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Cetacean Morbillivirus in Odontocetes Stranded along the Central California Coast, USA, 2000–15

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ABSTRACT: Effects of cetacean morbillivirus (CeMV) on dolphins vary from causing epidemics to subclinical infections. The former have been documented in the North Atlantic Ocean and Mediterranean Sea but not in the North Pacific Ocean, and the reasons for this are unknown. To explore the distribution of this virus in areas that have not experienced epidemics, we reviewed evidence for morbilliviral infection in odontocetes stranded along the California coast, US from 2000–15. Nine of 212 animals examined histologically had lesions compatible with morbilliviral infection, and 11 were tested for CeMV via reverse transcriptase-PCR. One striped dolphin (*Stenella coeruleoalba*) was PCR positive, and the sequenced product was most closely related to sequences in two strains found in cetaceans in Hawaii. This study suggests that CeMV may be a cause of morbidity and a rare contributor to mortality in cetaceans stranding along the California coast. Additional work is needed to understand CeMV distribution and host species susceptibility in this region.

Key words: *Brucella,* cetacean, marine mammal, meningitis, morbillivirus, PCR.

Cetacean morbillivirus (CeMV) causes bronchointerstitial pneumonia, lymphoid depletion, and nonsuppurative encephalitis in a variety of cetacean species. In the North Atlantic Ocean and Mediterranean Sea, epidemics with mass mortality have occurred, whereas in other regions, including the North Pacific, only sporadic mortalities or subclinical infection have been documented (Reidarson et al. 1998; West et al. 2013; van Bressem et al. 2014). The reasons for these differences are unknown. To explore the distribution of this virus in cetaceans inhabiting regions that have not experienced epidemics to date, we reviewed pathology records for evidence of morbilliviral infection in cetaceans stranding along the California coast, US.

Between February 2000 and August 2015, a full necropsy with histopathology was completed at the Marine Mammal Center on 212 odontocete cetaceans that stranded along the central California coast, from Mendocino County to Santa Barbara County (between 40°0′2″N, 124°1′14″W and 34°58′27″N, 120°38′54″W). A standard tissue set was collected in 10% neutral buffered formalin for histopathology and tissues frozen for ancillary diagnostic testing.

Archived histopathology records in the Marine Mammal Center database were reviewed to identify cases with brain, meningeal, lymph node, or lung lesions. Further testing for CeMV, or other pathogens, was elected on cases with archived frozen lung, lymph node, or brain if serology was positive for CeMV or if histopathologic findings included meningoencephalitis, lymphoid depletion, bronchointerstitial pneumonia suspicious for viral infection, syncytial cells, or inclusions, or if the animal had a secondary infection suggestive of immunosuppression.

Eleven cases met the case criteria and tissues were submitted for CeMV screening by PCR (Table 1): two harbor porpoises (*Phocoena phocoena*), three long-beaked common dolphins (*Delphinus capensis*), two Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), one striped dolphin (*Stenella coeruleoalba*), and three Risso's dolphins (*Grampus griseus*). The 11 cases included eight females and three males: two juveniles, three subadults, and six adults. All animals stranded alive but died either prior to rescue or during transport. One harbor porpoise (C-383) was pregnant.

Frozen sections of brain (either cerebrum or cerebellum) and lymph node were tested for cetacean morbillivirus by reverse transcriptase-PCR (RT-PCR) at the University of California at Davis's Marine Ecosystem Health Diagnostic and Surveillance Laboratory (n=8). Morbillivirus testing was performed using universal morbillivirus primers targeting a 429 base pair fragment of the phosphoprotein (P) gene (Barrett et al. 1993). Nucleotide sequences were obtained from the GenBank database for phylogenetic analysis, and alignments were performed using the Multiple Sequence Comparison alignment tool in Geneious Pro (Biomatters, Auckland, New Zealand). A neighbor-joining bootstrap tree (1000 replicates, Tamura-Nei model) comparing the corresponding P-gene fragments (389 bp) of known morbilliviruses was produced using PAUP* (version 4.0 software, Sinauer Associates, Sunderland, Massachusetts, USA). In addition, for the three adult Risso's dolphins, serology for CeMV using virus neutralization was completed (Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, Georgia, USA) as per previously published methods (Saliki and Lehenbauer 2001). The RT-PCR for CeMV was also completed at the Athens laboratory on lung, spleen, and lymph node from Risso's dolphin C-411 as per previously described methods (Sierra et al. 2014). The RT-PCR for CeMV was completed on brain and lymph node from Risso's dolphins C-332 and C-333 (Marine Animal Disease Laboratory, University of Florida, Gainesville Florida, USA) based on previously described methods (Tong et al. 2008). Frozen cerebrospinal fluid or brain were tested for *Brucella* sp. by real-time PCR in three animals with histologic lesions suggestive of brucellosis (Colegrove et al. 2016).

Amplifiable RNA was obtained from all samples tested. A PCR product of the expected size was amplified from the cerebellum of striped dolphin C-355 using the pan morbilliviral assay, and sequencing of 420 base pairs confirmed the presence of a cetacean morbillivirus sequence (GenBank Accession No. KX669681). The sequence was more similar to the CeMV strains detected in cetaceans from Hawaii than to strains from the Atlantic Ocean and Mediterranean Sea (99% nucleotide similarity; Fig. 1). One of three Risso's dolphins with positive serologic titers to morbillivirus had mild chronic meningoencephalitis; however, brain samples from all three dolphins were negative for CeMV via PCR.

Gross necropsy findings for striped dolphin C-355 were unremarkable, aside from an increased amount of clear cerebrospinal fluid. Histopathologic findings included moderate to marked chronic lymphocytic meningitis and ependymitis with hydrocephalus, as well as moderate lymphoid hyperplasia and plasmacytosis noted in the spleen and lymph nodes (Fig. 2). Brain was positive for *Brucella* sp. via PCR (Sierra et al. 2014). Encephalitis was not a feature in this case. Immunohistochemistry was negative on affected brain using a monoclonal antibody to canine distemper virus known to cross-react with CeMV (Stone et al. 2011).

The brain lesions in striped dolphin C-355 were highly consistent with lesions previously reported for *Brucella* sp. infections in cetaceans, with inflammation predominately located in the meninges and ependyma and little inflammation within the parenchyma (Guzmán-Verri et al. 2012). In CeMV infections, inflammation is typically more severe within the brain parenchyma (van Bressem et al. 2014). Thus, while PCR results indicated CeMV co-infection, brain lesions were more likely directly due to *Brucella* sp. infection. Lymphoid hyperplasia was consistent with chronic antigenic stimulation from brucellosis. During unusual mortality events in both the Gulf of Mexico and on the US Atlantic coast, we have noted animals with chronic CeMV infection that had few or no histologic lesions directly attributable to the viral infection, but in which viral RNA remained and was amplified by RT-PCR, as was the case for striped dolphin C-355 (van Bressem et al. 2014).

TABLE 1. Cetaceans stranded along central California coast, USA between 2000 and 2015, fitting case criteria suspicious for morbillivirus infection following necropsy. Cetaceans examined: striped dolphin (*Stenella coeruleoalba*), harbor porpoise (*Phocoena phocoena*), Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), Risso's dolphin (*Grampus griseus*), and long-beaked common dolphin (*Delphinus capensis*). Dashes indicate either that significant lesions were not found or testing was not performed for the specified animal. CSF = cerebrospinal fluid.

ID	Species	Sex	Age class	Brain lesions	Lung lesions	Lymph node lesions	Morbillivirus PCR	Additional tests
C-355	Striped dolphin	Female	Subadult	Marked lymphocytic meningitis	—	Lymphoid hyperplasia	Positive	Brucella PCR positive (CSF)
C-301	Harbor porpoise	Female	Juvenile	Focal lymphoplasmacytic encephalitis	Granulomatous eosinophilic verminous pneumonia	Lymphoid hyperplasia	Negative	
C-326	Pacific white-sided dolphin	Male	Adult	Severe suppurative and fibrinous meningoencephalitis	Moderate necrotizing interstitial pneumonia		Negative	
C-332	Risso's dolphin	Male	Adult	Moderate lymphocytic and histiocytic encephalitis	Mild granulomatous verminous pneumonia		Negative[a]	Morbillivirus serum neutralization positive CMV (1:256)
C-333	Risso's dolphin	Male	Adult	Neuronal lipofuscinosis	Mild fibrosis	Sinus histiocytosis	Negative[a]	Morbillivirus serum neutralization positive CMV (1:64)
C-339	Long-beaked common dolphin	Female	Juvenile	Moderate lymphoplasmacytic and histiocytic meningitis	—	Lymphoid hyperplasia	Negative	*Brucella* PCR negative (brain, lymph node)
C-375	Long-beaked common dolphin	Female	Subadult	Lymphoplasmacytic meningoencephalitis	Mild granulomatous verminous pneumonia	Lymphoid hyperplasia	Negative	Brucella PCR positive (CSF)
C-383	Harbor porpoise	Female	Adult	Lymphoplasmacytic and histiocytic meningoencephalitis	Eosinophilic and histiocytic bronchopneumonia	Lymph node fibrosis	Negative	—
C-411	Risso's dolphin	Female	Adult		Mild neutrophilic and histiocytic bronchopneumonia	Lymphoid hyperplasia	Negative[b]	Morbillivirus serum neutralization positive CMV (1:512)

Table 1. Continued.

ID	Species	Sex	Age class	Brain lesions	Lung lesions	Lymph node lesions	Morbillivirus PCR	Additional tests
C-453	Pacific white-sided dolphin	Female	Subadult	Severe lymphoplasmacytic meningoencephalitis	Mild pulmonary edema (agonal)	Lymphoid hyperplasia	Negative	Brucella PCR positive
C-460	Long-beaked common dolphin	Female	Adult	Mild lymphocytic encephalitis			Negative	Brucella PCR negative

[a] Previously tested negative via reverse transcription-PCR at Marine Animal Disease Laboratory.
[b] Previously tested negative via reverse transcription-PCR at University of Georgia Athens Veterinary Diagnostic Laboratory.

Morbilliviruses are highly contagious and cause serious disease with immunosuppression in their hosts (van Bressem et al. 2014). This immunosuppression may allow opportunistic or co-infection to occur with pathogens such as with *Brucella* sp. and *Toxoplasma* sp. The role of CeMV in striped dolphin C-355 was unknown, and there was no overt histologic evidence of immunosuppression or lesions that could be directly attributed to CeMV. While *Brucella* and CeMV co-infection has been previously reported in cetaceans, the potential interplay between these two organisms is poorly understood (West et al. 2015).

Our review of 212 odontocete records over the past 16 yr, and a previous study by Reidarson et al. (1998), suggested that while CeMV may play a role in cetacean morbidity in the eastern North Pacific, its direct role in mortality remains to be proven. The one case in this study that tested positive via RT-PCR did not have pathognomonic histologic lesions, nor was an organism detected by immunohistochemistry. Because only suspect cases received additional screening, RT-PCR positive animals may have been missed if they did not have lesions suggestive of morbilliviral infection, as may have occurred with chronic infection (Stephens et al. 2014). How morbillivirus contributes to morbidity and stranding in cetaceans in this region remains unclear. As has been described previously, CeMV strains are circulating in cetacean species in the North Pacific (Reidarson et al. 1998; West et al. 2013, 2015). The positive serologic titers noted in the three Risso's dolphins further suggest exposure to CeMV in some species in the North Pacific. No reported cases from California or Hawaii have had morbillivirus lesions typical of an acute infection. The eastern North Pacific is unusual in that large mortality events associated with morbillivirus have not been documented.

The morbillivirus sequence from striped dolphin C-355 was most similar to two sequences in strains detected in cetaceans in Hawaii (Fig. 1). This would be expected, as striped dolphins are a widely distributed

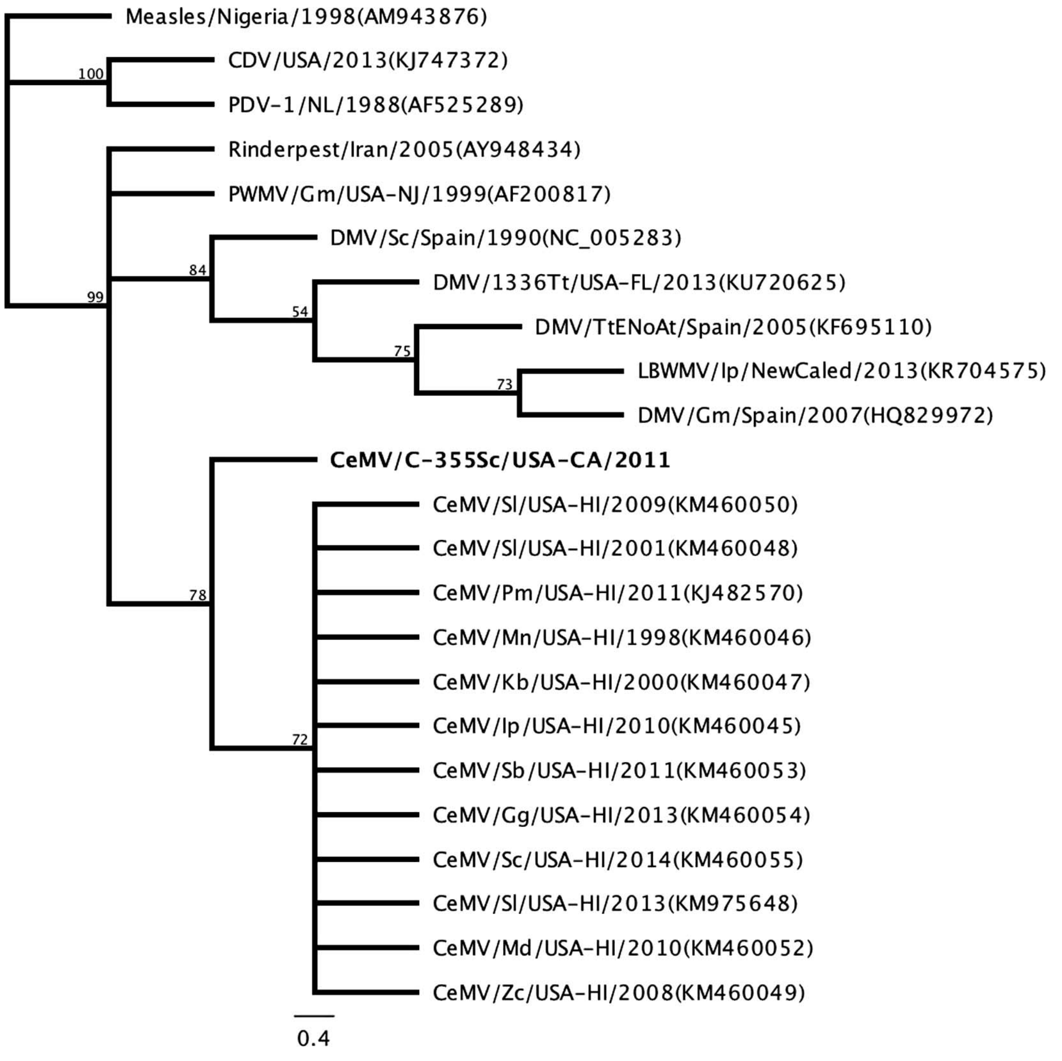

FIGURE 1. A phylogenetic tree comparing the 420 base pair sequence of the phosphoprotein gene fragment from the morbillivirus sequenced from the striped dolphin (*Stenella coeruleoalba*) C-355 (in bold, GenBank Accession No. KX669681) with other marine mammal morbilliviruses (Pestes des Petits is used as an outgroup for comparison). Sequence names include the virus name (CDV=canine distemper virus; PDV=phocine distemper virus; PWMV=pilot whale morbillivirus; DMV=dolphin morbillivirus; LBWMV=Longman's beaked whale morbillivirus; CeMV=cetacean morbillivirus), species (Gm=*Globicephala melas*; Sc=*Stenella coeruleoalba*; Tt=*Tursiops truncates*; Ip=*Indopacetus pacificus*; Sb=*Steno bredanensis*; Gg=*Grampus griseus*; Sl=*Stenella longirostris*; Pm=*Physeter microcephalus*; Mn=*Megaptera novaeangliae*; Kb=*Kogia breviceps*; Md=*Mesoplodon densirostris*; Zc=*Ziphius cavirostris*), country, year, and GenBank accession number. Scale bar indicates the nucleotide substitutions per site.

pelagic species, found in tropical and warm-temperate waters of the Atlantic, Pacific, and Indian oceans (Hammond et al. 2008). They were the principal species impacted by recurring morbillivirus epidemics in the Mediterranean beginning in 1990 (van Bressem et al. 2014). However, striped dolphin strandings are rare in California, making up only five of the 212 cetaceans screened. A better understanding is needed on the effect of CeMV on cetacean health in the North Pacific, including the distribution of virus and susceptibility of hosts in this region. Screening of tissues via RT-PCR is important in cases that show

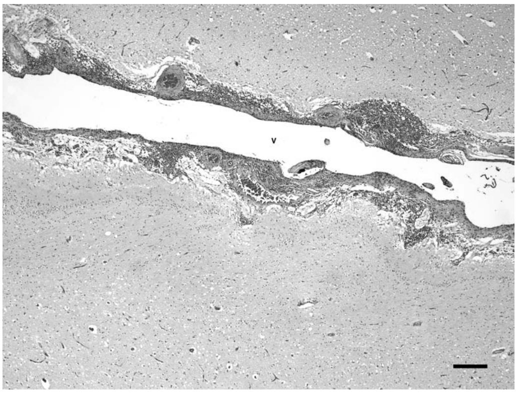

FIGURE 2. H&E stained section of cerebrum from subadult female striped dolphin (*Stenella coeruleoalba*) C-355 with lymphocytic inflammation extending along the ependyma of the entire lateral ventricle (labeled "v"). Bar=100 μm.

evidence of potential immunosuppression with opportunistic infections, because lesions associated with CeMV are often absent or are masked by the lesions caused by opportunist pathogens. However, in considering the role of CeMV in a region where the virus has not previously been confirmed, multiple diagnostic tests should be evaluated before the diagnosis of CeMV disease is accepted. Ideally there should be at least two of the following: pathognomonic lesions in at least one animal, positive immunohistochemistry, virus isolation or PCR with sequencing, or positive serology (van Bressem et al. 2014). While these criteria have not been fulfilled for any one individual animal, or even a species, in this retrospective survey, positive serology and PCR results have been obtained in two separate species, Risso's dolphins and a striped dolphin. Although not confirming the role of CeMV in cetacean morbidity and mortality on the North American Pacific coast, this study expands the known range for the virus based on a previous study (Reidarson et al. 1998). More significantly, the results provide an epidemiological link between the eastern Pacific and the mid-Pacific where a phylogenetically similar CeMV strain is known to cause cetacean mortality.

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