdV-1 was detected in 56% of northern elephant seals, in 64% and 43% of individuals with and without ocular lesions, respectively (Table 1, Fig. 1). PhAdV-1 viral DNA was detected in ocular swabs and three corneal tissue samples. BLAST analysis indicated that PhAdV-1 was most closely related to both canine adenovirus 1 (CAU55001) and common noctule adenovirus (GU198877) with 76% amino acid identity. However, pairwise comparisons showed the fragment was most similar to the novel Pacific harbor seal adenovirus detected in this study, phocine adenovirus 2 (PhAdV-2; JX244192), with 83% amino acid identity. PhAdV-2 was detected in 9% of Pacific harbor seals from all three sample types, in 9% and 8% of individuals with and without ocular lesions, respectively (Table 1, Fig. 1). Phylogenetic analysis confirmed the classification of the novel adenoviruses within the *Mastadenovirus* genus (Fig. 2). OtAdV-2 remained equally distant from the other *Mastadenovirus* species (immediate neighbors included AlAdV-1 and Bovine adenovirus 3 [AF061654]). PhAdV-1 and PhAdV-2 formed a monophyletic group within the tree (nearest neighbors included TsAdV-1, Phocoena adenovirus 1 [JN377908], and OtAdV-1).

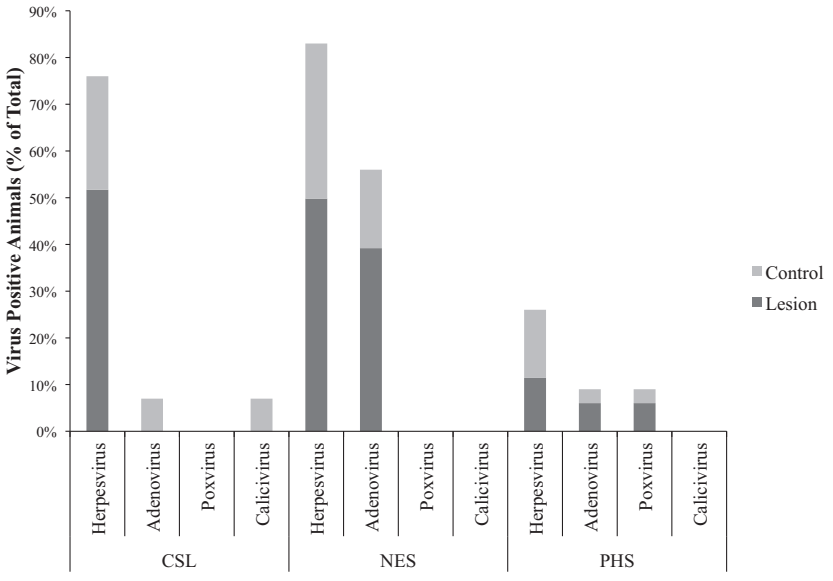

Figure 1. The prevalence of viruses detected in each host species, including both individuals with ocular lesions and controls, in California sea lions (CSL), northern elephant seals (NES), and Pacific harbor seals (PHS). The *x*-axis is pinniped species, and the *y*-axis is percentage of viral positive animals of all the animals of that species that were sampled.

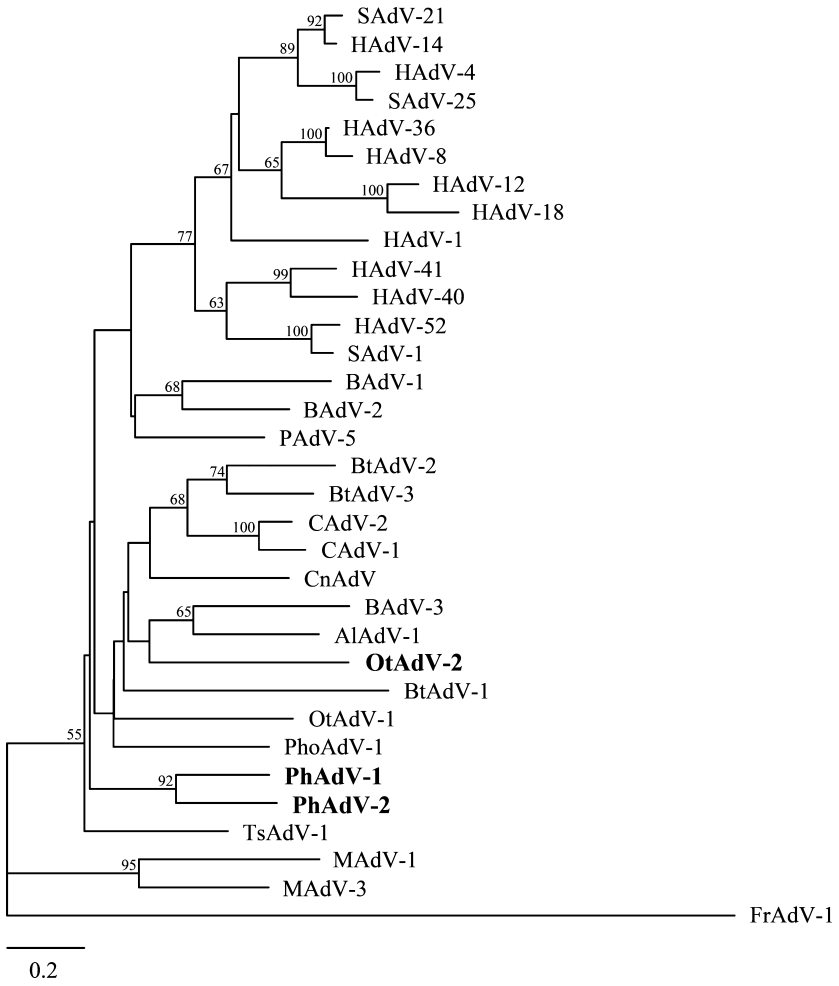

Figure 2. Phylogenetic analysis of 33 partial adenovirus sequences. The phylogenetic tree was constructed based on an alignment of adenovirus fragments 90 amino acids in length that were derived from 272 nucleotides of the DNA polymerase gene. Novel sequences from this study are bolded. Corresponding bootstrap values above 50% are shown, based on 1000 resamplings of the data. The scale bar indicates evolutionary distances based on 0.2 substitutions per position. Sequences obtained from GenBank include the following: Alpaca AdV (AAdV; FJ711592), Bovine AdV 1 (BAdV-1; NC_006324), BAdV-2 (AF252854), BAdV-3 (AF061654), Bat AdV 1 (BtAdV-1; AB303301), BtAdV-2 (FJ983127), BtAdV-3 (GU226970), Canine AdV 1 (CAdV-1; CAU55001), CAdV-2 (CAU77082), Common noctule AdV (CnAdV; GU198877), Human AdV 1 (HAdV-1; AF534906), HAdV-4 (AY458656), HAdV-8 (AB448769), HAdV-12 (M14785), HAdV-14 (AY803294), HAdV-18 (GU191019), HAdV-36 (GQ384080), HAdV-40 (NC_001454), HAdV-41 (DQ315364), HAdV-52 (DQ923122), Murine AdV 1 (MAdV-1; AC_000012), MAdV-3 (EU835513), Otarine AdV 1 (OtAdV-1; GU979536), Porcine AdV 5 (PAdV-5; AF289262), Phocoena AdV 1 (PhoAdV-1; JN377908), Simian AdV 1 (SAdV-1; AY771780), SAdV-21 (AC000010), SAdV-25 (AF394196), Tree shrew AdV 1 (TsAdV-1; AF258784). Frog AdV 1 (FrAdV-1; AF224336) was designated as the outgroup.

Herpesviral DNA was detected in ocular samples from all species, with a higher prevalence in California sea lions (76%, 22/29) ($P < 0.001$) and northern elephant seals (72%, 13/18) ($P < 0.001$) compared to Pacific harbor seals (26%, 9/34) (Fig. 1). Herpesviral infection was more prevalent in the adult ($P < 0.01$) and juvenile ($P < 0.01$) age classes compared to pups. Otariid herpesvirus 1 (OtHV-1; AF193617) was not detected in any California sea lions. Otariid herpesvirus 2 (OtHV-2; GQ429148) was detected in 72% of California sea lions, in 78% and 64% of individuals with and without ocular lesions (Table 1). OtHV-2 DNA was amplified in ocular swabs from all but one individual, corneal tissue in six, and aqueous humor in two. A novel California sea lion herpesvirus, Otariid herpesvirus 4 (OtHV-4; JX244190), was detected in an ocular swab from one California sea lion (3%) with ocular lesions (6%, Table 1) and was most closely related to phocid herpesvirus 3 (PhHV-3; DQ093191) and phocid herpesvirus 4 (PhHV-4; DQ183057) with 59% and 62% amino acid identity.

Phocid herpesvirus 1 (PhHV-1; U92269) was detected in 21% of Pacific harbor seals from all three sample types, in 14% and 33% of individuals with and without ocular lesions (Table 1). Phocid herpesvirus 2 (GQ429152) was amplified from an ocular swab from one Pacific harbor seal (3%) without ocular lesions (8%, Table 1). PhHV-3 was detected in 67% of northern elephant seals, in 64% and 71% of individuals with and without ocular lesions (Table 1). PhHV-3 DNA was amplified from ocular swabs and two corneal tissue samples. A novel herpesvirus, phocid herpesvirus 6 (PhHV-6; JX244194), was detected in ocular swabs from three (17%) northern elephant seals in 18% and 14% of individuals with and without ocular lesions (Table 1). PhHV-6 was also detected in one (3%) Pacific harbor seal with ocular lesions (5%, Table 1). BLAST analysis indicated that PhHV-6 was most closely related to phocid herpesvirus 5 (PhHV-5; GQ429153) with 80% amino acid identity.

Bayesian phylogenetic analysis of the herpesviral sequences confirmed the classification of both novel viruses within the Gammaherpesvirus subfamily (Fig. 3). OtHV-4 clustered with PhHV-3 and PhHV-4 found in northern elephant seals and Hawaiian monk seals. PhHV-6 clustered with PhHV-5, a virus found in Pacific harbor seals and classified as a *Macavirus*.[46]

Pinniped parapoxviruses were not detected in California sea lions and northern elephant seals. Ocular swabs from three (9%) Pacific harbor seals tested positive for harbor

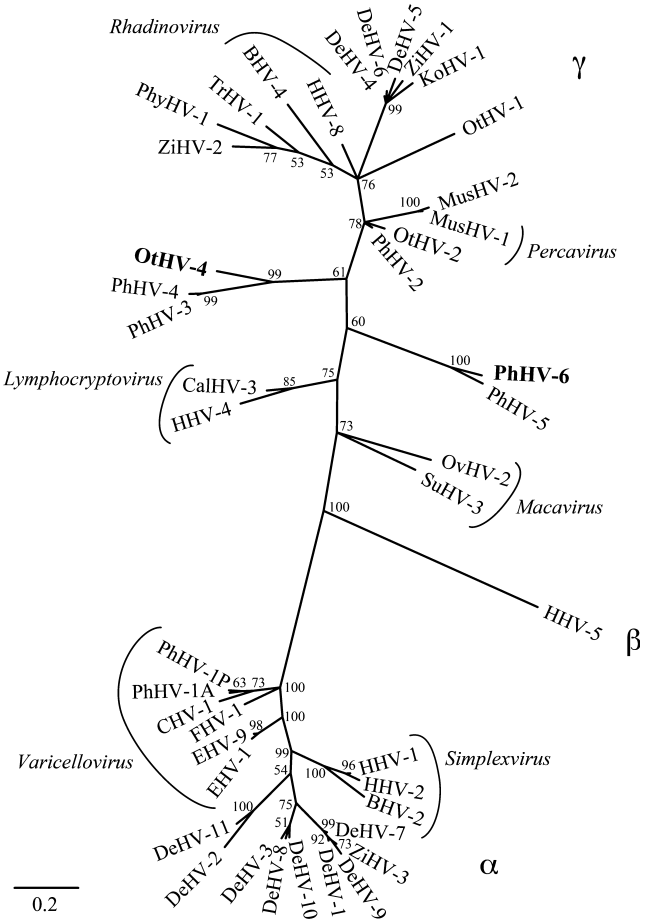

Figure 3. Phylogenetic analysis of 43 partial herpesvirus sequences. The phylogenetic tree was constructed based on an alignment of marine mammal and representative herpesvirus fragments ranging from 54 to 64 amino acids in length derived from 163 to 192 nucleotides of the DNA polymerase gene. Novel sequences from this study are bolded. Posterior probabilities for branching patterns are shown as percentages. The scale bar indicates an evolutionary distance of 0.2 substitutions per amino acid position. Sequences obtained from GenBank include the following: Bovine HV 2 (BHV-2; AF181249), BHV-4 (AF318573), Callitichine HV 3 (CalHV-3; AF091058), Canine HV 1 (CHV-1; AY949827), Delphinid HV 1 (DeHV-1; AF196646), DeHV-2 (GQ429149), DeHV-3 (AY757301), DeHV-4 (AY952777), DeHV-5 (AY952776), DeHV-6 (DQ288666), DeHV-7 (AY608707), DeHV-8 (GQ429250), DeHV-9 (GQ429151), DeHV-10 (AB510473), DeHV-11 (AB510474), Equid HV 1 (EHV-1; NC_001491), EHV-9 (NC_011644), Feline HV 1 (FHV-1; AJ224971), Human HV 1 (HHV-1; X14112), HHV-2 (Z86099), HHV-4 (DQ279927), HHV-8 (U93872), Kogiid HV 1 (KoHV-1; AY949830), Mustelid HV 1 (MusHV-1; GU799569), MusHV-2 (GU979535), Otariid HV 1 (OtHV-1; AF193617), OtHV-2 (GQ429148), Ovine HV 2 (OHV-2; DQ198083), Phocid HV 1 Atlantic isolate (PhHV-1A; T. Goldstein, personal communication), PhHV-1P (Pacific isolate; PHU92269), PhHV-2 (GQ429152), PhHV-3 (DQ093191), PhHV-4 (DQ183057), PhHV-5 (GQ429153), Physeterid HV 1 (PhyHV-1; AB510475), Suid HV 3 (SuHV-3; AF478169), Trichechid HV 1 (TrHV-1; DQ238847), Ziphid HV 1 (ZiHV-1; AY949828), ZiHV-2 (AB510476), and ZiHV-3 (GU066291). Human HV 5 (HHV-5; AY304063) was designated as the outgroup.

seal parapoxvirus 2 (DQ273136), in 9% and 8% of individuals with and without ocular lesions (Table 1).

Caliciviruses were not detected in northern elephant seals or Pacific harbor seals. Ocular swabs and one corneal tissue from two (7%) California sea lions without ocular lesions (18%) tested positive for a San Miguel sea lion virus of unknown serotype (Table 1). The viral fragment, designated isolate 9221 (SMSV isolate 9221; JX244193), was most closely related to Walrus calicivirus (WCV; AF321298) with 95% amino acid identity and San Miguel sea lion virus serotype 2 (DQ666629) with 92% amino acid identity. Phylogenetic analysis confirmed the classification of this viral fragment as a San Miguel sea lion virus, within the genus *Vesivirus*, and showed that the virus formed a monophyletic group with WCV, vesicular exanthema of swine virus serotype B (DQ666632), rabbit vesivirus (AJ866991), and San Miguel sea lion virus serotype 7 (SMU76887) (Fig. 4).

When evaluating viral infection among the categories of ocular lesions, California sea lions with keratoconjunctivitis were slightly more likely to test positive for a herpesvirus (85%, 11/13) than the control animals (64%, 7/11), although this difference was not significant. No California sea lions with ocular lesions tested positive for a virus other than herpesviruses. Two control California sea lions tested positive for adenovirus, and two were positive for calicivirus. There were high percentages of herpesvirus- and adenovirus-positive northern elephant seals including animals with keratoconjunctivitis (78%, 7/9; 67%, 6/9, respectively) and controls (71%, 5/7; 43% 3/7, respectively). No difference was found between the prevalence of herpesvirus in Pacific harbor seals with keratoconjunctivitis (25%, 4/16) compared to controls (33%, 4/12).

DISCUSSION

Results from this study showed no significant differences in the prevalence of viral infection or coinfection between groups of individuals with or without corneal lesions, and no association of infection with lesion type or onset, or presence of concurrent disease. Nonetheless, the results indicated a high overall prevalence of viruses and suggested that viral presence in ocular tissues in these species was common. The species differences were notable as northern elephant seals and California sea lions had a very high percentage of herpes-positive animals (over 70%), whereas <30% of Pacific harbor seals were positive.

Interestingly, PhHV-1, adenoviruses in California sea lions, and caliciviruses were more common, although not statistically significant, in animals without ocular lesions compared to animals of the same species with ocular lesions. This is an important result for managers screening animals for infectious diseases to make animal disposition decisions. These results implied that the seals and sea lions had ocular tissues infected with several viruses with and without having lesions.

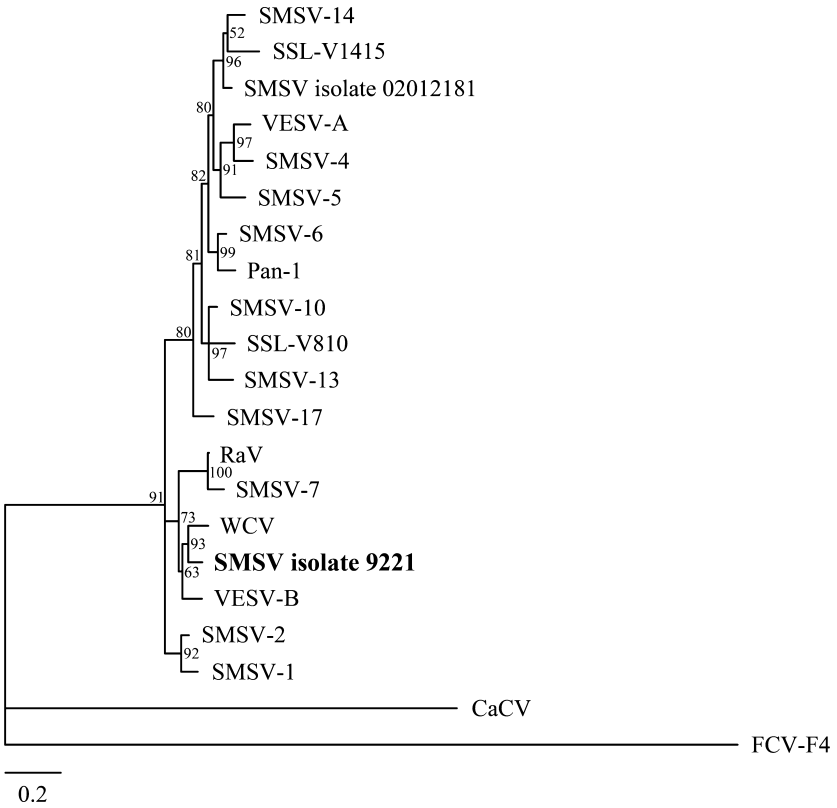

Figure 4. Phylogenetic analysis of 21 partial calicivirus sequences. The phylogenetic tree was constructed using an alignment based on a 513-nucleotide fragment of the capsid protein gene region A of the San Miguel sea lion virus serotypes available in GenBank and corresponding regions of other representative vesiviruses. The sequence identified in this study is bolded. The scale bar indicates evolutionary distances based on 0.2-nucleotide substitutions per position. Sequences obtained from GenBank include the following: Canine calicivirus (CaCV; AF053720), Rabbit vesivirus (RaV; AJ866991), San Miguel sea lion virus serotype 1 (SMSV-1; U15301), SMSV-2 (DQ666630), SMSV-4 (DQ666630), SMSV-5 (U76883), SMSV-6 (U76885), SMSV-7 (U76887), SMSV-10 (DQ666631), SMSV-13 (DQ666636), SMSV-14 (U76879), SMSV-17 (U52005), SMSV isolate 02012181 (DQ666635), Steller sea lion vesivirus V810 (SSLV-V810; EF193004), SSLV-V1415 (EF195384), Vesicular exanthema of swine virus serotype A48 (VESV-A; U76874), VESV serotype B51 (VESV-B; DQ666632), VESV-like calicivirus strain Pan-1 (Pan-1; AF091736), and Walrus calicivirus (WCV; AF321298). Feline calicivirus serotype F4 (FCV-F4; D31836) was designated as the outgroup.

Coinfection with two or more viruses was most prevalent in northern elephant seals and did not correlate with presence of a lesion, type, onset, or presence of concurrent disease. Coinfection in other species is often thought to be associated with immunosuppression, but there was no clinical evidence of immune suppression in these elephant seals. This species does have a low degree of genetic diversity, which could contribute to species differences in viral recognition and susceptibility to infection.

Despite the lack of association between ophthalmic lesions and adenovirus infection, one known and three novel adenoviruses were detected, including the first identified in northern elephant seals and Pacific harbor seals. All three novel adenoviruses were classified within the *Mastadenovirus* genus, which includes other mammalian adenoviruses.[47] PhAdV-1 from northern elephant seals and PhAdV-2 from Pacific harbor seals were more closely related to each other compared with known *Mastadenoviruses*, including OtAdV-1, a previously identified California sea lion adenovirus.[30] OtAdV-2 was equally distant from all *Mastadenovirus* species, which confirmed that the fragment was novel and also distinct from OtAdV-1.

Adenoviruses have recently been identified and characterized in pinnipeds, and the pathogenicity and prevalence in free-ranging populations are unknown. Adenoviruses are known to cause a range of lesions,[15] such as corneal damage and anterior uveitis in approximately 20% of dogs recovering from natural infection with canine adenovirus 1 (CAdV-1) and occasionally in dogs after immunization.[48] In a study where a novel adenovirus was isolated from two California sea lions and associated with hepatitis and endothelial cell infection, the ocular lesions of one of the two sea lions were consistent with lesions seen in dogs naturally infected with CAdV-1.[30]

Results indicated that herpesviruses were the most common virus present among all three pinniped species and that keratoconjunctivitis was the most common lesion present. Various herpesviruses in terrestrial species are associated with keratoconjunctivitis. Feline herpesvirus 1 ulcerates corneal and conjunctival epithelium and is a common cause of feline ocular disease.[49–51] Children and puppies can also develop keratitis during infection with herpesvirus.[51–53] Two novel herpesviruses were detected from ocular samples: OtHV-4 in a California sea lion and PhHV-6 in three northern elephant seals and a Pacific harbor seal. Both novel viruses were classified within the gammaherpesvirus subfamily and clustered closely with other known pinniped herpesviruses. It is interesting that PhHV-6 was amplified in both elephant seal and harbor seals. While interspecies transmission was not explicitly demonstrated, the results suggested that individuals from the two species were infected with the same potential pathogen and therefore indicated the potential for interspecies transmission.

An increased number of California sea lions and northern elephant seals had herpesviral DNA in ocular samples, due in particular to the high prevalence of OtHV-2 and PhHV-3. OtHV-2 has been detected in ocular lesions in California sea lions with necrotizing keratitis[9]; therefore, it was not surprising to find this virus. However, the virus

was also present in 64% of animals without corneal lesions. Similarly, PhHV-3 was detected in both animals with and without corneal lesions. Thus, it is not known whether the presence of herpesviruses indicated a primary infection or the reactivation of a latent infection, as herpesviruses frequently enter latency after initial infection and may reactivate during periods of stress.[54] Viral DNA has been detected in the corneas of cats with feline herpesvirus 1 and humans with herpes simplex virus 1 without the presence of active lesions, indicating that herpesviruses may be detected during periods of latency and without signs of ocular disease.[18,55] Similarly, studies on equine herpesvirus 2 have shown contradicting results with some identifying viral DNA in a greater proportion of horses without keratoconjunctivitis than in those with clinical signs.[56] Herpesvirus in pinnipeds may behave similarly as in other species. Therefore, it is likely that herpesvirus is ubiquitous in pinnipeds and causes keratitis, conjunctivitis, or both in many animals, which can range from minor to severe, and in some animals never causes clinical signs.

While no bacteria were observed by histopathology in any samples, the three Pacific harbor seals with active keratitis had a neutrophilic response that could indicate an initial primary or secondary bacterial infection that may have resolved post-treatment. The use of systemic and topical ophthalmic antibiotics could have affected the results of the bacterial cultures and thus may explain the low number of animals from which bacteria were cultured. Some of the bacteria cultured from Pacific harbor seals (*Pseudomonas*, *E. coli*, *M. morganii*, and *Moraxell*a) have been described in previous studies on conjunctival swabs,[31,33,57] but some were less common, including *Klebsiella*, *Corynebacterium*, *B. hudsonensis*, and *Psychrobacter*.

The presence of *Moraxella*-like sp. warrants further investigation as *Moraxella bovis* is the cause of infectious bovine keratitis (IBK)[58] and *Moraxella ovis* causes infectious keratoconjunctivitis (IKC) in sheep, goats, and cattle.[59] One study found that ultraviolet light or corneal damage prior to inoculation with *M. bovis* was often necessary for infection to occur and that isolation increased during spring and summer with peak prevalence when ultraviolet radiation was the highest.[58] Thus further studies investigating the prevalence of *Moraxella* sp. in combination with predisposing factors such as UV light, trauma, or water quality may be of clinical value.

Although environmental factors were not assessed in this study, their potential impact on the development of ocular lesions in stranded and captive pinnipeds cannot be discounted. Excessive exposure to sunlight, poor water quality, and the use of oxidants in life support systems have been identified in previous studies as potential causes and complications of ocular problems in pinnipeds in captivity.[3,4,60] However, our results were not suggestive of an environmental cause for the development of ocular lesions as some animals were admitted with lesions and others developed lesions during rehabilitation, but had no spatial or temporal clustering of cases suggestive of an environmental cause. The focus of this study was to investigate viral epidemiology in ocular lesions, although environmental factors could have been involved in the pathogenesis of the varying ocular lesions investigated in this study. Future studies should examine the potential interplay of environmental factors and infectious disease in the development of ocular lesions in captive and stranded pinnipeds.

One limitation of this study was that ocular exams were performed by experienced marine mammal veterinarians, but not by a veterinary ophthalmologist or with the use of a slit-lamp biomicroscope and special stains such as Rose Bengal that can aid with the detection of probable virus-induced lesions. Therefore, subtle lesions including dendritic ulcerations often caused by herpesviruses may have been missed. Examinations were performed in a brightly lit environment, which causes significant miosis and thus precludes examination of the lens, vitreous, and retina and makes assessment of flare difficult. Consequently, both anterior uveitis and other intraocular diseases may have been missed. Another limitation was that the controls were animals that died, often due to a systemic underlying disease, which was likely a significant stressor and potentially resulted in reactivation of a latent herpesvirus infection. Also, undoubtedly, nearly all animals were stressed due to being in a captive environment. Therefore, the presence of underlying disease and the captive environment potentially contributed to the high detection rate of herpesvirus. Although animal handling and disinfectant procedures were in place to limit spread between animals, contact between animals in the same pens could have also contributed to the high detection rate of viruses. Future studies should collect samples from these species in the wild to determine the prevalence of these viruses in free-ranging populations.

In conclusion, viral infection did not correlate with the presence of ocular lesions. The high prevalence of several viruses in ocular samples indicated that viruses in the eyes of pinnipeds were common and should be considered in future evaluations of corneal lesions in these species. However, a positive viral test should not necessarily preclude release of an otherwise healthy animal, as viral infection may not be the cause of disease. Further data are needed on the prevalence of viruses in wild pinniped populations, and a correlation with other potential sources of systemic infection that may be associated with ocular disease to address this issue. The comparison of infectious agents in ocular tissues of individuals with and without lesions provided contrasting results and highlighted the difficulty of determining whether an infectious agent acts as primary pathogen in ocular lesions. As the loss of sight is a factor that may preclude an animal from being released and treatment of ocular lesions is challenging and stressful to the animal, it is imperative to continue to pursue an understanding of the pathogenesis of pinniped corneal lesions.

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REFERENCES

1. Greenwood A. Prevalence of ocular anterior segment disease in captive pinnipeds. *Aquatic Mammals* 1985; **1**: 13–15.
2. Stoskopf MK, Zimmerman S, Hirst LW *et al.* Ocular anterior segment disease in northern fur seals. *Journal of the American Veterinary Medical Association* 1985; **187**: 1141–1144.
3. Colitz C, Renner M, Manire C *et al.* Characterization of progressive keratitis in Otariids. *Veterinary Ophthalmology* 2010; **13**: 47–53.
4. Colitz CMH, Saville WJA, Renner MS *et al.* Risk factors associated with cataracts and lens luxations in captive pinnipeds in the United States and the Bahamas. *Journal of the American Veterinary Medical Association* 2010; **237**: 429–436.
5. Miller S, Colitz CMH, St Leger J *et al.* A retrospective survey of the ocular histopathology of the pinniped eye with emphasis on corneal disease. *Veterinary Ophthalmology* 2013; **16**: 119–129.
6. Gerber JA, Roletto J, Morgan LE *et al.* Findings in pinnipeds stranded along the central and northern California coast, 1984–1990. *Journal of Wildlife Diseases* 1993; **3**: 423–433.
7. Braun R, Paulson M, Omphroy C *et al.* Corneal opacities in Hawaiian monk seals (abstract). *Annual Meeting of the IAAAM* 1996; **27**: 97–99.
8. Haulena M, McKnight C, Gulland F. Acute necrotizing keratitis in California sea lions (*Zalophus californianus*) housed at a rehabilitation facility (abstract). *Annual Meeting of the IAAAM* 2003; **34**: 193–194.
9. Nollens H, Jacobson E, Beusse D *et al.* A novel herpesvirus associated with necrotizing keratitis in the California sea lion (abstract). *Annual Meeting of the IAAAM* 2004; **35**: 109.
10. Levine G, Braun R, Gulland F *et al.* Idiopathic corneal edema in a Monk seal pup hand reared from birth (abstract). *Annual Meeting of the IAAAM* 2009; **40**: 177–183.
11. Davidson MG, Nelms SR. Diseases of the canine lens and cataract formation. In: *Veterinary Ophthalmology*, 4th edn. (ed. Gelatt KN) Blackwell Publishing, Ames, IA, 2007; 859–887.
12. Gelatt KN. *Essentials of Veterinary Ophthalmology*. Lippincott Williams and Wilkins, Philadelphia, PA, 2000.
13. Tolar EL, Hendrix DVH, Rohrbach BW *et al.* Evaluation of clinical characteristics and bacterial isolates in dogs with bacterial keratitis: 97 cases (1993–2003). *Journal of the American Veterinary Medical Association* 2006; **228**: 80–85.
14. Townsend W. Food and fiber-producing animal ophthalmology. In: *Veterinary Ophthalmology*, 4th edn. (ed. Gelatt KN) Lippincott Williams and Wilkins, Philadelphia, PA, 2007; 1282–1287.
15. Woods L. Adenoviral diseases. In: *Infectious Diseases of Wild Mammals*, 3rd edn (eds Williams E, Barker I) Manson Publishing, London, 2001; 202–212.
16. Bartha A, Juhasz M, Liebermann H. Isolation of a bovine herpesvirus from calves with respiratory disease and keratoconjunctivitis. A preliminary report. *Acta Veterinaria Academiae Scientiarum Hungaricae* 1966; **16**: 357–358.
17. Stiles J. Ocular manifestations of systemic disease. Part 3. The horse. In: *Veterinary Ophthalmology*, 3rd edn. (ed. Gelatt KN) Lippincott Williams and Wilkins, Philadelphia, PA, 1999; 1473–1492.
18. Kaye S, Choudhary A. Herpes simplex keratitis. *Progress in Retinal and Eye Research* 2006; **25**: 355–380.
19. Gaskell R, Dawson S, Radford A *et al.* Feline herpesvirus. *Veterinary Research* 2007; **38**: 337–354.
20. Lenghaus C, Studdert M, Gavier-Widén D. Calicivirus infections. In: *Infectious Diseases of Wild Mammals*, 3rd edn. (eds Williams E, Barker I) Manson Publishing, London, 2001; 280–291.
21. Gerriets W, Joy N, Huebner-Guthardt J *et al.* Feline Calicivirus: a neglected cause of feline ocular infections? *Veterinary Ophthalmology* 2012; **15**: 172–179.
22. Robinson A, Kerr P. Poxvirus infections. In: *Infectious Diseases of Wild Mammals*, 3rd edn. (eds Williams E, Barker I). Manson Publishing, London, 2001; 179–201.
23. Kitching RP, Taylor WP. Clinical and antigenic relationship between isolates of sheep and goat pox viruses. *Tropical Animal Health and Production* 1985; **17**: 64–74.
24. Davies FG. Lumpy skin disease, an African Capripox virus disease of cattle. *British Veterinary Journal* 1991; **6**: 489–503.
25. Hailat N, Al-Rawashdeh O, Lafi S *et al.* An outbreak of sheep pox associated with unusual winter conditions in Jordan. *Tropical Animal Health and Production* 1994; **26**: 79–80.
26. Dunn JL, Overstrom NA, St. Aubin DJ. An epidemiologic survey to determine factors associated with corneal and lenticular lesions in captive Harbor seals and California sea lions (abstract). *Annual Meeting of the IAAAM* 1996; **27**: 108–109.
27. Carmichael LE. The pathogenesis of ocular lesions of infectious canine hepatitis: II. Experimental ocular hypersensitivity produced by the virus. *Veterinary Pathology* 1965; **2**: 344–359.
28. Ledbetter EC, Riis RC, Kern TJ *et al.* Corneal ulceration associated with naturally occurring canine herpesviruse-1 infection in two adult dogs. *Journal of the American Veterinary Medical Association* 2006; **229**: 376–384.
29. Ishiko H, Shimada Y, Konno T *et al.* Novel human adenovirus causing nosocomial epidemic keratoconjunctivitis. *Journal of Clinical Microbiology* 2008; **46**: 2002–2008.
30. Goldstein T, Colegrove KM, Hanson M *et al.* Isolation of a novel adenovirus from California sea lions *Zalophus californianus*. *Diseases of Aquatic Organisms* 2011; **94**: 243–248.
31. Johnson SP, Nolan S, Gulland FMD. Antimicrobial susceptibility of bacteria isolated from pinnipeds stranded in central and northern California. *Journal of Zoo and Wildlife Medicine* 1998; **29**: 288–294.
32. Thornton SM, Nolan S, Gulland FMD. Bacterial isolates from California sea lions (*Zalophus californianus*), Harbor seals (*Phoca vitulina*), and Northern elephant seals (*Mirounga angustirostris*) admitted to a rehabilitation center along the central California coast, 1994–1995. *Journal of Zoo and Wildlife Medicine* 1998; **29**: 171–176.
33. Lockwood SK, Chovan JL, Gaydos JK. Aerobic bacterial isolations from harbor seals (*Phoca vitulina*) stranded in Washington: 1992–2003. *Journal of Zoo and Wildlife Medicine* 2006; **37**: 281–291.
34. Goldstein T, Mazet JAK, Gulland FMD *et al.* The transmission of phocine herpesvirus-1 in rehabilitating and free-ranging Pacific harbor seals (*Phoca vitulina*) in California. *Veterinary Microbiology* 2004; **103**: 131–141.
35. Nollens HH, Jacobson ER, Gulland FMD *et al.* Pathology and preliminary characterization of a parapoxvirus isolated from a

California sea lion (*Zalophus californianus*). *Journal of Wildlife Diseases* 2006; **42**: 23–32.
36. McClenahan SD, Burek KA, Beckman KB *et al.* Genomic characterization of novel marine vesiviruses from Steller sea lions (*Eumetopias jubatus*) from Alaska. *Virus Research* 2008; **138**: 26–35.
37. Grieg DJ, Gulland FMD, Kreuder C. A decade of live California sea lion (*Zalophus californianus*) strandings along the central california coast: causes and trends, 1991–2000. *Aquatic Mammals* 2005; **31**: 11–22.
38. VanDevanter DR, Warrener P, Bennet L *et al.* Detection and analysis of diverse herpesviral species by consensus primer PCR. *Journal of Clinical Microbiology* 1996; **34**: 1666–1671.
39. Reid SM, Ansell DM, Ferris NP *et al.* Development of a reverse transcription polymerase chain reaction procedure for the detection of marine caliciviruses with potential application for nucleotide sequencing. *Journal of Virological Methods* 1999; **82**: 99–107.
40. Wellehan JFX, Johnson AJ, Harrach B *et al.* Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. *Journal of Virology* 2004; **78**: 13366–13369.
41. Goldstein T, Lowenstine LJ, Lipscomb TP *et al.* Infection with a novel gammaherpesvirus in Northern elephant seals (*Mirounga angustirostris*). *Journal of Wildlife Diseases* 2006; **42**: 830–835.
42. Buckles EL, Lowenstine LJ, Funke C *et al.* Otarine Herpesvirus-1, not papillomavirus, is associated with endemic tumours in California sea lions (*Zalophus californianus*). *Journal of Comparative Pathology* 2007; **135**: 183–189.
43. Felsenstein J. PHYLIP – phylogenetic inference package (version 3.2). *Cladistics* 1989; **5**: 164–166.
44. Ronquist F, Huelsinback J. MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics* 2003; **19**: 1572–1574.
45. R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2011. ISBN 3-900051-07-0, [WWW document]. URL http://www.R-project.org (accessed June 2011)
46. Maness HTD, Nollen HH, Jensen ED *et al.* Phylogenetic analysis of marine mammal herpesviruses. *Veterinary Microbiology* 2011; **149**: 23–29.
47. Davison AJ, Benkö M, Harrach B. Genetic content and evolution of adenoviruses. *Journal of General Virology* 2003; **84**: 2895–2908.
48. Greene CE. Infectious canine hepatitis and canine acidophil cell hepatitis. In: *Infectious Diseases of the Dog and Cat*, 3rd edn. (ed. Greene CE) Saunders Elsevier, St. Louis, 2006; 41–47.
49. Andrew SE. Ocular manifestations of feline herpesvirus. *Journal of Feline Medicine and Surgery* 2001; **3**: 9–16.
50. Maggs DJ. Update on pathogenesis, diagnosis, and treatment of feline herpesvirus. *Clinical Techniques in Small Animal Practice* 2005; **20**: 94–101.
51. Stiles J. Ocular infections. In: *Infectious Diseases of the Dog and Cat*, 3rd edn. (ed. Greene CE) Saunders Elsevier, St. Louis, 2006; 974–991.
52. Albert DM, Lahav M, Carmichael LE *et al.* Canine herpes-induced retinal dysplasia and associated ocular anomalies. *Investigative Ophthalmology* 1976; **15**: 267–278.
53. Schwartz GS, Holland EJ. Oral acyclovir for the management of herpes simplex virus keratitis in children. *Ophthalmology* 2000; **107**: 278–282.
54. Roizman B. Herpesviridae. In: *Field's Virology*, 3rd edn. (eds Fields BN, Knipe DM, Howley PM) Lippincott–Raven Publishers, Philadelphia, PA, 1996; 2221–2229.
55. Townsend WM, Stiles J, Guptill-Yoran L *et al.* Development of a reverse transcriptase-polymerase chain reaction assay to detect feline herpesvirus-1 latency-associated transcripts in the trigeminal ganglia and corneas of cats that did not have clinical signs of ocular disease. *American Journal of Veterinary Research* 2004; **65**: 314–319.
56. Borchers K, Ebert M, Fetsch A *et al.* Prevalence of equine herpesvirus type 2 (EHV-2) DNA in ocular swabs and its cell tropism in equine conjunctiva. *Veterinary Microbiology* 2006; **118**: 260–266.
57. Siebert U, Wohlsein P, Lehnert K *et al.* Pathological findings in harbor seals (*Phoca vitulina*): 1996–2005. *Journal of Comparative Pathology* 2007; **137**: 47–58.
58. Brown MH, Brightman AH, Fenwick BW *et al.* Infectious bovine keratoconjunctivitis: a review. *Journal of Veterinary Internal Medicine* 1998; **12**: 259–266.
59. Dubay SA, Williams ES, Mills K *et al.* Association of Moraxella ovis with keratoconjunctivitis in mule deer and moose in Wyoming. *Journal of Wildlife Diseases* 2000; **36**: 241–247.
60. Gage LJ. Captive pinniped eye problems, we can do better!. *Journal of Marine Animals and Their Ecology* 2008; **4**: 25–28.