DOI: 10.7589/JWD-D-19-00008

*Journal of Wildlife Diseases*, 56(4), 2020, pp. 873–883
© Wildlife Disease Association 2020

# CANINE DISTEMPER VIRUS IN THE SEA OTTER (*ENHYDRA LUTRIS*) POPULATION IN WASHINGTON STATE, USA

**Nancy Thomas,[1] C. LeAnn White,[1] Jeremiah Saliki,[2] Krysten Schuler,[3] Deanna Lynch,[4] Ole Nielsen,[5] J. P. Dubey,[6] and Susan Knowles[1,7]**

[1] US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA
[2] Athens Veterinary Diagnostic Laboratory, University of Georgia, 501 D. W. Brooks Drive, Athens, Georgia 30602, USA
[3] Animal Health Diagnostic Center, Cornell University College of Veterinary Medicine, 240 Farrier Road, Ithaca, New York 14850, USA
[4] US Fish and Wildlife Service, Washington Fish and Wildlife Office, 510 Desmond Drive, Suite 102, Lacey, Washington 98503, USA
[5] Department of Fisheries and Oceans Canada, Central and Arctic Region, 501 University Crescent, Winnipeg, Manitoba, Canada, R3T 2N6
[6] US Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, Maryland 20705, USA
[7] Corresponding author (email: sknowles@usgs.gov)

ABSTRACT: Before 2001, all serosurveys for morbilliviruses in sea otters (*Enhydra lutris*) in California, Washington, and Alaska, US, documented a 0% seroprevalence. The first published serologic detections of morbillivirus in sea otters occurred in 2001–02 in live-captured Washington sea otters, with a documented 80% seroprevalence. We conducted a retrospective study of sea otter cases from 1989 to 2010 compiled at the US Geological Survey, National Wildlife Health Center to identify cases of morbilliviral disease in Washington sea otters and to characterize the disease using immunohistochemistry, reverse transcription (RT)-PCR, genetic sequencing, virus isolation, and serology. We identified six cases of morbilliviral disease and 12 cases of morbilliviral infection in this population of sea otters during 2000–10. Significant histologic findings included inflammation in the white and gray matter of the brain characterized by lymphoplasmacytic perivascular cuffing, neuronal necrosis, and satellitosis in gray matter and by spongiosis, myelin degeneration, spheroids, and gemistocytes in white matter. Intranuclear and intracytoplasmic viral inclusion bodies were found in neurons, Purkinje cells, and glia. Immunohistochemistry for canine distemper virus (CDV) showed positive staining in neurons, glial cells, and cell processes. A pan-morbillivirus RT-PCR with subsequent restriction endonuclease digestion or sequencing identified CDV. Virus isolation was not successful. Two sea otters with morbilliviral encephalitis showed greater antibody titers to CDV than phocine distemper virus. Histologic changes were confined to the central nervous system and resembled neurologic canine distemper in domestic dogs. Cases of sea otters with morbilliviral infection without histologic changes could represent early infections or incompletely cleared sublethal infections. We found that morbillivirus was present in the Washington sea otter population as early as 2000, and we provide a description of the pathology of canine distemper in sea otters.

*Key words:* Encephalitis, *Enhydra lutris kenyoni*, immunohistochemistry, morbillivirus, mortality, neurologic manifestation, reverse transcription PCR.

## INTRODUCTION

Morbilliviruses first gained prominence as a cause of mass mortality in marine mammals when phocine distemper virus (PDV) decimated European harbor seal (*Phoca vitulina vitulina*) populations in the North Atlantic Ocean in 1988 (Heide-Jørgensen et al. 1992). Epidemics due to canine distemper virus (CDV) of terrestrial origin had occurred previously in land-locked Baikal seals (*Pusa sibirica*) in 1987 (Grachev et al. 1989) and subsequently in Caspian seals (*Phoca caspica*) in 2000 (Kennedy et al. 2000), but PDV, although related, was found to be antigenically and genetically distinct from CDV (Duignan et al. 2014). Other morbilliviruses known to cause disease and mortality in cetaceans, including dolphin morbillivirus and porpoise morbillivirus, are considered cetacean morbilliviruses (CMVs), with dolphin morbillivirus as the prototype (International Committee on Taxonomy of Viruses 2019).

Morbilliviral disease, whether caused by PDV, CMV, CDV, or human measles virus,



Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024

results in acute epithelial and lymphoid system damage, potentially followed by subacute-to-chronic neurologic disease and opportunistic infections. The gross and microscopic lesions and diagnostic techniques of PDV and CMV are similar to those of canine distemper in domestic dogs (Duignan et al. 2014; Van Bressem et al. 2014).

Although mass die-offs of marine mammals with morbillivirus mainly have been confined to the North Atlantic Ocean, Gulf of Mexico, and Mediterranean Sea (Duignan et al. 2014; Van Bressem et al. 2014), morbilliviruses have long been a concern for sea otters (*Enhydra lutris*), the largest North American mustelid that lives in the near-shore marine habitat of the North Pacific Ocean (Estes and Palmisano 1974). The first serodetection of morbillivirus in a marine mammal in the Pacific Ocean occurred in common dolphins (*Delphinus delphis*) that stranded in California from 1995 to 1997 (Reidarson et al. 1998). Free-ranging northern sea otters sampled in Washington in 1992–97 ($n$=14), Alaska in 1997 ($n$=72), and California from 1995 to 2000 ($n$=75) were seronegative for morbillivirus (Ham-Lammé et al. 1999; Hanni et al. 2003). The first published serodetection of morbillivirus in sea otters occurred in live-captured Washington otters in 2001–02 (Brancato et al. 2009). Serum virus neutralization showed 24 of 30 (80%) of the otters had antibodies to morbillivirus, with higher titers to CDV and PDV than to the CMV. In south-central Alaskan sea otters, PDV nucleic acid was detected in 8 of 77 (10%) of nasal swabs collected in 2004–06, and serology indicated 30 of 73 (41%) of the otters had antibodies to PDV (Goldstein et al. 2011).

Cases of morbillivirus encephalitis causing mortality were documented in Washington sea otters collected during 2004–08 (White et al. 2018). Here, we report results of a retrospective analysis of beachcast Washington sea otters submitted for necropsy to the US Geological Survey, National Wildlife Health Center (Madison, Wisconsin, USA) to identify the virus associated with morbilliviral disease in Washington sea otters and document morbillivirus-associated pathology.

## MATERIALS AND METHODS

### Case review

Cases collected during 1989–2010 ($n$=71) were reviewed for histopathologic findings compatible with morbilliviral disease, specifically for nonsuppurative encephalitis of morbilliviral or unknown origin, interstitial pneumonia, or an unexplained cause of death. An additional criterion for selection was otter deaths associated in time or place with an otter with morbilliviral disease. Beginning in 2009, necropsy tissues were routinely screened by a reverse transcription PCR (RT-PCR) technique for morbilliviruses in brain and kidney, so RT-PCR–positive cases from 2009 to 2010 ($n$=2) were also selected for this study. The selected cases were screened for evidence of morbillivirus infection by immunohistochemistry (IHC), RT-PCR, and sequencing, virus isolation, and serology.

### Immunohistochemistry

Sections of lung, kidney, and at least two sections of brain including cerebellum, brainstem, and cerebrum from each otter were examined for morbillivirus antigens by using an avidin-biotin complex IHC technique. Liver (cases 10, 13, and 19), pancreas (cases 3–5 and 18), and tongue (case 3) were examined for select cases. Sections (4 µm in thickness) were cut from paraffin blocks for IHC at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL; Stillwater, Oklahoma, USA) or Athens Veterinary Diagnostic Laboratory (AVDL; Athens, Georgia, USA). A subset of the sections was processed at both laboratories to ensure that the results were comparable. At OADDL, following heat-induced epitope retrieval with citrate buffer (pH 6.0), nonspecific binding sites were blocked with a commercially available avidin-biotin blocking kit (Zymed, San Francisco, California, USA) and normal rabbit serum (Vector Laboratories, Burlingame, California, USA). Tissue sections were incubated with a 1:1,000 dilution of commercial monoclonal antibody to CDV (cross-reactions with PDV and human measles virus; clone DV2-12, Custom Monoclonals, Sacramento, California, USA), followed by the secondary antibody biotinylated rabbit anti-mouse immunoglobulin G (IgG; DAKO, Carpenteria, California, USA). The conjugate was streptavidin–horseradish peroxidase, and the chromagen was the Nova RED substrate kit for peroxidase (Vector Laboratories). Slides were counterstained with Mayer's hematoxylin (Sigma, St. Louis, Missouri, USA). A positive control using confirmed CDV-positive tissue was included in each run of the immunohistochemical assay, and an assay control for each tissue was a duplicate slide stained using a substituted control (mouse

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024

IgG) for the primary antibody. The IHC technique at AVDL was similar, but different reagents were used. Nonspecific binding was blocked with 3% hydrogen peroxide and a commercially available blocking agent (Power Block, Biogenex, San Ramon, California, USA). The primary antibody used was a mouse monoclonal against CDV specific for nucleoprotein (VMRD, Inc., Pullman, Washington, USA) at a final concentration of 1:400. The secondary antibody was biotinylated horse anti-mouse IgG, rat absorbed (Vector Laboratories), and the conjugate streptavidin–horseradish peroxidase (DAKO). The substrate-chromogen system used 3,3′-diaminobenzidine (DAKO) to visualize the antigen-antibody complex. Tissue sections were counterstained with Gill's hematoxylin. Positive tissue controls consisted of CDV-positive canine brain; as an assay control, the primary antibody was eliminated and substituted with buffered saline containing 0.05% Tween 20.

Because protozoal infections are a common cause of encephalitis in sea otters, IHC to detect *Sarcocystis neurona* and *Toxoplasma gondii* was also conducted on cases with microscopic evidence of encephalitis. At least two brain sections from each suspect sea otter were tested at the US Department of Agriculture, Animal Parasitic Diseases Laboratory (Beltsville, Maryland, USA) by using previously described methods (Thomas et al. 2007).

### RT-PCR and sequencing

For each case meeting the criteria identified earlier, frozen brain and kidney samples were tested by RT-PCR using pan-morbillivirus primer sets previously described at OADDL (Saliki et al. 2002) and AVDL (Sierra et al. 2014). From a subset of suspect cases, other available tissues including thymus, spleen, lymph node, and lung (cases 7 and 15), pancreas (cases 4 and 5), and urinary bladder (case 16) were tested by RT-PCR. When a positive PCR result was obtained, the viral species was determined by either restriction endonuclease digestion (Saliki et al. 2002) or sequencing of the PCR products (Sierra et al. 2014).

### Virus isolation and serology

Virus isolation using SLAM-transfected Vero cells (Vero.DogSLAMtag, Kyushu University, Fukuoka, Japan; Nielsen et al. 2008) was attempted on kidney tissue that was RT-PCR positive from 10 sea otters (cases 1–8, 10, and 12) and brain (cases 1–8 and 10), liver (cases 1–5 and 7), pancreas (cases 4 and 5), spleen (case 3), or heart (case 1) from those cases.

Briefly, tissues were thawed and homogenized to give a 10% (w/v) cell-free suspension in Dulbecco's Modified Eagle's Medium/Nutrient Medium F-12 containing antibiotics (penicillin, 200 IU/mL; streptomycin, 200 μg/mL; and gentamycin, 50 μg/mL). An aliquot of 0.5 mL served as inoculum for each 75-$cm^2$ tissue culture flask containing Vero.DogSLAMtag cells. This cell line was stably transfected and expresses the canine SLAM molecule, the cell receptor for CDV attachment (Nielsen et al. 2008). Any virus present was allowed to adsorb for 1 h at 37 C before 10 mL of fresh medium containing 2% fetal calf serum was added to each flask. Flasks were incubated at 37 C, and cells were subcultured at a ratio of 1:2 every week with replenishment with fresh media for at least 6 wk or until signs of cytopathic effect consistent with morbillivirus infection (rounding up of cells, giant cell formation, and syncytia formation) were detected. Serum was submitted to AVDL or OADDL for serum neutralization assays (Garner et al. 2000) to detect antibodies to CDV and PDV.

## RESULTS

### Case review: Histopathology, IHC, and associated gross findings

Nineteen of 71 sea otter necropsy cases met the selection criteria for further investigation for morbilliviruses (Table 1). Six of the 19 sea otters (cases 1–6) had histopathologic findings consistent with morbilliviral disease, all limited to the brain (Table 2). Inflammation was widespread in both gray and white matter in the six sea otters, and most common in the brainstem, cerebellum, and cerebral medulla, or more specifically the pons, cerebellar medulla, and the corpus callosum and corona radiata. Lesions generally were more severe around ventricles or beneath the leptomeninges, such as the fourth ventricle near the rostral medullary vellum, and in the pontine nucleus. Inflammation also was present in gray matter of the spinal cord in the two sea otters (cases 5 and 6) in which this tissue was examined. Inflammation was often found near the junctions of gray and white matter or where gray and white matter were mixed (Fig. 1A). In gray matter, neuronal necrosis and satellitosis could be found (Fig. 1B), but often the predominant lesions were areas of nonspecific, diffuse-to-multifocal mononuclear

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024

TABLE 1. Necropsy summary collection information for Washington state, USA population of northern sea otters (*Enhydra lutris kenyoni*) examined during 1989–2010 for evidence of morbillivirus infection based on postmortem findings of nonsuppurative encephalitis of morbilliviral or unknown origin, interstitial pneumonia, unexplained cause of death, or associated in place or time with other morbilliviral cases.

| Case | Collection date | Collection location | Sex[a] | Length (cm) | Mass (kg) | Age class[b] | Body condition[c] |
|---|---|---|---|---|---|---|---|
| 1 | October 2004 | Ocean Shores | F | 134 | 24 | Adult | Fair |
| 2 | September 2006 | Ocean Shores | M | 147 | 29 | Adult | Poor |
| 3 | May 2007 | Rialto Beach | M | 140 | 24 | Adult | Poor |
| 4 | July 2007 | Kalaloch | M | 145 | 26 | Adult | Fair |
| 5 | July 2008 | Hobuck Beach | F | 125 | 16 | Adult | Poor |
| 6 | October 2006 | Hobuck Beach | F | 134 | 25 | Adult | Excellent |
| 7 | July 2005 | Pacific Beach | M | 138 | 22 | Adult | Emaciated |
| 8 | February 2010 | Hobuck Beach | M | 57 | 2 | Neonate | Fair |
| 9 | August 2000 | Copalis River | M | 124[d] | 38 | Adult | Good |
| 10 | July 2000 | Copalis River | M | 114 | 15 | Subadult | Good |
| 11 | August 2000 | Copalis River | F | 117 | 17 | Adult | Good |
| 12 | March 2010 | Hobuck Beach | F | 109 | 12 | Immature | Poor |
| 13 | July 2000 | Roosevelt Beach | M | 144 | 42 | Adult | Good |
| 14 | August 2003 | Kayostia Beach | F | 131 | 27 | Adult | Good |
| 15 | July 2005 | Ocean Shores | M | 138 | 26 | Adult | Emaciated |
| 16 | July 2005 | Pacific Beach | M | 143 | 29 | Adult | Fair |
| 17 | May 2007 | Ocean City | M | 122 | 20 | Subadult | Poor |
| 18 | May 2007 | Moclips River | M | 147 | 26 | Adult | Emaciated |
| 19 | July 2000 | Ocean Park | F | 125 | 20 | Adult | Good |

[a] M = male; F = female.
[b] Geraci and Lounsbury (2005).
[c] Qualitative assessment of subcutaneous, visceral, and perirenal adipose stores.
[d] Tail removed before submission, so total length could not be recorded.

cell infiltrates with variably thick perivascular cuffs of lymphocytes, astrocytes, or macrophages, and plasma cells. In white matter or mixed gray and white matter, spongy gliotic foci contained ballooning, degenerated myelin sheaths, occasional spheroids, and numerous swollen reactive astrocytes with glassy, homogenous, or finely granular cytoplasm (gemistocytes; Fig. 1C). Dense myelin tracts, such as the corpus callosum, had increased numbers of astrocytes and other glia that formed small foci or chains, and perivascular cuffs of lymphocytes, astrocytes, and plasma cells could be very thick. Inclusion bodies were few in some cases, but large glassy eosinophilic intranuclear and intracytoplasmic inclusion bodies (Fig. 1D) could be found in neurons, Purkinje cells, and glia, particularly if large foci of intense inflammation were present in gray matter. Inclusion bodies could also be found in perivascular cells in both gray and white matter. Some intracytoplasmic inclusion bodies were so large that they filled the cell. The leptomeninges had mild perivascular lymphocyte aggregates when located adjacent to parenchymal lesions. In two of the six sea otters, a small number of syncytia were found in the leptomeninges (Fig. 1E).

Immunohistochemistry for CDV antigen was positive in neurons, glial cells, and cell processes in all six of these sea otters (Fig. 1F, inset). The CDV antigen was detected in few to many cells within inflammatory foci in brain from five of the six sea otters but in only three isolated neurons in the sixth sea otter (Table 2). Brain from two of the sea otters with morbilliviral encephalitis contained single foci of protozoal encephalitis: in one sea otter, inflammation surrounded several protozoal schizonts (*Sarcocystis* sp.) in the cerebellar

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024

TABLE 2. Histology, immunohistochemistry (IHC), PCR results, and diagnosis for Washington state, USA population of northern sea otters (*Enhydra lutris kenyoni*) examined during 1989–2010 for evidence of morbillivirus infection.

| | Severity of lesions[a,b] | | | | IHC label abundance[c] | | | PCR[d] | | SN titer[e] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case | Polioencephalitis | Leukoencephalitis | Astrocytosis | Inclusion bodies | Brain | Kidney | Lung | Brain | Kidney | CDV[f] | PDV[g] | Diagnosis[h] |
| Morbillivirus encephalitis[i] | | | | | | | | | | | | |
| 1 | ++++ | ++ | + | +++ | ++++ | + | – | + | + | 64 | 32 | ME, SN |
| 2 | +++ | + | ++++ | +++ | ++++ | – | – | + | + | U | U | ME |
| 3 | +++ | +++ | +++ | + | ++ | – | – | + | + | 64 | 16 | ME |
| 4 | ++ | ++++ | ++ | + | ++ | – | – | + | + | U | U | ME |
| 5 | ++++ | ++++ | ++++ | ++ | ++++ | – | – | + | – | U | U | ME |
| 6 | ++ | ++++ | +++ | + | + | – | – | + | + | U | U | ME, TR |
| Morbillivirus infection[j] | | | | | | | | | | | | |
| 7 | +++ | + | – | – | + | – | – | + | + | U | U | SN |
| 8 | – | – | – | – | + | – | – | + | + | – | – | UN |
| 9 | – | – | – | – | + | – | – | – | – | NA | NA | UN, TR |
| 10 | NA | NA | NA | NA | – | – | – | + | + | NA | NA | TG |
| 11 | NA | NA | NA | NA | – | – | – | + | – | NA | NA | UN |
| 12 | NA | NA | NA | NA | – | – | – | – | + | NA | NA | SN |
| 13 | NA | NA | NA | NA | – | – | – | – | + | U | U | SN, TG |
| 14 | NA | NA | NA | NA | – | – | – | – | + | NA | NA | TR |
| 15 | NA | NA | NA | NA | – | – | – | – | + | U | U | SN |
| 16 | NA | NA | NA | NA | – | – | – | – | + | U | U | SN |
| 17 | NA | NA | NA | NA | – | – | – | – | + | – | – | SN |
| 18 | NA | NA | NA | NA | – | – | – | – | + | U | U | SN |
| Morbillivirus negative[k] | | | | | | | | | | | | |
| 19 | NA | NA | NA | NA | – | – | – | – | – | U | U | UN |

[a] None (–); mild (+) to severe (++++).

[b] NA = not applicable; inflammation in brain was not attributed to morbillivirus.

[c] Negative (–); weak (+) to strong (++++).

[d] Morbillivirus PCR: negative (–), positive (+).

[e] Serum neutralization titers against CDV or PDV that were greater than or equal to were considered positive; (–) = negative; U = unsuitable due to hemolysis; NA = not applicable; case 8 was considered negative, but the sample was very hemolyzed and could not be read at lower dilutions.

[f] CDV = canine distemper virus.

[g] PDV = phocine distemper virus.

[h] ME = morbillivirus encephalitis; SN = *Sarcocystis neurona* encephalitis; TR = trauma; UN = undetermined; TG = *Toxoplasma gondii* encephalitis.

[i] Morbillivirus encephalitis = animal is PCR and IHC positive for morbillivirus and has encephalitis caused by morbillivirus.

[j] Morbillivirus infection = animal is PCR or IHC positive for morbillivirus and has encephalitis not caused by morbillivirus or does not have encephalitis.

[k] Morbillivirus negative = animal tested negative for morbillivirus by PCR and IHC.

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024

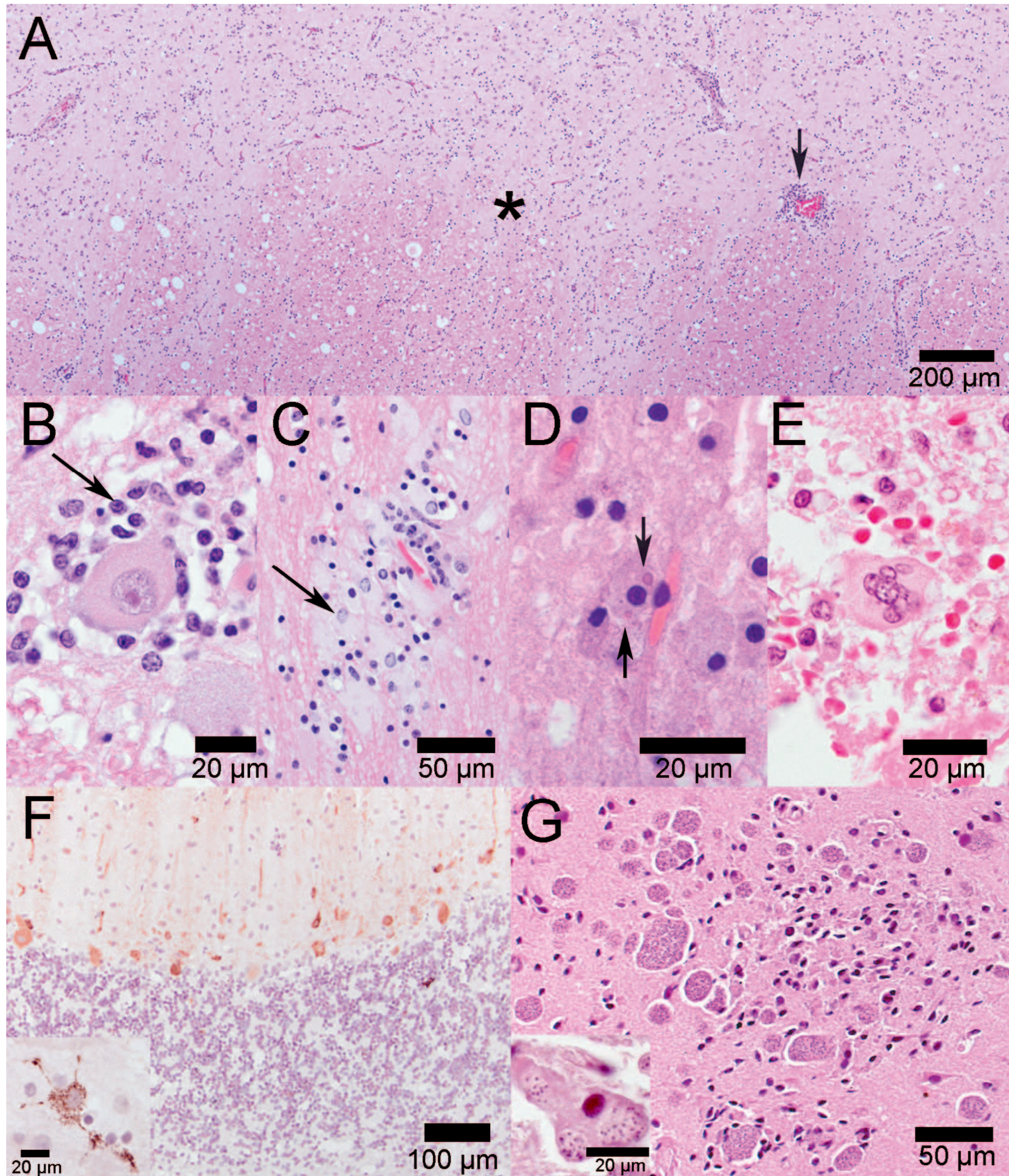


FIGURE 1. Photomicrographs of brain collected from sea otters (*Enhydra lutris kenyoni*) found moribund or dead in Grays Harbor and Clallam counties, Washington, USA. (A) Lymphoplasmacytic perivascular cuffing (arrow) and gliosis are evident near the junctions of gray and white matter (*). Vacuolation is present in the white matter. H&E stain. (B) In gray matter, neurons are occasionally surrounded by glial cells (arrow; satellitosis). H&E stain. (C) In white matter, gliotic foci contain numerous swollen reactive astrocytes with glassy, homogenous, or finely granular cytoplasm (arrow; gemistocytes). H&E stain. (D) Intracytoplasmic eosinophilic inclusion bodies (arrows) are in a glial cell. H&E stain. (E) A syncytial cell is observed within the leptomeninges. H&E stain. (F) There is positive immunoreactivity to CDV within Purkinje cells in the cerebellum. The inset shows a higher magnification of a Purkinje cell and its processes with positive immunoreactivity to CDV. 4plus Streptavidin Horseradish Peroxidase technique. (G) In the cerebrum of a sea otter with severe disseminated toxoplasmosis, there is an inflammatory focus with many neurons containing intracytoplasmic *Toxoplasma gondii* tachyzoites. The inset shows a higher magnification of a neuron with intracytoplasmic *T. gondii* tachyzoites. H&E stain.

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024

molecular layer (case 1); in another, a small protozoal tissue cyst morphologically resembling *T. gondii* was embedded within an inflammatory focus (case 6; Thomas et al. 2007).

No gross lesions other than inadequate body fat were found in five of the six sea otters with encephalitis, but the sixth case had a peracute puncture wound in the skull and thoracic blunt traumatic injuries and plentiful body fat, and this was the only encephalitis case that had ingesta in its gastrointestinal tract. Gross or microscopic pulmonary edema was the only consistent abnormality in lung tissue (five of six animals), and in all six animals CDV IHC was negative in lung (Table 2). A qualitative assessment of lymphoid tissues showed lymphoid depletion in three sea otters, but no lymphocyte necrosis was found. Eosinophilic cytoplasmic inclusion bodies were present in transitional epithelium in the renal pelvis in one sea otter (case 1), and CDV IHC was positive in three renal tubular epithelial cells in the kidney section from that animal but negative in the other five animals (Table 2). Hepatic biliary tubular epithelium in three sea otters (cases 10, 13, and 19) and pancreatic duct epithelium in four sea otters (cases 3–5 and 18) also had eosinophilic cytoplasmic inclusions, but all of these sections were negative by CDV IHC. One sea otter had multiple small gross and microscopic foci of hyperkeratosis and dyskeratosis on the ventral aspect of the tongue (case 3), but this lesion was negative by CDV IHC. One sea otter (case 1) had lesions of acute septicemia; *Alcaligenes* sp. bacteria (opportunistic organism common in the marine environment) were isolated in pure culture from multiple organs. Mature protozoal tissue cysts in skeletal muscle of five of the six sea otters had the morphology of *Sarcocystis* sp. (five of six) or *T. gondii* (one of six).

Thirteen additional cases met the selection criteria of nonsuppurative encephalitis, interstitial pneumonia, or unexplained cause of death. The primary diagnoses in the cases with nonsuppurative encephalitis included *S. neurona* ($n$=6), dual *S. neurona* and *T. gondii* infection ($n$=1; Lindsay et al. 2001), disseminated toxoplasmosis ($n$=1), or trauma ($n$=1). One sea otter (case 14) with fatal head trauma and mild encephalitis also had interstitial pneumonia of unknown cause. The cause of death was undetermined for four otters, one of which also had trauma (Table 2). One sea otter (case 18) with *S. neurona* encephalitis also had pneumonia and sepsis due to *Streptococcus* sp., *Escherichia coli*, and *Pasteurella multocida*. The CDV IHC was positive in brain from three of these 13 cases (Table 2), including one sea otter with *S. neurona* encephalitis and two sea otters with no gross or microscopic brain lesions. However, in these three cases only one to three individual cell bodies in brain were IHC positive, and these were not associated with inflammation. One of these otters (case 9) was RT-PCR negative for morbillivirus, but tissues were decomposed, which could account for the negative result. Lung and kidney sections from all 13 cases were negative by CDV-IHC (Table 2).

Immunohistochemistry for *S. neurona* and *T. gondii* detected actively dividing stages of *S. neurona* (schizonts and merozoites) widespread in brain sections from seven sea otters infected with morbillivirus, including one case (case 13) in which actively dividing *T. gondii* (tachyzoites) was also widespread (Table 2). One sea otter had a severe disseminated *T. gondii* infection that included tachyzoites and small groups of *T. gondii* organisms in inflammatory foci in all areas of the brain (case 10). More than 40 small clusters were associated with one cerebral focus, and multiple groups could be found within single neurons (Fig. 1G, inset). No microscopic lesions could be distinguished in other organs in this moderately decomposed, previously frozen sea otter carcass. However, IHC demonstrated that tachyzoites and small groups of *T. gondii* were present in all tissues tested (brain, heart, lung, liver, kidney, skeletal muscle, adrenal gland, pancreas, lymph node, and intestine), including a striking number of tachyzoites in the lamina propria of the intestine.

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024

### RT-PCR and sequencing

Brain tissues from 10 of the selected 19 cases were morbilliviral RT-PCR positive (Table 2). The morbillivirus was identified as CDV in six sea otters (cases 1–5 and 7) by sequencing of the PCR products. An attempt to sequence the PCR product from case 8 was unsuccessful.

Kidney tissue from 15 of the 19 selected sea otters was RT-PCR positive for morbillivirus, including most of the sea otters with morbilliviral encephalitis (5 of 6) and most sea otters with PCR-positive brain tissue (8 of 10; Table 2). All of the additional tissues tested were RT-PCR negative, including tissue pools with lymphoid organs from three cases (two of which also included lung), pancreas from two cases, and urinary bladder from one case.

### Virus isolation and serology

No virus was isolated from any of the 29 frozen tissue samples from 10 sea otters submitted for testing. Sera from two otters with morbilliviral encephalitis contained antibodies against both viruses with the anti-CDV antibody titer greater than the anti-PDV titer by one and two dilutions (Table 2).

### Case review: Field data

No sea otter necropsy cases before 2000 (*n*=14) met the selection criteria for inclusion in this study; however, only a small proportion of dead-stranded sea otters in Washington were necropsied during this time. Morbilliviral encephalitis mortalities occurred from 2004 to 2008: one in 2004, two in 2006, two in 2007, and one in 2008 (Table 2). Morbillivirus infections unaccompanied by lesions were detected from 2000 to 2010, including a cluster of four infections in 2000: one in 2003, three in 2005, two in 2007, and two in 2010.

The sea otters with morbilliviral encephalitis were found in four locations on the outer Washington coast (Table 1). Sea otters with morbilliviral infections without disease were found primarily south of the offshore range with the exception of three in Clallam County (Table 1). The cluster of four infections in 2000 occurred at the Copalis River (*n*=3) and Roosevelt Beach (*n*=1).

Three adult male and three adult female sea otters were diagnosed with morbilliviral encephalitis. The 12 sea otters with morbilliviral infections without disease included eight adults, two subadults, one immature, and one neonatal pup; only three females were present in this group, but the sex distribution in all sea otter necropsied during this period was similar (White et al. 2013).

Three sea otters with morbilliviral encephalitis were found alive on beaches (cases 1, 3, and 5); they were described as lethargic with labored breathing and progressive weakness. Hind leg paresis was noted for two of these animals (cases 3 and 5), but the seizures or tremors that may accompany protozoal encephalitis in sea otters (Thomas et al. 2007) were not observed.

## DISCUSSION

This study describes the pathology of morbillivirus in sea otters and identifies CDV as the causative virus by RT-PCR, sequencing, and IHC, although virus isolation was unsuccessful. Serology was also supportive. Pathology was confined to the central nervous system and resembled neurologic CDV infection in other wild and domestic species (Williams 2001). The principal diagnostic features were inflammation that was prominent in white as well as gray matter of the brain, particularly affecting the brainstem, cerebellar medulla, and corpus callosum, characterized by astrocyte hypertrophy and spongy change in white matter and the presence of intracytoplasmic and intranuclear inclusion bodies. The virus could be detected by RT-PCR in brain, and viral antigen could be visualized by IHC in inflammatory foci and sometimes was widespread in neurons, glia, and their processes. We found no lesions in the respiratory tract, lymphoid system, or renal system; however, virus was detected by RT-PCR in kidney from most sea otters with neurologic lesions and by IHC in renal tubular cells in one case. Syncytia were seen

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024

occasionally but only in the leptomeninges. Viral inclusion bodies usually were few, intracytoplasmic, and found in the brain; they were usually large but highly variable in size and number per cell. Intracytoplasmic viral inclusion bodies were present in kidney from one sea otter. We found no evidence that epithelial cytoplasmic inclusion bodies in pancreatic ducts and bile ducts were viral. Pancreatic tissue was negative for morbilliviruses by CDV-IHC and RT-PCR in the two sea otters with pancreatic inclusions. Virus isolation was negative for all submitted tissues. It is possible that these cases represented later stage infections where the viral load was decreased compared with acute-stage infections and was not sufficient for isolation. Virus isolation was conducted in 2009 and 2010, and some tissues were held frozen for up to 9 yr, which also could have decreased the viral load.

Pathology associated with morbilliviral disease in sea otters differed from that observed in pinnipeds and cetaceans dying during morbilliviral epizootics (Duignan et al. 2014; Van Bressem et al. 2014). In those outbreaks, severe systemic disease accompanies neurologic lesions so that viral inclusions and antigen are readily detected within the lung, in lymphoid tissue, and in other epithelial sites. Morbillivirus-associated pneumonia includes syncytial cells and intracytoplasmic and intranuclear inclusion bodies as diagnostic features. Nonsuppurative encephalitis often accompanies pneumonia, and astrocytosis, neuronal necrosis, foci of demyelination, and intracytoplasmic and intranuclear inclusion bodies are characteristic. Lymphocytolysis and lymphoid depletion are evident in the lymphoid organs. Viral antigen can be detected in the lesions as well as other epithelial sites by IHC and molecular techniques such as RT-PCR (Kennedy 2001; Di Guardo et al. 2005). Localized neurologic morbilliviral disease in the absence of systemic lesions has been reported in individual cetacean mortalities, most notably in Mediterranean striped dolphins (*Stenella coeruleoalba*) in years following the first epizootic in that species and region (Domingo et al. 1995). In striped dolphin cases, lesions were confined to the central nervous system and resembled chronic localized morbillivirus encephalitis in dogs and terrestrial wildlife that survive the acute systemic stage of distemper (Deem et al. 2000).

Concurrent infections occur commonly in dogs and wildlife as a complication of morbillivirus infections, as opportunistic organisms gain footholds after lymphoid destruction in the systemic stage of the disease (Williams 2001). One sea otter that died among a cluster of mortality in 2000 had a severe *T. gondii* infection, and another had reactivated latent toxoplasmosis encephalitis (Lindsay et al. 2001), suggesting these animals were immunocompromised. Morbillivirus also was detected in kidney from several other sea otters with protozoal encephalitis; however, protozoal encephalitis is found at necropsy in Washington sea otters in the same prevalence as the morbillivirus-positive sea otters in this study (White et al. 2018), so these concurrent infections may have been coincidental.

Morbillivirus was detected in tissues from sea otters with no lesions of morbilliviral disease. Five sea otters had PCR or IHC evidence of morbillivirus in brain tissue, although no morbilliviral lesions were found. These include a neonatal sea otter that still retained meconium in its colon, suggesting the possibility of vertical transmission (case 8). Any IHC-positive cells in these cases were few and remote from inflammation or other abnormalities.

Morbillivirus also was detected by RT-PCR in kidneys from 10 of 13 sea otters with no evidence of morbilliviral disease, and this was the only positive tissue in seven of these animals. It is not known whether these sea otters were very early in the course of infection or whether they had incompletely cleared sublethal infections.

No antibodies to CDV and PDV were detected in the Washington sea otter population during 1992–97, and the authors of that study suggested that such naïve sea otter populations may be vulnerable to high mortality from the morbilliviruses' introduction (Ham-Lammé et al. 1999). A large mortality

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024

event did occur in the population in 2000 with 18 reported carcasses in July and August, most too decomposed for necropsy. When the population was sampled again in 2001–02, morbillivirus exposure was found in 80% of the clinically healthy sea otters throughout their primary range (Brancato et al. 2009). High prevalence of morbilliviral antibodies following morbilliviral epizootics has been reported in other marine mammal species (Hall et al. 2006; Raga et al. 2008). In our retrospective analysis, morbillivirus was detected in four of the five sea otters examined from the 2000 mortality event (cases 9–11 and 13), including one sea otter with severe disseminated toxoplasmosis (case 10), but lesions indicative of morbillivirus were not found in any cases until 2004. In summary, morbillivirus was present in Washington sea otters dying during the 2000 event, but we have no definitive evidence that infection was associated with disease.

Molecular evidence indicates that the CDV strain that caused Baikal seal mortality was of terrestrial origin (Mamaev et al. 1995). A terrestrial origin also has been proposed for the CDV strains that caused Caspian seal deaths (Forsyth et al. 1998) and seroconversion in polar bears (*Ursus maritimus*; Garner et al. 2000). Distemper was reported in river otter (*Lontra canadensis*) pups from a coastal British Columbia island that neighbors Washington (Mos et al. 2003), and encounters between sea otters and river otters or terrestrial carnivores are possible. Regardless of the origin of the virus affecting the Washington sea otter population, CDV seroprevalence declined to 3 of 30 (10%) in this population as of 2011 (White et al. 2013), potentially leaving a large portion of the population again susceptible should a morbillivirus be reintroduced.

## ACKNOWLEDGMENTS

The specimens included in this dataset were reported to the Washington Sea Otter Stranding Network by members of the public, staff of the Olympic National Park, and members of the Makah Tribe and Quinault Indian Nation; we thank them for their time and efforts. We also thank the staff of the US Fish and Wildlife Service's Washington Field Office and the Washington Department of Fish and Wildlife for assistance with retrieval and submission of carcasses. We thank Darlene Giracello (Oklahoma State Veterinary Diagnostic Laboratory) and Abbie Butler (College of Veterinary Medicine, University of Georgia) for technical assistance in IHC and the staff of the National Wildlife Health Center Wildlife Disease Diagnostic Laboratories Branch for diagnostic support. We thank Yasuke Yanagi (Department of Virology, Kyushu University, Fukuoka, Japan) for providing the Vero.Dog-SLAMtag cell line. The use of trade, firm or product names is for descriptive purposes only and does not imply endorsement by the US Government. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the US Fish and Wildlife Service.

## LITERATURE CITED

Brancato MS, Milonas L, Bowlby CE, Jameson R, Davis JW. 2009. Chemical contaminants, pathogen exposure and general health status of live and beach-cast Washington sea otters (*Enhydra lutris kenyoni*). *Marine Sanctuaries Conservation Series ONMS-09-01*. US Department of Commerce, National Oceanic and Atmospheric Administration, Office of National Marine Sanctuaries, Silver Spring, Maryland, 181 pp.

Deem SL, Spelman LH, Yates RA, Montali RJ. 2000. Canine distemper in terrestrial carnivores: A review. *J Zoo Wildl Med* 31:441–451.

Di Guardo G, Marruchella G, Agrimi U, Kennedy S. 2005. Morbillivirus infections in aquatic mammals: A brief overview. *J Vet Med A Physiol Pathol Clin Med* 52:88–93.

Domingo M, Vilafranca M, Visa J, Prats N, Trudgett A, Visser I. 1995. Evidence for chronic morbillivirus infection in the Mediterranean striped dolphin (*Stenella coeruleoalba*). *Vet Microbiol* 44:229–239.

Duignan PJ, Van Bressem MF, Baker JD, Barbieri M, Colegrove KM, De Guise S, De Swart RL, Di Guardo G, Dobson A, Duprex WP, et al. 2014. Phocine distemper virus: Current knowledge and future directions. *Viruses* 6:5093–5134.

Estes JA, Palmisano JF. 1974. Sea otters: Their role in structuring nearshore communities. *Science* 185: 1058–1060.

Forsyth MA, Kennedy S, Wilson S, Eybatov T, Barrett T. 1998. Canine distemper virus in a Caspian seal. *Vet Rec* 143:662–664.

Garner GW, Evermann JF, Saliki JT, Follmann EH, McKeirnan AJ. 2000. Morbillivirus ecology in polar bears (*Ursus maritimus*). *Polar Biol* 23:474–478.

Geraci JR, Lounsbury VJ. 2005. *Marine mammals ashore: A field guide for strandings*. 2nd Ed. National Aquarium in Baltimore, Baltimore, Maryland, pp. 308.

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024

Goldstein T, Gill VA, Tuomi P, Monson D, Burdin A, Conrad PA, Dunn JL, Field C, Johnson C, Jessup DA, et al. 2011. Assessment of clinical pathology and pathogen exposure in sea otters (*Enhydra lutris*) bordering the threatened population in Alaska. *J Wildl Dis* 47:579–592.

Grachev MA, Kumarev VP, Mamaev LV, Zorin VL, Baranova LV, Denikina NN, Belikov SI, Petrov EA, Kolesnik VS, Kolesnik RS, et al. 1989. Distemper virus in Baikal seals. *Nature* 338:209–210.

Hall AJ, Jepson PD, Goodman SJ, Häarkönen T. 2006. Phocine distemper virus in the North and European Seas — Data and models, nature and nurture. *Biol Conserv* 131:221–229.

Ham-Lammé KD, King DP, Taylor BC, House C, Jessup DA, Jeffries S, Yochem PK, Gulland FMD, Ferrick DA, Stott JL. 1999. The application of immuno-assays for serological detection of morbillivirus exposure in free ranging harbor seals (*Phoca vitulina*) and sea otters (*Enhydra lutris*) from the western coast of the United States. *Mar Mamm Sci* 15:601–608.

Hanni KD, Mazet JA, Gulland FM, Estes J, Staedler M, Murray MJ, Miller M, Jessup DA. 2003. Clinical pathology and assessment of pathogen exposure in southern and Alaskan sea otters. *J Wildl Dis* 39:837–850.

Heide-Jørgensen MP, Härkönen T, Dietz R, Thompson PM. 1992. Retrospective of the 1988 European seal epizootic. *Dis Aquat Org* 13:37–62.

International Committee on Taxonomy of Viruses. 2019. *Genus: Morbillivirus*. https://talk.ictvonline.org ictv-reports/ictv_online_report/negative-sense-rna-viruses/mononegavirales/w/paramyxoviridae/1183/genus-morbillivirus. Accessed November 2019.

Kennedy S. 2001. Morbillivirus infections in aquatic mammals. In: *Infectious diseases of wild mammals*, 3rd Ed., Williams ES, Barker IK, editors. Iowa State University Press, Ames, Iowa, pp. 64–76.

Kennedy S, Kuiken T, Jepson PD, Deaville R, Forsyth M, Barrett T, Van de Bildt MW, Osterhaus AD, Eybatov T, Duck C, et al. 2000. Mass die-off of Caspian seals caused by canine distemper virus. *Emerg Infect Dis* 6: 637–639.

Lindsay DS, Thomas NJ, Rosypal AC, Dubey JP. 2001. Dual *Sarcocystis neurona* and *Toxoplasma gondii* infection in a northern sea otter from Washington state, USA. *Vet Parasitol* 97:319–327.

Mamaev LV, Denikina NN, Belikov SI, Volchkov VE, Visser IKG, Fleming M, Kai C, Harder TC, Liess B, Osterhaus ADME, et al. 1995. Characterisation of morbilliviruses isolated from Lake Baikal seals (*Phoca sibirica*). *Vet Microbiol* 44:251–259.

Mos L, Ross PS, McIntosh D, Raverty S. 2003. Canine distemper virus in river otters in British Columbia as an emergent risk for coastal pinnipeds. *Vet Rec* 152: 237–239.

Nielsen O, Smith G, Weingartl H, Lair S, Measures L. 2008. Use of a SLAM transfected Vero cell line to isolate and characterize marine mammal morbilliviruses using an experimental ferret model. *J Widl Dis* 44:600–611.

Raga JA, Banyard A, Domingo M, Corteyn M, Van Bressem MF, Fernández M, Aznar FJ, Barrett T. 2008. Dolphin morbillivirus epizootic resurgence, Mediterranean Sea. *Emerg Infect Dis* 14:471–473.

Reidarson TH, McBain J, House C, King DP, Stott JL, Krafft A, Taubenberger JK, Heyning J, Lipscomb TP. 1998. Morbillivirus infection in stranded common dolphins from the Pacific Ocean. *J Wildl Dis* 34:771–776.

Saliki JT, Cooper EJ, Gustavson JP. 2002. Emerging morbillivirus infections of marine mammals: Development of two diagnostic approaches. *Ann N Y Acad Sci* 969:51–59.

Sierra E, Sánchez S, Saliki JT, Blas-Machado U, Arbelo M, Zucca D, Fernández A. 2014. Retrospective study of etiologic agents associated with nonsuppurative meningoencephalitis in stranded cetaceans in the Canary Islands. *J Clin Microbiol* 52:2390–2397.

Thomas NJ, Dubey JP, Lindsay DS, Cole RA, Meteyer CU. 2007. Protozoal meningoencephalitis in sea otters (*Enhydra lutris*): A histopathological and immunohistochemical study of naturally occurring cases. *J Comp Pathol* 137:102–121.

Van Bressem MF, Duignan PJ, Banyard A, Barbieri M, Colegrove KM, De Guise S, Di Guardo G, Dobson A, Domingo M, Fauquier D, et al. 2014. Cetacean morbillivirus: Current knowledge and future directions. *Viruses* 6:5145–5181.

White CL, Lankau EW, Lynch D, Knowles S, Schuler KL, Dubey JP, Shearn-Bochsler VI, Isidoro-Ayza M, Thomas NJ. 2018. Mortality trends in northern sea otters (*Enhydra lutris kenyoni*) collected from the coasts of Washington and Oregon, USA (2002–15). *J Wildl Dis* 54:238–247.

White CL, Schuler KL, Thomas NJ, Webb JL, Saliki JT, Ip HS, Dubey JP, Frame ER. 2013. Pathogen exposure and blood chemistry in the Washington, USA population of northern sea otters (*Enhydra lutris kenyoni*). *J Wildl Dis* 49:887–899.

Williams ES. 2001. Canine distemper. In: *Infectious diseases of wild mammals*, 3rd Ed., Williams ES, Barker IK, editors. Iowa State University Press, Ames, Iowa, pp. 50–59.

*Submitted for publication 29 November 2019.*
*Accepted 18 March 2020.*

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/56/4/873/2622039/jwd-d-19-00008.pdf by guest on 14 August 2024